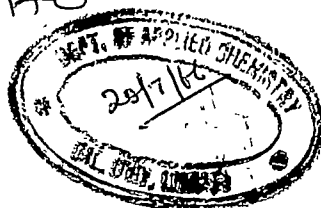


NATURE

A WEEKLY JOURNAL OF SCIENCE

COR - HO 6731-212-RP342



Volume 208

OCTOBER 2, 1965, to DECEMBER 25, 1965

212

LONDON

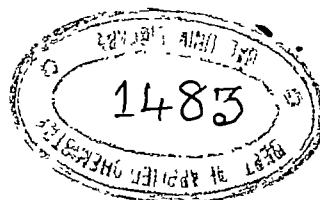
MACMILLAN (JOURNALS), LTD.
NEW YORK: ST. MARTIN'S PRESS, INC.

DATES and PAGINATION of WEEKLY ISSUES

<i>Serial No.</i>		<i>Date of issue</i>		<i>Pagination</i>
5005	...	October	2	1-100
5006	...	"	9	101-208
5007	...	"	16	209-310
5008	"	23	311-414
5009	...	"	30	415-512
5010	...	November	6	513-612
5011	"	13	613-714
5012	...	"	20	715-818
5013	...	"	27	819-920
5014	...	December	4	921-1024
5015	...	"	11	1025-1132
5016	...	"	18	1133-1240
5017		"	25	1241-1354

RP342

INDEX



- ABABEL, L. et al.** : Enzyme Regulatory Mechanisms at the Level of Lactate-oxidoreductase in Erythrocytes and Ascites Tumour Cells, 185
- Abbot, A., and Mitchell, J.** : Ultrastructure of the Antigen-retaining Reticulum of Lymph Node Follicles as shown by High-resolution Autoradiography, 500
- Abbott, Dr. D. C., Harrison, R. B., Tatton, J. O'G., and Thomson, Dr. J.** : Organochlorine Pesticides in the Atmospheric Environment, 1317
- Abe, M., Monig, H., and Koch, R.** : Influence of Oxygen and Cysteine on the Radical State of X-irradiated Lyophilized Liver, 499
- Abelson, Dr. D. M.** : Ultra-violet Spectroscopy in the Analysis of Chromatograms, 784
- Aberdeen, University of, Appointments, 436**
Chair of Genetics, 436, 833
Professor of Inorganic Chemistry, 20
- Aberdeenshire** : Corona Structures in the Basic Igneous Masses of East (Dr. D. S. Weedon), 885
- Abnormal Fibrinogen (Fibrinogen Baltimore)** : New Inherited Coagulation Disorder caused by an (Dr. E. A. Beck et al.), 143
- Aborigines** : Haptoglobin Johnson in Australian (D. W. Cooper et al.), 694
- Hereditary Ovalocytosis and Haemoglobin E-Ovalocytosis in Malayan (Dr. Lie-Injo Luan Eng), 1329**
- Portraits of Tasmanian, 230**
- Abraham, Prof. E. P.** : Antimicrobial Agents and Chemotherapy—1964, review, 980
- Absence of Diffusion Control of Reaction Rate in the Thermal Decomposition of Amorphous Polymers (D. A. Smith), 1200**
- Absolute Determination of the Acceleration due to Gravity at the National Physical Laboratory** : New (Dr. A. H. Cook), 279
- Absorption and Emission** : Anomalous Intensity Ratios of the Interstellar Lines of OH in (R. X. McGee et al.), 1193
- Absorption by the Perfused Small Intestine of Amphibia** : Hydrolysis of Disaccharides during (Dr. D. S. Parsons and J. S. Prichard), 1097
- Absorption of Carbon Dioxide by Plants** : Influence of Hexadecane on (Prof. J. M. Riedhart), 300
- Absorption of Fat** : Use of Polyethylene Glycol in Investigations of (Dr. H. Vavřínková and Dr. A. Kroudl), 293
- Absorption Spectra of Aromatic Nitrenes and Dinitrenes** : Ultra-violet (A. Reiser and V. Frazer), 682
- Absorption Spectrum of the CH₃O-radical** : Electronic (J. F. Ogilvie), 1315
- Absorption** : Transport of Methionine and Sodium Butyrate by Intracellular Plasma Mechanism of Intestinal (S. L. Hart and Dr. J. A. Nissim), 145
- (Acanthocytosis)** : Precipitin Activity in the Sera of Patients with Deficiency of β -Lipoproteins (Dr. R. F. Murray, jun., and Prof. B. S. Blumberg), 357
- Acceleration due to Gravity at the National Physical Laboratory** : New Absolute Determination of the (Dr. A. H. Cook), 279
- Accidents** : Cerebrovascular, 734
- Accretion in the Solar Nebula** : Enrichment of Iron during (Prof. S. R. Taylor), 886
- Accumulation and Incorporation of Amino-acid in Rat Intestine in vitro (Dr. J. R. Bronk and Dr. D. S. Parsons), 785**
- Accumulation of Keto-acids in Tissues during Diphtheria Intoxication (Dr. S. C. Agarwal), 914**
- Acetohydroxamate** : Bacterial Urease Inhibitor with Therapeutic Potential in Hyperammonaemic States (Dr. W. N. Fishbein and P. P. Carbone), and (H. D. Hochstein), 46
- N-Acetyl Neuraminic Acid Terminating Receptors for Myxovirus Attachment** : Nuclear Surface (Prof. P. I. Marcus et al.), 1122
- Acetylcholine on the Heart of the Mollusc *Tapes turrida*** : Biphasic Dose-response Relationship with (P. R. Carroll and G. B. Chesher), 1220
- Achondrites** : Abundances of Rare Earth Elements in Eclogitic Rocks and Basaltic (J. W. Morgan and Dr. J. F. Lovering), 1311
- Acid Mucopolysaccharide and Hexosamine Metabolism in Dermal Connective Tissue of Adrenalectomized Rats (Prof. J. Fabianek, Dr. A. Herp and Dr. L. Martinez), 1212**
- Acid Mucopolysaccharides** : Chemical Physiology of the (J. E. Scott), 1270
- Acid Phosphatases (Dr. C. A. Vernon, J. Gaudie, J. M. Hanson, J. M. Humphreys, P. E. Smith, A. J. Lawrence and Dr. B. E. C. Banks), 382**
- Acid Soils** : Measurement of Exchangeable Aluminium in (J. B. Skeen and Dr. M. E. Sumner), 712
- Ackman, Dr. R. G.** : Occurrence of Odd-numbered Fatty Acids in the Mullet *Mugil cephalus*, 1213
- Acoustic Velocity Dispersion in some Non-associated Organic Liquids (Dr. S. E. A. Hakim and W. J. Comley), 1082**
- Acridine Orange by Polyribonucleotides** : Heterogeneous (Prof. R. F. Beers, jun., and G. Armile), 466
- Acrylamide-gel Electrophoresis of β -Lactoglobulins stored in Solutions at pH 8.7 (Dr. P. Akroyd), 488**
- Acrylamide-gel Electrophoresis** : Quantitative Determination of Haemoglobin A₂ by (Lieut.-Commander T. G. Ferris et al.), 1103
- Acrylamide Gels** : Taxonomic Investigation of Several Brassica Species using Serology and the Separation of Proteins by Electrophoresis on (Dr. J. G. Vaughan et al.), 704
- Actinomycin D** : An *in vitro* Action of Human Growth Hormone in the Presence of (T. E. Martin and Prof. F. G. Young), 684
- Rapidly Labelled Synthesis of Ribonucleic Acid in Ehrlich Ascites C-2 Tumour Cells treated with (D. A. Popović), 49**
- Stimulation of Sendai Virus Multiplication by Puromycin and (Dr. D. O. White and I. M. Cheyne), 813**
- Activation of Muscle Differentiation** : Replication and the (Dr. P. Pietsch and S. B. McCollister), 1170
- Activation of Spores of *Penicillium roqueforti* (R. C. Lawrence), 801**
- Acute Radio-sensitivity as a Function of Age in Mice (Dr. J. F. Spalding, O. S. Johnson and R. F. Archuleta), 905**
- Acylating Agent** : Thalidomide as a Possible Biological (S. Fabro et al.), 1208
- Adams, Dr. D. M., and Morris, D. M.** : Far-infra-red Spectra of some Square-planar Ions, 283
- Adams, Roy M. (edited by)** : Boron, Metallo-Boron Compounds and Boranes, review by Dr. L. H. Long, 968
- Adaptive Growth (Prof. R. J. Goss), review by Dr. R. A. Weiss, 107**
- Aden Protectorate** : Stratigraphy and Structure of the Eastern (Dr. Z. R. Beydoun), review by E. E. Wellings, 615
- Adenine-specific Antibodies with Denatured Deoxyribonucleic Acid** : Reaction of (Dr. S. Estrada-Parra et al.), 1010
- Adenosine Di- and Tri-phosphates in the Presence of Calcium Phosphate Precipitates** : Transphosphorylation of (Dr. R. W. Burley), 683
- Adenosine Diphosphate-induced Aggregation of Human Platelets** : Plasma Co-factors in (Dr. D. Deykun et al.), 296
- Adenosine Monophosphate and Melanocyte-stimulating Hormone on Frog Skin in vitro** : Effects of Cylic (Dr. M. W. Bitensky and S. R. Burstein), 1282
- Adenosine Triphosphatase Systems of Rat Liver Mitochondria** : Stoichiometry of Proton Translocation through the Respiratory Chain and (Dr. P. Mitchell and Dr. J. Moyle), 147
- Adenosine Triphosphate-Metal-Norapinephrine Ternary Complexes and Catecholamine Binding (Dr. R. W. Colburn and Dr. J. W. Maas), 37**
- Adenovirus** : Association of, with Bovine Respiratory Disease (Dr. J. H. Darbyshire et al.), 307
- Adenovirus 7 and SV 40** : Mixed Infection of African Green Monkey Cells by (Dr. R. K. Wertz et al.), 1350
- Adgie, R. L., Gent, H., Slee, O. B., Frost, Prof. A. D., Palmer, Dr. H. P., and Rowson, Dr. B.** : New Limits to the Angular Sizes of Some Quasars, 275
- Adrenal Cortex in the Appearance of Rat Slow Alpha-Globulin** : Involvement of the (Prof. W. G. Ham and S. R. Ellenson), 1330
- Adrenal Medulla by Muscarinic and Pilocarpine** : Preferential Release of Adrenaline from the (Prof. W. W. Douglas and Dr. A. M. Poisner), 1102
- Adrenalectomized Rats** : Acid Mucopolysaccharide and Hexosamine Metabolism in Dermal Connective Tissue of (Prof. J. Fabianek et al.), 1212
- Adrenaline from the Adrenal Medulla by Muscarinic and Pilocarpine** : Preferential Release of (Prof. W. W. Douglas and Dr. A. M. Poisner), 1102
- Adrenals** : Aldosterone Secretion : Differences in Direct Effects of Rubidium and Potassium Perfusion into Dog (Prof. J. G. Hilton et al.), 1099
- Adrenergic Vasodilation** : Pronethalol-induced Reversal of (Dr. M. G. Moreira and Dr. W. Osswald), 1006
- Adrian, The Lord** : A History of the Rockefeller Institute 1901-1953, review, 1244
- Adsorption and the Differential Capacitance of the Electrical Double-layer at Platinum/Halide Metal Interfaces (A. D. Graves and Dr. D. Inman), 481**
- Adult Behaviour in Rats** : Effect of Foster-mothers' Strain and Pre-natal Experience on (Dr. J. M. Joffe), E15
- Adults and pre-School Children's Scribbles** : Form-similarity between Phenomena of (R. Kellogg et al.), 1129
- Advances in Enzyme Regulation, Vol. 2, (edited by G. Weber), review by C. J. R. Thorne, 319**
- Aeronomy and Ice Sheets** : Antarctic (Dr. G. de Q. Robin), 439
- 'Aerosil'** : Infra-red Spectra of Quaternary Ammonium Compounds adsorbed on Silica-gel (Dr. L. C. F. Blackman and R. Harrop), 777
- Aerosol Particles** : Ultra-purification by Separation of (Dr. S. Behrendt), 581
- Aetiology for Down's Syndrome (Mongolism)** : Virus (A. Stoller and R. D. Collmann), 903
- Aflatoxin** : Effect on Cultured Heteroploid Human Embryonic Lung Cells (Dr. M. S. Legator, S. M. Zuffante and A. R. Harp), 345
- Africa** : Introduction to the Flowering Plants of West (Mrs. M. Steentoft Nielsen), review by F. N. Hepper, 976
- Malaria and the Opening-up of Central (R. Weatherall), 1267**
- Meat from Wild Animals in, 122**
- 'Africa'** : "There is all (Dr. A. S. Thomas), 16
- African Green Monkey Kidney Cells by Adenovirus 7 and SV 40** : Mixed Infection of (Dr. R. K. Wertz et al.), 1350
- African Mammals, 1046**
- African (South) Prehistory, 22**
- African Students in Britain, review by Lord Twining, 957**
- African Wildlife** : Research and Management in East (Dr. J. Morton Boyd), 828
- Agar-Agar Diffusion Procedure to Pesticide Residue Analysis and to the Cholinesterase Screening of Candidate Pesticides** : Application of an (Dr. K. I. Beynon and G. Stoydn), 748
- Agar-gel Electrophoresis of Soluble Lens Proteins in Galactose-fed Rats (E. Wittgenstein and Dr. K. W. Rowe, jun.), 386**
- Agarwal, Dr. S. C.** : Accumulation of Keto-acids in Tissues during Diphtheria Intoxication, 914
- Age** : Altered Electrophoretic Mobilities of some Erythrocytic Enzymes as a Function of their (Dr. H. Walter et al.), 76
- Age** : Memory Changes with (Prof. D. Schonfield), 918
- Age and of Hypoxia** : Life-shortening in Mice exposed to Radiation Effects of (Dr. P. J. Lindop and Prof. J. Rotblat), 1070
- Age Determinations of North American Rocks, 435**
- Age in Mice** : Acute Radio-sensitivity as a Function of (Dr. J. F. Spalding et al.), 905
- Age of the Weardale Granite (F. J. Fitch and Dr. J. A. Miller), 743**
- Aged Mice** : Multiple Thyroid Grafts in (Dr. D. Metcalf), 87
- Ageing and in Prolapse** : In efferential Matrix of the Intervertebral Disk in (Dr. T. K. F. Taylor and Dr. K. Little), 384
- Ageing in Eastern Europe** : International Conference on Gerontology, review by Dr. David A. Hall, 981
- Ageing** : Transplantation Experiments on Placental (R. L. Simmons and J. Weintraub), 82
- Ages and Andean Uplift** : Isotopic (Dr. R. W. R. Rutland et al.), 677
- Ages** : Wilson's Theory of (J. T. Hollin), 12

- Agfa-Gevaert AG Leverkusen-München : Mitteilungen aus den Forschungslaboratorien der, Band IV, (edited by Prof. E. Klein and R. Matejec), review by K. O. Ganguin, 720
- Agglutination : Mathematical Model for the Process of Aggregation in Immune (T. K. Ming *et al.*), 84
- Agglutinin in the Snail *Helix (Helix) lactea* : A Specific (Prof. W. C. Boyd and R. Brown), 593
- Aggregates as a Cause of Failure in Concrete : Shrinking, 1261
- Aggregating and Take-off in Anthophorid Bees, *Anthophora acraensis* Fab. : Periodic (Prof. L. K. H. Goma), 807
- Aggregation and Electric Shock in Mice treated with Cocaine : Lethal Effects of (H. Lal and Dr. R. D. Chessick), 295
- Aggregation in Immune Agglutination : Mathematical Model for the Process of (T. K. Ming *et al.*), 84
- Aggregation of Human Platelets : Plasma 60-factors in Adenosine Diphosphate-induced (Dr. D. Deykin *et al.*), 296
- Aggressive Behaviour, Mental Subnormality and the XYY Male (P. A. Jacobs, M. Bruntom, M. M. Melville, Dr. R. P. Brittain and Dr. W. F. McClelland), 1351
- Agricultural Engineering : Component Failure Investigation : National Institute of, 1262
- Agricultural History, 1939-64 : Dutch Study Circle for, review by G. E. Fussell, 1138
- Agricultural Problems in Co. Limerick, Eire : Regional Geochemical Reconnaissance applied to some (Prof. J. S. Webb and W. J. Atkinson), 1056
- Agriculture, Shambat : University of Khartoum, Sudan : Silver Jubilee of the Faculty of (Prof. M. A. Nour), 620
- Aid Overseas : British Government, 1153
- Aid to Developing Countries : British, 128
- Aid to Education Overseas, 120
- (*Alurupoda melanoleuca*) : Bursa Pharyngea in the Giant Panda (Prof. A. J. E. Cave), 865
- Air Ion Effects on the Oxygen Consumption of Barley Seedlings (S. Kotaka, Prof. A. P. Krueger, P. C. Andriese, K. Nishizawa, T. Ohuchi, M. Takenobu and Y. Kozure), 1112
- Air : Towards a University of the, 715
- Aircraft : Anglo-French Military, Project Director for *Jaguar* Strike/Trainer, 527
- Aitken, Prof. A. C. : Retirement from Chair of Mathematics in the University of Edinburgh, 832
- Aiya, Prof. S. V. C. : Some Characteristics of Tropical Thunderstorms, 641
- Akroyd, Dr. P. : Acrylamide-gel Electrophoresis of β -Lactoglobulins stored in Solutions at pH 8.7, 488
- Alais and Orgueil Carbonaceous Meteorites : Evidence for Microfossils in the (S. L. Van Landingham), 947
- (α 5 Alanine \rightarrow Aspartic Acid) : A New Haemoglobin, J Toronto (Dr. J. H. Crookston *et al.*), 1059
- Alaskan Mammals, 936
- Albert Lasker Medical Research Awards, 1263
- Albumin Antibody : Rapid Detection and Titration of Antihuman Serum (Dr. M. S. Tobin), 1338
- Alcohol and Evoked Potentials in the Cat (A. K. Schweigert, Dr. A. R. David, A. H. Stewart and Dr. H. E. Himwich), 688
- Alderson, Dr. T., and Khan, A. H. : Mutagenic Effect of Irradiated and Unirradiated DNA in *Drosophila*, 700
- Aldosterone in Myocardial Infarction, 836
- Aldosterone Secretion : Differences in Direct Effects of Rubidium and Potassium Perfusion into Dog Adrenals (Prof. J. G. Hilton, Dr. S. Alter and Dr. P. Cushman), 1099
- Aldrin or Dieldrin : Metabolites in Urine of Rats on Diets containing (P. R. Datta *et al.*), 289
- Alexander, W. M., *et al.* : Measured Velocities of Interplanetary Dust Particles, 673
- Alfred, Lawrence J. : Differential Inhibition of Deoxyribonucleic Acid and Ribonucleic Acid Synthesis induced in Cultured Mammalian Cells by 7-12-Dimethylbenzanthracene, 1339
- Algae : Biology of the (Dr. F. E. Round), review by Dr. M. Parke and G. T. Boalch, 824
- Algard, Prof. F. T., Dodge, A. H., and Kirkman, Prof. H. : Androgen-Gestrogen-induced Tumours : Lactic Dehydrogenase Relative to Chaeptithelioma Induction in the Syrian Hamster, 567
- ALGOL 60 Implementation (B. Randall and L. J. Russell), review by I. M. Khabaza, 217
- Alkali, etc. Works : Inspection of, One Hundred and First Annual Report on, 1964, 234
- Alkaline Earth Metals, Handbuch der Experimentellen Pharmakologie. Band 17. Two Volumes in French (Zénon-Marcel Bacq, sub-editor), review by Prof. E. Zaimis, 720
- Alkaline Hydrolysis of Glycine Ethyl Ester : Protonation Equilibria and (Dr. M. Robson), 265
- n-Alkan-1-ols in Butter after Oxidative Deterioration (W. Stark and D. A. Forss), 190
- Allan, Dr. D. : Manual for the Identification of Medical Bacteria, review, 213
- Theory and Practice in Experimental Bacteria, review, 213
- Allan, Dr. N., Beale, D., Irvine, D., and Lehmann, Dr. H. : Three Haemoglobins K : Woolwich, an Abnormal, Cameron and Ibadan, Two Unusual Variants of Human Haemoglobin A, 658
- Allatectomized Males and Females on the Sexual Maturation of Young Male Adults of *Schistocerca gregaria* (Forsk.) (Orthoptera : Acrididae) : an Inhibitory Effect of (M. J. Norris and M. P. Pender), 1122
- Alleles in Different Botanical Varieties of *Brassica oleracea* : Identical S (K. F. Thompson and J. P. Taylor), 306
- Allen, Dr. Gordon : A Search for Mutation in Chemical Systems at Steady State, 258
- Allen, H. W., and Jolley, Dr. W. B. : Formation of Complexes between Basic Proteins of Leucocytes and Plasma Globulins, 390
- Allen, Dr. John : Laminar and Turbulent Flow in Open Channels, 1196
- Allen, J. R. L. : Atlas and Glossary of Primary Sedimentary Structures, review, 315
- Allen, Prof. P., and Keith, Prof. M. L. : Carbon Isotope Ratios and Palaeosalinities of Purbeck-Walden Carbonates, 1278
- Allergy : Direct Reaction of Penicillin with ϵ -Amino-groups : New Chemical Aspect of Penicillin (Dr. C. H. Schneider and Dr. A. L. de Weck), 57
- Allergy : Fresh Approach to the Problem of, 530
- Allogenic Inhibition of Tumour Cells by *in vitro* Contact with Cells containing Foreign H-2 Antigens (K. E. Hellstrom, I. Hellstrom and C. Bergheden), 458
- Allografts in Thymectomized X-irradiated Dogs : Bone Marrow (I. L. Chertkov *et al.*), 399
- Alloys : Dispersion-softened Zinc (W. H. McCarthy *et al.*), 579
- Allsopp, Dr. A. : Strasburger's Textbook of Botany, New English Edition, review, 618
- Alpha Activity of Drinking Water using a Nuclear Emulsion : Determination of the Total (M. W. Tyrrell *et al.*), 1074
- Alps : Oil Pipeline over the, 435
- Alter, Dr. S., *et al.* : Aldosterone Secretion : Differences in Direct Effects of Rubidium and Potassium Perfusion into Dog Adrenals, 1099
- Alteration of Hamster Cells by Nucleic Acid *in vitro* (K. Shepley, Dr. E. J. Ambrose and Dr. K. S. Kirby), 1072
- Altered Electrophoretic Mobilities of Some Erythrocytic Enzymes as a Function of their Age (Dr. H. Walter, F. W. Selby and J. R. Francisco), 76
- Altered Perfusion Rates : Effect of, on the Retention of Noradrenaline by the Spleen (Dr. D. M. Paton and Prof. C. N. Gillis), 391
- A.C. Resistivity of Anodic Oxide Films on Valve Metals (Dr. G. C. Wood and C. Pearson), 547
- Alternating Current Stimulation : Squid Giant Axon : Repetitive Responses to (Prof. H. R. Hirsch), 1218
- Aluminium, Germanium and Silicon to 60 kilobars : Fusion Curves of Zinc, (J. Lees and B. H. J. Williamson), 278
- Aluminium in Acid Soils : Measurement of Exchangeable (J. B. Skeen and Dr. M. E. Sumner), 712
- Aluminium-Silicon Eutectic : Interconnexion of Silicon in Modified (J. A. E. Bell and Prof. W. C. Winegar), 177
- Amazon : A Visit to the Lower (I. R. Bishop and Prof. G. E. H. Foxon), 114
- Ambrose, Dr. E. J., *et al.* : Alteration of Hamster Cells by Nucleic Acid *in vitro*, 1072
- American Forms : Marsupial Spermatozoa Pairing in the Epididymis of (Dr. J. D. Biggers and Dr. E. D. DeLamater), 402
- American Geographical Society : Awards, 1047
- America (North) : Bark Canoes and Skin Boats of, 22
- American (North) Rocks : Age Determinations of, 435
- American Society of Plant Physiologists : Awards, 231
- Officers, 231
- Amides : Slow Inversion at the Nitrogen Atom in Certain (Dr. T. H. Siddall and Dr. C. A. Prohaska), 582
- Amines in the Gastric Mucosa of the Rat : Formation of (R. Håkanson), 793
- Amino-acid in Rat Intestine *in vitro* : Accumulation and Incorporation of (Dr. J. R. Bronk and Dr. D. S. Parsons), 785
- Amino-acid Sequence of β -LPH from Sheep Pituitary Glands : Isolation and (Prof. Choh Hao Li *et al.*), 1093
- Amino-acids as Trimethylsilyl Derivatives : Quantitative Gas Chromatography of (Prof. E. D. Smith and H. Sheppard, jun.), 878
- Amino-acids from Proteins : Freeing of (Dr. L. J. Hastewell and R. N. Priestland), 387
- Amino-acids from the Resting Human Forearm : Evidence for the Release of Individual (Dr. D. R. London and Dr. T. H. Foley), and (C. G. Webb), 588
- Amino acids : Polyamide Layer Chromatography of DNP (Prof. Kung-Tsung Wang and J. Mei-Kuo Huang), 281
- ϵ -Amino Group of Collagen : Reactivity of the (J. H. Bowes *et al.*), 1094
- ϵ -Amino-groups : New Chemical Aspect of Penicillin Allergy : Direct Reaction of Penicillin with (Dr. C. H. Schneider and Dr. A. L. de Weck), 57
- Aminoacyl Naphthylamidase in the Rat : Source of Blood (Dr. B. Monis), 587
- 2-Aminoethanephosphonic Acid : a Phosphoramidic Acid Rearrangement : Biosynthesis of (Dr. W. Segal), 1284
- 2-Aminoethyl-guanidine and of a Eugenol as Virus Inhibitors : Derivatives of (S. Pácsa *et al.*), 409
- Amino-nitrogen Content of Solutions of γ -Keratoase from Wool Keratin : Changes in (Dr. R. S. Asquith and P. A. Watson), 786
- (Aminopeptidase) Isozymes of Arylamidase (Dr. B. Monis), 587
- Ammonium Compounds adsorbed on Silica-gel 'Aerosil' : Infra-red Spectra of Quaternary (Dr. L. C. F. Blackman and R. Harrop), 777
- Amodio, Dr. A., *et al.* : Distribution of the Intestinal Factor in Normal Mice which inactivates Murine Virus Hepatitis, 1009
- Amorphous Polymers : Absence of Diffusion Control of Reaction Rate in the Thermal Decomposition of (D. A. Smith), 1200
- Amos, T. G. : Kinesis, 908
- Amount and Composition of the Serum Seromucoid Fraction : Factors affecting the (Dr. A. J. Anderson), 491
- Amphibia : Hydrolysis of Disaccharides during Absorption by the Perfused Small Intestine of (Dr. D. S. Parsons and J. S. Prichard), 1097
- Amphibians : Enzymatic Synthesis of the Skin-lightening Agent, Melatonin, in (Dr. J. Axelrod *et al.*), 386
- Ampicillin : Crystalline Modifications of (K. W. B. Austin *et al.*), 999
- Amylase-levels in the Human Male and Female : Comparison of Salivary (Prof. R. L. McGeachin *et al.*), 1211
- Anaemia? : A Sex-dependent Factor in Aplastic (Dr. D. E. Pegg and J. E. Burns), 1187
- Analogy : Biological and Mental Evolution : an Exercise in (Arthur Koestler), 1033
- Analysis : Concentration Effects in Chromatographic (Prof. I. D. Rattee *et al.*), 269
- Counterexamples in (Prof. B. R. Gelbaum and Prof. J. M. H. Olmsted), review by Prof. T. A. Broadbent, 218
- Analysis in the Sub-sieve Range by Electronic Counter : Size (Prof. C. C. Harris and Dr. A. Jowett), 175
- Analysis of Chromatograms : Ultra-violet Spectroscopy in the (Dr. D. M. Abelson), 784
- Analysis of Organic Acids by Tritiating Methylation : Quantitative Chromatographic (G. K. Koch and [the late] G. Jurriens), 1312
- Analysis of Surface-active Agents by Infrared and Chemical Methods : Identification and (Prof. D. Hummel), Two Volumes, review by Dr. P. Robson, 617
- Analysis of Vibration : Matrix (Prof. R. E. D. Bishop *et al.*), review by Prof. W. A. Tuplin, 616
- Analysis : Structure, Behavior and Application to Inorganic, Organic Complexing Reagents (D. D. Perrin), review by Dr. T. S. West, 3
- Analytical Chemistry : Organic Complexing Reagents (D. D. Perrin), review by Dr. T. S. West, 3
- Anaphylactic Release of Vascular Permeability Factor or Histamine by Specific Protease Inhibitor in Tissue Culture : Inhibition of (Prof. H. Hayashi *et al.*), 1007
- Anatomical Changes associated with Juvenile-to-Mature Growth Phase Transition in *Hedera* (J. R. Goodin), 504
- Anatomie des Menschen : Histologie und Mikroskopische (Dr. W. Bargmann), 5th German Edition, review by Dr. G. Sander, 109
- Ancestry of Sea-stars (Dr. G. M. Philip) : (Prof. H. B. Fell), 766
- Andean Uplift : Isotopic Ages and (Dr. R. W. R. Rutland *et al.*), 677
- Andersen, A. J. : Uptake by Plants of Radiotracer from Contaminated Soils, 195
- Anderson, Dr. E. S. : A Rapid Screening Test for Transfer Factors in Drug-sensitive Enterobacteriaceae, 1016
- Anderson, Dr. E. S., and Lewis, Dr. M. J. : Characterization of a Transfer Factor associated with Drug Resistance in *Salmonella typhimurium*, 843
- Anderson, Dr. A. J. : Factors affecting the Amount and Composition of the Serum Seromucoid Fraction, 491
- Anderson-Berry Prize, 1966 : David, 530

INDEX

- Anderson, Dr. Mary R., and Green, Prof. H. N.: Implications of the Immunological Theory of Cancer to Multiple Causes, 338
- Anderson, Ruth, and Burdette, Prof. W. J.: Puffing of Salivary Gland Chromosomes after Treatment with Carbon Dioxide, 409
- Anderses and the Growth of Continents: Geochemistry of (Prof. S. R. Taylor and Dr. A. J. R. White), 271
- Andrews, Dr. E. H.: Thermal Stress, review, 517
- Plenum Press Handbooks of High Temperature Materials, Three Volumes, review, 517
- Andriese, P. C., et al.: Air Ion Effects on the Oxygen Consumption of Barley Seedlings, 1112
- Androgen-Oestrogen-induced Tumours: Lactic Dehydrogenase Relative to Chaetepithelioma Induction in the Syrian Hamster (Prof. F. T. Algard); (A. H. Dodge and Prof. H. Kirkman), 567
- Androgenic Steroid Complexes with β -Bromophenol (Dr. C. Eger and Dr. D. A. Norton), 997
- Angaut, Pierre, and Bowsher, Dr. D.: Cerebello-rubral Connexions in the Cat, 1002
- Angel, A., Magni, F., and Strata, P.: Evidence for Pre-synaptic Inhibition in the Lateral Geniculate Body, 495
- Anghileri, L. J.: Oleic Acid labelled with Iodine-131, and Triolein Deiodination *in vitro*, 896
- Anglo-African Joint Research Schemes and Recruitment, 333
- Anglo-French Military Aircraft: Appointment of Project Director, 527
- Anglo-Romanian Agreement on Co-operation, 328
- Angular Momenta of Eclipsing Binaries and the Fission Theory of their Origin (I. W. Roxburgh), 65
- Angular Sizes of Some Quasars: New Limits to the (R. L. Adge et al.), 275
- Animal Communication: Mimicry and the Evolution of (Dr. W. Wickler), 519
- Animal Ecology Division of the New Zealand D.S.I.R., Director, 326
- Animals: Care of Laboratory, 630
- Drug Dosage in Laboratory (C. D. Barnes and L. G. Eltherington), review by Dr. J. A. Parsons, 1028
- International Committee on Laboratory (Prof. J. A. B. Gray), 738
- Lung Tumours in (Prof. L. Severi), 632
- Animals for Rheumatism Research: Laboratory, 1046
- Animals in Africa: Meat from Wild, 122
- Anodic Oxide Films on Valve Metals: A.C. Resistivity of (Dr. G. C. Wood and C. Pearson), 547
- Anolis (Sauria: Iguanidae): Interspecific Karyotypic Variation as a Systematic Character in the Genus (G. C. Gorman), 95
- Anomalous Intensity Ratios of the Interstellar Lines of OH in Absorption and Emission (R. X. McGee, Dr. B. J. Robinson, F. F. Gardner and J. G. Bolton), 1193
- Antagonists: Anticoccidial Activity of Nicotinamide (Dr. S. J. Ball et al.), 397
- Antarctic Aeronomy and Ice Sheets (G. de Q. Robin), 439
- Anthophora acraensis Fab.: Periodic Aggregating and Take-off in Anthophorid Bees, (Prof. L. K. H. Goma), 807
- Anthophorid Bees, *Anthophora acraensis* Fab.: Periodic Aggregating and Take-off in (Prof. L. K. H. Goma), 807
- Anthropology: A Hundred Years of (T. K. Penniman et al.), Third Edition, revised, review by Prof. M. Fortes, 215
- Anti-auxin 2,3,5-Tri-iodobenzoic acid: Induction of Tension Wood with (Prof. R. W. Kennedy and Prof. J. L. Farrar), 406
- Antibiotic which retards Fouling: *Sargassum* Tannin, an (Prof. J. McN. Sieburth and Prof. J. T. Conover), 52
- Antibodies on Polyoma Tumour Cells: Distinction between the Effects of Antiviral and Anticellular Polyoma (I. Hellstrom), 652
- Antibodies with Denatured Deoxyribonucleic Acid: Reaction of Adenine-specific (Dr. S. Estrada-Parra et al.), 1010
- Antibody: a Localized Haemolysis in Gel Method for the Detection of Cells producing 7S (Dr. J. Sterzl et al.), 858
- Rapid Detection and Titration of Antihuman Serum Albumin (Dr. M. S. Tobin), 1338
- Antibody and Opsonin by a Macrophage Phagocytic System: Differentiation between Cytophilic (W. E. Parish), 594
- Antibody Localization in the Mouse: Antithymic (Dr. B. Hintz and Prof. M. M. Webber), 797
- Antibody Method: A New Fluorescent (Prof. E. H. Beutner et al.), 353
- Antibody-plaque-forming Cells: Thymus and the Production of (Dr. J. F. A. P. Miller et al.), 1332
- Antibody Response in Thymectomized and Sham-thymectomized Mice: Time-course Studies on (Dr. N. R. Sinclair), 1104
- Anticellular Polyoma Antibodies on Polyoma Tumour Cells: Distinction between the Effects of Antiviral and (I. Hellstrom), 652
- Anticoccidial Activity of Nicotinamide Antagonists (Dr. S. J. Ball, E. W. Warren and Dr. E. W. Parnell), 397
- Anticorps de Transplantation (André Govaerts), review by Dr. R. B. Taylor, 988
- Antidiuretic Hormone and Bile Flow (Earl Homsher and Dr. G. C. Cotzias), 687
- I Antigen in Leukaemic Patients (Dr. J. Ducos, J. Ruffie, P. Colombes, Y. Marty and E. Ohayon), 1329
- Antigen Indirect Immunofluorescence Staining: Mixed Antiglobulin Immunofluorescence or Labelled (Prof. E. H. Beutner et al.), 353
- Antigen-retaining Reticulum of Lymph Node Follicles as shown by High-resolution Autoradiography: Ultrastructure of the (J. Mitchell and A. Abbot), 500
- Antigenic Pattern of *Azotobacter* grown on Different Nitrogen Sources: Differences in the (Prof. T. Holme and B. Zacharias), 1235
- Antigenic Variation in Chronic Infections of *Plasmodium knowlesi*: Immunity to Malaria: (Dr. K. N. Brown and I. N. Brown), 1286
- Antigénique de Tumeurs Expérimentales: Structure (Dr. C. Deckers), review by R. R. Dourmashkin, 1140
- Antigens: Allogeneic Inhibition of Tumour Cells by *in vitro* Contact with Cells containing Foreign H-2 (K. E. Hellstrom et al.), 458
- Antigens in Transplanted Mouse Eggs: Histocompatibility (Dr. R. L. Simmons and P. S. Russell), 698
- Anti-gestagenic Properties: Specific Uptake in Corpora Lutea of a Non-steroid Substance with (A. Hanngren et al.), 461
- Antiglobulin Immunofluorescence or Labelled Antigen Indirect Immunofluorescence Staining: Mixed (Prof. E. H. Beutner et al.), 353
- Antihuman Serum Albumin Antibody: Rapid Detection and Titration of (Dr. M. S. Tobin), 1338
- Anti-inflammatory Drugs: Inhibition of Conjugation by (Dr. T. Hargreaves), 1101
- Antimalarial Prophylaxis in Eastern Nigeria, 1154
- Antimatter and Tree Rings (V. S. Venkatavardan), 772
- Antimicrobial Agents and Chemotherapy—1964 (edited by J. C. Sylvester), review by Prof. E. P. Abraham, 980
- Antioxidants based on Protection of *Tetrahymena pyriformis* from the Photodynamic Toxicity of Benzo(a)pyrene: A Simple Bioassay for (Dr. S. S. Epstein et al.), 655
- Antiphonal Singing of Birds: Origin and Significance of the Power of Vocal Imitation: with Special Reference to the (Dr. W. H. Thorpe and M. E. W. North), 219
- Antithymic Antibody Localization in the Mouse (Dr. B. Hintz and Prof. M. M. Webber), 797
- Antiviral Action of Oxidized Spermine—Inactivation of Plant Viruses (U. Bachrach, S. Rabina, G. Loebenstein and G. Elion), 1095
- Antiviral and Anticellular Polyoma Antibodies on Polyoma Tumour Cells: Distinction between the Effects of (I. Hellstrom), 652
- Anton, Prof. Aaron H., and Gennaro, jun., Prof. J. F.: Norepinephrine and Serotonin in the Tissues and Venoms of Two Pit Vipers, 1174
- Anvil Apparatus: Pressure Distribution in the Tetrahedral (S. Lees), 31
- ap Rees, Dr. T., and Graham D.: Occurrence of Fructose-1-phosphate in Tissues of Higher Plants, 88
- Aplastic Anaemia? A Sex-dependent Factor in (Dr. D. E. Pegg and J. E. Burns), 1187
- Apoferitin Synthesis in Human Erythroid Cells in Thalassaemia (Dr. E. H. Eylar and Dr. G. Matoli), 661
- Apparent Suppression of Mutation Rates in Bacteria by Spermine (Dr. H. G. Johnson and Dr. M. h. Bach), 408
- Application of an Agar-Agar Diffusion Procedure to Pesticide Residue Analysis and to the Cholinesterase Screening of Candidate Pesticides (Dr. K. I. Beynon and G. Stoydin), 748
- Application of Continued Fractions and Their Generalizations to Problems in Approximation Theory (A. N. Khovanskii), review by I. M. Khabaza, 318
- Application of the Materials Scientist: Education and (Dr. G. F. Freeguard), 939
- Applied Research: the Case for, 1025
- Applied Science in the Schools (T. A. Burdett), 1269
- Approximation Theory: Application of Continued Fractions and Their Generalizations to Problems in (A. N. Khovanskii), review by I. M. Khabaza, 318
- Approximation to the Equation of Thermogravimetric Data: Tschibychew (Dr. E. L. Jones), 1306
- Aquatic Microbiology Group, 734
- Aqueous Solutions: Electric Potentials generated by Freezing Dilute (H. C. Parreira and A. J. Eytch), 33
- Arc Track Oxide Layers: Surface Charges on (Dr. W. P. Jayasekara et al.), 774
- Archaeological Sites: Soil and Landscape at, 530
- Archer, Dr. S., et al.: A New, Active Metabolite of 'Miracid', 1005
- Archer of Science Award: Pacific Science Center Foundation, 326
- Architecture of Molecules (Prof. Linus Pauling and Roger Hayward), review by Dame Kathleen Lonsdale, 957
- Archuleta, R. F., et al.: Actin Radio-sensitivity as a Function of Age in Mice, 905
- Ardan, Dr. G. B., and Ikeda, H.: A New Property of the Early Receptor Potential of Rat Retina, 1100
- Arendt, P. R.: Comparison of Experimental Data of the Effective Ion Mass in the Upper Ionosphere with a Theoretical Structure, 443
- Argid Sawflies (Hymenoptera, Symphyta): Bilateral Asymmetry: Larval Polymorphism in some (Dr. M. F. Claridge and Dr. J. M. Edington), 301
- Arginine and Urea Biosynthesis in the Land Planarian: Its Significance in Biochemical Evolution (Prof. J. W. Campbell), 1299
- Arithmetic: Basic Laws of (G. Frege), review by Prof. T. A. A. Broadbent, 318
- Arizona Meteorite Crater: Origin of Atypical Meteorites from the (Prof. M. E. Lipschutz), 636
- Armilei, G., and Beers, jun., Prof. R. F.: Heterogeneous Binding of Acridine Orange by Polyribonucleotides, 466
- Armytage, Prof. W. H. G.: Education and the Labour Movement 1870-1920, review, 960
- Arnett, Dr. G. M., and Lal, Dr. R. B.: Effect of Ultra-violet Irradiation on the Electrical Conductivity of Zinc Oxide Single Crystals, 1305
- Arnott, Prof. H. J., Pautard, Dr. F. G. E., and Steinink, Prof. H.: Structure of Calcium Oxalate Monohydrate, 1197
- Aromatic Hydrocarbons with the Ionization Potential: Correlation of the Third Excited State of Polycyclic (Dr. M. A. Slifkin), 380
- Aromatic Nitrenes and Dinitrates: Ultra-violet Absorption Spectra of (A. Reiser and V. Frazer), 682
- Arrangement of Hours on Night Work: Shift Working: the (Mrs. G. de la Mare and Dr. J. Walker), 1127
- Art, 1740-70: British, 122
- Artichop Host: Microcrystal Formation: Plant Tumour Virus In (E. Shikata and Dr. K. Maramorash), 507
- Arthropods and Man: Evolutionary Tendency of Relative Growth In (Dr. R. Matsuda), 1228
- Arthropods: Feeding Mechanism of Blood-sucking (M. M. J. Lavoipierre), 302
- Artificial Insemination of Dystrophic Mice with Mixtures of Spermatozoa (J. L. Southard, H. G. Wolfe and Dr. E. S. Russell), 1126
- Artificial Membranes: Effect of Trans-Solutes on the Fluxes of Chloride Ions across (S. Kitahara et al.), 187
- Arylamidase (Aminopeptidase): Isozymes of (Dr. B. Monis), 587
- Ascent of Sap in Trees: Effect of Negative Absolute Pressure on Flow Resistance (Prof. B. Johnson and Prof. J. R. Dixon), 1347
- Asclites Cell Extracts: Nicotinamide Mononucleotide Pyrophosphorylase Activity in (Prof. L. S. Dietrich et al.), 347
- Asclites Hepatoma Cells: Formation of Intra-mitochondrial Glucosyl-oligosaccharides from Glucose by Intact Novikoff (Dr. V. N. Nigam and Dr. G. de Lamirande), 6-4
- Asclites Tumour Cells: Enzyme Regulatory Mechanisms at the Level of Lactate-oxidoreductase in Erythrocytes and (S. Rapoport et al.), 185
- Asclites C-2 Tumour Cells treated with Actinomycin D: Rapidly Labelled Synthesis of Ribonucleic Acid in Ehrlich (D. A. Popović), 49
- Asclitic Form: Conversion of the Inconvertible L-I Lymphosarcoma to (Prof. M. R. Sikov), 902
- Ascorbic Acid Content of Pseudo-pregnant Immature Rats used in Parlow's Test: Diurnal Variations in Ovarian (Dr. M. de la Lastra and Dr. H. Craxatto), 789
- Asiatic Antiquities: British Museum: Department of Western, 434
- Askonas, Dr. B., Butler, Prof. J. A. V., Jacob, Prof. F., Phillips, Dr. D. C., Sachs, Dr. L., and Sherbet, Dr. G. V.: Biological Systems at the Molecular Level, 1048
- Asquith, Dr. R. S., and Watson, P. A.: Changes in Amino-nitrogen Content of Solutions of γ -Keratin from Wool Keratin, 786
- Assay for MTV: Immunology of the Mouse Mammary Tumour Virus (MTV): a Qualitative *in vitro* (P. B. Blair), 165
- Assay in the Chick Embryo: Comparison of the Chorionallantoic Membrane and Splenomegaly Systems of Graft-versus-host (L. W. Coppleston and Dr. D. Michle), 53
- Assessment of Partition Data for correlating Structure and Biological Activity using Thin-layer Chromatography: Simple (Dr. C. B. C. Boyce and Dr. B. V. Millborrow), 537
- Assimilation of Glucose: Maintenance of *Escherichia coli* and the (S. B. McGrew and Prof. M. F. Mallette), 1096
- Association of Adenoviruses with Bovine Respiratory Disease (Dr. J. H. Darbyshire), (A. R. Jennings and A. R. Omar), (P. S. Dawson and P. H. Lamont), 307
- Association of Clinical Biochemists, Election of Officers, 631
- Associations: Industrial Research and the Research, 1

INDEX

- Aston, Prof. J. G., Misra, Dr. D. N., and Cresswell, K. M.: Simultaneous Oxidation and Reduction of Diphenyl Picrylhydrazyl on Surfaces: Evidence for Catalytic Activity of F-centres, 181
- Astronautics Award: 1965 Guggenheim International, 119
- Asymmetry: Bilateral: a Larval Polymorphism in some Argid Sawflies (Hymenoptera, Symphyta) (Dr. M. F. Claridge and Dr. J. M. Edington), 301
- Atkins, Prof. Hedley: The Surgeon's Craft, review by N. W. Nisbet, 7
- Atkinson, W. J., and Webb, Prof. J. S.: Regional Geochemical Reconnaissance applied to Some Agricultural Problems in Co. Limerick, Eire, 1056
- Atlantic Deep-sea Sediments: Pleistocene Glacial-marine Zones in North (Dr. J. R. Conolly and Dr. M. Ewing), 135
- Atlas and Glossary of Primary Sedimentary Structures (Prof. F. J. Pettijohn and Prof. P. E. Potter), review by J. R. L. Allen, 315
- Atmosphere: Physics of the Marine (Dr. H. U. Roll), review by Prof. J. Darbyshire, 419
- Atmospheric Environment: Organo-chlorine Pesticides in the (Dr. D. C. Abbott et al.), 1317
- Atmospheric Moisture in Nozzles: Effects of Seeding on the Condensation of (Dr. E. R. Buckle and Dr. A. A. Pouring), 367
- Atom Reaction Rates with Electron Capture Cross-sections: Correlation of Sodium (K. R. Wilson and Prof. D. R. Herschbach), 182
- Atomic Collisions in Irradiated Crystal Lattices: Correlated (Dr. L. T. Chaderton and I. McC. Torrens), 880
- Atomic Energy Authority and Central Electricity Generating Board Co-operation Scheme: U.K., 120
- Atomic Nuclei: Structural Basis of Neutron and Proton Magic Numbers in (Prof. L. Pauling), 174
- Atomphysik: Einführung in die (Prof. W. Finkelburg), review by Dr. E. Bretscher, 217
- Atoms in Solids: Evidence of Thermal and Hot Processes in the 'Hot-zone' produced by Copper-64 Recoil (K. Yoshihara and H. Ebihara), 482
- Atropine or Histamine in a Silicone Rubber Implant: Prolonged Administration of (P. Bass, R. A. Purdon and J. N. Wiley), 591
- Auditory System: Neural Volleying: Upper Frequency Limits detectable in the (Capt. J. C. Boudreau), 1237
- Audley, Dr. A., and Fursey, A.: Examination of a Polysaccharide Flocculant and Flocculated Kaolinite by Electron Microscopy, 753
- Augenstein, L. G., Mason, R., and Quastler, H.: Advances in Radiation Biology, Volume 1, review by J. St. L. Philpot, 1032
- Aumann, G. D., and Emlen, Prof. J. T.: Relation of Population Density to Sodium Availability and Sodium Selection by Microtine Rodents, 198
- Auroral Zone: Variation according to the Latitude of the Electron Content of the Ionosphere near the (L. Liszka), 280
- Austin, K. W. B., Marshall, A. C., and Smith H.: Crystalline Modifications of Ampicillin, 999
- Austin Miller, Prof. A.: Retirement from the Chair of Geography in the University of Reading, 628
- Australia: Great Barrier Reef, Facilities for Research, 230
- Australia: Iron Ore Production and Research at Broken Hill, 22
- Australia: Spectroscopy in (Dr. G. C. Barraclough et al.), 125
- Australian Aborigines: Haptoglobin Johnson in (D. W. Cooper et al.), 694
- Australian Biochemical Society Meeting (Dr. F. J. R. Hird), 837
- Australian Conference on Electrochemistry, 1963: Proceedings of the First (edited by J. A. Friend and F. Gutmann, assisted by J. W. Hayes), review by Dr. R. F. Phillips and N. A. Hampson, 104
- Australian Echidna (*Tachyglossus aculeatus*): Immune System in a Monotreme: Studies on the (Dr. E. Diener and Dr. E. H. M. Ealey), 950
- Australian Museum: South, 1046
- Australian Prehistory, 835
- Automation: Social Implications of, 230
- Autodiagnostic Identification of the D (13-15) Chromosome responsible for Dr. Trisomic Patau's Syndrome (Dr. F. Giannelli), 669
- Autoradiography: Ultrastructure of the Antigen-retaining Reticulum of Lymph Node Follicles as shown by High-resolution (J. Mitchell and A. Abbot), 500
- Autumn Phenomena in Woody Plants: Some Postulated Relationships among (R. B. Van Huystee et al.), 89
- Auxin Transport: Action of Gibberellic Acid on (Prof. P. E. Pilet), 1344
- Auxins: Promotion by Kinetin of the Polar Transport of Two (Dr. C. C. McCready et al.), 1065
- Avena Coleoptile by Indolyl-3-acetic Acid: Stimulation of Synthesis of Ribonucleic Acid in Subapical Sections of (Prof. T. H. Hamilton et al.), 1180
- Avian Embryos: Development of *Emeria tenella* in (P. L. Long), 509
- Aviation: Ministry of, Appointments, 19
- Aviation: Ministry of, Anglo-French Military Aircraft: Project Director, 527
- Avron, Dr. M., and Zweig, G.: Dependence of Photophosphorylation by Isolated Chloroplasts on the Oxidation-Reduction State of N-Methylphenazinium Methyl Sulphate (Phenazine Methosulphate), 190
- Awakening: Thinning of the Human Cornea on (Dr. R. B. Mandell and Prof. I. Fatt), 292
- Award: Feldberg Foundation, 1263
- Award: 1965 Guggenheim International Astronautics, 119
- Award: Pacific Science Centre Foundation Archives of Science, 326
- Award of Royal Medals, Royal Society of London, 832
- Awards: Albert Lasker Medical Research, 1263
- American Geographical Society, 1047
- American Society of Plant Physiologists, 231
- Gairdner Foundation, 530
- Royal Society of London: Medal, 934
- Awards for Science and Mathematics Teachers: Guinness, 631
- Axelrod, Dr. J., Quay, Prof. W. B., and Baker, P. C.: Enzymatic Synthesis of the Skin-lightening Agent, Melatonin, in Amphibians, 386
- Axon: Repetitive Responses to Alternating Current Stimulation: Squid Giant (Prof. H. R. Hirsch), 1218
- Axons: Intracellular Chloride Activity of Crayfish Giant (Dr. A. Strickholm and B. G. Wallin), 790
- Azetidine-2-carboxylic Acid: Synthesis of Ribonucleic Acid by *Escherichia coli* during Inhibition by (Dr. J. M. Midgley and Dr. D. G. Wild), 585
- Azotobacter grown on Different Nitrogen Sources: Differences in the Antigenic Pattern of (Prof. T. Holme and B. Zacharias), 1235
- BACH, Dr. M. K., and Johnson, Dr. H. G.: Apparent Suppression of Mutation Rates in Bacteria by Spermine, 408
- Bachrach, U., Rabina, S., Loebenstein, G., and Eilon, G.: Antiviral Action of Oxidized Spermine—Inactivation of Plant Viruses, 1095
- Bacillus cereus Strain T: Timing of Protein Synthesis during Germination and Outgrowth of Spores of (W. Steinberg et al.), 710
- 'Baconia': Method: Ethology and the (Dr. W. P. C. Wightman), 918
- Bacq, Prof. Zénon-Marcel, (sub-editor): Handbuch der Experimentellen Pharmakologie, Band 17: Ions Alcalino-Terreux. Two Volumes in French, review by Prof. E. Zaimis, 720
- Bacteria: An Example for New Type of Regulatory Mechanism in (Dr. A. Tomasz), 155
- Bacteria: Manual for the Identification of Medical (S. T. Cowan and K. J. Steel), review by Dr. D. Allan, 213
- Metabolism of Nucleic Acids and Protein in Satrving (B. Brdar et al.), 303
- Photosynthetic (E. N. Kondrat'eva), review by Prof. T. W. Goodwin, 977
- Theory and Practice in Experimental (G. G. Meynell and E. Meynell), review by Dr. D. Allan, 213
- Typography of, 332
- Bacteria able to metabolize Fluoroacetate or Fluoroacetamide: Isolation of (Dr. M. Kelly), 809
- Bacteria by Spermine: Apparent Suppression of Mutation Rates in (Dr. H. G. Johnson and Dr. M. K. Bach), 408
- Bacteria: Interaction of Cephaloridine with Penicillinase-producing Gram-negative (Dr. J. M. T. Hamilton-Miller et al.), 235
- Bacteria, or Viruses in Mice pretreated with *Escherichia coli* Endotoxin or infected with *Mycobacterium tuberculosis*: Interferon Appearance stimulated by Endotoxin, (Prof. J. S. Youngner and Prof. W. R. Stinebring), 456
- Bacterial Count and Cell Differentiation in Milk (C. L. Duitschaeffer), 200
- Bacterial Urease Inhibitor with Therapeutic Potential in Hyperammonaemic States: Acetohydroxamate: (Dr. W. N. Fishbein et al.), 46
- Bacteriology: The Brilliant and Tragic Life of W. M. K. Haffkine (S. A. Waksman), review by Dr. H. I. Jhala, 418
- Bacteriophage by Sensitized Leucocytes in vitro: Inactivation of T-2 (R. Young and Dr. F. H. Ruddle), 1105
- Bader, G., Stiller, D., and Ruffert, K.: Fluorochrome Stains for Histological Diagnosis of Visceral Mycoses, 796
- Bafna, Dr. S. L., and Shah, R. S.: Separation of Coumarins, 76
- Bafort, Dr. J., Vincke, I. H., and Timperman, G.: Gametogenesis of *Plasmodium vinckei*, 1230
- Bagley, B. G.: Dense Packing of Hard Spheres with Five-fold Symmetry, 674
- Baikie, Dr. A. G., and Spiers, Dr. A. S. D.: Chronic Granulocytic Leukaemia: Demonstration of the Philadelphia Chromosome in Cultures of Spleen Cells, 497
- Bailey, R. W.: Oligosaccharides, review by Prof. M. Stacey, 971
- Bailey, Dr. R. W., and Russell, G. B.: Disruption of Protozoa by Indole and Related Compounds, 1001
- Baines, Dr. M. J., and Williams, I. P.: Growth of Interstellar Grains, 1191
- Baker, Dr. Bridget I.: Direct Action of Thyroxine on the Trout Pituitary in vitro, 1234
- Baker, F. N., Salisbury, Prof. G. W., and Fehcheimer, Prof. N. S.: Measurements of Bovine Chromosomes, 97
- Baker, P. C., et al.: Enzymatic Synthesis of the Skin-lightening Agent, Melatonin, in Amphibians, 386
- Balance: Electromagnetic Recording Diver (S. Løvtrup and S. Larsson), 1116
- Balázs, A. (edited by): International Conference on Gerontology, review by Dr. David A. Hall, 981
- Baliga, Dr. B. S., Srinivasan, Prof. P. R., and Borek, Prof. E.: Changes in the t-RNA Methylating Enzymes during Insect Metamorphosis, 555
- Ball, Dr. S. J., Warren, E. W., and Parnell, Dr. E. W.: Anticoccidial Activity of Nicotinamide Antagonists, 397
- Ballhausen, C. J., and Gray, Dr. H. B.: Molecular Orbital Theory, review by E. W. T. Richards, 110
- Baltimore): New Inherited Coagulation Disorder caused by an Abnormal Fibrinogen ('Fibrinogen (Dr. E. A. Beck et al.), 143
- Bamm, Dr. R. K.: 'Complement Fractionation' in a Natural Hybrid between *Rubus procerus* Muell., and *R. laciniatus* Willd., 608
- Bangham, Dr. A. D., Standish, Dr. M. M., and Miller, N.: Cation Permeability of Phospholipid Model Membranes: Effect of Narcotics, 1295
- Banks, Dr. Barbara E. C., et al.: Acid Phosphatases, 382
- Banner, Dr. F. T., and Blow, Dr. W. H.: Progress in the Planktonic Foraminiferal Biostratigraphy of the Neogene, 1164
- Barbiturate: Effects of Propylthiouracil and Thyroid Feeding on the Response of Mice to Injected Convulsant (Prof. A. J. Prange, jun., and Prof. M. A. Lipton), 791
- Barbiturate Hypnosis: Regional Changes in Brain Serotonin after Head X-irradiation and its Significance in the Potentiation of (Dr. V. Nair), 1293
- Bargmann, Dr. W.: Histologie und Mikroskopische Anatomie des Menschen, 5th German Edition, review by Dr. G. Sander, 109
- Bark Canoes and Skin Boats of North America, 22
- Barley Committee, Trials 1963: European Brewery Convention: Report of the, review by A. H. Cook, 9
- Barley Seedlings: Air Ion Effects on the Oxygen Consumption of (S. Kotaka et al.), 1112
- Barnafi, L., et al.: Isolation and Amino-acid Sequence of β LPH from Sheep Pituitary Glands, 1093
- Barnes, C. D., and Eltherington, L. G.: Drug Dosage in Laboratory Animals, review by Dr. J. A. Parsons, 1028
- Barnicot, Prof. N. A.: Natural Selection and Transmissible Disease, 535
- Barr Bodies in the Mouse (Dr. J. D. Kenney and Dr. U. Miltwch), 93
- Barraclough, Dr. G. C., Black, Dr. P. J., David, D. J., Pullin, Dr. A. D. E., and Shannon, J. S.: Spectroscopy in Australia, Fifth Australian Spectroscopy Conference, 125
- Barrage: Solway Firth, 629
- Barrell, Dr. H.: Retirement as Superintendent of Standards Division, National Physical Laboratory, Teddington, 118
- Barrett, Prof. A. H., et al.: Observations of Polarized OH Emission, 440
- Barrow, Dr. R. F.: Spectroscopy and Molecular Structure, review, 214
- Barry, Dr. T. I., and Lay, L. A.: Cation Sites in Synthetic Zeolites, 1312
- Bary, Prof. Nina K.: Treatise on Trigonometric Series, Vols. 1 and 2, review by Prof. T. A. Broadbent, 106
- Basaltic Achondrites: Abundances of Rare Earth Elements in Eclogitic Rocks and (J. W. Morgan and Dr. J. F. Lovering), 1311
- Basic Component of Solar Radio Emission at Centimetre and Decimetre Wavelengths (Dr. M. K. Das Gupta and D. Basu), 739
- Basic Igneous Masses of East Aberdeenshire: Corona Structures in the (Dr. D. S. Weedon), 885
- Basic Laws of Arithmetic (G. Frege), translated by M. Furth, review by Prof. T. A. Broadbent, 318
- Bass, P., Purdon, R. A., and Wiley, J. N.: Prolonged Administration of Atropine or Histamine in a Silicone Rubber Implant, 591
- Basu, D., and Das Gupta, Dr. M. K.: Basic Component of Solar Radio Emission at Centimetre and Decimetre Wavelengths, 739
- Bate, Dr. R. H.: Principles of Zoological Micropalaentology, Volume 2, review, 974
- Bates, B., et al.: Subcutaneous Sarcomata in Hamsters Induced by Rous Sarcoma Virus (Bryan), 336

- Bates, Dr. C. A.: Theory of Magnetism, review, 616
 Physics of Magnetism, review, 616
- Bates, Dr. Roger G.: Determination of pH, review by R. Sawyer, 318
- Bates, Dr. W. J.: Mathematical Theory of Optics, review, 971
- Bats: Technique for the Removal of the Brain in (A. M. Greenhall), 1014
- Battersea College of Technology, Chair of Chemistry, 119
- Bawn, Prof. C. E. H.: *Obituary of Prof. H. Staudinger*, 626
- Beale, D., et al.: A New Haemoglobin, J. Toronto (α5 Alanine→Aspartic Acid), 1059
- Beale, D., et al.: Three Haemoglobins K: Woolwich, an Abnormal, Cameroon and Ibadan, Two Unusual Variants of Human Haemoglobin A, 658
- Bear Skull recovered from a Late Glacial Deposit near Lake Simcoe, Ontario: A Well-preserved Grizzly (R. L. Peterson), 1233
- Bearse, G. E., et al.: Effects of Starvation and Protein Depletion on Mercury Retention in Two Strains of Chickens, 706
- Beck, A., and Orgel, L. E.: Photo-chemical Synthesis of Condensed Phosphates, 1000
- Beck, Dr. E. A., Charache, P., and Jackson, Dr. D. P.: New Inherited Coagulation Disorder caused by an Abnormal Fibrinogen ('Fibrinogen Baltimore'), 143
- Beckman, D. L., et al.: Delayed Mortality in Adult Chickens exposed to 1 Atmosphere Oxygen, 1003
- Bee Orchids: Conservation of the, 936
- Beef Thyroid *in vitro*: Biosynthesis of Triiodothyronine Sulphate by (G. L. Cohn), 80
- Beers, jun., Prof. R. F., and Armilei, G.: Heterogeneous Binding of Acridine Orange by Polyribonucleotides, 466
- Bees, *Anthophora acaensis* Fab.: Periodic Aggregating and Take-off in Anthophorid (Prof. L. K. H. Goma), 807
- Beet Cyst Nematode, *Heterodera schachtii* Schm.: Zinc and Other Metallic Ions as Hatching Agents for the (Dr. A. J. Clarke and Dr. A. M. Shepherd), 502
- Behaviour: A General Theory for the Evolution of Intelligent (Dr. D. Stenhouse), 815
- Behaviour in Rats: Effect of Foster-mothers' Strain and Pre-natal Experience on Adult (Dr. J. M. Joffe), 815
- Behaviour of Strontium and Calcium: Effects of Dietary Levels of Phosphorus and Calcium on the Comparative (K. Kostial et al.), 1110
- Behaviour of the Two Sexes in Certain Birds with Special Reference to Phalaropes: Prolactin and the Incidence of Brood Patch Formation and Incubation (Prof. E. O. Hohn and S. C. Cheng), 197
- Behaviour of Yttrium Ions and their Structure in Solution: Nomadic (Dr. R. C. Vickery), 678
- Behaviour Therapy: Experiments in (edited by Prof. H. J. Eysenck), review by Dr. B. Semeonoff, 1027
- Behavioural Deficit in the Rat induced by feeding Phenylalanine (Dr. R. C. Leaf, Dr. P. L. Carlton and Dr. S. M. Hess), 1021
- Behrendt, Dr. Stefan: Ultra-purification by Separation of Aerosol Particles, 581
- Beier, G., et al.: Long-spacing Segments from Renatured α1 Sub-units of Collagen, 685
- Belenky, M. A., and Polenov, Dr. A. L.: Electron Microscope Observations of Neurosecretory Elements in the Neuro-intermediate Lobe in Skates, 94
- Belfast, University of, Appointments, 836, 1047
- Belgium: Research and Economic Growth in, 834
- Science In, 1045
- Bell, Prof. D. A.: Appointment to Chair of Electronic Engineering in the University of Hull, 732
- Bell, Dr. G. D. H.: Wheat, review, 976
- Bell, J. A. E., and Winegard, Prof. W. C.: Interconnexion of Silicon in Modified Aluminium-Silicon Eutectic, 177
- Bender, Dr. A. E.: Dictionary of Nutrition and Food Technology, Second Edition, review by Dr. A. M. Copping, 987
- Benham, Dr. P. H., and Hoyle, Dr. R. (edited by): Thermal Stress, review by Dr. E. H. Andrews, 517
- Benigni, R., Capra, C., and Cattorini, P. E.: *Pianta Medicinali: Chimica Farmacologia e Terapia*, Vol. 2, review by Dr. F. N. Howes, 218
- Benjamin, D. C., et al.: Synthesis of α-Glycoprotein (Darcy) of Rat Serum by the Liver, 1221
- Bennett, Dr. A.: Effect of Gastrin on Isolated Smooth Muscle Preparations, 170
- A Pharmacological Investigation of Human Isolated Ileum, 1289
- Benzene by Electron Capture Gas Chromatography: Determination of p.p.m. Concentrations of Carbon Disulphide in (D. W. Grant and G. A. Vaughan), 75
- Benzo(a)pyrene: A Simple Bioassay for Antioxidants based on Protection of *Tetrahymena pyriformis* from the Photodynamic Toxicity of (Dr. S. S. Epstein et al.), 655
- Berberian, Prof. D. A., et al.: A New, Active Metabolite of 'Miracid D', 1005
- Berg, Dr. K., and Gedde-Dahl, jun., Dr. T.: Linkage in Man: the Inv and the Lp Serum Type Systems, 1126
- Berg, O. E., et al.: Measured Velocities of Interplanetary Dust Particles, 673
- Bergheden, C., et al.: Allotenic Inhibition of Tumour Cells by *in vitro* Contact with Cells containing Foreign H-2 Antigens, 458
- Berlyne, Dr. G. M., and Nilwargkur, S.: Deterioration of Stored Inulin Solutions, 77
- Bernal, Prof. J. D.: Science in History, Third Edition, review by R. Brightman, 986
- Bernstein, Prof. M. H., et al.: 'Ring-forms' of Reovirus Particles, 606
- Berry, Dr. Roger J.: A Reduced Oxygen Enhancement Ratio for X-ray Survival of HeLa Cells *in vitro*, after Treatment with 'Methotrexate', 1108
- Berthelina limax (Kawaguti and Baba 1959) from the Indian Ocean: Record of the Bivalve Gasteropod (K. P. Rao), 404
- Beryllium Oxide (edited by R. Smith and J. P. Howe), Proceedings of First International Conference, review by Dr. M. F. Lappert, 9
- Beta-lipoprotein with Tritium: Labelling of (T. Gosztanyi et al.), 381
- Beutner, Prof. E. H., Holborow, Dr. E. G., and Johnson, G. D.: A New Fluorescent Antibody Method: Mixed Anticglobulin Immunofluorescence or Labelled Antigen Indirect Immunofluorescence Staining, 353
- Bewley, Dr. D. K., et al.: Increased Sensitivity of *in vitro* Murine Leukaemia Cells to Fractionated X-rays and Fast Neutrons, 168
- Beydon, Dr. K. I., and Stoydin, G.: Application of an Agar-Agar Diffusion Procedure to Pesticide Residue Analysis and to the Cholinesterase Screening of Candidate Pesticides, 748
- Beydoun, Dr. Z. R.: Stratigraphy and Structure of the Eastern Aden Protectorate, review by F. E. Wellings, 615
- Beyond the Edge of Certainty, Essays in Contemporary Science and Philosophy (edited by R. G. Golodny), review by F. I. G. Rawlins, 314
- Bhowani, S. S., and Johri, Prof. B. M.: Growth Responses of Mature Endosperm in Cultures, 1345
- Bhowal, J. G., and Choudhury, A. K. R.: Estimation of the Frequency of Functioning Gametes in Monosomics, 917
- Bibliographical Material: A Guide to Irish (A. R. Eager), review by H. J. Heaney, 1139
- Biggers, Dr. J. D., and Dr. E. D. DeLamater: Marsupial Spermatozoa Pairing in the Epididymis of American Frogs, 402
- Bigley, R. H., et al.: Kinetic Differences between Human Red Cell and Leucocyte Pyruvate Kinase, 194
- Bilateral Asymmetry: a Larval Polymorphism in some Argid Sawflies (Hymenoptera, Symphyta) (Dr. M. F. Claridge and Dr. J. M. Edington), 301
- Bile Flow: Antidiuretic Hormone and (E. Homsher and Dr. G. C. Cotzias), 687
- Bilek, Dr. J., et al.: Introduction of a Permanent Electrode into the Pituitary Gland of the Cow: Influence of Electrical Stimulation on Milk Ejection, 690
- Binaries and the Fission Theory of their Origin: Angular Momenta of Eclipsing (L. W. Roxburgh), 65
- Binding of Acridine Orange by Polyribonucleotides: Heterogeneous (Prof. R. F. Beers, jun., and G. Armilei), 466
- Binding of Tetracyclines to Bone (Dr. D. D. Perrin), 787
- Binding of Xenon to Horse Haemoglobin (Dr. B. P. Schoenborn), 760
- Binding to DNA Loops or to DNA Helices with the Effect on RNA Synthesis: Correlation of the (Prof. J. H. Frenster), 1093
- Binocular Rivalry and Saccadic Suppression: Suppression of the Pupillary Light Reflex in (M. Lorber et al.), 558
- Binocular Visual Acuity: Monocular versus (Dr. F. W. Campbell and Dr. D. G. Green), 191
- Bioassay for Antioxidants based on Protection of *Tetrahymena pyriformis* from the Photodynamic Toxicity of Benzo(a)pyrene: A Simple (Dr. S. S. Epstein, I. B. Saporoschetz, M. Small, W. Park and N. Mantel), 655
- Biochemical Evolution: Arginine and Urea Biosynthesis in the Land Planarian: its Significance in (Prof. J. W. Campbell), 1299
- Biochemical Society Meeting: Australian (Dr. F. J. R. Hird), 837
- Biochemistry and Mental Function (Prof. S. S. Kety), 1252
- Biochemistry: Essays in, Vol. 1, (edited by P. N. Campbell and G. D. Greville), review by Dr. D. T. Etnoro, 316
- Biochemistry of Phenolic Compounds (edited by J. B. Harborne), review by Sir Robert Robinson, 515
- Biochemistry: Rapid Mixing and Sampling Techniques in (edited by B. Chance et al.), review by Prof. S. Dagley, 823
- Biochemists: Association of, Clinical, 631
- Biochemists in Spain: Meeting of (Dr. J. R. Villanueva), 632
- Biogenesis of Ethylene in *Pericallium digitatum* (Dr. B. A. Sprayberry, Prof. W. C. Hall and Dr. C. S. Miller), 1322
- Biological Activity using Thin-layer Chromatography: Simple Assessment of Partition Data for correlating Structure and (Dr. C. B. C. Boyce and Dr. B. V. Milborrow), 537
- Biological Acylating Agent: Thalidomide as a Possible (S. Fabro et al.), 1208
- Biological and Mental Evolution: An Exercise in Analogy (Arthur Koestler), 1033
- Biological Development: Control of, 838
- Biological Programme: International (Dr. E. B. Worthington), 223
- Biological Rhythm Research (Prof. A. Sollberger), review by Dr. A. D. Lees, 973
- Biological Specimens: Simple Technique for embedding and supporting Delicate (R. H. Harris), 199
- Biological Systems at the Molecular Level (Dr. B. Askonas, Prof. J. A. V. Butler, Prof. F. Jacob, Dr. D. C. Phillips, Dr. L. Sachs and Dr. G. V. Sherber), 1048
- Biology of the Algae (Dr. F. E. Round), review by Dr. M. Parke and G. T. Bolach, 824
- Biology of the Cytoplasm (Dr. O. A. Scheide, R. G. McCandless and R. J. Munn), 247
- Biology: Volume 1: Advances in Radiation (edited by L. G. Augenstein et al.), review by J. St. L. Philpot, 1032
- Bioluminescence in the Sea: Measurements of Spontaneous (Dr. B. P. Boden et al.), 1078
- Biomedical Research: Use of Pigs in (Dr. L. K. Bustad and R. O. McClellan), 531
- Biosphere: Evolution in the (Prof. H. F. Blum), 324
- Biostratigraphy of the Neogene: Progress in the Planktonic Foraminiferal (Dr. F. T. Banner and Dr. W. H. Blow), 1164
- Biosynthesis of 2-Aminoethanephosphonic Acid: A Phosphoramidic Acid Rearrangement? (Dr. W. Segal), 1284
- Biosynthesis of Triiodothyronine Sulphate by Beef Thyroid *in vitro* (G. L. Cohn), 80
- Biosynthetic Utilization by Human Spermatozoa of a Metabolite of the Female Reproductive Tract: Oxidative and (Prof. C. Turner), 1115
- Biphasic Dose-response Relationship with Acetylcholine on the Heart of the Mollusc *Tapes turgida* (P. R. Carroll and G. E. Chesher), 1220
- Birch, G.: Radiation Preservation of Foods, review, 973
- Birch, Prof. L. Charles: Nature and God, review, 985
- Birds: Origin and Significance of the Power of Vocal Imitation: with Special Reference to the Amphiphil Singing of (Dr. W. H. Thorpe and M. E. W. North), 219
- Birds with Special Reference to Phalaropes: Prolactin and the Incidence of Brood Patch Formation and Incubation Behaviour of the Two Sexes in Certain (Prof. E. O. Hohn and S. C. Cheng), 197
- Birmingham, University of, Appointments, 231, 436, 836
- Bisalbuminaemic Family: A New (Dr. G. Sander et al.), 1222
- Biscoe, Dr. T. J.: Some Effects of Drugs on the Isolated Superfused Carotid Body, 294
- Biscoe, Dr. T. J., and Stehbens, Dr. W. E.: Electron Microscopic Observations on the Carotid Body, 708
- Bishop, I. R., and Foxon, Prof. G. E. H.: A Visit to the Lower Amazon, 114
- Bishop, Dr. J. O.: Reticulocyte Ribosome Fraction with an Exceptional Capacity for Polyphenylalanine Synthesis, 361
- Bishop, Prof. R. E. D.: Vibration, review by K. R. Weiss, 964
- Bishop, Prof. R. E. D., Gladwell, G. M. L., and Michaelson, S.: Matrix Analysis of Vibration, review by Prof. W. A. Tuplin, 616
- Bitensky, Dr. M. W., and Kurstein, S. R.: Effects of Cyclic Adenosine Monophosphate and Melanocyte-stimulating Hormone on Frog Skin *in vitro*, 1282
- Bitó, Dr. J., and Lakatos, G.: Dependence of the Properties of Moving Striations in the Plasma of the Positive Column on some External and Internal Parameters, 445
- 'Bitometer': An Insect (P. Kashin and H. G. Wakeley), 462
- Bivalve Gastropod *Berthelina limax* (Kawaguti and Baba 1959) from the Indian Ocean: Record of the (K. P. Rao), 404
- Black, Dr. M., and Richardson, M.: Promotion of Germination in Light-requiring Seed by Chloramphenicol, 1114
- Black, M. Kay, et al.: Promotion by Kinetin of the Polar Transport of Two Auxins, 1065
- Black, Dr. P. J., et al.: Spectroscopy in Australia, 125
- Black, R. F., et al.: Palaeomagnetism of the Isachsen Diabasic Rocks, 179
- Black and White: Tactile-vision: Thermal and Texture Cues in the Discrimination of (C. N. French), 1352
- Black Sea: *Relictogobius kryzhanorskii* and the Penetration of Mediterranean Gobies into the (Dr. P. J. Miller), 474
- Blackett, Prof. P. M. S.: Election as President of the Royal Society in succession to Lord Florey, 1044
- Blackman, Dr. L. C. F., and Harrop, R.: Infra-red Spectra of Quaternary Ammonium Compounds adsorbed on Silica-gel 'Aerosil', 777
- Blackwood, Miss Beatrice, et al.: Hundred Years of Anthropology, Third Edition, revised, review by Prof. M. Fortes, 215
- Blair, Phyllis B.: Immunology of the Mouse Mammary Tumour Virus (MTV): A Qualitative *in vitro* Assay for MTV, 165

- Blake, A., and Peacocke, Dr. A. R. : Optical Rotatory Dispersion and Secondary Structure of Ribonucleic Acid in Mammalian Ribosomes, 1319
- Blanco, A., et al. : Polymorphism of Lactate Dehydrogenase Isozymes in Pigeons, 284
- Blau, Dr. J. N. : Phagocytic Function of Hassall's Corpuscles, 564
- Bloat in Ruminants : Possible Relationship between Trace Metals and (P. J. Harris and Prof. F. Sebba), 869
- Blood Aminoacyl Naphthylamidase in the Rat : Source of (Dr. B. Monis), 587
- Blood Cells : Rate of Chromosome Duplication at the End of the Deoxyribonucleic Acid Synthetic Period in Human (Dr. C. W. Gilbert et al.), 159
- Blood Flow in Turtles (F. N. White and G. Ross), 759
- Blood Group Secretion Factors in Bronchogenic Carcinoma (Prof. C. McNeil, E. F. Treutelman, J. N. Ladle, W. M. Helmick and Prof. H. P. Plenk), 299
- Blood *in vitro* : Carbon Dioxide Production of Whole (L. H. Capel et al.), 82
- Blood : Inhibition of Haemolysis by 'Cremophor' in Conserved (Dr. K. Reber), 195
- Blood Supply in Liver Regeneration : Role of Portal (Dr. R. Y. Thomson and A. M. Clarke), 392
- Blood-sucking Arthropods : Feeding Mechanism of (M. M. J. Lavoipierre), 302
- Blotched Kenya Genet : Sulphocysteine in the Urine of the (J. C. Crawhall and Dr. S. Segal), 1320
- Blow, Dr. W. H., and Banner, Dr. F. T. : Progress in the Planktonic Foraminiferal Biostratigraphy of the Neogene, 1164
- Blum, Prof. Harold F. : Evolution in the Biosphere, 324
- Blumberg, Prof. B. S., and Murray, jun., Dr. R. F. : Precipitin Activity in the Sera of Patients with Deficiency of β -Lipoproteins (Acanthocytosis), 357
- Blunden, G., and Challen, Dr. S. B. : Red Pigment in Leaf Galls of *Salix fragilis* L., 388
- Blunt, Prof. M. J., et al. : Possible Role of Neuroglia, 600
- Boalch, G. T., and Parke, Dr. M. : Biology of the Algae, review, 824
- Boats of North America : Bark Canoes and Skin, 22
- Bock, Dr. F. G., Shamberger, R. J., and Myers, H. K. : Tumour-promoting Agents in Unburned Cigarette Tobacco, 584
- Boden, Dr. B. P., Kampa, Dr. E. M., and Snodgrass, J. M. : Measurements of Spontaneous Bioluminescence in the Sea, 1078
- Bodleian Library, 1153
- Bodsworth, Prof. C. : Appointment as Professor and Head of the Department of Metallurgy, Brunel College, London, 433
- Body-weight and Resistance to Exhaustion in the Rat : Effect of Infantile Treadmill Experience on (R. A. Levitt and Prof. W. B. Webb), 1128
- Bohm, Prof. David : Special Theory of Relativity, review by Prof. C. W. Kilmister, 986
- Boiteux, Dr. H., and Mavrodineanu, Dr. R. : Flame Spectroscopy, review by Dr. D. T. Lewis, 5
- Bokkenheuser, V., et al. : Prevention of Cholesterol-induced Cholelithiasis by Neomycin, 1226
- Bolker, H. J. : *Obituary* of Prof. C. B. Purves, 731
- Bolton, J. G., et al. : Anomalous Intensity Ratios of the Interstellar Lines of OH in Absorption and Emission, 1193
- Bombarding Electron Energy : Relative Ionization Efficiencies of Hydrocarbon Molecules as a Function of (J. A. Takacs and Prof. G. R. Freeman), 996
- Bond Strengths in Borazoles : Boron-Nitrogen (Dr. B. C. Smith and L. Thakur), 74
- Bone : Binding of the Tetracyclines to (Dr. D. D. Perrin), 787
- Bone Marrow Allografts in Thymectomized X-irradiated Dogs (I. L. Chertkov, A. S. Maximenko, M. N. Novikova and M. O. Raushenbach), 399
- Bone Marrow as the Major Source of Potential Immunologically Competent Cells in the Adult Mouse (Dr. M. L. Tian and Dr. L. J. Cole), 1223
- Bone Marrow in Chronic Myelogenous Leukaemia : Return of Leucocytes to the (Dr. J. H. Moxley et al.), 1281
- Bone : Validity of Radiocarbon Dates on (Dr. M. A. Tamers and F. J. Pearson, jun.), 1053
- Bonsall, Prof. F. F. : Appointed to McLaurin Chair of Mathematics in the University of Edinburgh, 19
- Boocock, G., and Popplewell, D. S. : Distribution of Plutonium in Serum Proteins following Intravenous Injection into Rats, 282
- Books : Storage of, 434
- Boranes : Boron, Metallo-Boron Compounds and (edited by Roy M. Adams), review by Dr. L. H. Long, 968
- Borazoles : Boron-Nitrogen Bond Strengths in (Dr. B. C. Smith and L. Thakur), 74
- Borek, Prof. E., et al. : Changes in the t-RNA Methylating Enzymes during Insect Metamorphosis, 555
- Borgstrom, Dr. Georg, (edited by) : Fish as Food, Vol. 3 and Vol. 4, Processing, review by Dr. G. R. Wadsworth, 317
- Boron, Metallo-Boron Compounds and Boranes (edited by Roy M. Adams), review by Dr. L. H. Long, 968
- Boron-Nitrogen Bond Strengths in Borazoles, (Dr. B. C. Smith and L. Thakur), 74
- Borum, Dr. Kirstine : Labelling of Mouse Thymocytes *in vivo* with Tritiated Thymidine for Cell Transfer Experiments, 253
- Botany : Strasburger's Textbook of (rewritten by R. Harder et al.), review by Dr. A. Allsopp, 618
- Boudreau, Capt. James C. : Neural Volleying : Upper Frequency Limits detectable in the Auditory System, 1237
- Boulter, Dr. D., et al. : Taxonomic Investigation of Several *Brassica* Species using Serology and the Separation of Proteins by Electrophoresis on Acrylamide Gels, 704
- Bourdillon, R. E., Clarke, Prof. C. A., Ridges, A. P., Sheppard, Prof. P. M., Harper, Dr. P., and Leslie, Dr. S. A. : 'Pink Spot' in the Urine of Schizophrenics, 453
- Bovine Chromosomes : Measurements of (F. N. Baker et al.), 923
- Bovine Lung and from *Mycoplasma mycoides* : Serological Relationship between Galactans from Normal (Dr. M. Shifrine and R. N. Gourlay), 498
- Bovine Respiratory Disease : Association of Adenoviruses with (Dr. J. H. Darbyshire et al.), 307
- Bovine Synovial Fluid : Electron Microscope Observations on the Free Protein and the Protein-polysaccharide Complex of (Dr. J. W. Smith and Dr. J. Frame), 867
- Bowen, E. J. (edited by) : Recent Progress in Photobiology, review by Dr. A. Knowles, 974
- Bowes, J. H., Raistrick, A. S., and Moss, Dr. J. A. : Reactivity of the α -Amino Group of Collagen, 1094
- Bourne, G. H. (edited by) : World Review of Nutrition and Dietetics, Vol. 5, review by Dr. A. M. Copping, 421
- Bowsher, Dr. David and Angaut, P. : Cerebello-rubral Connexions in the Cat, 1002
- Boyce, Dr. C. B. C., and Milborrow, Dr. B. V. : Simple Assessment of Partition Data for correlating Structure and Biological Activity using Thin-layer Chromatography, 537
- Boyd, Prof. W. C., and Brown, R. : A Specific Agglutinin in the Snail *Otala* (*Helix*) *lactea*, 593
- Brachet, Prof. J. : Emission of Feulgen-positive Particles during the *in vitro* Maturation of Toad Oocytes, 596
- Bracken, Dr. E. C. : Appointment as Assistant to the Chief, U.S. Medical Research Fellowship Analyses, 433
- Bracken : Carcinogenic Activity of (Dr. I. Antice Evans and J. Mason), 913
- Bradley, Dr. D. J., Engwell, M., and Komatsu, H. : Laser-induced Damage in Diamond, 1081
- Bradley, Dr. R. S. : Physics of High Pressures and the Condensed Phase, review, 515
- Brain, The Right Hon. Lord : Drug-dependence, 825
- Brain in Bats : Technique for the Removal of the (A. M. Greenhall), 1014
- Brain Serotonin after Head X-irradiation and its Significance in the Potentiation of Barbiturate Hypnosis : Regional Changes in (Dr. V. Nair), 1293
- Brammer, Dr. K. W., et al. : Molecular Weight of Urokinase, 894
- Brant, Prof. D. H., and Kavanau, Prof. J. L. : Wheel-running Preferences of *Peromyscus*, 597
- Brassica oleracea* : Identical S Alleles in Different Botanical Varieties of (K. F. Thompson and J. P. Taylor), 306
- Brassica* Species using Serology and the Separation of Proteins by Electrophoresis on Acrylamide Gels : Taxonomic Investigation of Several (Dr. J. G. Vaughan et al.), 704
- Breakdown of Simple Hydrocarbons subjected to a Silent Electric Discharge (P. E. Cross), 892
- Breakdown Rate of Serum γ -Globulins in Mice (Dr. D. E. H. Tee, Dr. J. Watkins and M. Wang), 251
- Brdar, B., Kos, E., and Drakulic, M. : Metabolism of Nucleic Acids and Protein in Starving Bacteria, 303
- Breeding a Pelagic Copepod, *Euterpina acutifrons* (Dana), in the Laboratory (H. W. Neunies and G.-F. Pongolini), 571
- Breeding : Genetical Principles and Plant (Prof. W. Williams), review by Dr. K. Jones, 7
- Brenner, Dr. F. C. : Appointment as Assistant Chief of the National Bureau of Standards Textile and Apparel Technology Center, 628
- Bretschger, Dr. E. : Einführung in die Atomphysik, review, 217
- Brewery Convention : European, Report of the Barley Committee : Trials, 1963, review by A. H. Cook, 9
- Brickwork : Load-bearing, 121
- Briggs, Dr. D. E., and Millin, Dr. D. J. : Formation of Hydroxamates during Proteolysis by an Enzyme System from Yeast, 287
- Brightman, R. : The Government Explains : a Study of the Information Services, review, 1139
- The Modern University, review, 518
- Science in History, Third Edition, review, 986
- Brightness at 14.1 Mc/s : Sky (K. W. Yates and Dr. R. Wiebeinski), 64
- Brimble, L. J. F. : Sudden Death of the Editor of Nature on November 15th, 1965, 732
- Brimble, Jack, 819
- Bristol Channel : Evolution of the (Dr. F. J. North), Third Edition, review by Dr. A. R. MacGregor, 110
- Bristol, University of, Appointments, 1047
- Britain : Commercial Nuclear Power Stations in (S. Weintraub), 25
- Energy Forecasts for, 229
- Fuel Policy for, 24
- National Instrument Service in, 528
- Nature Conservancy in, 635
- New Commonwealth Students in, review by Lord Twining, 957
- Social and Industrial Gerontology in Great (C. E. Fleming), 623
- Soil Survey of Great, 123
- University Entrance Requirements in, 433
- Volcanological Research in, 435
- Britain, France and Western Germany : Comparative Study of Housing in, 233
- British Aid to Developing Countries, *Overseas Development : The Work of the New Ministry*, 128
- British Art, 1740-70, 122
- British Council, Science Education Officer, 432
- British Glass Industry Research Association, Tenth Annual Report, 1965, 733
- British Hovercraft, 833
- British Industry : Research and Development in, 101
- British Isles : Freshwater Life of the (John Clegg), Third Edition, review by W. J. Rees, 988
- British Museum : Department of Western Asiatic Antiquities, 434
- British Museum (Natural History) : Catalogue of Type Specimens of Microlepidoptera in the, described by E. Meyrick, (Dr. J. F. Gates Clarke), Vol. 5, review by Prof. G. C. Varley, 108
- British Productivity Council, 935
- Brittain, Dr. R. P., et al. : Aggressive Behaviour, Mental Sub-normality and the XYY Male, 1351
- Broadbent, Prof. T. A. A. : Basic Laws of Arithmetic (G. Frege), review, 318
- Counterexamples in Analysis, review, 218
- Introduction to Lattice Theory, review, 984
- Mathematical Analysis : Functions, Limits, Series and Continued Fractions, review, 1140
- Mathematical Discovery : On Understanding, Learning, and Teaching Problem Solving, Volume 2, review, 986
- Treatise on Trigonometric Series, Two Volumes, review, 106
- Brockington, Prof. C. F. : Public Health in the Nineteenth Century, review by Sir Arthur MacNalty, 977
- Broda, Prof. E., and Findenegg, G. : Mechanism of Uptake of Trace Elements by Plant Roots, 196
- Brody, Dr. J. A., and Huntley, B. : Human Lymphocytes cultured in Microplates, 1232
- Broken Hill, Australia : Iron Ore Production and Research at, 22
- p-Bromophenol : Androgenic Steroid Complexes with (Dr. C. Eger and Dr. D. A. Norton), 997
- Bronchogenic Carcinoma : Blood Group Secretion Factors in (Prof. C. McNeil et al.), 299
- Bronk, Dr. J. R., and Parsons, Dr. D. S. : Accumulation and Incorporation of Amino-acid in Rat Intestine *in vitro*, 785
- Brood Patch Formation and Incubation Behaviour of the Two Sexes in Certain Birds with Special Reference to Phalaropes : Prolactin and the Incidence of (Prof. E. O. Hohn and S. C. Cheng), 197
- Brook, Prof. G. L. : The Modern University, review by R. Brightman, 518
- Brown, Barry, et al. : Mathematical Model for the Process of Aggregation in Immune Agglutination, 84
- Brown, Dr. D. M. : Nucleotides and Coenzymes, review, 987
- Brown, Dr. F., and Martin, Dr. S. J. : A New Model for Virus Ribonucleic Acid Replication, 861
- Brown, I. N., and Brown, Dr. K. N. : Immunity to Malaria : Antigenic Variation in Chronic Infections of *Plasmodium knowlesi*, 1286
- Brown, Prof. J. : Appointed to Chair of Psychology in the University of Newcastle upon Tyne, 21
- Brown, Dr. K. N., and Brown, I. N. : Immunity to Malaria : Antigenic Variation in Chronic Infections of *Plasmodium knowlesi*, 1286
- Brown, Dr. P. E. : Geology of Granite, review, 1030
- Brown, Dr. R. A. : X-ray Studies on Highly Pure Magnesium Oxide, 481
- Brown, Rebecca, and Boyd, Prof. W. C. : A Specific Agglutinin in the Snail *Otala* (*Helix*) *lactea*, 593

- Bruce, M., et al.: Social Companions and the Mother-Infant Relationship in Rhesus Monkeys, 301
- Brumm, D., et al.: Reconstruction of Phase Objects by Holography, 1159
- Brunel College, London, Professor of Metallurgy, 433
- Brunton, Muriel, et al.: Aggressive Behaviour, Mental Sub-normality and the XY Male, 1351
- Bryozoa Research: First International Conference, "Post-Palaeozoic Bryozoa", 1965, 124
- Bubbles and Liquid Drops in an Electrically Stressed Insulating Liquid: Deformation of Gas (Dr. K. C. Kao), 279
- Buckle, Dr. E. R., and Pouring, Dr. A. A.: Effects of Seeding on the Condensation of Atmospheric Moisture in Nozzles, 367
- Bud Formation in the Hypotrichida: Endogenous (Dr. C. R. Curds), 90
- Budak, Dr. Hayati: Pure Quadrupole Resonance Frequency Shift and Internal Stress in Gamma-ray Irradiated Sodium Chlorate, 382
- Budd, R. E., et al.: Quantitative Determination of Haemoglobin A₂ by Acrylamide-gel Electrophoresis, 1103
- Building and Civil Engineering Industries: Research and Development in, 629
- Building Material in Hot Climates: Timber as, 1153
- Building Research: Director of, Ministry of Technology, 228
- Building Research Institute, Roorkee, India: Central, 629
- Bukrinskaya, A. G., Gitelman, A. K., and Shapiro, I. M.: Influence of Heterologous Histone on the Synthesis of Cellular and Viral Ribonucleic Acid and Production of Haemagglutinin, 557
- Bulbar Gustatory Relay of the Rat: Chemotopic Organization in the (B. P. Halpern), 393
- Bullard Forest Research Fellowships, 631
- Bunn, Dr. C. W.: Optical Transforms, review, 1031
- Burdett, T. A.: Applied Science in the Schools, 1269
- Burdette, Prof. W. J., and Anderson, R.: Puffing of Salivary Gland Chromosomes after Treatment with Carbon Dioxide, 409
- Burges, N. A., et al., (edited by): Flora Europaea, Vol. 1, review by Prof. J. A. Nannfeldt, 8
- Burges, R. A., Brammer, Dr. K. W., and Coombes, J. D.: Molecular Weight of Urokinase, 894
- 'Buried' Tyrosyl Residues and the Activity of Trypsin (J. F. Riordan, Dr. W. E. C. Wacker and Prof. B. L. Yaloe), 1209
- Burke, Dr. D. C., and Ross, Dr. J.: Molecular Weight of Chick Interferon, 1297
- Burkhill, Dr. J. C.: Funktionentheorie, Fourth Edition, review, 963
- Burkitt, M. C.: Earliest Civilizations of the Near East, review, 984
- The Royal Hordes: Nomad Peoples of the Steppes, review, 1138
- Burley, Dr. R. W.: Transphosphorylation of Adenosine Di- and Tri-phosphates in the Presence of Calcium Phosphate Precipitates, 683
- Burnet, Sir Macfarlane: Retirement from Directorship of the Walter and Eliza Hall Institute of Medical Research, Melbourne, 729
- Burniston Brown, Dr. G.: Physics Nobel Lectures Including Presentation Speeches and Laureates' Biographies 1922-1941, review, 103
- Burns, J. E., and Pegg, Dr. D. E.: A Sex-dependent Factor in Aplastic Anaemia?, 1187
- Burrows, Dr. Robert B.: Microscopic Diagnosis of the Parasites of Man, review by Dr. G. Lapage, 1029
- Bursa Pharyngea in the Giant Panda (*Ailuropoda melanoleuca*) (Prof. A. J. E. Cave), 865
- Burstein, S. R., and Bitensky, Dr. M. W.: Effects of Cyclic Adenosine Monophosphate and Melanocyte-stimulating Hormone on Frog Skin *in vitro*, 1282
- Burton, Dr. D. E., Lambie, Dr. A. J., Ludgate, J. C. L., Newbold, Dr. G. T., Percival, Dr. A., and Siggers, D. T.: 2-Trifluoromethylbenzimidazoles: a New Class of Herbicidal Compounds, 1166
- Bustad, Dr. L. K., and McClellan, R. O.: Use of Pigs in Biomedical Research, 531
- Butler, Prof. J. A. V., et al.: Biological Systems at the Molecular Level, 1048
- Butlin, K. R.: *Obituary* by Prof. J. R. Postgate, 831
- Butter after Oxidative Deterioration: *n*-Alkan-1-ols in (W. Stark and D. A. Fors), 190
- Butterfield, Prof. Herbert: History as the Organization of Man's Memory, 1036
- C**ACAO Seed Cotyledon coincident with Seed Death: Respiratory Changes in the (L. A. Casas et al.), 1348
- Caesium-137 in Columbia River Plume, July 1964: Strontium-90 and (Prof. K. Park et al.), 1084
- Calozoic Volcanic Succession: Re-classification of the Ethiopian (P. A. Mohr), 177
- Calcium Cyclamate: Feeding and Reproduction of Rats fed (P. O. Nees and P. H. Derse), 81
- Calcium on the Comparative Behaviour of Strontium and Calcium: Effects of Dietary Levels of Phosphorus and (K. Kostial et al.), 1110
- Calcium Oxalate Monohydrate: Structure of (Prof. H. J. Arnott et al.), 1197
- Calcium Phosphate Precipitates: Transphosphorylation of Adenosine Di- and Tri-phosphates in the Presence of (Dr. R. W. Burley), 683
- Calculation of Surface Area and Average Pore Radius: Errors in the (Dr. D. Dollimore and Dr. G. R. Heal), 1092
- Calculations with Two Nearly Equal Quantities (Dr. J. H. Fremlin; Dr. F. T. Worrell), 1306
- Caldwell, Dr. W. L., Lamerton, Prof. L. F., and Bewley, Dr. D. K.: Increased Sensitivity of *in vitro* Murine Leukaemia Cells to Fractionated X-rays and Fast Neutrons, 168
- Calibration of the Tetrahedral Anvil Apparatus: Combined Very High Pressure/High Temperature (J. Lees and B. H. J. Williamson), 278
- California?: Oligocene in (J. H. Lipps), 885
- Callingham, Dr. B. A.: Natural History of the Chromaffin Cell, review, 6
- Calnan, Prof. C. D.: The Epidermis, review, 213
- Calorimetry: Modern Low-temperature (Dr. I. M. Firth), 1162
- Cambridge Engineering Freshmen, 528
- Cambridge, University of, Churchill College Fellowships, 936
- Cameroon and Ibadan, Two Unusual Variants of Human Haemoglobin A: Three Haemoglobins K: Woolwich, an Abnormal, (Dr. N. Allan et al.), 658
- Campbell, Dr. F. W., and Green, Dr. D. G.: Monocular versus Binocular Visual Acuity, 191
- Campbell, Prof. J. W.: Arginine and Urea Biosynthesis in the Land Planarian: its Significance in Biochemical Evolution, 1299
- Campbell, P. N., and Greville, G. D., (edited by): Essays in Biochemistry, Vol. 1, review by Dr. T. Elmore, 316
- Campos, J. O., Koler, D. R., and Bigley, R. H.: Kinetic Differences between Human Red Cell and Leucocyte Pyruvate Kinase, 194
- Canada: Plant Physiology in (Prof. J. A. Webb), 27
- Cancer Coagulative Factor: A Screening Test for Substances Inhibiting the (Dr. E. M. Glaser et al.), 1008
- Cancer Invasion: Principles of Cell Motility: The Direction of Cell Movement and (Dr. S. B. Carter), 1183
- Cancer: 1964: Proceedings of the International Symposium on Chemotherapy of (edited by Prof. P. A. Plattner), review by Dr. D. C. Roberts, 1032
- Cancer to Multiple Causes: Implications of the Immunological Theory of (Dr. M. R. Anderson and Prof. H. N. Green), 338
- Canoes and Skin Boats of North America: Bark, 22
- Capacitance of the Electrical Double-layer at Platinum/Halide Metal Interfaces: Adsorption and the Differential (A. D. Graves and Dr. D. Inman), 481
- Capel, L. H., Fletcher, E. C., and Nunn, Prof. J. F.: Carbon Dioxide Production of Whole Blood *in vitro*, 82
- Capp, B., and Hill, R. M.: Ionization Times in Shock-heated Potassium Vapour, 176
- Capra, C., et al.: *Plante Medicinali: Chimica Farmacologia e Terapia*, Vol. 2, review by Dr. F. N. Howes, 218
- Carathéodory's Principle and the Kelvin Statement of the Second Law (J. Dunning-Davies), 576
- Carbohydrate Chemistry: Methods in (edited by Prof. R. L. Whistler), Vol. 4, Starch, and Vol. 5, General Polysaccharides, review by Prof. M. Stacey, 971
- Carbohydrates: Fixation of Metabolic Products in the Soil during Decomposition of (H. Sørensen), 97
- Carbon and Graphite Group, 735
- Carbon Dioxide by Plants: Influence of Hexadecane on Absorption of (Prof. J. M. Riedhart), 300
- Carbon Dioxide Production of Whole Blood *in vitro* (L. H. Capel, E. C. Fletcher and Prof. J. F. Nunn), 82
- Carbon Dioxide: Puffing of Salivary Gland Chromosomes after Treatment with (Prof. W. J. Burdette and R. Anderson), 409
- Carbon Disulphide in Benzene by Electron Capture Gas Chromatography: Determination of p.p.m. Concentrations of (D. W. Grant and G. A. Vaughan), 75
- Carbon Isotope Ratios and Palaeosalinities of Purbeck-Wealden Carbonates (Prof. P. Allen and Prof. M. L. Keith), 1278
- Carbonaceous Meteorites: Evidence for Microfossils in the Alais and Orgueil (S. L. Van Landingham), 947
- Carbonates: Carbon Isotope Ratios and Palaeosalinities of Purbeck-Wealden (Prof. P. Allen and Prof. M. L. Keith), 1278
- Carbone, P. P., et al.: Acetohydroxamate: Bacterial Urease Inhibitor with Therapeutic Potential in Hyperammonaemic States, 46
- Carbonell, Dr. L. M., and Kanetsuna, Dr. F.: Glycogen in Yeast Form of *Paracoccidioides brasiliensis*, 686
- Carboniferous (Upper) of South-west England: Recent Finds in, and their Significance (Dr. H. G. Reading); (Dr. J. E. Prentice), 745
- Carbons at the Active Centre of Enzymes: Evidence for Structures resembling Crystalline (Dr. E. D. Wachsmuth), 942
- Carcinogenesis influenced by Vegetable Extracts: Methylcholanthrene (V. Vendég), 598
- Carcinogenic Activity of Bracken (Dr. I. Antice Evans and J. Mason), 913
- Carcinogenic 4-Nitro-quinoline N-Oxide of Tryptophan and Indole Uptake in *Escherichia coli*: Interference by the (A. C. Zahalsky and Prof. S. L. Marcus), 296
- Carcinoma: Blood Group Secretion Factors in Bronchogenic (Prof. C. McNeil et al.), 299
- Cardiac Contraction: Heat Production in a (Dr. N. V. Ricchiuti and Dr. C. L. Gibbs), 897
- Care of Laboratory Animals, 630
- Careers with Instruments, 733
- Carlton, Dr. P. L., et al.: Behavioural Deficit in the Rat induced by feeding Phenylalanine, 1021
- Carmichael, Prof. J. W., Julius, Prof. R. S., and Martin, Dr. P. M. D.: Relative Similarities in One Dimension, 544
- Carmichael, Dr. Leonard: James Smithson: Pathfinder in Science and Philanthropy, 321
- Carnaghan, R. B. A.: Hepatic Tumours in Ducks fed a Low Level of Toxic Groundnut Meal, 308
- Carotenoid by a Green Alga: Production of (Prof. F. Dentice di Accadia et al.), 1342
- Carotid Body: Electron Microscopic Observations on the (Dr. T. J. Biscoe and Dr. W. E. Stehbens), 708
- Carotid Body: Some Effects of Drugs on the Isolated Superfused (Dr. T. J. Biscoe), 294
- Carp, R. I., and Krlitchevsky, Dr. D.: Influence of Heavy Water (Deuterium Oxide) on Plaque Size of Western Equine Encephalitis Virus, 93
- Carr, Dr. Jean, and Wilhelm, Prof. D. L.: Interspecies Differences in Response to Polypeptides as Permeability Factors, 653
- Carrier of Palaeomagnetism: Cassiterite as a (V. Hanuš and M. Krs), 675
- Carroll, P. R., and Cheshier, G. B.: Biphasic Dose-response Relationship with Acetylcholine on the Heart of the Mollusc *Tapes turrida*, 1220
- Carter, J. C., et al.: Observations of Polarized OH Emission, 440
- Carter, Dr. S. B.: Principles of Cell Motility: The Direction of Cell Movement and Cancer Invasion, 1183
- Cartridges for Field Sampling of Iodine-131 in Milk: Evaluation of Ion Exchange (R. H. Johnson, jun., and T. C. Reavey), 750
- Casas, Iván A., Redshaw, E. S., and Ibáñez, M. L.: Respiratory Changes in the Cacao Seed Cotyledon coincident with Seed Death, 1348
- Casas-Builla, M., and Gómez-Campo, C.: Radiation-induced Sex Reversal in *Ecballium elaterium*, 805
- Case, R., et al.: Labelled Desolarizing Drugs in Normal and Denervated Muscle, 901
- Case for Applied Research, 1025
- Cassiterite as a Carrier of Palaeomagnetism (V. Hanuš and M. Krs), 675
- Cat: Alcohol and Evoked Potentials in the (A. K. Schweigert et al.), 688
- Cerebello-rubral Connections in the (P. Angaut and Dr. D. Bowsher), 1002
- Correlation between Unit Activity and Slow Potential Changes in the Unanaesthetized Cerebral Cortex of the (A. D. J. Robertson), 757
- Differences in Variability of Discharge Frequency between Primary and Secondary Muscle Spindle Afferent Endings of the (R. B. Stein and Dr. P. B. C. Matthews), 1217
- Catalan, Dr. R. E., and Munido, Dr. A. M.: *In vitro* Effect of Gamma-radiation on Different Cholinesterase Preparations, 1227
- Catalytic Activity for F-Centres: Evidence for (Prof. J. G. Aston et al.), 181
- Catecholamine Binding: Adenosine Triphosphate-Metal-Norepinephrine Ternary Complexes and (Dr. R. W. Colburn and Dr. J. W. Maas), 37
- Catecholamine Transport: Co-ordination Chemistry and Membrane Function with Particular Reference to the Synapse and (Dr. J. W. Maas and Dr. R. W. Colburn), 41
- Catecholamines or Serotonin: Thromboses in Large Veins and Pulmonary Embolisms induced by (Prof. H. Selye et al.), 900
- Catenas: Soil, 123
- Cathodes: Diffusion or Hydride Component of Hydrogen Overpotential at Platinized Platinum and Palladized Palladium (M. N. Hull and Dr. F. A. Lewis), 887
- Cation Permeability of Phospholipid Model Membranes: Effect of Narcotics, (Dr. A. D. Bangham, Dr. M. M. Standish and N. Miller), 1295
- Cation Sites in Synthetic Zeolites (Dr. T. I. Barry and L. A. Lay), 1312
- Cattle fed on High-energy Diets: Phosphorus Excretion of (W. D. Reed et al.), 953

INDEX

- Cattle Hair : Cysteine Content of (P. H. Springell and S. J. Leach), 1326
- Cattle with *Fasciola hepatica* : A Comparison of Low and High Infection Rates : Experimental Infections of (J. G. Ross), 907
- Cattorini, P. E., et al. : *Plante Medicinali* : *Chimica Farmacologia e Terapia*, Vol. 2, review by Dr. F. N. Howes, 218
- Caulfield, H. J. : Transmission through a Tapered Quartz Tube in the Laser Near Field, 773
- Causes and Cures of Neurosis (Prof. H. J. Eysenck and Dr. S. Rachman), review by Dr. B. Semonoff, 1027
- Cave, Prof. A. J. E. : The Bursa Pharyngea in the Giant Panda (*Ailuropoda melanoleuca*), 865
- Cawley, Mr. R. W. : Appointment as Director of the Wheat Research Institute New Zealand D.S.I.R., 327
- Cell : Circulation in the (Prof. C. C. Lindegren), 304
- Cell Differentiation in Milk : Bacterial Count and (C. L. Duitschaeffer), 200
- Cell Growth in Root Caps : Cytoplasmic Organelles and (Dr. B. E. Juniper and Dr. F. A. L. Clowes), 864
- 'Cell Killing' in Radiobiology (Dr. D. M. Robinson), 500
- Cell Membrane : Heterogeneity of Water-soluble Structural Components of Human Red (Prof. M. D. Poulik and Dr. P. K. Lauf), 874
- Cell Motility : the Direction of Cell Movement and Cancer Invasion : Principles of (Dr. S. B. Carter), 1183
- Cell Nuclei : Computer Recognition of Cervical (G. W. McMaster), 485
- Cell Physiology : Methods in, Vol. 1, (edited by D. M. Prescott), review by Dr. J. H. Humphrey, 6
- Cell Product : Control of the Competent State in *Pneumococcus* by a Hormone-like (Dr. A. Tomasz), 155
- Cell Transfer Experiments : Labelling of Mouse Thymocytes *in vivo* with Tritiated Thymidine for (Dr. K. Borum), 253
- Cell Wall Mucopeptide : 2,6-Diamino-3-hydroxypimelic Acid in Microbial (H. R. Perkins), 872
- Cell-walls of *Pseudomonas aeruginosa* : 'Lipid A' Component from the (Dr. K. Clarke et al.), 586
- Cells : Pectic Polysaccharides in the Growth of Plant (S. E. B. Gould et al.), 876
- Cells : Thymus and the Production of Antibody-plaque-forming (Dr. J. F. A. P. Miller et al.), 1332
- Cells containing Foreign H-2 Antigens : Allogeneic Inhibition of Tumour Cells by *in vitro* Contact with (K. E. Hellstrom et al.), 458
- Cells producing 7S Antibody : A Localized Haemolysis in Gel Method for the Detection of (Dr. J. Sterzl et al.), 858
- Celtic Customs : Traditional, 1263
- Cementite : A Preparation of (J. Freil and Dr. A. K. Galwey), 183
- Centenary of the Quekett Microscopical Club (Prof. G. J. Cunningham), 937
- Centimetre and Decimetre Wavelengths : Basic Component of Solar Radio Emission at (Dr. M. K. Das Gupta and D. Basu), 739
- Central Action of Hemicholinium (Dr. D. M. J. Quastel and Prof. D. R. Curtis), 192
- Central Africa : Malaria and the Opening-up of (R. Weatherall), 1267
- Central Building Research Institute, Roorkee, India, 629
- Central Stimulant Properties of Compounds with Peripheral Muscarinic Properties (G. B. Leslie), 1291
- 'Centron' : Concept of the (Dr. D. W. Menzies), 163
- Centropomidae from Lake Chad : Winter Scale Rings in *Lates niloticus* (Pisces : (A. J. Hopson), 1013
- Cephaloridine to Staphylococcal Penicillinase : Relative Instability of (Dr. M. Ridley and Dr. I. Phillips), 1076
- Cephaloridine with Penicillinase-producing Gram-negative Bacteria : Interaction of (Dr. J. M. T. Hamilton-Miller et al.), 235
- Cerebellar Cortex : Cholinergic Excitation and Inhibition in the (P. Kása and Dr. B. Csillik), 695
- Cerebello-rubral Connections in the Cat (P. Angaut and Dr. D. Bowsher), 1002
- Cerebral Cortex of the Cat : Correlation between Unit Activity and Slow Potential Changes in the Unanesthetized (A. D. J. Robertson), 757
- Cerebral Cortex on the Cuneate Nucleus of the Monkey : Influence of the (Dr. F. Harris et al.), 1215
- 'Cerebral Death' by Hypoxia : Modification of Radiation (Dr. E. A. Wright and J. Shewell), 904
- Cerebrovascular Accidents, 734
- Ceric Salts : Oxidation of Some 2,4-Dinitrophenylamines with (Miss S. Haworth and Dr. J. R. Holker), 184
- Ceriodaphnia quadrangula* (O. F. Muller) : Haemoglobin in (B. Czeczuga), 907
- Certainty : Beyond the Edge of (edited by R. G. Colodny), review by F. I. G. Rawlins, 314
- Cervical Cell Nuclei : Computer Recognition of (G. W. McMaster), 485
- Cetus Arc : Filamentary Nebulosity in the Vicinity of the (Dr. J. Meaburn), 575
- Chadderton, Dr. L. T., and Torrens, I. McC. : Correlated Atomic Collisions in Irradiated Crystal Lattices, 880
- Chaetepithelioma Induction in the Syrian Hamster : Lactic Dehydrogenase Relative to (Prof. F. T. Algard et al.), 567
- Chain Configurations : Hexagonal-mesh Structures and Stable Polypeptide (Prof. R. E. Dickerson), 139
- Chain Reaction under an Ultrasonic Wave Effect : Initiation of (Prof. I. E. Elpiner et al.), 945
- Challen, Dr. S. B., and Blunden, G. : Red Pigment in Leaf Galls of *Salix fragilis* L., 388
- Chamberlain, Dr. J. E., and Gebbie, Dr. H. A. : Sub-millimetre Dispersion and Rotational Line Strengths of the Hydrogen Halides, 480
- Chance, B., Eisenhardt, R. H., Gibson, Q. H., and Longberg-Holm, K. K. (edited by) : Rapid Mixing and Sampling Techniques in Biochemistry, review by Prof. S. Dagley, 823
- Changes associated with Juvenile-to-Mature Growth Phase Transition in *Hedera* : Anatomical (J. R. Goodin), 504
- Changes in Amino-nitrogen Content of Solutions of γ -Keratoses from Wool Keratin (Dr. R. S. Asquith and P. A. Watson), 786
- Changes in Size of the Corpus Allatum in a Polymorphic Insect (D. White), 807
- Changes in the Histological Structure of the Spinal Column of *Sorex araneus* (L.) : Seasonal (L. Saure and H. Hyvarinen), 705
- Changes in the t-RNA Methylating Enzymes during Insect Metamorphosis (Dr. B. S. Baliga, Prof. P. R. Srinivasan and Prof. E. Borek), 555
- Changes in the Unanesthetized Cerebral Cortex of the Cat : Correlation between Unit Activity and Slow Potential (A. D. J. Robertson), 757
- Channels : Laminar and Turbulent Flow in Open (Dr. J. Allen), 1196
- Chantry, Dr. G. W., and Gebbie, Dr. H. A. : Sub-millimetre Wave Spectra Polar Liquids, 378
- Chapman, Dr. D. : Structure of Lipids by Spectroscopic and X-ray Techniques, review by Dr. F. D. Gunstone, 1246
- Charache, Patricia, et al. : New Inherited Coagulation Disorder caused by an Abnormal Fibrinogen ('Fibrinogen Baltimore'), 143
- Character Recognition by Holography (Prof. D. Gabor), 422
- Characterization of a Transfer Factor associated with Drug Resistance in *Salmonella typhimurium* (Dr. E. S. Anderson and Dr. M. J. Lewis), 843
- Characterization of Glucose-6-phosphate Dehydrogenase among Chinese (P. W. Wong, Ling-yu Shih, Prof. D. Y.-Y. Hsia and Dr. Y. C. Tsao), 1323
- Charge Transfer Properties of *dl*-Thioctic Acid and Other Disulphides (Wayne M. Moreau and Prof. K. Weiss), 1203
- Charged Particle to its Relative Biological Effectiveness : Relationship of the Velocity of a (Dr. J. E. Turner and H. Hollister), 36
- Charges on Arc Track Oxide Layers : Surface (Dr. W. P. Jayasekara et al.), 774
- Charing Cross Hospital Medical School : Chair of Biochemistry and Organic Chemistry, 1045
- Charnock, Dr. J. S., and Optit, Dr. L. J. : Molecular Model for a Sodium Pump, 471
- Chattopadhyay, D. P., et al. : Prevention of Cholesterol-induced Cholelithiasis by Neomycin, 1226
- Cheese (Dr. J. G. Davis), Two Volumes, review by Dr. J. A. B. Smith, 975
- Chemical Analyses of Rocks and Minerals, 1261
- Chemical and the Chemiosmotic Mechanisms of Electron Transport Phosphorylation : Evidence discriminating between the (Dr. P. Mitchell and Dr. J. Moyle), 1205
- Chemical Aspect of Penicillin Allergy : Direct Reaction of Penicillin with α -Amino-groups : New (Dr. C. H. Schneider and Dr. A. L. de Weck), 57
- Chemical Education, 1257
- Chemical Industries Association, Ltd., 630
- Chemical Industry : Melville on Macromolecule, 836
- Chemical Methods : Identification and Analysis of Surface-active Agents by Infrared and (Prof. D. Hummel), Two Volumes, review by Dr. P. Robson, 617
- Chemical Physiology of the Acid Mucopolysaccharides (J. E. Scott), 1270
- Chemical Shifts in Secondary and Tertiary Phosphines : Phosphorus-31 (Prof. S. O. Grim and W. McFarlane), 995
- Chemical Systems at Steady State : A Search for Mutation in (Dr. J. Allen), 258
- Chemiosmotic Mechanisms of Electron Transport Phosphorylation : Evidence discriminating between the Chemical and the (Dr. P. Mitchell and Dr. J. Moyle), 1205
- Chemistry : Analytical, Organic Complexing Reagents (D. D. Perrin), review by Dr. T. S. West, Elements of (A.-L. Lavoisier), review by Dr. Roger Partington, 987
- A Hundred Years of (Prof. A. Findlay), review by Dr. R. G. Partington, 969
- Methods in Carbohydrate (edited by Prof. R. L. Whistler), Vols. 4 and 5, review by Prof. M. Stacey, 971
- Optical Rotatory Dispersion and Circular Dichroism in Organic (Dr. P. Crabbe), review by Prof. W. Klyne, 970
- Chemistry and Membrane Function with Particular Reference to the Synapse and Catecholamine Transport : Co-ordination (Dr. J. W. Maas and Dr. R. W. Colburn), 41
- Chemistry and Refining : Advances in Petroleum, Vol. 10 (edited by J. J. McKetta, jun.), review by H. B. Milner, 969
- Chemistry and Technology of Leather (edited by Fred O'Flaherty, W. T. Roddy and R. M. Lollar), Vol. 4, review by Prof. A. G. Ward, 617
- Chemistry of Heterocyclic Compounds, Vol. 19, Parts 1 and 2 : Heterocyclic Compounds with Three- and Four-membered Rings (edited by Dr. A. Weissberger), review by Dr. F. G. Mann, 2
- Chemistry of Plant Pigments (Dr. J. B. Harborne), 1051
- Chemotherapy—1964 : Antimicrobial Agents and (edited by J. C. Sylvester), review by Prof. E. P. Abraham, 980
- Chemotherapy of Cancer : 1964 : Proceedings of the International Symposium on (edited by Prof. P. A. Plattner), review by Dr. D. C. Roberts, 1032
- Chemotopic Organization in the Bulbar Gustatory Relay of the Rat (B. P. Halpern), 393
- Cheng, S. C., and Hohn, Prof. E. O. : Prolactin and the Incidence of Brood Patch Formation and Incubation Behaviour of the Two Sexes in Certain Birds with Special Reference to Phalaropes, 197
- Chernick, J., and Levine, Dr. M. M. : A Numerical Model of Random Packing of Spheres, 68
- Cherry, Prof. Colin : Communication Processes, NATO Conference Series, Vol. 4, review, 717
- Chertkov, I. L., Maximenko, A. S., Novikova, M. N., and Raushenbach, M. O. : Bone Marrow Allografts in Thymectomized X-irradiated Dogs, 399
- Chervinski, J. : Sexual Dimorphism in *Tilapia*, 703
- Chesher, G. B., and Carroll, P. R. : Biphasic Dose-response Relationship with Acetylcholine on the Heart of the Mollusc *Tapes turrida*, 1220
- Chessick, Dr. R. D., and Lal, H. : Lethal Effects of Aggregation and Electric Shock in Mice treated with Cocaine, 295
- Chessin, Prof. M., and Duff, R. : Rapid Method for Selenium Assay of Plant Material, 1001
- Cheyne, I. M., and White, Dr. D. O. : Stimulation of Sendai Virus Multiplication by Puromycin and Actinomycin D, 813
- Chick Embryo : Chromosome Marker Studies on the Development of the Haemopoietic System in the (M. A. S. Moore and Dr. J. J. T. Owen), 956
- Chick Embryo : Comparison of the Chorioallantoic Membrane and Splenomegaly Systems of Graft-versus-host Assay in the (L. W. Coppleston and Dr. D. Michie), 53
- Chick Interferon : Molecular Weight of (Dr. D. C. Burke and Dr. J. Ross), 1297
- Chicken Embryo : A New Syndrome : Hyperlipaemia following Viral Infection in the (Dr. S. E. Grossberg and Prof. W. M. O'Leary), 954
- Chickens : Effects of Starvation and Protein Depletion on Mercury Retention in Two Strains of (D. V. Larkin et al.), 706
- Chickens exposed to 1 Atmosphere Oxygen : Delayed Mortality in Adult (Prof. Harold S. Weiss et al.), 1003
- Chieco-Bianchi, L., et al. : Induction of Malignant Lymphomas by Urethane in Adult Mice bearing Syngeneic Thymus Grafts, 398
- Chieco-Bianchi, L., Fiore-Donati, Dr. L., Tridente, G., and Pennelli, N. : Lymphatic Neoplastic Transformation of Thymus in Mice with Myeloid Virus-induced Leukaemia, 697
- Chikazumi, Prof. S. : Physics of Magnetism, review by Dr. C. A. Bates, 616
- Child : Early Growth of Logic in the (B. Inhelder and J. Piaget), review by Dr. J. H. Duthie, 1246
- Children with Haemolytic Disease of the Newborn : Low Concentrations of Serum Haptoglobin in Mothers of (J. Pinter), 694
- Children's Scribbles : Form-similarity between Phosphenes of Adults and pre-School (R. Kellogg et al.), 1129
- Chimerical Nature of the Entire-leaf Variant in the Potato Variety 'Majestic' (Dr. H. W. Howard), 917
- Chinese : Characterization of Glucose-6-phosphate Dehydrogenase among (P. W. K. Wong et al.), 1323
- Chisman, Mr. D. G. : Appointment as Science Education Officer, British Council, 432
- Chiswick-Langley Section of the M.4 Motorway, 1261
- Chloramphenicol, Dihydrostreptomycin-, and Kanamycin-inactivating Enzymes from Multiple Drug-resistant *Escherichia coli* carrying Episome 'R' (S. Okamoto and Y. Suzuki), 1301
- Chloramphenicol : Promotion of Germination in Light-requiring Seed by (Dr. M. Black and M. Richardson), 1114
- Chlorexolone (M and B 8430) : Metabolism of 5-Chloro-2-cyclo-hexyl-1-oxo-6-Sulphamoyl Isoindoline, (K. Corbett et al.), 286
- Chloride Activity of Crayfish Giant Axons : Intracellular (Dr. A. Strickholm and B. G. Wallin), 790

- Chloride and Water Excretion in Single Nephrons of the Kidney of the Rat : Effect of 'Furosemid' on (G. Malnic *et al.*), 80
- Chloride Ions across Artificial Membranes : Effect of *Trans*-Solute on the Fluxes of (S. Kikahara *et al.*), 187
- 5-Chloro-2-cyclohexyl-1-oxo-6-sulphamoyl Isoindoline, Chlorexolone (M. and B. 8430) : Metabolism of (K. Corbett *et al.*), 286
- (2-Chloroethyl) trimethylammonium Chloride and Gibberellic Acid on Growth, Fruit Bud Formation and Frost Resistance in One-year-old Pear Trees : Effects of (Dr. I. Modliwowska), 503
- Chlorophyll and Hydroquinone : Interaction between (E. Fujimori and M. Tavlá), 78
- Chloroplast Preparation : Optical Rotatory Dispersion of a (Dr. Bacon Ke), 573
- Chloroplasts on the Oxidation-Reduction State of *N*-Methylphenazinium Methyl Sulphate (Phenazine Methosulphate) : Dependence of Photophosphorylation by Isolated (G. Zweig and Dr. M. Avron), 190
- Chloroquine Resistance and the Nature of Malarial Pigment (Prof. I. W. Sherman, J. B. Mudd and Prof. W. Trager), 691
- CH₃O-radical : Electronic Absorption Spectrum of the (J. F. Ogilvie), 1315
- Choh Hao Li, Prof. Barnali, L., Chrétien, M., and Chung, D. : Isolation and Amino-acid Sequence of β -LPH from Sheep Pituitary Glands, 1093
- Cholelithiasis by Neomycin : Prevention of Cholesterol-induced (Dr. E. H. Mosbach *et al.*), 1226
- Cholesterol-induced Cholelithiasis by Neomycin : Prevention of (Dr. E. H. Mosbach *et al.*), 1226
- Cholinergic Excitation and Inhibition in the Cerebellar Cortex (P. Kása and Dr. B. Csillik), 695
- Cholinesterase Preparations : *In vitro* Effect of Gamma-radiation on Different (Dr. R. E. Catalan and Dr. A. M. Municio), 1227
- Cholinesterase : Proposed Physiological Function for Plasma (H. S. Funnell and Prof. W. T. Oliver), 689
- Cholinesterase Screening of Candidate Pesticides : Application of an Agar-Agar Diffusion Procedure to Pesticide Residue Analysis and to the (Dr. K. I. Beynon and G. Stoydini), 748
- Chondritic Meteorites : Metal Grains in (J. A. Wood), 1085
- Chopra, V. L. : Tests on *Drosophila* for the Production of Mutations by Irradiated Medium or Irradiated DNA, 699
- Chorioallantoic Membrane and Splenomegaly Systems of Graft-versus-host Assay in the Chick Embryo : Comparison of the (L. W. Coppleson and Dr. D. Michie), 53
- Choudhury, A. K. R., and Bhowal, J. G. : Estimation of the Frequency of Functioning Gametes in Monosomics, 917
- Chrétien, M., *et al.* : Isolation and Amino-acid Sequence of β -LPH from Sheep Pituitary Glands, 1093
- Christian, J. W. : Theory of Crystal Dislocations, review, 110
- Christie, Dr. George A. : Teratogenic Effects of Synthetic Compounds related to Trypan Blue : The Effect of 1,7-Diamino-8-naphthol-3,6-disulphonic Acid on Pregnancy in the Rat, 1219
- Christmas Holiday Films and Talks on Geology, 1155
- Chromaffin Cell : Natural History of the (Prof. R. E. Coupland), review by Dr. B. A. Callingham, 6
- Chromatograms : Ultra-violet Spectroscopy in the Analysis of (Dr. D. M. Abelson), 784
- Chromatographic Analysis : Concentration Effects in (Prof. I. D. Rattee *et al.*), 269
- Chromatographic Analysis of Organic Acids by Trinitating Methylation : Quantitative (G. K. Koch and [the late] G. Jurrrens), 1312
- Chromatography : Advances in Liquid (Dr. B. Makin and F. P. Speakman), 1087
- Determination of p.p.m. Concentrations of Carbon Disulphide in Benzene by Electron Capture Gas (D. W. Grant and G. A. Vaughan), 75
- Determination of Nicotine in Smokers' Urine by Gas (Dr. N. L. McNiven *et al.*), 788
- Heterogeneity of Titan Yellow demonstrated by Thin-layer (R. J. Hall and L. R. Flynn), 1202
- Resolution of the Optical Isomers of DL-Tryptophan, 5-Hydroxy-DL-tryptophan and 6-Hydroxy-DL-tryptophan by Paper and Thin-layer (Dr. S. F. Contractor and J. Wragg), 71
- Simple Assessment of Partition Data for correlating Structure and Biological Activity using Thin-layer (Dr. C. B. C. Boyce and Dr. B. V. Milborrow), 537
- Chromatography and the Vent Pea Test : Presence of Plant Growth Substances in Earthworms demonstrated by Paper (R. L. Nielson), 1113
- Chromatography of Amino-acids as Trimethylsilyl Derivatives : Quantitative Gas (Prof. E. D. Smith and H. Sheppard, jun.), 878
- Chromatography of DNP Amino acids : Polyamide Layer (Prof. Kung-Tsung Wang and J. Mei-Kuo Huang), 281
- Chromatography of α -Gestrogens : Polyamide Layer (Y. C. Tung and Prof. K.-T. Wang), 581
- Chronic Acid : Inhibition of Corrosion of Copper in (K. F. Lorking), 778
- Chromium Diffusion Coatings on Iron : Mechanism of Oxidation of (Dr. I. A. Menzies and D. Mortimer), 1307
- Chromium Diffusion Coatings on Plain Carbon Steels : Mechanism of Oxidation of (Dr. I. A. Menzies and D. Mortimer), 1309
- Chromium (IV) Peroxo Compound : A New (Dr. D. A. House and Prof. C. S. Garner), 776
- Chromosome Duplication at the End of the Deoxyribonucleic Acid Synthetic Period in Human Blood Cells : Rate of (Dr. C. W. Gilbert *et al.*), 159
- Chromosome in Cultures of Spleen Cells : Demonstration of the Philadelphia (Dr. A. S. D. Spiers and Dr. A. G. Baikie), 497
- Chromosome Marker Studies on the Development of the Haemopoietic System in the Chick Embryo (M. A. S. Moore and Dr. J. J. T. Owen), 956
- Chromosome Reduction in a Tetraploid *Solanum* : Double (G. E. Marks *et al.*), 359
- D(13-15) Chromosome responsible for Di Trisomic Patau's Syndrome : Autoradiographic Identification of the (Dr. F. Giannelli), 669
- Chromosomes : Demonstration of the Spiral Structure of Human (Dr. Y. Ohnuki), 916
- Distribution of Deoxyribonucleic Acid along Human (Dr. A. Ward), 486
- Measurements of Bovine (F. N. Baker *et al.*), 97
- Chromosomes after Treatment with Carbon Dioxide : Puffing of Salivary Gland (Prof. W. J. Burdette and R. Anderson), 409
- Chronic Granulocytic Leukaemia : Demonstration of the Philadelphia Chromosome in Cultures of Spleen Cells (Dr. A. S. D. Spiers and Dr. A. G. Baikie), 497
- Chung, D., *et al.* : Isolation and Amino-acid Sequence of β -LPH from Sheep Pituitary Glands, 1093
- Chwojnik, A., *et al.* : Use of Technetium-99m in Hepatic Scintigraphy, 86
- CIBA Resins in Ireland : Use of, 21
- Cichlidae : Signal Value of the Genital Tassel in the Male *Tilapia macrochir* Blgr. (Pisces) (Dr. W. W. Wickler), 595
- Cigarette Tobacco : Tumour-promoting Agents in Unburned (Dr. F. G. Bock *et al.*), 584
- Ciliate *Tetrahymena pyriformis* GL : Isolation of Macronuclei from the (Dr. Y. C. Lee and Prof. O. H. Scherbaum), 1350
- Cinnamic Acid in Gherkin Hypocotyls : Photo-induced Hydroxylation of (Dr. G. Engelsma), 1117
- Circuits and Microminiaturization : Solid (edited by G. W. A. Dummer), review by Dr. J. R. Tillman, 1249
- Circular Dichroism in Organic Chemistry : Optical Rotatory Dispersion and (Dr. P. Crabbé), review by Prof. W. Klyne, 970
- Circulation in the Cell (Prof. C. C. Lindgren), 304
- Cissus : Development of the Ovary in a Species of (N. C. Nair), 402
- Citation Index 1964 Annual Cumulation : Science. Eight Parts (edited by E. Garfield), review by C. W. Cleverdon, 717
- Civil Service : Management Training in the, 934
- Organization of the Scientific, 1133
- Professional and Technical Staff in the, 120
- Recruitment to the, 327
- Civil Service Image : Improving the, 311
- Civil Service Policy : Rethinking, 415
- Civilizations of the Near East : Earliest (James Mellaart), review by M. C. Burkitt, 984
- Claridge, Dr. M. F., and Elington, Dr. J.-M. : Bilateral Asymmetry : a Larval Polymorphism in some Argid Sawflies (Hymenoptera, Symphyta), 301
- Clark, Prof. R. B. : Appointment to Chair of Zoology in the University of Newcastle upon Tyne, 43
- Clark, R. W. : Tizard, review, 110
- Clarke, Dr. A. J., and Shepherd, Dr. A. M. : Zinc and Other Metallic Ions as Hatching Agents for the Beet Cyst Nematode, *Heterodera schachtii* Schm., 502
- Clarke, A. M. : Relationship between the Electromyogram and the Force of the Isometric Reflex Response of Normal Human Subjects, 551
- Clarke, A. M., and Thomson, Dr. R. Y. : Role of Portal Blood Supply in Liver Regeneration, 392
- Clarke, Prof. C. A., *et al.* : 'Pink Spot' in the Urine of Schizophrenics, 453
- Clarke, Dr. K., Gray, Dr. G. W., and Reaveley, D. A. : 'Lipid A' Component from the Cell-walls of *Pseudomonas aeruginosa*, 586
- Clarke, Dr. K. U., and Gillett, C. : Relationship between the Removal of the Frontal Ganglion and Protein Starvation in *Locusta migratoria* L., 808
- Classification and Terminology for Enzyme Mutants in Micro-organisms : A Suggested Phenotypic (Dr. J. F. Collins *et al.*), 841
- Claxton, Dr. T. A. : Polarization and Hybridization, 891
- Clay : Holothurian Sclerite from the Speeton (B. N. Fletcher), 281
- Clayton, W. D. : Methods for the Examination of Root Systems and Roots, review, 319
- Clayton, Dr. Y. M., *et al.* : Isolation of *Cryptococcus neoformans* from Pigeon Habitats in London, 301
- 'Clean Room' Technology, Conference, 231
- Clegg, John : Freshwater Life of the British Isles, Third Edition, review by Dr. W. J. Rees, 988
- Clegg, Dr. J. B., *et al.* : Globin Synthesis in Thalassemia : an *in vitro* Study, 1061
- Clemenson, Prof. C. A. B. : Effect of Certain Types of Paper on Sexual Maturation of the Insect *Pyrrhocoris apterus*, 510
- Cleverdon, C. W. : Science Citation Index 1964 Annual Cumulation, Eight Parts, review, 717
- Clinical Application : Demonstration of Lactic Dehydrogenase Isozymes and its (Dr. A. Kontinen and S. Lindy), 782
- Clinical Biochemists : Association of, 631
- Cloud Altitudes from Gemini-Titan-5 : Determination of (F. Sledy *et al.*), 775
- Clough Scholarship : Emma, 735
- Clowes, Dr. F. A. L., and Juniper, Dr. B. E. : Cytoplasmic Organelles and Cell Growth in Root Caps, 864
- Cluster Galaxies : Hydrogen Content of Virgo (Dr. B. J. Robinson and J. A. Koehler), 993
- Coagulation Disorder caused by an Abnormal Fibrinogen ('Fibrinogen Baltimore'), New Inherited (Dr. E. A. Beck *et al.*), 143
- Coagulative Factor : A Screening Test for Substances Inhibiting the Cancer (Dr. E. M. Glaser *et al.*), 1008
- Cobalt : Epileptogenic Effect of Extradural and Intracranial (H. Payan *et al.*), 792
- Cobalt-58 in Habrobracon : Distribution and Effective Half-life of (Prof. D. S. Grosch), 506
- Cocaine : Lethal Effects of Aggregation and Electric Shock in Mice treated with (H. Lal and Dr. R. D. Chessick), 295
- Cockcroft, Sir John : The Journals of David E. Lilienthal, Vol. 1, 1939-45, Vol. 2, 1945-1950, review, 962
- Cockroach, *Leucophaeamaderae* : Guanase Activity of the Symbionts and Fat Bodies of the (Dr. L. L. Pierre), 666
- Cod (*Gadus morhua*) : a Single Peptide Difference : Haemoglobin Polymorphism in the (M. C. Rattazzi and C. Piki), 489
- Coefficient for Compressed Nitrogen : Townsend's First Ionization (Dr. B. W. Ward), 994
- Coenzymes : Nucleotides and (Dr. D. W. Hutchinson), review by Dr. D. M. Brown, 987
- Cohen, Prof. Marshall H. : Scintillations on the Small Diameter Radio Sources, 277
- Cohen, Prof. Sydney : Appointment to Chair of Chemical Pathology, Guy's Hospital Medical School, 934
- Cohn, George L. : Biosynthesis of Triiodothyronine Sulphate by Beef Thyroid *in vitro*, 80
- Colburn, Dr. R. W., and Maas, Dr. J. W. : Adenosine Triphosphate-Metal-Norepinephrine Ternary Complexes and Catecholamine Binding, 37
- Co-ordination Chemistry and Membrane Function with Particular Reference to the Synapse and Catecholamine Transport, 41
- Cole, Prof. B. N. : Introduction to Plate and Shell Theory, review, 1137
- Cole, Dr. L. J., and Tyan, Dr. M. L. : Bone Marrow as the Major Source of Potential Immunologically Competent Cells in the Adult Mouse, 1223
- Coleman, C. E., and Hardie, Dr. D. : Slow-bend Testing of Hydrized Zirconium, 69
- Coleoptile by Indolyl-3-acetic Acid : Stimulation of Synthesis of Ribonucleic Acid in Sub-apical Section of *Avena* (Prof. T. H. Hamilton *et al.*), 1180
- Collagen : Long-spacing Segments from Renatured α 1 Sub-units of (K. Kühn *et al.*), 685
- Collagen : Reactivity of the ϵ -Amino Group of (J. H. Bowes *et al.*), 1094
- Collagenase in Rat Tissues : Distribution of (J. F. Woods and Prof. G. Nichols, jun.), 1325
- Colliculus Neurons : Somesthetic and Visual Responses of Superior (Dr. D. Jassik-Gerschenfeld), 898
- Collins, Dr. J. F., Mandelstam, Dr. J., Pollock, Prof. M. R., Richmond, Dr. M. H., and Sneath, Dr. P. A. : A Suggested Phenotypic Classification and Terminology for Enzyme Mutants in Micro-organisms, 841
- Collins, R., and Deutsch, Prof. J. A. : Facilitation as a Function of Temporal Spacing of Stimuli in Intracranial Self-stimulation, 592
- Collinson, A. J. L., *et al.* : Determination of the Total Alpha Activity of Drinking Water using a Nuclear Emulsion, 1074
- Collisionless Shock Waves in a Magnetized Plasma : Experimental Observations on the Structure of (Dr. J. W. M. Paul *et al.*), 133
- Collisions in Irradiated Crystals : Correlated Atomic (Dr. L. T. Chadderton and I. McC. Torrens), 880
- Collmann, R. D., and Stedler, A. : Virus Aetiology for Down's Syndrome (Mongolism), 903

- Colodny, R. G., (edited by): *Beyond the Edge of Certainty: Essays in Contemporary Science and Philosophy*, review by F. I. G. Rawlins, 314
- Colombes, P., et al.: I Antigen in Leukaemic Patients, 1329
- Colombo, Dr. G.: Rotational Period of the Planet Mercury, 575
- Colour of 'Pure' Water (Dr. J. E. Tyler), 549
- Colour Television, 1153
- Columbia River Plume, July 1964: Strontium-90 and Caesium-137 in (Prof. K. Park et al.), 1084
- Comar, Prof. C. L., et al.: Effects of Dietary Levels of Phosphorus and Calcium on the Comparative Behaviour of Strontium and Calcium, 1110
- Combined Very High Pressure/High Temperature Calibration of the Tetrahedral Anvil Apparatus, Fusion Curves of Zinc, Aluminium, Germanium and Silicon to 60 Kilobars (L. Lees and B. H. J. Williamson), 278
- Comfort in Industry: Thermal, 122
- Comley, W. J., and Hakim, Dr. S. E. A.: Acoustic Velocity Dispersion in some Non-associated Organic Liquids, 1082
- Commentariolus of Copernicus, 1263
- Commercial Nuclear Power Stations in Britain (S. Weintraub), 25
- Common Features of the Optical Continuum associated with Violent Cosmic Events (Prof. L. Searle, Dr. A. W. Rodgers, W. L. W. Sargent and J. B. Oke), 1190
- Commonwealth Students in Britain: New, review by Lord Twining, 957
- Communication: Mimicry and the Evolution of Animal (Dr. W. Wickler), 519
- Communication Processes (edited by F. A. Geldard), NATO Conference Series, Vol. 4, review by Prof. C. Cherry, 717
- Companions and the Mother-Infant Relationship in Rhesus Monkeys: Social (Y. Spencer-Booth et al.), 301
- Comparison of European Health Services, 438
- Comparison of Experimental Data of the Effective Ion Mass in the Upper Ionosphere with a Theoretical Structure (P. R. Arendt), 443
- Comparison of Salivary Amylase-levels in the Human Male and Female (Prof. R. L. McGeachin, P. A. Hay and P. A. Prell), 1211
- Comparison of the Chorioallantoic Membrane and Splenomegaly Systems of Graft-versus-host Assay in the Chick Embryo, (L. W. Coppieson and Dr. D. Michie), 53
- Competent State in *Pneumococcus* by a Hormone-like Cell Product: Control of the (Dr. A. Tomasz), 155
- Complement-fixation Test: Rubella Virus (Dr. H. Stern), 200
- 'Complement Fractionation' in a Natural Hybrid between *Rubus procerus* Muell. and *R. laciniatus* Willd (Dr. R. K. Bammi), 608
- Complement System: Effects of Hydrocortisone Succinate on the (Dr. H. Gewurz et al.), 755
- Complexes between Basic Proteins of Leucocytes and Plasma Globulins: Formation of (Dr. W. B. Jolley and H. W. Allen), 390
- Complexing Reagents: Organic (D. D. Perrin), review by Dr. T. S. West, 3
- Component of Normal Adult Connective Tissue: Inhibition of Growth of Malignant Cells *in vitro* by a (Prof. M. S. Parshley and I. Mandl), 800
- Component of Solar Radio Emission at Centimetre and Decimetre Wavelengths: Basic (Dr. M. K. Das Gupta and D. Basu), 739
- Components of Human Red Cell Membrane: Heterogeneity of Water-soluble Structural (Prof. M. D. Poulik and Dr. P. K. Lauf), 874
- Composition of the Serum Seromucoid Fraction: Factors affecting the Amount and (Dr. A. J. Anderson), 491
- Compounds: Biochemistry of Phenolic (edited by J. B. Harborne), review by Sir Robert Robinson, 515
- Compressed Nitrogen: Townsend's First Ionization Coefficient for (Dr. B. W. Ward), 994
- Computer Recognition of Cervical Cell Nuclei (G. W. McMaster), 485
- Concentration Effects in Chromatographic Analysis (Prof. I. D. Rattee, D. M. Lewis and Dr. C. B. Stevens), 269
- Concentration Profiles in Oxide Scales on Nickel-10.9 per cent Cobalt Alloy: Metal (Dr. G. C. Wood and J. M. Ferguson), 369
- Concentrations of Carbon Disulphide in Benzene by Electron Capture Gas Chromatography: Determination of p.p.m. (D. W. Grant and G. A. Vaughan), 75
- Concept of the 'Centron' (Dr. D. W. Menzies), 163
- Concrete: Shrinking Aggregates as a Cause of Failure in, 1261
- Concrete Surfacings: Reconditioning, 834
- Condensation of Atmospheric Moisture in Nozzles: Effects of Seeding on the (Dr. E. R. Buckle and Dr. A. A. Pouring), 367
- Condensed Phase: Physics of High Pressures and the (edited by A. van Itterbeek), review by Dr. R. S. Bradley, 515
- Condensed Tannins: Structure of (L. L. Creasy and Dr. T. Swain), 151
- Cones and Male Flowers of the Hop (*Humulus lupulus*, L.): Relationships between Constituents of (Dr. R. D. Hartley and Dr. R. A. Neve), 804
- Congo at Elisabethville: Official University of the, 124
- Congo River: Occurrence of *Salvinia auriculata* Aublet on the (Dr. E. C. S. Little), 1111
- Conjugation, Inhibition of, by Anti-inflammatory Drugs (Dr. T. Hargreaves), 1101
- Conn, Prof. E. E., et al.: Enzymatic Formation of β -Cyanalanine from Cyanide, 1207
- Connective Tissue: Inhibition of Growth of Malignant Cells *in vitro* by a Component of Normal Adult (Prof. M. S. Parshley and I. Mandl), 800
- Connective Tissue of Adrenalectomized Rats: Acid Mucopolysaccharide and Hexosamine Metabolism in Dermal (Prof. J. Fabianek et al.), 1212
- Conolly, Dr. J. R., and Ewing, Dr. M.: Pleistocene Glacial-marine Zones in North Atlantic Deep-sea Sediments, 135
- Conover, Prof. J. T., and Sieburth, Prof. J. McN.: *Sargassum* Tannin, an Antibiotic which retards Fouling, 52
- Conservancy in Britain: Nature, 635
- Conservation and Man's Environment (Dr. I. McTaggart Cowan), 1145
- Conservation of the Bee Orchis, 936
- Conserved Blood: Inhibition of Haemolysis by 'Cremophor' in (Dr. K. Reber), 195
- Conserving the Natural Heritage, 1241
- Constants for Both Phases: Equation of State of Liquid-vapour Equilibrium with the Same (Dr. I. Kuhn), 1315
- Constituents of Cones and Male Flowers of the Hop (*Humulus lupulus*, L.): Relationships between (Dr. R. D. Hartley and Dr. R. A. Neve), 804
- Contaminated Soils: Uptake by Plants of Radiostromium from (A. J. Andersen), 195
- Continental Crust: Radioactive Elements in the (Dr. K. S. Heier), 479
- Continents: Geochemistry of Andesites and the Growth of (Prof. S. R. Taylor and Dr. A. J. R. White), 271
- Continuum associated with Violent Cosmic Events: Common Features of the Optical (Prof. L. Searle et al.), 1190
- Continued Fractions and their Generalizations to Problems in Approximation Theory: Application of (A. N. Khovanskii), review by I. M. Khabaza, 318
- Continuous Irradiation of HeLa Cells at -196°C (A. H. W. Nias and Dr. L. G. Lajtha), 400
- Continuum Background at High Galactic Latitudes: Structure of the Radio (Dr. M. J. S. Quigley and Dr. C. G. T. Haslam), 741
- Contractile Activity in Skeletal Muscle: Enzymatic Adaptation to (J. Kendrick-Jones and Prof. S. V. Perry), 1068
- Contraction: Heat Production in a Cardiac (Dr. N. V. Raghunath and Dr. C. L. Gibbs), 897
- Contraction in Twigs: Thermal (I. J. McCracken and Prof. T. T. Kozlowski), 910
- Contractions ('Catch') in an Invertebrate Muscle: Energy Requirements for Relaxation from Tonic (Dr. K. Minihan and Prof. R. E. Davies), 1327
- Contractor, Dr. S. F., and Wragg, J.: Resolution of the Optical Isomers of DL-Tryptophan, 5-Hydroxy-DL-tryptophan and 6-Hydroxy-DL-tryptophan by Paper and Thin-layer Chromatography, 71
- Control and the Respiratory System: Self-adaptive (Dr. I. P. Priban and Dr. W. F. Fincham), 339
- Control: Electrical Machinery and (Prof. I. L. Kosow), review by J. C. West, 9
- Control of Biological Development, 838
- Control of Furunculosis in Impounded Adult Salmon (A. C. Leaman), 1344
- Control of Lipid Concentration in Rat Heart and Gastrocnemius: Hormonal (R. M. Denton and Prof. P. J. Randle), 488
- Control of Reaction Rate in the Thermal Decomposition of Amorphous Polymers: Absence of Diffusion (D. A. Smith), 1200
- Control of the Competent State in *Pneumococcus* by a Hormone-like Cell Product: An Example for a New Type of Regulatory Mechanism in Bacteria (Dr. A. Tomasz), 155
- Controlled Rectifiers: Semiconductor (F. E. Gentry et al.), review by Prof. W. Fishwick, 719
- Conversion of the Inconvertible L-I Lympho-sarcoma to Ascitic Form (Prof. M. R. Sikov), 902
- Convulsant Barbiturate: Effects of Propylthiouracil and Thyroid Feeding on the Response of Mice to Injected (Prof. A. J. Prange, jun., and Prof. M. A. Lipton), 791
- Conway, Dr. E., and Young, Dr. E. G.: Seaweed Research, 840
- Cook, Dr. A. H.: Appointment as Superintendent of the Standards Division, National Physical Laboratory, Teddington, 118
- Cook, Dr. A. H.: European Brewery Convention, Report of Barley Committee: Trials, 1963, review, 9
- Cook, Dr. A. H.: A New Absolute Determination of the Acceleration due to Gravity at the National Physical Laboratory, 279
- Cook, Sir James: Eighteen Plus, Unity and Diversity in Higher Education, review, 313
- Coombes, J. D., et al.: Molecular Weight of Urokinase, 894
- Cooper, Dr. W., Lewis, H. B. M., and Nicholls, Dr. E. M.: Haptoglobin Johnson in Australian Aborigines, 694
- Co-operation: Anglo-Romanian Agreement on, 328
- Co-operation Scheme: U.K. Atomic Energy Authority and Central Electricity Generating Board, 120
- Co-ordination Chemistry and Membrane Function with Particular Reference to the Synapse and Catecholamine Transport (Dr. J. W. Maas and Dr. R. W. Colburn), 41
- Copepod, *Euterpina acutifrons* (Dana), in the Laboratory: Breeding a Pelagic (H. W. Neunues and G.-F. Pongolini), 571
- Copernicus: *Commentariolus* of, 1263
- Copland, J. W.: Establishment of a Pure Infection of the Nematode *Ostertagia circumcincta* in the Sheep, 1225
- Copley, A. L., and Lee, E. H. (edited by): Proceedings of the Fourth International Congress on Rheology, 1963, review by Dr. G. W. Scott Blair, 965
- Copper in Chromic Acid: Inhibition of Corrosion of (K. F. Lorking), 778
- Cu⁺⁺ in Diamine Oxidase: Electron Spin Resonance Spectrum of (Prof. A. van Heuvelen), 888
- Copper-64 Recoil Atoms in Solids: Evidence of Thermal and Hot Processes in the 'Hot-zone' produced by (K. Yoshihara and H. Ebihara), 482
- Copping, Dr. A. M.: Dictionary of Nutrition and Food Technology, Second Edition, review, 987
- World Review of Nutrition and Dietetics, Vol. 5, review, 421
- Coppleson, L. W., and Michie, Dr. D.: Comparison of the Chorioallantoic Membrane and Splenomegaly Systems of Graft-versus-host Assay in the Chick Embryo, 53
- Corbett, K., Edwards, Miss S. A., Lee, G. E., and Threlfall, T. L.: Metabolism of 5-Chloro-2-cyclohexyl-1-oxo-6-sulphamoyl Isoindoline, Chlorexolone (M 8430), 286
- Cormack, Prof. D. V., and Morrison, H. G.: Variation of the X-ray Sensitivity of *Serratia marcescens* with Relative Humidity, 91
- Cornea on Awakening: Thinning of the Human (Dr. R. B. Mandell and Prof. I. Fatt), 292
- Corner, Dr. George W.: A History of the Rockefeller Institute 1901-1953, Origins and Growth, review by the Lord Adrian, 1244
- Cornfield, Dr. A. H., and Ekpete, D. M.: Effect of pH and Addition of Organic Materials on Denitrification Losses from Soil, 1200
- Corona Structures in the Basic Igneous Masses of East Aberdeenshire (Dr. D. S. Weedon), 885
- Corpora Lutea of a Non-steroid Substance with Anti-gestagenic Properties: Specific Uptake in (A. Hanngren et al.), 461
- Corpus Allatum in a Polymorphic Insect: Changes in Size of the (D. White), 807
- Corpuscles: A Phagocytic Function of Hassall's (Dr. J. N. Blau), 564
- Correlated Atomic Collisions in Irradiated Crystal Lattices (Dr. L. T. Chadderton and I. McC. Torrens), 880
- Correlation between Unit Activity and Slow Potential Changes in the Unanaesthetized Cerebral Cortex of the Cat (A. D. J. Robertson), 757
- Correlation of Sodium Atom Reaction Rates with Electron Capture Cross-sections (K. R. Wilson and Prof. D. R. Herschbach), 182
- Correlation of the Binding to DNA Loops or to DNA Helices with the Effect on RNA Synthesis (Prof. J. H. Frenster), 1093
- Correlation of the Third Excited State of Polycyclic Aromatic Hydrocarbons with the Ionization Potential (Dr. M. A. Slikin), 380
- Corrosion of Copper in Chromic Acid: Inhibition of (K. F. Lorking), 778
- Cortex: Cholinergic Excitation and Inhibition in the Cerebellar (P. Kása and Dr. B. Csillik), 695
- Cortex of the Cat: Correlation between Unit Activity and Slow Potential Changes in the Unanaesthetized Cerebral (A. D. J. Robertson), 757
- Cosmic Events: Common Features of the Optical Continuum associated with Violent (Prof. L. Searle et al.), 1190
- Cosmic Ray Physics (A. E. Sandstrom), review by Dr. J. J. Quenby, 315
- Cosmic Rays: Daily Variation of (J. B. Mercer and Prof. B. G. Wilson), 477
- Cosmology: Recent Developments in (Prof. F. Hoyle), 111
- Cottrell, Prof. A. H.: Appointed Deputy Chief Scientific Adviser to the Ministry of Defence, 19
- Cottrell, Prof. A. H.: Theory of Crystal Dislocations, review by J. W. Christian, 110
- Cotyledon coincident with Seed Death: Respiratory Changes in the Cacao Seed (I. A. Casas et al.), 1348
- Cotzias, Dr. G. C., and Homsher, Earl: Antidiuretic Hormone and Bile Flow, 687
- Cotzias, Dr. G. C., et al.: Functional Similarities between the Cytoplasmic Organelles of Melanocytes and the Mitochondria of Hepatocytes, 810
- Coulson, Dr. C. B., and Sim, Dr. A. K.: Proteins of Wheat Flour, 583

- Coumarins : Separation of (R. S. Shah and Dr. S. L. Bafna), 76
 Counter : Size Analysis in the Sub-seve Range by Electronic (Prof. C. C. Harris and Dr. A. Jowett), 175
 Counterexamples in Analysis (Prof. B. R. Gelbaum and Prof. J. M. H. Olmsted), review by Prof. T. A. A. Broadbent, 218
 Countries : British Aid to Developing, 128
 Countryside in 1970 (E. M. Nicholson), 1265
 Countryside in 1970 : Second Conference on the, 629
 Coupland, Prof. Rex E. : Natural History of the Chromaffin Cell, review by Dr. B. A. Callingham, 6
 Courant, R., et al. : Funktionentheorie, Fourth Edition, review by Dr. J. C. Burkill, 963
 Cow : Influence of Electrical Stimulation on Milk Ejection : Introduction of a Permanent Electrode into the Pituitary Gland of the (B. Hynek et al.), 690
 Cow, Pig, Rhesus Monkey and Human Ovarian Oocytes : Maturation in vitro of Mouse, Sheep, (Dr. R. G. Edwards), 349
 Cowan, S. T., and Steel, K. J. (the late) : Manual for the Identification of Medical Bacteria, review by Dr. D. A. Allan, 213
 Cox, Dr. J. S. G., et al. : Iron Dextran Complex, 237
 Crabbe, Dr. Pierre : Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry, review by Prof. W. Klyne, 970
 Cranial Capacity of the Hominine from Olduvai Bed I (Prof. R. L. Holloway, jun.; Prof. P. V. Tobias), 205
 Crater : Origin of Atypical Meteorites from the Arizona Meteorite (Prof. M. E. Lipschutz), 636
 Crater photographed by Ranger VII : Structures Inside a Lunar (Captain V. G. Smalley and L. B. Ronca), 930
 Crawford, D. V., and Hallsworth, E. G. (edited by) : Experimental Pedology : Proceedings of 11th Easter School in Agricultural Science, 1964, review by Dr. A. M. Smith, 718
 Crawlhall, J. C., and Segal, Dr. S. : Sulphocysteine in the Urine of the Blotched Kenya Genet, 1320
 Crayfish Giant Axons : Intracellular Chloride Activity of (Dr. A. Strickholm and B. G. Wallin), 790
 Creasy, L. L., and Swain, Dr. T. : Structure of Condensed Tannins, 151
 Creativity : Science, Inventiveness and, 921
 Creese, R., et al. : Labelled Depolarizing Drugs In Normal and Denervated Muscle, 901
 'Cremophor' in Conserved Blood : Inhibition of Haemolysis by (Dr. K. Reber), 195
 Cresswell, Katherine M., et al. : Simultaneous Oxidation and Reduction of Diphenyl Picrylhydrazyl on Surfaces : Evidence for Catalytic Activity of F-Centres, 181
 Cronstedt, Axel Fredrick : Discoverer of Nickel, 229
 Crookston, Dr. J. H., Beale, D., Irvine, D., and Lehmann, Dr. H. : A New Haemoglobin, J Toronto (α Alanine→Aspartic Acid), 1059
 Cross, P. E. : Breakdown of Simple Hydrocarbons subjected to a Silent Electric Discharge, 892
 Cross-sections : Correlation of Sodium Atom Reaction Rates with Electron Capture (K. R. Wilson and Prof. D. R. Herschbach), 182
 Crossing-over in *Drosophila* Males by Means of Ovarian Extract : Induction of (O. S. Reddi et al.), 203
 Croxatto Dr. H., and de la Lastra, Dr. M. : Diurnal Variations In Ovarian Ascorbic Acid Content of Pseudopregnant Immature Rats used in Parlow's Test, 789
 Cruickshank, Prof. R. (edited by) : Modern Trends in Immunology, I, review by Dr. F. T. Perkins, 9
 Crust : Radioactive Elements in the Continental (Dr. K. S. Heier), 479
Cryptococcus neoformans from Pigeon Habitats in London : Isolation of (Dr. H. S. Randhawa et al.), 801
 Crystal Dislocations : Theory of (Prof. A. H. Cottrell), review by J. W. Christian, 110
 Crystal Lattices : Correlated Atomic Collisions in Irradiated (Dr. L. T. Chadderton and I. McC. Torrens), 880
 Crystalline Carbons at the Active Centre of Enzymes : Evidence for Structures resembling (Dr. E. D. Wachsmuth), 942
 Crystalline Modifications of Ampicillin (K. W. B. Austin, A. C. Marshall and H. Smith), 999
 Crystals : Delayed Fluorescence from Organic (G. F. Moore and Dr. I. H. Munro), 772
 Crystals : Effect of Environment on the Orientation of the Nitrate Ions In (Prof. N. R. Kunchur), 778
 Crystals : Effect of Ultra-violet Irradiation on the Electrical Conductivity of Zinc Oxide Single (Dr. R. B. Lal and Dr. G. M. Arnett), 1305
 Crystals : Pairs of Spiral Etch Pits in Ice (J. Muguruma), 180
 Csillik, Dr. B., and Kása, P. : Cholinergic Excitation and Inhibition in the Cerebellar Cortex, 695
 Cues in the Discrimination of Black and White : Tactile-Vision : Thermal and Texture (C. N. French), 1352
 Cultures : A Factor stimulating DNA Synthesis derived from the Medium of Leucocyte (Dr. S. Kasakura and Prof. L. Lowenstein), 794
 Cultures : Growth and Cytopathogenicity of *H*-viruses in Human and Simian Cell (Dr. H. Toolan and Dr. N. Ledinko), 812
 Cultures of Spleen Cells : Demonstration of the Philadelphia Chromosome In (Dr. A. S. D. Spiers and Dr. A. G. Baikie), 497
 Cuneate Nucleus of the Monkey : Influence of the Cerebral Cortex on the (Dr. F. Harris et al.), 1215
 Cunningham, Prof. G. J. : Centenary of the Quekett Microscopical Club, 937
 Curds, Dr. Colin R. : Endogenous Bud Formation in the Hypotrichida, 90
 Cures of Neurosis : Causes and (Prof. H. J. Eysenck and Dr. S. Rachman), review by Dr. B. Semeonoff, 1027
 Curtin, D. Y., et al. : Systematic Identification of Organic Compounds, Fifth Edition, review by Dr. D. T. Lewis, 1032
 Curtis, Prof. D. R., and Quastel, Dr. D. M. J. : Central Action of Hemicholinium, 192
 Cushman, Dr. P., et al. : Aldosterone Secretion : Differences In Direct Effects of Rubidium and Potassium Perfusion into Dog Adrenals, 1099
 Customs : Traditional Celtic, 1263
 Cutaneous Elastin In Ehlers-Danlos Syndrome (Dr. D. P. Varadi and Dr. D. A. Hall), 1224
 Cyanide by *Escherichia coli* Extracts : Enzymatic Formation of β-Cyanoalanine from (Dr. P. M. Dunnill and Prof. L. Fowden), 1206
 Cyanide : Enzymatic Formation of β-Cyanoalanine from (Dr. H. G. Floss et al.), 1207
 β-Cyanoalanine from Cyanide by *Escherichia coli* Extracts : Enzymatic Formation of (Dr. P. M. Dunnill and Prof. L. Fowden), 1206
 β-Cyanoalanine from Cyanide : Enzymatic Formation of (Dr. H. G. Floss et al.), 1207
 Cyclamate : Feeding and Reproduction of Rats fed Calcium (P. O. Nees and P. H. Derse), 81
 Cyclic Adenosine Monophosphate and Melanocyte-stimulating Hormone on Frog Skin in vitro : Effects of (Dr. M. W. Birensky and S. R. Burstein), 1282
 Cycloacetate In the Prevention of Fatty Infiltration in the Liver : Effect of Hydrolysed Glucose (Prof. N. Nach et al.), 1001
 Cysteine Content of Cattle Hair (P. H. Springell and S. J. Leach), 1326
 Cysteine on the Radical State of X-irradiated Lyophilized Liver : Influence of Oxygen and (M. Abe et al.), 499
 Cytopathogenicity of *H*-viruses in Human and Simian Cell Cultures : Growth and (Dr. H. Toolan and Dr. N. Ledinko), 812
 Cytophilic Antibody and Opsonin by a Macrophage Phagocytic System : Differentiation between (V. E. Parish), 594
 Cytoplasm : Biology of the (Dr. O. A. Scheide et al.), 247
 Cytoplasmic Labelling with Uridine-5-³H in Human Lymphocytes cultured with Phytohaemagglutinin (Dr. G. C. B. Winter and Prof. J. M. Yoffey), 1018
 Cytoplasmic Organelles and Cell Growth in Root Caps (Dr. B. E. Juniper and Dr. F. A. L. Clowes), 864
 Cytoplasmic Organelles of Melanocytes and the Mitochondria of Hepatocytes : Functional Similarities between the (Dr. M. H. Van Woert et al.), 810
 Czczuga, B. : Haemoglobin in *Ceriodaphnia quadrangula* (O. F. Muller), 907
- DAGLEY, Prof. S. : Rapid Mixing and Sampling Techniques in Biochemistry, review, 823
 Daily Variation of Cosmic Rays (J. B. Mercer and Prof. B. G. Wilson), 477
 Daly, Dr. J. J., and Maier, Dr. L. : Structure of Phosphobenzene, 383
 Daly, Olive, et al. : Experimentation with Plants at Sub-atmospheric Oxygen-levels : Effects of Oxygen Pressure and Salts on Germination of Winter Rye, 1012
 Damage in Diamond : Laser-Induced (Dr. D. J. Bradley et al.), 1081
 Damage in Natural White Diamond : Laser-induced (P. Whiteman and Dr. G. W. Wilson), 66
 Dangor, A. E. : Plasma Diagnostics with Microwaves, review, 965
 Daniel, Prof. E. E., et al. : Electrical Activity in Human Uterine Muscle Strips, 290
 Damping of S Waves (Sir Harold Jeffreys), 675
 Darbyshire, Prof. J. : Physics of the Marine Atmosphere, review, 419
 Darbyshire, Dr. J. H., Jennings, A. R., Omar, A. R., Dawson, P. S., and Lamont, P. H. : Association of Adenoviruses with Bovine Respiratory Disease, 307
 Darcy, D. A., et al. : Synthesis of α-Glyco-protein (Darcy) of Rat Serum by the Liver, 1221
 D'Arcy Hart, Dr. P. : Immunology for Students of Medicine, Second Edition, review, 1028
 Das Gupta, Dr. M. K., and Basu, D. : Basic Component of Solar Radio Emission at Centimetre and Decimetre Wavelengths, 739
 Dates on Bone : Validity of Radiocarbon (Dr. M. A. Tamers and F. J. Pearson, jun.), 1053
 Datta, Dr. N., and Kontomichalou, Dr. P. : Penicillinase Synthesis controlled by Infectious R Factors in Enterobacteriaceae, 239
 Datta, P. R., Lang, Dr. E. P., Watts, J. O., Klein, A. K., and Nelson, M. J. : Metabolites in Urine of Rats on Diets containing Aldrin or Dieldrin, 289
 Davey, A. B., and Payne, A. R. : Rubber in Engineering Practice, review by Dr. L. R. G. Treloar, 825
 David, D. J., et al. : Spectroscopy in Australia, 125
 David Anderson-Berry Prize, 1966, 530
 Davies, Dr. K. : Ionospheric Radio Propagation (National Bureau of Standards Monograph 80), review by Dr. H. Rishbeth, 419
 Davies, Prof. R. E., and Mirihan, Dr. K. : Energy Requirements for Relaxation from Tonic Contractions ('Catch') in an Invertebrate Muscle, 1327
 Davies, Dr. William : Grassland and Fodder Resources in India, review, 821
 Davis, Dr. J. G. : Cheese, Two Volumes, review by Dr. J. A. B. Smith, 975
 Davis, Dr. J. H. : Proceedings of the International Conference on Magnetism, Nottingham, September 1964, 1030
 Davis, M. A., et al. : Mammals of the World, Three Volumes, review by Dr. L. Harrison Matthews, 210
 Davison, Prof. A. N. : Appointment to Chair of Biochemistry and Organic Chemistry at Charing Cross Hospital Medical School, 1045
 Davisson-Germer Prize, 1263
 Dawson, P. S., et al. : Association of Adenoviruses with Bovine Respiratory Disease, 307
 Dawson, Prof. William W. : Quantum Energy Specificity of Simple Photoreceptor Units, 589
 Day, N. E. : Statistics and Experimental Design in Engineering and the Physical Sciences, Two Volumes, review, 214
 de Burgh, P. M., et al. : Thymus and the Production of Antibody-plaque-forming Cells, 1332
 de la Lastra, Dr. M., and Croxatto, Dr. H. : Diurnal Variations In Ovarian Ascorbic Acid Content of Pseudopregnant Immature Rats used in Parlow's Test, 789
 de la Mare, Mrs. Gwynneth, and Walker, Dr. J. : Shift Working : the Arrangement of Hours on Night Work, 1127
 DeLamater, Dr. E. D., and Eggers, Dr. J. D. : Marsupial Spermatozoa Pairing in the Epididymis of American Forms, 402
 de Lamiandre, Dr. G., and Nigam, Dr. V. N. : Formation of Intra-mitochondrial Glucosyloligosaccharides from Glucose by Intact Novikoff Ascites Hepatoma Cells, 664
 Dead Insect Larvae in Nigeria : Micro-organisms associated with (Dr. N. Okafor), 1015
 Death : Respiratory Changes In the Cacao Seed Cotyledon coincident with Seed (I. A. Casas et al.), 1348
 Decametric Region : Possible Contribution of Jupiter's Magnetospheric Tail to the Radio Emissions of the Planet in the (G. M. Gruber), 1271
 Decay in Irradiated Polyethylene : Mechanism of Radical (Dr. D. O. Geymer and Dr. C. D. Wagner), 72
 Decay of Immunological Responsiveness after Thymectomy in Adult Life (R. B. Taylor), 1334
 December : Night Sky In, 735
 Decimetre Wavelengths : Basic Component of Solar Radio Emission at Centimetre and (Dr. M. K. Das Gupta and D. Basu), 739
 Deckers, Dr. C. : Structure Antigénique de Tumeurs Expérimentales, review by R. R. Dourmaskhin, 1140
 Decomposition of Amorphous Polymers : Absence of Diffusion Control of Reaction Rate in the Thermal (D. A. Smith), 1200
 Decomposition of Carbohydrates : Fixation of Metabolic Products in the Soil during (H. Sørensen), 97
 Deem, H. W., et al. : Thermal Radiative Properties, review by Dr. E. H. Andrews, 517
 Deep-sea Sediments : Pleistocene Glacial-marine Zones in North Atlantic (Dr. J. R. Conolly and Dr. M. Ewing), 135
 Defence : Ministry of, Deputy Chief Scientific Adviser, 19

- Deficiency of α -Lipoproteins (Acanthocytosis) : Precipitin Activity in the Sera of Patients with (Dr. R. F. Murray, jun., and Prof. B. S. Blumberg), 357
- Deformation of Gas Bubbles and Liquid Drops in an Electrically Stressed Insulating Liquid (Dr. K. C. Kao), 279
- Degrossi, Dr. O., Seiber, J. M., Gotta, H., Olivari, A., and Chwojnik, A. : Use of Technetium-99m in Hepatic Scintigraphy, 85
- Dehydrogenase Isozymes in Pigeons : Polymorphism of Lactate (Prof. Wm. H. Zinkham et al.), 284
- Deiodination *in vitro* : Oleic Acid labelled with Iodine-131, and Triolein (L. J. Angileri), 896
- Delayed Effect of Thymectomy in Adult Life on Immunological Competence (Dr. D. Metcalf), 1336
- Delayed Fluorescence from Organic Crystals (G. F. Moore and Dr. I. H. Munro), 772
- Delayed Hypersensitivity : Isolation of a Lymphoid-cell Protein with Relation to (U. Rothman and S. Lidén), 389
- Delayed Mortality in Adult Chickens exposed to 1 Atmosphere Oxygen (Prof. H. S. Weiss, D. L. Beckman and R. A. Wright), 1003
- Delicate Biological Specimens : Simple Technique for embedding and supporting (R. H. Harris), 199
- Demetsopoulos, I. C., et al. : Determination of the Total Alpha Activity of Drinking Water using a Nuclear Emulsion (M. W. Tyrrell, I. C. Demetsopoulos and A. J. L. Collinson, 1074
- Denaturation of Lactic Dehydrogenase Isozymes and its Clinical Application (Dr. Aarne Kontinen and S. Lindy), 782
- Denatured Deoxyribonucleic Acid : Reaction of Adenine-specific Antibodies with (Dr. S. Estrada-Parra et al.), 1010
- Denitrification Losses from Soil : Effect of pH and Addition of Organic Materials on (D. M. Ekpete and Dr. A. H. Cornfield), 1200
- Dennis, Dr. E. W., et al. : A New Active Metabolite of 'Miracid D', 1005
- Dennis, R. W. G. : Seed-borne Fungi, review, 319
- Dense Packing of Hard Spheres with Five-fold Symmetry (B. G. Bagley), 674
- Density Functions of Hydromagnetic Emissions at High Latitude : Spectral (Dr. A. Egeland), 539
- Denticite di Accadia, Prof. F., Gribanovski-Sassu, O., Romagnoli, A., and Tuttobello, L. : Production of Carotenoid by a Green Alga, 1342
- Denton, R. M., and Randle, Prof. P. J. : Hormonal Control of Lipid Concentration in Rat Heart and Gastrocnemius, 488
- DNA : Tests on *Drosophila* for the Production of Mutations by Irradiated Medium or Irradiated (V. L. Chopra), 699
- DNA in *Drosophila* : Mutagenic Effect of Irradiated and Unirradiated (A. H. Khan and Dr. T. Alderson), 700
- DNA Loops or to DNA Helices with the Effect on RNA Synthesis : Correlation of the Binding to (Prof. J. H. Frenster), 1093
- DNA Synthesis derived from the Medium of Leucocyte Cultures : A Factor Stimulating (Dr. S. Kasakura and Prof. L. Lowenstein), 794
- Deoxyribonucleic Acid : Electron Spin Resonance in X-irradiated Oriented (Dr. J. P. Elliott and Dr. S. J. Wyard), 483
- Deoxyribonucleic Acid : Reaction of Adenine-specific Antibodies with Denatured (Dr. S. Estrada-Parra et al.), 1010
- Deoxyribonucleic Acid along Human Chromosomes : Distribution of (Dr. A. Ward), 486
- Deoxyribonucleic Acid and Ribonucleic Acid : Separation of High-molecular (Dr. O. Klamerth), 1318
- Deoxyribonucleic Acid and Ribonucleic Acid Synthesis Induced in Cultured Mammalian Cells by 7:12-Dimethylbenzanthracene . Differential Inhibition of (L. J. Alfred), 1339
- Deoxyribonucleic Acid during Selective Transcription : Localized Strand Separations within (Prof. J. H. Frenster), 894
- Deoxyribonucleic Acid on Hydroxyapatite : Fractionation of Mouse (Dr. P. M. B. Walker and Dr. A. McLaren), 1175
- Deoxyribonucleic Acid Replication in the Giant Foot-pad Cells of *Sarcophaga bullata* : Differential (Dr. J. M. Whitten), 1019
- Deoxyribonucleic Acid Synthesis in Cultured Human Cells and its Bearing on the Concepts of Endoreduplication and Polyploidy (Dr. U. Mittwoch, Mrs. K. P. Lele and W. S. Webster), 242
- Deoxyribonucleic Acid Synthetic Period in Human Blood Cells : Rate of Chromosome Duplication at the End of the (Dr. C. W. Gilbert et al.), 159
- Dependence of Photophosphorylation by Isolated Chloroplasts on the Oxidation-Reduction State of N-Methylphenazinium Methyl Sulphate (Phenazine Methosulphate) (G. Zweig and Dr. M. Avron), 190
- Dependence of the Properties of Moving Striations in the Plasma of the Positive Column on Some External and Internal Parameters (G. Lakatos and Dr. J. Bitó), 445
- Depletion by Dietary Leucine : Serotonin (Dr. A. Yuwiler and Dr. E. Geller), 83
- Depolarizing Drugs in Normal and Denervated Muscle Labelled (Prof. D. B. Taylor et al.), 901
- Depressant and Dual Stimulant-depressant Action of Glutaramide Homologues in Mice : Titration Curves depicting Central Stimulant (Dr. A. Shulman et al.), 568
- Derivatives of 2-Aminoethyl-guanidine and of a Eugenol as Virus Inhibitors (S. Pácsa, O. H. Hankovsky and K. Hudeg), 409
- Dermal Connective Tissue of Adrenalectomized Rats : Acid Mucopolysaccharide and Hexosamine Metabolism in (Prof. J. Fabianek et al.), 1212
- Dermis and Epidermis to Lightening Agents : Response of Melanocytes of (Prof. J. McGuire and H. Moller), 493
- Derse, P. H., and Nees, P. O. : Feeding and Reproduction of Rats fed Calcium Cyclamate, 81
- Desalination of Sea-water, 629
- Design in Engineering and the Physical Sciences : Statistics and Experimental, Two Volumes (Prof. N. L. Johnson and Prof. F. C. Leone), review by N. E. Day, 214
- Design Method, 1052
- De-spin of Planets and Satellites : Tidal (Dr. P. Golreich), 375
- Destin : L'homme Face à Son (C. L. Mayer), review by F. I. G. Rawlins, 318
- Detection and Titration of Antihuman Serum Albumin Antibody : Rapid (Dr. M. S. Tobin), 1338
- Deterioration : n-Alkan-1-ols in Butter after Oxidative (W. Stark and D. A. Forss), 190
- Deterioration of Stored Inulin Solutions (S. Nilwarangkur and Dr. G. M. Berlyne), 77
- Determination of Cloud Altitudes from Gemini-Titan-S (F. Saiedy, W. A. Morgan and Dr. D. Q. Wark), 775
- Determination of Nicotine in Smokers' Urine by Gas Chromatography (Dr. N. L. McNiven, K. H. Ralsingham, S. Pacashnik and Prof. R. I. Dorfman), 788
- Determination of pH : Theory and Practice (Dr. R. G. Bates), review by R. Sawyer, 318
- Determination of p.p.m. Concentrations of Carbon Disulphide in Benzene by Electron Capture Gas Chromatography (Dr. W. Grant and G. A. Vaughan), 75
- Determination of the Acceleration due to Gravity at the National Physical Laboratory : New Absolute (Dr. A. H. Cook), 279
- Determination of the Total Alpha Activity of Drinking Water using a Nuclear Emulsion (M. W. Tyrrell, I. C. Demetsopoulos and A. J. L. Collinson, 1074
- Detonability of Nitroglycerin contained in Porous Rock (J. E. Hay and F. H. Scott), 1197
- Deuterium Oxide Exchange : An Infra-red Investigation of the Effect of Pressure on Silica Powders, as revealed by (F. H. Hambleton et al.), 138
- Deuterium Oxide in Mice : Histological Investigations of the Mechanism of Sterility Induced by (A. M. Hughes and Dr. L. E. Glass), 1119
- (Deuterium Oxide) : Influence of Heavy Water, on Plaque Size of Western Equine Encephalitis Virus (R. I. Carp and Dr. D. Kruschevsky), 93
- Deutsch, Prof. J. A., and Collins, R. : Facilitation as a Function of Temporal Spacing of Stimuli in Intracranial Self-stimulation, 592
- Developing Countries : British Aid to, 128
- Development : Control of Biological, 838
- Development in British Industry : Research and, 101
- Development of *Eimeria tenella* in Avian Embryos (P. L. Long), 509
- Development of the Eye (G. V. Lopashov and O. G. Stroevea), review by Dr. R. A. Weale, 979
- Development of the Haemopoietic System in the Chick Embryo Chromosome Marker Studies on the (M. A. S. Moore and Dr. J. J. T. Owen), 956
- Development of the Ovary in a Species of *Cissus* (N. C. Nair), 402
- Developments in Cosmology : Recent (Prof. F. Hoyle), 111
- De Weck, Dr. A. L., and Schneider, Dr. C. H. : New Chemical Aspect of Penicillin Allergy : the Direct Reaction of Penicillin with ϵ -Amino-groups, 57
- Dextran Complex : Iron (Dr. C. R. Ricketts et al.), 237
- Deykin, Dr. D., Pritzker, C. R., Scolnick, E. M. : Plasma Co-factors in Adenosine Diphosphate-Induced Aggregation of Human Platelets, 296
- Diabasic Rocks : Palaeomagnetism of the Isachsen (A. Larochelle et al.), 179
- Diagenesis : Organic Matter in Recent and Ancient Limestones and its Role in their (D. J. Shearman and Sir Patrick A. d'E. Skipwith), 1310
- Diagnosis of the Parasites of Man : Microscopic (Dr. R. B. Burrows), review by Dr. G. Lapege, 1029
- Diagnosis of Visceral Mycoses : Fluorochrome Stains for Histological (G. Bader et al.), 796
- Diagnostics with Microwaves : Plasma (Dr. M. A. Heald and C. B. Wharton), review by A. E. Dangor, 965
- Diamantopoulos, Dr. E., and Gassel, Dr. M. M. : Nerve Potential Recordings during electrically and mechanically evoked Monosynaptic Reflexes in Man, 1004
- Diamine Oxidase : Electron Spin Resonance Spectrum of Cu^{++} in (Prof. A. van Heuvelen), 888
- 2,6-Diamino-3-hydroxyphenylacetic Acid in Microbial Cell Wall Mucopolysaccharide (H. R. Perkins), 872
- 1,7-Diamino-8-naphthol-3,6-disulphonic Acid on Pregnancy in the Rat : the Effect of (Dr. G. A. Christie), 1219
- Diamond : Laser-induced Damage in (Dr. D. J. Bradley et al.), 1081
- Diamond : Laser-induced Damage in Natural White (P. Whiteman and Dr. G. W. Wilson), 66
- Dickerson, Prof. R. E. : Hexagonal-mesh Structures and Stable Polypeptide Chain Configurations, 139
- Dickmann, Z., and Dzuik, Prof. P. J. : Failure of the Zona Reaction in Five Pig Eggs, 502
- Dictionary of Nutrition and Food Technology (Dr. A. E. Bender), Second Edition, review by Dr. A. M. Copping, 987
- Dictionary of Organic Compounds (edited by Dr. J. R. A. Pollock and Dr. R. Stevens), Fourth Edition, Five Volumes and Supplement, review by Dr. F. G. Mann, 417
- Dieldrin : Metabolites in Urine of Rats on Diets containing Aldrin or (P. R. Datta et al.), 289
- Diener, Dr. E., and Ealey, Dr. E. H. M. : Immune System in a Monotreme : Studies on the Australian Echidna (*Tachyglossus aculeatus*), 950
- Dietary Leucine : Serotonin Depletion by (Dr. A. Yuwiler and Dr. E. Geller), 83
- Dietary Levels of Phosphorus and Calcium on the Comparative Behaviour of Strontium and Calcium : Effects of (K. Kostial, S. Vojvodic and Prof. C. L. Comar), 1110
- Dieter, Dr. N. H., et al. : Observations of a Strong Unidentified Microwave Line and of Emission from the OH Molecule, 29
- Dietetics, Vol. 5 : World Review of Nutrition and (edited by G. H. Bourne), review by Dr. A. M. Copping, 421
- Dietrich, Prof. L. S., Fuller, L., Yero, I. L., and Martinez, L. : Nicotinamide Mononucleotide (NMN) Pyrophosphorylase Activity in Ascites Cell Extracts, 347
- Diets containing Aldrin or Dieldrin : Metabolites in Urine of Rats on (P. R. Datta et al.), 289
- Differences between Human Red Cell and Leucocyte Pyruvate Kinase : Kinetic (J. O. Campos et al.), 194
- Differences in Response to Polypeptides as Permeability Factors : Interspecies (Dr. J. Carr and Prof. D. L. Wilhelm), 653
- Differences in the Antigenic Pattern of *Azotobacter* grown on different Nitrogen Sources (Prof. T. Holme and B. Zacharias), 1235
- Differences in the Rate of Tissue Regeneration in the Rabbit's Ear : Sex (Dr. J. Joseph and Dr. M. Dyson), 599
- Differences in Variability of Discharge Frequency between Primary and Secondary Muscle Spindle Afferent Endings of the Cat (R. B. Stein and Dr. P. B. C. Matthews), 1217
- Different Molecular Species of Mouse Interferon induced by Statolon (Prof. T. C. Merigan and Dr. W. J. Kleneschmidt), 667
- Differential Capacitance of the Electrical Double-layer at Platinum/Halide Metal Interfaces : Adsorption and the (A. D. Graves and Dr. D. Inman), 481
- Differential Deoxyribonucleic Acid Replication in the Giant Foot-pad Cells of *Sarcophaga bullata* (Dr. J. M. Whitten), 1019
- Differential Inhibition of Deoxyribonucleic Acid and Ribonucleic Acid Synthesis induced in Cultured Mammalian Cells by 7:12-Dimethylbenzanthracene (L. J. Alfred), 1339
- Differentiation between Cytophilic Antibody and Opsonin by a Macrophage Phagocytic System (W. E. Parish), 594
- Diffusion Control of Reaction Rate in the Thermal Decomposition of Amorphous Polymers : Absence of (D. A. Smith), 1200
- Diffusion or Hydride Component of Hydrogen Overpotential at Platinized Platinum and Palladized Palladium Cathodes (M. N. Hull and Dr. F. A. Lewis), 887
- Diffusion Procedure to Pesticide Residue Analysis and to the Cholinesterase Screening of Candidate Pesticides : Application of an Agar-Agar (Dr. K. I. Breenon and G. Stoydin), 748
- Diffusive Transport Rates in Structured Media (Prof. A. B. Metzner), 267
- Diggle, W. R., and Saxon, J. : An Unusually Radioactive Fossil Fish from Thurso, Scotland, 400
- Dihydrostreptomycin-, and Kanamycin-inactivating Enzymes from Multiple Drug-resistant *Escherichia coli* carrying Episome 'R'. Chloramphenicol-, (S. Okamoto and Y. Suzuki), 1301
- Dijksterhuis, Prof. E. J. : *Obituary* by Prof. H. Freudenthal, 1258

- Dilute Aqueous Solutions: Electric Potentials generated by Freezing (H. C. Parreira and A. J. Eydé), 33
- Dimension of Management: Third (John J. Haywood), 928
- Dimension: Relative Similarities in One (Prof. J. W. Carmichael *et al.*), 544
- Dimethyl Sulphoxide in Liquid Scintillation Counting: Use of (E. Wright and K. R. Millar), 889
- 7:12-Dimethylbenzanthracene: Differential Inhibition of Deoxyribonucleic Acid and Ribonucleic Acid Synthesis induced in Cultured Mammalian Cells by (L. J. Alfred), 1339
- 2,4-Dimethylheptanoic Acid: Urinary Metabolites in the ω -Oxidation of (Dr. S. R. Tannenbaum and Prof. S. A. Miller), 452
- Dimond, Sister Marie Therese: Hatching Time of Turtle Eggs, 401
- Dimorphic Forms of *Mycobacterium phlei*: Occurrence of (D. E. S. Stewart-Tull), 603
- Dimorphism in *Tilapia*: Sexual (J. Chervinski), 703
- Dinitrenes: Ultra-violet Absorption Spectra of Aromatic Nitrenes and (A. Reiser and V. Frazer), 682
- Dinitro-octylphenols in Certain Commercial Fungicides: Identification of (Dr. A. H. M. Kirby and L. D. Hunter), 189
- 2,4-Dinitrophenylamines with Ceric Salts: Oxidation of Some (Miss S. Haworth and Dr. J. R. Holker), 184
- Diolefine Complex of Platinum (II) $C_{10}H_{14}O_2PtCl_2$: Structure of a (D. Gibson *et al.*), 580
- Diperoxodithylenetriammine Chromium (IV)I-hydrate: A New Chromium(IV) Peroxo Compound (Dr. D. A. House and Prof. C. S. Garner), 776
- Diphenyl Picrylhydrazyl on Surfaces: Simultaneous Oxidation and Reduction of (Prof. J. G. Aston *et al.*), 181
- Diphtheria Intoxication: Accumulation of Keto-acids in Tissues during (Dr. S. C. Agarwal), 914
- Dipole Magnetic Field: Laboratory Observations of Plasma Instabilities in a (Prof. R. G. Quinn), 376
- Direct Ultramicroscopic Observation of Thermal Scattering in Sodium Chloride (Dr. G. Heilmann), 884
- Direction of Cell Movement and Cancer Invasion: Principles of Cell Motility: (Dr. S. B. Carter), 1183
- Disaccharides during Absorption by the Perfused Small Intestine of Amphibia: Hydrolysis of (Dr. D. S. Parsons and J. S. Prichard), 1097
- Disk Electrophoresis: Simplified Technique for Preparative (Prof. D. Sultzeanu and W. F. Goldman), 1120
- Discharge Frequency between Primary and Secondary Muscle Spindle Afferent Endings of the Cat: Differences in Variability of (R. B. Stein and Dr. P. B. C. Matthews), 1217
- Discontinuity in the S/N Fatigue Curve of a Stainless Steel: Effect of Temperature on the (T. R. G. Williams and C. R. Shurmer), 379
- Discoverer of Nickel: Axel Fredrick Cronstedt, 229
- Discrimination of Black and White: Tactile-Vision Thermal and Texture Cues in the (C. N. French), 1352
- Disease: Association of Adenoviruses with Bovine Respiratory (Dr. J. H. Darbyshire), 307
- Disease: Mitral Valve, 631
- Disease: Natural Selection and Transmissible (Prof. N. A. Barnicot), 535
- Disease of the Newborn: Low Concentrations of Serum Haptoglobin in Mothers of Children with Haemolytic (J. Pintera), 694
- Diseases: Immunological Tests in Helminth (Dr. R. Elsdon-Dew), 696
- Disinfection as Applied to Medical, Industrial and Laboratory Practice: A Review of Sterilization and (Prof. S. D. Rubbo and Dr. J. F. Gardner), review by R. Hare, 1249
- Dislocations: Theory of Crystal (Prof. A. H. Cottrell), review by J. W. Christian, 110
- Disorder caused by an Abnormal Fibrinogen ('Fibrinogen Baltimore'): New Inherited Coagulation (Dr. E. A. Beck *et al.*), 143
- Disperse Systems: Relaxation Effects in the Electrophoresis of (J. N. Shaw and Dr. R. H. Ottewill), 681
- Dispersion and Rotational Line Strengths of the Hydrogen Halides: Sub-millimetre (Dr. J. E. Chamberlain and Dr. H. A. Gebbie), 480
- Dispersion in some Non-associated Organic Liquids: Acoustic Velocity (Dr. S. E. A. Hakim and W. J. Comley), 1082
- Dispersion of a Chloroplast Preparation: Optical Rotatory (Dr. Bacon Ke), 573
- Dispersion-softened Zinc Alloys (W. H. McCarthy, Prof. J. C. Shyne and Prof. O. D. Sherby), 579
- Disruption of Protozoa by Indole and Related Compounds (Dr. R. W. Bailey and G. B. Russell), 1001
- Dissociation Energy of the N-H Bond in Hydrazine (Dr. I. P. Fisher and Dr. G. A. Heath), 1199
- Distance Measurement: Electromagnetic, 837
- Distinction between the Effects of Antiviral and Anticellular Polyoma Antibodies on Polyoma Tumour Cells (I. Hellstrom), 652
- Distribution and Effective Half-life of Cobalt-58 in *Habrobracon* (Prof. D. S. Grosch), 906
- Distribution of Collagenase in Rat Tissues (J. F. Woods and Prof. G. Nichols, jun.), 1325
- Distribution of Deoxyribonucleic Acid along Human Chromosomes (Dr. A. Ward), 486
- Distribution of Plutonium in Serum Proteins following Intravenous Injection into Rats (G. Boocock and D. S. Popplewell), 282
- Distribution of the Intestinal Factor of Normal Mice which inactivates Murine Virus Hepatitis (Prof. M. Piazza, Dr. A. Amodio and Dr. G. Pane), 1009
- Disturbances: Coincidence of Magnetic, with Local Earthquakes recorded from the Ethiopian Rift System (Prof. P. Goun), 541
- Disulphides: Charge Transfer Properties of *dl*-Thioctic Acid and Other (W. M. Moreau and Prof. K. Weiss), 1203
- Ditmarsh, R., and Mynlieff, P. F.: Rate of Micelle Formation of Sodium Alkyl Sulphates in Water, 889
- Diurnal Variations in Ovarian Ascorbic Acid Content of Pseudopregnant Immature Rats used in Parlow's Test (Dr. M. de la Lanza and Dr. H. Croxatto), 789
- Diver Balance: Electromagnetic Recording (S. Lovtrup and S. Larsson), 1116
- Dixon, Prof. John R., and Johnson, Prof. B.: Ascend of Sap in Trees: Effect of Negative Absolute Pressure on Flow Resistance, 1347
- Djerassi, Prof. Carl, and Sample, S. D.: Mass Spectrometric Fragmentation of Nitrophenylhydrazones, 1314
- Dobzhansky, Dr. T.: Heredity and the Nature of Man, review by Prof. C. H. Waddington, 959
- Documentation Services: Science Information and, 122
- Dodge, A. H., *et al.*: Androgen-Oestrogen-induced Tumours: Lactic Dehydrogenase Relative to Chatepithelioma Induction in the Syrian Hamster, 567
- Dog Adrenals: Aldosterone Secretion: Differences in Direct Effects of Rubidium and Potassium Perfusion Into (Prof. J. G. Hilton *et al.*), 1099
- Dog Kidney: Release of Plasminogen Activator by the Isolated Perfused (Dr. R. Holmans *et al.*), 291
- Dogs: Bone Marrow Allografts in Thymectomized X-irradiated (I. L. Chertkov *et al.*), 399
- Doi, Prof. Roy H.: Purine Rich Regulatory Sequences in Messenger Ribonucleic Acid, 552
- Dollimore, Dr. D., and Heal, Dr. G. R.: Errors in the Calculation of Surface Area and Average Pore Radius, 1092
- Donovan, Prof. D. T.: Handbook of Palaeontological Techniques, review, 968
- Doppler Shift at Low Elevations: Effects of Pronounced Electron-content Variations on (H. Socher), 644
- Doppler-shifted Whistler-mode Signals: Frequency-Spectrum of (R. A. Morris and N. Poletti), 479
- Dorfman, Prof. R. L., *et al.*: Determination of Nicotine in Smokers' Urine by Gas Chromatography, 788
- Dorset and their Similarity to Orf: Milker's Nodula Virus Infections in (Dr. J. Nagington *et al.*), 505
- Dosage in Laboratory Animals: Drug (C. D. Barnes and L. G. Eltherington), review by Dr. J. A. Parsons, 1028
- Dose-response Relationship with Acetylcholine on the Heart of the Mollusc *Tapes turrida*: Biphasic (P. R. Carroll and G. B. Chesher), 1220
- Double Chromosome Reduction in a Tetraploid *Solanum* (G. E. Marks, Dr. R. K. McKee and Dr. J. B. Harborne), 359
- Double-layer at Platinum/Halide Metal Interfaces: Adsorption and the Differential Capacitance of the Electrical (A. D. Graves and Dr. D. Inman), 481
- Douglas, Prof. W. W., and Pcsiner, Dr. A. M.: Preferential Release of Adrenaline from the Adrenal Medulla by Muscarine and Pilocarpine, 1102
- Dourmashkin, R. R.: Structure Antigénique de Tumeurs Expérimentales, review, 1140
- Down's Syndrome (Mongolism): Virus Aetiology for (A. Stoller and R. D. Collmann), 903
- Downes, Dr. D., Maxwell, Prof. A., and Meeks, Prof. M. L.: Radio Structure of the Galactic Centre Region, 1189
- Drakulic, M., *et al.*: Metabolism of Nucleic Acids and Protein in Starving Bacteria, 303
- Draught of Fishes (Dr. F. D. Ommanney), review by Dr. L. Harrison Matthews, 517
- Dravid, Dr. A. R., *et al.*: Alcohol and Evoked Potentials in the Cat, 688
- Dresser, Dr. D. W., *et al.*: A Localized Haemolysis in Gel Method for the Detection of Cells producing 7S Antibody, 858
- Drinking Water using a Nuclear Emulsion: Determination of the Total Alpha Activity of (M. W. Tyrrell *et al.*), 1074
- Drosophila* for the Production of Mutations by Irradiated Medium or Irradiated DNA: Tests on (V. L. Chopra), 699
- Drosophila* Males by Means of Ovarian Extract: Induction of Crossing-over in (O. S. Reddi *et al.*), 203
- Drosophila* Medium: Lack of Mutagenic Effect of Irradiated (Dr. O. S. Reddi *et al.*), 702
- Drosophila melanogaster*: Phenocopies of the *ma-l* and *ry* Mutants of (E. C. Keller, jun., and Dr. E. Glaszmann), 202
- Drosophila*: Mutagenic Effect of Irradiated and Unirradiated DNA in (A. H. Khan and Dr. T. Alderson), 700
- Drug-dependence (Lord Brain), 825
- Drug Dosage in Laboratory Animals: A Handbook, (C. D. Barnes and L. G. Eltherington), review by Dr. J. A. Parsons, 1028
- Drug Resistance in *Salmonella typhimurium*: Characterization of a Transfer Factor associated with (Dr. E. S. Anderson and Dr. M. J. Lewis), 843
- Drug-resistant *Escherichia coli* carrying Episome 'R': Chloramphenicol-, Dihydrostreptomycin-, and Kanamycin-inactivating Enzymes from Multiple (S. Okamoto and Y. Suzuki), 1301
- Drug-sensitive Enterobacteriaceae: A Rapid Screening Test for Transfer Factors in (Dr. E. S. Anderson), 1016
- Drugs in Normal and Denervated Muscle: Labelled Depolarizing (Prof. D. B. Taylor *et al.*), 901
- Drugs: Micromethod for Measuring Microbial Sensitivity to (Dr. W. Koch and Dr. D. Kaplan), 50
- Drugs on the Isolated Superfused Carotid Body: Some Effects of (Dr. T. J. Biscoe), 294
- Duchenne Muscular Dystrophy: Lactic Dehydrogenase Isoenzymes in Muscle from Patients with (Prof. T. H. Shepard *et al.*), 1107
- Duck Egg-white: Isolation of a Histidine-less Lysozyme: Lysozymes Characterized in (Dr. J. Jøllès *et al.*), 1204
- Ducks fed a Low Level of Toxic Groundnut Meal: Hepatic Tumours in (R. B. A. Carnaghan), 308
- Ducos, Dr. J., Ruffie, J., Colombes, P., Marty, Y., and Ohayon, E.: I Antigen in Leukaemic Patient., 1329
- Duddy, Mr. R. R.: Appointed Deputy Chief Scientific Officer and Director of the Concord Project in the Ministry of Aviation, 19
- Duff, R., and Chessin, Prof. M.: Rapid Method for Selenium Assay of Plant Material, 1001
- Duitschaever, C. L.: Bacterial Count and Cell Differentiation in Milk, 200
- Dummer, G. W. A. (edited by): Solid Circuits and Microminiaturization, review by Dr. J. R. Tillman, 1249
- Dunning-Davies, J.: Carathéodory's Principle and the Kelvin Statement of the Second Law, 576
- Dunnill, Dr. Patricia M., and Fowden, Prof. L.: Enzymatic Formation of β -Cyanoalanine from Cyanide by *Escherichia coli* Extracts, 1206
- Dunworth, Dr. J. V.: Appointment as Director of the National Physical Laboratory, 732
- Duplication at the End of the Deoxyribonucleic Acid Synthetic Period in Human Blood Cells: Rate of Chromosome (Dr. C. W. Gilbert *et al.*), 159
- Durney, B., and Roxburgh, W.: Stability of Rotating Massive Stars in General Relativity, 1304
- Dust Particles: Measured Velocities of Interplanetary (C. S. Nilsson *et al.*), 673
- Dutch Study Circle for Agricultural History, 1939-64, review by G. E. Fussell, 1138
- Duthie, Dr. J. H.: Early Growth of Logic in the Child, review, 1246
- Dyer, Dr. A. J., and Hicks, B. B.: Stratospheric Transport of Volcanic Dust inferred from Solar Radiation Measurements, 131
- Dymysa, Dr. H. A., Stoewand, G. S., Enright, Dr. J. J., Trexler, Prof. P. C., and Gall, L. C.: Human Indigenous Microflora in Gnotobiotic Rats, 1236
- Dynamics of Rockets and Satellites (edited by G. V. Groves), review by Dr. G. King-Hele, 516
- Dynamics: Research Frontiers in Fluid (edited by Dr. R. J. Seeger and Dr. G. Temple), review by Prof. K. Stewartson, 967
- Dyson, Dr. Mary, and Joseph, Dr. J.: Sex Differences in the Rate of Tissue Regeneration in the Rabbit's Ear, 599
- Dystrophic Mice with Mixtures of Spermatozoa: Artificial Insemination of (J. L. Southard *et al.*), 1125
- Dzulk, Prof. P. J., and Dickmann, Z.: Failure of the Zona Reaction in Five Pig Eggs, 502
- EAGER, A. R.: A Guide to Irish Bibliographical Material, review by H. J. Heaney, 1139
- Eager, Prof. R. L., and Mahadevappa, Dr. D. S.: Radiolysis of Sodium Tetrachlorate Dihydrate, 781

- Ealey, Dr. E. H. M., and Diener, Dr. E.: Immune System in a Monotreme: Studies on the Australian Echidna (*Tachyglossus aculeatus*), 950
- Eanes, Prof. E. D., Gillissen, Dr. I. H., and Posner, Prof. A. S.: Intermediate States in the Precipitation of Hydroxyapatite, 365
- Ear: Physics of the (Dr. T. S. Littler), review by Prof. I. G. Taylor, 1136
- Ear: Sex Differences in the Rate of Tissue Regeneration in the Rabbit's (Dr. J. Joseph and Dr. M. Dyson), 599
- Earliest Civilizations of the Near East (James Meilaart), review by M. C. Burkitt, 984
- Early Growth of Logic in the Child, Classification and Seriation (B. Inhelder and J. Piaget), review by Dr. J. H. Duchie, 1246
- Early Receptor Potential of Rat Retina: A New Property of the (Dr. G. B. Arden and H. Ikeda), 1100
- Early Seventeenth Century Scientists (edited by R. Harré), review by Dr. W. L. Sumner, 985
- Earth: A Planet Called (Prof. George Gamow), review by Patrick Moore, 961
- Earth Elements in Eclogitic Rocks and Basaltic Achondrites: Abundances of Rare (J. W. Morgan and Dr. J. F. Lovering), 1311
- Earthquakes recorded from the Ethiopian Rift System: Coincidence of Magnetic Disturbances with Local (Prof. P. Goulin), 541
- Earthworms demonstrated by Paper Chromatography and the Went Pea Test: Presence of Plant Growth Substances in (R. L. Nielson), 1113
- East Aberdeenshire: Corona Structures in the Basic Igneous Masses of (Dr. D. S. Weedon), 885
- East African Wildlife: Research and Management in (Dr. J. Morton Boyd), 828
- East Midlands Regional Office of the Ministry of Technology, 833
- Easterling, R. E., et al.: Quantitative Determination of Haemoglobin A₂ by Acrylamide-gel Electrophoresis, 1103
- Eastern Europe: Ageing In, review by Dr. David A. Hall, 981
- Ebenzer, D. N., et al.: Lack of Mutagenic Effect of Irradiated *Drosophila* Medium 702
- Ebihara, H., and Yoshihara, K.: Evidence of Thermal and Hot Processes in the "Hot-zone" produced by Copper-64 Recoil Atoms in Solids, 482
- Ecballium elaterium: Radiation-induced Sex Reversal in (C. Gómez-Campo and M. Casas-Builla), 805
- Echidna (*Tachyglossus aculeatus*): Immune System in a Monotreme: Studies on the Australian (Dr. E. Diener and Dr. E. H. M. Ealey), 950
- Eclipsing Binaries and the Fission Theory of their Origin: Angular Momenta of (I. W. Roxburgh), 65
- Eclogitic Rocks and Basaltic Achondrites: Abundances of Rare Earth Elements in (J. W. Morgan and Dr. J. F. Lovering), 1311
- Ecology and the Industrial Society (edited by G. T. Goodman, R. W. Edwards and Dr. J. M. Lambert), Symposium of British Ecological Society, 1964, review by Dr. A. Macfadyen, 618
- Ecology in Tropical High Forest: Production, 22
- Economic Development: Regional, 935
- Economic Growth in Belgium: Research and, 834
- Economic Growth: Science and, 1260
- Ecosystem: Man's Place in the Island (edited by Dr. F. R. Fosberg), review by Sir L. Dudley Stamp, 109
- Edey, T. N.: Undernutrition and Early Embryonic Mortality in Merino Ewes, 1232
- Edinburgh: Royal Society of, 1260, Elections, 627
- Edinburgh, University of, Appointments, 936, Chair of Mathematics, 19, 832
- Edington, Dr. J. M., and Claridge, Dr. M. F.: Bilateral Asymmetry: a Larval Polymorphism in some Argid Sawflies (Hymenoptera, Symphyta), 301
- Editor of Nature: Sudden Death of Mr. L. J. F. Brimble on November 15th, 1965, 732
- Education and Application of the Materials Scientist (Dr. G. F. Freeguard), 939
- Education and the Labour Movement 1870-1920 (Brian Simon), review by Prof. W. H. G. Armytage, 960
- Education: Chemical, 1257
- Education: Eighteen Plus, Unity and Diversity in Higher (edited by Marjorie Reeves), review by Sir James Cook, 313
- Education Overseas: Aid to, 120
- Education: Physics (Dr. J. M. A. Lenihan), 26
- Edwards, Dr. R. G.: Maturation in vitro of Mouse, Sheep, Cow, Pig, Rhesus Monkey and Human Ovarian Oocytes, 349
- Edwards, R. W., et al. (edited by): Ecology and the Industrial Society, review by Dr. A. Macfadyen, 618
- Edwards, Miss S. A., et al.: Metabolism of 5-Chloro-2-cyclohexyl-1-oxo-6-sulphamoyl Isoindoline, Chlorexolone (M and B 8430), 286
- Eel: A Dual Neurosecretory Innervation of the Pars Distalis of the Pituitary of the (Sir Francis Knowles and L. Vollrath), 1343
- Eel: A Functional Relationship between Neurosecretory Fibres and Pituitary Cells in the (Sir Francis Knowles and L. Vollrath), 1343
- Effects of (2-Chloroethyl) trimethyl-ammonium Chloride and Gibberellic Acid on Growth, Fruit Bud Formation and Frost Resistance in One-year-old Pear Trees (Dr. I. Modlibowska), 503
- Efficiencies of Hydrocarbon Molecules as a Function of Bombarding Electron Energy: Relative Ionization (J. A. Takacs and Prof. G. R. Freeman), 996
- Efremov, Dr. G.: A Modification System for Fingerprinting Haemoglobin, 298
- Egeland, Dr. A.: Spectral Density Functions of Hydromagnetic Emissions at High Latitude, 539
- Eger, Dr. Chaim, and Norton, Dr. D. A.: Androgenic Steroid Complexes with p-Bromophenol, 997
- Egg-white: Isolation of a Histidine-less Lysozyme: Lysozymes characterized in Duck (Dr. J. Jollès et al.), 1204
- Eggs: Hatching Time of Turtle (Sister Marie Therese Dimond), 401
- Eggs: Histocompatibility Antigens in Transplanted Mouse (Dr. R. L. Simmons and P. S. Russell), 698
- Ehlers-Danlos Syndrome: Cutaneous Elastin in (Dr. D. P. Varadi and Dr. D. A. Hall), 1224
- Ehrlich Ascites C-2 Tumour Cells treated with Actinomycin D: Rapidly Labelled Synthesis of Ribonucleic Acid in (D. A. Popović), 49
- Eidinoff, Prof. M. L., Bates, B., Seinglass, M., and Haddad, Prof. J. R.: Subcutaneous Sarcomata in Hamsters Induced by Rous Sarcoma Virus (Bryan), 336
- Eighteen Plus: Unity and Diversity in Higher Education (edited by Marjorie Reeves), review by Sir James Cook, 313
- Eilon, G., et al.: Antiviral Action of Oxidized Spermine—Inactivation of Plant Viruses, 1095
- Eimeria tenella in Avian Embryos: Development of (P. L. Long), 509
- Einer-Jensen, N., et al.: Specific Uptake in Corpora Lutea of a Non-steroid Substance with Anti-gestagenic Properties, 461
- Einführung in die Atomphysik (Prof. W. Finkelnburg) 19th and 20th reprint, review by Dr. E. Bretscher, 217
- Eire: Observations on the Mont Pelée Eruption of 1902 and their Significance to Ordovician Welded Tuffs of County Mayo, (Dr. J. McManus), 578
- Eire: Regional Geochemical Reconnaissance applied to some Agricultural Problems in Co. Limerick, (Prof. J. S. Webb and W. J. Atkinson), 1056
- Eisenhardt, R. H., et al. (edited by): Rapid Mixing and Sampling Techniques in Biochemistry, review by Prof. S. Dayley, 823
- Ekpote, D. M., and Cornfield, Dr. A. H.: Effect of pH and Addition of Organic Materials on Denitrification Losses from Soil, 1200
- Elastin in Ehlers-Danlos Syndrome: Cutaneous (Dr. D. P. Varadi and Dr. D. A. Hall), 1224
- Electric Discharge: Breakdown of Simple Hydrocarbons Subjected to a Silent (P. E. Cross), 892
- Electric Potentials generated by Freezing Dilute Aqueous Solutions (H. C. Parreira and A. J. Eydtt), 33
- Electric Shock in Mice treated with Cocaine: Lethal Effects of Aggregation and (H. Lal and Dr. R. D. Chessick), 295
- Electrical Activity in Human Uterine Muscle Strips (Dr. T. B. Machachlan, Prof. E. E. Daniel and Prof. W. M. Paul), 290
- Electrical Conductivity of Zinc Oxide Single Crystals: Effect of Ultra-Violet Irradiation on the (Dr. R. B. Lal and Dr. G. M. Arnett), 1305
- Electrical Double-layer at Platinum/Halide Metal Interfaces: Adsorption and the Differential Capacitance of the (A. D. Graves and Dr. D. Inman), 481
- Electrical Machinery and Control (Prof. I. L. Kosow), review by Prof. J. C. West, 9
- Electrical Power Generation: Magneto-hydrodynamic (edited by J. A. Satkowski and H. B. Smets), review by Prof. M. W. Thring, 966
- Electrical Stimulation on Milk Ejection: Introduction of a Permanent Electrode into the Pituitary Gland of the Cow: Influence of (B. Hynek et al.), 690
- Electrically Stressed Insulating Liquid: Deformation of Gas Bubbles and Liquid Drops in an (Dr. K. C. Kao), 279
- Electricity Generating Board Co-operation Scheme: U.K. Atomic Energy Authority and Central, 120
- Electrochemistry: Encyclopedia of (edited by C. A. Hampel), review by Dr. R. F. Phillips and N. A. Hampson, 104
- Electrochemistry: Proceedings of the First Australian Conference on (edited by J. A. Friend et al.), review by Dr. R. F. Phillips and N. A. Hampson, 104
- Electrode into the Pituitary Gland of the Cow: Influence of Electrical Stimulation on Milk Ejection: Introduction of a Permanent (B. Hynek et al.), 690
- Electromagnetic Distance Measurement, 837
- Electromagnetic Recording Diver Balance (S. Løvtrup and Sune Larsson), 1116
- Electromyogram and the Force of the Isometric Reflex Response of Normal Human Subjects: Relationship between the (Alex M. Clarke), 551
- Electron Capture Cross-sections: Correlation of Sodium Atom Reaction Rates with (K. R. Wilson and Prof. D. R. Herschbach), 182
- Electron Capture Gas Chromatography: Determination of p.p.m. Concentrations of Carbon Disulphide in Benzene by (D. W. Grant and G. A. Vaughan), 75
- Electron Content of the Ionosphere near the Auroral Zone: Variation according to the Latitude of the (L. Liszka), 280
- Electron-content Variations on Doppler Shift at Low Elevations: Effects of Pronounced (H. Soicher), 644
- Electron Energy: Relative Ionization Efficiencies of Hydrocarbon Molecules as a Function of Bombarding (J. A. Takacs and Prof. G. R. Freeman), 996
- Electron-irradiated Graphite: Mechanism of Stored-energy Release at 200°C in (Prof. E. W. J. Mitchell and M. R. Taylor), 638
- Electron Microscope Observations on the Free Protein and the Protein-poly-saccharide Complex of Bovine Synovial Fluid (Dr. J. W. Smith and Dr. J. Frame), 867
- Electron Microscope Observations of Neurosecretory Elements in the Neuro-intermediate Lobe in Skates (Dr. A. L. Polenov and M. A. Belenky), 94
- Electron Microscopic Observations on the Carotid Body (Dr. T. J. Biscoe and Dr. W. E. Stehbens), 708
- Electron Microscopy: Examination of a Polysaccharide Flocculant and Flocculated Kaolinite by (Dr. A. Audsley and A. Fursy), 753
- Electron Microscopy: Freeze-fixation, a New Method for (Prof. O. Nilsson), 90
- Electron Spin Resonance in X-irradiated Oriented Deoxyribonucleic Acid (Dr. J. P. Elliott and Dr. S. J. Wyard), 483
- Electron Spin Resonance Investigations of Ultra-violet and X-irradiated Homocysteine-thiolactone (Prof. R. Koch and Dr. H. Monig), 86
- Electron Spin Resonance Spectrum of Cu²⁺ in Diamine Oxidase (Prof. A. van Heuvelen), 888
- Electron Transport Phosphorylation: Evidence discriminating between the Chemical and the Chemiosmotic Mechanisms of (Dr. P. Mitchell and Dr. J. Moyle), 1205
- Electronic Absorption Spectrum of the CH₃O-radical (J. F. Ogilvie), 1315
- Electronic Counter: Size Analysis in the Sub-sieve Range by (Prof. C. C. Harris and Dr. A. Jowett), 175
- Electrophoresis of Disperse Systems: Relaxation Effects in the (J. N. Shaw and Dr. R. H. Ottewill), 681
- Electrophoresis of β -Lactoglobulins stored in Solutions at pH 8.7; Acrylamide-gel (Dr. P. Akroyd), 488
- Electrophoresis of Soluble Lens Proteins in Galactose-fed Rats: Agar-gel (E. Wittgenstein and Dr. K. W. Rowe, jun), 386
- Electrophoresis on Acrylamide Gels: Taxonomic Investigation of Several Brassica Species using Serology and the Separation of Proteins by (Dr. J. G. Vaughan et al.), 704
- Electrophoresis: Quantitative Determination of Haemoglobin A₂ by Acrylamide-gel (Lieut-Commander T. G. Ferris et al.), 1103
- Electrophoresis: Simplified Technique for Preparative Disc (Prof. D. Sulitzzeanu and W. F. Goldman), 1120
- Electrophoretic Mobilities of some Erythrocytic Enzymes as a Function of their Age: Altered (Dr. H. Walter et al.), 76
- Electrophoretic Mobility of an Immune Globulin from Rainbow Trout Serum (H. O. Hodgins, Dr. G. J. Ridgway and F. M. Utter), 1106
- Elementary Particle Physics, 23
- Elements by Plant Roots: Mechanism of Uptake of Trace (G. Findenegg and Prof. E. Broda), 196
- Elements in the Continental Crust: Radioactive (Dr. K. S. Heier), 479
- Elements of Chemistry (Antoine-Laurent Lavoisier, translated by Robert Kerr. Introduction by Prof. D. McKie), review by Roger Partington, 987
- Elevations: Effects of Pronounced Electron-content Variations on Doppler Shift at Low (H. Soicher), 644
- Eleventh Pacific Science Congress, 328
- Elisabethville: Official University of the Congo at, 124
- Elithorn, Dr. A., and Jones, D.: Interstimulus Interval and Number Judgment, 153
- Ellenson, Sandra R., and Heim, Prof. W. G.: Involvement of the Adrenal Cortex in the Appearance of Rat Slow Alpha-Globulin, 1330
- Elliott, Dr. J. P., and Wyard, Dr. S. J.: Electron Spin Resonance in X-irradiated Oriented Deoxyribonucleic Acid, 483
- Elliott, Dr. R. C., et al.: Phosphorus Excretion of Cattle fed on High-energy Diets, 953
- Elmore, Dr. D. T.: Essays in Biochemistry, Vol. 1, review, 316
- Structure and Activity of Enzymes, Symposium, 1964, review, 972
- Elpner, Prof. I. E., Sokolskaya, A. V., and Margulis, M. A.: Initiation of Chain Reaction under an Ultrasonic Wave Effect, 945
- Elsdon-Dew, Dr. R.: Immunological Tests in Helminthic Diseases, 696
- Etherington, L. G., and Barnes, C. D.: Drug Dosage in Laboratory Animals, review by Dr. J. A. Parsons, 1028
- Elvidge, Prof. J. A.: Appointed to Chair of Chemistry at Battersea College of Technology, 119
- Elworthy, Prof. P. H.: Appointment to New Chair of Pharmaceutical Technology in the University of Strathclyde, 934

- Emanuel, N. M. (edited by): Oxidation of Hydrocarbons in the Liquid Phase, review by Dr. H. Steiner, 420
- Embedding and Supporting Delicate Biological Specimens: Simple Technique for (R. H. Harris), 199
- Embolisms induced by Catecholamines or Serotonin: Thromboses in Large Veins and Pulmonary (Prof. H. Selye et al.), 900
- Embrittlement of Low-boron Type 304 Stainless Steel: Irradiation (W. R. Martin et al.), 73
- Embryo Cells: Plaque-formation by Non-immune and X-irradiated Lymphoid Cells on Monolayers of Mouse (G. Moller and E. Moller), 260
- Embryo: Chromosome Marker Studies on the Development of the Haemopoietic System in the Chick (M. A. S. Moore and Dr. J. J. T. Owen), 956
- Comparison of the Chorioallantoic Membrane and Splenomegaly Systems of Graft-versus-host Assay in the Chick (L. W. Coppleston and Dr. D. Michie), 53
- A New Syndrome: Hyperlipaemia following Viral Infection in the Chicken (Dr. S. E. Grossberg and Prof. W. M. O'Leary), 954
- Embryonic Lung Cells: Aflatoxin: Effect on Cultured Heteroploid Human (Dr. M. S. Legator et al.), 345
- Embryonic Mortality in Merino Ewes: Undernutrition and Early (T. N. Edey), 1232
- Embryos: Development of *Eimeria tenella* in Avian (P. L. Long), 509
- Emission: Anomalous Intensity Ratios of the Interstellar Lines of OH in Absorption and (R. X. McGee et al.), 1193
- Emission at Centimetre and Decimetre Wavelengths: Basic Component of Solar Radio (Dr. M. K. Das Gupta and D. Basu), 739
- Emission from the OH Molecule: Observations of a Strong Unidentified Microwave Line and of (Prof. H. Weaver et al.), 29
- Emission in the Direction of Radio Source W49: OH (B. Zuckerman et al.), 441
- Emission: Observations of Polarized OH (Dr. S. Weinreb et al.), 440
- Emission of Feulgen-positive Particles during the *in vitro* Maturation of Toad Oocytes (Prof. J. Brachet), 596
- Emission Spectra of Minerals: Infra-red (Prof. M. J. D. Low), 1089
- Emissions at High Latitude: Spectral Density Functions of Hydromagnetic (Dr. A. Egeland), 539
- Emlen, Prof. J. T., and Aumann, G. D.: Relation of Population Density to Sodium Availability and Sodium Selection by Microtine Rodents, 198
- Emmie Clough Scholarship, 735
- Emmons, Dr. P. R., Harrison, M. J. G., Honour, Dr. A. J., and Mitchell, Dr. J. R. A.: Effect of a Pyrimidopyrimidine Derivative on Thrombus Formation in the Rabbit, 255
- Encephalitis Virus: Influence of Heavy Water (Deuterium Oxide) on Plaque Size of Western Equine (R. I. Carp and Dr. D. Kritchevsky), 93
- Encyclopedia of Electrochemistry (edited by C. A. Hampel), review by Dr. R. F. Phillips and N. A. Hampson, 104
- Encyclopedia of Plant Physiology (edited by W. Ruhland), Vol. 15: Differentiation and Development, Two Parts, review by Dr. C. R. Metcalfe, 619
- Endocrinology: European Society for Comparative, 436
- Endogenous Bud Formation in the Hypotrachida (Dr. C. R. Curds), 90
- Endoreplication and Polyploidy: Deoxyribonucleic Acid Synthesis in Cultured Human Cells and its Bearing on the Concepts of (Dr. U. Mittwoch et al.), 242
- Endosperm in Cultures: Growth Responses of Mature (Prof. B. M. Johri and S. S. Bhowani), 1345
- Endotoxin, Bacteria, or Viruses in Mice pretreated with *Escherichia coli* Endotoxin or infected with *Mycobacterium tuberculosis*: Interferon Appearance stimulated by (Prof. J. S. Youngner and Prof. W. R. Stinebring), 456
- Endplate Potentials and some Implications: Evidence for a Poisson Distribution of Miniature (Dr. P. W. Gage and Dr. J. I. Hubbard), 395
- Energy Forecasts for Britain, 229
- Energy of Moisture in Unsaturated Soils: Thermistor Hygrometer for determining the Free (B. G. Richards), 608
- Energy of the N-H Bond in Hydrazine: Dissociation (Dr. I. P. Fisher and Dr. G. A. Heath), 1199
- Energy: Relative Ionization Efficiencies of Hydrocarbon Molecules as a Function of Bombarding Electron (J. A. Takacs and Prof. G. R. Freeman), 996
- Energy Requirements for Relaxation from Tonic Contractions ('Catch') in an Invertebrate Muscle (Dr. K. Minihan and Prof. R. E. Davies), 1327
- Energy Specificity of Simple Photoreceptor Units: Quantum (Prof. W. W. Dawson), 589
- Engelsma, Dr. G.: Photo-induced Hydroxylation of Cinnamic Acid in Gherkin Hypocotyls, 1117
- Engineering and the Physical Sciences: Statistics and Experimental Design in (Prof. N. L. Johnson and Prof. F. C. Leone), Two Vols., review by N. E. Day, 214
- Engineering at the Massachusetts Institute of Technology: Materials Science and, 726
- Engineering Freshmen: Cambridge, 528
- Engineering Industries: Research and Development in the Building and Civil, 629
- Engineering: Mechanical and Electrical, 1152
- Engineering Practice: Rubber in (A. B. Davey and A. R. Payne), review by Dr. L. R. G. Treloar, 823
- England and their Significance: Recent finds in the Upper Carboniferous of South-west (Dr. H. G. Reading); (Dr. J. E. Prentice), 745
- Engler, A.: Syllabus der Pflanzenfamilien, Vol. 2, Angiosperms, Twelfth Edition (edited by Prof. H. Melchior), review by Prof. T. G. Tutin, 316
- Engwell, M., et al.: Laser-induced Damage in Diamond, 1081
- Enokibara, H., et al.: Effect of 'Furosemid' on Chloride and Water Excretion in Single Nephrons of the Kidney of the Rat, 80
- Enrichment of Iron during Accretion in the Solar Nebula (Prof. S. R. Taylor), 886
- Enright, Dr. J. J., et al.: Human Indigenous Microflora in Gnotobiotic Rats, 1236
- Enterobacteriaceae: Penicillinase Synthesis controlled by Infectious R Factors in (Dr. N. Datta and Dr. P. Kontomichailou), 239
- Enterobacteriaceae: A Rapid Screening Test for Transfer Factors in Drug-sensitive (Dr. E. S. Anderson), 1016
- Entire-leaf Variant in the Potato Variety 'Majestic': Chimerical Nature of the (Dr. H. W. Howard), 917
- Entrance Requirements in Britain: University, 433
- Environment: Conservation and Man's (Dr. I. McTaggart Cowan), 1145
- Ergonomics: Man in his Working (K. F. H. Murrell), review by C. E. Fleming, 106
- Organochlorine Pesticides in the Atmospheric (Dr. D. C. Abbott et al.), 1317
- Environment on the Orientation of the Nitrate Ions in Crystals: Effect of (Prof. N. R. Kunchur), 778
- Enzymatic Adaptation to Contractile Activity in Skeletal Muscle (J. Kendrick-Jones and Prof. S. V. Perry), 1068
- Enzymatic Formation of β -Cyanoalanine from Cyanide (Dr. H. G. Floss, Dr. L. Hadurger and Prof. E. E. Conn), 1207
- Enzymatic Formation of β -Cyanoalanine from Cyanide by *Escherichia coli* Extracts (Dr. P. M. Dunnill and Prof. L. Fowden), 1206
- Enzymatic Synthesis of the Skin-lightening Agent, Melatonin, in Amphibians (Dr. J. Axelrod, Prof. W. B. Quay and P. C. Baker), 386
- Enzyme Mutants in Micro-organisms: A Suggested Phenotypic Classification and Terminology for (Dr. I. F. Collins et al.), 841
- Enzyme Nomenclature, 736
- Enzyme Regulation, Vol. 2: Advances in (edited by G. Weber), review by Dr. C. J. R. Thorne, 319
- Enzyme Regulatory Mechanisms at the Level of Lactate-oxidoreductase in Erythrocytes and Ascites Tumour Cells (S. Rapoport, L. Ababei, C. Wagenknecht and S. R. Sarkar), 185
- Enzyme System from Yeast: Formation of Hydroxamates during Proteolysis by an (Dr. D. E. Briggs and Dr. D. J. Millin), 287
- Enzymes: Evidence for Structures resembling Crystalline Carbons at the Active Centre of (Dr. E. D. 'Wachsmuth'), 942
- Enzymes: Structure and Activity of (edited by T. W. Goodwin et al.), review by Dr. D. T. Elmore, 572
- Enzymes as a Function of their Age: Altered Electrophoretic Mobilities of some Erythrocytic (Dr. H. Walter et al.), 76
- Enzymes during Insect Metamorphosis: Changes in the t-RNA Methylating (Dr. B. S. Baliga et al.), 555
- Enzymes from Multiple Drug-resistant *Escherichia coli* carrying Episome 'R': Chloramphenicol-, Dihydrostreptomycin-, and Kanamycin-inactivating (S. Okamoto and Y. Suzuki), 1301
- Epacridaceae: Warded Pollen Grains of the (Dr. J. M. Matthews), 803
- Epidermis: The (edited by W. Montagna and W. C. Lobitz), review by Prof. C. D. Calnan, 213
- Epidermis: Presumed Sensory Cells in Fish (Dr. Mary Whitear), 703
- Epidermis to Lightening Agents: Response of Melanocytes of Dermis and (Prof. J. McGuire and H. Moller), 493
- Epididymis of American Forms: Marsupial Spermatozoa Pairing in the (Dr. J. D. Biggers and Dr. E. D. DeLamater), 402
- Epileptogenic Effect of Extracranial and Extracranial Cobalt (H. Payan, Dr. R. Strebel and Prof. S. Levine), 792
- Episome 'R': Chloramphenicol-, Dihydrostreptomycin-, and Kanamycin-inactivating Enzymes from Multiple Drug-resistant *Escherichia coli* carrying (S. Okamoto and Y. Suzuki), 1301
- Epstein, Dr. S. S., Saporoschetz, I. B., Small, M., Park, W., and Mantel, N.: A Simple Bioassay for Antioxidants based on Protection of *Tetrahymena pyriformis* from the Photodynamic Toxicity of Benzo(a)pyrene, 655
- Equal Quantities: Calculations with Two Nearly (Dr. J. H. Fremlin; Dr. F. T. Worrell), 1306
- Equation of State of Liquid-vapour Equilibrium with the Same Constants for both Phases (Dr. I. Kuhn), 1315
- Equation of Thermogravimetric Data: Tschibyschev Approximation to the (Dr. E. L. Jones), 1306
- Equine Encephalitis Virus: Influence of Heavy Water (Deuterium Oxide) on Plaque Size of Western (R. I. Carp and Dr. D. Kritchevsky), 93
- Erdey-Grúz, Dr. T., and Tencsényi-Valdappal, Dr. I. (edited by): Science in Hungary, review by Prof. D. Gabor, 983
- Erdmann, D., et al.: Morphacins, a Novel Group of Plant-growth Regulators, 1013
- Ergonomics: Man in his Working Environment, (K. F. H. Murrell), review by C. E. Fleming, 106
- Errors in the Calculations of Surface Area and Average Pore Radius (Dr. D. Dollimore and Dr. G. R. Heal), 1092
- Eruption of 1902 and their Significance to Ordovician Welded Tuffs of County Mayo, Eire: Observations on the Mont Pelée (Dr. J. McManus), 578
- Erythrocytes and Ascites Tumour Cells: Enzyme Regulatory Mechanisms at the Level of Lactate-oxidoreductase in (S. Rapoport et al.), 185
- Erythrocytes: Stable Messenger RNA in Nucleated (Dr. R. B. Scott and R. A. Malt), 497
- Erythrocytic Enzymes as a Function of their Age: Altered Electrophoretic Mobilities of some (Dr. H. Walter et al.), 76
- Erythroid Cells in Thalassemia: Apoferritin Synthesis in Human (Dr. E. H. Eylar and Dr. G. Matelli), 661
- Escherichia coli* and the Assimilation of Glucose: Maintenance of (S. B. McGrew and Prof. M. F. Mallerie), 1096
- Escherichia coli* carrying Episome 'R': Chloramphenicol-, Dihydrostreptomycin-, and Kanamycin-inactivating Enzymes from Multiple Drug-resistant (S. Okamoto and Y. Suzuki), 1301
- Escherichia coli* during Inhibition by Azetidine-2-carboxylic Acid: Synthesis of Ribonucleic Acid by (Dr. J. M. Midgley and Dr. D. G. Wild), 585
- Escherichia coli* Endotoxin or infected with *Mycobacterium tuberculosis*: Interferon Appearance stimulated by Endotoxin, Bacteria, or Viruses in Mice pretreated with (Prof. J. S. Youngner and Prof. W. R. Stinebring), 456
- Escherichia coli* Extracts: Enzymatic Formation of β -Cyanoalanine from Cyanide by (Dr. P. M. Dunnill and Prof. L. Fowden), 1206
- Escherichia coli*: Interference by the Carcinogenic 4-Nitro-quinoline N-Oxide of Tryptophan and Indole Uptake in (A. C. Zahalsky and Prof. S. L. Marcus), 296
- Esperanto Congress: International, 1264
- Essays in Biochemistry: Vol. 1 (edited by P. N. Campbell and G. D. Greville), review by Dr. D. T. Elmore, 316
- Estrada-Parra, Dr. S., Schmil, A., and Martinez, R.-M.: Reaction of Adenine-specific Antibodies with Denatured Deoxyribonucleic Acid, 1010
- Etch Pits in Ice Crystals: Pairs of Spiral (J. Muguruma), 180
- Ethiopian Cainozoic Volcanic Succession: Re-classification of the (P. A. Mohr), 177
- Ethiopian Rift System: Coincidence of Magnetic Disturbances with Local Earthquakes recorded from the (Prof. P. Gounin), 541
- Ethology and the 'Baconian' Method (Dr. W. P. D. Wightman), 918
- Ethyl-chlorophenoxy-isobutyrate in the Rat: Nature of the Hepatomegalic Effect produced by (Dr. R. Hess et al.), 856
- N-Ethyl-N-nitrosourea: Induction of Intestinal Tumours by (Dr. R. Schoental), 300
- Ethylene in *Penicillium digitatum*: Biogenesis of (Dr. B. A. Sprayberry et al.), 1322
- Eugenol as Virus Inhibitors: Derivatives of 2-Aminoethyl-guanidine and of a (S. Pácsa et al.), 409
- Europaeva: Flora (edited by T. G. Tutin et al.), Vol. 1, review by Prof. J. A. Nannfeldt, 8
- European-American Committee on Reactor Physics, 1259
- European Brewery Convention, Report of the Barley Committee: Trials 1963, review by A. H. Cool, 9
- European Health Services: Comparison of, 438
- European Society for Comparative Endocrinology, Officers and Council, 436
- Eutectic: Interconnection of Silicon in Modified Aluminium-Silicon (J. A. E. Bell and Prof. W. C. Wiregard), 177
- Euterpe acutifrons* (Dana), in the Laboratory: Breeding a Pelagic Copepod, (H. W. Neunes and G.-F. Pongolini), 571
- Evaluation of Ion Exchange Cartridges for Field Sampling of Iodine-131 in Milk (R. H. Johnson, jun., and T. C. Reavey), 750
- Evaluation of Quality in Poultry Meat: Objective (Dr. A. W. Khan), 204
- Evans, D., Osborn, J. A., Jurdine, F. H., Wilkinson, Prof. G.: Homogeneous Hydrogenation and Hydroformylation using Ruthenium Complexes, 1203
- Evans, Prof. H. J.: Appointment to Chair of Genetics in the University of Aberdeen, 833

- Evans, Dr. I. Antice, and Mason, J.: Carcinogenic Activity of Bracken, 913
- Evaporation Studies: Water Balance and (M. R. Rao), 776
- Evidence for Microfossils in the Alais and Orgueil Carbonaceous Meteorites (S. L. Van Landingham), 947
- Evidence for a Second 'Structural' Locus determining Human Phosphoglucose mutase (Dr. D. A. Hopkinson and Prof. H. Harris), 410
- Evidence for Structures resembling Crystalline Carbons at the Active Centre of Enzymes (Dr. E. D. Wachsmuth), 942
- Evolution: An Exercise in Analogy: Biological and Mental (Arthur Koestler), 1033
- Arginine and Urea Biosynthesis in the Land Planarian: its Significance in Biochemical (Prof. J. W. Campbell), 1299
- Fossil Evidence for Human (Sir Wilfrid E. Le Gros Clark), Second Edition, review by Dr. W. E. Swinton, 1140
- Evolution in the Biosphere (Prof. H. F. Blum), 324
- Evolution of Animal Communication: Mimicry and the (Dr. W. Wickler), 519
- Evolution of Intelligent Behaviour: A General Theory for the (Dr. D. Stenhouse), 815
- Evolution of the Bristol Channel (Dr. F. J. North), Third Edition, review by Dr. A. R. MacGregor, 110
- Evolutionary Tendency of Relative Growth in Arthropods and Man (Dr. R. Matsuda), 1228
- Ewes: Undernutrition and Early Embryonic Mortality in Merino (T. N. Edey), 1232
- Ewing, Dr. M., and Conolly, Dr. J. R.: Pleistocene Glacial-marine Zones in North Atlantic Deep-sea Sediments, 135
- Examination of a Polysaccharide Flocculant and Flocculated Kaolinite by Electron Microscopy (Dr. A. Audsley and A. Fursey), 753
- Exchangeable Aluminium in Acid Soils: Measurement of (J. B. Skeen and Dr. M. E. Sumner), 712
- Excitation and Inhibition in the Cerebellar Cortex: Cholinergic (P. Kása and Dr. B. Csillik), 695
- Excitation and Inhibition in Single Mechanoreceptors (Pacini Corpuscles): Processes of (Dr. O. B. Ilyinsky), 351
- Excited State of Polycyclic Aromatic Hydrocarbons with the Ionization Potential: Correlation of the Third (Dr. M. A. Slifkin), 380
- Excretion in Single Nephrons of the Kidney of the Rat: Effect of 'Furosemid' on Chloride and Water (G. Malnic et al.), 80
- Excretion of Cattle fed on High-energy Diets: Phosphorus (W. D. C. Reed et al.), 953
- Exhaustion in the Rat: Effect of Infantile Treadmill Experience on Body-weight and Resistance to (R. A. Levitt and Prof. W. B. Webb), 1128
- Experimental Data of the Effective Ion Mass in the Upper Ionosphere: Comparison of, with a Theoretical Structure (P. R. Arendt), 443
- Experimental Observations on the Structure of Collisionless Shock Waves in a Magnetized Plasma (Dr. J. W. M. Paul, L. S. Holmes, Dr. M. J. Parkinson and J. Sheffield), 133
- Experimental Pedology, Proceedings of 11th Easter School in Agricultural Science, 1964, (edited by E. G. Hallsworth and D. V. Crawford), review by Dr. A. M. Smith, 718
- Experiments in Behaviour Therapy (edited by Prof. H. J. Eysenck), review by Dr. B. Semeonoff, 1027
- Extracranial Cobalt: Epileptogenic Effect of Extracranial and (H. Payan et al.), 792
- Extracranial and Extracranial Cobalt: Epileptogenic Effect of (H. Payan et al.), 792
- Eyde, A. J., and Parrela, H. C.: Electric Potentials generated by Freezing Dilute Aqueous Solutions, 33
- Eye: Development of the (G. V. Lopashov and O. G. Stroeve), review by Dr. R. A. Weale, 979
- Eylar, Dr. E. H., and Matoli, Dr. G.: Apoferritin Synthesis in Human Erythroid Cells in Thalassemia, 661
- Eysenck, Prof. H. J. (edited by): Experiments in Behaviour Therapy, review by Dr. B. Semeonoff, 1027
- Eysenck, Prof. H. J., and Rachman, Dr. S.: Causes and Cures of Neurosis, review by Dr. B. Semeonoff, 1027
- FABIANEK, Prof. J., Herp, Dr. A., and Martinez, Dr. L.: Acid Mucopolysaccharide and Hexosamine Metabolism in Dermal Connective Tissue of Adrenalectomized Rats, 1212**
- Fabro, S., Smith, R. L., and Williams, Prof. R. T.: Thalidomide as a Possible Biological Acylating Agent, 1208
- Facilitation as a Function of Temporal Spacing of Stimuli in Intracranial Self-stimulation (Prof. J. A. Deutsch and R. Collins), 592
- Factor associated with Drug Resistance in *Salmonella typhimurium*: Characterization of a Transfer (Dr. E. S. Anderson and Dr. M. J. Lewis), 843
- Factor produced in vitro: A Lymphocyte-stimulating (Dr. J. Gordon and Prof. L. D. MacLean), 795
- Factor Stimulating DNA Synthesis derived from the Medium of Leucocyte Cultures (Dr. S. Kasakura and Prof. L. Lowenstein), 794
- Factors affecting the Amount and Composition of the Serum Seromucoid Fraction (Dr. A. J. Anderson), 491
- Failure of the Zona Reaction in Five Pig Eggs (Prof. P. J. Dziuk and Z. Dickmann), 502
- Faiman, Dr. C.: Fasting Levels of Growth Hormone in Men and Women, 687
- Faith and Society: Science, (Prof. M. Polanyi), review, 985
- Fall-out in London Rain-water during 1964 (Dr. R. Wood, J. R. W. Kerr and L. A. Richards), 1083
- Fallout, Soil, Plants, Foods, Man: Radioactive (edited by E. B. Fowler), review, 218
- Families: Prisoners and their (Mrs. P. Morris), review by Prof. D. C. Marsh, 216
- Far-infrared Spectra of some Square-planar Ions (Dr. D. M. Adams and D. M. Morris), 283
- Farrar, Prof. J. L., and Kennedy, Prof. R. W.: Induction of Tension Wood with the Anti-auxin 2,3,5-Tri-iodobenzoic acid, 406
- Fasciola hepatica: A Comparison of Low and High Infection Rates: Experimental Infections of Cattle with (J. G. Ross), 907
- Fast Neutrons: Increased Sensitivity of *in vitro* Murine Leukaemia Cells to Fractionated X-rays and (Dr. W. L. Caldwell et al.), 168
- Fast Reactor Agreement, 1152
- Fasting Levels of Growth Hormone in Men and Women (Dr. C. Faiman), 687
- Fat Bodies of the Cockroach, *Leucophaea maderae*: Guanase Activity of the Symbionts and (Dr. L. L. Pierre), 666
- Fat: Use of Polyethylene Glycol in Investigations of Absorption of (Dr. H. Vavrínková and Dr. A. Krondl), 293
- S/N Fatigue Curve of a Stainless Steel: Effect of Temperature on the Discontinuity in the (T. R. G. Williams and C. R. Shurmer), 379
- Fatt, Prof. I., and Mandell, Dr. R. B.: Thinning of the Human Cornea on Awakening, 292
- Fatty Acids in the Mullet *Mugil cephalus*: Occurrence of Odd-numbered (Dr. R. G. Ackman), 1213
- Fatty Infiltration in the Liver: Effect of Glucose Cycloacetate in the Prevention of (Prof. N. Nath et al.), 1001
- Fauna: Selective Effect of Particulate Insecticides on *Simulium* among Stream (Prof. W. E. Kershaw et al.), 199
- F-Centres: Evidence for Catalytic Activity of (Prof. J. G. Aston et al.), 181
- Fearnley, G. R.: Fibrinolysis, review by Dr. D. Ogston, 1247
- Fechheimer, Prof. N. S., et al.: Measurements of Bovine Chromosomes, 97
- Feeding and Reproduction of Rats fed Calcium Cyclamate (P. O. Nees and P. H. Dorse), 81
- Feeding Mechanism of Blood-sucking Arthropods (M. M. J. Lavoipierre), 302
- Feigen, Prof. G. A., et al.: Presynaptic Effects of Potassium Ion on the Mammalian Neuromuscular Junction, 590
- Feldberg Foundation Award, 1263
- Fell, Prof. H. Barradough, and Philip, Dr. G. M.: Ancestry of Sea-stars, 766
- Fellowship: New Shell Research, 1263
- Fellowships: Bullard Forest Research, 631
- South-East Asia Treaty Organization: Research, 1263
- Female: Comparison of Salivary Amylase-levels in the Human Male and (Prof. R. L. McGeachin et al.), 1211
- Female Reproductive Tract: Oxidative and Biosynthetic Utilization by Human Spermatzoa of a Metabolite of the (Prof. C. Turner), 1115
- Ferguson, J. M., and Wood, Dr. G. C.: Metal Concentration Profiles in Oxide Scales on Nickel-10.9 per cent Cobalt Alloy, 369
- Ferredoxin and Rubredoxin: Nature of the Non-haem Iron in (Dr. R. D. Gillard et al.), 769
- Ferris, Lieut.-Commander T. G., Easterling, Robert E., and Budd, R. E.: Quantitative Determination of Haemoglobin A₂ by Acrylamide-gel Electrophoresis, 1103
- Ferrites: Re-entrant Hysteresis Loop and Multistable (Dr. R. M. Glaister and Dr. E. W. Lee), 67
- Fertilizer Corporation of India, Ltd., 734
- Feulgen-positive Particles: Emission of, during the *in vitro* Maturation of Toad Oocytes (Prof. J. Brachet), 596
- Fibrinogen ('Fibrinogen Baltimore'): New Inherited Coagulation Disorder caused by an Abnormal (Dr. E. A. Beck et al.), 143
- Fibrinolysis (G. R. Fearnley), review by Dr. D. Ogston, 1247
- Fibrous Sulphur: Microstructure of (A. Jaskolska and A. E. B. Presland), 1088
- Field Sampling of Iodine-131 in Milk: Evaluation of Ion Exchange Cartridges for (R. H. Johnson, jun., and T. C. Reavey), 750
- Fieldier, Dr. G.: Lunar and Planetary Surface Conditions, review, 961
- Filamentary Nebulosity in the Vicinity of the Cetus Arc (Dr. J. Meaburn), 575
- Films on Valve Metals: A.C. Resistivity of Anodic Oxide (Dr. G. C. Wood and C. Pearson), 547
- Fincham, Prof. J. R. S.: Microbial and Molecular Genetics, review by Dr. S. W. Glover, 1029
- Fincham, Dr. W. F., and Priban, Dr. I. P.: Self-adaptive Control and the Respiratory System, 339
- Findenegg, G., and Broda, Prof. E.: Mechanism of Uptake of Trace Elements by Plant Roots, 196
- Findlay, Prof. A.: A Hundred Years of Chemistry, Third Edition revised by Dr. T. I. Williams, review by Dr. R. G. Partington, 969
- Finds in the Upper Carboniferous of South-West England and their Significance: Recent (Dr. H. G. Reading); (Dr. J. E. Prentice), 745
- Fine Structure of Wood (Dr. P. Rudman), 55
- Fingerprinting Haemoglobin: Modification System for (Dr. G. Efremov), 298
- Finkelburg, Prof. W.: Einführung in die Atomphysik, review by Dr. E. Bretscher, 217
- Finney, Prof. D. J.: Probability and Statistics, review, 217
- Fiore-Donati, Dr. L., et al.: Lymphatic Neoplastic Transformation of Thymus in Mice with Myeloid Virus-induced Leukaemia, 697
- Fiore-Donati, Dr. L., Chieco-Bianchi, L., Tridante, G., Mazzarella, L.: Induction of Malignant Lymphomas by Urethane in Adult Mice bearing Syngeneic Thymus Grafts, 398
- Firbas, F., et al. (rewritten by): Strasburger's Textbook of Botany, review by Dr. A. Allsopp, 618
- Firth, Dr. I. M.: Modern Low-temperature Calorimetry, 1162
- Fish as Food (edited by Dr. G. Borgstrom), Vol. 3 and Vol. 4, Processing, review by Dr. G. R. Wadsworth, 317
- Fish Epidermis: Presumed Sensory Cells in (Dr. M. Whitear), 703
- Fish from Thurso, Scotland: An Unusually Radioactive Fossil (W. R. Diggle and J. Saxon), 400
- Fishbein, Dr. W. N., Carbone, Paul P., and Hochstein, H. D.: Acetohydroxamate: Bacterial Urease Inhibitor with Therapeutic Potential in Hyperammonaemic States, 46
- Fishenden, R. M.: *Obituary* of Mr. K. E. B. Jay, 625
- Fisher, Dr. I. P., and Heath, Dr. G. A.: Dissociation Energy in the N-H Bond in Hydrazine, 1199
- Fishes: A Draught of (Dr. F. D. Ommann), review by Dr. L. Harrison Matthews, 517
- Fishwick, Prof. W.: Semiconductor Controlled Rectifiers, review, 719
- Fisk, Sir Ernest Thomas: *Obituary* by Sir Lionel Hooke, 526
- Fission Theory of their Origin: Angular Momenta of Eclipsing Binaries and the (I. W. Roxburgh), 65
- Fitch, F. J., and Miller, Dr. J. A.: Age of the Weardale Granite, 743
- Fitzmaurice, C., et al.: The Iron Dextran Complex, 237
- Five-fold Symmetry: Dense Packing of Hard Spheres with (B. G. Bagley), 674
- Fixation of Metabolic Products in the Soil during Decomposition of Carbohydrates (H. Sørensen), 97
- Flagellation of Gram-negative Rods: Effect of Incubation Temperature on the (D. A. Orr and Dr. M. M. Taylor), 1017
- Flame Spectroscopy (Dr. R. Mavrodineanu and Dr. H. Boiteux), review by Dr. D. T. Lewis, 5
- Flames: Methane-rich Perchloric Acid (Dr. G. S. Pearson), 283
- Fleming, C. E.: Ergonomics: Man in his Working Environment, review, 106
- Social and Industrial Gerontology in Great Britain, 623
- Fleming, Dr. R. J., and Kerr, R. M.: Thermoluminescence and Ultra-violet Phosphorescence of the Nucleic Acid Bases, 1198
- Fletcher, B. N.: Holothurian Sclerites from the Speeton Clay, 281
- Fletcher, E. C., et al.: Carbon Dioxide Production of Whole Blood *in vitro*, 82
- Flewett, Dr. T. H., et al.: Inhibition of Influenza Virus Haemagglutination by Polymerized Orosomucoid, 915
- Flocculant and Flocculated Kaolinite by Electron Microscopy: Examination of a Polysaccharide (Dr. A. Audsley and A. Fursey), 753
- Flocculated Kaolinite by Electron Microscopy: Examination of a Polysaccharide Flocculant and (Dr. A. Audsley and A. Fursey), 753
- Flora Europaea (edited by T. G. Tutin, V. H. Heywood, N. A. Burges, D. H. Valentine, S. M. Walters and D. A. Webb assisted by P. W. Ball and A. O. Chater), Vol. 1: Lycopodiaceae to Plantaginaceae, review by Prof. J. A. Nannfeldt, 8
- Flory, Lord: Anniversary Address to the Royal Society on November 30th, 1965, 1141

- Floss, Dr. H. G., Hadwiger, Dr. L., and Conn, Prof. E. E.: Enzymatic Formation of β -Cyanoalanine from Cyanide, 1207
- Flour: Proteins of Wheat (Dr. C. B. Coulson and Dr. A. K. Sim), 583
- Flow in Open Channels: Laminar and Turbulent (Dr. J. Allen), 1196
- Flow Resistance: Ascent of Sap in Trees: Effect of Negative Absolute Pressure on (Prof. B. Johnson and Prof. J. R. Dixon), 1347
- Flowering Plants of West Africa: Introduction to the (Mrs. M. Steentoft Nielsen), review by F. N. Hepper, 976
- Flowering Plants: Shade Tolerance in (Dr. J. P. Grime), 161
- Fluid Dynamics: Research Frontiers in (edited by Dr. R. J. Seeger and Dr. G. Temple), review by Prof. K. Stewartson, 967
- Fluorescence from Organic Crystals: Delayed (G. F. Moore and Dr. I. H. Munro), 772
- Fluorescent Antibody Method: A New (Prof. E. H. Beutner et al.), 353
- Fluorescent Pseudomonads: Formation of Protocatechuic from Quinic Acid by (Dr. D. J. Stewart), 200
- Fluoroacetamide: Isolation of Bacteria able to metabolize Fluoroacetate or (Dr. M. Kelly), 809
- Fluoroacetate or Fluoroacetamide: Isolation of Bacteria able to metabolize (Dr. M. Kelly), 809
- Fluorochrome Stains for Histological Diagnosis of Visceral Mycoses (G. Bader, D. Stiller and K. Ruffert), 796
- Fluxes of Chloride Ions across Artificial Membranes: Effect of *Trans*-Solute on the (S. Kitahara et al.), 187
- Flynn, L. R., and Hall, R. J.: Heterogeneity of Titan Yellow demonstrated by Thin-layer Chromatography, 1202
- Fodder Resources in India: Grassland and (R. O. Whyte), Revised Edition, review by Dr. W. Davies, 821
- Foley, Dr. T. H., et al.: Evidence for the Release of Individual Amino-acids from the Resting Human Forearm, 588
- Food: Fish as (edited by Dr. G. Borgstrom), Vols. 3 and 4, review by Dr. G. R. Wadsworth, 317
- Food Poisoning, 630
- Food Technology: Dictionary of Nutrition and (Dr. A. E. Bender), Second Edition, review by A. M. Copping, 987
- Foods: Radiation Preservation of, review by G. Birch, 973
- Foot-pad Cells of *Sarcophaga bullata*: Differential Deoxyribonucleic Acid Replication in the Giant (Dr. J. M. Whitten), 1019
- Foraminiferal Biostratigraphy of the Neogene: Progress in the Planktonic (Dr. F. T. Banner and Dr. W. H. Blow), 1164
- Force of the Isometric Reflex Response of Normal Human Subjects: Relationship between the Electromyogram and the (A. M. Clarke), 551
- Forearm: Evidence for the Release of Individual Amino-acids from the Resting Human (Dr. D. R. London et al.), 588
- Forecasts for Britain: Energy, 229
- Forest Hydrology (J. C. Rodda), 1157
- Forest: Production Ecology in Tropical High, 22
- Forest Research Fellowships: Bullard, 631
- Formaldehyde: Nitroso (I. M. Napier and Prof. R. G. W. Norrish), 1090
- Formation of Amines in the Gastric Mucosa of the Rat (R. Håkanson), 793
- Formation of Complexes between Basic Proteins of Leucocytes and Plasma Globulins (Dr. W. B. Jolley and H. W. Allen), 390
- Formation of β -Cyanoalanine from Cyanide: Enzymatic (Dr. H. G. Floss et al.), 1207
- Formation of β -Cyanoalanine from Cyanide by *Escherichia coli* Extracts: Enzymatic (Dr. P. M. Dunnill and Prof. L. Fowden), 1206
- Formation of Hydroxamates during Proteolysis by an Enzyme System from Yeast (Dr. D. E. Briggs and Dr. D. J. Milin), 287
- Formation of Hydroxyl Molecules in Interstellar Space (Dr. J. L. Symonds), 1195
- Formation of Protocatechuic from Quinic Acid by Fluorescent Pseudomonads (Dr. D. J. Stewart), 200
- Formation of the Monoacetylacetonato-Iron (III) Complex: Kinetics of (W. K. Ong and Dr. R. H. Prince), 1201
- Form-similarity between Phosphenes of Adults and pre-School Children's Scribbles (R. Kellogg, Prof. M. Knoll and Dr. J. Kugler), 1129
- Forss, D. A., and Stark, W.: *n*-Alkan-1-ols in Butter after Oxidative Deterioration, 190
- Fortes, Prof. M.: A Hundred Years of Anthropology, Third Edition, revised, review, 215
- Fosberg, Dr. F. R. (edited by) Man's Place in the Island Ecosystem, a Symposium, review by Sir Dudley Stamp, 109
- Fossil Evidence for Human Evolution (Sir Wilfrid E. Le Gros Clark), Second Edition, review by Dr. W. E. Swinton, 1140
- Fossil Fish from Thurso, Scotland: An Unusually Radioactive (W. R. Diggie and J. Saxon), 400
- Fossil 'Zoophycos': Origin of (Dr. M. Plička), 579
- Fossilifères: Les Temps (Prof. H. Termier et G. Termier), Vol. 1, Paléozoïque Inférieur, review by Prof. A. Williams, 614
- Foster, J. R., et al.: Inhibition of Influenza Virus Haemagglutination by Polymerized Orosomucoid, 915
- Foster, W. D.: History of Parasitology, review by Dr. G. Lapage, 109
- Foster-mothers' Strain and Pre-natal Experience on Adult Behaviour in Rats: Effect of (Dr. J. M. Joffe), 815
- Fouling: *Sargassum* Tannin, an Antibiotic which retards (Prof. J. McN. Sleiburth and Prof. J. T. Conover), 52
- Foundation Grants for 1966: Lalor, 436
- Foundations: Soils and, 1153
- Fourier Theory: Treatise on Trigonometric Series, review by Prof. T. A. A. Broadbent, 106
- Fowden, Prof. L., and Dunnill, Dr. P. M.: Enzymatic Formation of β -Cyanoalanine from Cyanide by *Escherichia coli* Extracts, 1206
- Fowler, E. B. (edited by): Radioactive Fallout, Soils, Plants, Foods, Man, review, 218
- Fowles, Prof. G. W.: Appointed Professor of Inorganic Chemistry in the University of Reading, 20
- Foxon, Prof. G. E. H., and Bishop, I. R.: A Visit to the Lower Amazon, 114
- Fractionated X-rays and Fast Neutrons: Increased Sensitivity of *in vitro* Murine Leukaemia Cells to (Dr. W. L. Caldwell et al.), 168
- Fractionation of Mouse Deoxyribonucleic Acid on Hydroxyapatite (Dr. P. M. B. Walker and A. McLaren), 1175
- Fractionation of Ribonucleic Acids in the Zonal Ultracentrifuge (Dr. J. R. B. Hastings, Dr. J. H. Parish, Dr. K. S. Kirby and E. S. Klucis), 645
- Fractions and Their Generalizations to Problems in Approximation Theory: Application of Continued (A. N. Khovanskii), review by I. M. Khabaza, 318
- Fragmentation of Nitrophenylhydrazones: Mass Spectrometric (Prof. C. Djerassi and S. D. Sample), 1314
- Fraisse, Prof. Paul: Psychology of Time, review by Dr. W. Gooddy, 822
- Frame, Dr. John, and Smith, Dr. J. W.: Electron Microscope Observations on the Free Protein and the Protein-polysaccharide Complex of Bovine Synovial Fluid, 867
- France and Western Germany: Comparative Study of Housing in Britain, 233
- France: Industrial Research and Development in, 834
- Science in, 733
- Francisco, Juanita R., et al.: Altered Electrophoretic Mobilities of some Erythrocytic Enzymes as a Function of their Age, 76
- Franklin, J. C., et al.: Irradiation Embrittlement of Low-boron Type 304 Stainless Steel, 73
- Frazer, V., and Reiser, A.: Ultra-violet Absorption Spectra of Aromatic Nitrenes and Dinitrenes, 682
- Free Protein and the Protein polysaccharide Complex of Bovine Synovial Fluid: Electron Microscope Observations on the (Dr. J. W. Smith and Dr. J. Frame), 867
- Freeguard, Dr. G. F.: Education and Application of the Materials Scientist, 939
- Freeing of Amino-acids from Proteins (Dr. L. J. Hastewell and R. N. Priestland), 387
- Free, J., and Galwey, Dr. A. K.: A Preparation of Cementite, 183
- Freele, H., et al.: A New Active Metabolite of 'Miracil' D, 1005
- Freeman, Dr. David H.: Appointment to the Analytical Chemistry Division of the Institute for Materials Research at the National Bureau of Standards, 1259
- Freeman, Prof. G. R., and Talocs, J. A.: Relative Ionization Efficiencies of Hydrocarbon Molecules as a Function of Bombarding Electron Energy, 996
- Freeze-fixation, a New Method for Electron Microscopy (Prof. O. Nilsson), 90
- Freezing Dilute Aqueous Solutions: Electric Potentials Generated by (H. C. Ferreira and A. J. Eyd), 33
- Frege, G.: Basic Laws of Arithmetic, review by Prof. T. A. A. Broadbent, 318
- Fremlin, Dr. J. H.; Worrell, Dr. F. T.: Calculations with Two Nearly Equal Quantities, 1306
- French, C. N.: Tactile-vision: Thermal and Texture Cues in the Discrimination of Black and White, 352
- French Oceanography, 1261
- Frester, Prof. John H.: Co-relation of the Binding to DNA Loops or to DNA Helices with the Effect on RNA Synthesis, 1093
- Localized Strand Separations within Deoxyribonucleic Acid during Selective Transcription, 894
- Frequency and Time Services: U.S. Standard (Dr. R. L. Smith-Rose), 25
- Frequency Limits detectable in the Auditory System: Neural Volleying: Upper (Capt. J. C. Boudreau), 1237
- Frequency of Functioning Gametes in Monosomics: Estimation of the (A. K. R. Choudhury and J. G. Showal), 917
- Frequency Shift and Internal Stress in Gamma-ray Irradiated Sodium Chlorate: Pure Quadrupole Resonance (Dr. H. Budak), 382
- Frequency Spectrum of Doppler-shifted Whistler-mode Signals (R. A. Morris and N. Poletti), 479
- Freshwater Life of the British Isles (John Clegg), Third Edition, review by W. J. Rees, 988
- Freudenthal, Prof. H.: Obituary of Prof. E. J. Dijksterhuis, 1258
- Probability and Statistics, review by Prof. D. J. Finney, 217
- Frey, Dr. H. M.: Appointment as Professor of Physical Chemistry in the University of Reading, 20
- Friedlander, Dr. G., Kennedy, Prof. J. W., and Miller, Prof. J. M.: Nuclear and Radiochemistry, Second Edition, review by Dr. H. A. C. McKay, 105
- Friend, J. A., Gutmann, F., and Hayes, J. W. (edited by): Proceedings of First Australian Conference on Electrochemistry, 1963, review by Dr. R. F. Phillips and N. A. Hampson, 104
- Friesen, Dr. Henry: Further Purification and Characterization of a Placental Protein with Immunological Similarity to Human Growth Hormone, 1214
- Frog Skin *in vitro*: Effects of Cyclic Adenosine Monophosphate and Melanocyte-stimulating Hormone on (Dr. M. W. Bitensky and S. R. Burstein), 1282
- Frolich, E., and Wallace, Prof. A.: Phosphorus-deficiency Symptoms in Tobacco and Transpirational Water Loss, 1231
- Frontal Ganglion and Protein Starvation in *Locusta migratoria* L.: Relationship between the Removal of the (Dr. K. U. Clarke and C. Gillott), 808
- Frost, Prof. A. D., et al.: New Limits to the Angular Sizes of Some Quasars, 275
- Frost, S., et al.: Selective Effect of Particulate Insecticides on *Simulium* among Stream Fauna, 199
- Frost Resistance in One-year-old Pear Trees: Effects of (2-Chloroethyl)-trimethyl ammonium Chloride and Gibberellic Acid on Growth, Fruit Bud Formation and (Dr. I. Modlibowska), 503
- Fructose-1-phosphate in Tissues of Higher Plants: Occurrence of (D. Graham and Dr. T. ap Rees), 38
- Fruit Bud Formation and Frost Resistance in One-year-old Pear Trees: Effects of (2-Chloroethyl)-trimethylammonium Chloride and Gibberellic Acid on Growth, (Dr. I. Modlibowska), 503
- Fruit-tree Rootstocks: Increase during Winter in Capacity for Root Regeneration in Detached Shoots of (Dr. B. H. Howard), 912
- Fuel Policy for Britain, 24
- Fujimori, E., and Tavia, M.: Interaction between Chlorophyll and Hydroquinone, 78
- Fuller, L., et al.: NMN-Pyrophosphorylase Activity in Ascites Cell Extracts, 347
- Function of Hassall's Corpuscles: A Phagocytic (Dr. J. N. Blau), 564
- Functional Significance of Sweat Glands and Sebaceous Glands in Seals (J. K. Ling), 560
- Functional Similarities between the Cytoplasmic Organelles of Melanocytes and the Mitochondria of Hepatocytes (Dr. M. H. Van Woert, A. R. Nicholson and Dr. G. C. Cotzias), 810
- Fundamentals of Information Retrieval: Some (J. R. Sharp), review by P. C. Williams, 822
- Fungi: Seed-borne (J. P. Malone and A. E. Muskett), review by R. W. G. Dennis, 319
- Fungicides: Identification of Dinitro-octylphenols in Certain Commercial (Dr. A. H. M. Kirby and L. D. Hunter), 189
- Funkhouser, A., et al.: Reconstruction of Phase Objects by Holography, 1159
- Funktionentheorie (A. Hurvitz and R. Courant), Fourth Edition (H. Rohrl), review by Dr. J. C. E. Kirkill, 963
- Funnell, H. S., and Oliver, Prof. W. T.: Proposed Physiological Function for Plasma Cholinesterase, 689
- 'Furosemid' on Chloride and Water Excretion in Single Nephrons of the Kidney of the Rat: Effect of (G. Malnic, F. L. Vieira and H. Enokibara), 80
- Fursey, Anita, and Audsley, Dr. A.: Examination of a Polysaccharide Flocculant and Flocculated Kaolinite by Electron Microscopy, 753
- Furunculosis in Impounded Adult Salmon: Control of (A. C. Leaman), 1344
- Fusion Curves of Zinc, Aluminium, Germanium and Silicon to 60 kilobars (J. Lees and B. H. J. Williamson), 278
- Fuson, R. C., et al.: Systematic Identification of Organic Compounds, Fifth Edition, review by Dr. D. T. Lewis, 1032
- Fussell, G. E.: Dutch Study Circle for Agricultural History, 1939-64, review, 1138
- Fyfe, Prof. W. S.: Appointment to Royal Society Research Professorship, 527

- GABOR, Prof. D.: Character Recognition by Holography, 422
Science in Hungary, review, 983
- Gabor, Prof. D., Stroke, Prof. G. W., Brumm, D., Funkhouser, A., and Labeyrie, A.: Reconstruction of Phase Objects by Holography, 1159
- Gabrielsen, A. E., and Good, R. A. (edited by): The Thymus in Immunobiology, review by Dr. R. B. Taylor, 318
- (*Gadus morhua*): Haemoglobin Polymorphism in the Cod (M. C. Rattazzi and C. Pék), 489
- Gage, Dr. P. W., and Hubbard, Dr. J. I.: Evidence for a Poisson Distribution of Miniature Endplate Potentials and some Implications, 395
Gairdner Foundation Awards, 530
- Galactans from Normal Bovine Lung and from *Mycoplasma mycoides*: Serological Relationship between (Dr. M. Shifrine and R. N. Gourlay), 498
- Galactic Centre Region: Radio Structure of the (Dr. D. Downes *et al.*), 1189
- Galactic Latitudes: Structure of the Radio Continuum Background at High (Dr. M. J. S. Quigley and Dr. C. G. T. Haslam), 741
- Galactose-fed Rats: Agar-gel Electrophoresis of Soluble Lens Proteins in (E. Wittgenstein and Dr. K. W. Rowe, jun.), 386
- Galaxies: Hydrogen Content of Virgo Cluster (Dr. B. J. Robinson and J. A. Koehler), 993
- Galaxies: Radio Observations of Spiral (Prof. B. Y. Mills and J. R. Glanfield), 10
- Gall, L. C., *et al.*: Human Indigenous Microflora in Gnotobiotic Rats, 1236
- Galls of *Salix fragilis* L.: Red Pigment in Leaf (G. Blunden and Dr. S. B. Challen), 388
- Galwey, Dr. A. K., and Freil, J.: A Preparation of Cementite, 183
- Gametes in Monosomics: Estimation of the Frequency of Functioning (A. K. R. Choudhury and J. G. Bhowal), 917
- Gametogenesis of *Plasmodium vinckei* (Dr. J. Bafort, I. H. Vincke and G. Timperman), 1230
- Gamma-radiation on Different Cholinesterase Preparations: *In vitro* Effect of (Dr. R. E. Catalan and Dr. A. M. Municio), 1227
- Gamma-ray Irradiated Sodium Chlorate: Pure Quadrupole Resonance Frequency Shift and Internal Stress in (Dr. H. Budak), 382
- Gamow, Prof. George: A Planet Called Earth, review by Patrick Moore, 961
- Ganguin, K. O.: Mitteilungen aus den Forschungslaboratorien der Agfa-Gevaert AG Leverkusen-München, Band IV, review, 720
- Garattini, Prof. S., and Valzelli, Prof. L.: Serotonin, review by Dr. G. B. West, 1247
- Gardner, F. F., *et al.*: Anomalous Intensity Ratios of the Interstellar Lines of OH in Absorption and Emission, 1193
- Gardner, Dr. Joan F., and Rubbo, Prof. S. D.: A Review of Sterilization and Disinfection as Applied to Medical, Industrial and Laboratory Practice, review by R. Hare, 1249
- Garfield, E. (edited by): Science Citation Index 1964 Annual Cumulation, Eight Parts, review by C. W. Cleverdon, 717
- Garner, Prof. C. S., and House, Dr. D. A.: Diperoxodiethylenetriammine-chromium(IV) 1-hydrate: A New Chromium(IV) Peroxo Compound, 776
- Garnham, Prof. P. C. C.: Second International Conference on Protozoology, 330
- Gas Bubbles and Liquid Drops in an Electrically Stressed Insulating Liquid: Deformation of (Dr. K. C. Kao), 279
- Gas Chromatography: Determination of Nicotine in Smokers' Urine by (Dr. N. L. McNiven *et al.*), 788
- Gas Chromatography: Determination of p.p.m. Concentrations of Carbon Disulphide in Benzene by Electron Capture (D. W. Grant and G. A. Vaughan), 75
- Gas Chromatography of Amino-acids as Trimethylsilyl Derivatives: Quantitative (Prof. E. D. Smith and H. Sheppard, jun.), 878
- Gassel, Dr. M. M., and Diamantopoulos, Dr. E.: Nerve Potential Recordings during electrically and mechanically evoked Monosynaptic Reflexes in Man, 1004
- Gasteropod *Berthelina limax* from the Indian Ocean: Record of the Bivalve (K. P. Rao), 404
- Gastric Mucosa of the Rat: Formation of Amines in the (R. Håkanson), 793
- Gastrin on Isolated Smooth Muscle Preparations: Effect of (Dr. A. Bennett), 170
- Gastrocnemius: Hormonal Control of Lipid Concentration in Rat Heart and (R. M. Denton and Prof. P. J. Randle), 488
- Gates Clarke, Dr. J. F.: Catalogue of Type Specimens of Microlepidoptera in the British Museum (Natural History) described by Edward Meyrick, review by Prof. G. C. Varley, 108
- Gauldie, J., *et al.*: Acid Phosphatases, 382
- Gebbie, Dr. H. A. and Chamberlain, Dr. J. E.: Sub-millimetre Dispersion and Rotational Line Strengths of the Hydrogen Halides, 480
- Gebbie, Dr. H. A., and Chantry, Dr. G. W.: Sub-millimetre Wave Spectra of Polar Liquids, 378
- Gedde-Dahl, jun. Dr. T., and Berg, Dr. T.: Linkage in Man: the Inv and the Lp Serum Type Systems, 1126
- Geddes, Prof. J. D.: Appointment as Professor of Civil Engineering in the Welsh College of Advanced Technology, Cardiff, 1152
- Gel Method for the Detection of Cells producing 7S Antibody: A Localized Haemolysis in (Dr. J. Sterzl *et al.*), 858
- Gelbaum, Prof. B. R., and Olmsted, Prof. J. M. H.: Counterexamples in Analysis, review by Prof. T. A. Broadbent, 218
- Geldard, F. A. (edited by): Communication Processes, NATO Conference Series, Vol. 4, review by Prof. C. Cherry, 717
- Geller, Dr. E., and Yuwiler, Dr. A.: Serotonin Depletion by Dietary Leucine, 83
- Gemini-Titan-5*: Determination of Cloud Altitudes from (F. Siedy *et al.*), 775
- Generation of Nutritional Movements (D. G. Heathcote), 909
- Genet. Sulphocysteine in the Urine of the Blotched Kenya (J. C. Crawhall and Dr. S. Segal), 1320
- Genetic Morphism: Schizophrenia as a (Prof. A. Hoffer), 306
- Genetic Principles and Plant Breeding (Prof. W. Williams), Botanical Monographs, Vol. 5, review by Dr. K. Jones, 7
- Genetics and Studies of Twins: Research in Human, 230
- Genetics: Microbial and Molecular (Prof. J. R. S. Fincham), review by Dr. S. W. Glover, 1029
- Geniculate Body: Evidence for Presynaptic Inhibition in the Lateral (A. Angel *et al.*), 495
- Genital Tassel in the Male *Tilapia macrochir* Blgr. (Pisces: Cichlidae): Signal Value of the (Dr. W. Wickler), 595
- Gennaro, jun., Prof. J. F., and Anton, Prof. A. H.: Norepinephrine and Serotonin in the Tissues and Venoms of Two Pit Vipers, 1174
- Gent, H., *et al.*: New Limits to the Angular Sizes of Some Quasars, 275
- Gentry, F. E., Gutzwiller, F. W., Holonyak, jun., N., and Von Zastrow, E. E.: Semiconductor Controlled Rectifiers, review by Prof. W. Fishwick, 719
- Geochemical Group: New Zealand, 123
- Geochemical Reconnaissance against some Agricultural Problems in Co. Limerick, Eire: Regional (Prof. J. S. Webb and W. J. Atkinson), 1056
- Geochemistry of Andestites and the Growth of Continents (Prof. S. R. Taylor and Dr. A. J. R. White), 271
- Geoelectric Effect in Plants: Origin of the (Prof. M. B. Wilkins and A. E. R. Woodcock), 990
- Geographical Society: Awards: American, 1047
- Geological Provinces: Seismicity and other Properties of (Prof. E. R. Kanase-wich), 1275
- Geological Thesis on Hadhrumut, review by F. E. Wellings, 615
- Geology: Christmas Holiday Films and Talks on, 1155
- Geology of Grange (Prof. E. Ragun), Translated from the Second French Edition, review by Dr. P. E. Brown, 1030
- Geology of the Pacific: Marine (Prof. H. W. Menard), review by Dr. A. S. Laughton, 215
- Geophysical Survey, Geophysics Division, New Zealand D.S.I.R., Superintendent, 327
- George, Dr. F. H.: *Obituary* of Prof. K. R. L. Hall, 432
- George, Marilyn J., *et al.*: Strontium-90 and Caesium-137 in Columbia River Plume, July 1964, 1084
- Germanium and Silicon to 60 kilobars: Fusion Curves of Zinc, Aluminium, (J. Lees and B. H. J. Williamson), 278
- Germany: Comparative Study of Housing in Britain, France and Western, 233
- Germination and Outgrowth of Spores of *Bacillus cereus* Strain T: Timing of Protein Synthesis during (W. Steinberg *et al.*), 710
- Germination in Light-requiring Seed by Chloramphenicol: Promotion of (Dr. M. Black and M. Richardson), 1114
- Germination of White Mustard: Molecular Structural Factors and their Role in the (S. E. B. Gould *et al.*), 876
- Germination of Winter Rye: Effects of Oxygen Pressure and Salts on (Dr. S. M. Siegel *et al.*), 1012
- Germes and Ideas, Routes of Epidemics and Ideologies, (Dr. A. Siegfried), review by F. Hawking, 1249
- Gerontology in Great Britain: Social and Industrial (C. E. Fleming), 623
- Gerontology: International Conference on (edited by A. Balázs), review by Dr. David A. Hall, 981
- Gesche idenis van de Landbouw, 1939-1964: De Studiekring roer de, review by G. E. Fussell, 1138
- Gewurz, Dr. H., Wernick, P. R., Quie, Dr. P. G., and Good, Prof. R. A.: Effects of Hydrocortisone Succinate on the Complement System, 755
- Geymer, Dr. D. O., and Wagner, Dr. C. D.: Mechanism of Radical Decay in Irradiated Polyethylene, 72
- Gherkin Hypocotyls: Photo-induced Hydroxylation of Cinnamic Acid in (Dr. G. Engelsma), 1117
- Ghosh, S. S., *et al.*: Indian Woods, Vol. 2: Linaceae to Moringaceae, review by Dr. C. R. Metcalfe, 212
- Giannelli, Dr. F.: Autoradiographic Identification of the D(13-15) Chromosome responsible for Di Trisomic Patau's Syndrome, 669
- Giant Axon: Repetitive Responses to Alternating Current Stimulation: Squid (Prof. H. R. Hirsch), 1218
- Giant Axons: Intracellular Chloride Activity of Crayfish (Dr. A. Strickholm and B. G. Wallin), 790
- Giant Foot-pad Cells of *Sarcophaga bullata*: Differential Deoxyribonucleic Acid Replication in the (Dr. J. M. Whitten), 1019
- Giant Panda (*Ailuropoda melanoleuca*): Bursa Pharyngea in the (Prof. A. J. E. Cave), 865
- Gibb, Dr. J. A.: Appointment as Director of the Animal Ecology Division of the New Zealand D.S.I.R., 327
- Gibberellin Acid on Auxin Transport: Action of (Prof. P. E. Pilet), 1344
- Gibberellin Acid on Growth, Fruit Bud Formation and Frost Resistance in One-year-old Pear Trees: Effects of (2-Chloroethyl)-trimethylammonium Chloride and (Dr. I. Modilbowski), 503
- Gibbs, Dr. C. L., and Ricciuti, Dr. N. V.: Heat Production in a Cardiac Contraction, 897
- Gibson, D., Oldham, C., Lewis, Prof. J., Lawton, D., Mason, Prof. R., and Robertson, G. B.: Structure of a Diolefin Complex of Platinum(II) $C_{10}H_{16}O_8PtCl_2$, 580
- Gibson, Q. H., *et al.* (edited by): Rapid Mixing and Sampling Techniques in Biochemistry, review by Prof. S. Dagley, 823
- Giese, Prof. A. C. (edited by): Photophysiology, Two Volumes, review by Dr. Edith Harrison, 978
- Gilbert, Dr. C. W., Muldal, S., and Lajtha, Dr. L. G.: Rate of Chromosome Duplication at the End of the Deoxyribonucleic Acid Synthetic Period in Human Blood Cells, 159
- Gilbert, Dr. D. A., and McCabe, Dr. M.: Kinetic Parameters for Oxygen in Reactions Involving Purified Oxidases and Tissue Slices, 450
- Gill, Dr. S. S.: *Obituary* of Prof. Wilfred Merchant, 1043
- Gillam, J. P.: *Obituary* of Sir Ian Richmond, 831
- Gillard, Dr. R. D., McKenzie, Dr. E. D., Mason, Prof. R., Mayhew, S. G., Peel, Dr. J. L., and Stangroom, J. E.: Nature of the Non-haem Iron in Ferredoxin and Rubredoxin, 769
- Gillespie, P. J.: Ionizing Radiation: a Potential Lightning Hazard?, 577
- Gillissen, Dr. I. H., *et al.*: Intermediate States in the Precipitation of Hydroxyapatite, 365
- Gillis, Prof. C. N., and Paton, Dr. D. M.: Effect of Altered Perfusion Rates on the Retention of Noradrenaline by the Spleen, 391
- Gillott, C., and Clarke, Dr. K. U.: Relationship between the Removal of the Frontal Ganglion and Protein Starvation in *Locusta migratoria* L., 808
- Gitelman, A. K., *et al.*: Influence of Heterologous Histone on the Synthesis of Cellular and Viral Ribonucleic Acid and Production of Haemagglutinin, 557
- Githens III, Dr. S., and Glick, Dr. J. L.: Role of Sialic Acid in Potassium Transport of L 1210 Leukaemia Cells, 88
- Gittins, J. F.: Power Travelling-wave Tubes, review by Dr. P. A. Lindsay, 1140
- Giunarro, Constance, *et al.*: Experimentation with Plants at Sub-atmospheric Oxygen-levels: Effects of Oxygen Pressure and Salts on the Germination of Winter Rye, 1012
- Gjessing, E. T.: Use of 'Sephadex' Gel for the Estimation of Molecular Weight of Humic Substances in Natural Water, 1091
- Glacial-marine Zones in North Atlantic Deep-sea Sediments: Pleistocene (Dr. J. R. Conolly and Dr. M. Ewing), 135
- Gladwell, G. M. L., *et al.*: Matrix Analysis of Vibration, review by Prof. W. A. Tuplin, 616
- Glaister, Dr. R. M., and Lee, Dr. E. W.: Re-entrant Hysteresis Loop and Multi-stable Ferrites, 67
- Glands in Seals: Functional Significance of Sweat Glands and Sebaceous (J. K. Ling), 560
- Glanfield, J. R., and Mills, Prof. B. Y.: Radio Observations of Spiral Galaxies, 10
- Glaser, Dr. E. M., Spink, P., and O'Meara, Prof. R. A. Q.: A Screening Test for Substances Inhibiting the Cancer Coagulative Factor, 1008
- Glass, Dr. L. E., and Hughes, A. M.: Histological Investigations of the Mechanism of Sterility induced by Deuterium Oxide in Mice, 1119
- Glass Industry Research Association: British, 733
- Glassman, Dr. E., and Keller, jun., E. C.: Phenocopies of the *ma-1* and *ry*-Mutants of *Drosophila melanogaster*: Inhibition *in vivo* of Xanthine Dehydrogenase by 4-Hydroxypyrazolo(3,4-d)pyrimidine, 202
- Glick, Dr. J. L., and Githens III, Dr. S.: Role of Sialic Acid in Potassium Transport of L 1210 Leukaemia Cells, 88
- Globin Synthesis in Thalassaemia: An *in vitro* Study (Dr. D. J. Weatherall, Dr. J. B. Clegg and Dr. M. A. Naughton), 1061

- Globulin from Rainbow Trout Serum: Electrophoretic Mobility of an Immune (H. O. Hodgins et al.), 1106
- Globulins: Formation of Complexes between Basic Proteins of Leucocytes and Plasma (Dr. W. B. Jolley and H. W. Allen), 390
- 7S γ -Globulins in Mice: Breakdown Rate of Serum (Dr. D. E. H. Tee et al.), 251
- Glossary of Primary Sedimentary Structures: Atlas and (Prof. F. J. Pettijohn and Prof. P. E. Potter), review by J. R. L. Allen, 315
- Glove Damage as a Cause of Wound Infection, 836
- Glover, Dr. S. W.: Microbial and Molecular Genetics, review, 1029
- Glucose by Intact Novikoff Ascites Hepatoma Cells: Formation of Intra-mitochondrial Glucosyl-oligosaccharides from (Dr. V. N. Nigam and Dr. G. de Lamiandé), 664
- Glucose Cycloacetate in the Prevention of Fatty Infiltration in the Liver: Effect of Hydrolysed (Prof. N. Nath, V. D. Yawalkar and Prof. M. C. Nath), 1001
- Glucose: Maintenance of *Escherichia coli* and the Assimilation of (S. B. McGrew and Prof. M. F. Mallette), 1096
- Glucose-filled Phospholipid/Cholesterol Structures: Effect of Steroids and 'Triton X-100' on (Dr. G. Weissmann et al.), 649
- Glucose-6-phosphate Dehydrogenase among Chinese Characterization of (P. W. K. Wong et al.), 1323
- Glucosyl-oligosaccharides from Glucose by Intact Novikoff Ascites Hepatoma Cells: Formation of Intra-mitochondrial (Dr. V. N. Nigam and Dr. G. de Lamiandé), 664
- Glutarimide Homologues in Mice: Titration Curves depicting Central Stimulant, Depressant and Dual Stimulant-depressant Action of (Dr. A. Shulman et al.), 568
- Glycine Ethyl Ester: Protonation Equilibria and Alkaline Hydrolysis of (Dr. M. Robson), 265
- Glycogen in Yeast Form of *Paracoccidioides brasiliensis* (Dr. F. Kanetsuna and Dr. L. M. Carbonell), 686
- Glycol in Investigations of Absorption of Fat. Use of Polyethylene (Dr. H. Vavřínková and Dr. A. Krondl), 293
- α -Glycoprotein (Darcy) of Rat Serum by the Liver: Synthesis of (Prof. H. E. Weimer et al.), 1221
- Glycosidases: Hydrolysis of Two 17 α -Oestradiol Glycosides by Rabbit Tissue (K. S. Whittemore and Dr. D. S. Layne), 288
- Glycosides: Hydrolysis of Two 17 α -Oestradiol, by Rabbit Tissue Glycosidases (K. S. Whittemore and Dr. D. S. Layne), 288
- Gnetalean Pollen Grains in the Katrol Formation (Upper Jurassic) of Kutch, India: Occurrence of (Y. K. Mathur and Mrs. K. Mathur), 912
- Gnotobiotic Rats: Human Indigenous Microflora in (Dr. H. A. Dymysa et al.), 1236
- Gobies into the Black Sea: *Relictogobius kryzhanovskii* and the Penetration of Mediterranean (Dr. P. J. Miller), 474
- God Beyond Time (J. H. Reynier), review by F. I. G. Rawlins, 985
- God: Nature and (Prof. L. Charles Birch), review, 985
- Godwin, E. R., Rees, Dr. K. R., Rowland, Dr. G. F., and Varcoe, J. S.: Nucleocytoplasmic Interactions in the Rat Liver Cell, 246
- Goedewaagen, Dr. M. A. J., and Schuurman, Dr. J. J.: Methods for the Examination of Root Systems and Roots, review by W. D. Clayton, 319
- Goldman, W. F., and Sulitzanu, Prof. D.: Simplified Technique for Preparative Disc Electrophoresis, 1120
- Goldreich, Dr. Peter: Tidal De-spin of Planets and Satellites, 375
- Goma, Prof. L. K. H.: Periodic Aggregating and Take-off in Anthrophorid Bees, *Anthophora acraensis* Fab., 807
- Gomba, Sz., et al.: Vital Staining of the Granulated Juxtaglomerular Cells in the Mouse Kidney, 1331
- Gómez-Campo, C., and Casas-Builla, M.: Radiation-induced Sex Reversal in *Echallium elatium*, 805
- Gonatas, Dr. N. K., and Shy, Prof. G. M.: Virus-like Particles in Sub-acute Sclerosing Encephalitis, 1338
- Good, Dr. I. J.: Regression of a Phenotypic Value on the Values for Parents and Grandparents, 203
- Good, R. A., and Gabrielsen, A. E. (edited by) The Thymus in Immunobiology, review by Dr. R. B. Taylor, 318
- Good, Prof. R. A., et al.: Effects of Hydrocortisone Succinate on the Complement System, 755
- Goody, Dr. W.: Psychology of Time, review, 822
- Goodin, J. R.: Anatomical Changes associated with Juvenile-to-Mature Growth Phase Transition in *Hedera*, 504
- Goodman, G. T., Edwards, R. W., and Lambert, Dr. J. M., (edited by): Ecology and the Industrial Society, Symposium, review by Dr. A. Macfadyen, 618
- Goodman, Dr. H. S., et al.: Mathematical Model for the Process of Aggregation in Immune Agglutination, 84
- Goodwin, Prof. T. W.: Photosynthetic Bacteria, review, 977
- Goodwin, Prof. T. W., Harris, J. I., and Hartley, B. S. (edited by): Structure and Activity of Enzymes, Symposium 1964, review by Dr. D. T. Elmore, 972
- Gordon, Dr. J., and Maclean, Prof. L. D.: A Lymphocyte-stimulating Factor produced *in vitro*, 795
- Gordon, Dr. L. H., et al.: Lactic Dehydrogenase Isoenzymes in Muscle from Patients with Duchenne Muscular Dystrophy, 1107
- Gorman, G. C.: Interspecific Karyotypic Variation as a Systematic Character in the Genus *Anolis* (Sauria: Iguanidae), 95
- Goss, Prof. R. J.: Adaptive Growth, review by Dr. R. A. Weiss, 107
- Gosztonyi, T., Márton, J., and Kovács, A.: Labelling of Beta-lipoprotein with Tritium, 381
- Gotta, H., et al.: Use of Technetium-99m in Hepatic Scintigraphy, 86
- Gounin, Prof. Pierre: Coincidence of Magnetic Disturbances with Local Earthquakes recorded from the Ethiopian Rift System, 541
- Gould, Sheila E. B., Rees, Dr. D. A., Richardson, N. G., and Steele, I. W.: Pectic Polysaccharides in the Growth of Plant Cells: Molecular Structural Factors and their Role in the Germination of White Mustard, 876
- Gourlay, R. N., and Shifrine, Dr. M.: Serological Relationship between Galactans from Normal Bovine Lung and from *Mycoplasma mycoides*, 498
- Govaerts, André: Les Anticorps de Transplantation, review by R. B. Taylor, 988
- Government Explains: A Study of the Information Services (Mrs. M. Ogilvy-Webb), review by R. Brightman, 1139
- Graft-versus-host Assay in the Chick Embryo: Comparison of the Chorioallantoic Membrane and Splenomegaly Systems of (L. W. Coppleston and Dr. D. Michie), 53
- Grafts in Aged Mice: Multiple Thymus (Dr. D. Metcalf), 87
- Grafts: Induction of Malignant Lymphomas by Urethane in Adult Mice bearing Syngeneic Thymus (Dr. L. Fiore-Donati et al.), 398
- Graham, D., and ap Rees, Dr. T.: Occurrence of Fructose-1-phosphate in Tissues of Higher Plants, 88
- Grains: Growth of Interstellar (Dr. M. J. Baines and I. P. Williams), 1191
- Gram-negative Bacteria: Interaction of Cephaloridine with Penicillinase-producing (Dr. J. M. T. Hamilton-Miller et al.), 235
- Gram-negative Rods: Effect of Incubation Temperature on the Flagellation of (D. A. Orr and Dr. M. M. Taylor), 1017
- Grandparents: Regression of a Phenotypic Value on the Values for the Parents and (Dr. I. J. Good), 203
- Granite: Age of the Wearale (F. J. Fitch and Dr. J. A. Miller), 743
- Granite: Geology of (Prof. E. Raguin), Second Edition, review by Dr. P. E. Brown, 1030
- Grant, Prof. C. K.: My View of the World (E. Schrodinger), review, 5
- Grant, D. W., and Vaughan, G. A.: Determination of p.p.m. Concentrations of Carbon Disulphide in Benzene by Electron Capture Gas Chromatography, 75
- Grant, G. A., et al.: Thymus and the Production of Antibody-plaque-forming Cells, 1332
- Grant, Dr. J.: Pulp Processes, review, 719
- Grants for 1966: Lalor Foundation, 436
- Granulated Juxtaglomerular Cells in the Mouse Kidney: Vital Staining of the (V. Szokoly et al.), 1331
- Granulocytic Leukaemia: Chronic (Dr. A. S. D. Spiers and Dr. A. G. Baikie), 497
- Graphite Group: Carbon end, 735
- Graphite: Mechanism of Stored-energy Release at 200°C in Electron-irradiated (Prof. E. W. J. Mitchell and M. R. Taylor), 638
- Grassland and Fodder Resources in India (R. O. Whyte), Revised Edition, review by Dr. W. Davies, 821
- Grasty, Dr. R. L., et al.: Isotopic Ages and Andean Uplift, 677
- Graves, A. D., and Inman, Dr. D.: Adsorption and the Differential Capacitance of the Electrical Double-layer at Platinum/Halide Metal Interfaces, 481
- Gravitational Collapse: Quasi-Stellar Sources and (edited by Prof. I. Robinson et al.), review by Sir Bernard Lovell, 963
- Gravity at the National Physical Laboratory: New Absolute Determination of the Acceleration due to (Dr. A. H. Cook), 279
- Gray, Dr. G. W., et al.: 'Lipid A' Component from the Cell-walls of *Pseudomonas aeruginosa*, 506
- Gray, Dr. H. B., and Balhuzen, C. J.: Molecular Orbital Theory, review by E. W. T. Richards, 10
- Gray, Prof. J. A. B.: International Committee on Laboratory Animals, 738
- Gray, Dr. L. H.: *Obituary* by Dr. O. C. A. Scott, 226
- Great Barrier Reef, Australia, Facilities for Research, 230
- Grebenik, E.: Elements of the Natural Movement of Population, review, 1032
- Green, Dr. D. G., and Campbell, Dr. F. W.: Monocular versus Binocular Visual Acuity, 191
- Green, Prof. H. N., and Anderson, Dr. M. R.: Implications of the Immunological Theory of Cancer to Multiple Causes, 338
- Green Alga: Production of Carotenoid by a (Prof. F. Dentice di Accadia et al.), 1342
- Greenhall, A. M.: Technique for the Removal of the Brain in Bats, 1014
- Greenwood, F. C., et al.: Plasma Growth-hormone Levels in Chronic Starvation in Man, 686
- Gregor, Dr. J. W.: Retirement as Director of the Scottish Plant Breeding Station, 228
- Greville, G. D., and Campbell, P. N. (edited by): Essays in Biochemistry, Vol. 1, review by Dr. D. T. Elmore, 316
- Gribanovskii-Sassu: Olga, et al.: Production of Carotenoid by a Green Alga, 1342
- Grim, Prof. S. O., and McFarlane, W.: Phosphorus-31 Chemical Shifts in Secondary and Tertiary Phosphines, 995
- Grime, Dr. J. P.: Shade Tolerance in Flowering Plants, 161
- Grizzly Bear Skull recovered from a Late Glacial Deposit near Lake Simcoe, Ontario: A Well-preserved (R. L. Peterson), 1233
- Groenman, S. T.: A Register of Scientific Research in the Netherlands, 28
- Grosch, Prof. D. S.: Distribution and Effective Half-life of Cobalt-58 in Habron, 906
- Grose, Dr. F. J., Bernstein, Prof. M. H., and Lerner, Dr. A. M.: 'Ring-forms' of Reovirus Particles, 636
- Gross, Dr. S. H., et al.: Rotation Period of the Planet Mercury, 375
- Grossberg, Dr. S. E., and O'Leary, Prof. W. M.: Hyperlipaemia following Viral Infection in the Chicken Embryo: a New Syndrome, 954
- Groundnut Meal: Hepatic Tumours in Ducks fed a Low Level of Toxic (R. B. A. Carnaghan), 308
- Groves, G. V. (edited by): Dynamics of Rockets and Satellites, review by D. G. King-Hele, 516
- Growth: Adaptive (Prof. F. J. Goss), review by Dr. R. A. Weiss, 107
- Growth and Cytopathogenicity of H-viruses in Human and Simian Cell Cultures (Dr. H. Toolan and Dr. N. Ledinko), 812
- Growth and Growth Induction: Direct and Indirect Effects of Radiation on Plant Cells: their Relation to (Dr. R. D. Holsten et al.), 850
- Growth, Fruit Bud Formation and Frost Resistance in One-year-old Pear Trees: Effects of (2-Chloroethyl)-trimethylammonium Chloride and Gibberellic Acid on (Dr. I. Modlibowska), 503
- Growth Hormone: Further Purification and Characterization of a Placental Protein with Immunological Similarity to Human (Dr. H. Friesen), 1214
- Growth Hormone in Men and Women: Fasting Levels of (Dr. C. Faiman), 687
- Growth Hormone in the Presence of Actinomycin D: An *in vitro* Action of Human (T. E. Martin and Prof. F. G. Young), 684
- Growth-hormone Levels in Chronic Starvation in Man: Plasma (Dr. V. Marks et al.), 686
- Growth in Arthropods and Man: Evolutionary Tendency of Relative (Dr. R. Matsuda), 1228
- Growth in Root Caps: Cytoplasmic Organelles and Cell (Dr. B. E. Juniper and Dr. F. A. L. Clowes), 864
- Growth of B.H.K. Cells in the Hamster Cheek Pouch: Effect of β -Propiolactone and Thiomersal on (G. A. Keeble and C. S. Heymann), 1125
- Growth of Continents: Geochemistry of Andesites and the (Prof. S. R. Taylor and Dr. A. J. R. White), 271
- Growth of Interstellar Grains (Dr. M. J. Baines and I. P. Williams), 1191
- Growth of Logic in the Child: Early (B. Inhelder and J. Piaget), review by Dr. J. H. Duthie, 1246
- Growth of Malignant Cells *in vitro* by a Component of Normal Adult Connective Tissue: Inhibition of (Prof. M. S. Parsley and I. Mandl), 800
- Growth of Plant Cells: Pectic Polysaccharides in the (S. E. B. Gould et al.), 876
- Growth Phase Transition in *Hedera*: Anatomical Changes associated with Juvenile-to-Mature (J. R. Goodin), 504
- Growth Rates of Micro-organisms: Measurement of Unsteady State (Prof. R. I. Mateles et al.), 263
- Growth Responses of Mature Endosperm in Cultures (Prof. B. M. Johri and S. S. Bhojwani), 1345
- Gruber, G. M.: Possible Contribution of Jupiter's Magnetospheric Tail to the Radio Emissions of the Planet in the Decametric Region, 1271
- Guam and Oriboca Viruses by Naturally Infected Mosquitoes: Transmission of (A. Toda and R. E. Snopce), 304
- Guanase Activity of the Symbionts and Fat Bodies of the Cockroach, *Leucophaea maderae* (Dr. L. L. Pierre), 666
- Guest, Dr. J. E., et al.: Isotopic Ages and Andean Uplift, 677
- Guggenheim International Astronautics Award, 1965, 119
- Guile, Dr. A. E., et al.: Surface Charges on Arc Track Oxide Layers, 774
- Gunnies Awards for Science and Mathematics Teachers, 631
- Gunstone, Dr. F. D.: Structure of Lipids by Spectroscopic and X-ray Techniques, review, 1246

- Gustatory Relay of the Rat : Chemotopic Organization in the Bulbar (B. P. Halpern), 393
- Gut : Role of the, in Synthesizing the Protein Component of Low-density (Beta) Lipoprotein (P. J. Scott and C. C. Winterbourn), 494
- Gutmann, E., and Hnik, P. (edited by) : Effect of Use and Disuse on Neuromuscular Functions, Symposium, 1962, review by Prof. K. Krnjević, 1248
- Gutmann, F., et al. (edited by) : Proceedings of the First Australian Conference on Electrochemistry, 1963, review by Dr. R. F. Phillips and N. A. Hampson, 104
- Gutzwiller, F. W., et al. : Semiconductor Controlled Rectifiers, review by Prof. W. Fishwick, 719
- Guy's Hospital Medical School : Chair of Chemical Pathology, 934
- HABROBRACON** : Distribution and Effective Half-life of Cobalt-58 in (Prof. D. S. Grosch), 906
- Haddad, Prof. J. R., et al. : Subcutaneous Sarcomata in Hamsters induced by Rous Sarcoma Virus (Bryan), 336
- Hadramut : Geological Thesis on, review by F. E. Wellings, 615
- Hadwiger, Dr. L., et al. : Enzymatic Formation of β -Cyanoalanine from Cyanide, 1207
- Haemagglutination by Polymerized Orosomucoid : Inhibition of Influenza Virus (P. H. Whitehead et al.), 915
- Haemagglutinin : Influence of Heterologous Histone on the Synthesis of Cellular and Viral Ribonucleic Acid and Production of (A. G. Bukrinskaya et al.), 557
- Haemoglobin A₁ by Acrylamide-gel Electrophoresis : Quantitative Determination of (Lieut.-Commander T. G. Ferris et al.), 1103
- Haemoglobin : Binding of Xenon to Horse (Dr. B. P. Schoenborn), 760
- Haemoglobin : Modification System for Fingerprinting (Dr. G. Efremov), 298
- Haemoglobin E-Ovalocytosis in Malayan Aborigines : Hereditary Ovalocytosis and (Dr. Lie-Injo Luan Eng), 1329
- Haemoglobin in *Ceriodaphnia quadrangula* (O. F. Muller) (B. Czeczuga), 907
- Haemoglobin, J. Toronto (a5 Alanine-Aspartic Acid) - A New (Dr. J. H. Crookston et al.), 1059
- Haemoglobin Polymorphism in the Cod (*Gadus morhua*) : A Single Peptide Difference (M. C. Rattazzi and C. P. Kik), 489
- Haemoglobins K : Woolwich, an Abnormal, Cameroon and Ibadan, Two Unusual Variants of Human Haemoglobin A : Three (Dr. N. Allan et al.), 658
- Haemolysis in Gel Method for the Detection of Cells producing 7S Antibody : A Localized (Dr. J. Sterzl et al.), 858
- Haemolysis : Inhibition of, by 'Cremophor' in Conserved Blood (Dr. K. Reber), 195
- Haemolytic Disease of the Newborn : Low Concentrations of Serum Haptoglobin in Mothers of Children with (J. Pintera), 694
- Haemopoietic System in the Chick Embryo : Chromosome Marker Studies on the Development of the (M. A. S. Moore and Dr. J. J. T. Owen), 956
- Haffkine, W. M. W., The Brilliant and Tragic Life of (S. A. Waksman), review by Dr. H. I. Jhalal, 418
- Hair Colour of the Population of Tristan da Cunha (Dr. E. Sunderland), 412
- Hair : Cysteine Content of Cattle (P. H. Springell and S. J. Leach), 1326
- Håkanson, R. : Formation of Amines in the Gastric Mucosa of the Rat, 793
- Hakim, Dr. S. E. A., and Comley, W. J. : Acoustic Velocity Dispersion in some Non-associated Organic Liquids, 1082
- Haldane, Prof. J. B. S., 1264
- Half-life of Cobalt-58 in Habrobrakon : Distribution and Effective (Prof. D. S. Grosch), 906
- Halides : Sub-millimetre Dispersion and Rotational Line Strengths of the Hydrogen (Dr. J. E. Chamberlain and Dr. H. A. Gebbie), 480
- Hall, Dr. David A. : International Conference on Gerontology, review, 981
- Hall, Dr. David A., and Varadi, Dr. D. P. : Cutaneous Elastin in Ehlers-Danlos Syndrome, 1224
- Hall, Prof. K. R. L. : *Obituary* by Dr. F. H. George, 432
- Hall, R. J., and Flynn, L. R. : Heterogeneity of Titan Yellow demonstrated by Thin-layer Chromatography, 1202
- Hall, Prof. W. C., et al. : Biogenesis of Ethylene in *Penicillium digitatum*, 1322
- Hallett, Dr. J. : Physics of Ice, review, 986
- Hallsworth, E. G., and Crawford, D. V. (edited by) : Experimental Pedology, Proceedings of 11th Easter School in Agricultural Science, 1964, review by Dr. A. M. Smith, 718
- Halpern, B. P. : Chemotopic Organization in the Bulbar Gustatory Relay of the Rat, 393
- Halvorson, Prof. H. O., et al. : Timing of Protein Synthesis during Germination and Outgrowth of Spores of *Bacillus cereus* Strain T, 710
- Hambleton, F. H., Hockey, Dr. J. A., and Taylor, J. A. G. : An Infra-red Investigation of the Effect of Pressure on Silica Powders, as revealed by Deuterium Oxide Exchange, 138
- Hamilton, Dr. C. M. : Effects of Starvation and Protein Depletion on Mercury Retention in Two Strains of Chickens, 706
- Hamilton, Mr. J. A. : Appointment as Project Director for Anglo-French Military Aircraft : Ministry of Aviation, 527
- Hamilton, Prof. T. H., Moore, R. J., Rumsey, A. F., Means, A. R., Schrank, Prof. A. R. : Stimulation of Synthesis of Ribonucleic Acid in Sub-apical Sections of *Avena Coleoptile* by Indolyl-3-acetic Acid, 1190
- Hamilton, Prof. W. J. : *Obituary* of Prof. Jocelyn Patterson, 1042
- Hamilton-Miller, Dr. J. M. T., Smith, Dr. J. T., and Knox, Prof. R. : Interaction of Cephaloridine with Penicillinase-producing Gram-negative Bacteria, 235
- Hamlet, S. E., et al. : Mammals of the World, Three Volumes, review by Dr. L. Harrison Matthews, 210
- Hampel, C. A. (edited by) : Encyclopedia of Electrochemistry, review by Dr. R. F. Phillips and N. A. Hampson, 104
- Hampson, N. A., and Phillips, Dr. R. F. : Encyclopedia of Electrochemistry, review, 104
- Proceedings of the First Australian Conference on Electrochemistry, 1963, review, 104
- Hamster Cells by Nucleic Acid *in vitro* : Alteration of (K. Shepley et al.), 1072
- Hamster Cheek Pouch : Effect of β -Propiolactone and Thiomersal on Growth of B.H.K. Cells in the (S. A. Keeble and C. S. Heymann), 1125
- Hamster : Lactate Dehydrogenase Relative to Chaetepithelioma Induction in the Syrian (Prof. F. T. Algard et al.), 567
- Hamsters : Subcutaneous Sarcomata in, Induced by Rous Sarcoma Virus (Bryan) (Prof. M. L. Eldinoff et al.), 336
- Hankowsky, Olga H., et al. : Derivatives of 2-Aminoethyl-guanidine and of a Eugenol as Virus Inhibitors, 409
- Hanngren, A., Einer-Jensen, N., and Ullberg, S. : Specific Uptake in Corpora Lutea of a Non-steroid Substance with Antigenic Properties, 461
- Hanson, Jennifer M., et al. : Acid Phosphatases, 382
- Hanus, V., and Krs, M. : Cassiterite as a Carrier of Palaeomagnetism, 675
- Haptoglobin in Mothers of Children with Haemolytic Disease of the Newborn : Low Concentrations of Serum (J. Pintera), 694
- Haptoglobin Johnson in Australian Aborigines (D. W. Cooper, Dr. H. B. M. Lewis and Dr. E. M. Nicholls), 694
- Harborne, Dr. J. B., (edited by) : Biochemistry of Phenolic Compounds, review by Sir Robert Robinson, 515
- Chemistry of Plant Pigments, 1051
- Harborne, Dr. J. B., et al. : Double Chromosome Reduction in a Tetraploid *Solanum*, 359
- Harden, A. (1865-1940), 732
- Harder, R., Schumacher, W., Firas, F., and Von Denffer, D., (rewritten by) : Strasburger's Textbook of Botany, New English Edition, review by Dr. A. Allsopp, 618
- Hardie, Dr. D., and Coleman, C. E. : Slow-bend Testing of Hydrided Zirconium, 69
- Hare, R. : A Review of Sterilization and Disinfection as Applied to Medical, Industrial and Laboratory Practice, review, 1249
- Hargreaves, Dr. T. : Inhibition of Conjugation by Anti-inflammatory Drugs, 1101
- Harp, A. R., et al. : Aflatoxin : Effect on Cultured Heteroploid Human Embryonic Lung Cells, 345
- Harper, Dr. P., et al. : 'Pink Spot' in the Urine of Schizophrenics, 453
- Harré, R. (edited by) : Early Seventeenth Century Scientists, review by Dr. W. L. Sumner, 985
- Harris, Prof. C. C., and Jowett, Dr. A. : Size Analysis in the Sub-sieve Range by Electronic Counter, 175
- Harris, Dr. F., Jabbur, Dr. S. J., Morse, Dr. R. W., and Towe, Dr. A. L. : Influence of the Cerebral Cortex on the Cuneate Nucleus of the Monkey, 1215
- Harris, Dr. G. P. : Horticultural Science at the University of Reading, 437
- Harris, Prof. Harry, and Hopkinson, Dr. D. A. : Evidence for a Second 'Structural' Locus determining Human Phosphoglucomutase, 410
- Harris, J. L., et al. (edited by) : Structure and Activity of Enzymes, Symposium 1964, review by Dr. D. T. Elmore, 972
- Harris, P. J., and Sebba, Prof. F. : Possible Relationship between Trace Metals and Bloat in Ruminants, 869
- Harris, R. H. : Simple Technique for embedding and supporting Delicate Biological Specimens, 199
- Harrison, Dr. Edith : Photophysiology, Two Volumes, review, 978
- Harrison, M. J. G., et al. : Effect of a Pyrimidopyrimidine Derivative on Thrombus Formation in the Rabbit, 255
- Harrison, R. B., et al. : Organochlorine Pesticides in the Atmospheric Environment, 1317
- Harrison Matthews, Dr. L. : A Draught of Fishes, review, 517
- Mammals of the World, Three Volumes, review, 210
- Harrop, R., and Blackman, Dr. L. C. F. : Infra-red Spectra of Quaternary Compounds adsorbed on Silica-gel 'Aerosil', 777
- Hart, S. L., and Nissim, Dr. J. A. : Mechanism of Intestinal Absorption : Transport of Methionine and Sodium Butyrate by Intracellular Plasma, 145
- Hartley, B. S., et al. (edited by) : Structure and Activity of Enzymes, Symposium 1964, review by Dr. D. T. Elmore, 972
- Hartley, Dr. Frank : Pharmacy in History, review, 982
- Hartley, Dr. G. S. : *Obituaries* of Dr. W. E. Ripper and Dr. E. P. Jones, 431
- Two Pioneers of Pest Control, 430
- Hartley, Dr. R. A., and Neve, Dr. R. A. : Relationships between Constituents of Cones and Male Flowers of the Hop (*Humulus lupulus*, L.), 804
- Haslam, Dr. C. G. T., and Quigley, Dr. M. J. S. : Structure of the Radio Continuum Background at High Galactic Latitudes, 741
- Hassall's Corpuscles : Phagocytic Function of (Dr. J. N. Blau), 564
- Hastowell, Dr. L. J., and Priestland, R. N. : Freeing of Amino-acids from Proteins, 387
- Hastings, Dr. J. R. B., Parish, Dr. J. H., Kirby, Dr. K. S., and Klucis, E. S. : Fractionation of Ribonucleic Acids in the Zonal Ultracentrifuge, 645
- Hatching Agents for the Beet Cyst Nematode, *Heterodera schachtii* Schm. : Zinc and Other Metallic Ions as (Dr. A. J. Clarke and Dr. A. M. Shepherd), 502
- Hatching Time of Turtle Eggs (Sister Marie Therese Dimond), 401
- Hatheren, Dr. T. : Appointment as Superintendent of the Geophysical Survey, Geophysics Division, New Zealand D.S.I.R., 327
- Hatton, Sir Ronald : *Obituary* by Prof. H. G. H. Kearns, 1258
- Hawking, F. : Germs and Ideas : Routes of Epidemics and Ideologies, review, 1249
- Haworth, Miss Susan, and Holker, Dr. J. R. : Oxidation of some 2,4-Dinitrophenylamines with Ceric Salts, 184
- Hay, J. E., and Scott, F. H. : Detonability of Nitroglycerin contained in Porous Rock, 1197
- Hay, Patricia A., et al. : Comparison of Salivary Amylase-levels in the Human Male and Female, 1211
- Hayashi, Prof. H., Kinuwaki, Y., and Yoshinaga, M. : Inhibition of Anaphylactic Release of Vascular Permeability Factor or Histamine by Specific Protease Inhibitor in Tissue Culture, 1007
- Hayward, Roger, and Pauling, Prof. Linus : The Architecture of Molecules, review by Dame Kathleen Lonsdale, 957
- Haywood, John J. : Third Dimension of Management, 928
- Hazard, J. B., and Smith, David E. (edited by) : The Thyroid, review by Dr. E. E. Pochin, 979
- Head X-irradiation and its Significance in the Potentiation of Barbiturate Hypnosis : Regional Changes in Brain Serotonin after (Dr. V. Nair), 1293
- Heal, Dr. G. R., and Dollimore, Dr. D. : Errors in the Calculations of Surface Area and Average Pore Radius, 1092
- Heald, Dr. M. A., and Wharton, C. B. : Plasma Diagnostics with Microwaves, review by A. E. Dangor, 965
- Health in the Nineteenth Century : Public (Prof. C. F. Brockington), review by Sir Arthur MacNalty, 977
- Health Officials : Training of Public, 1045
- Health Organization, 1964 : World, 437
- Health Services : Comparison of European, 438
- Healy, W. B., and Ludwig, T. G. : Ingestion of Soil by Sheep in New Zealand in Relation to Wear of Teeth, 806
- Heaney, H. J. : A Guide to Irish Bibliographical Material, review, 1139
- Heart and Gastrocnemius : Hormonal Control of Lipid Concentration in Rat (R. M. Denton and Prof. P. J. Randle), 488
- Heart of the Mollusc *Tapes turrida* : Biphasic Dose-response Relationship with Acetylcholine on the (P. R. Carroll and G. B. Chesher), 1220
- Heart Production in a Cardiac Contraction (Dr. N. V. Riciuti and Dr. C. L. Gibbs), 897
- Heath, Dr. G. A., and Fisher, Dr. I. P. : Dissociation Energy of the N-H Bond in Hydrazine, 1199
- Heathcote, D. G. : Generation of Nutational Movements, 909
- Heavy Water (Deuterium Oxide) : Influence of, on Plaque Size of Western Equine Encephalitis Virus (R. I. Carp and Dr. D. Kritchevsky), 93
- Heider : Anatomical Changes associated with Juvenile-to-Mature Growth Phase Transition in (J. R. Goodin), 504
- Heier, Dr. K. S. : Radioactive Elements in the Continental Crust, 479
- Heilmann, Dr. G. : Direct Ultramicroscopic Observation of Thermal Scattering in Sodium Chloride, 884
- Helm, Prof. Werner G., and Ellenson, S. R. : Involvement of the Adrenal Cortex in the Appearance of Rat Slow Alpha-Globulin, 1330

- Heinz, Dr. E., et al. : Effect of *Trans*-Solutcs on the Fluxes of Chloride Ions across Artificial Membranes, 187
- HeLa Cells at -196°C : Continuous Irradiation of (A. H. W. Nias and Dr. L. G. Lajtha), 400
- HeLa Cells *in vitro*, after Treatment with 'Methotrexate' : A Reduced Oxygen Enhancement Ratio for X-ray Survival of (Dr. R. J. Berry), 1108
- HeLa Cells : Synthesis of Ribosomal RNA in Synchronized (Prof. M. D. Scharff and Dr. E. Robbins), 464
- (*Helix*) *lactea* : A Specific Agglutinin in the Snail *Otala* (Prof. W. C. Boyd and R. Brown), 593
- Hellstrom, I., et al. : Allogeneic Inhibition of Tumour Cells by *in vitro* Contact with Cells containing Foreign H-2 Antigens, 458
- Hellstrom, Ingegerd : Distinction between the Effects of Antiviral and Anticellular Polyoma Antibodies on Polyoma Tumour Cells, 652
- Hellstrom, K. E., Hellstrom, I., and Bergheden, C. : Allogeneic Inhibition of Tumour Cells by *in vitro* Contact with Cells containing Foreign H-2 Antigens, 458
- Helmick, W. M., et al. : Blood Group Secretion Factors in Bronchogenic Carcinoma, 299
- Helmintic Diseases : Immunological Tests in (Dr. R. Elsdon-Dew), 696
- Hemicholinium : Central Action of (Dr. D. M. J. Quastel and Prof. D. R. Curtis), 192
- Henry, J. A., et al. : Titration Curves depicting Central Stimulant, Depressant and Dual Stimulant-depressant Action of Glutaramide Homologues in Mice, 568
- Hepatic Scintillography : Use of Technetium-99m in (Dr. O. Degrossi et al.), 86
- Hepatic Tumours in Ducks fed a Low Level of Toxic Groundnut Meal (R. B. A. Carnaghan), 308
- Hepatitis : Distribution of the Intestinal Factor of Normal Mice which inactivates Murine Virus (Prof. M. Piazza et al.), 1009
- Hepatitis Virus : Propagation and Properties of (Dr. Z. F. Ch. Kachani), 605
- Hepatocytes : Functional Similarities between the Cytoplasmic Organelles of Melanocytes and the Mitochondria of (Dr. M. H. Van Woert et al.), 810
- Hepatoma Cells : Formation of Intramitochondrial Glucosyl-oligosaccharides from Glucose by Intact Novikoff Ascites (Dr. V. N. Nigam and Dr. G. de Lamirande), 664
- Hepatomegalic Effect produced by Ethyl-chlorophenoxy-isobutyrate in the Rat : Nature of the (Dr. R. Hess et al.), 856
- Hepper, F. N. : Introduction to the Flowering Plants of West Africa, review, 976
- Herbicide Compounds : 2-Trifluoromethylbenzimidazoles : a New Class of (Dr. D. E. Burton et al.), 1166
- Hereditary Ovalocytosis and Haemoglobin E-Ovalocytosis in Malayan Aborigines (Dr. Lie-Injo Luan Eng), 1329
- Heredity and the Nature of Man (Dr. T. Dobzhansky), review by Prof. C. H. Waddington, 959
- Heritage : Conserving the Natural, 1241
- Herp, Dr. Anthony, et al. : Acid Mucopolysaccharide and Hexosamine Metabolism in Dermal Connective Tissue of Adrenalectomized Rats, 1212
- Herrmann, Dr. R., and Stoss, Dr. B. : Immunogenic Activity of Lipids of *M. tuberculosis*, 1224
- Herschbach, Prof. D. R., and Wilson, K. R. : Correlation of Sodium Atom Reaction Rates with Electron Capture Cross-sections, 182
- Hertz, Dr. L., et al. : Possible Role of Neuroglia, 600
- Herzberger, M. (Supplementary Notes by) : Mathematical Theory of Optics (Dr. R. K. Luneburg), review by Dr. W. J. Bates, 971
- Hesp, R., and Ramsbottom, B. : Effect of Sodium Alginate in inhibiting the Uptake of Radiostrontium by the Human Body, 1341
- Hess, Dr. R., Staubli, W., and Riess, W. : Nature of the Hepatomegalic Effect produced by Ethyl-chlorophenoxy-isobutyrate in the Rat, 856
- Hess, Dr. S. M., et al. : Behavioural Deficit in the Rat induced by feeding Phenylalanine, 1021
- Heterocyclic Compounds with Three- and Four-membered Rings (edited by Dr. A. Weissberger), Parts 1 and 2, review by Dr. F. G. Mann, 2
- Heterodera schachtii* Schm. : Zinc and Other Metallic Ions as Hatching Agents for the Beet Cyst Nematode, (Dr. A. J. Clarke and Dr. A. M. Shepherd), 502
- Heterogeneity of Titan Yellow demonstrated by Thin-layer Chromatography (R. J. Hall and L. R. Flynn), 1202
- Heterogeneity of Water-soluble Structural Components of Human Red Cell Membrane (Prof. M. D. Poulik and Dr. P. K. Lauf), 874
- Heterogeneous Binding of Acridine Orange by Polyribonucleotides (Prof. R. F. Beers, jun., and G. Armilei), 466
- Heterologous Histone on the Synthesis of Cellular and Viral Ribonucleic Acid and Production of Haemagglutinin : Influence of (A. G. Bukrinskaya et al.), 557
- Heteroploid Human Embryonic Lung Cells : Aflatoxin : Effect on Cultured (Dr. M. S. Legator et al.), 345
- Hexadecane on Absorption of Carbon Dioxide by Plants : Influence of (Prof. J. M. Riedhart), 300
- Hexagonal-mesh Structures and Stable Polypeptide Chain Configurations (Prof. R. E. Dickerson), 139
- Hexosamine Metabolism in Dermal Connective Tissue of Adrenalectomized Rats : Acid Mucopolysaccharide and (Prof. J. Fabianek et al.), 1212
- Heymann, C. S., and Keeble, S. A. : Effect of β -Propiolactone and Thiomerol on Growth of B.H.K. Cells in the Hamster Cheek Pouch, 1125
- Heywood, V. H., et al. (edited by) : Flora Europaea, Vol. I, review by Prof. J. A. Nannfeldt, 8
- Hicks, B. B., and Dyer, Dr. A. J. : Stratospheric Transport of Volcanic Dust inferred from Solar Radiation Measurements, 131
- Hideg, K., et al. : Derivatives of 2-Aminoethyl-guanidine and of a Eugenol as Virus Inhibitors, 409
- High-energy Diets : Phosphorus Excretion of Cattle fed on (W. D. C. Reed et al.), 953
- High Galactic Latitudes : Structure of the Radio Continuum Background at (Dr. M. J. S. Quigley and Dr. C. G. T. Haslam), 741
- High Latitude : Spectral Density Functions of Hydromagnetic Emissions at (Dr. A. Egeland), 539
- High-molecular Deoxyribonucleic Acid and Ribonucleic Acid : Separation of (Dr. O. Klammerth), 1318
- High Pressure/High Temperature Calibration of the Tetrahedral Anvil Apparatus : Combined Very (J. Lees and B. H. J. Williamson), 278
- High-pressure Science and Technology, 328
- High Pressures and the Condensed Phase : Physics of (edited by A. van Itterbeek), review by Dr. R. S. Bradley, 515
- High-resolution Autoradiography : Ultrastructure of the Antigen-retaining Reticulum of Lymph Node Follicles as shown by (J. Mitchell and A. Abbot), 500
- High Temperature Materials : Plenum Press Handbooks of, Three Volumes, (Dr. P. T. B. Shaffer et al.), review by Dr. E. H. Andrews, 517
- High-velocity Particle Impact : Welding by (Dr. R. F. Rolsten et al.), 1273
- Higher Education : Eighteen Plus : Unity and Diversity In (edited by Marjorie Reeves), review by Sir James Cook, 313
- Highways : New Zealand State, 835
- Hijaz Railway, Saudi Arabia : Reactivation of the, 1262
- Hilditch, Prof. J. P. : *Obituary* by P. N. Williams, 730
- Hill, Dr. C. R. : Polonium-210 in Man, 423
- Hill, R. M., and Capp, B. : Ionization Times in Shock-heated Potassium Vapour, 176
- Hilton, Prof. J. G., Alter, Dr. S., and Cushman, Dr. P. : Aldosterone Secretion : Differences in Direct Effects of Rubidium and Potassium Perfusion into Dog Adrenals, 1099
- Himwich, Dr. H. E., et al. : Alcohol and Evoked Potentials in the Cat, 688
- Hinde, Prof. R. A., et al. : Social Companions and the Mother-Infant Relationship in Rhesus Monkeys, 331
- Hintz, Dr. B., and Webber, Prof. M. M. : Antithymic Antibody Localization in the Mouse, 797
- Hirai, Hidematsu : Isoprecipitin in the Sera of Rats resistant to Tumour Transplantation, 798
- Hird, Dr. F. J. R. : Australian Biochemical Society Meeting, 837
- Hirsch, Prof. H. R. : Squid Giant Axon : Repetitive Responses to Alternating Current Stimulation, 1218
- Hirsch, Prof. P. B. : Election to Isaac Wolfson Professorship of Metallurgy in the University of Oxford, 628
- Hirsch, R. L., et al. : Prevention of Cholesterol-induced Cholelithiasis by Neomycin, 1226
- Hirst, Prof. R. J., et al. : Human Senses and Perception, review by Prof. O. L. Zangwill, 981
- Histamine by Specific Protease Inhibitor in Tissue Culture : Inhibition of Anaphylactic Release of Vascular Permeability Factor or (Prof. H. Hayashi et al.), 1007
- Histamine in a Silicon Rubber Implant : Prolonged Administration of Atropine or (P. Bass et al.), 59
- Histidine-less Lysozyme : Lysozymes characterized in Duck Egg-white : Isolation of a (Dr. J. Jollès et al.), 1204
- Histocompatibility Antigens in Transplanted Mouse Eggs (Dr. R. L. Simmons and P. S. Russell), 693
- Histological Diagnosis of Visceral Mycoses : Fluorochrome Stains for (G. Bader et al.), 796
- Histological Investigations of the Mechanism of Sterility induced by Deuterium Oxide in Mice (A. M. Hughes and Dr. L. E. Glass), 1119
- Histological Structure of the Spinal Column of *Sorex araneus* (L.) : Seasonal Changes in the (L. Säure and H. Hyvärinen), 705
- Histologie und Mikroskopische Anatomie des Menschen (Dr. W. Bargmann), 5th German Edition, review by Dr. G. Sander, 109
- Histone on the Synthesis of Cellular and Viral Ribonucleic Acid and Production of Haemagglutinin : Influence of Heterologous (A. G. Bukrinskaya et al.), 557
- History and Philosophy of Science : Teaching in the (Dr. W. Mays), 937
- History as the Organizer of Man's Memory (Prof. Herbert Butterfield), 1036
- History, 1939-64 : Dutch Study Circle for Agricultural, review by G. E. Fussell, 1138
- History of Parasitology (W. D. Foster), review by Dr. G. Lapege, 109
- History of the Rockefeller Institute 1901-1953, Origins and Growth, (Dr. G. W. Corner), review by the Lord Adrian, 1244
- History : Pharmacy in (Prof. G. E. Trease), review by Dr. Frank Hartley, 982
- History : Science in (Prof. J. D. Bernal), Third Edition, review by R. Brightman, 986
- Hnik, Pavel, and Gutmann, E. (edited by) : Effect of Use and Disuse on Neuromuscular Functions, Symposium, 1962, review by Prof. K. Krnjević, 1248
- Hoare, Dr. C. A. : *Obituary* of Academician Eugene N. Pavlovsky, 1151
- Hobson, Prof. A. D. : Retirement from Chair of Zoology in the University of Newcastle upon Tyne, 432
- Hochstein, H. D., et al. : Acetohydroxamate : Bacterial Urease Inhibitor with Therapeutic Potential in Hyperammonaemic States, 46
- Hockey, Dr. J. A., et al. : An Infra-red Investigation of the Effect of Pressure on Silica Powders, as revealed by Deuterium Oxide Exchange, 138
- Hodgins, H. O., Ridgway, C. G. J., and Utter, F. M. : Electrophoretic Mobility of an Immune Globulin from Rainbow Trout Serum, 1106
- Hodgkin, Prof. A. L. : Award of Copley Medal of the Royal Society, 1141
- Hoffer, Prof. A. : Schizophrenia as a Genetic Morphism, 306
- Hofmann, Dr. A. F. : Prevention of Cholesterol-induced Cholelithiasis by Neomycin, 1226
- Hofmann, Prof. W. W., et al. : Presynaptic Effects of Potassium Ion on the Mammalian Neuromuscular Junction, 590
- Höhn, Prof. E. O., and Cheag, S. C. : Prolactin and the Incidence of Brood Patch Formation and Incubation Behaviour of the Two Sexes in Certain Birds with Special Reference to Phalaropes, 197
- Holborow, Dr. E. J., et al. : A New Fluorescent Antibody Method : Mixed Anti-globulin Immunofluorescence or Labelled Antigen Indirect Immunofluorescence Staining, 353
- Holemans, Dr. R., Johnston, Dr. J. G., and Reddick, Dr. R. L. : Release of Plasminogen Activator by the Isolated Perfused Dog Kidney, 291
- Holker, Dr. J. R., and Haworth, Miss S. : Oxidation of some 2,4-Dinitrophenylamines with Ceric Salts, 184
- Hollin, John J. : Wilson's Theory of Ice Ages, 12
- Hollister, Hal, and Turner, Dr. J. E. : Relationship of the Velocity of a Charged Particle to its Relative Biological Effectiveness, 36
- Holloway, jun., Prof. R. L., and Tobias, Prof. P. V. : Cranial Capacity of the Hominine from Olduvai Bed I, 205
- Holme, Prof. Tor, and Zacharias, B. : Differences in the Antigenic Pattern of *Azotobacter* grown on Different Nitrogen Sources, 1235
- Holmes, Prof. Arthur : *Obituary* by Prof. F. H. Stewart, 525
- Holmes, D. E., et al. : Site of Attack in the Radiolysis of Pyrimidine Compounds, 1086
- Holmes, Dr. E. G. : *Obituary* of Dr. H. R. Marston, 524
- Holmes, L. S., et al. : Experimental Observations on the Structure of Collisionless Shock Waves in a Magnetized Plasma, 133
- Holography : Character Recognition by (Prof. D. Gabor), 422
- Holography : Reconstruction of Phase Objects by (Prof. D. Gabor et al.), 1159
- Holonyak, jun., N., et al. : Semiconductor Controlled Rectifiers, review by Prof. W. Fishwick, 719
- Holothurian Sclerites from the Speeton Clay (B. N. Fletcher), 281
- Holsten, Dr. R. D., Sugii, Dr. M., and Steward, Prof. F. C. : Direct and Indirect Effects of Radiation on Plant Cells : their Relation to Growth and Growth Induction, 850
- Hominid and Pebble-industry in Hungary : Lower Palaeolithic (Dr. M. Kretzoi and Dr. L. Vértess), 205
- Hominine from Olduvai Bed I : Cranial Capacity of the (Prof. R. L. Holloway, jun., and Prof. P. V. Tobias), 205
- L'homme Face à Son Destin (C. L. Mayer), review by F. I. G. Rawlins, 318
- Homocysteine-thiolactone : Electron Spin Resonance Investigations of Ultra-violet and X-irradiated (Prof. R. Koch and Dr. H. Monig), 86
- Homogeneous Hydrogenation and Hydroformylation using Ruthenium Complexes (D. Evans, J. A. Osborn, F. H. Jardine and Prof. G. Wilkinson), 1203
- Homsher, Earl, and Cozias, Dr. G. C. : Antidiuretic Hormone and Bile Flow, 687
- Honey-bee : Osmotropotaxis in the (Dr. H. Martin), 59

- Honour, Dr. A. J., et al. : Effect of a Pyrimidopyrimidine Derivative on Thrombus Formation in the Rabbit, 255
- Hooke, Sir Lionel : *Obituary of Sir Ernest Thomas Fisk*, 526
- Hop (*Humulus lupulus*, L.) : Relationships between Constituents of Cones and Male Flowers of the (Dr. R. D. Hartley and Dr. R. A. Neve), 804
- Hop Mosaic Virus : Mechanical Transmission of (Dr. J. T. Legg), 1017
- Hopkins, Alan K., et al. : Welding by High-velocity Particle Impact, 1273
- Hopkins, Prof. R. H. : *Obituary by Dr. F. W. Norris*, 1259
- Hopkinson, Dr. D. A., and Harris, Prof. H. : Evidence for a Second 'Structural' Locus determining Human Phosphoglucomutase, 410
- Hopson, A. J. : Winter Scale Rings in *Lates niloticus* (Pisces : Centropomidae) from Lake Chad, 1013
- Hormonal Control of Lipid Concentration in Rat Heart and Gastrocnemius (R. M. Denton and Prof. P. J. Randle), 488
- Hormone : Further Purification and Characterization of a Placental Protein with Immunological Similarity to Human Growth (Dr. H. Friesen), 1214
- Hormone : The Juvenile (Sir Vincent Wigglesworth), 522
- Hormone and Bile Flow : Antidiuretic (E. Homsher and Dr. G. C. Cotzias), 687
- Hormone in Men and Women : Fasting Levels of Growth (Dr. C. Faiman), 687
- Hormone in the Presence of Actinomycin D : An *in vitro* Action of Human Growth (T. E. Martin and Prof. F. G. Young), 684
- Hormone-like Cell Product : Control of the Competent State In *Pneumococcus* by a (Dr. A. Tomasz), 155
- Horse Haemoglobin : Binding of Xenon to (Dr. B. P. Schoenborn), 760
- Horticultural Science at the University of Reading (Dr. G. P. Harris), 437
- Horvat, Jozef, et al. : Effect of a Lipid Fraction from Rat Thymus on Delayed Hypersensitivity Reactions of Neonatally Thymectomized Rats, 356
- Hospital Lighting, 835
- Hot Climates : Timber as Building Material in, 1153
- Hot Processes in the 'Hot-zone' produced by Copper-64 Recoil Atoms in Solids : Evidence of Thermal and (K. Yoshihara and H. Ebihara), 482
- 'Hot-zone' produced by Copper-64 Recoil Atoms in Solids : Evidence of Thermal and Hot Processes in the (K. Yoshihara and H. Ebihara), 482
- Hours on Night Work : Shift Working : the Arrangement of (Mrs. G. de la Mare and Dr. J. Walker), 1127
- House, Dr. D. A., and Garner, Prof. C. S. : Diperoxodithylenetriammine-chromium(IV) *l*-hydrate : A New Chromium(IV) Peroxo Compound, 776
- Housing in Britain, France and Western Germany : Comparative Study of, P.E.P. Broadsheet, No. 490 (E. G. Howes), review, 233
- Hovercraft : British, 833
- Howard, Dr. B. H. : Increase during Winter in Capacity for Root Regeneration in Detached Shoots of Fruit-tree Rootstocks, 912
- Howard, Dr. H. W. : Chimerical Nature of the Entire-leaf Variant in the Potato Variety 'Majestic', 917
- How and Why do we Learn ? (edited by W. R. Niblett), review by A. D. C. Peterson, 314
- Howe, J. P., and Smith, R. (edited by) : Beryllium Oxide, review by Dr. M. F. Lappert, 9
- Howell, S. L., Parry, D. G., and Taylor, Dr. K. W. : Secretion of Newly Synthesized Insulin *in vitro*, 487
- Howes, Dr. F. N. : *Piante Medicinali : Chimica Farmacologia e Terapia*, review, 218
- Howorth, N., et al. : Plasma Growth-hormone Levels in Chronic Starvation in Man, 686
- Hoyle, Dr. R., and Benham, Dr. P. P. (edited by) : Thermal Stress, review by Dr. E. H. Andrews, 517
- Hoyle, Prof. F. : Recent Developments in Cosmology, 111
- Hsia, Prof. David Yi-Yung, et al. : Characterization of Glucose-6-phosphate Dehydrogenase among Chinese, 1323
- Huang, Joyce Mei-Kuo, and Wang, Prof. Kung-Tsung : Polyamide Layer Chromatography of DNP Amino-acids, 281
- Hubbard, Dr. J. I., and Gage, Dr. P. W. : Evidence for a Poisson Distribution of Miniature Endplates Potentials and some Implications, 395
- Hughes, Ann M., and Glass, Dr. L. E. : Histological Investigations of the Mechanism of Sterility induced by Deuterium Oxide in Mice, 1119
- Hull, M. N., and Lewis, Dr. F. A. : Diffusion or Hydride Component of Hydrogen Overpotential at Platinized Platinum and Palladized Palladium Cathodes, 887
- Hull : University of, Chair of Electronic Engineering, 732
- Hullelt, Mr. E. W. : Retirement as Director of the Wheat Research Institute of the New Zealand D.S.I.R., 327
- Human and Simian Cell Cultures : Growth and Cytopathogenicity of *H*-viruses in (Dr. H. Toolan and Dr. N. Ledinko), 812
- Human Blood Cells : Rate of Chromosome Duplication at the End of the Deoxyribonucleic Acid Synthetic Period in (Dr. C. W. Gilbert et al.), 159
- Human Body : Effect of Sodium Alginate in inhibiting the Uptake of Radiostrontium by the (R. Hesp and B. Ramsbottom), 1341
- Human Cells and its Bearing on the Concepts of Endoreduplication and Polyploidy : Deoxyribonucleic Acid Synthesis in Cultured (Dr. U. Mittwoch et al.), 242
- Human Chromosomes : Demonstration of the Spiral Structure of (Dr. Y. Ohnuki), 916
- Human Chromosomes : Distribution of Deoxyribonucleic Acid along (Dr. A. Ward), 486
- Human Cornea on Awakening : Thinning of the (Dr. R. B. Mandell and Prof. I. Fatt), 292
- Human Embryonic Lung Cells : Aflatoxin : Effect on Cultured Heteroploid (Dr. M. S. Legator et al.), 345
- Human Erythroid Cells in Thalassemia : Apoferritin Synthesis in (Dr. E. H. Eylar and Dr. G. Miatoli), 661
- Human Evolution : Fossil Evidence for (Sir Wilfrid E. Le Gros Clark), Second Edition, review by Dr. W. E. Swinton, 1140
- Human Forearm : Evidence for the Release of Individual Amino-acids from the Resting (Dr. D. R. London et al.), 588
- Human Genetics and Studies of Twins : Research in, 230
- Human Growth Hormone : Further Purification and Characterization of a Placental Protein with Immunological Similarity to (Dr. H. Friesen), 1214
- Human Growth Hormone in the Presence of Actinomycin D : An *in vitro* Action of (T. E. Martin and Prof. F. G. Young), 684
- Human Haemoglobin A : Three Haemoglobins K : Woolwich, an Abnormal, Cameroon and Ibadan, Two Unusual Variants of (Dr. N. Allan et al.), 658
- Human Indigenous Microflora in Gnotobiotic Rats (Dr. H. A. Dymasz, G. S. Stoewsand, Dr. J. J. Enright, Prof. P. C. Trexler and L. C. Gall), 1236
- Human Intelligence, 1154
- Human Isolated Ileum : A Pharmacological Investigation of (Dr. A. Bennett), 1289
- Human Lymphocytes cultured in Microplates (Dr. J. A. Brody and B. Huntley), 1232
- Human Lymphocytes cultured with Phytohaemagglutinin : Cytoplasmic Labelling with Uridine-5-³H in (Dr. G. C. B. Winter and Prof. J. M. Yoffey), 1018
- Human Lymphocytes in Culture : Reaction of, to Components of the Medium (G. J. Johnson and Prof. P. S. Russell), 343
- Human Male and Female : Comparison of Salivary Amylase-levels in the (Prof. R. L. McGeachin et al.), 1211
- Human Myxosarcoma : Properties of Hyaluronic Acid from a (Dr. M. L. Marcante), 896
- Human Ovarian Oocytes : Maturation *in vitro* of Mouse, Shepp, Cow, Pig, Rhesus Monkey and (Dr. R. G. Edwards), 349
- Human Phosphoglucomutase : Evidence for a Second 'Structural' Locus determining (Dr. D. A. Hopkinson and Prof. H. Harris), 410
- Human Platelets : Plasma Co-factors in Adenosine Diphosphate-induced Aggregation of (Dr. D. Deykin et al.), 296
- Human Red Cell and Leucocyte Pyruvate Kinase : Kinetic Differences between (J. O. Campos et al.), 194
- Human Red Cell Membrane : Heterogeneity of Water-soluble Structural Components of (Prof. M. D. Poulik and Dr. P. K. Lauf), 874
- Human Senses and Perception (Prof. G. M. Wyburn, R. W. Pickford and R. J. Hirst), review by Prof. O. L. Zangwill, 981
- Human Spermatozoa of a Metabolite of the Female Reproductive Tract : Oxidative and Biosynthetic Utilization by (Prof. C. Terner), 1115
- Human Subjects : Relationship between the Electromyogram and the Force of the Isometric Reflex Response of Normal (A. M. Clarke), 551
- Human Uterine Muscle Strips : Electrical Activity in (Dr. T. B. MacLachlan et al.), 290
- Humanistic Technology (Vice-Admiral H. G. Rickover), 721
- Hume-Rothery, Prof. W. : Retirement from Isaac Wolfson Chair of Metallurgy in the University of Oxford, 627
- Humic Substances in Natural Water : Use of 'Sephadex' Gel for the Estimation of Molecular Weight of (E. T. Gjessing), 1091
- Humidity : Variation of the X-ray Sensitivity of *Serratia marcescens* with Relative (H. G. Morrison and Prof. D. V. Cormack), 91
- Hummel, Prof. Dieter : Identification and Analysis of Surface-active agents by Infrared and Chemical Methods, Two Volumes, review by Dr. P. Robson, 617
- Humphrey, Dr. J. H. : Methods in Cell Physiology, Vol. 1, review, 6
- Humphrey, Dr. J. H., and White, Prof. R. G. : Immunology for Students of Medicine, Second Edition, review by Dr. P. D'Arcy Hart, 1028
- Humphreys, J. M., et al. : Acid Phosphatases, 382
- Humphries, Mr. G. J. : Retirement as Director of Overseas Surveys of the Ministry of Overseas Development, and Surveys Adviser to the Minister, 118
- (*Humulus lupulus*, L.) : Relationships between Constituents of Cones and Male Flowers of the Hop (Dr. R. D. Hartley and Dr. R. A. Neve), 804
- Hundred Years of Anthropology (T. K. Penniman, Dr. J. S. Weiner and Miss B. Blackwood), Third Edition, revised, review by Prof. M. Fortes, 215
- Hundred Years of Chemistry (Prof. A. Findlay), Third Edition (revised by Dr. T. I. Williams), review by Dr. R. G. Partington, 969
- Hungary : Lower Palaeolithic Hominid and Pebble-industry in (Dr. M. Kretzoi and Dr. L. Vértes), 205
- Hungary, Science in (edited by Dr. T. Erdey-Grúz and Dr. I. Trencsényi-Waldapfel), review by Prof. D. Gabor, 983
- Hunt, Dr. D. R. : Native Vegetation of Nebraska, review, 1249
- Hunt, Harold H., et al. : Welding by High-velocity Particle Impact, 1273
- Hunter, L. D., and Kirby, Dr. A. H. M. : Identification of Dinitro-octylphenols in Certain Commercial Fungicides, 189
- Huntley, Bob, and Brody, Dr. J. A. : Human Lymphocytes Cultured in Microplates, 1232
- Hurwitz, A., and Courant, R., *Funktionentheorie*, Fourth Edition, review by Dr. J. C. Burkill, 963
- Husband, Dr. H. C. : Award of Royal Medal of the Royal Society, 1142
- Hutchinson, Dr. D. W. : Nucleotides and Coenzymes, review by D. M. Brown, 987
- Hyalomma dromedarii* (Ixodidae) : An Improved Method for obtaining *in vitro* Uniform Cell Monolayer Sheets from Tissues of the Tick, (Dr. M. G. R. Varma and Miss W. Wallers), 602
- Hyaluronic Acid from a Human Myxosarcoma : Properties of (Dr. M. L. Marcante), 896
- Hybrid between *Rubus procerus* Muell. and *R. laciniatus* Willd. : 'Complement Fractionation' in a Natural (Dr. R. K. Bamm), 608
- Hybridization : Polarization and (Dr. T. A. Claxton), 891
- Hydrazine : Dissociation Energy of the N-H Bond in (Dr. I. P. Fisher and Dr. G. A. Heath), 1199
- Hydride Component of Hydrogen Overpotential at Platinized Platinum and Palladized Palladium Cathodes : Diffusion or (M. N. Hull and Dr. F. A. Lewis), 887
- Hydrided Zirconium : Slow-bend Testing of (C. E. Coleman and Dr. D. Hardle), 69
- Hydrocarbon Molecules as a Function of Bombarding Electron Energy : Relative Ionization Efficiencies of (J. A. Takacs and Prof. G. R. Freeman), 996
- Hydrocarbons in the Liquid Phase : Oxidation of (edited by N. M. Emanuel), review by Dr. H. Steiner, 420
- Hydrocarbons subjected to a Silent Electric Discharge : Breakdown of Simple (P. E. Cross), 892
- Hydrocarbons with the Ionization Potential : Correlation of the Third Excited State of Polycyclic Aromatic (Dr. M. A. Slikin), 380
- Hydrocortisone Succinate on the Complement System : Effects of (Dr. H. Gewurz, P. R. Wernick, Dr. P. G. Que and Prof. R. A. Good), 755
- Hydroelectric Schemes : Scottish, 1153
- Hydroformylation using Ruthenium Complexes : Homogeneous Hydrogenation and (D. Evans et al.), 1203
- Hydrogen Content of Virgo Cluster Galaxies (Dr. B. J. Robinson and J. A. Koehler), 993
- Hydrogen Halides : Sub-millimetre Dispersion and Rotational Line Strengths of the (Dr. J. E. Chamberlain and Dr. H. A. Gebbie), 480
- Hydrogen Overpotential at Platinized Platinum and Palladized Palladium Cathodes : Diffusion or Hydride Component of (M. N. Hull and Dr. F. A. Lewis), 887
- Hydrogenation and Hydroformylation using Ruthenium Complexes : Homogeneous (D. Evans et al.), 1203
- Hydrology : Forest (J. C. Rodda), 1157
- Hydrolysis of Disaccharides during Absorption by the Perfused Small Intestine of Amphibia, (Dr. D. S. Parsons and J. S. Prichard), 1097
- Hydrolysis of Glycine Ethyl Ester : Protonation Equilibria and Alkaline (Dr. M. Robson), 265
- Hydrolysis of Two 17 α -Oestradiol Glycosides by Rabbit Tissue Glycosidases (K. S. Whittemore and Dr. D. S. Layne), 288
- Hydromagnetic Emissions at High Latitude : Spectral Density Functions of (Dr. A. Egeland), 539
- Hydroquinone : Interaction between Chlorophyll and (E. Fujimori and M. Tavia), 78
- Hydroxamates during Proteolysis by an Enzyme System from Yeast : Formation, of (Dr. D. E. Briggs and Dr. D. J. Millin), 287
- Hydroxyapatite : Fractionation of Mouse Deoxyribonucleic Acid on (Dr. P. M. B. Walker and Dr. A. McLaren), 1175
- Hydroxyapatite : Intermediate States in the Precipitation of (Prof. E. D. Eanes et al.), 365
- 5-Hydroxy-DL-tryptophan and 6-Hydroxy-DL-tryptophan by Paper and Thin-layer Chromatography : Resolution of the Optical Isomers of DL-Tryptophan, (Dr. S. F. Contracto and J. Wragg), 71
- Hydroxyl Molecules in Interstellar Space : Formation of (Dr. J. L. Symonds), 1195

- Hydroxylation of Cinnamic Acid in Gherkin Hypocotyls : Photo-induced (Dr. G. Engelsma), 1117
- 4-Hydroxypyrazolo(3,4-d)pyrimidine : Inhibition *in vivo* of Xanthine Dehydrogenase by (E. C. Keller, jun., and Dr. E. Glassman), 202
- Hygrometer for determining the Free Energy of Moisture in Unsaturated Soils : Thermistor (B. G. Richards), 608
- Hymenoptera, Symphyta) : Bilateral Asymmetry : a Larval Polymorphism in some Argid Sawflies (Dr. M. F. Claridge and Dr. J. M. Edington), 301
- Hynek, B., Bilek, Dr. J., and Lis, M. : Introduction of a Permanent Electrode into the Pituitary Gland of the Cow : Influence of Electrical Stimulation on Milk Ejection, 690
- Hynes, Prof. H. B. N. : Selective Effect of Particulate Insecticides on *Simulium* among Stream Fauna, 199
- Hyperammonaemic States : Acetohydroxamate : Bacterial Urease Inhibitor with Therapeutic Potential in (Dr. W. N. Fishbein *et al.*), 46
- Hyperlipaemia following Viral Infection in the Chicken Embryo : A New Syndrome (Dr. S. E. Grossberg and Prof. W. M. O'Leary), 954
- Hypersensitivity : Isolation of a Lymphoid-cell Protein with Relation to Delayed (U. Rothman and S. Lidén), 389
- Hypersensitivity Reactions of Neonatally Thymectomized Rats : Effect of a Lipid Fraction from Rat Thymus on Delayed (Prof. B. D. Janković *et al.*), 356
- Hypnosis : Regional Changes in Brain Serotonin after Head X-irradiation and its Significance in the Potentiation of Barbiturate (Dr. V. Nair), 1293
- Hypocotyls : Photo-induced Hydroxylation of Cinnamic Acid in Gherkin (Dr. G. Engelsma), 1117
- Hypotrichidia : Endogenous Bud Formation in the (Dr. C. R. Curds), 90
- Hypoxia : Life-shortening in Mice exposed to Radiation : Effects of Age and of (Dr. P. J. Lindop and Prof. J. Rotblat), 1070
- Hypoxia : Modification of Radiation Cerebral Death by (Dr. E. A. Wright and J. Shewell), 904
- Hysteresis Loop and Multistable Ferrites : Re-entrant (Dr. R. M. Glaister and Dr. E. W. Lee), 67
- Hyvärinen, H., and Saure, L. : Seasonal Changes in the Histological Structure of the Spinal Column of *Sorex araneus* (L.), 705
- I**
- IBADAN, Two Unusual Variants of Human Haemoglobin A : Three Haemoglobins K : Woolwich, an Abnormal, Cameroon and (Dr. N. Allan *et al.*), 658
- Ibáñez, Manuel L., *et al.* : Respiratory Changes in the Cacao Seed Cotyledon coincident with Seed Death, 1348
- Ice Ages : Wilson's Theory of (J. T. Hollin), 12
- Ice Crystals : Pairs of Spiral Etch Pits in (J. Muguruma), 180
- Ice : Physics of (Prof. E. R. Pounder), review by Dr. J. Hallett, 986
- Ice Sheets : Antarctic Aeronomy and (Dr. G. de Q. Robin), 439
- Ideas : Routes of Epidemics and Ideologies : Germs and (Dr. A. Siegfried), review by F. Hawking, 1249
- Identical S Alleles in Different Botanical Varieties of *Brassica oleracea* (K. F. Thompson and J. P. Taylor), 306
- Identification and Analysis of Surface-active Agents by Infrared and Chemical Methods (Prof. D. Hummel), Two Volumes, review by Dr. P. Robson, 617
- Identification of Dinitro-octylphenols in Certain Commercial Fungicides (Dr. A. H. M. Kirby and L. D. Hunter), 189
- Identification of Medical Bacteria : Manual for the (S. T. Cowan and K. J. Steel), review by Dr. D. Allan, 213
- Identification of Organic Compounds : Systematic, Fifth Edition (R. L. Shriner, R. C. Fuson and D. Y. Curtin), review by Dr. D. T. Lewis, 1032
- Identification of the D(13-15) Chromosome responsible for Di Trisomic Patan's Syndrome : Autoradiographic (Dr. F. Giannelli), 669
- Igneous Masses of East Aberdeenshire : Corona Structures in the Basic (Dr. D. S. Weedon), 885
- Iguanidae : Interspecific Karyotypic Variation as a Systematic Character in the Genus *Anolis* (Sauria) (G. C. Gorman), 95
- Ikeda, H., and Arden, Dr. G. B. : A New Property of the Early Receptor Potential of Rat Retina, 1100
- Ileum : A Pharmacological Investigation of Human Isolated (Dr. A. Bennett), 1289
- Illite and Montmorillonite : Interstratified Mineral of (Prof. M. Sato *et al.*), 179
- Ilyinsky, Dr. O. B. : Processes of Excitation and Inhibition in Single Mechanoreceptors (Pacinian Corpuscles), 351
- Imitation : with Special Reference to the Antiphonal Singing of Birds : Origin and Significance of the Power of Vocal (Dr. W. H. Thorpe and M. E. W. North), 219
- Immune Agglutination : Mathematical Model for the Process of Aggregation in (T. K. Ming *et al.*), 84
- Immune Globulin from Rainbow Trout Serum : Electrophoretic Mobility of an (H. O. Hodgins *et al.*), 1106
- Immune System in a Monotreme : Studies on the Australian Echidna (*Tachyglossus aculeatus*) (Dr. E. Diener and Dr. E. H. M. Ealey), 950
- Immunity to Malaria : Antigenic Variation in Chronic Infections of *Plasmodium knowlesi* (Dr. K. N. Brown and I. N. Brown), 1286
- Immunobiology : Thymus in (edited by R. A. Good and A. E. Gabrielsen), review by Dr. R. B. Taylor, 318
- Immunofluorescence or Labelled Antigen Indirect Immunofluorescence Staining : Mixed Antiglobulin (Prof. E. H. Beutner *et al.*), 353
- Immunogenic Activity of Lipids of *M. tuberculosis* (Dr. B. Stoss and Dr. R. Herrmann), 1224
- Immunological Competence : Delayed Effect of Thymectomy in Adult Life on (Dr. D. Metcalf), 1336
- Immunological Responsiveness after Thymectomy in Adult Life : Decay of (R. B. Taylor), 1334
- Immunological Responsiveness : Effect of Thymectomy in Adult Mice on (Dr. J. F. A. P. Miller), 1337
- Immunological Similarity to Human Growth Hormone : Further Purification and Characterization of a Placental Protein with (Dr. H. Friesen), 1214
- Immunological Tests in Helminthic Diseases (Dr. R. Elsdon-Dew), 696
- Immunological Theory of Cancer to Multiple Causes : Implications of the (Dr. M. R. Anderson and Prof. H. N. Green), 338
- Immunologically Competent Cells in the Adult Mouse Bone Marrow as the Major Source of Potential (Dr. M. L. Tyan and Dr. L. J. Cole), 1223
- Immunology for Students of Medicine (J. H. Humphrey and Prof. R. G. White), Second Edition, review by Dr. P. D'Arcy Hart, 1028
- Immunology, I : Modern Trends in (edited by Prof. R. Cruickshank), review by Dr. F. T. Perkins, 9
- Immunology of the Mouse Mammary Tumor Virus (MTV) : A Qualitative *in vitro* Assay for MTV (P. B. Blair), 165
- Impact : Welding by High-velocity Particle (Dr. R. F. Rolsten *et al.*), 1273
- Implications of the Immunological Theory of Cancer to Multiple Causes (Dr. M. R. Anderson and Prof. H. N. Green), 338
- Impounded Adult Salmon : Control of Furunculosis in (A. C. Leaman), 1344
- Improving the Civil Service Image, 311
- Inactivation of T-2 Bacteriophage by Sensitized Leucocytes *in vitro* (R. Young and Dr. F. H. Ruddle), 1105
- Inactivation of Plant Viruses : Antiviral Action of Oxidized Spermine- (U. Bachrach *et al.*), 1095
- Inconvertible L-1 Lymphosarcoma to Ascitic Form : Conversion of the (Prof. M. R. Sikov), 902
- Incorporation of Amino-acid in Rat Intestine *in vitro* : Accumulation and (Dr. J. R. Bronk and Dr. C. S. Parsons), 785
- Increase during Winter in Capacity for Root Regeneration in Detached Shoots of Fruit-tree Rootstocks (Dr. B. H. Howard), 912
- Increased Sensitivity of *in vitro* Murine Leukaemia Cells to Fractionated X-rays and Fast Neutrons (Dr. W. L. Caldwell and Prof. L. F. Lamerton) and (Dr. K. Bewley), 168
- Incubation Behaviour of the Two Sexes in Certain Birds with Special Reference to Phalaropes : Prolactin and the Incidence of Brood Patch Formation and (Prof. E. O. Hohn and S. C. Cheng), 197
- Incubation Temperature, Effect of, on the Flagellation of Gram-negative Rods (D. A. Orr and Dr. M. M. Taylor), 1017
- Index 1964 Annual Cumulation : Science Citation, Eight Parts (edited by E. Garfield), review by C. W. Cleverdon, 717
- India : Central Building Research Institute, Roorkee, 629
- Grassland and Fodder Resources in (R. O. Whyte), Revised Edition, review by Dr. W. Davies, 821
- National Institute of Sciences of, 936
- Occurrence of Gnatalezn Pollen Grains in the Katrol Formation (Upper Jurassic) of Kutch, (Y. K. Mathur and Mrs. K. Mathur), 912
- India, Ltd. : Fertilizer Corporation of, 734
- Indian Ocean : Record of the Bivalve Gasteropod *Berthellina limax* (Kawaguti and Baba 1959) from the (K. P. Rao), 404
- Indian Scientific Periodicals : Directory of, 434
- Indian Woods, Identification, Properties and Uses, Vol. 2 : Linaceae to Moringaceae (edited by S. S. Ghosh, K. R. Rao, S. K. Purkayastha, Dr. K. A. Chowdhury *et al.*), review by C. R. Metcalfe, 212
- Indirect Immunofluorescence Staining : Mixed Antiglobulin Immunofluorescence or Labelled Antigen (Prof. E. H. Beutner *et al.*), 353
- Indole and Related Compounds : Disruption of Protozoa by (Dr. R. W. Bailey and G. B. Russell), 1001
- Indole Uptake in *Escherichia coli* : Interference by the Carcinogenic 4-Nitroquinoline N-Oxide of Tryptophan and (A. C. Zahalsky and Prof. S. L. Marcus), 296
- Indolyl-3-acetic Acid : Stimulation of Synthesis of Ribonucleic Acid in Subapical Sections of *Avena Col-optile* by (Prof. T. H. Hamilton *et al.*), 1180
- Induction of Crossing-over in *Drosophila* Males by Means of Ovarian Extract (O. S. Reddi, G. M. Raddy and M. S. Rao), 203
- Induction of Intestinal Tumours by N-Ethyl-N-nitrosourea (Dr. R. Schoental), 300
- Induction of Malignant Lymphomas by Urethane in Adult Mice bearing Syngeneic Thymus Grafts (Dr. L. Fiore-Donati, L. Chicco-Bianchi, G. Tridente and L. Mazzarella), 398
- Induction of Tension Wood with the Anti-auxin 2,3,5-Tri-iodobenzoic acid (Prof. R. W. Kennedy and Prof. J. L. Farrar), 406
- Industrial Gerontology in Great Britain : Social and (C. E. Fleming), 623
- Industrial Research and Development in France, 834
- Industrial Research in New Zealand : Scientific and, 1156
- Industrial Research and the Research Associations, 1
- Industrial Society : Ecology and the (edited by G. T. Goodman *et al.*), review by Dr. A. Macfadyen, 613
- Industries : Research and Development in the Building and Civil Engineering, 629
- Industry : Melville on Macromolecule Chemical, 836
- Pharmaceutical, 121
- Research and Development in British, 101
- Scientific Research in the Development of Polish, 120
- Thermal Comfort in, 122
- Training for Management in, 613
- Infantile Treadmill Experience on Body-weight and Resistance to Exhaustion in the Rat : Effect of (R. A. Levitt and Prof. W. B. Webb), 1128
- Infarction : Aldosterone in Myocardial, 836
- Infected Mosquitoes : Transmission of Guamá and Oriboca Viruses by Naturally (A. Toda and R. E. Slope), 304
- Infection : Glove Damage a Cause of Wound, 836
- Infection of African Green Monkey Kidney Cells by Adenovirus 7 and SV 40 : Mixed (Dr. R. K. Wertz, C. C. O'Connor, Dr. A. S. Rabson, and G. T. O'Connor), 1350
- Infection of the Nematode *Caetartagia circumcincta* in the Sheep : Establishment of a Pure (J. W. Copland), 1229
- Infection Rates : Experimental Infections of Cattle with *Fasciola hepatica* : A Comparison of Low and High (J. G. Ross), 907
- Infections of *Plasmodium knowlesi* : Immunity to Malaria : Antigenic Variation in Chronic (Dr. K. N. Brown and I. N. Brown), 1286
- Infectious R Factors in Enterobacteriaceae : Penicillinase Synthesis controlled by (Dr. N. Datta and Dr. P. Kontomichalou), 239
- Infectious Laryngotracheitis Virus : Nucleic Acid of (G. A. Tannock), 711
- Inference : Statistical (Prof. J. C. R. Li), Two Volumes, review by Prof. D. V. Lindley, 420
- Influence of Heavy Water (Deuterium Oxide) on Plaque Size of Western Equine Encephalitis Virus (R. I. Carp and Dr. D. Kritchevsky), 93
- Influence of Heterologous Histone on the Synthesis of Cellular and Viral Ribonucleic Acid and Production of Haemagglutinin (A. G. Bukrinskaya, A. K. Gitelman and I. M. Slapiro), 557
- Influence of the Cerebral Cortex on the Cuneate Nucleus of the Monkey (Dr. F. Harris, Dr. S. J. Jabbar, Dr. R. W. Morse and Dr. A. L. Towe), 1215
- Influenza Virus Haemagglutination by Polymerized Orosomucoid : Inhibition of (P. H. Whitehead *et al.*), 915
- Information Activities of the U.S. National Academy of Sciences, 1260
- Information and Documentation Services : Science, 122
- Information in Parliament : Scientific and Technological, 209
- Information Retrieval : Some Fundamentals of (J. R. Sharp), review by P. C. Williams, 822
- Information Services : The Government Explains : a Study of the (Mrs. M. Ogilvy-Webb), review by R. Brightman, 1139
- Infrared and Chemical Methods : Identification and Analysis of Surface-active Agents by (Prof. D. Hummel), Two Volumes, review by Dr. P. Robson, 617
- Infrared Emission Spectra of Minerals (Prof. M. J. D. Low), 1089
- Infrared Investigation of the Effect of Pressure on Silica Powders, as revealed by Deuterium Oxide Exchange, (F. H. Hambleton, Dr. J. A. Hockey and J. A. G. Taylor), 138
- Infrared Spectra of Quaternary Ammonium Compounds adsorbed on Silica-gel 'Aerosil' (Dr. L. C. E. Blackman and R. Harrop), 777
- Infra-red Spectroscopy to Determine the Orientation of Pyridine sorbed on Montmorillonite : Use of (J. M. Serratos), 679

- Ingestion of Soil by Sheep in New Zealand in Relation to Wear of Teeth (W. B. Healy and T. G. Ludwig), 806
- Inhelder, Barbel, and Piaget, J.: Early Growth of Logic in the Child, review by Dr. J. H. Duthie, 1246
- Inherited Coagulation Disorder, caused by an Abnormal Fibrinogen ('Fibrinogen Baltimore'): New (Dr. E. A. Beck *et al.*), 143
- Inhibition by Azetidine-2-carboxylic Acid: Synthesis of Ribonucleic Acid by *Escherichia coli* during (Dr. J. M. Midgley and Dr. D. G. Wild), 585
- Inhibition in Single Mechanoreceptors (Pacinian Corpuscles). Processes of Excitation and (Dr. O. B. Ilyinsky), 351
- Inhibition in the Cerebellar Cortex: Cholinergic Excitation and (P. Kása and Dr. B. Csillik), 695
- Inhibition in the Lateral Geniculate Body: Evidence for Pre-synaptic (A. Angel *et al.*), 495
- Inhibition *in vivo* of Xanthine Dehydrogenase by 4-Hydroxypyrazolo (3,4-d) pyrimidine (E. C. Keller, jun., and Dr. E. Glassman), 202
- Inhibition of Anaphylactic Release of Vascular Permeability Factor or Histamine by Specific Protease Inhibitor in Tissue Culture (Prof. H. Hayashi, Y. Kinuwaki and M. Yoshinaga), 1007
- Inhibition of Conjugation by Anti-inflammatory Drugs (Dr. T. Hargreaves), 1101
- Inhibition of Corrosion of Copper in Chromic Acid (K. F. Lorking), 778
- Inhibition of Deoxyribonucleic Acid and Ribonucleic Acid Synthesis Induced in Cultured Mammalian Cells by 7:12-Dimethylbenzanthracene: Differential (L. J. Alfred), 1339
- Inhibition of Growth of Malignant Cells *in vitro* by a Component of Normal Adult Connective Tissue (Prof. M. S. Parsley and I. Mandl), 800
- Inhibition of Haemolysis by 'Cremophor' in Conserved Blood (Dr. K. Reber), 195
- Inhibition of Influenza Virus Haemagglutination by Polymerized Orosomucoid (P. H. Whitehead, Dr. T. H. Flewett, J. R. Foster and Dr. H. G. Sammons), 915
- Inhibition of Transplantable and Spontaneous Murine Tumours by the M-P Virus (Dr. N. Molomut and Dr. M. Padnos), 948
- Inhibition of Tumour Cells by *in vitro* Contact with Cells containing Foreign H-2 Antigens. Allogeneic (K. E. Hellstrom *et al.*), 458
- Inhibitor in Tissue Culture: Inhibition of Anaphylactic Release of Vascular Permeability Factor or Histamine by Specific Protease (Prof. H. Hayashi *et al.*), 1007
- Inhibitor with Therapeutic Potential in Hyperammonaemic States: Acetohydroxamate: Bacterial Urease (Dr. W. N. Fishbein *et al.*), 46
- Inhibitory Effect of Allatecomized Males and Females on the Sexual Maturation of Young Male Adults of *Schistocerca gregaria* (Forsk.) (Orthoptera: Acrididae) (M. J. Norris and M. P. Pender), 1122
- Initiation of Chain Reaction under an Ultrasonic Wave Effect (Prof. I. E. Elpiner, A. V. Sokolskaya and M. A. Margulis), 945
- Injected Convulsant Barbiturate: Effects of Propylthiouracil and Thyroid Feeding on the Response of Mice to (Prof. A. J. Prange, jun., and Prof. M. A. Lipton), 791
- Injection into Rats: Distribution of Plutonium in Serum Proteins following Intravenous (G. Boocock and D. S. Popplewell), 282
- Inman, Dr. D., and Graves, A. D.: Adsorption and the Differential Capacitance of the Electrical Double-layer at Platinum/Halide Metal Interfaces, 481
- Innervation of the Pars Distalis of the Pituitary of the Eel: A Dual Neurosecretory (Sir Francis Knowles and L. Vollrath), 1343
- Inorganic Analysis: Structure, Behavior and Application to Organic Complexing Reagents (D. D. Perrin), review by Dr. T. S. West, 3
- Insect 'Bitometer' (P. Kashin and H. G. Wakeley), 462
- Insect: Changes in Size of the Corpus Allatum in a Polymorphic (D. White), 807
- Insect Larvae in Nigeria: Micro-organisms associated with Dead (Dr. N. Okafor), 1015
- Insect Metamorphosis: Changes in the t-RNA Methylating Enzymes during (Dr. B. S. Baliga), 555
- Insect *Pyrrhocoris apterus*: Effect of Certain Types of Paper on Sexual Maturation of the (Prof. C. A. B. Clemenson), 510
- Insecticides on *Simulium* among Stream Fauna: Selective Effect of Particulate (Prof. W. E. Kershaw *et al.*), 199
- Instabilities in a Dipole Magnetic Field: Laboratory Investigations of Plasma (Prof. R. G. Quinn), 376
- Institute of Medical Research, Melbourne: Walter and Eliza Hall, 729
- Institute of Physics and the Physical Society (S. Weintraub), 232
- Institute of Technology: Materials Science and Engineering at the Massachusetts, 726
- Instrument Service in Britain: National, 528
- Instruments with: Careers with, 733
- Insulating Liquid: Deformation of Gas Bubbles and Liquid Drops in an Electrically Stressed (Dr. K. C. Kao), 279
- Insulin *in vitro*: Secretion of Newly Synthesized (S. L. Howell *et al.*), 487
- Intelligence: Human, 1154
- Intelligent Behaviour: A General Theory for the Evolution of (Dr. D. Stenhouse), 815
- Intensity Ratios of the Interstellar Lines of OH in Absorption and Emission: Anomalous (R. X. McGee *et al.*), 1193
- Interaction between Chlorophyll and Hydroquinone (E. Fujimori and M. Tavia), 78
- Interaction of Cephaloridine with Penicillinase-producing Gram-negative Bacteria (Dr. J. M. T. Hamilton-Miller, Dr. J. T. Smith and Prof. R. Knox), 235
- Interactions in the Rat Liver Cell: Nucleo-cytoplasmic (E. R. Godwin *et al.*), 246
- Intercellular Matrix of the Intervertebral Disk in Ageing and in Prolapse (Dr. T. K. F. Taylor and Dr. K. Little), 384
- Interconnection of Silicon in Modified Aluminium-Silicon Eutectic (J. A. E. Bell and Prof. W. C. Winegard), 177
- Interfaces: Adsorption and the Differential Capacitance of the Electrical Double-layer at Platinum/Halide Metal (A. D. Graves and Dr. D. Inman), 481
- Interference by the Carcinogenic 4-Nitroquinoline N-Oxide of Tryptophan and Indole Uptake in *Escherichia coli* (A. C. Zahalsky and Prof. S. L. Marcus), 296
- Interferometric Estimation of Effects of Testosterone and Methandrostenolone on the Dry Mass of Various Rat Muscles (Dr. W. Staubli and Dr. T. Okada), 562
- Interferon Appearance Stimulated by Endotoxin, Bacteria, or Viruses in Mice pre-treated with *Escherichia coli* Endotoxin or infected with *Mycobacterium tuberculosis* (Prof. J. S. Youngner and Prof. W. R. Stinebring), 456
- Interferon induced by Staolon: Different Molecular Species of Mouse (Prof. T. C. Merigan and Dr. W. J. Kleinschmidt), 667
- Interferon: Molecular Weight of Chick (Dr. D. C. Burke and Dr. J. Ross), 1297
- Intermediate States in the Precipitation of Hydroxyapatite (Prof. E. D. Eanes, Dr. I. H. Gillessen and Prof. A. S. Pcsner), 365
- International Astronautics Award: 1965 Guggenheim, 119
- International Biological Programme (Dr. E. B. Worthington), 223
- International Commission on Zoological Nomenclature, 1154
- International Committee on Laboratory Animals (Prof. J. A. B. Gray), 738
- International Commission on Radiological Protection: Officers and Members 1965-69, 936
- International Conference on Gerontology (edited by A. Balázs), review by Dr. David A. Hall, 981
- International Conference on Magnetism, Nottingham, September 1964: Proceedings of the, review by Dr. J. H. Davis, 1030
- International Conference on Mechanisms and Machines, 231
- International Conference on Operational Research, Oslo 1963: Proceedings of the 3rd (edited by G. Kreweras and G. Morlat), review by S. J. Morrison, 1245
- International Conference on Protozoology: Second (Prof. P. C. C. Garnham), 330
- I.C.S.U. Bulletin (No. 4; July 1965), 528
- International Directory of Isotopes, Third Edition (International Atomic Energy Agency), review by C. B. G. Taylor, 987
- International Esperanto Congress, 1264
- International Paper Size, 528
- International Recognition: Nutrition and its (Sir Rudolph A. Peters), 117
- International Symposium on Chemotherapy of Cancer, 1964: Proceedings of the (edited by Prof. P. A. Plattner), review by D. C. Roberts, 1032
- Interplanetary Dust Particles: Measured Velocities of (C. S. Nilsson *et al.*), 673
- Interspecies Differences in Response to Polypeptides as Permeability Factors (Dr. J. Carr and Prof. D. L. Wilhelm), 653
- Interspecific Karyotypic Variation as a Systematic Character in the Genus *Anolis* (Sauria: Iguanidae) (G. C. Gorman), 95
- Interstellar Grains: Growth of (Dr. M. J. Baines and I. P. Williams), 1191
- Interstellar Lines of OH in Absorption and Emission: Anomalous Intensity Ratios of the (R. X. McGee *et al.*), 1193
- Interstellar Obscuration (Dr. K. Nandy), 274
- Interstellar Space: Formation of Hydroxyl Molecules in (Dr. J. L. Symonds), 1195
- Interstimulus Interval and Number Judgment (D. Jones and Dr. A. Elithorn), 153
- Interstratified Mineral of Illite and Montmorillonite (Prof. M. Sato, K. Oinuma and K. Kobayashi), 179
- Interstratified (Mixed-layer) Minerals: Structure of (Dr. M. Sato), 70
- Interval and Number Judgment: Interstimulus (D. Jones and Dr. A. Elithorn), 153
- Intervertebral Disk in Ageing and in Prolapse: Intercellular Matrix of the (Dr. T. K. F. Taylor and Dr. K. Little), 384
- Intestinal Absorption: Transport of Methionine and Sodium Butyrate by Intracellular Plasma: Mechanism of (S. L. Hart and Dr. J. A. Nissim), 145
- Intestinal Factor of Normal Mice which inactivates Murine Virus Hepatitis: Distribution of the (Prof. M. Piazza *et al.*), 1009
- Intestinal Tumours by N-Ethyl-N-nitrosourea: Induction of (Dr. R. Schoental), 300
- Intestine *in vitro*: Accumulation and Incorporation of Amino-acid in Rat (Dr. J. R. Bronk and Dr. D. S. Parsons), 785
- Intestine of Amphibia: Hydrolysis of Disaccharides during Absorption by the Perfused Small (Dr. D. S. Parsons and J. S. Prichard), 1097
- Intracellular Chloride Activity of Crayfish Giant Axons (Dr. A. Strickholm and B. G. Wallin), 790
- Intracellular Plasma: Mechanism of Intestinal Absorption: Transport of Methionine and Sodium Butyrate by (S. L. Hart and Dr. J. A. Nissim), 145
- Intracranial Self-stimulation: Facilitation as a Function of Temporal Spacing of Stimuli in (Prof. J. A. Deutsch and R. Collins), 592
- Intra-mitochondrial Glucosyl-oligosaccharides from Glucose by Intact Novikoff Ascites Hepatoma Cells: Formation of (Dr. V. N. Nigam and Dr. G. de Lamiandé), 664
- Intravenous Injection into Rats: Distribution of Plutonium in Serum Proteins following (G. Boocock and Dr. S. Popplewell), 282
- Introduction of a Permanent Electrode into the Pituitary Gland of the Cow: Influence of Electrical Stimulation on Milk Ejection (B. Hynek, Dr. J. Bilek and M. Lis), 690
- Introduction to Lattice Theory (Prof. D. E. Rutherford), review by Prof. T. A. A. Broadbent, 984
- Introduction to Plate and Shell Theory (C. E. Turner), review by Prof. B. N. Cole, 1137
- Inulin Solutions: Deterioration of Stored (S. Nilwarangkur and Dr. G. M. Berlyne), 77
- Inventiveness and Creativity: Science, 921
- Inversion at the Nitrogen Atom in Certain Amides: Slow (Dr. T. H. Siddall and Dr. C. A. Prohaska), 582
- Invertebrate Muscle: Energy Requirements for Relaxation from Tonic Contractions ('Catch') in an (Dr. K. Minihan and Prof. R. E. Davies), 1327
- Iodine-131, and Triolein Deiodination *in vitro*: Oleic Acid labelled with (L. J. Angliger), 896
- Iodine-131 in Milk: Evaluation of Ion Exchange Cartridges for Field Sampling of (R. H. Johnson, jun., and T. C. Reavey), 750
- Ion Exchange Cartridges for Field Sampling of Iodine-131 in Milk: Evaluation of (R. H. Johnson, jun., and T. C. Reavey), 750
- Ion Mass in the Upper Ionosphere with a Theoretical Structure: Comparison of Experimental Data of the Effective (P. R. Arendt), 443
- Ionization Coefficient for Compressed Nitrogen: Townsend's First (Dr. B. W. Ward), 994
- Ionization Efficiencies of Hydrocarbon Molecules as a Function of Bombarding Electron Energy: Relative (J. A. Takacs and Prof. G. R. Freeman), 996
- Ionization Potential: Correlation of the Third Excited State of Polycyclic Aromatic Hydrocarbons with the (Dr. M. A. Slifkin), 380
- Ionization Times in Shock-heated Potassium Vapour (R. M. Hill and B. Capp), 176
- Ionizing Radiation: A Potential Lightning Hazard? (P. J. Gillespie), 577
- Ionosphere, and X-ray Images of the Sun (Dr. C. M. Minnis), 476
- Ionosphere near the Auroral Zone: Variation according to Latitude of the Electron Content of the (L. Liszka), 280
- Ionosphere with a Theoretical Structure: Comparison of Experimental Data of the Effective Ion Mass in the Upper (P. R. Arendt), 443
- Ionospheric Radio Propagation (Dr. K. Davies), National Bureau of Standards Monograph 80, review by Dr. H. Rishbeth, 419
- Ions across Artificial Membranes: Effect of Trans-Solutes on the Fluxes of Chloride (S. Kitahara *et al.*), 187
- Ions: Far-infrared Spectra of some Square-planar (Dr. D. M. Adams and D. M. Morris), 283
- Ireland: Use of CIBA Resins in, 21
- Irish Bibliographical Material: A Guide to (A. R. Eager), review by H. J. Heaney, 1139
- Iron Dextran Complex (Dr. C. R. Ricketts), (Dr. J. S. G. Cox, C. Fitzmaurice and G. F. Moss), 237
- Iron during Accretion in the Solar Nebula: Enrichment of (Prof. S. R. Taylor), 886
- Iron in Ferredoxin and Rubredoxin: Nature of the Non-haem (Dr. R. D. Gillard *et al.*), 769
- Iron: Mechanism of Oxidation of Chromium Diffusion Coatings on (Dr. I. A. Menzies and D. Mortimer), 1307
- Iron Meteorite: The Wolf Creek (Prof. S. R. Taylor), 944
- Iron Ore Production and Research at Broken Hill, Australia, 22
- Irradiated and Unirradiated DNA in *Drosophila*: Mutagenic Effect of (A. H. Khan and Dr. T. Alderson), 700
- Irradiated Crystal Lattices: Correlated Atomic Collisions in (Dr. L. T. Chadderton and I. McC. Torrens), 880
- Irradiated *Drosophila* Medium: Lack of Mutagenic Effect of (Dr. O. S. Reddi *et al.*), 702

- Irradiated Medium or Irradiated DNA : Tests on *Drosophila* for the Production of Mutations-by (V. L. Chopra), 699
- Irradiated Polyethylene : Mechanism of Radical Decay in (Dr. D. O. Geymer and Dr. C. D. Wagner), 72
- Irradiated Sodium Chlorate : Pure Quadrupole Resonance Frequency Shift and Internal Stress in Gamma-ray (Dr. H. Budak), 382
- Irradiation Embrittlement of Low-boron Type 304 Stainless Steel (W. R. Martin, J. R. Weir, R. E. McDonald and J. C. Franklin), 73
- Irradiation of HeLa Cells at -196°C : Continuous (A. H. W. Nias and Dr. L. G. Lajtha), 400
- Irvine, D., et al. : A New Haemoglobin, J Toronto ($\alpha 5$ Alanine \rightarrow Aspartic Acid), 1059
- Irvine, D., et al. : Three Haemoglobins K : Woolwich, an Abnormal, Cameroon and Ibadan, Two Unusual Variants of Human Haemoglobin A, 658
- Isachsen Diabasic Rocks : Palaeomagnetism of the (A. Laroche et al.), 179
- Isaković, Dr. Katarina, et al. : Effect of a Lipid Fraction from Rat Thymus on Delayed Hypersensitivity Reactions of Neonatally Thymectomized Rats, 356
- Istense, H., et al. : Polymorphism of Lactate Dehydrogenase Isozymes in Pigeons, 284
- Island Ecosystem : Man's Place in the (edited by Dr. F. R. Fosberg), review by Sir L. Dudley Stamp, 109
- Isoindoline, Chloroxolone (M. and B. 8430) : Metabolism of 5-Chloro-2-cyclohexyl-1-oxo-6-sulphamoyl (K. Corbett et al.), 286
- Isolation and Amino-Acid Sequence of β -LPH from Sheep Pituitary Glands (Prof. Choh Hao Li, L. Barnafi, M. Chrétien and D. Chung), 1093
- Isolation of a Histidine-less Lysozyme : Lysozymes Characterized in Duck Egg-white : (Dr. J. Jollès et al.), 1204
- Isolation of a Lymphoid-cell Protein with Relation to Delayed Hypersensitivity (U. Rothman and S. Lidén), 389
- Isolation of Bacteria able to metabolize Fluoroacetate or Fluoroacetamide (Dr. M. Kelly), 809
- Isolation of *Cryptococcus neoformans* from Pigeon Habitats in London (Dr. H. S. Randhawa, Dr. Y. M. Clayton and Dr. R. W. Riddell), 801
- Isolation of Macronuclei from the Ciliate *Tetrahymena pyriformis* GL (Dr. Y. C. Lee and Prof. O. H. Scherbaum), 1350
- Isomers of DL-Tryptophan, 5-Hydroxy-DL-tryptophan and 6-Hydroxy-DL-tryptophan by Paper and Thin-layer Chromatography : Resolution of the Optical (Dr. S. F. Contractor and J. Wragg), 71
- Isometric Reflex Response of Normal Human Subjects : Relationship between the Electromyogram and the Force of the (A. M. Clarke), 551
- Isoelectricity in the Sera of Rats resistant to Tumour Transplantation (H. Hirai), 798
- Isozymes : Third Edition : International Directory of, review by C. B. G. Taylor, 987
- Isotopic Ages and Andean Uplift (Dr. R. W. R. Rutland, Dr. J. E. Guest) and (Dr. R. L. Grasty), 677
- Isozymes in Pigeons : Polymorphism of Lactate Dehydrogenase (Prof. Wm H. Zinkham et al.), 284
- Isozymes of Arylamidase (Aminopeptidase) : Source of Blood Aminoacyl Naphthylamide in the Rat (Dr. B. Monis), 587
- (Ixodidae) : An Improved Method for obtaining, *in vitro*, Uniform Cell Monolayer Sheets from Tissues of the Tick, *Hyalomma dromedarii* (Dr. M. G. R. Varma and Miss W. Wallers), 602
- JABBUR, Dr. S. J., et al. : Influence of the Cerebral Cortex on the Cuneate Nucleus of the Monkey, 1215
- Jack Brimble, 819
- Jackson, Prof. D. P., et al. : New Inherited Coagulations Disorder caused by an Abnormal Fibrinogen ('Fibrinogen Baltimore'), 143
- Jacob, Prof. F., et al. : Biological Systems at the Molecular Level, 1048
- Jacobs, Patricia A., Brunton, M., Melville, M. M., Brittain, Dr. R. P., and McClelland, Dr. W. F. : Aggressive Behaviour, Mental Subnormality and the XYY Male, 1351
- Jakolska, A., and Presland, A. E. B. : Microstructure of Fibrous Sulphur, 1088
- James Smithson Medal : Royal Society of London, 324
- James Smithson : Pathfinder in Science and Philanthropy (Dr. L. Carmichael), 321
- Janković, Prof. B. D., Isaković, Dr. K., and Horvat, J. : Effect of a Lipid Fraction from Rat Thymus on Delayed Hypersensitivity Reactions of Neonatally Thymectomized Rats, 356
- January : Night Sky in, 1264
- Jardine, F. H., et al. : Homogeneous Hydrogenation and Hydroformylation using Ruthenium Complexes, 1203
- Jassik-Gerschenfeld, Dr. Dora : Somesthetic and Visual Responses of Superior Colliculus Neurons, 898
- Jay, Mr. K. E. : Obituary by R. M. Fishenden, 625
- Jayasekara, Dr. W. P. Guile, Dr. A. E., and Naylor, Dr. K. A. : Surface Charges on Arc Track Oxide Layers, 774
- Jeffries, Sir Harold : Damping of S Waves, 675
- Jeffries, Dr. T. O. : Appointed Professor of Industrial Engineering in the University College of Swansea, 19
- Jennings, A. R., et al. : Association of Adenoviruses with Bovine Respiratory Disease, 307
- Jhala, Dr. H. I. : The Brilliant and Tragic Life of W. M. H. Haffkine, Bacteriologist, review, 418
- Joffe, Dr. J. M. : Effect of Foster-mothers' Strain and Pre-natal Experience on Adult Behaviour in Rats, 815
- Johnson, Prof. Bruce, and Dixon, Prof. J. R. : Ascent of Sap in Trees : Effect of Negative Absolute Pressure on Flow Resistance, 1347
- Johnson, G. D., et al. : A New Fluorescent Antibody Method : Mixed Antigen-antibody Immunofluorescence or Labelled Antigen Indirect Immunofluorescence Staining, 353
- Johnson, G. J., and Russell, Prof. P. S. : Reaction of Human Lymphocytes in Culture to Components of the Medium, 343
- Johnson, Dr. H. G., and Bach, Dr. M. K. : Apparent Suppression of Mutation Rates in Bacteria by Spermine, 408
- Johnson, Prof. N. L., and Leone, Prof. F. C. : Statistics and Experimental Design in Engineering and the Physical Sciences, Two Volumes, review by N. E. Day, 214
- Johnson, O. S., et al. : Acute Radio-sensitivity as a Function of Age in Mice, 905
- Johnson, jun., R. H., and Reavey, T. C. : Evaluation of Ion Exchange Cartridges for Field Sampling of Iodine-131 in Milk, 750
- Johnston, Dr. J. G., et al. : Release of Plasminogen Activator by the Isolated Perfused Dog Kidney, 291
- Johri, Prof. B. M., and Bhojwani, S. S. : Growth Responses of Mature Endosperm in Cultures, 1345
- Jollès, Dr. J., Spotorno, G., and Jollès, P. : Lysozymes Characterized in Duck Egg-white : Isolation of a Histidine-less Lysozyme, 1204
- Jollès, P., et al. : Lysozymes characterized in Duck Egg-white : Isolation of a Histidine-less Lysozyme, 1204
- Jolley, Dr. W. B., and Aller, H. W. : Formation of Complexes between Basic Proteins of Leucocytes and Plasma Globulins, 390
- Jones, D., and Elithorn, Dr. A. : Interstimulus Interval and Number Judgment, 153
- Jones, Dr. Eric L. : Tschetchev Approximation to the Equation of Thermogravimetric Data, 135
- Jones, Dr. E. P. : Obituary by Dr. G. S. Hartley, 431
- Jones, Dr. K. : Genetical Principles and Plant Breeding, review, 7
- Jones, Dr. O. T. G., and Watson, W. A. : Activity of 2-Trifluoromethylbenzimidazoles as Uncouplers of Oxidative Phosphorylation, 1169
- Joseph, Dr. J., and Dyson, Dr. M. : Sex Differences in the Rate of Tissue Regeneration in the Rabbit's Ear, 599
- Journals of David E. Lilienthal : Two Volumes, review by Sir John Cockcroft, 962
- Jowett, Dr. A., and Harris, Prof. C. C. : Size Analysis in the Sub-sieve Range by Electronic Counter, 175
- Judgment : Interstimulus Interval and Number (D. Jones and Dr. A. Elithorn), 153
- Julius, Prof. R. S., et al. : Relative Similarities in One Dimension, 544
- Juniper, Dr. B. E., and Clowes, Dr. F. A. L. : Cytoplasmic Organelles and Cell Growth in Root Cap, 864
- Jupiter and Saturn : Radiation Balance of (T. Owen and T. E. Walsh), 476
- Jupiter's Magnetospheric Tail to the Radio Emissions of the Planet in the Decametric Region : Possible Contribution of (G. M. Gruber), 1271
- Jurriens, G. [the late], and Koch, G. K. : Quantitative Chromatographic Analysis of Organic Acids by Tritiating Methylation, 1312
- Juvenile Hormone (Sir Vincent Wigglesworth), 522
- Juvenile-to-Mature Growth Phase Transition in *Hedera* : Anatomical Changes associated with (J. R. Goodin), 504
- Juxtaglomerular Cells in the Mouse Kidney : Vital Staining of the Granulated (V. Szokoly et al.), 1331
- KACHANI, Dr. Z. F. Ch. : Propagation and Properties of Hepatitis Virus, 605
- Kampa, Dr. Elizabeth M., et al. : Measurements of Spontaneous Bioluminescence in the Sea, 1078
- Kanamycin-inactivating Enzymes from Multiple Drug-resistant *Escherichia coli* carrying Episome 'F' : Chloramphenicol-, Dihydrostreptomycin-, and (S. Okamoto and Y. Suzuki), 1301
- Kanasewich, Prof. E. R. : Seismicity and other Properties of Geological Provinces, 1275
- Kanazawa, T., et al. : Strontium-90 and Caesium-137 in Columbia River Plume, July 1964, 1084
- Kanetsuna, Dr. F., and Carbonell, Dr. L. M. : Glycogen in Yeast Form of *Paracoccidioides brasiliensis*, 686
- Kao, Dr. K. C. : Deformation of Gas Bubbles and Liquid Drops in an Electrically Stressed Insulating Liquid, 279
- Kaolinite by Electron Microscopy : Examination of a Polysaccharide Flocculant and Flocculated (Dr. A. Audsley and A. Fursey), 753
- Kaplan, Dr. Deborah, and Koch, Dr. W. : Micromethod for Measuring Microbial Sensitivity to Drugs, 50
- Karyotypic Variation as a Systematic Character in the Genus *Anolis* (Sauria : Iguanidae) : Interspecific (G. C. Gorman), 95
- Kása, P., and Csillik, Dr. B. : Cholinergic Excitation and Inhibition in the Cerebellar Cortex, 695
- Kasakura, Dr. S., and Lowerstein, Prof. L. : A Factor stimulating DNA Synthesis derived from the Medium of Leucocyte Cultures, 794
- Kashin, P., and Wakeley, H. G. : An Insect 'Bitometer', 462
- Katrol Formation (Upper Jurassic) of Kutch, India : Occurrence of Gnetalean Pollen Grains in the Y. K. Mathur and Mrs K. Mathur), 912
- Katsuragi, Y., et al. : Strontium-90 and Caesium-137 in Columbia River Plume, July 1964, 1084
- Kavanau, Prof. J. L., and Erant, Prof. D. H. : Wheel-running Preferences of *Peromyscus*, 597
- Ke, Dr. Bacon : Optical Rotatory Dispersion of a Chloroplast Preparation, 573
- Kearns, Prof. H. G. H. : Obituary of Sir Ronald Hatton, 1258
- Keeble, S. A., and Heymann, C. S. : Effect of β -Propiolactone and Thiomersal on Growth of B.H.K. Cells in the Hamster Cheek Pouch, 1125
- Keith, Prof. M. L., and Allen, Prof. P. : Carbon Isotope Ratios and Palaeosalinities of Purbeck-Wealden Carbonates, 1278
- Keldysh, Prof. M. V. : Coferment of 1965 Guggenheim International Astronautics Award, 119
- Keller, jun., E. C., and Glassman, Dr. E. : Phenocopies of the *mal*- and *ry* Mutants of *Drosophila melanogaster* : Inhibition *in vivo* of Xanthine Dehydrogenase by 4-Hydroxypyrazolo (3,4-d)pyrimidine, 202
- Kellogg, Rhoda, Knoll, Prof. M., and Kugler, Dr. J. : Form-similarity between Phenocopies of Adult- and pre-School Children's Scribbles, 1129
- Kelly, Dr. M. : Isolation of Bacteria able to metabolize Fluoroacetate or Fluoroacetamide, 809
- Kelvin Statement of the Second Law : Carathéodory's Principle and the (J. Dunning-Davies), 574
- Kendrew, Dr. J. C. : Award of Royal Medal of the Royal Society, 1141
- Kendrick-Jones, J., and Perry, Prof. S. V. : Enzymatic Adaptation to Contractile Activity in Skeletal Muscle, 1058
- Kennedy, Prof. J. W., et al. : Nuclear and Radiochemistry, Second Edition, review by Dr. H. A. C. McKay, 105
- Kennedy, Prof. R. W., and Farrar, Prof. J. L. : Induction of Tension Wood with the Anti-auxin 2,3,5-Tri-iodobenzoic Acid, 406
- Kenney, Dr. J. D., and Mittwoch, Dr. U. : Barr Bodies in the Mouse, 93
- Kenya Genet : Sulphocysteine in the Urine of the Bloated (J. C. Crawhall and Dr. S. Segal), 1320
- Kepler's Dream (J. Lear), review by Dr. B. Pagel, 960
- Keratin : Changes in Amino-nitrogen Content of Solutions of γ -Keratin from Wool (Dr. R. S. Asquith and P. A. Watson), 786
- γ -Keratin from Wool Keratin : Changes in Amino-nitrogen Content of Solutions of (Dr. R. S. Asquith and P. A. Watson), 786
- Kerr, J. R. W., et al. : Fall-out in London Rain-water during 1964, 1083
- Kerr, R. M., and Fleming, Dr. R. J. : Thermoluminescence and Ultra-violet Phosphorescence of the Nucleic Acid Bases, 1198
- Kershaw, Prof. W. E., Williams, T. R., Frost, S., and Hynes, Prof. H. B. N. : Selective Effect of Particulate Insecticides on *Simulium* among Stream Fauna, 199
- Keto-acids in Tissues during Diphtheria Intoxication : Accumulation of (Dr. S. C. Agarwal), 914
- Kety, Prof. Seymour S. : Biochemistry and Mental Function, 1252
- Keynan, A., et al. : Timing of Protein Synthesis during Germination and Outgrowth of Spores of *Bacillus cereus* Strain T, 710
- Khabaza, I. M. : ALGOL 64 Implementation, review, 217
- Khabaza, I. M. : Application of Continued Fractions and their Generalizations to Problems in Approximation Theory, review, 318
- Khan, A. H., and Aldersoe, Dr. T. : Mutagenic Effect of Irradiated and Unirradiated DNA in *Drosophila*, 700

- Khan, Dr. A. W. : Objective Evaluation of Quality in Poultry Meat, 204
 Khartoum, Sudan : University of, Faculty of Agriculture, Shambat, (Prof. M. A. Nour), 620
- Khovanskii, A. N. : Application of Continued Fractions and their Generalizations to Problems in Approximation Theory, review by I. M. Khabaza, 318
- Kidney : Release of Plasminogen Activator by the Isolated Perfused Dog (Dr. R. Holemans *et al.*), 291
- Kidney : Vital Staining of the Granulated Juxtaglomerular Cells in the Mouse (V. Szokoly *et al.*), 1331
- Kidney Cells by Adenovirus 7 and SV 40 : Mixed Infection of African Green Monkey (Dr. R. K. Wertz *et al.*), 1350
- Kidney of the Rat : Effect of 'Furosemid' on Chloride and Water Excretion in Single Nephrons of the (G. Malnic *et al.*), 80
- Kilmister, Prof. C. W. : Special Theory of Relativity, review, 986
- Kinesis (T. G. Amos), 908
- Kinetic Differences between Human Red Cell and Leucocyte Pyruvate Kinase (J. O. Campos, R. D. Koler and R. H. Bigley), 194
- Kinetic Parameters for Oxygen in Reactions Involving Purified Oxidases and Tissue Slices (Dr. M. McCabe and Dr. D. A. Gilbert), 450
- Kinetics of Formation of the Monoacetylacetonato-Iron (III) Complex (W. K. Ong and Dr. R. H. Prince), 1201
- Kinetin of the Polar Transport of Two Auxins : Promotion by (Dr. C. C. McCready *et al.*), 1065
- King, Dr. G. W. : Spectroscopy and Molecular Structure, review by Dr. R. F. Barrow, 214
- King-Hele, D. G. : Dynamics of Rockets and Satellites, review, 516
- Visual Observation of Satellites, 1156
- Kinuaki, Y., *et al.* : Inhibition of Anaphylactic Release of Vascular Permeability Factor or Histamine by Specific Protease Inhibitor in Tissue Culture, 1007
- Kirby, Dr. A. H. M., and Hunter, L. D. : Identification of Dinitro-octylphenols in Certain Commercial Fungicides, 189
- Kirby, Dr. K. S., *et al.* : Alteration of Hamster Cells by Nucleic Acid *in vitro*, 1072
- Fractionation of Ribonucleic Acids in the Zonal Ultracentrifuge, 645
- Kirkman, Prof. H., *et al.* : Androgen-Oestrogen-induced Tumours : Lactic Dehydrogenase Relative to Chaetepithelioma Induction in the Syrian Hamster, 567
- Kltahara, S., Hienz, Dr. E., and Stahlmann, C. : Effect of *Trans*-Solutes on the Fluxes of Chloride Ions across Artificial Membranes, 187
- Klammer, Dr. O. : Separation of High-molecular Deoxyribonucleic Acid and Ribonucleic Acid, 1318
- Klein, A. K., *et al.* : Metabolites in Urine of Rats on Diets containing Aldrin or Dieldrin, 289
- Klein, Prof. E., and Matejec, R. : Mitteilungen aus den Forschungslaboratorien der Agfa-Gevaert AG Leverkusen-Munchen, Band IV, review by K. O. Ganguin, 720
- Kleinschmidt, Dr. W. J., and Merigan, Prof. T. C. : Different Molecular Species of Mouse Interferon Induced by Statolon, 667
- Klucis, E. S., *et al.* : Fractionation of Ribonucleic Acids in the Zonal Ultracentrifuge, 645
- Klyne, Prof. W. : Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry, review, 970
- Knoll, Prof. M., *et al.* : Form-similarity between Phosphenes of Adults and pre-School Children's Scribbles, 1129
- Knowles, Dr. A. : Recent Progress in Photobiology, review, 974
- Knowles, Sir Francis, and Vollrath, L. : A Dual Neurosecretory Innervation of the Pars Distalis of the Pituitary of the Eel, 1343
- A Functional Relationship between Neurosecretory Fibres and Pituitary Cells in the Eel, 1343
- Knox, Prof. R., *et al.* : Interaction of Cephaloridine with Penicillinase-producing Gram-negative Bacteria, 235
- Kobayashi, K., *et al.* : Interstratified Mineral of Illite and Montmorillonite, 179
- Koch, G. K., and [the late] Jurrrens, G. : Quantitative Chromatographic Analysis of Organic Acids by Tritiating Methylation, 1312
- Koch, Prof. R., and Mong, Dr. H. : Electron Spin Resonance Investigations of Ultra-violet and X-irradiated Homocysteine-thiolactone, 86
- Koch, R., *et al.* : Influence of Oxygen and Cysteine on the Radical State of X-irradiated Lyophilized Liver, 499
- Koch, Dr. W., and Kaplan, Dr. D. : Micromethod for Measuring Microbial Sensitivity to Drugs, 50
- Koehler, J. A., and Robinson, Dr. B. J. : Hydrogen Content of Virgo Cluster Galaxies, 993
- Koestler, Arthur : Biological and Mental Evolution : an Exercise in Analogy, 1033
- Koler, R. D., *et al.* : Kinetic Differences between Human Red Cell and Leucocyte Pyruvate Kinase, 194
- Komatsu, H., *et al.* : Laser-induced Damage in Diamond, 1081
- Kondrat'eva, E. N. : Photosynthetic Bacteria, review by Prof. T. W. Goodwin, 977
- Kontomichalou, Dr. P., and Datta, Dr. N. : Penicillinase Synthesis controlled by Infectious R Factors in Enterobacteriaceae, 239
- Kontinen, Dr. A., and Lindy, S. : Denaturation of Lactic Dehydrogenase Isozymes and its Clinical Application, 782
- Kos, E., *et al.* : Metabolism of Nucleic Acids and Protein in Starving Bacteria, 303
- Kosow, Prof. I. L. : Electrical Machinery and Control, review by Prof. J. C. West, 9
- Kostial, K., Vojvodic, S., and Comar, Prof. C. L. : Effects of Dietary Levels of Phosphorus and Calcium on the Comparative Behaviour of Strontium and Calcium, 1110
- Kotaka, S., Krueger, Prof. A. P., Andriese, P. C., Nishizawa, K., Ohuchi, T., Takenobu, M., and Kozure, Y. : Air Ion Effects on the Oxygen Consumption of Barley Seedlings, 1112
- Kovács, A., *et al.* : Labelling of Beta-lipoprotein with Tritium, 381
- Kozlowski, Prof. T. T., and McCracken, I. J. : Thermal Contraction in Twigs, 910
- Kozure, Y., *et al.* : Air Ion Effects on the Oxygen Consumption of Barley Seedlings, 1112
- Kretzoi, Dr. M., and Vértés, Dr. L. : Lower Palaeolithic Hominid and Pebble-industry in Hungary, 205
- Kreweras, G., and Morlat, G. (edited by) : Proceedings of the 3rd International Conference on Operational Research, Oslo 1963, review by S. J. Morrison, 1245
- Kritchevsky, Dr. D., and Carp, R. I. : Influence of Heavy Water (Deuterium Oxide) on Plaque Size of Western Equine Encephalitis Virus, 93
- Krnjević, Prof. K. : Effect of Use and Disuse on Neuromuscular Functions, review, 1248
- Mechanisms of Neural Regeneration, review, 1248
- Kronld, Dr. A., and Vavrínková, Dr. H. : Use of Polyethylene Glycol in Investigations of Absorption of Fat, 293
- Krs, M., and Hanuš, V. : Cassiterite as a Carrier of Palaeomagnetism, 675
- Krueger, Prof. A. P., *et al.* : Air Ion Effects on the Oxygen Consumption of Barley Seedlings, 1112
- Kugler, Dr. J., *et al.* : Form-similarity between Phosphenes of Adults and pre-School Children's Scribbles, 1129
- Kuhn, Dr. István : Equation of State of Liquid-vapour Equilibrium with the Same Constants for Both Phases, 1315
- Kuhn, K., Tkocz, C., Zimmermann, B., and Beier, G. : Long-spacing Segments from Renatured α 1 Sub-units of Collagen, 685
- Kummel, B., and Raup, D. (edited by) : Handbook of Palaeontological Techniques, review by Prof. D. T. Donovan, 968
- Kunchur, Prof. N. R. : Effect of Environment on the Orientation of the Nitrate Ions in Crystals, 778
- Kupchik, L., *et al.* : Polymorphism of Lactate Dehydrogenase Isozymes in Pigeons, 284
- Kuppusami, J., and Suryanarayana, C. V. : Ultrasonic Method of Determining the Critical Micelle Concentration of Surfactants, 780
- Kurbanovic, M., and Lafeber, D. : Photographic Reproduction of Soil Fabric Patterns, 609
- Kutch, India : Occurrence of Gnetalean Pollen Grains in the Katrol Formation (Upper Jurassic) of (Y. K. Mathur and Mrs. K. Mathur), 912
- LA TROBE University, Melbourne, 434
- Labelled Antigen Indirect Immunofluorescence Staining Mixed Antiglobulin Immunofluorescence or (Prof. E. H. Beutner *et al.*), 353
- Labelled Depolarizing Drugs in Normal and Denervated Muscle (Prof. D. B. Taylor, R. Creese, O. A. Nedergaard and R. Case), 901
- Labelling of Beta-lipoprotein with Tritium (T. Gosztonyi, J. Márton and A. Kovács), 381
- Labelling of Mouse Thymocytes *in vivo* with Tritiated Thymidine for Cell Transfer Experiments (Dr. K. Borum), 253
- Labelling with Uridine-5-³H in Human Lymphocytes cultured with Phytohaemagglutinin : Cytoplasmic (Dr. G. C. B. Winter and Prof. J. M. Yoffey), 1018
- Labeyrie, A., *et al.* : Reconstruction of Phase Objects by Holography, 1159
- Laboratory Animals : Care of, 630
- Laboratory Animals : Drug Dosage in (C. D. Barnes and L. G. Eltherington), review by Dr. J. A. Parsons, 1028
- Laboratory Animals : International Committee on (Prof. J. A. B. Gray), 738
- Laboratory Animals for Rheumatism Research, 1046
- Laboratory Photochemical Reactor, 935
- Labour Movement 1870-1920 : Education and the (Brian Simon), review by Prof. W. H. G. Armytage, 960
- Lacaz Vieira, F., *et al.* : Effect of 'Furosemid' on Chloride and Water Excretion in Single Nephrons of the Kidney of the Rat, 80
- Lack of Mutagenic Effect of Irradiated *Drosophila* Medium (Dr. O. S. Reddi, G. M. Reddy, J. J. Rao, D. N. Ebenezer and M. S. Rao), 702
- Lactate Dehydrogenase Isozymes in Pigeons : Polymorphism of (Prof. Wm. H. Zinkham *et al.*), 284
- Lactate Dehydrogenase Variant in the Mouse (Mrs. L. Riles), 814
- Lactate-oxidoreductase in Erythrocytes and Ascites Tumour Cells : Enzyme Regulatory Mechanisms at the Level of (S. Rapoport *et al.*), 185
- Lactic Dehydrogenase Isoenzymes in Muscle from Patients with Duchenne Muscular Dystrophy (Prof. T. H. Shepard, Dr. L. H. Gordon and Dr. J. E. Wollen-Weber), 1107
- Lactic Dehydrogenase Isozymes : Denaturation of, and its Clinical Application (Dr. A. Kontinen and S. Lindy), 782
- Lactic Dehydrogenase Relative to Chaetepithelioma Induction in the Syrian Hamster (Prof. F. T. Algard *et al.*), 567
- β -Lactoglobulins Stored in Solutions at pH 8.7 : Acrylamide-gel Electrophoresis of (Dr. P. Akroyd), 488
- Ladle, J. N., *et al.* : Blood Group Secretion Factors in Bronchogenic Carcinoma, 299
- Lafeber, D., and Kurbanovic, M. : Photographic Reproduction of Soil Fabric Patterns, 609
- Lajtha, Dr. L. G., *et al.* : Rate of Chromosome Duplication at the End of the Deoxyribonucleic Acid Synthetic Period in Human Blood Cells, 159
- Lajtha, Dr. G., and Nias, A. H. W. : Continuous Irradiation of HeLa Cells at -196°C , 400
- Lakatos, G., and Bitó, Dr. J. : Dependence of the Properties of Moving Striations in the Plasma of the Positive Column on some External and Internal Parameters, 445
- Lake Chad : Winter Scale Rings In *Lates niloticus* (Pisces Centropomidae) from (A. J. Hopson), 1013
- Lake District : Preserving the, 1260
- Lake Simcoe, Ontario : A Well preserved Grizzly Bear Skull recovered from a Late Glacial Deposit near (R. L. Peterson), 1233
- Lakes : Man-made (Dr. R. L. McConnell and Dr. E. B. Worthington), 1039
- Lal, Harbans, and Chessick, Dr. R. D. C. : Lethal Effects of Aggregation and Electric Shock In Mice treated with Cocaine, 295
- Lal, Dr. R. B., and Arnett, Dr. G. M. : Effect of Ultra-violet Irradiation on the Electrical Conductivity of Zinc Oxide Single Crystals, 1305
- Lalor Foundation Grants for 1966, 436
- Lambert, Dr. J. M., *et al.* (edited by) : Ecology and the Industrial Society, review by Dr. A. Macfadyen, 618
- Lambie, Dr. A. J., *et al.* : 2-Trifluoromethylbenzimidazoles : a New Class of Herbicidal Compounds, 1166
- Lamerton, Prof. L. F., *et al.* : Increased Sensitivity of *in vitro* Murine Leukaemia Cells to Fractionated X-rays and Fast Neutrons, 168
- Laminar and Turbulent Flow in Open Channels (Dr. J. Allen), 1196
- Lamont, P. H., *et al.* : Association of Adenoviruses with Bovine Respiratory Disease, 307
- Land Planarian : Its Significance in Biochemical Evolution : Arginine and Urea Biosynthesis in the (Prof. J. W. Campbell), 1299
- Landbou, 1939-1964 : De Studiekring voor de Gescheiedenis van de, review by G. E. Fussell, 1138
- Landsberg, P. T. : Physique des Semiconducteurs : Comptes Rendus du 7e Congrès International, review, 217
- Landscape at Archaeological Sites : Soil and, 530
- Lange, K. I., *et al.* : Mammals of the World, Three Volumes, review by Dr. L. Harrison Matthews, 210
- Lapage, Dr. G. : History of Parasitology, review, 109
- Microscopic Diagnosis of the Parasites of Man, review, 1029
- Lappert, M. F. : Beryllium Oxide, review, 9
- Larkin, Donna V., Miller, V. L., Barse, G. E., and Hamilton, Dr. C. M. : Effects of Starvation and Protein Depletion on Mercury Retention in Two Strains of Chickens, 706
- Laroche, A., Black, R. F., and Wanless, R. K. : Palaeomagnetism of the Isachsen Diabasic Rocks, 179
- Larsson, S., and Løvtrup, S. : Electromagnetic Recording Diver Balance, 1116
- Larvae in Nigeria : Micro-organisms associated with Dead Insect (Dr. N. Okafor), 1015
- Larval Polymorphism in some Argid Sawflies (Hymenoptera, Symphyta) : Bilateral Asymmetry (Dr. M. F. Claridge and Dr. J. M. Edington), 301
- Laryngotracheitis Virus : Nucleic Acid of Infectious (G. A. Tannock), 711
- Laser-Induced Damage in Diamond (Dr. D. J. Bradley, M. Engwell and H. Komatsu), 1081

- Laser-induced Damage in Natural White Diamond (P. Whiteman and Dr. G. W. Wilson), 66
- Laser Near Field: Transmission through a Tapered Quartz Tube in the (H. J. Caulfield), 773
- Lates niloticus (Pisces: Centropomidae) from Lake Chad: Winter Scale Rings in (A. J. Hopson), 1013
- Latitude: Spectral Density Functions of Hydromagnetic Emissions at High (Dr. A. Egeland), 539
- Latitude: Variation according to, of the Electron Content of the Ionosphere near the Auroral Zone (L. Liszka), 280
- Latitudes: Structure of the Radio Continuum Background at High Galactic. (Dr. M. J. S. Quigley and Dr. C. G. T. Haslam), 741
- Lattice Theory: Introduction to (Prof. D. E. Rutherford), review by Prof. T. A. A. Broadbent, 984
- Lattices: Correlated Atomic Collisions in Irradiated Crystal (Dr. L. T. Chad-derton and I. McC. Torrens), 880
- Lauf, Dr. P. K., and Poulik, Prof. M. D.: Heterogeneity of Water-soluble Struc-tural Components of Human Red Cell Membrane, 874
- Laug, Dr. E. P., et al.: Metabolites in Urine of Rats on Diets containing Aldrin or Dieldrin, 289
- Laughton, Dr. A. S.: Marine Geology of the Pacific, review, 215
- Lavoipierre, M. M. J.: Feeding Mechanism of Blood-sucking Arthropods, 302
- Lavoisier, Antoine-Laurent: Elements of Chemistry. Translated by Robert Kerr, Introduction by Prof. D. McKie, review by Dr. Roger Partington, 987
- Lawrence, A. J., et al.: Acid Phosphatases, 382
- Lawrence, R. C.: Activation of Spores of *Penicillium roqueforti*, 801
- Lawton, D., et al.: Structure of a Diolefine Complex of Platinum(II) $C_{10}H_{16}O_2PtCl_2$, 580
- Lay, L. A., and Barry, Dr. T. I.: Cation Sites in Synthetic Zeolites, 1312
- Laycock, Glenda M., et al.: Titration Curves depicting Central Stimulant, Depressant and Dual Stimulant-depressant Action of Glutaramide Homolo-gues in Mice, 568
- Layer Chromatography of DNP Amino-acids: Polyamide (Prof. Kung-Tsung Wang and J. Mei-Kuo Huang), 281
- Layne, Dr. D. S., and Whittemore, K. S.: Hydrolysis of Two 17 α -Oestradiol Glycosides by Rabbit Tissue Glycosidases, 288
- Le Gros Clark, Sir Wilfrid E.: Fossil Evidence for Human Evolution, Second Edition, review by Dr. W. E. Swinton, 1140
- Lea, Dr. F. M.: Retirement as Director of Building Research, Ministry of Tech-nology, 228
- Leach, S. J., and Springell, P. H.: Cysteine Content of Cattle Hair, 1326
- Leaf, Dr. R. C., Carlton, Dr. P. L., and Hess, Dr. S. M.: Behavioural Deficit in the Rat induced by feeding Phenylalanine, 1021
- Leaf Galls of *Salix fragilis* L.: Red Pigment in (G. Blunden and Dr. S. B. Challen), 388
- Leaman, Angela C.: Control of Furunculosis in Impounded Adult Salmon, 1344
- Lear, John: Kepler's Dream, review by Dr. B. Pagel, 960
- Learn: How and Why do we (edited by W. R. Niblett), review by A. D. C. Peterson, 314
- Leather: Chemistry and Technology of (edited by F. O'Flaherty et al.), Vol. 4, review by Prof. A. G. Ward, 617
- Ledinko, Dr. N., and Toolan, Dr. H.: Growth and Cytopathogenicity of H-viruses in Human and Simian Cell Cultures, 812
- Lee, E. H., and Copley, A. L. (edited by): Proceedings of the Fourth International Congress on Rheology, 1963, review by Dr. G. W. Scott Blair, 965
- Lee, Dr. E. W., and Glaister, Dr. R. M.: Re-entrant Hysteresis Loop and Multi-stable Ferrites, 67
- Lee, G. E., et al.: Metabolism of 5-Chloro-2-cyclohexyl-1-oxo-6-sulphamoyl isondoline, Chlorexolone (M and B 8430), 286
- Lee, Dr. Y. C., and Scherbaum, Prof. O. H.: Isolation of Macronuclei from the Ciliate *Tetrahymena pyriformis* GL, 1350
- Leeds, University of, Appointments, 1047
- Lees, Dr. A. D.: Biological Rhythm Research, review, 973
- Lees, J.: Pressure Distribution in the Tetrahedral Anvil Apparatus, 31
- Lees, J., and Williamson, B. H. J.: Combined Very High Pressure/High Tempera-ture Calibration of the Tetrahedral Anvil Apparatus, Fusion Curves of Zinc, Aluminium, Germanium and Silicon to 60 kilobars, 278
- Legator, Dr. M. S., Zuffante, S. M., and Harp, A. R.: Aflatoxin: Effect on Cultured Heteroploid Human Embryonic Lung Cells, 345
- Legg, Dr. J. T.: Mechanical Transmission of Hop Mosaic Virus, 1017
- Lehmann, Dr. H., et al.: A New Haemoglobin J Toronto (as Alanine \rightarrow Aspartic Acid), 1059
- Three Haemoglobins K: Woolwich, an Abnormal, Cameroon and Ibadan, Two Unusual Variants of Human Haemoglobin A, 658
- Leicester, Department of Museum Studies: University of, 122
- Lele, Mrs. Kusum P., et al.: Deoxyribonucleic Acid Synthesis in Cultured Human Cells and its Bearing on the Concepts of Endoreduplication and Polyploidy, 242
- Lenihan, Dr. J. M. A.: Physics Education, 26
- Lens Proteins in Galactose-fed Rats: Agar-gel Electrophoresis of Soluble (E. Wittgenstein and Dr. K. W. Rowe, jun.), 386
- Leone, Prof. F. C., and Johnson, Prof. N. L.: Statistics and Experimental Design in Engineering and the Physical Sciences, Two Volumes, review by N. E. Day, 214
- Lepiota konradii: Spore Discharge in (Dr. M. H. Zoberl), 405
- Lerner, Dr. A. M., et al.: 'Ring-forms' of Reovirus Particles, 606
- Leslie, G. B.: Central Stimulant Properties of Compounds with Peripheral Muscarinic Properties, 1291
- Leslie, Dr. Shirley A., et al.: 'Pink Spot' in the Urine of Schizophrenics, 453
- Lethal Effects of Aggregation and Electric Shock in Mice treated with Cocaine (H. Lal and Dr. R. D. Chessick), 295
- Leucine: Serotonin Depletion by Dietary (Dr. A. Yuwiler and Dr. E. Geller), 83
- Leucoanthocyanins of White Pine in relation to Weevil Attack (F. S. Santamour, jun.), 407
- Leucocyte Cultures: A Factor stimulating DNA Synthesis derived from the Medium of (Dr. S. Kasakura and Prof. L. Lowenstein), 794
- Leucocyte Pyruvate Kinase: Kinetic Differences between Human Red Cell and (J. O. Campos et al.), 194
- Leucocytes and Plasma Globulins: Formation of Complexes between Basic -Proteins of (Dr. W. B. Jolley and H. W. Allen), 390
- Leucocytes in vitro: Inactivation of T-2 Bacteriophage by Sensitized (R. Young and Dr. F. H. Ruddle), 1105
- Leucocytes to the Bone Marrow in Chronic Myelogenous Leukaemia: Return of (Dr. J. H. Moxley et al.), 1281
- Leucophaea maderae: Guanase Activity of the Symbionts and Fat Bodies of the Cockroach (Dr. L. L. Plerre), 666
- Leukaemia: Chronic Granulocytic (Dr. A. S. D. Spiers and Dr. A. G. Baikie), 497
- Leukaemia: Lymphatic Neoplastic Transformation of Thymus in Mice with Myeloid Virus-induced (L. Chicco-Bianchi et al.), 697
- Leukaemia: Return of Leucocytes to the Bone Marrow in Chronic Myelogenous (Dr. J. H. Moxley et al.), 1281
- Leukaemia Cells: Role of Sialic Acid in Potassium Transport of L1210 (Dr. J. L. Glick and Dr. S. Gichens III), 88
- Leukaemia Cells to Fractionated X-rays and Fast Neutrons: Increased Sensitivity of *in vitro* Murine (Dr. W. L. Caldwell et al.), 168
- Leukaemic Patients: 1 Anzigen in (Dr. J. Ducos et al.), 1329
- Leverkusen-Munchen: Mitteilungen aus den Forschungslaboratorien der Agfa-Gevaert AG (edited by Prof. E. Klein and R. Matejec), Vol. 4, review by K. O. Gangun, 720
- Levine, Dr. M. M., and CERNICK, J.: A Numerical Model of Random Packing of Spheres, 68
- Levine, Prof. S., et al.: Epileptogenic Effect of Extradural and Extracranial Cobalt, 792
- Levitt, R. A., and Webb, Prof. W. B.: Effect of Infantile Treadmill Experience on Body-weight and Resistance to Exhaustion in the Rat, 1128
- Lewis, D. M., et al.: Concentration Effects in Chromatographic Analysis, 269
- Lewis, Dr. D. T.: Flame Spectroscopy, review, 5
- Systematic Identification of Organic Compounds, Fifth Edition, review, 1032
- Lewis, Dr. F. A., and Hull, A. N.: Diffusion or Hydride Component of Hydrogen Overpotential at Platinized Platinum and Palladized Palladium Cathodes, 887
- Lewis, Dr. H. B. M., et al.: Haptoglobin Johnson in Australian Aborigines, 694
- Lewis, Prof. J., et al.: Structure of a Diolefine Complex of Platinum (II) $C_{10}H_{16}O_2PtCl_2$, 580
- Lewis, Dr. M. J., and Anderson, Dr. E. S.: Characterization of a Transfer Factor associated with Drug Resistance in *Salmonella typhimurium*, 843
- Li, Prof. J. C. R.: Statistical Inference, Two Volumes, review by Prof. D. V. Lindley, 420
- Li, P. H., et al.: Some Postulated Relationships among Autumn Phenomena in Woody Plants, 89
- Lidén, S., and Rothman, S.: Isolation of a Lymphoid-cell Protein with Relation to Delayed Hypersensitivity, 389
- Lie-Injo Luan Eng, Dr.: Hereditary Ovalocytosis and Haemoglobin E-Ovalocytosis in Malayan Aborigines, 1329
- Life-shortening in Mice exposed to Radiation: Effects of Age and of Hypoxia (Dr. P. J. Lindop and Prof. J. Rotblat), 1070
- Light Reflex in Binocular Eulvalry and Saccadic Suppression: Suppression of the Pupillary (M. Lorber et al.), 558
- Light-requiring Seed by Chloramphenicol: Promotion of Germination in (Dr. M. Black and M. Richardson), 1114
- Lightening Agents: Response of Melanocytes of Dermis and Epidermis to (Prof. J. McGuire and H. Zoller), 493
- Lighting: Hospital, 835
- Lightning Hazard: Ionizing Radiation: A Potential (P. J. Gillespie), 577
- Lilienthal: Journals of David E.: Two Volumes, review by Sir John Cockcroft, 962
- Lilley, Prof. A. E., et al.: OH Emission in the Direction of Radio Source W49, 441
- Limerick, Eire: Regional Geochemical Reconnaissance applied to some Agri-cultural Problems in Co. (Prof. J. S. Webb and W. J. Atkinson), 1056
- Limestones and its Role in their Diagenesis: Organic Matter in Recent and Ancient (Dr. J. Shearman and Sir Patrick A. d'E Skipwith), 1310
- Limits to the Angular Sizes of some Quasars: New (R. L. Adgie et al.), 275
- Lindgren, Prof. Carl C.: Circulation in the Cell, 304
- Lindop, Prof. D. V.: Statistical Inference, Two Volumes, review, 420
- Lindop, Dr. Patricia J., and Rotblat, Prof. J.: Life-shortening in Mice exposed to Radiation: Effects of Age and of Hypoxia, 1070
- Lindsay, Dr. P. A.: Power Travelling-wave Tubes, review, 1140
- Lindy, S., and Kontinen, Dr. A.: Denaturation of Lactic Dehydrogenase Iso-zymes and its Clinical Application, 782
- Line Strengths of the Hydrogen Halides: Sub-millimetre Dispersion and Rota-tional (Dr. J. E. Chamberlain and Dr. H. A. Gebbie), 480
- Lines of OH in Absorption and Emission: Anomalous Intensity Ratios of the Interstellar (R. X. McGee et al.), 1193
- Ling, John K.: Functional Significance of Sweat Glands and Sebaceous Glands in Seals, 560
- Linkage in Man: The Inv and the Lp Serum Type Systems (Dr. T. Gedde-Dahl, jun., and Dr. K. Berg), 1126
- Linskens, Prof. H. F. (edited by): Pollen Physiology and Fertilization, a sym-posium at the University of Nijmegen, 1963, review by Dr. P. A. Thompson, 218
- 'Lipid A' Component from the Cell-walls of *Pseudomonas aeruginosa* (Dr. K. Clarke, Dr. G. W. Gray and D. A. Reaveley), 586
- Lipid Concentration in Rat Heart and Gastrocnemius: Hormonal Control of (R. M. Denton and Prof. P. J. Randle), 488
- Lipid Fraction from Rat Thymus: Effect of a, on Delayed Hypersensitivity Reactions of Neonatally Thymectomized Rats (Prof. B. D. Jankovic, Dr. K. Isakovic and J. Torvat), 356
- Lipids by Spectroscopic and X-ray Techniques: Structure of (Dr. D. Chapman), review by Dr. F. D. Gunstone, 1246
- Lipids of *M. tuberculosis*: Immunogenic Activity of (Dr. B. Stoss and Dr. R. Herrmann), 1224
- Lipoprotein: Role of the Gut in Synthesizing the Protein Component of Low-density (Beta) (P. L. Scott and C. C. Winterbourn), 494
- β -Lipoproteins (Acanthocytosis): Precipitin Activity in the Sera of Patients with Deficiency of (Dr. A. F. Murray, jun., and Prof. B. S. Blumberg), 357
- Lipps, J. H.: Oligocene in California, 885
- Lipschutz, Prof. Michael E.: Origin of Atypical Meteorites from the Arizona Meteorite Crater, 536
- Lipson, Prof. H., and Taylor, Dr. C. A.: Optical Transforms: Their Preparation and Application to X-ray Diffraction Problems, review by Dr. C. W. Bunn, 1031
- Lipton, Prof. M. A., and Pange, jun., Prof. A. J.: Effects of Propylthiouracil and Thyroid Feeding on the Response of Mice to Injected Convulsant Barbiturate, 791
- Liquid Chromatography: Advances in (Dr. B. Makin and F. P. Speakman), 1087
- Liquid Drops in an Electrically Stressed Insulating Liquid: Deformation of Gas Bubbles and (Dr. F. C. Kao), 279
- Liquid Phase: Oxidation of Hydrocarbons in the (edited by N. M. Emanuel), review by Dr. H. Zeiner, 420
- Liquid Scintillation Counting: Use of Dimethyl Sulphoxide in (E. Wright and K. R. Millar), 889
- Liquid-vapour Equilibrium with the Same Constants for Both Phases: Equation of State of (Dr. I. Ekin), 1315
- Liquids: Acoustic Velocity Dispersion in some Non-associated Organic (Dr. S. E. A. Hakim and W. J. Comley), 1082
- Liquids: Sub-millimetre Wave Spectra of Polar (Dr. G. W. Chantry and Dr. H. A. Gebbie), 378
- Lis, M., et al.: Introduction of a Permanent Electrode into the Pituitary Gland, of the Cow: Influence of Electrical Stimulation on Milk Ejection, 690
- Liszka, Ludwik: Variation according to Latitude of the Electron Content of the Ionosphere near the Auroral Zone, 280
- Little, Dr. E. C. S.: Occurrence of *Salvinia auriculata* Aublet on the Congo River, 1111

- Little, Dr. K., and Taylor, Dr. T. K. F. : Intercellular Matrix of the Intervertebral Disk in Ageing and in Prolapse, 384
- Littler, Dr. T. S. : Physics of the Ear, review by Prof. I. G. Taylor, 1136
- Liu, Prof. V. C. : A Wave Mechanical Approach to the Plasma Interaction Problems, 883
- Liver : Effect of Hydrolysed Glucose Cycloacetate in the Prevention of Fatty Infiltration in the (Prof. N. Nath et al.), 1001
- Influence of Oxygen and Cysteine on the Radical State of X-irradiated Lyophilized (M. Abe et al.), 499
- Synthesis of α -Glycoprotein (Darcy) of Rat Serum by the (Prof. H. E. Weimer et al.), 1221
- Liver Cell : Nucleo-cytoplasmic Interactions in the Rat (E. R. Godwin et al.), 246
- Liver Mitochondria : Stoichiometry of Proton Translocation through the Respiratory Chain and Adenosine Triphosphatase Systems of Rat (Dr. P. Mitchell and Dr. J. Moyle), 147
- Liver Regeneration : Role of Portal Blood Supply in (Dr. R. Y. Thomson and A. M. Clarke), 392
- Livingstone's Contributions to Medicine, 1050
- Load-bearing Brickwork, 121
- Lobitz, W. C., and Montagna, W. (edited by) : The Epidermis, review by Prof. C. D. Calnan, 213
- Localized Haemolysis in Gel Method for the Detection of Cells producing 7S Antibody (Dr. J. Sterzl and Dr. I. Rihá) ; (Dr. D. W. Dresser and Dr. H. H. Wortis), 858
- Localized Strand Separations within Deoxyribonucleic Acid during Selective Transcription (Prof. J. H. Frentzer), 894
- Lock, Dr. R. C. : Promotion to Senior Principal Scientific Officer (Special Merit) Aerodynamics Division, National Physical Laboratory, 527
- Locus determining Human Phosphoglucomutase : Evidence for a Second 'Structural' (Dr. D. A. Hopkinson and Prof. H. Harris), 410
- Locusta migratoria* L. : Relationship between the Removal of the Frontal Ganglion and Protein Starvation in (Dr. K. U. Clarke and C. Gillott), 808
- Loebenstein, G., et al. : Antiviral Action of Oxidized Spermine—Inactivation of Plant Viruses, 1095
- Logic in the Child : Early Growth of (B. Inhelder and J. Piaget), review by Dr. J. H. Duthie, 1246
- Lollar, R. M., et al. (edited by) : Chemistry and Technology of Leather, Vol. 4, review by Prof. A. G. Ward, 617
- London, Dr. D. R., Foley, Dr. T. H., and Webb, C. G. : Evidence for the Release of Individual Amino-acids from the Resting Human Forearm, 588
- London : Isolation of *Cryptococcus neoformans* from Pigeon Habitats in (Dr. H. S. Randhawa et al.), 801
- London Rain-water during 1964 : Fall-out in (Dr. R. Wood et al.), 1083
- London, University of, Appointments, 735, 1047
- Long, Dr. L. H. : Boron, Metallo-Boron Compounds and Boranes, review, 968
- Long, P. L. : Development of *Emeria tenella* in Avian Embryos, 509
- Longberg-Holm, K. K., et al. (edited by) : Rapid Mixing and Sampling Techniques in Biochemistry, review by Prof. S. Dagley, 823
- Long-spacing Segments from Renatured α Sub-units of Collagen (K. Kuhn, C. Tkocz, B. Zimmermann and G. Beier), 685
- Lonsdale, Dame Kathleen : The Architecture of Molecules, review, 957
- Loop and Multistable Ferrites : Re-entrant Hysteresis (Dr. R. M. Glaister and Dr. E. W. Lee), 67
- Lopashov, G. V., and Stroeve, O. G. : Development of the Eye, review by Dr. R. A. Weale, 979
- Lorber, M., Zuber, Dr. B. L., and Stark, Dr. L. : Suppression of the Pupillary Light Reflex in Binocular Rivalry and Saccadic Suppression, 558
- Lord Nuffield's Benefactions for the Advance of Medicine, 1153
- Lorking, K. F. : Inhibition of Corrosion of Copper in Chromic Acid, 778
- Lovell, Sir Bernard : Quasi-Stellar Sources and Gravitational Collapse, review, 963
- Lovering, Dr. J. F., and Morgan, J. W. : Abundances of Rare Earth Elements in Eclogite Rocks and Basaltic Achondrites, 1311
- Løvtrup, S., and Larsson, S. : Electromagnetic Recording Diver Balance, 1116
- Low, Prof. M. J. D. : Infra-red Emission Spectra of Minerals, 1089
- Low-boron Type 304 Stainless Steel : Irradiation Embrittlement of (W. R. Martin et al.), 73
- Low Concentrations of Serum Haptoglobin in Mothers of Children with Haemolytic Disease of the Newborn (J. Pintera), 694
- Low Elevations : Effects of Pronounced Electron-content Variations on Doppler Shift at (H. Socher), 644
- Low-temperature Calorimetry : Modern (Dr. I. M. Firth), 1162
- Lowenstein, Prof. L., and Kasakura, Dr. S. : A Factor stimulating DNA Synthesis derived from the Medium of Leucocyte Cultures, 794
- Lower Palaeolithic Hominid and Pebble-industry in Hungary (Dr. M. Kretzoi and Dr. L. Vértess), 205
- Lower Palaeozoic Stratigraphy : Les Temp. Fossilifères (Prof. H. Termier and G. Termier), Vol. 1, review by Prof. A. Williams, 614
- β -LPH from Sheep Pituitary Glands : Isolation and Amino-acid Sequence of (Prof. Choh Hao Li et al.), 1093
- Lucks, C. F., et al. : Thermal Radiative Properties, review by Dr. E. H. Andrews, 517
- Ludgate, J. C. L., et al. : 2-Trifluoromethylbenzimidazoles : a New Class of Herbicidal Compounds, 1166
- Ludwig, T. G., and Healy, W. B. : Ingestion of Soil by Sheep in New Zealand in Relation to Wear of Teeth, 806
- Lum, W. T., et al. : Observations of a Strong Unidentified Microwave Line and of Emission from the OH Molecule, 29
- Lunar and Planetary Surface Conditions (Dr. N. A. Weil), review by Dr. G. Fielder, 961
- Lunar Crater photographed by *Ranger VII* : Structures inside a (Captain V. G. Smalley and L. B. Ronca), 930
- Luneburg, Dr. R. K. : Mathematical Theory of Optics, review by Dr. W. J. Bates, 971
- Lung and from *Mycoplasma mycoides* : Serological Relationship between Galactans from Normal Bovine (Dr. M. Shifrine and R. N. Gourlay), 498
- Lung Cells : Aflatoxin : Effect on Cultured Heteroploid Human Embryonic (Dr. M. S. Legator et al.), 345
- Lung Tumours in Animals (Prof. L. Severi), 632
- Lunn, G. H. : *Obituary* of Prof. H. Schardin, 832
- Lust, S., et al. : Morphactins, a Novel Group of Plant-growth Regulators, 1013
- Lymph Node Follicles as shown by High-resolution Autoradiography : Ultrastructure of the Antigen-retaining Reticulum of (J. Mitchell and A. Abbot), 500
- Lymphatic Neoplastic Transformation of Thymus in Mice with Myeloid Virus-induced Leukaemia (L. Chicco-Bianchi, Dr. L. Fiore-Donati, G. Tridente and N. Pennelli), 697
- Lymphocyte-stimulating Factor produced *in vitro* (Dr. J. Gordon and Prof. L. D. MacLean), 795
- Lymphocytes cultured in Microplates : Human (Dr. J. A. Brody and B. Huntley), 1232
- Lymphocytes cultured with Phytohaemagglutinin : Cytoplasmic Labelling with Uridine-5-³H in Human (Dr. G. C. B. Winter and Prof. J. M. Yoffey), 1018
- Lymphocytes in Culture to Components of the Medium : Reaction of Human (G. J. Johnson and Prof. F. S. Russell), 343
- Lymphoid-cell Protein with Relation to Delayed Hypersensitivity : Isolation of a (U. Rothman and S. Lidén), 389
- Lymphoid Cells on Monolayers of Mouse Embryo Cells : Plaque-formation by Non-immune and X-irradiated (G. Moller and E. Moller), 260
- Lymphomas : Induction of Malignant, by Urethane in Adult Mice bearing Syngeneic Thymus Grafts (Dr. L. Fiore-Donati et al.), 398
- L-I Lymphosarcoma to Ascitic Form : Conversion of the Inconvertible (Prof. M. R. Sikov), 902
- Lyophilized Liver : Influence of Oxygen and Cysteine on the Radical State of X-irradiated (M. Abe et al.), 499
- Lysozymes Characterized in Duck Egg-white : Isolation of a Histidine-less Lysozyme (Dr. J. Jollès, G. Spotorno and P. Jollès), 1204
- Lytletton, Dr. R. A. : Award of Royal Medal of the Royal Society, 1141
- Lyssternik, L. A., and Yanpol'skii, A. R. (edited by) : Mathematical Analysis Functions, Limits, Series and Continued Fractions, review by Prof. T. A. A. Broadbent, 1140
- McCABE, Dr. M., and Gilbert, Dr. D. A. : Kinetic Parameters for Oxygen in Reactions Involving Purified Oxidases and Tissue Slices, 450
- McCandless, Ruth G., et al. : Biology of the Cytoplasm, 247
- McCarthy, W. H., Shyne, Prof. J. C., and Sherby, Prof. O. D. : Dispersion-softened Zinc Alloys, 579
- McClellan, R. O., and Bustad, Dr. L. K. : Use of Pigs in Biomedical Research, 531
- McClelland, Dr. W. F., et al. : Aggressive Behaviour, Mental Subnormality and the XYY Male, 1351
- McCollister, Susan B., and Pietsch, Dr. Paul : Replication and the Activation of Muscle Differentiation, 1170
- McCombie, Prof. C. W. : Solid State Physics. Advances in Research and Applications, Vol. 15, review, 4
- McConnell, Dr. Rosemary L., and Worthington, Dr. E. B. : Man-made Lakes, 1039
- McCormick, Prof. N. G. : A Proposal on Nature's Time-scale, 334
- McCracken, C. W., et al. : Measured Velocities of Interplanetary Dust Particles, 673
- McCracken, I. J., and Kozlowski, Prof. T. T. : Thermal Contraction in Twigs, 910
- McCreedy, Dr. C. C., Osborne, Dr. D. J., and Black, M. K. : Promotion by Kinetin of the Polar Transport of Two Auxins, 1065
- McDonald, R. E., et al. : Irradiation Embrittlement of Low-boron Type 304 Stainless Steel, 73
- Macfadyen, Dr. A. : Ecology and the Industrial Society, review, 618
- McFarlane, W., and Grim, Prof. S. O. : Phosphorus-31 Chemical Shifts in Secondary and Tertiary Phosphines, 995
- McGeachin, Prof. R. L., Hay, P. A., and Prell, P. A. : Comparison of Salivary Amylase-levels in the Human Male and Female, 1211
- McGee, R. X., Robinson, Dr. B. J., Gardner, F. F., and Bolton, J. G. : Anomalous Intensity Ratios of the Interstellar Lines of OH in Absorption and Emission, 1193
- McGovern, W. E., Gross, Dr. S. H., and Rasool, Dr. S. I. : Rotation Period of the Planet Mercury, 375
- MacGregor, Dr. A. R. : Evolution of the Bristol Channel, Third Edition, revised and enlarged, review, 110
- McGrew, Sarah B., and Mallette, Prof. M. F. : Maintenance of *Escherichia coli* and the Assimilation of Glucose, 1096
- McGuire, Prof. J., and Moller, H. : Response of Melanocytes of Dermis and Epidermis to Lightening Agents, 493
- MacInnes, Dr. Duncan A. : *Obituary*, 626
- McKay, Dr. H. A. C. : Nuclear and Radiochemistry, Second Edition, review, 105
- McKee, Dr. R. K., et al. : Double Chromosome Reduction in a Tetraploid *Solanum*, 359
- McKenzie, Dr. E. D., et al. : Nature of the Non-haem Iron in Ferredoxin and Rubredoxin, 769
- McKetta, jun., John J. (edited by) : Advances in Petroleum Chemistry and Refining, Vol. 10, review by H. B. Milner, 969
- McKie, Prof. D. : Introduction to Lavoisier's Elements of Chemistry, review by Roger Partington, 987
- MacLachlan, Dr. T. B., Daniel, Prof. E. E., and Paul, Prof. W. M. : Electrical Activity in Human Uterine Muscle Strips, 290
- McLaren, Dr. Anne, and Walker, Dr. P. M. B. : Fractionation of Mouse Deoxyribonucleic Acid on Hydroxyapatite, 1175
- MacLean, Prof. L. D., and Gordon, Dr. J. : A Lymphocyte-stimulating Factor produced *in vitro*, 795
- McManus, Dr. J. : Observations on the Mont Pelée Eruption of 1902 and their Significance to Ordovician Welded Tuffs of County Mayo, Eire, 578
- McMaster, G. W. : Computer Recognition of Cervical Cell Nuclei, 485
- McMaster, G. W., and Ward, Dr. A. : Pattern Measurement, 428
- MacNalty, Sir Arthur : Public Health in the Nineteenth Century, review, 977
- Spencer Wells, the Life and Work of a Victorian Surgeon, review, 1135
- McNeil, Prof. Crichton, Trentelman, E. F., Ladle, J. N., Helmick, W. M., and Plenk, Prof. H. P. : Blood Group Secretion Factors in Bronchogenic Carcinoma, 299
- McNiven, Dr. N. L., Raisinghani, K. H., Patashnik, S., and Dorfman, Prof. R. I. : Determination of Nicotine in Smokers' Urine by Gas Chromatography, 788
- MacRobbie, Dr. E. A. C. : Photosynthetic Mechanisms of Green Plants, Papers presented at a Symposium, 1963, review, 212
- McTaggart Cowan, Dr. Ian : Conservation and Man's Environment, 1145
- Maas, Dr. J. W., and Colburn, Dr. R. W. : Adenosine Triphosphate-Metal-norepinephrine Ternary Complexes and Catecholamine Binding, 37
- Co-ordination Chemistry and Membrane Function with Particular Reference to the Synapse and Catecholamine Transport, 41
- Machinery and Control : Electrical (Prof. I. L. Kosow), review by Prof. J. C. West, 9
- Machines : International Conference on Mechanisms and, 231
- Macromolecule Chemical Industry : Melville on, 836
- Macronuclei from the Ciliate *Tetrahymena pyriformis* GL : Isolation of (Dr. Y. C. Lee and Prof. O. H. Scherbaum), 1350
- Macrophage Phagocytic System : Differentiation between Cytophilic Antibody and Opsonin by a (V. E. Parish), 594
- Magic Numbers in Atomic Nuclei : Structural Basis of Neutron and Proton (Prof. L. Pauling), 174
- Magnesium Oxide : X-ray Studies on Highly Pure (Dr. R. A. Brown), 481
- Magnetic Disturbances with Local Earthquakes recorded from the Ethiopian Rift System : Coincidence of (Prof. P. Gouin), 541
- Magnetic Field : Laboratory Observations of Plasma Instabilities in a Dipole (Prof. R. G. Quinn), 376
- Magnetism, Nottingham, September 1964 : Proceedings of the International Conference on, review by Dr. J. H. Davis, 1030
- Magnetism : Theory of (D. C. Mattis), review by Dr. C. A. Bates, 616
- Physics of (Prof. S. Chikazumi), review by Dr. C. A. Bates, 616

- Magnetized Plasma: Experimental Observations on the Structure of Collisionless Shock Waves in a (Dr. J. W. M. Paul et al.), 133
- Magnetohydrodynamic Electrical Power Generation, Symposium 1964, Four Volumes (edited by J. A. Satkowski and H. B. Smets), review by Prof. M. W. Thring, 966
- Magnetospheric Tail to the Radio Emissions of the Planet in the Decametric Region: Possible Contribution of Jupiter's (G. M. Gruber), 1271
- Magnus, Prof. D. K.: Vibrations, review by K. R. Weiss, 964
- Magui, F., et al.: Evidence for Pre-synaptic Inhibition in the Lateral Geniculate Body, 495
- Mahadevappa, Dr. D. S., and Eager, Prof. R. L.: Radiolysis of Sodium Tetrahydroxide Dihydrate, 781
- Maier, Dr. Ludwig, and Daly, Dr. J. J.: Structure of Phosphobenzene, 383
- Maintenance of *Escherichia coli* and the Assimilation of Glucose (S. B. McGrew and Prof. M. F. Mallette), 1096
- 'Majestic': Chimerical Nature of the Entire-leaf Variant in the Potato Variety (Dr. H. W. Howard), 917
- Makin, Dr. B., and Speakman, F. P.: Advances in Liquid Chromatography, 1087
- Malaria and the Opening-up of Central Africa (R. Weatherall), 1267
- Malaria: Antigenic Variation in Chronic Infections of *Plasmodium knowlesi* Immunity to (Dr. K. N. Brown and I. N. Brown), 1286
- Malarial Pigment: Chloroquine Resistance and the Nature of (Prof. I. W. Sherman et al.), 691
- Malayan Aborigines: Hereditary Ovalocytosis and Haemoglobin E-Ovalocytosis in (Dr. Lie-Injo Luan Eng), 1329
- Male: Aggressive Behaviour, Mental Sub-normality and the XYY (P. A. Jacobs et al.), 1351
- Male and Female: Comparison of Salivary Amylase-levels in the Human (Prof. R. L. McGeehan et al.), 1211
- Male Flowers of the Hop (*Humulus lupulus*, L.): Relationships between Constituents of Cones and (Dr. R. D. Hartley and Dr. R. A. Neve), 804
- Male *Tilapia macrochir* Blgr. (Pisces: Cichlidae): Signal Value of the Genital Tassel in the (Dr. W. Wickler), 595
- Males by Means of Ovarian Extract: Induction of Crossing-over in *Drosophila* (O. S. Reddi et al.), 203
- Malignant Cells *in vitro* by a Component of Normal Adult Connective Tissue: Inhibition of Growth of (Prof. M. S. Parsley and I. Mandl), 800
- Malignant Lymphomas: Induction of, by Urethane in Adult Mice bearing Syngeneic Thymus Grafts (Dr. L. Fiore-Donati et al.), 398
- Mallette, Prof. M. F., and McGrew, S. B.: Maintenance of *Escherichia coli* and the Assimilation of Glucose, 1096
- Malnic, G., Vieira, F. L., and Enokibara, H.: Effect of 'Furosemid' on Chloride and Water Excretion in Single Nephrons of the Kidney of the Rat, 80
- Malone, J. P., and Muskett, A. E.: Seed-borne Fungi, review by R. W. G. Dennis, 319
- Malt, R. A., and Scott, Dr. R. B.: Stable Messenger RNA in Nucleated Erythrocytes, 497
- Mammalian Cells by 7:12-Dimethylbenzanthracene: Differential Inhibition of Deoxyribonucleic Acid and Ribonucleic Acid Synthesis induced in Cultured (L. J. Alfred), 1339
- Mammalian Neuromuscular Junction: Presynaptic Effects of Potassium Ion on the (Dr. R. L. Parsons et al.), 590
- Mammalian Ribosomes: Optical Rotatory Dispersion and Secondary Structure of Ribonucleic Acid in (A. Blake and Dr. A. R. Peacocke), 1319
- Mammalogy: Physiological (edited by W. V. Mayer and R. G. Van Gelder), Volumes 1 and 2, review by H. N. Southern, 1136
- Mammals: African, 1046, Alaskan, 936
- Mammals of the World (E. P. Walker, F. Warnick, K. J. Lange, H. E. Uible, S. E. Hamlet, M. A. Davis, and P. F. Wright), Vols. 1, 2 and 3, review by Dr. L. Harrison Matthews, 210
- Mammary Tumour Virus (MTV): A Qualitative *in vitro* Assay for MTV. Immunology of the Mouse (P. B. Blair), 165
- Man: Evolutionary Tendency of Relative Growth in Arthropods and (Dr. R. Matsuda), 1228
- Hereditry and the Nature of (Dr. T. Dobzhansky), review by Prof. C. H. Waddington, 959
- Microscopic Diagnosis of the Parasites of (Dr. R. B. Burrows), review by Dr. G. Lapage, 1029
- Nerve Potential Recordings during electrically and mechanically evoked Monosynaptic Reflexes in (Dr. M. M. Gassel and Dr. E. Diamantopoulos), 1004
- Plasma Growth-hormone Levels in Chronic Starvation in (Dr. V. Marks et al.), 686
- Polonium-210 in (Dr. C. R. Hill), 423
- Technics and the Nature of (Lewis Mumford), 923
- The Inv and the Lp Serum Type Systems: Linkage in (Dr. T. Gedde-Dahl, jun., and Dr. K. Berg), 1126
- Man in his Working Environment: Ergonomics (K. F. H. Murrell), review by C. E. Fleming, 106
- Man-made Lakes (Dr. R. L. McConnell and Dr. E. B. Worthington), 1039
- Man's Environment: Conservation and (Dr. I. McTaggart Cowan), 1145
- Man's Memory: History as the Organization of (Prof. Herbert Butterfield), 1036
- Man's Place in the Island Ecosystem, A Symposium (edited by Dr. F. R. Fosberg), review by Sir L. Dudley Stamp, 109
- Management in East African Wildlife: Research and (Dr. J. Morton Boyd), 828
- Management in Industry: Training for, 613
- Management Training in the Civil Service, 934
- Management: Third Dimension of (John J. Haywood), 928
- Manchester Literary and Philosophical Society, 1260
- Manchester: University of, Chair of Hydrodynamics and Hydrology, 436
- Appointments, 436, 1047
- Mandell, Dr. R. B., and Fatt, Prof. I.: Thinning of the Human Cornea on Awakening, 292
- Mandelstam, Dr. J., et al.: A Suggested Phenotypic Classification and Terminology for Enzyme Mutants in Micro-organisms, 841
- Mandl, Ines, and Parsley, Prof. M. S.: Inhibition of Growth of Malignant Cells *in vitro* by a Component of Normal Adult Connective Tissue, 800
- Mann, Dr. F. G.: Dictionary of Organic Compounds, Fourth Edition, Five Volumes and Supplement, review, 417
- Heterocyclic Compounds with Three- and Four-membered Rings, Parts 1 and 2, review, 2
- Mantel, N., et al.: A Simple Bioassay for Antioxidants based on Protection of *Tetrahymena pyriformis* from the Photodynamic Toxicity of Benzo(a)pyrene, 655
- Maramorosch, Dr. K., and Shikata, E.: Plant Tumour Virus in Arthropod Host: Microcrystalline Formation, 507
- Marcante, Dr. Maria Luisa: Properties of Hyaluronic Acid from a Human Myxosarcoma, 896
- Marcus, Prof. Philip I., Salb, Jesse M., and Schwartz, V. G.: Nuclear Surface N-Acetyl Neuraminic Acid Terminating Receptors for Myxovirus Attachment, 1122
- Marcus, Prof. S. L., and Zahalsky, A. C.: Interference by the Carcinogenic 4-Nitroquinoline N-Oxide of Tryptophan and Indole Uptake in *Escherichia coli*, 296
- Margulis, M. A., et al.: Initiation of Chain Reaction under an Ultrasonic Wave Effect, 945
- Marine Atmosphere: Physics of the (Dr. H. U. Roll), review by Prof. J. Darbyshire, 419
- Marine Geology of the Pacific (Prof. H. W. Menard), review by Dr. A. S. Loughton, 215
- Marks, G. E., McKee, Dr. R. K., and Harborne, Dr. J. B.: Double Chromosome Reduction in a Tetraploid *Solanum*, 359
- Marks, Dr. V., Howorth, H., and Greenwood, F. C.: Plasma Growth-hormone Levels in Chronic Starvation in Man, 686
- Marrow Allografts in Thymectomized X-irradiated Dogs: Bone (I. L. Chertkov et al.), 399
- Marsh, Prof. D. C.: Prisceners and their Families, review, 216
- Marshall, A. C., et al.: Crystalline Modifications of Ampicillin, 999
- Marston, Dr. H. R.: Obituary by Dr. E. G. Holmes, 524
- Marsupial Spermatozoa Faring in the Epididymis of American Forms (Dr. J. D. Biggers and Dr. E. J. Delamater), 402
- Martin, Dr. H.: Osmotricpotaxis in the Honey-bee, 59
- Martin, Dr. Louis, et al.: A New Bis-albuminamic Family, 1222
- Martin, Dr. P. M. D., et al.: Relative Similarities in One Dimension, 544
- Martin, Dr. Rene, et al.: A New Bis-albuminamic Family, 1222
- Martin, Dr. S. J., and Brown, Dr. F.: A New Model for Virus Ribonucleic Acid Replication, 861
- Martin, T. E., and Young, Prof. F. G.: An *in vitro* Action of Human Growth Hormone in the Presence of Actinomycin D, 684
- Martin, W. R., Weir, J. E., McDonald, R. E., and Franklin, J. C.: Irradiation Embrittlement of Low-boron Type 304 Stainless Steel, 73
- Martinez, Laura, et al.: NMN-Pyrophosphorylase Activity in Ascites Cell Extracts, 347
- Martinez, Dr. Leonor, et al.: Acid Mucopolysaccharide and Hexosamine Metabolism in Dermal Connective Tissue of Adrenalectomized Rats, 1212
- Martinez, Rosa-Maria, et al.: Reaction of Adenine-specific Antibodies with Denatured Deoxyribonucleic Acid, 1010
- Márton, J., et al.: Labelling of Beta-lipoprotein with Tritium, 381
- Marty, Y., et al.: 1 Antigen in Leukaemic Patients, 1329
- Mason, J., and Evans, Dr.: Anticarcinogenic Activity of Bracken, 913
- Mason, R., et al.: Advances in Radiation Biology, Volume 1, review by J. St. L. Philpot, 1032
- Mason, Prof. R., et al.: Nature of the Non-haem Iron in Ferredoxin and Rubredoxin, 769
- Structure of a Dioxole Complex of Platinum(II) $C_{10}H_{14}O_2PtCl_2$, 580
- Mass Spectrometric Fragmentation of Nitrophenylhydrazones (Prof. C. Djerassi and S. D. Sample), 1314
- Massachusetts Institute of Technology: Materials Science and Engineering at the, 726
- Massive Stars in General Relativity: Stability of Rotating (B. Durney and I. W. Roxburgh), 1304
- Matejcek, R., and Klein, Prof. E.: Mitteilungen aus den Forschungslaboratorien der Agfa-Gevaert AG Leverkusen-München, Band IV, review by K. O. Ganguin, 720
- Mateles, Prof. R. J., Ryu, C. Y., and Yasuda, T.: Measurement of Unsteady State Growth Rates of Micro-organisms, 263
- Materials Science and Engineering at the Massachusetts Institute of Technology, 726
- Materials Scientist: Education and Application of the (Dr. G. F. Freeguard), 939
- Mathematical Analysis: Functions, Limits, Series and Continued Fractions (edited by L. A. Lyusternik and A. R. Yanpol'skii), review by Prof. T. A. A. Broadbent, 1140
- Mathematical Discovery: On Understanding, Learning and Teaching Problem Solving, Volume 2 (G. Polya), review by Prof. T. A. A. Broadbent, 986
- Mathematical Model for the Process of Aggregation in Immune Agglutination (T. K. Ming, Dr. H. S. Goodman and B. Brown), 84
- Mathematical Theory of Optics (Dr. R. K. Luneburg and M. Herzberger), review by Dr. W. J. Bates, 971
- Mathematics Teachers: Guinness Awards for Science and, 631
- Mathur, Mrs. K., and Mathur, Y. K.: Occurrence of Gnathalean Pollen Grains in the Katol Formation (Upper Jurassic) of Kutch, India, 912
- Mathur, Y. K., and Mathur, Mrs. K.: Occurrence of Gnathalean Pollen Grains in the Katol Formation (Upper Jurassic) of Kutch, India, 912
- Matioli, Dr. G., and Eylar, Dr. E. H.: Apoferritin Synthesis in Human Erythroid Cells in Thalassemia, 661
- Matrix Analysis of Vibration (Prof. R. E. D. Bishop, G. M. L. Gladwell and S. Michaelsen), review by Prof. W. A. Tuplin, 616
- Matrix of the Intervertebral Disk in Ageing and in Prolapse: Intercellular (Dr. T. K. F. Taylor and Dr. K. Little), 384
- Matsuda, Dr. Ryuichi: Evolutionary Tendency of Relative Growth in Arthropods and Man, 1228
- Matthews, Dr. J. M.: Worted Pollen Grains of the Epacridaceae, 803
- Matthews, Dr. P. B. C., and Stein, R. B.: Differences in Variability of Discharge Frequency between Primary and Secondary Muscle Spindle Afferent Endings of the Cat, 1217
- Mattis, D. C.: Theory of Magnetism, review by Dr. C. A. Bates, 616
- Maturation *in vitro* of Mouse, Sheep, Cow, Pig, Rhesus Monkey and Human Ovarian Oocytes (Dr. R. G. Edwards), 349
- Maturation of the Insect *Pyrhocris apterus*: Effect of Certain Types of Paper on Sexual (Prof. C. A. B. Clemetson), 510
- Maturation of Toad Oocytes: Emission of Feulgen-positive Particles during the *in vitro* (Prof. J. Brachet), 596
- Mavrodineanu, Dr. R., and Boiteux, Dr. H.: Flame Spectroscopy, review by Dr. D. T. Lewis, 5
- Maximenko, A. S., et al.: Bone Marrow Allografts in Thymectomized X-irradiated Dogs, 399
- Maxwell, Prof. A., et al.: Radio Structure of the Galactic Centre Region, 1189
- Mayer, C. L.: L'homme face à son Destin, review by F. I. G. Rawlins, 318
- Mayer, W. V., and Van Gelder, R. G. (edited by): Physiological Mammalogy, Volumes 1 and 2, review by H. N. Southern, 1136
- Mayhew, S. G., et al.: Nature of the Non-haem Iron in Ferredoxin and Rubredoxin, 769
- Mayo, E.: Observations on the Mont Pelée Eruption of 1902 and their Significance to Ordovician Welded Tuffs of County (Dr. J. McManus), 578
- Mays, Dr. W.: Teaching in the History and Philosophy of Science, 937
- Mazzarella, L., et al.: Induction of Malignant Lymphomas by Urethane in Adult Mice bearing Syngeneic Thymus Grafts, 598
- Meaburn, Dr. J.: Filamentary Nebulosity in the Vicinity of the Cetus Arc, 575
- Means, A. R., et al.: Stimulation of Synthesis of Ribonucleic Acid in Subapical Sections of *Avena Coleoptile* by Indolyl-3-acetic Acid, 1180
- Measured Velocities of Interplanetary Dust Particles (C. S. Nilsson, W. M. Alexander, C. W. McCracken, O. E. Berg and L. Secretan), 673
- Measurement: Electromagnetic Distance, 837

- Measurement of Exchangeable Aluminium in Acid Soils (J. B. Skeen and Dr. M. E. Sumner), 712
- Measurement of Unsteady State Growth Rates of Micro-organisms (Prof. R. I. Mateles, D. Y. Ryu and T. Yasuda), 263
- Measurement: Particle Size, 529
- Measurement: Pattern (Dr. A. Ward and G. W. McMaster), 428
- Measurements of Bovine Chromosomes (F. N. Baker, Prof. G. W. Salisbury and Prof. N. S. Fechner), 97
- Measurements of Spontaneous Bioluminescence in the Sea (Dr. B. P. Boden, Dr. E. M. Kampa and J. M. Snodgrass), 1078
- Measurements: Stratospheric Transport of Volcanic Dust inferred from Solar Radiation (Dr. A. J. Dyer and B. B. Hicks), 131
- Measuring Microbial Sensitivity to Drugs: Micromethod for (Dr. W. Koch and Dr. D. Kaplan), 50
- Meat from Wild Animals in Africa, 122
- Meat: Objective Evaluation of Quality in Poultry (Dr. A. W. Khan), 204
- Mechanical and Electrical Engineering, 1152
- Mechanical Transmission of Hop Mosaic Virus (Dr. J. T. Legg), 1017
- Mechanism of Intestinal Absorption: Transport of Methionine and Sodium Butyrate by Intracellular Plasma (S. L. Hart and Dr. J. A. Nissim), 145
- Mechanism of Oxidation of Chromium Diffusion Coatings on Iron (Dr. I. A. Menzies and D. Mortimer), 1307
- Mechanism of Oxidation of Chromium Diffusion Coatings on Plain Carbon Steels (Dr. I. A. Menzies and D. Mortimer), 1309
- Mechanism of Radical Decay in Irradiated Polyethylene (Dr. D. O. Geymer and Dr. C. D. Wagner), 72
- Mechanism of Sterility induced by Deuterium Oxide in Mice: Histological Investigations of the (A. M. Hughes and Dr. L. E. Glass), 1119
- Mechanism of Stored-energy Release at 200°C in Electron-irradiated Graphite (Prof. E. W. J. Mitchell and M. R. Taylor), 638
- Mechanism of Uptake of Trace Elements by Plant Roots (G. Findenegg and Prof. E. Broda), 196
- Mechanisms and Machines: International Conference on, 231
- Mechanisms of Electron Transport Phosphorylation: Evidence discriminating between the Chemical and the Chemiosmotic (Dr. P. Mitchell and Dr. J. Moyle), 1205
- Mechanisms of Neural Regeneration (edited by M. Singer and J. P. Schade), review by Prof. K. Krnjevic, 1248
- Mechanoreceptors (Pacini Corpuscles) Processes of Excitation and Inhibition in Single (Dr. O. B. Ilyinsky), 351
- Medal Awards: Royal Society of London, 934
- Royal Society of London, James Smithson, 324, Royal, 832
- Medical Bacteria: Manual for the Identification of (S. T. Cowan and K. J. Steel), review by Dr. D. Allan, 213
- Medical Research Awards: Albert Lasker, 1263
- Medical Research, Melbourne: Walter and Eliza Hall Institute of, 729
- Medical School: Planning a New, 634
- Medicinal: Piante: *Chimica Farmacologia e Terapia*, Vol. 2, (R. Benigni et al.), review by Dr. F. N. Howes, 218
- Medicine: Livingstone's Contributions to, 1050
- Lord Nuffield's Benefactions for the Advance of, 1153
- 1965 Nobel Prize for (Prof. M. R. Pollock), 1250
- Mediterranean Gobies into the Black Sea: *Relictogobius kryzhanovskii* and the Penetration of (Dr. P. J. Miller), 474
- Medium of Leucocyte Cultures: A Factor stimulating DNA Synthesis derived from the (Dr. S. Kasakura and Prof. L. Lowenstein), 794
- Meeks, Prof. M. L., et al.: Observations of Polarized OH Emission, 440
- Radio Structure of the Galactic Centre Region, 1189
- 141 Mc/s: Sky Brightness at (K. W. Yates and Dr. R. Wielebinski), 64
- Melanocyte-stimulating Hormone on Frog Skin *in vitro*: Effects of Cyclic Adenosine Monophosphate and (Dr. M. W. Bitensky and S. R. Burstein), 1282
- Melanocytes and the Mitochondria of Hepatocytes: Functional Similarities between the Cytoplasmic Organelles of (Dr. M. H. Van Woert et al.), 810
- Melanocytes of Dermis and Epidermis to Lightening Agents: Response of (Prof. J. McGuire and H. Moller), 493
- Melatonin, in Amphibians: Enzymatic Synthesis of the Skin-lightening Agent, (Dr. J. Axelrod et al.), 386
- Melatonin in Pineal Extracts: Separation Method for (Dr. F. C. G. van de Veerdonk), 1324
- Melbourne: La Trobe University, 434
- Walter and Eliza Hall Institute of Medical Research, Directorship, 729
- Melchior, Prof. H. (edited by): *Syllabus der Pflanzenfamilien*, Vol. 2, Twelfth Edition (A. Engler), review by Prof. T. G. Tutin, 316
- Mellaart, James: Earliest Civilizations of the Near East, review by M. C. Burkitt, 984
- Melville, Marie M., et al.: Aggressive Behaviour, Mental Sub-normality and the XYY Male, 1351
- Melville on Macromolecule Chemical Industry, 836
- Membrane Function with Particular Reference to the Synapse and Catecholamine Transport: Co-ordination Chemistry and (Dr. J. W. Maas and Dr. R. W. Colburn), 41
- Membranes: Effect of Narcotics: Cation Permeability of Phospholipid Model (Dr. A. D. Bangham et al.), 1295
- Membranes: Effect of *Trans*-Solutos on the Fluxes of Chloride Ions across Artificial (S. Kitahara et al.), 187
- Memory Changes with Age (Prof. D. Schonfield), 918
- Memory: History as the Organization of Man's (Prof. Herbert Butterfield), 1036
- Men and Women: Fasting Levels of Growth Hormone in (Dr. C. Faiman), 687
- Menard, Prof. H. W.: Marine Geology of the Pacific, review by Dr. A. S. Laughton, 215
- Mendip District of Somerset: Soils of the, 328
- Menschen: Histologie und Mikroskopische Anatomie des (Dr. W. Bargmann), 5th German Edition, review by G. Sander, 109
- Mental Evolution: an Exercise in Analogy: Biological and (Arthur Koestler), 1033
- Mental Function: Biochemistry and (Prof. S. S. Kety), 1252
- Mental Sub-normality and the XYY Male: Aggressive Behaviour, (P. A. Jacobs et al.), 1351
- Menzies, Dr. D. W.: Concept of the 'Centron', 163
- Menzies, Dr. I. A., and Mortimer, D.: Mechanism of Oxidation of Chromium Diffusion Coatings on Iron, 1307
- Mechanism of Oxidation of Chromium Diffusion Coatings on Plain Carbon Steels, 1309
- Mepacrine- and Primaquine-resistant Strains of *Plasmodium berghei*, Vincke and Lips, 1948 (Dr. W. Peters), 693
- Mercer, J. B., and Wilson, Prof. B. G.: Daily Variation of Cosmic Rays, 477
- Merchant, Prof. Wilfred: *Obituary* by Dr. S. S. Gill, 1043
- Mercury Retention in Two Strains of Chickens: Effects of Starvation and Protein Depletion on (D. V. Larkin et al.), 706
- Mercury: Rotation Period of the Planet (W. E. McGovern et al.), 375
- Mercury: Rotational Period of the Planet (Dr. G. Colombo), 575
- Merigan, Prof. T. C., and Kleinschmidt, Dr. W. J.: Different Molecular Species of Mouse Interferon induced by Statolon, 667
- Merino Ewes: Undernutrition and Early Embryonic Mortality in (T. N. Edey), 1232
- Messenger Ribonucleic Acid: Purine Rich Regulatory Sequences in (Prof. R. H. Doi), 552
- Messenger RNA in Nucleated Erythrocytes: Stable (Dr. R. B. Scott and R. A. Malt), 497
- Metabolic Products in the Soil during Decomposition of Carbohydrates: Fixation of (H. Sorensen), 97
- Metabolism of 5-Chloro-2-cyclohexyl-1-oxo-6-sulphamoyl Isoindoline, Chloexolone (M. and B. 8430) (K. Corbett, Miss S. A. Edwards, G. E. Lee and T. L. Threlfall), 286
- Metabolism of Nucleic Acids and Protein in Starving Bacteria (B. Brdar, E. Kos and M. Drakulic), 303
- Metabolite of 'Miracil D': A New Active (D. Rosi et al.), 1005
- Metabolite of the Female Reproductive Tract: Oxidative and Biosynthetic Utilization by Human Spermatozoa of a (Prof. C. Terner), 1115
- Metabolites in the ω -Oxidation of 2,4-Dimethylheptanoic Acid: Urinary (Dr. S. R. Tannenbaum and Prof. S. A. Miller), 452
- Metabolites in Urine of Rats on Diets containing Aldrin or Dieldrin (P. R. Datta, Dr. E. P. Lang, J. O. Watts, A. K. Klein and M. J. Nelson), 289
- Metal Concentration Profiles in Oxide Scales on Nickel-10.9 per cent Cobalt Alloy (Dr. G. C. Wood and J. M. Ferguson), 369
- Metal Grains in Chondritic Meteorites (J. A. Wood), 1085
- Metal Interfaces: Adsorption and the Differential Capacitance of the Electrical Double-layer at Platinum/Halide (A. D. Graves and Dr. D. Inman), 481
- Metallic Ions as Hatching Agents for the Beet Cyst Nematode, *Heterodera schachtii* Schm.: Zinc and Other (Dr. A. J. Clarke and Dr. A. M. Shepherd), 502
- Metallo-Boron Compounds and Boranes: Boron, (edited by Roy M. Adams), review by Dr. L. H. Long, 968
- Metals: Alkaline Earth, Handbuch der Experimentellen Pharmakologie, Band 17, Two Volumes in French (sub-editor: Prof. Z.-M. Bacq), review by Prof. E. Zaizms, 720
- Metals and Bloat in Ruminants: Possible Relationship between Trace (P. J. Harris and Prof. F. Sebba), 869
- Metals: A.C. Resistivity of Anodic Oxide Films on Valve (Dr. G. C. Wood and C. Pearson), 547
- Metamorphosis: Changes in the t-RNA Methylating Enzymes during Insect (Dr. B. S. Baliga et al.), 555
- Metaphysik der Naturwissenschaft (W. Schapp), review, 988
- Metcalf, Dr. Donald: Delayed Effect of Thymectomy in Adult-life on Immunological Competence, 1336
- Multiple Thymus Grafts in Aged Mice, 87
- Metcalf, Dr. C. R.: *Encyclopedia of Plant Physiology*, Vol. 15, Two Parts, review, 619
- Indian Woods, Vol. 2, Linaceae to Moringaceae, review, 212
- Meteorite: The Wolf Creek Iron (Prof. S. R. Taylor), 944
- Meteorites: Evidence for Microfossils in the Alais and Orgueil Carbonaceous (S. L. VanLandingham), 947
- Meteorites from the Arizona Meteorite Crater: Origin of Atypical (Prof. M. E. Lipschutz), 636
- Meteorites: Metal Grains in Chondritic (J. A. Wood), 1085
- Methandrostenolone on the Dry Mass of Various Rat Muscles: Interferometric Estimation of Effects of Testosterone and (Dr. W. Staubli and Dr. T. Okada), 562
- Methane-rich Perchloric Acid Flames (Dr. G. S. Pearson), 283
- Methionine and Sodium Butyrate by Intracellular Plasma: Mechanism of Intestinal Absorption: Transport of (S. L. Hart and Dr. J. A. Nissim), 145
- Method: Design, 1052
- Method for obtaining, *in vitro*, Uniform Cell Monolayer Sheets from Tissues of the Tick, *Hyalomma dromedarii* (Ixodidae): Improved (Dr. M. G. R. Varma and Miss W. Wallers), 602
- Methods for the Examination of Root Systems and Roots (Dr. J. J. Schuurman and Dr. M. A. J. Goedewaagen), review by W. D. Clayton, 319
- Methods in Carbohydrate Chemistry (edited by Prof. R. L. Whistler), Vols. 4 and 5, review by Prof. M. Stacey, 971
- Methods in Cell Physiology, Vol. 1 (edited by D. M. Prescott), review by Dr. J. H. Humphrey, 6
- 'Methoxytrexate': a Reduced Oxygen Enhancement Ratio for X-ray Survival of HeLa Cells *in vitro*, after Treatment with (Dr. R. J. Berry), 1108
- Methyl Sulphate (Phenazine Methosulphate): Dependence of Photophosphorylation by Isolated Chloroplasts on the Oxidation-Reduction State of N-Methylphenazinium (G. Zweig and Dr. M. Avron), 190
- Methylating Enzymes during Insect Metamorphosis: Changes in the t-RNA (Dr. B. S. Baliga et al.), 555
- Methylation: Quantitative Chromatographic Analysis of Organic Acids by Triacetic (G. K. Koch and [the late] G. Jurriens), 1312
- Methylcholanthrene Carcinogenesis influenced by Vegetable Extracts (V. Vendég), 598
- N-Methylphenazinium Methyl Sulphate (Phenazine Methosulphate): Dependence of Photophosphorylation by Isolated Chloroplasts on the Oxidation-Reduction State of (G. Zweig and Dr. M. Avron), 190
- Metzner, Prof. A. B.: Diffusive Transport Rates in Structured Media, 267
- Meynell, Elinor, and Meynell, G. G.: Theory and Practice in Experimental Bacteria, review by Dr. D. Allan, 213
- Meynell, G. G., and Meynell, E.: Theory and Practice in Experimental Bacteria, review by Dr. D. Allan, 213
- Meyrick, Edward, (described by): Catalogue of Type Specimens of Microlepidoptera in the British Museum (Natural History) (Dr. J. F. Gates Clarke), Vol. 5, review by Prof. G. C. Varley, 108
- Mice: Acute Radio-sensitivity as a Function of Age in (Dr. J. F. Spalding et al.), 905
- Breakdown Rate of Serum 7S γ -Globulins in (Dr. D. E. H. Tee et al.), 251
- Histological Investigations of the Mechanism of Sterility induced by Deuterium Oxide in (A. M. Hughes and Dr. L. E. Glass), 1119
- Multiple Thymus Grafts in Aged (Dr. D. Metcalf), 87
- Time-course Studies on Antibody Response in Thymectomized and Sham-thymectomized (Dr. N. R. Sinclair), 1104
- Titration Curves depicting Central Stimulant, Depressant and Dual Stimulant-depressant Action of Glutaramide Homologues In (Dr. A. Shulman et al.), 568
- Mice bearing Syngeneic Thymus Grafts: Induction of Malignant Lymphomas by Urethane in Adult (Dr. L. Fiore-Donati et al.), 398
- Mice exposed to Radiation: Effects of Age and of Hypoxia: Life-shortening in (Dr. P. J. Lindop and Prof. J. Rotblat), 1070
- Mice on Immunological Responsiveness: Effect of Thymectomy in Adult (Dr. J. F. A. P. Miller), 1337
- Mice pretreated with *Escherichia coli* Endotoxin or infected with *Mycobacterium tuberculosis*: Interferon Appearance stimulated by Endotoxin, Bacteria, or Viruses in (Prof. J. S. Youngner and Prof. W. R. Stinebring), 456

- Mice to Injected Convulsant Barbiturate: Effects of Propylthiouracil and Thyroid Feeding on the Response of (Prof. A. J. Prange, jun., and Prof. M. A. Lipton), 791
- Mice treated with Cocaine: Lethal Effects of Aggregation and Electric Shock in (H. Lal and Dr. R. D. Chessick), 295
- Mice with Mixtures of Spermatozoa: Artificial Insemination of Dystrophic (S. L. Southard et al.), 1126
- Mice with Myeloid Virus-induced Leukaemia: Lymphatic Neoplastic Transformation of Thymus in (L. Chicco-Bianchi et al.), 697
- Micelle Concentration of Surfactants: Ultrasonic Method of Determining the Critical (J. Kuppasami and C. V. Suryanarayana), 780
- Micelle Formation of Sodium Alkyl Sulphates in Water: Rate of (P. F. Mijnlief and R. Ditmarsch), 889
- Michaelson, S., et al.: Matrix Analysis of Vibration, review by Prof. W. A. Tuplin, 616
- Michie, Dr. D., and Coppleson, L. W.: Comparison of the Chorioallantoic Membrane and Splenomegaly Systems of Graft-versus-host Assay in the Chick Embryo, 53
- M. tuberculosis*: Immunogenic Activity of Lipids of (Dr. B. Stoss and Dr. R. Herrmann), 1224
- Microbial and Molecular Genetics (Prof. J. R. S. Fincham), review by Dr. S. W. Glover, 1029
- Microbial Cell Wall Mucopeptide 2,6-Diamino-3-hydroxypimelic Acid in (H. R. Perkins), 872
- Microbial Sensitivity to Drugs: Micro-method for Measuring (Dr. W. Koch and Dr. D. Kaplan), 50
- Microbiology Group: Aquatic, 734
- Microcrystal Formation: Plant Tumour Virus in Arthropod Host: (E. Shikata and Dr. K. Maramorosch), 507
- Micro-fauna: Soil, 435
- Microflora in Gnotobiotic Rats: Human Indigenous (Dr. H. A. Dymsha et al.), 1236
- Microfossils in the Alais and Orgueil Carbonaceous Meteorites: Evidence for (S. L. VanLandingham), 947
- Microlepidoptera in the British Museum (Natural History) described by Edward Meyrick Catalogue of the Type Specimens of (Dr. J. F. Gates Clarke), Vol. 5, review by Prof. G. C. Varley, 108
- Micromethod for Measuring Microbial Sensitivity to Drugs (Dr. W. Koch and Dr. D. Kaplan), 50
- Microminutization: Solid Circuits and (edited by G. W. A. Dummer), review by Dr. J. R. Tillman, 1249
- Micro-organisms associated with Dead Insect Larvae in Nigeria (Dr. N. Okafor), 1015
- Micro-organisms: I. Measurement of Unsteady State Growth Rates of (Prof. R. I. Mateles et al.), 263
- Note on Typography, 332
- A Suggested Phenotypic Classification and Terminology for Enzyme Mutants in (Dr. J. F. Collins et al.), 841
- Micropalaeontology: Principles of Zoological, Volume 2 (Dr. V. Pokorny), review by Dr. R. H. Bate, 974
- Microplates: Human Lymphocytes Cultured in (Dr. J. A. Brody and B. Huntley), 1232
- Microscopic Diagnosis of the Parasites of Man (Dr. R. B. Burrows), review by Dr. G. Lapage, 1029
- Microscopical Club: Centenary of the Quekett (Prof. G. J. Cunningham), 937
- Microtine Rodents: Relation of Population Density to Sodium Availability and Sodium Selection by (G. D. Aumann and Prof. J. T. Emlen), 198
- Microstructure of Fibrous Sulphur (A. Jaskolska and A. E. B. Presland), 1088
- Microwave Line and of Emission from the OH Molecule: Observations of a Strong Unidentified (Prof. H. Weaver et al.), 29
- Microwaves: Plasma Diagnostics with (Dr. M. A. Heald and C. B. Wharton), review by A. E. Dangor, 965
- Midgley, Dr. Joan M., and Wild, Dr. D. G.: Synthesis of Ribonucleic Acid by *Escherichia coli* during Inhibition by Azetidine-2-carboxylic Acid, 585
- Midlands Regional Office of the Ministry of Technology: East, 833
- Mijnlieff, P. F., and Ditmarsch, R.: Rate of Micelle Formation of Sodium Alkyl Sulphates in Water, 889
- Mikroskopische Anatomie des Menschen: Histologie und (Dr. W. Bargmann), 5th German Edition, review by Dr. G. Sander, 109
- Milborrow, Dr. B. V., and Boyce, Dr. C. B. C.: Simple Assessment of Partition Data for correlating Structure and Biological Activity using Thin-layer Chromatography, 537
- Milk Ejection: Introduction of a Permanent Electrode into the Pituitary Gland of the Cow: Influence of Electrical Stimulation on (B. Hynek et al.), 690
- Milk: Bacterial Count and Cell Differentiation in (C. L. Duitschaefer), 200
- Milk: Evaluation of Ion Exchange Cartridges for Field Sampling of Iodine-131 in (R. H. Johnson, jun., and T. C. Reavey), 750
- Milker's Nodule Virus Infections in Dorset and their Similarity to Orf (Dr. J. Nagington, Dr. G. H. Tee and J. S. Smith), 505
- Millar, K. R., and Wright, E.: Use of Dimethyl Sulphoxide in Liquid Scintillation Counting, 889
- Millboard Manufacture, 529
- Miller, Dr. C. S., et al.: Biogenesis of Ethylene in *Penicillium digitatum*, 1322
- Miller, Dr. J. A., and Fitch, F. J.: Age of the Weardale Granite, 743
- Miller, Dr. J. F. A. P.: Effect of Thymectomy in Adult Mice on Immunological Responsiveness, 1337
- Miller, Dr. J. F. A. P., de Burgh, P. M., and Grant, G. A.: Thymus and the Production of Antibody-plaque-forming Cells, 1332
- Miller, Prof. J. M., et al.: Nuclear and Radiochemistry, Second Edition, review by Dr. H. A. C. McKay, 105
- Miller, Nigel, et al.: Cation Permeability of Phospholipid Model Membranes: Effect of Narcotics, 1295
- Miller, Dr. P. J.: *Relictogobius kryzhanovskii* and the Penetration of Mediterranean Gobies into the Black Sea, 474
- Miller, Prof. S. A., and Tannenbaum, Dr. S. R.: Urinary Metabolites in the ω -Oxidation of 2,4-Dimethylheptanoic Acid, 452
- Miller, Prof. T. G.: Appointment to Chair of Geography in the University of Reading, 628
- Miller, V. L., et al.: Effects of Starvation and Protein Depletion on Mercury Retention in Two Strains of Chickens, 706
- Millin, Dr. D. J., and Briggs, Dr. D. E.: Formation of Hydroxamates during Proteolysis by an Enzyme System from Yeast, 287
- Millin, Dr. D. J., and Saltmarsh-Andrew, M. J.: Nucleotide Peptides in Yeast, 468
- Mills, Prof. B. Y., and Gianfield, J. R.: Radio Observations of Spiral Galaxies, 10
- Miner, H. B.: Advances in Petroleum Chemistry and Refining, Vol. 10, review, 969
- Mimicry and the Evolution of Animal Communication (Dr. W. Vickler), 519
- Mineral of Illite and Montmorillonite: Interstratified (Prof. M. Sato et al.), 179
- Minerals: Chemical Analyses of Rocks and, 1261
- Infra-red Emission Spectra of (Prof. M. J. D. Low), 1089
- Structure of Interstratified (Mixed-layer) (Dr. M. Sato), 70
- Ming, Tao Kuang, Goodman, Dr. H. S., and Brown, B.: Mathematical Model for the Process of Aggregation in Immune Agglutination, 84
- Miniature Endplate Potentials and some Implications: Evidence for a Poisson Distribution of (Dr. P. W. Gage and Dr. J. I. Hubbard), 395
- Minihan, Dr. Kathleen, and Davies, Prof. R. E.: Energy Requirements for Relaxation from Tonic Contractions ('Catch') in an Invertebrate Muscle, 1327
- Minister of Power's Advisory Council on Research and Development, 433
- Ministry of Aviation: Appointments, 19, Project Director Anglo-French Military Aircraft, 527
- Ministry of Defence: Deputy Chief Scientific Adviser, 19
- Ministry of Overseas Development, Directorate of Overseas Surveys, 118
- Ministry of Technology, 228, 833, 1152
- Minnis, Dr. C. M.: Ionosphere, and X-ray Images of the Sun, 476
- 'Miracid D': A New, Active Metabolite of (D. Rosi et al.), 1005
- Misra, Dr. D. N., et al.: Simultaneous Oxidation and Reduction of Diphenyl Picrylhydrazyl on Surfaces: Evidence for Catalytic Activity of F-Centres, 181
- Mitchell, Prof. E. W. J., and Taylor, M. R.: Mechanism of Stored-energy Release at 200°C in Electron-irradiated Graphite, 638
- Mitchell, Judith, and Abbot, A.: Ultrastructure of the Antigen-retaining Reticulum of Lymph Node Follicles as shown by High-resolution Autoradiography, 500
- Mitchell, Dr. J. R. A., et al.: Effect of Pyrimidopyrimidine Derivative on Thrombus Formation in the Rabbit, 255
- Mitchell, Dr. Peter, and Moyle, Dr. J.: Evidence discriminating between the Chemical and the Chemiosmotic Mechanisms of Electron Transport Phosphorylation, 1205
- Stoichiometry of Protein Translocation through the Respiratory Chain and Adenosine Triphosphatase Systems of Rat Liver Mitochondria, 147
- Mitochondria of Hepatocytes: Functional Similarities between the Cytoplasmic Organelles of Melanocytes and the (Dr. M. H. Van Woert et al.), 810
- Mitochondria: Stoichiometry of Proton Translocation through the Respiratory Chain and Adenosine Triphosphatase Systems of Rat Liver (Dr. P. Mitchell and Dr. J. Moyle), 147
- Mitochondrial Activity in Sleeping Sickness Trypanosomes: Polymorphism and (Dr. K. Vickerman), 762
- Mitral Valve Disease, 631
- Mittwoch, Dr. Ursula, and Kenney, Dr. J. D.: Barr Bodies in the Mouse, 93
- Mittwoch, Dr. U., Lele, Mrs. K. P., and Webster, W. S.: Deoxyribonucleic Acid Synthesis in Cultured Human Cells and its Bearing on the Concepts of Endoreduplication and Polyploidy, 242
- Mixed Antiglobulin Immunofluorescence or Labelled Antigen Indirect Immunofluorescence Staining (Prof. E. H. Beutner et al.), 353
- Mixtures of Spermatozoa: Artificial Insemination of Dystrophic Mice with (J. L. Southard et al.), 1126
- Miyake, Y., et al.: Strontium-90 and Caesium-137 in Columbia River Plume, July 1964, 1084
- Mobilities of some Erythrocytic Enzymes as a Function of their Age: Altered Electrophoretic (Dr. H. Walter et al.), 76
- Mobility of an Immune Globulin from Rainbow Trout Serum: Electrophoretic (H. O. Hodgins et al.), 1106
- Model for a Sodium Pump: Molecular (Dr. L. J. Opit and Dr. J. S. Charnock), 471
- Model for the Process of Aggregation in Immune Agglutination: Mathematical (T. K. Ming et al.), 84
- Model for Virus Ribonucleic Acid Replication: A New (Dr. F. Brown and Dr. S. J. Martin), 861
- Model of Random Packing of Spheres: A Numerical (Dr. M. M. Levine and J. Chernick), 68
- Modern Low-temperature Calorimetry (Dr. I. M. Firth), 1162
- Modern Technique in Restoration of Roman Mosaics, 334
- Modern Trends in Immunology, I (edited by Prof. R. Cruickshank), review by Dr. F. T. Perkins, 9
- Modern University (Prof. G. L. Brook), review by R. Brightman, 518
- Modification of Radiation 'Cerebral Death' by Hypoxia (Dr. E. A. Wright and J. Shewell), 904
- Modification System for Fingerprinting Haemoglobin (Dr. G. Efremov), 298
- Modlibowska, Dr. Irena: Effects of (2-Chloroethyl)trimethyl-ammonium Chloride and Gibberellic Acid on Growth, Fruit Bud Formation and Frost Resistance in One-year-old Pear Trees, 503
- Modrinski, Mr. N.: Retirement as Superintendent of the Geophysical Survey, Geophysics Division, New Zealand D.S.I.R., 327
- Mohr, G., et al.: Morphactins, a Novel Group of Plant-growth Regulators, 1013
- Mohr, P. A.: Re-classification of the Ethiopian Cainozoic Volcanic Succession, 177
- Moisture in Nozzles: Effects of Seeding on the Condensation of Atmospheric (Dr. E. R. Buckle and Dr. A. A. Pouring), 367
- Moisture in Unsaturated Soils: Thermistor Hygrometer for determining the Free Energy of (B. G. Richards), 608
- Molecular Genetics: Microbial and (Prof. J. R. S. Fincham), review by Dr. S. W. Glover, 1029
- Molecular Level: Biological Systems at the (Dr. B. Askonas et al.), 1048
- Molecular Model for a Sodium Pump (Dr. L. J. Opit and Dr. J. S. Charnock), 471
- Molecular Orbital Theory (C. J. Ballhausen and Dr. H. B. Gray), review by E. W. T. Richards, 110
- Molecular Pharmacology (I, No. 1, July 1965, edited by A. Goldstein), 21
- Molecular Species of Mouse Interferon induced by Statolon: Different (Prof. T. C. Merigan and Dr. W. J. Kleinschmidt), 667
- Molecular Structural Factors and their Role in the Germination of White Mustard (S. E. B. Gould et al.), 876
- Molecular Structure: Spectroscopy and (Dr. G. W. King), review by Dr. R. F. Barrow, 214
- Molecular Weight of Chick Interferon (Dr. D. C. Burke and Dr. J. Ross), 1297
- Molecular Weight of Humic Substances in Natural Water: Use of 'Sephadex' Gel for the Estimation of (E. T. Giesing), 1091
- Molecular Weight of Urokinase (R. A. Burges, Dr. K. W. Brammer and J. D. Coombes), 894
- Molecule: Observations of a Strong Unidentified Microwave Line and of Emission from the OH (Prof. H. Weaver et al.), 29
- Molecules: Architecture of (Prof. Linus Pauling and Roger Hayward), review by Dame Kathleen Lonsdale, 957
- Möller, Erna, and Möller, G.: Plaque-formation by Non-immune and X-irradiated Lymphoid Cells on Monolayers of Mouse Embryo Cells, 260
- Möller, Goran, and Möller, E.: Plaque-formation by Non-immune and X-irradiated Lymphoid Cells on Monolayers of Mouse Embryo Cells, 260
- Möller, H., and McGuire, Prof. J.: Response of Melanocytes of Dermis and Epidermis to Lightening Agents, 493
- Möller, Dr. J. V.: Tubular Site of Urate Secretion in the Rabbit, 492
- Mollusc *Tapes turgida*: Biphasic Dose-response Relationship with Acetylcholine on the Heart of the (P. R. Carroll and G. B. Cheshier), 1220
- Molomut, Dr. N., and Padnos, Dr. M.: Inhibition of Transplantable and Spontaneous Murine Tumours by the M-P Virus, 948
- Momenta of Eclipsing Binaries and the Fission Theory of their Origin: Angular (I. W. Roxburgh), 65
- (Mongolian): Virus Aetiology for Down's Syndrome (A. Stoller and R. D. Collmann), 903

- Monig, H., et al.: Influence of Oxygen and Cysteine on the Radical State of X-irradiated Lyophilized Liver, 499
- Monig, Dr. H., and Koch, Prof. R.: Electron Spin Resonance Investigations of Ultra-violet and X-irradiated Homocysteinethiolactone, 86
- Monis, Dr. Benito: Isozymes of Arylamidase (Aminopeptidase): Source of Blood Aminoacyl Naphthylamidase in the Rat, 587
- Monkey: Influence of the Cerebral Cortex on the Cuneate Nucleus of the (Dr. F. Harris et al.), 1215
- Monkey Kidney Cells by Adenovirus 7 and SV 40: Mixed Infection of African Green (Dr. R. K. Wertz et al.), 1350
- Monkeys: Social Companions and the Mother-Infant Relationship in Rhesus (Y. Spencer-Booth et al.), 301
- Monoacetylacetonato-Iron(III) Complex: Kinetics of Formation of the (W. K. Ong and Dr. R. H. Prince), 1201
- Monocular versus Binocular Visual Acuity (Dr. F. W. Campbell and Dr. D. G. Green), 191
- Monolayer Sheets from Tissues of the Tick, *Hyalomma dromedarii* (Ixodidae): An Improved Method for obtaining, *in vitro*, Uniform Cell (Dr. M. G. R. Varma and Miss W. Wallers), 602
- Monolayers of Mouse Embryo Cells: Plaque-formation by Non-immune and X-irradiated Lymphoid Cells on (G. Moller and E. Moller), 260
- Monosomics: Estimation of the Frequency of Functioning Gametes in (A. K. R. Choudhury and J. G. Bhowal), 917
- Monosynaptic Reflexes in Man: Nerve Potential Recordings during electrically and mechanically evoked (Dr. M. M. Gassel and Dr. E. Diamantopoulos), 1004
- Monotreme: Studies on the Australian Echidna (*Tachyglossus aculeatus*): Immune System in a (Dr. E. Diener and Dr. E. H. M. Ealey), 950
- Mont Pelée Eruption of 1902 and their Significance to Ordovician Welded Tuffs of County Mayo, Eire: Observations on the (Dr. J. McManus), 578
- Montagna, W., and Lobitz, W. C. (edited by): The Epidermis, review by Prof. C. D. Calnan, 213
- Montague Napier Trust Fund, Royal Society of London, 1155
- Montmorillonite: Interstratified Mineral of Illite and (Prof. M. Sato et al.), 179
- Montmorillonite: Use of Infra-red Spectroscopy to determine the Orientation of Pyridine sorbed on (J. M. Serratos), 679
- Moore, G. F., and Munro, Dr. I. H.: Delayed Fluorescence from Organic Crystals, 772
- Moore, M. A. S., and Owen, Dr. J. J. T.: Chromosome Marker Studies on the Development of the Haemopoietic System in the Chick Embryo, 956
- Moore, Patrick: A Planet Called Earth, review, 961
- Moore, R. J., et al.: Stimulation of Synthesis of Ribonucleic Acid in Sub-apical Sections of *Avena* Coleoptile by Indolyl-3-acetic Acid, 1180
- Moreau, W. M., and Weiss, Prof. K.: Charge Transfer Properties of *dl*-Thioctic Acid and Other Disulphides, 1203
- Moreira, Dr. M. G., and Osswald, Dr. W.: Pronethalol-Induced Reversal of Adrenergic Vasodilation, 1006
- Morgan, J. W., and Lovering, Dr. J. F.: Abundances of Rare Earth Elements in Eclogitic Rocks and Basaltic Achondrites, 1311
- Morgan, W. A., et al.: Determination of Cloud Albedos from Gemini-Titan-5, 775
- Morlat, G., and Kreweras, G. (edited by): Proceedings of the 3rd International Conference on Operational Research, Oslo 1963, review by S. J. Morrison, 1245
- Morphactins, a Novel Group of Plant-growth Regulators (G. Schneider, D. Erdmann, S. Lust, G. Mohr and K. Niehammer), 1013
- Morphism: Schizophrenia as a Genetic (Prof. A. Hoffer), 306
- Morris, D. M., and Adams, Dr. D. M.: Far-infra-red Spectra of some Square-planar Ions, 283
- Morris, Mrs. Pauline: Prisoners and their Families, review by Prof. D. C. Marsh, 216
- Morris, R. A., and Poletti, N.: Frequency Spectrum of Doppler-shifted Whistler-mode Signals, 479
- Morrison, H. G., and Cormack, Prof. D. V.: Variation of the X-ray Sensitivity of *Serratia marcescens* with Relative Humidity, 91
- Morrison, S. J.: Proceedings of the 3rd International Conference on Operational Research, Oslo 1963, review, 1245
- Morse, Dr. R. W., et al.: Influence of the Cerebral Cortex on the Cuneate Nucleus of the Monkey, 1215
- Mortality in Adult Chickens exposed to 1 Atmosphere Oxygen: Delayed (Prof. H. S. Weiss et al.), 1003
- Mortality in Merino Ewes: Undernutrition and Early Embryonic (T. N. Edey), 1232
- Mortimer, D., and Menzies, Dr. I. A.: Mechanism of Oxidation of Chromium Diffusion Coatings on Iron, 1307
- Mechanism of Oxidation of Chromium Diffusion Coatings on Plain Carbon Steels, 1309
- Morton Boyd, Dr. J.: Research and Management in East African Wildlife, 828
- Mosaic Virus: Mechanical Transmission of Hop (Dr. J. T. Legg), 1017
- Mosaics: Modern Technique in Restoration of Roman, 334
- Mosbach, Dr. E. H., Bokkenheuser, V., Chattopadhyay, D. P., Schmidt, M., Hirsh, R. L., and Hofmann, Dr. A. F.: Prevention of Cholesterol-induced Cholelithiasis by Neomycin, 1226
- Mosquitoes: Transmission of Guama and Oriboca Viruses by Naturally Infected (A. Toda and R. E. Shope), 304
- Moss, G. F., et al.: The Iron Dextran Complex, 237
- Moss, Dr. J. A., et al.: Reactivity of the α -Amino Group of Collagen, 1094
- Most, Prof. H., et al.: Primary Tissue Phase of *Plasmodium bergheri* in Different Experimental Hosts, 903
- Mother-Infant Relationship in Rhesus Monkeys: Social Companions and the (Y. Spencer-Booth et al.), 301
- Mothers of Children with Haemolytic Disease of the Newborn: Low Concentrations of Serum Haptoglobin in (J. Pintera), 694
- Motility: The Direction of Cell Movement and Cancer Invasion: Principles of Cell (Dr. S. B. Carter), 1183
- Motion and Sunspot Comparison: Solar (Dr. R. M. Wood and Prof. K. D. Wood), 129
- Motorway: Chiswick-Langley Section of the M4, 1261
- Mouse: Antithymic Antibody Localization in the (Dr. B. Hintz and Prof. M. M. Webber), 797
- Barr Bodies in the (Dr. J. D. Kenney and Dr. U. Mittwoch), 93
- Bone Marrow as the Major Source of Potential Immunologically Competent Cells in the Adult (Dr. M. L. Tyman and Dr. L. J. Cole), 1223
- A Lactate Dehydrogenase Variant in the (Mrs L. Riles), 814
- Mouse Deoxyribonucleic Acid on Hydroxyapatite: Fractionation of (Dr. P. M. B. Walker and Dr. A. McLaren), 1175
- Mouse Eggs: Histocompatibility Antigens in Transplanted (Dr. R. L. Simmons and P. S. Russell), 698
- Mouse Embryo Cells: Plaque-formation of Non-immune and X-irradiated Lymphoid Cells on Monolayers of (G. Moller and E. Moller), 260
- Mouse Interferon induced by Statolon: Different Molecular Species of (Prof. T. C. Merigan and Dr. W. J. Kleinschmidt), 667
- Mouse Kidney: Vital Staining of the Granulated Juxtaglomerular Cells in the (V. Szokoly et al.), 1331
- Mouse Mammary Tumour Virus (MTV): A Qualitative *in vitro* Assay for MTV: Immunology of the (P. B. Blair), 165
- Mouse, Sheep, Cow, Pig, Rhesus Monkey and Human Ovarian Oocytes: Maturation *in vitro* of (Dr. R. G. Edwards), 349
- Mouse Thymocytes *in vivo* with Tritiated Thymidine for Cell Transfer Experiments: Labelling of (Dr. K. Borum), 253
- Movements: Generation of Nutational (D. G. Heathcote), 909
- Moxley, Dr. J. H., Perry, Prof. S., Weiss, Prof. G. H., and Zelen, Dr. M.: Return of Leucocytes to the Bone Marrow in Chronic Myelogenous Leukaemia, 1281
- Moyle, Dr. Jennifer, and Mitchell, Dr. P.: Evidence discriminating between the Chemical and the Chemiosmotic Mechanisms of Electron Transport Phosphorylation, 1205
- Stoichiometry of Proton Translocation through the Respiratory Chain and Adenosine Triphosphatase Systems of Rat Liver Mitochondria, 147
- Mucopolysaccharide: 2,6-Diamino-3-hydroxypimelic Acid in Microbial Cell Wall (H. R. Perkins), 872
- Mucopolysaccharide and Hexosamine Metabolism in Dermal Connective Tissue of Adrenalectomized Rats: Acid (Prof. J. Fabianek et al.), 1212
- Mucopolysaccharides: Chemical Physiology of the Acid (J. E. Scott), 1270
- Mucosa of the Rat: Formation of Amines in the Gastric (R. Håkanson), 793
- Mudd, J. B., et al.: Chloroquine Resistance and the Nature of Malarial Pigment, 691
- Mugil cephalus: Occurrence of Odd-numbered Fatty Acids in the Mullet (Dr. R. G. Ackman), 1213
- Muguruma, J.: Pairs of Spiral Etch Pits in Ice Crystals, 180
- Muldal, S., et al.: Rate of Chromosome Duplication at the End of the Deoxyribonucleic Acid Synthetic Period in Human Blood Cells, 159
- Muller, Dr. Paul: *Obituary*, 1043
- Mullet *Mugil cephalus*: Occurrence of Odd-numbered Fatty Acids in the (Dr. R. G. Ackman), 1213
- Multiple Causes: Implications of the Immunological Theory of Cancer to (Dr. M. R. Anderson and Prof. H. N. Green), 338
- Multiple Thymus Grafts in Aged Mice (Dr. D. Metcalf), 87
- Multistable Ferrites: Re-entrant Hysteresis Loop and (Dr. R. M. Glaister and Dr. E. W. Lee), 67
- Mumford, Lewis: Technics and the Nature of Man, 923
- Municio, Dr. A. M., and Catalan, Dr. R. E.: *In vitro* Effect of Gamma-radiation on Different Cholinesterase Preparations, 1227
- Munn, R. J., et al.: Biology of the Cytoplasm, 247
- Munro, Dr. I. H., and Moore, G. F.: Delayed Fluorescence from Organic Crystals, 772
- Murine Leukaemia Cells: Fractionated X-rays and Fast Neutrons: Increased Sensitivity of *in vitro* (Dr. W. L. Caldwell et al.), 168
- Murine Tumours by the M-P Virus: Inhibition of Transplantable and Spontaneous (Dr. N. Molomut and Dr. M. Padnos), 948
- Murine Virus Hepatitis: Distribution of the Intestinal Factor of Normal Mice which inactivates (Prof. M. Piazza et al.), 1009
- Murray, jun., Dr. R. F., and Blumberg, Prof. B. S.: Precipitin Activity in the Sera of Patients with Deficiency of β -Lipoproteins (Acanthocytosis), 357
- Murrell, K. F. H.: Ergonomics: Man in his Working Environment, review by C. E. Fleming, 106
- Muscarine and Pilocarpine: Preferential Release of Adrenaline from the Adrenal Medulla of (Prof. W. W. Douglas and Dr. A. M. Poisner), 1102
- Muscarinic Properties: Central Stimulant Properties of Compounds with (G. B. Leslie), 1291
- Muscle: Energy Requirements for Relaxation from Tonic Contractions ('Catch') in an Invertebrate (Dr. K. Minihan and Prof. R. E. Davies), 1327
- Enzymatic Adaptation to Contractile Activity in Skeletal (J. Kendrick-Jones and Prof. S. V. Perry), 1068
- Labelled Depolarizing Drugs in Normal and Denervated (Prof. D. B. Taylor et al.), 901
- Muscle Differentiation: Replication and the Activation of (Dr. P. Pietsch and S. B. McCollister), 1170
- Muscle from Patients with Duchenne Muscular Dystrophy: Lactic Dehydrogenase Isoenzymes in (Prof. T. H. Shepard et al.), 1107
- Muscle Preparations: Effect of Gastrin on Isolated Smooth (Dr. A. Bennett), 170
- Muscle Spindle Afferent Endings of the Cat: Differences in Variability of Discharge Frequency between Primary and Secondary (R. B. Stein and Prof. P. B. C. Matthews), 1217
- Muscles: Interferometric Estimation of Effects of Testosterone and Methandrostenolone on the Dry Mass of Various Rat (Dr. W. Staubli and Dr. T. Okada), 562
- Muscular Dystrophy: Lactic Dehydrogenase Isoenzymes in Muscle from Patients with Duchenne (Prof. T. H. Shepard et al.), 1107
- Museum: British, Department of Western Asiatic Antiquities, 434
- Museum: South Australian, 1046
- Museum Studies: University of Leicester, Department of, 122
- Museums Journal, (Vol. 65, No. 2), 434
- Museums of Southern Rhodesia: National, 122
- Musket, A. E., and Malone, J. P.: Seed-borne Fungi, review by R. W. G. Dennis, 319
- Mustard: Molecular Structural Factors and their Role in the Germination of White (S. E. B. Gould et al.), 876
- Mutagenic Effect of Irradiated and Unirradiated DNA in *Drosophila* (A. H. Khan and Dr. T. Alderson), 700
- Mutagenic Effect of Irradiated *Drosophila* Medium: Lack of (Dr. O. S. Reddi et al.), 702
- Mutants in Micro-organisms: A Suggested Phenotypic Classification and Terminology for Enzyme (Dr. J. F. Collins et al.), 841
- Mutants of *Drosophila melanogaster*: Phenocopies of the *ma-l* and *ry* (E. C. Keller, jun., and Dr. E. Glassman), 202
- Mutation in Chemical Systems at Steady State: A Search for (Dr. G. Allen), 258
- Mutation Rates in Bacteria by Spermine: Apparent Suppression of (Dr. H. G. Johnson and Dr. M. K. Bach), 408
- Mutations by Irradiated Medium or Irradiated DNA: Tests on *Drosophila* for the Production of (V. L. Chopra), 699
- My View of the World (Erwin Schrödinger), review by Prof. C. K. Grant, 5
- Mycobacterium phlei*: Occurrence of Dimorphic Forms of (D. E. Stewart-Tull), 603
- Mycobacterium tuberculosis*: Interferon appearance stimulated by Endotoxin, Bacteria, or Viruses in Mice pretreated with *Escherichia coli* Endotoxin or infected with (Prof. J. S. Youngner and Prof. W. R. Stinebring), 456
- Mycoplasma mycoides*: Serological Relationship between Galactans from Normal Bovine Lung and from (Dr. M. Shifrine and R. N. Gourlay), 498
- Mycoses: Fluorochrome Stains for Histological Diagnosis of Visceral (G. Bader et al.), 796
- Myelogenous Leukaemia: Return of Leucocytes to the Bone Marrow in Chronic (Dr. J. H. Moxley et al.), 1281

- Myeloid Virus-induced Leukaemia: Lymphatic Neoplastic Transformation of Thymus in Mice with (L. Chicco-Bianchi *et al.*), 697
- Myers, H. K., *et al.*: Tumour-promoting Agents in Unburned Cigarette Tobacco, 584
- Myers, jun., Prof. L. S., Ward, J. F., Tsukamoto, W. T., and Holmes, D. E.: Site of Attack in the Radiolysis of Pyrimidine Compounds, 1086
- Myocardial Infarction: Aldosterone in, 836
- Myxosarcoma: Properties of Hyaluronic Acid from a Human (Dr. M. L. Marcante), 896
- Myxovirus Attachment: Nuclear Surface N-Acetyl Neuaminic Acid Terminating Receptors for (Prof. P. I. Marcus *et al.*), 1122
- NAGINGTON, Dr. J., Tee, Dr. G. H., and Smith, J. S.: Milker's Nodule Virus Infections in Dorset and their Similarity to Orf, 505
- Nair, N. C.: Development of the Ovary in a Species of *Cissus*, 402
- Nair, Dr. V.: Regional Changes in Brain Serotonin after Head X-irradiation and its Significance in the Potentiation of Barbiturate Hypnosis, 1293
- Nandy, Dr. K.: Interstellar Oscillation, 274
- Nannfeldt, Prof. J. A.: Flora Europaea, Vol. I, review, 8
- Napier, I. M., and Norrish, Prof. R. G. W.: Nitroso Formaldehyde, 1090
- Narcotics: Cation Permeability of Phospholipid Model Membranes: Effect of (Dr. A. D. Bangham *et al.*), 1295
- Nath, Prof. M. C., *et al.*: Effect of Hydrolysed Glucose Cycloacetate in the Prevention of Fatty Infiltration in the Liver, 1001
- Nath, Prof. N., Yawalkar, V. D., and Nath, Prof. M. C.: Effect of Hydrolysed Glucose Cycloacetate in the Prevention of Fatty Infiltration in the Liver, 1001
- National Bureau of Standards: Appointment of Dr. D. H. Freeman to the Analytical Chemistry Division, 1259
- Textile and Apparel Technology Center, Appointment of Assistant Chief, 628
- National Institute of Agricultural Engineering: Component Failure Investigation, 1262
- National Institute of Sciences of India: Members of the Council for 1966, 936
- National Instrument Service in Britain, 528
- National Museums of Southern Rhodesia, 122
- National Physical Laboratory: New Absolute Determination of the Acceleration due to Gravity at the (Dr. A. H. Cook), 279
- National Physical Laboratory: Resignation of Director, Sir Gordon Sutherland, 732
- Special Merit Promotions, 527
- Retirement of the Superintendent of the Standards Division, 118
- National Trust, Seventeenth Annual Report, 834
- Natural Heritage: Conserving the, 1241
- Natural History of the Chromaffin Cell (Prof. R. E. Coupland), review by Dr. B. A. Callingham, 6
- Natural Selection and Transmissible Disease (Prof. N. A. Barnicot), 535
- Nature and God (Prof. L. Charles Birch), review, 985
- Nature Conservancy in Britain, 635
- Nature of the Hepatomegalic Effect produced by Ethyl-chlorophenoxyisobutyrate in the Rat (Dr. R. Hess, W. Staubi, and W. Röss), 856
- Nature of Man: Heredity and the (Dr. T. Dobzhansky), review by Prof. C. H. Waddington, 959
- Nature of Man: Technics and the (Lewis Mumford), 923
- Nature: Sudden Death of Mr. L. J. F. Brimble on November 15th, 1965; the Editor of, 732
- Nature's Time-scale: A Proposal on (Prof. N. G. McCormick), 334
- Naturwissenschaft: Metaphysik der (W. Schapp), review, 988
- Naughton, Dr. M. A., *et al.*: Globin Synthesis in Thalassemia: an *in vitro* Study, 1061
- Naylor, Dr. K. A., *et al.*: Surface Charges on Arc Track Oxide Layers, 774
- Near East: Earliest Civilizations of the (J. Mellaart), review by M. C. Burkitt, 984
- Near Field: Transmission through a Tapered Quartz Tube in the Laser (H. J. Caulfield), 773
- Nearly Equal Quantities: Calculations with Two (Dr. J. H. Fremlin: Dr. F. T. Worrell), 1306
- Nebraska, Native Vegetation of (Prof. J. E. Weaver), review by Dr. D. R. Hunt, 1249
- Nebula: Enrichment of Iron during Accretion in the Solar (Prof. S. R. Taylor), 886
- Neulosity in the Vicinity of the Cetus Arc: Filamentary (Dr. J. Meaburn), 575
- Nedergaard, O. A., *et al.*: Labelled Depolarizing Drugs in Normal and Denervated Muscle, 901
- Nees, P. O., and Dörse, P. H.: Feeding and Reproduction of Rats fed Calcium Cyclamate, 81
- Negative Absolute Pressure on Flow Resistance: Ascent of Sap in Trees: Effect of (Prof. B. Johnson and Prof. J. R. Dixon), 1347
- Nelson, M. J., *et al.*: Metabolites in Urine of Rats on Diets containing Aldrin or Dieldrin, 289
- Nematode, *Heterodera schachtii* Schm.: Zinc and Other Metallic Ions as Hatching Agents for the Beet Cyst (Dr. A. J. Clarke and Dr. A. M. Shepherd), 502
- Nematode *Ostertagia circumcincta* in the Sheep: Establishment of a Pure Infection of the (J. W. Copland), 1229
- Neogene: Progress in the Planktonic Foraminiferal Biostratigraphy of the (Dr. F. T. Banner and Dr. W. H. Blow), 1164
- Neomycin: Prevention of Cholesterol-induced Cholelithiasis by (Dr. E. H. Mosbach *et al.*), 1226
- Neonatal Thymectomized Rats: Effect of a Lipid Fraction from Rat Thymus on Delayed Hypersensitivity Reactions of (Prof. B. D. Janković *et al.*), 356
- Neoplastic Transformation of Thymus in Mice with Myeloid Virus-induced Leukaemia: Lymphatic (L. Chicco-Bianchi *et al.*), 697
- Nephrons of the Kidney of the Rat: Effect of 'Furosemid' on Chloride and Water Excretion in Single (G. Malnic *et al.*), 80
- Nerve Potential Recordings during electrically and mechanically evoked Monosynaptic Reflexes in Man (Dr. M. M. Gassel and Dr. E. Diamantopoulos), 1004
- Nerve: Ultrastructure of the Phrenic (Dr. A. S. Wilson and D. G. Silva), 707
- Netherlands: Register of Scientific Research in the (S. T. Groenman), 28
- Neunes, H. W., and Pongolini, G.-F.: Breeding a Pelagic Copepod, *Euterpina acutifrons* (Dana), in the Laboratory, 571
- Neural Regeneration: Mechanisms of (edited by M. Singer and J. P. Schade), review by Prof. K. Krnjević, 1248
- Neural Volleys: Upper Frequency Limits detectable in the Auditory System (Capt. J. C. Boudreau), 1237
- Neuroglia: Possible Role of (Prof. C. P. Wendell-Smith and Prof. M. J. Blunt), and (Dr. L. Hertz), 600
- Neurointermediate Lobe in Skates: Electron Microscope Observations of Neurosecretory Elements in the (Dr. A. L. Polenov and M. A. Belenky), 94
- Neuromuscular Functions: Effect of Use and Disuse on (edited by E. Gutmann and P. Hnik) Symposium 1962, review by Prof. K. Krnjević, 1248
- Neuromuscular Junction: Presynaptic Effects of Potassium Ion on the Mammalian (Dr. R. L. Parsons *et al.*), 590
- Neurons: Somesthetic and Visual Responses of Superior Colliculus (Dr. D. Jassik-Gerschenfeld), 898
- Neurosecretory Elements in the Neurointermediate Lobe in Skates: Electron Microscope Observations of (Dr. A. L. Polenov and M. A. Belenky), 94
- Neurosecretory Fibres and Pituitaries in the Eel: A Functional Relationship between (Sir Francis Knowles and L. Vollrath), 1343
- Neurosecretory Innervation of the Pars Distalis of the Pituitary of the Eel: A Dual (Sir Francis Knowles and L. Vollrath), 1343
- Neurosis: Causes and Cures of (Prof. H. J. Eysenck and Dr. S. Rachman), review by Dr. B. Semeonoff, 1027
- Neutron and Proton Magic Numbers in Atomic Nuclei: Structural Basis of (Prof. L. Pauling), 174
- Neutrons: Increased Sensitivity of *in vitro* Murine Leukaemia Cells to Fractionated X-rays and Fast (Dr. W. L. Caldwell *et al.*), 168
- Neve, Dr. R. A., and Hartley, Dr. R. D.: Relationships between Constituents of Cones and Male Flowers of the Hop (*Humulus lupulus*, L.), 804
- New Absolute Determination of the Acceleration due to Gravity at the National Physical Laboratory, (Dr. A. H. Cook), 279
- New, Active Metabolite of Miracil D⁷ (D. Rosi, G. Peruzzotti, Dr. E. W. Dennis, Prof. D. A. Berberian, H. Freele and Dr. S. Archer), 1005
- New Bis albuminemic Family (Dr. G. Sandor, Dr. L. Martin, Dr. M. Porsin, Dr. A. Rousseau and Dr. R. Martin), 1222
- New Chemical Aspect of Penicillin Allergy: Direct Reaction of Penicillin with α -Amino-groups (Dr. C. H. Schneider and Dr. A. L. de Weck), 57
- New Chromium(IV) Peroxo Compound (Dr. D. A. House and Prof. C. S. Garner), 776
- New Class of Herbicidal Compounds: 2-Trifluoromethylbenzimidazoles: a (Dr. D. E. Burton *et al.*), 1166
- New Commonwealth Students in Britain (P.E.P. Publication), review by Lord Twining, 957
- New Fluorescent Antibody Method: Mixed Antiglobulin Immunofluorescence or Labelled Antigen Indirect Immunofluorescence Staining (Prof. E. H. Beutner, Dr. E. J. Holborow and G. D. Johnson), 353
- New Guinea: Pyrethrum Fom, 835
- New Haemoglobin, J Toranzo (α S Alanine \rightarrow Aspartic Acid) (Dr. J. H. Crookston), and (D. Beale, D. Irvine and Dr. H. Lehmann), 1059
- New Inherited Coagulation Disorder caused by an Abnormal Fibrinogen ('Fibrinogen Baltimore') (Dr. E. A. Beck, P. Charache and Prof. D. P. Jackson), 143
- New Limits to the Angular Sizes of Some Quasars (R. L. Adgie, H. Gent, O. B. Slee), (Prof. A. D. Frost, Dr. H. P. Palmer and Dr. B. Rowson), 275
- New Medical School: Planning a, 634
- New Method for Electron Microscopy: Freeze-fixation, a (Prof. O. Nilsson), 90
- New Model for Virus Ribonucleic Acid Replication (Dr. F. Brown and Dr. S. J. Martin), 861
- New Property of the Early Receptor Potential of Rat Retina (Dr. G. B. Arden and H. Ikeda), 1100
- New Shell Research Fellowship, 1263
- New Type of Regulatory Mechanism in Bacteria: An Example for a (Dr. A. Tomasz), 155
- New York State: Pollen Studies in, 1262
- New Zealand: Geochemical Group, 123
- National Art Gallery and Dominion Museum, 1261
- Scientific Research in, 1233
- Scientific and Industrial Research in, 1156
- State Highways, 835
- New Zealand Department of Scientific and Industrial Research, (N.Z.D.S.I.R.), 327
- Director of Animal Ecology Division, 326
- Director of the Wheat Research Institute, 327
- Superintendent of Geophysical Survey, Geophysics Division, 327
- New Zealand in Relation to Year of Teeth: Ingestion of Soil by Sheep in (W. B. Healy and T. G. Ludwig), 806
- Newbold, Dr. G. T., *et al.*: 2-Trifluoromethylbenzimidazoles: A New Class of Herbicidal Compounds, 1166
- Newborn: Low Concentrations of Serum Haptoglobin in Mothers of Children with Haemolytic Disease of the (J. Pintera), 694
- Newcastle upon Tyne: University of, Chair of Psychology, 21, Chair of Zoology, 432
- Newly Synthesized Insulin *in vitro*: Secretion of (S. L. Howell *et al.*), 487
- Nias, A. H. W., and Lajtha, Dr. L. G.: Continuous Irradiation of HeLa Cells at -196°C , 400
- Niblett, W. R. (edited by): How and Why do we Learn?, review by A. D. C. Peterson, 314
- Nicholls, Dr. E. M., *et al.*: Haptoglobin Johnson in Australian Aborigines, 694
- Nichols, jun., Prof. George, and Woods, J. F.: Distribution of Collagenase in Rat Tissues, 1325
- Nicholson, A. R., *et al.*: Functional Similarities between the Cytoplasmic Organelles of Melanocytes and the Mitochondria of Hepatocytes, 810
- Nicholson, E. M.: The Countryside in 1970, 1265
- Nickel: Discoverer of, A. = Cronstedt, 229
- Nickel-10.9 per cent Cobalt Alloy: Metal Concentration Profiles in Oxide Scales on (Dr. G. C. Wood and J. M. Ferguson), 369
- Nicol, Prof. T., Vernon-Roberts, Dr. B., and Quantock, Dr. D. C.: Protective Effect of Oestrogens against the Toxic Decomposition Products of Tri-nitroethanol, 1098
- Nicotinamide Antagonists: Anticoccidial Activity of (Dr. S. J. Ball *et al.*), 397
- Nicotinamide Mononucleotide (NMN) Pyrophosphorylase Activity in Asclites Cell Extracts (Prof. L. S. Dietrich, L. Fuller, I. L. Yero and L. Martinez), 347
- Nicotine in Smokers' Urine by Gas Chromatography: Determination of (Dr. N. L. McNiven *et al.*), 788
- Nielson, R. L.: Presence of Plant Growth Substances in Earthworms demonstrated by Paper Chromatography and the Went Pea Test, 1113
- Niehammer, K., *et al.*: Morphactins, a Novel Group of Plant-growth Regulators, 1013
- Nigam, Dr. V. N., and de Laminade, Dr. G.: Formation of Intra-mitochondrial Glucosyloligosaccharides from Glucose by Intact Novikoff Asclites Hepatoma Cells, 664
- Nigeria: Antimalarial Prophylaxis in Eastern, 1154
- Nigeria: Micro-organisms associated with Dead Insect Larvae in (Dr. N. Okafor), 1015
- Night Sky in November, 329, December, 735, January, 1264
- Night Work: Shift Working: the Arrangement of Hours on (Mrs. G. de la Mare and Dr. J. Walker), 1127
- Nilsson, C. S., Alexander, W. M., McCracken, C. W., Berg, O. E., and Secretan, L.: Measured Velocities of Interplanetary Dust Particles, 673
- Nilsson, Prof. O.: Freeze-fixation, a New Method for Electron Microscopy, 90
- Nilwarangkur, S., and Berline, Dr. G. M.: Deterioration of Stored Inulin Solutions, 77
- Nineteenth Century: Public Health in the (Prof. C. F. Brockington), review by Sir Arthur MacNalty, 377

- Nineteenth Century : Science in the (edited by René Taton), review by Dr. W. P. D. Wightman, 958
- Nisbet, N. W. : The Surgeon's Craft, review, 7
- Nishizawa, K., et al. : Air Ion Effects on the Oxygen Consumption of Barley Seedlings, 1112
- Nissim, Dr. J. A., and Hart, S. L. : Mechanism of Intestinal Absorption : Transport of Methionine and Sodium Butyrate by Intracellular Plasma, 145
- Nitrate Ions in Crystals : Effect of Environment on the Orientation of the (Prof. N. R. Kunchur), 778
- Nitrenes and Dinitrenes : Ultra-violet Absorption Spectra of Aromatic (A. Reiser and V. Frazer), 682
- N-H Bond in Hydrazine : Dissociation Energy of the (Dr. I. P. Fisher and Dr. G. A. Heath), 1199
- Nitrogen Atom in Certain Amides : Slow Inversion at the (Dr. T. H. Siddall and Dr. C. A. Prohaska), 582
- Nitrogen Sources : Differences in the Antigenic Pattern of *Azotobacter* grown on Different (Prof. T. Holme and B. Zacharias), 1235
- Nitrogen : Townsend's First Ionization Coefficient for Compressed (Dr. B. W. Ward), 994
- Nitroglycerin contained in Porous Rock : Detonability of (J. E. Hay and F. H. Scott), 1197
- Nitrophenylhydrazones : Mass Spectrometric Fragmentation of (Prof. C. Djerassi and S. D. Sample), 1314
- 4-Nitro-quinoline N-oxide of Tryptophan and Indole Uptake in *Escherichia coli* : Interference by the Carcinogenic (A. C. Zaholsky and Prof. S. L. Marcus), 296
- Nitroso Formaldehyde (I. M. Napier and Prof. R. G. W. Norrish), 1090
- Nobel Lectures including Presentation Speeches and Laureates' Biographies, 1922-1941 : Physics, review by Dr. G. Burniston Brown, 103
- Nobel Prize for Medicine : 1965 (Prof. M. R. Pollock), 1250
- Nodule Virus Infections in Dorset and their Similarity to Orf - Milker's (Dr. J. Nagington et al.), 505
- Nomad Peoples of the Steppes : The Royal Hordes, (E. D. Phillips), review by M. C. Burkitt, 1138
- Nomadic Behaviour of Yttrium Ions and their Structure in Solution (Dr. R. C. Vickery), 678
- Nomenclature : Enzyme, 736
- Non-haem Iron in Ferredoxin and Rubredoxin : Nature of the (Dr. R. D. Gillard, Dr. E. D. McKenzie, Prof. R. Mason, S. G. Mayhew, Dr. J. L. Peel and J. C. Stangroom), 769
- Non-immune and X-irradiated Lymphoid Cells on Monolayers of Mouse Embryo Cells : Plaque-formation by (G. Moller and E. Moller), 260
- Non-proprietary Names : Veterinary, 630
- Non-steroid Substance with Antiestrogenic Properties : Specific Uptake in Corpora Lutea of a (A. Hanngren et al.), 461
- Noradrenaline by the Spleen : Effect of Altered Perfusion Rates on the Retention of (Dr. D. M. Paton and Prof. C. N. Gillis), 391
- Norepinephrine and Serotonin in the Tissues and Venoms of Two Pit Vipers (Prof. A. H. Anton and Prof. J. F. Gennaro, jun.), 1174
- Norris, Dr. F. W. : *Obituary* of Prof. R. H. Hopkins, 1259
- Norris, M. J., and Perner, M. P. : An Inhibitory Effect of Allatectomized Males and Females on the Sexual Maturation of Young Male Adults of *Schistocerca gregaria* (Forsk.) (Orthoptera : Acrididae), 1122
- Norrish, Prof. R. G. W., and Napier, I. M. : Nitroso Formaldehyde, 1090
- North, Dr. F. J. : Evolution of the Bristol Channel, Third Edition, revised and enlarged, review by Dr. A. R. MacGregor, 110
- North, M. E. W., and Thorpe, Dr. W. H. : Origin and Significance of the Power of Vocal Imitation : with Special Reference to the Antiphonal Singing of Birds, 219
- North America : Bark Canoes and Skin Boats of, 22
- North American Rocks : Age Determinations of, 435
- North Atlantic Deep-sea Sediments : Pleistocene Glacial-marine Zones in (Dr. J. R. Conolly and Dr. M. Ewing), 135
- Norton, Dr. Dorita A., and Eger, Dr. C. : Androgenic Steroid Complexes with p-Bromophenol, 997
- Nossal, Prof. G. J. V. : Appointment as Director of the Walter and Eliza Hall Institute of Medical Research, Melbourne, 730
- Nour, Prof. M. A. : University of Khartoum, Sudan ; Silver Jubilee of the Faculty of Agriculture, Shambat, 620
- November : Night Sky in, 329
- Novikoff Asbestos Hepatoma Cells : Formation of Intra-mitochondrial Glucosyl-oligosaccharides from Glucose by Intact (Dr. V. N. Nigam and Dr. G. de Lamirolle), 664
- Novikova, M. N., et al. : Bone Marrow Allografts in Thymectomized X-irradiated Dogs, 399
- Nozzles : Effects of Seeding on the Condensation of Atmospheric Moisture in (Dr. E. R. Buckle and Dr. A. A. Pouring), 367
- Nuclear and Radiochemistry (Dr. G. Friedlander, Prof. J. W. Kennedy and Prof. J. M. Miller), Second Edition, review by H. A. C. McKay, 105
- Nuclear Emulsion : Determination of the Total Alpha Activity of Drinking Water using a (M. W. Tyrrell et al.), 1074
- Nuclear Power Stations in Britain : Commercial (S. Weintraub), 25
- Nuclear Surface N-Acetyl Neuraminic Acid Terminating Receptors for Myxovirus Attachment (Prof. P. I. Marcus, J. M. Salb and V. G. Schwartz), 1122
- Nucleated Erythrocytes : Stable Messenger RNA in (Dr. R. B. Scott and R. A. Malt), 497
- Nuclei : Computer Recognition of Cervical Cell (G. W. McMaster), 485
- Nuclei : Structural Basis of Neutron and Proton Magic Numbers in Atomic (Prof. L. Pauling), 174
- Nucleic Acid Bases : Thermoluminescence and Ultra-violet Phosphorescence of the (Dr. R. J. Fleming and R. M. Kerr), 1198
- Nucleic Acid *in vitro* : Alteration of Hamster Cells by (K. Shepley et al.), 1072
- Nucleic Acid of Infectious Laryngotracheitis Virus (G. A. Tannock), 711
- Nucleic Acids and Protein in Starving Bacteria : Metabolism of (B. Brdar et al.), 303
- Nucleo-cytoplasmic Interactions in the Rat Liver Cell (E. R. Godwin, Dr. K. R. Rees, Dr. G. F. Rowland and J. S. Varcoe), 246
- Nucleotide Peptides in Yeast (Dr. D. J. Millin and M. J. Saltmarsh-Andrew), 468
- Nucleotides and Coenzymes (Dr. D. W. Hutchinson), review by Dr. D. M. Brown, 987
- Number Judgment : Interstimulus Interval and (D. Jones and Dr. A. Elithorn), 153
- Numerical Model of Random Packing of Spheres (Dr. M. M. Levine and J. Chernick), 69
- Nunn, Prof. J. F., et al. : Carbon Dioxide Production of Whole Blood *in vitro*, 82
- Nutritional Movements : Generation of (D. G. Heathcote), 909
- Nutrition and Dietetics, Vol. 5 : World Review of (edited by G. H. Bourne), review by Dr. A. M. Copping, 421
- Nutrition and Food Technology : Dictionary of (Dr. A. E. Bender), Second Edition, review by Dr. A. M. Copping, 987
- Nutrition and its International Recognition (Sir Rudolph A. Peters), 117
- OBJECTIVE Evaluation of Quality in Poultry Meat (Dr. A. W. Khan), 204
- Obscuration : Interstellar (Dr. K. Nandy), 274
- Observation of Satellites : Visual (D. G. King-Hele), 1156
- Observations of a Strong Unidentified Microwave Line and of Emission from the OH Molecule (Prof. H. Weaver, Dr. D. R. V. Williams, Dr. N. H. Dieter and W. T. Lum), 29
- Observations of Polarized OH Emission (Dr. S. Weinreb, Prof. M. L. Meeks, J. C. Carter), and (Prof. A. H. Barrett and A. E. F. Rogers), 440
- Occurrence of Dimorphic Forms of *Mycobacterium phlei* (D. E. S. Stewart-Tull), 603
- Occurrence of Fructose-1-phosphate in Tissues of Higher Plants (D. Graham and Dr. T. ap Rees), 88
- Occurrence of Gnetalean Pollen Grains in the Katrol Formation (Upper Jurassic of Kutch, India (Y. K. Mathur and Mrs K. Mathur), 912
- Occurrence of Odd-numbered Fatty Acids in the Mullet *Mugil cephalus* (Dr. R. G. Ackman), 1213
- Occurrence of *Salvinia auriculata* Aublet on the Congo River (Dr. E. C. S. Little), 1111
- Oceanographic Institution : Woods Hole Promotions, 327
- Oceanography : French, 1261
- O'Connor, Cynthia C., et al. : Mixed Infection of African Green Monkey Kidney Cells by Adenovirus 7 and SV 40, 1350
- O'Connor, Gregory T., et al. : Mixed Infection of African Green Monkey Kidney Cells by Adenovirus 7 and SV 40, 1350
- Odd-numbered Fatty Acids in the Mullet *Mugil cephalus* : Occurrence of (Dr. R. G. Ackman), 1213
- 17 α -Oestradiol Glycosides by Rabbit Tissue Glycosidases : Hydrolysis of Two (K. S. Whittemore and Dr. D. S. Layne), 288
- Oestrogens : Polyamide Layer Chromatography of (Y. C. Tung and Prof. K.-T. Wang), 581
- Oestrogens, Protective Effect of, against the Toxic Decomposition Products of Tribromoethanol (Prof. T. Nicol et al.), 1098
- Official University of the Congo at Elisabethville, 124
- O'Flaherty, F., Roddy, W. T., and Lollar, R. M. (edited by) : Chemistry and Technology of Leather, Vol. 4, review by Prof. A. G. Ward, 617
- Ogilvie, J. F. : Electronic Absorption Spectrum of the CH₃O-radical, 1315
- Ogilvy-Webb, Mrs. Marjorie : The Government Explains : A Study of the Information Services, review by R. Brightman, 1139
- Ogston, Dr. D. : Fibrinolysis, review, 1247
- OH Emission in the Direction of Radio Source W49 (B. Zuckerman, Prof. A. E. Lilley and H. Penfield), 441
- OH Emission : Observations of Polarized (Dr. S. Weinreb et al.), 440
- OH in Absorption and Emission : Anomalous Intensity Ratios of the Interstellar Lines of (R. X. McGee et al.), 1193
- OH Molecule : Observations of a Strong Unidentified Microwave Line and of Emission from the (Prof. H. Weaver et al.), 29
- Ohayon, E., et al. : Antigen in Leukaemic Patients, 1329
- Ohnuki, Dr. Y. : Demonstration of the Spiral Structure of Human Chromosomes, 916
- Ohuchi, T., et al. : Air Ion Effects on the Oxygen Consumption of Barley Seedlings, 1112
- Oil Pipeline over the Alps, 435
- Onuma, K., et al. : Interstratified Mineral of Illite and Montmorillonite, 179
- Okada, Dr. T., and Stauble, Dr. W. : Interferometric Estimation of Effects of Testosterone and Methandrostenolone on the Dry Mass of Various Rat Muscles, 562
- Okafor, Dr. N. : Micro-organisms associated with Dead Insect Larvae in Nigeria, 1015
- Okamoto, Suehiko, and Suzuki, Y. : Chloramphenicol, Dihydrostreptomycin, and Kanamycin-inactivating Enzymes from Multiple Drug-resistant *Escherichia coli* carrying Episome 'R', 1301
- Oke, J. B., et al. : Common Features of the Optical Continua associated with Violent Cosmic Events, 1190
- Oldfield, Prof. R. C. : Pathology of Thinking, review, 982
- Oldham, C., et al. : Structure of a Diolefin Complex of Platinum(II), C₁₀H₁₆O₂PtCl₂, 580
- Olduvai Bed I : Cranial Capacity of the Hominine from (Prof. R. L. Holloway jun ; Prof. P. V. Tobias), 205
- O'Leary, Prof. W. M., and Grossberg, Dr. S. E. : Hyperlipaemia following Viral Infection in the Chicken Embryo - A New Syndrome, 954
- Oleic Acid labelled with Iodine-131, and Triolein Deiodination *in vitro* (L. J. Angileri), 896
- Oligocene in California ? (J. H. Lipps), 885
- Oligosaccharides (R. W. Bailey), review by Prof. M. Stacey, 971
- Oliver, A., et al. : Use of Technetium-99m in Hepatic Scintigraphy, 86
- Oliver, Prof. W. T., and Funnell, H. S. : Proposed Physiological Function for Plasma Cholinesterase, 689
- Olmsted, Prof. J. M. H., and Gelbaum, Prof. B. R. : Counterexamples in Analysis, reviewed by Prof. T. A. A. Broadbent, 218
- Omar, A. R., et al. : Association of Adenoviruses with Bovine Respiratory Disease, 307
- O'Meara, Prof. R. A. Q., et al. : A Screening Test for Substances inhibiting the Cancer Coagulant Factor, 1008
- Ommanney, Dr. F. D. : A Draught of Fishes, review by Dr. L. Harrison Matthews, 517
- One Dimension : Relative Similarities in (Prof. J. W. Carmichael et al.), 544
- Ong, W. K., and Prince, Dr. R. H. : Kinetics of Formation of the Monoacetyl-acetonato-Iron (III) Complex, 1201
- Ontario : A Well-preserved Grizzly Bear Skull recovered from a Late Glacial Deposit near Lake Simcoe, (R. L. Peterson), 1233
- Oocytes : Maturation *in vitro* of Mouse, Sheep, Cow, Pig, Rhesus Monkey and Human Ovarian (Dr. R. G. Edwards), 349
- Open Channels : Laminar and Turbulent Flow in (Dr. J. Allen), 1196
- Operational Research, Oslo 1963 : Proceedings of the 3rd International Conference (edited by G. Kreweras and G. Morlat), review by S. J. Morrison, 1245
- Opit, Dr. L. J., and Charnock, Dr. J. S. : Molecular Model for a Sodium Pump, 471
- Opsonin by a Macrophage Phagocytic System : Differentiation between Cytophilic Antibody and (W. E. Parish), 594
- Optical Continua associated with Violent Cosmic Events : Common Features of the (Prof. L. Searle et al.), 1190
- Optical Isomers of DL-Tryptophan, 5-Hydroxy-DL-tryptophan and 6-Hydroxy-DL-tryptophan by Paper and Thin-layer Chromatography : Resolution of the (Dr. S. F. Contractor and J. Wragg), 71
- Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry (Dr. P. Crabbe), review by Prof. W. Klyne, 970
- Optical Rotatory Dispersion and Secondary Structure of Ribonucleic Acid in Mammalian Ribosomes (A. Blake and Dr. A. R. Peacocke), 1319
- Optical Rotatory Dispersion of a Chloroplast Preparation (Dr. Bacon Ke), 573
- Optical Transforms : Their Preparation and Application to X-ray Diffraction Problems (Dr. C. A. Taylor and Prof. H. Lipson), review by Dr. C. W. Bunn, 1031

- Optics : Mathematical Theory of (Dr. R. K. Luneburg and M. Herzberger), review by Dr. W. J. Bates, 971
- Orbital Theory : Molecular (C. J. Ballhausen and Dr. H. B. Gray), review by E. W. T. Richards, 110
- Orchids : Conservation of the Bee, 936
- Ordovician Welded Tuffs of County Mayo, Eire : Observations on the Mont Pelée Eruption of 1902 and their Significance to (Dr. J. McManus), 578
- Orf : Milker's Nodule Virus Infections in Dorset and their Similarity to (Dr. J. Nagington *et al.*), 505
- Organelles and Cell Growth in Root Caps : Cytoplasmic (Dr. B. E. Juniper and Dr. F. A. L. Clowes), 864
- Organelles of Melanocytes and the Mitochondria of Hepatocytes : Functional Similarities between the Cytoplasmic (Dr. M. H. Van Woert *et al.*), 810
- Organic Acids by Tritiating Methylation : Quantitative Chromatographic Analysis of (G. K. Koch and the late G. Jurriens), 1312
- Organic Chemistry : Optical Rotatory Dispersion and Circular Dichroism in (Dr. P. Crabbe), review by Prof. W. Klyne, 970
- Organic Complexing Reagents : Structure, Behavior, and Application to Inorganic Analysis (D. D. Perrin), review by Dr. T. S. West, 3
- Organic Compounds : Dictionary of (edited by Dr. J. R. A. Pollock and Dr. R. Stevens), Fourth Edition, Five Volumes and Supplement, review by Dr. F. G. Mann, 417
- Organic Compounds : Systematic Identification of, Fifth Edition (R. L. Shriner *et al.*), review by Dr. D. T. Lewis, 1032
- Organic Crystals : Delayed Fluorescence from (G. F. Moore and Dr. I. H. Munro), 772
- Organic Liquids : Acoustic Velocity Dispersion in some Non-associated (Dr. S. E. A. Hakim and W. J. Comley), 1082
- Organic Matter in Recent and Ancient Limestones and its Role in their Diagenesis (D. J. Shearman and Sir Patrick A. d'E. Skipwith), 1310
- Organization of Man's Memory : History as the (Prof. Herbert Butterfield), 1036
- Organization of the Scientific Civil Service, 1133
- Organochlorine Pesticides in the Atmospheric Environment (Dr. D. C. Abbott, R. B. Harrison, J. O'G. Tatton and Dr. J. Thomson), 1317
- Organo-mineral Complex in Soils, 734
- Orgel, L. E., and Beck, A. : Photochemical Synthesis of Condensed Phosphates, 1000
- Orgueil Carbonaceous Meteorites : Evidence for Microfossils in the Alais and (S. L. Van Landingham), 947
- Oriboca Viruses by Naturally Infected Mosquitoes : Transmission of Guamá and (A. Toda and R. E. Shope), 304
- Orientation of Pyridine sorbed on Montmorillonite : Use of Infra-red Spectroscopy to determine the (J. M. Serratos), 679
- Orientation of the Nitrate Ions in Crystals : Effect of Environment on the (Prof. N. R. Kunchur), 778
- Oriented Deoxyribonucleic Acid : Electron Spin Resonance in X-irradiated (Dr. J. P. Elliott and Dr. S. J. Wyard), 483
- Origin of Apatite Meteorites from the Arizona Meteorite Crater (Prof. M. E. Lipschutz), 636
- Origin of Fossil 'Zoophytes' (Dr. M. Plička), 579
- Origin of the Geoelectric Effect in Plants (Prof. M. B. Wilkins and A. E. R. Woodcock), 990
- Orosomucoid : Inhibition of Influenza Virus Haemagglutination by Polymerized (P. H. Whitehead *et al.*), 915
- Orr, Dorothy A., and Taylor, Dr. M. M. : Effect of Incubation Temperature on the Flagellation of Gram-negative Rods, 1017
- (Orthoptera : Acrididae) : an Inhibitory Effect of Allatectomized Males and Females on the Sexual Maturation of Young Male Adults of *Schistocerca gregaria* (Forsk.), (M. L. Norris and M. P. Pender), 1122
- Osborn, J. A., *et al.* : Homogeneous Hydrogenation and Hydroformylation using Ruthenium Complexes, 1203
- Osborne, Dr. Daphne J., *et al.* : Promotion by Kinetin of the Polar Transport of Two Auxins, 1065
- Osmotropotaxis in the Honey-bee (Dr. H. Martin), 59
- Osswald, Dr. W., and Moreira, Dr. M. G. : Pronethalol-induced Reversal of Adrenergic Vasodepression, 1006
- Ostertagia circumcincta* in the Sheep : Establishment of a Pure Infection of the Nematode (J. W. Copland), 1229
- Otala (Helix) lactea* : a Specific Agglutinin in the Snail (Prof. W. C. Boyd and R. Brown), 593
- Ottewill, Dr. R. H., and Shaw, J. N. : Relaxation Effects in the Electrophoresis of Disperse Systems, 681
- Outgrowth of Spores of *Bacillus cereus* Strain T. : Timing of Protein Synthesis during Germination and (W. Steinberg *et al.*), 710
- Ovalocytosis and Haemoglobin E-Ovalocytosis in Malayan Aborigines : Hereditary (Dr. Lie-Injo Luan Eng), 1329
- Ovarian Ascorbic Acid Content of Pseudopregnant Immature Rats used in Parlow's Test : Diurnal Variations in (Dr. M. de la Lastra and Dr. H. Croxatto), 789
- Ovarian Extract : Induction of Crossing-over in *Drosophila* Males by means of (O. S. Reddi *et al.*), 203
- Ovarian Oocytes : Maturation *in vitro* of Mouse, Sheep, Cow, Pig, Rhesus Monkey and Human (Dr. R. G. Edwards), 349
- Overpotential at Platinized Platinum and Palladized Palladium Cathodes : Diffusion or Hydride Component of Hydrogen (M. N. Hull and Dr. F. A. Lewis), 887
- Overseas : Aid to Education, 120
- Overseas Development Institute, 935
- Overseas Development : Ministry of, Directorate of Overseas Surveys, 118
- Ovocytes : Emission of Feulgen-positive Particles during the *in vitro* Maturation of Toad (Prof. J. Brachet), 596
- Ovule in a Species of *Cissus* : Development of the (N. C. Nair), 402
- Owen, Dr. J. J. T., and Moore, M. A. S. : Chromosome Marker Studies on the Development of the Haemopoietic System in the Chick Embryo, 956
- Owen, T., and Walsh, T. E. : Radiation Balance of Jupiter and Saturn, 476
- Oxford : University of, Chair of Metallurgy, 627
- Vice-Chancellor's Oration, 1155
- Oxidases and Tissue Slices : Kinetic Parameters for Oxygen in Reactions involving Purified (Dr. M. McCabe and Dr. D. A. Gilbert), 450
- Oxidation and Reduction of Diphenyl Picrylhydrazyl on Surfaces : Simultaneous (Prof. J. G. Aston *et al.*), 181
- Oxidation of Chromium Diffusion Coatings on Iron : Mechanism of (Dr. I. A. Menzies and D. Mortimer), 1307
- Oxidation of Chromium Diffusion Coatings on Plain Carbon Steels : Mechanism of (Dr. I. A. Menzies and D. Mortimer), 1309
- ω -Oxidation of 2,4-Dimethylheptanoic Acid : Urinary Metabolites in the (Dr. S. R. Tannenbaum and Prof. S. A. Miller), 452
- Oxidation of Hydrocarbons in the Liquid Phase (edited by N. M. Emanuel), review by Dr. H. Steiner, 420
- Oxidation of some 2,4-Dinitrophenylamines with Ceric Salts (Miss S. Haworth and Dr. J. R. Holker), 184
- Oxidation-Reduction State of N-Methylphenazinium Methyl Sulphate (Phenazine Methosulphate) : Dependence of Photophosphorylation by Isolated Chloroplasts on the (G. Zweig and Dr. M. Avron), 190
- Oxidative and Biosynthetic Utilization by Human Spermatozoa of a Metabolite of the Female Reproductive Tract (Prof. C. Terner), 1115
- Oxidative Deterioration : n-Alkan-1-ols in Butter after (W. Stark and D. A. Fors), 190
- Oxidative Phosphorylation : Activity of 2-Trifluoromethylbenzimidazoles as Uncouplers of (Dr. O. T. G. Jones and W. A. Watson), 1169
- Oxide Films on Valve Metals : A.C. Resistivity of Anodic (Dr. G. C. Wood and C. Pearson), 547
- Oxide Layers : Surface Charges on Arc Track (Dr. W. P. Jayasekara *et al.*), 774
- Oxide Scales on Nickel-10.9 per cent Cobalt Alloy : Metal Concentration Profiles in (Dr. G. C. Wood and J. M. Ferguson), 369
- Oxidized Spermine—Inactivation of Plant Viruses : Antiviral Action of (U. Bachrach *et al.*), 1095
- Oxygen and Cysteine on the Radical State of X-irradiated Lyophilized Liver : Influence of (M. Abe, H. Monig and R. Koch), 499
- Oxygen Consumption of Barley Seedlings : Air Ion Effects on the (S. Kotaka *et al.*), 1112
- Oxygen : Delayed Mortality in Adult Chickens exposed to 1 Atmosphere (Prof. H. S. Weiss *et al.*), 1003
- Oxygen Enhancement Ratio for X-ray Survival of HeLa Cells *in vitro*, after Treatment with 'Methotrexate' : A Reduced (Dr. R. J. Berry), 1108
- Oxygen in Reactions involving Purified Oxidases and Tissue Slices : Kinetic Parameters for (Dr. M. McCabe and Dr. D. A. Gilbert), 450
- Oxygen-levels : Effects of Oxygen Pressure and Salts on Germination of Winter Rye : Experimentation with Plants at Sub-atmospheric (Dr. S. M. Siegel *et al.*), 1012

PACIFIC : Marine Geology of the (Prof. H. W. Menard), review by Dr. A. S. Laughton, 215

- Pacific Science Center Foundation Arches of Science Award, 326
- Pacific Science Congress : Eleventh, 328
- (Pacinian Corpuscles) : Processes of Excitation and Inhibition in Single Mechanoreceptors (Dr. O. B. Ilyinsky), 351
- Packing of Hard Spheres with Five-fold Symmetry : Dense (B. G. Bagley), 674
- Packing of Spheres : A Numerical Model of Random (Dr. M. M. Levine and J. Chernick), 68
- Pácsa, S., Hankovszky, O. H., and Hideg, K. : Derivatives of 2-Aminoethylguanidine and of a Eugenol as Virus Inhibitors, 409
- Padnos, Dr. M., and Molomut, Dr. N. : Inhibition of Transplantable and Spontaneous Murine Tumours by the M-P Virus, 948
- Pagel, Dr. Bernard : Kepler's Dream, review, 960
- Pairing in the Epididymis of American Forms : Marsupial Spermatozoa (Dr. J. D. Biggers and Dr. E. D. De Lamater), 402
- Pairs of Spiral Etch Pits in Ice Crystals (J. Muguruma), 180
- Palaeolithic (Lower) Hominid and Pebble-industry in Hungary (Dr. M. Kretzoi and Dr. L. Vértess), 205
- Palaeomagnetism : Cassiterite as a Carrier of (V. Hanuš and M. Krs), 675
- Palaeomagnetism of the Isachsen Diabasic Rocks (A. Laroche, R. F. Black and R. K. Wanless), 179
- Palaeontological Techniques : Handbook of (edited by B. Kummel and D. Raup), review by Prof. D. T. Donovan, 968
- Palaeosalinities of Purbeck-Wealden Carbonates : Carbon Isotope Ratios and (Prof. P. Allen and Prof. M. L. Keith), 1278
- Palaeozoic Stratigraphy : Lower, Les Temps Fossilières (Prof. H. Termier and G. Termier), Vol. 1, review by Prof. A. Williams, 614
- Palladium Cathodes : Diffusion or Hydride Component of Hydrogen Overpotential at Platinized Platinum and Palladized (M. N. Hull and Dr. F. A. Lewis), 887
- Palladized Palladium Cathodes : Diffusion or Hydride Component of Hydrogen Overpotential at Platinized Platinum and (M. N. Hull and Dr. F. A. Lewis), 887
- Palmer, Dr. H. P., *et al.* : New Limits to the Angular Sizes of Some Quasars, 275
- Panda (*Ailuropoda melanoleuca*) : Bursa Pharyngea in the Giant (Prof. A. J. E. Cave), 865
- Pane, Dr. G., *et al.* : Distribution of the Intestinal Factor in Normal Mice which inactivates Murine Virus Hepatitis, 1009
- Paper and Thin-layer Chromatography : Resolution of the Optical Isomers of DL-Tryptophan, 5-Hydroxy-DL-tryptophan and 6-Hydroxy-DL-tryptophan by (Dr. S. F. Contractor and J. Vragg), 71
- Paper Chromatography and the Went Pea Test : Presence of Plant Growth Substances in Earthworms demonstrated by (R. L. Nielson), 1113
- Paper : Effect of Certain Types of, on Sexual Maturation of the Insect *Pyrhocoris apterus* (Prof. C. A. B. Clemetson), 510
- Paper Size : International, 528
- Papers of the Victorian Era : Science, 935
- Paracoccidioides brasiliensis : Glycogen in Yeast Form of (Dr. F. Kanetsuma and Dr. L. M. Carbonell), 686
- Parameters : Dependence of the Properties of Moving Striations in the Plasma of the Positive Column on some External and Internal (G. Lakatos and Dr. J. Bitó), 445
- Parameters for Oxygen in Reactions involving Purified Oxidases and Tissue Slices : Kinetic (Dr. M. McCabe and Dr. D. A. Gilbert), 450
- Parasites of Man : Microscopic Diagnosis of the (Dr. R. B. Burrows), review by Dr. G. Lapage, 1029
- Parasitology, History of (W. D. Foster), review by Dr. G. Lapage, 109
- Parents and Grandparents : Regression of a Phenotypic Value on the Values for the (Dr. I. J. Good), 203
- Parish, Dr. J. H., *et al.* : Fractionation of Ribonucleic Acids in the Zonal Ultracentrifuge, 645
- Parish, W. E. : Differentiation between Cytophilic Antibody and Opsonin by a Macrophage Phagocytic System, 594
- Park, Prof. K., George, M. J., Miyake, Y., Saruhashi, K., Katsuragi, Y., and Kanazawa, T. : Strontium-90 and Caesium-137 in Columbia River Plume, July 1964, 1084
- Park, W., *et al.* : A Simple Bioassay for Antioxidants based on Protection of *Tetrahymena pyriformis* from the Photodynamic Toxicity of Benzo(a)pyrene, 655
- Parke, Dr. Mary, and Boalch, G. T. : Biology of the Algae, review, 824
- Parkinson, Dr. M. J., *et al.* : Experimental Observations on the Structure of Collisionless Shock Waves in a Magnetized Plasma, 133
- Parliament : Scientific and Technological Information in, 209
- Parlow's Test : Diurnal Variations in Ovarian Ascorbic Acid Content of Pseudopregnant Immature Rats used in (Dr. M. de la Lastra and Dr. H. Croxatto), 789
- Parnell, Dr. E. W., *et al.* : Anticoccidial Activity of Nicotinamide Antagonists, 397

- Parreira, H. C., and Eyd, A. J. : Electric Potentials generated by Freezing Dilute Aqueous Solutions, 33
- Parry, D. G., et al. : Secretion of Newly Synthesized Insulin *in vitro*, 487
- Pars Distalis of the Pituitary of the Eel : A Dual Neurosecretory Innervation of the (Sir Francis Knowles and L. Vollrath), 1343
- Parshley, Prof. Mary Stearns, and Mandl, I. : Inhibition of Growth of Malignant Cells *in vitro* by a Component of Normal Adult Connective Tissue, 800
- Parsons, Dr. D. S., and Bronk, Dr. J. R. : Accumulation and Incorporation of Amino-acid in Rat Intestine *in vitro*, 785
- Parsons, Dr. D. S., and Prichard, J. S. : Hydrolysis of Disaccharides during Absorption by the Perfused Small Intestine of Amphibia, 1097
- Parsons, Dr. J. A. : Drug Dosage in Laboratory Animals, review, 1028
- Parsons, Dr. R. L., Hofmann, Prof. W. W., and Feigen, Prof. G. A. : Presynaptic Effects of Potassium Ion on the Mammalian Neuromuscular Junction, 590
- Particle Impact : Welding by High-Velocity (Dr. R. F. Rolsten et al.), 1273
- Particle Physics : Elementary, 23
- Particle to its Relative Biological Effectiveness : Relationship of the Velocity of a Charged (Dr. J. E. Turner) and (H. Hollister), 36
- Particle Size Measurement, 529
- Particles : Measured Velocities of Interplanetary Dust (C. S. Nilsson et al.), 673
- 'Ring-forms' of Reovirus (Dr. F. J. Grose et al.), 606
- Ultra-purification by Separation of Aerosol (Dr. S. Behrendt), 581
- Particles in Sub-acute Sclerosing Encephalitis : Virus-like (Dr. N. K. Gonatas and Prof. G. M. Shy), 1338
- Particulate Insecticides on *Simulium* among Stream Fauna : Selective Effect of (Prof. W. E. Kershaw et al.), 199
- Partington, Dr. Roger : Lavoisier's Elements of Chemistry, review, 987
- Partington, Dr. R. G. : A Hundred Years of Chemistry, Third Edition, review, 969
- Partition Data for correlating Structure and Biological Activity using Thin-layer Chromatography : A Simple Assessment of (Dr. C. B. C. Boyce and Dr. B. V. Milborrow), 537
- Patashnik, S., et al. : Determination of Nicotine in Smokers' Urine by Gas Chromatography, 788
- Patau's Syndrome : Autoradiographic Identification of the D(13-15) Chromosome responsible for the Di Trisomic (Dr. F. Giannelli), 669
- Pathology of Thinking (Dr. B. V. Zeigarnik), review by Prof. R. C. Oldfield, 982
- Patients with Deficiency of β -Lipoproteins (Acanthocytosis) : Precipitin Activity in the Sera of (Dr. R. F. Murray, jun., and Prof. B. S. Blumberg), 357
- Patients with Duchenne Muscular Dystrophy : Lactic Dehydrogenase Isoenzymes in Muscle from (Prof. T. H. Shepard et al.), 1107
- Paton, Dr. D. M., and Gillis, Prof. C. N. : Effect of Altered Perfusion Rates on the Retention of Noradrenaline by the Spleen, 391
- Pattern Measurement (Dr. A. Ward and G. W. McMaster), 428
- Patterns : Photographic Reproduction of Soil Fabric (D. Lafeber and M. Kurbanovic), 609
- Patterson, Prof. Jocelyn : *Obituary* by Prof. W. J. Hamilton, 1042
- Paul, Dr. J. W., Holmes, L. S., Parkinson, Dr. M. J., and Sheffield, J. : Experimental Observations on the Structure of Collisionless Shock Waves in a Magnetized Plasma, 133
- Paul, Prof. W. M., et al. : Electrical Activity in Human Uterine Muscle Strips, 290
- Pauling, Prof. L. : Structural Basis of Neutron and Proton Magic Numbers in Atomic Nuclei, 174
- Pauling, Prof. Linus, and Hayward, Roger : The Architecture of Molecules, review by Dame Kathleen Lonsdale, 957
- Pautard, Dr. F. G. E., et al. : Structure of Calcium Oxalate Monohydrate, 1197
- Pavlovsky, Academician Eugene N. : *Obituary* by Dr. C. A. Hoare, 1151
- Payan, H., Strebel, Dr. R., and Levine, Prof. S. : Epileptogenic Effect of Extradural and Extracranial Cobalt, 792
- Payne, A. R., and Davey, A. B. : Rubber in Engineering Practice, review by Dr. L. R. G. Treloar, 823
- Peace : The Road to, 513
- Peacocke, Dr. A. R., and Blake, A. : Optical Rotatory Dispersion and Secondary Structure of Ribonucleic Acid in Mammalian Ribosomes, 1319
- Pear Trees : Effects of (2-Chloroethyl)trimethylammonium Chloride and Gibberellic Acid on Growth, Fruit Bud Formation and Frost Resistance in One-year-old (Dr. I. Modlibowska), 503
- Pearcey, Mr. H. H. : Special Merit Promotion to Deputy Chief Scientific Officer, Aerodynamics Division, National Physical Laboratory, 527
- Pearson, C., and Wood, Dr. G. C. : A.C. Resistivity of Anodic Oxide Films on Valve Metals, 547
- Pearson, jun., F. J., and Tamers, Dr. M. A. : Validity of Radiocarbon Dates on Bone, 1053
- Pearson, Dr. G. S. : Methane-rich Perchloric Acid Flames, 283
- Pebble-industry in Hungary : Lower Palaeolithic Hominid and (Dr. M. Kretzoi and Dr. L. Vértés), 205
- Pectic Polysaccharides in the Growth of Plant Cells : Molecular Structural Factors and their Role in the Germination of White Mustard (S. E. B. Gould, Dr. D. A. Rees, N. G. Richardson and I. W. Steele), 876
- Pedology : Experimental (edited by E. G. Hallsworth and D. V. Crawford), review by Dr. A. M. Smith, 718
- Peel, Dr. J. L., et al. : Nature of the Non-haem Iron in Ferredoxin and Rubredoxin, 769
- Pegg, Dr. D. E., and Burns, J. E. : A Sex-dependent Factor in Aplastic Anaemia, 1187
- Pegg, Dr. G. F. : Phytotoxin Production by *Verticillium albo-atrum* Reinke et Berthold, 1228
- Pelagic Copepod, *Euterpina acutifrons* (Dana), in the Laboratory : Breeding a (H. W. Neunes and G.-F. Pongolini), 571
- Pener, M. P., and Norris, M. J. : An Inhibitory Effect of Allatectomized Males and Females on the Sexual Maturation of Young Male Adults of *Schistocerca gregaria* (Forsk.) (Orthoptera : Acrididae), 1122
- Penetration of Mediterranean Gobies into the Black Sea : *Relictogobius kryzhanovskii* and the (Dr. P. J. Miller), 474
- Penfield, H., et al. : OH Emission in the Direction of Radio Source W49, 441
- Penicillin Allergy : Direct Reaction of Penicillin with α -Amino-groups : New Chemical Aspect of (Dr. C. H. Schneider and Dr. A. L. de Week), 57
- Penicillinase-producing Gram-negative Bacteria : Interaction of Cephaloridine with (Dr. J. M. T. Hamilton-Miller et al.), 235
- Penicillinase : Relative Instability of Cephaloridine to Staphylococcal (Dr. M. Ridley and Dr. I. Phillips), 1076
- Penicillinase Synthesis Controlled by Infectious R Factors in Enterobacteriaceae (Dr. N. Datta and Dr. P. Kontomichalou), 239
- Penicillium digitatum* : Biogenesis of Ethylene in (Dr. B. A. Sprayberry et al.), 1322
- Penicillium roqueforti* : Activation of Spores of (R. C. Lawrence), 801
- Pennelli, N., et al. : Lymphatic Neoplastic Transformation of Thymus in Mice with Myeloid Virus-induced Leukaemia, 697
- Penniman, T. K., et al. : A Hundred Years of Anthropology, Third Edition, revised, review by Prof. M. Fortes, 215
- Peoples of the Steppes : The Royal Hordes, Nomad (E. D. Phillips), review by M. C. Burkitt, 1138
- Peptide Difference : Haemoglobin Polymorphism in the Cod (*Gadus morhua*) : A Single (M. C. Rattazzi and C. P. Kik), 489
- Peptides in Yeast : Nucleotide (Dr. D. J. Millin and M. J. Saltmarsh-Andrew), 468
- Perception : Human Senses and (Prof. G. M. Wyburn et al.), review by Prof. O. L. Zangwill, 981
- Perchloric Acid Flames : Methane-rich (Dr. G. S. Pearson), 283
- Percival, Dr. A., et al. : 2-Trifluoromethylbenzimidazoles : a New Class of Herbicidal Compounds, 1166
- Perfused Dog Kidney : Release of Plasminogen Activator by the Isolated (Dr. R. Holemans et al.), 291
- Perfusion Rates : Effect of Altered, on the Retention of Noradrenaline by the Spleen (Dr. D. M. Paton and Prof. C. N. Gillis), 391
- Period of the Planet Mercury : Rotational (Dr. G. Colombo), 575
- Periodic Aggregating and Take-off in Anthophorid Bees, *Anthophora acroensis* Fab. (Prof. L. K. H. Goma), 807
- Periodicals : Directory of Indian Scientific, 434
- Periodicals from the U.S.S.R. : Scientific, 328
- Periods of General Relativistic Objects : Pulsation (J. P. Wright), 65
- Peripheral Muscarinic Properties : Central Stimulant Properties of Compounds with (G. B. Leslie), 1291
- Perkins, F. T. : Modern Trends in Immunology, I, review, 9
- Perkins, H. R. : 2,6-Diamino-3-hydroxypimelic Acid in Microbial Cell Wall Mucopolysaccharide, 872
- Permeability Factors : Interspecies Differences in Response to Polypeptides as (Dr. J. Carr and Prof. D. L. Wilhelm), 653
- Permeability of Phospholipid Model Membranes : Effect of Narcotics : Cation (Dr. A. D. Bangham et al.), 1295
- Peromyscus : Wheel-running Preferences of (Prof. J. L. Kavanau and Prof. D. H. Brant), 597
- Peroxo Compound : A New Chromium(IV) (Dr. D. A. House and Prof. C. S. Garner), 776
- Perrin, Dr. D. D. : Binding of Tetracyclines to Bone, 787
- Perrin, Dr. D. D. : Organic Complexing Reagents. Structure, Behaviour and Application to Inorganic Analysis, review by Dr. T. S. West, 3
- Perry, Prof. Seymour, et al. : Return of Leucocytes to the Bone Marrow in Chronic Myelogenous Leukaemia, 1281
- Perry, Prof. S. V., and Kendrick-Jones, J. : Enzymatic Adaptation to Contractile Activity in Skeletal Muscle, 1068
- Peruzzotti, G., et al. : A New, Active Metabolite of 'Miracil D', 1005
- Pest Control : Two Pioneers of (Dr. G. S. Hartley), 430
- Pesticide Residue Analysis and to the Cholinesterase Screening of Candidate Pesticides : Application of an Agar-Agar Diffusion Procedure to (Dr. K. I. Beynon and G. Stoydin), 748
- Pesticide-Wildlife Studies, 1046
- Pesticides : Application of an Agar-Agar Diffusion Procedure to Pesticide Residue Analysis and to the Cholinesterase Screening of Candidate (Dr. K. I. Beynon and G. Stoydin), 748
- Pesticides in the Atmospheric Environment : Organochlorine (Dr. D. C. Abbott et al.), 1317
- Peters : Sir Rudolph A. : Nutrition and its International Recognition, 117
- Peters, Dr. W. : Mepacrine- and Primaquine-resistant Strains of *Plasmodium berghei*, Vincke and Lips, 1948, 693
- Peterson, A. D. C. : How and Why do we Learn ?, review, 314
- Peterson, Dr. R. F. : Wheat, review by Dr. G. D. H. Bell, 976
- Peterson, R. L. : A Well-preserved Grizzly Bear Skull recovered from a Late Glacial Deposit near Lake Simcoe, Ontario, 1233
- Petroleum Chemistry and Refining : Advances in, Vol. 10 (edited by J. J. McKetta, jun.), review by H. B. Milner, 969
- Pettijohn, Prof. F. J., and Potter, Prof. P. E. : Atlas and Glossary of Primary Sedimentary Structures, review by J. R. L. Allen, 315
- Pflanzenfamilien : Syllabus der (A. Engler), Vol. 2, Angiosperms, Twelfth Edition, review by Prof. T. G. Tutin, 316
- pH : Determination of (Dr. R. G. Bates), review by R. Sawyer, 318
- Phagocytic Function of Hassall's Corpuscles (Dr. J. N. Blau), 564
- Phagocytic System : Differentiation between Cytophilic Antibody and Opsonin by a Macrophage (W. E. Parish), 594
- Phalaropes : Prolactin and the Incidence of Brood Patch Formation and Incubation Behaviour of the Two Sexes in Certain Birds with Special Reference to (Prof. E. O. Hohn and S. C. Cheng), 197
- Pharmaceutical Industry, 121
- Pharmacological Investigation of Human Isolated Ileum (Dr. A. Bennett), 1289
- Pharmacology : Molecular, 21
- Pharmacy in History (Prof. G. E. Trease), review by Dr. Frank Hartley, 982
- Pharmakologie : Handbuch der Experimentellen, Band 17 : Ions Alkalino-Terreux, Two Volumes in French (Sub-editor Prof. Z.-M. Bacq), review by Prof. E. Zaimis, 720
- Pharyngea in the Giant Panda (*Ailuropoda melanoleuca*) : Bursa (Prof. A. J. E. Cave), 865
- Phase Objects by Holography : Reconstruction of (Prof. D. Gabor et al.), 1159
- Phases : Equation of State of Liquid-Vapour Equilibrium with the Same Constants for Both (Dr. I. Kuhn), 1315
- (Phenazine Methosulphate) : Dependence of Photophosphorylation by Isolated Chloroplasts on the Oxidation-Reduction State of *N*-Methylphenazinium Methyl Sulphate (G. Zweig and Dr. M. Avron), 190
- Phenocopies of the *ma-1* and *ry* Mutants of *Drosophila melanogaster* : Inhibition *in vivo* of Xanthine Dehydrogenase by 4-Hydroxypyrazolo (3,4-*d*)pyrimidine (E. C. Keller, jun., and Dr. E. Glassman), 202
- Phenolic Compounds : Biochemistry of (edited by J. B. Harborne), review by Sir Robert Robinson, 515
- Phenotypic Classification and Terminology for Enzyme Mutants in Microorganisms : A Suggested (Dr. J. F. Collins, Dr. J. Mandelstam, Prof. M. R. Pollock, Dr. M. H. Richmond and Dr. P. H. A. Sneath), 841
- Phenotypic Value : Regression of a_0 on the Values for the Parents and Grand-parents (Dr. I. J. Good), 203
- Phenylalanine : Behavioural Deficit in the Rat induced by feeding (Dr. R. C. Leaf et al.), 1021
- Philadelphia Chromosome in Cultures of Spleen Cells : Demonstration of the (Dr. A. S. D. Spiers and Dr. A. G. Baikie), 497
- Philanthropy : James Smithson : Pathfinder in Science and (Dr. L. Carmichael), 321
- Philip, Dr. G. M., and Fell, Prof. H. B. : Ancestry of Sea-stars, 766
- Phillips, Dr. D. C., et al. : Biological Systems at the Molecular Level, 1048
- Phillips, E. D. : The Royal Hordes, Nomad Peoples of the Steppes, review by M. C. Burkitt, 1138
- Phillips, Dr. Ian, and Ridley, Dr. M. : Relative Instability of Cephaloridine to Staphylococcal Penicillinase, 1076
- Phillips, Dr. R. F., and Hampson, N. A. : Encyclopedia of Electrochemistry, review, 104
- Proceedings of the First Australian Conference on Electrochemistry, 1963, review, 104
- Philosophy : Beyond the Edge of Certainty : Essays in Contemporary Science and (edited by R. G. Colodny), review by F. I. G. Rawlins, 314
- Philosophy of Science : Teaching in the History and (Dr. W. Mays), 937
- Philpot, J. St. L. : Advances in Radiation Biology, Volume 1, review, 1032

- Phosphatases : Acid (Dr. C. A. Vernon *et al.*), 382
 Phosphate Precipitates : Transphosphorylation of Adenosine Di- and Tri-phosphates in the Presence of Calcium (Dr. R. W. Burley), 683
 Phosphates : Photochemical Synthesis of Condensed (A. Beck and L. E. Orgel), 1000
 Phosphenes of Adults and pre-School Children's Scribbles : Form-similarity between (R. Kellogg *et al.*), 1129
 Phosphines : Phosphorus Chemical Shifts in Secondary and Tertiary (Prof. S. O. Grim and W. McFarlane), 995
 Phosphobenzene : Structure of (Dr. J. J. Daly and Dr. L. Maier), 383
 Phosphoglucosylase : Evidence for a Second 'Structural' Locus determining Human (Dr. D. A. Hopkinson and Prof. H. Harris), 410
 Phospholipid/Cholesterol Structures : Effect of Steroids and 'Triton X-100' on Glucose-filled (Dr. G. Weissmann *et al.*), 649
 Phospholipid Model Membranes : Effect of Narcotics : Cation Permeability of (Dr. A. D. Bangham *et al.*), 1295
 Phosphoramidic Acid Re-arrangement : Biosynthesis of 2-Aminoethanephosphonic Acid (Dr. W. Segal), 1284
 Phosphorescence of the Nucleic Acid Bases : Thermoluminescence and Ultraviolet (Dr. R. J. Fleming and R. M. Kerr), 1198
 Phosphorus and Calcium on the Comparative Behaviour of Strontium and Calcium : Effects of Dietary Levels of (K. Kostial *et al.*), 1110
 Phosphorus-31 Chemical Shifts in Secondary and Tertiary Phosphines (Prof. S. O. Grim and W. McFarlane), 995
 Phosphorus-deficiency Symptoms in Tobacco and Transpirational Water Loss (Prof. A. Wallace and E. Frolich), 1231
 Phosphorus Excretion of Cattle fed on High-energy Diets (W. D. C. Reed, Dr. R. C. Elliott and Dr. J. H. Topps), 953
 Phosphorylation : Activity of 2-Trifluoromethylbenzimidazoles as Uncouplers of Oxidative (Dr. O. T. G. Jones and W. A. Watson), 1169
 Phosphorylation : Evidence discriminating between the Chemical and the Chemiosmotic Mechanisms of Electron Transport (Dr. P. Mitchell and Dr. J. Moyle), 1205
 Photobiology : Recent Progress in (edited by E. J. Bowen), Proceedings of an International Congress, 1964, review by Dr. A. Knowles, 974
 Photochemical Reactor : Laboratory, 935
 Photochemical Synthesis of Condensed Phosphates (A. Beck and L. E. Orgel), 1000
 Photodynamic Toxicity of Benzo(a)pyrene : A Simple Bioassay for Antioxidants based on Protection of *Tetrahymena pyriformis* from the (Dr. S. S. Epstein *et al.*), 655
 Photographic Reproduction of Soil Fabric Patterns (D. Lafeber and M. Kurbanovic), 609
 Photography : Research in, *Mitteilungen aus den Forschungslaboratorien der Agfa-Gevaert AG* Leverkusen-München, Band IV (edited by Prof. E. Klein and R. Matejec), review by K. O. Ganguin, 720
 Photo-induced Hydroxylation of Cinnamic Acid in Gherkin Hypocotyls (Dr. G. Engelsma), 1117
 Photophosphorylation by Isolated Chloroplasts on the Oxidation-Reduction State of N-Methylphenazinium Methyl Sulphate (Phenazine Methosulphate) : Dependence of (G. Zweig and Dr. M. Avron), 190
 Photophysiology (edited by Prof. A. C. Giese), Two Volumes, review by Dr. Edith Harrison, 978
 Photoreceptor Units : Quantum Energy Specificity of Simple (Prof. W. W. Dawson), 589
 Photosynthetic Bacteria (E. N. Kondrat'eva), review by Prof. T. W. Goodwin, 977
 Photosynthetic Mechanisms of Green Plants : Papers presented at a Symposium, 1963, review by Dr. E. A. C. MacRobbie, 212
 Phrenic Nerve : Ultrastructure of the (Dr. A. S. Wilson and D. G. Silva), 707
 Physical Laboratory : New Absolute Determination of the Acceleration due to Gravity at the National (Dr. A. H. Cook), 279
 Physical Sciences : Statistics and Experimental Design in Engineering and the (Prof. N. L. Johnson and Prof. F. C. Leone), Two Vols., review by N. E. Day, 214
 Physical Society : Institute of Physics and the (S. Weintraub), 232
 Physics : Cosmic Ray (A. E. Sandstrom), review by Dr. J. J. Quenby, 315
 Elementary Particle, 23
 Nobel Lectures including Presentation Speeches and Laureates' Biographies 1922-1941, review by Dr. G. Burniston Brown, 103
 Solid State : Advances in Research and Applications, Vol. 15 (edited by F. Seitz and D. Turnbull), review by Prof. C. W. McCombie, 4
 Physics and the Physical Society : Institute of (S. Weintraub), 232
 Physics Education (Dr. J. M. A. Lenihan), 26
 Physics of High Pressures and the Condensed Phase (edited by A. van Itterbeek), review by Dr. R. S. Bradley, 515
 Physics of Ice (Prof. E. R. Ponder), review by Dr. J. Hallett, 986
 Physics of Magnetism (Prof. S. Chikazumi), review by Dr. C. A. Bates, 616
 Physics of the Ear (Dr. T. S. Littler), review by Prof. I. G. Taylor, 1136
 Physics of the Marine Atmosphere (Dr. H. U. Roll), International Geophysics Series, Vol. 7, review by Prof. J. Darbyshire, 419
 Physiological Basis of Salt Tolerance in Plants (Dr. B. P. Stroganov), review by Dr. P. A. Thompson, 211
 Physiological Function for Plasma Cholinesterase : Proposed (H. S. Funnell and Prof. W. T. Oliver), 689
 Physiological Mammalogy (edited by W. V. Mayer and R. G. Van Gelder), Vol. 1 : Mammalian Populations, Vol. 2 : Mammalian Reactions to Stressful Environments, review by H. N. Southern, 1136
 Physiologists : American Society of Plant, Awards, 231 ; Officers, 231
 Physiology : Encyclopedia of Plant (edited by W. Ruhland), Vol. 15, Two Parts, review by Dr. C. R. Metcalfe, 619
 Physiology in Canada : Plant (Prof. J. A. Webb), 27
 Physiology : Methods in Cell, Vol. 1 (edited by D. M. Prescott), review by Dr. J. H. Humphrey, 6
 Physiology of the Acid Mucopolysaccharides : Chemical (J. E. Scott), 1270
 Physique des Semiconducteurs : Comptes Rendus du 7e Congrès International, review by P. T. Landsberg, 217
 Phytohaemagglutinin : Cytoplasmic Labelling with Uridine-5-³H in Human Lymphocytes cultured with (Dr. G. C. B. Winter and Prof. J. M. Yoffey), 1018
 Phytotoxin Production by *Verticillium albo-atrum* Reinke et Berthold (Dr. G. F. Pegg), 1228
 Piaget, Jean, and Inhelder, B. : Early Growth of Logic in the Child, review by Dr. J. H. Duthie, 1246
 Pianta Medica : Chimica Farmacologia e Terapia, Vol. 2 (R. Benigni, C. Capra and P. E. Cattorini), review by Dr. F. N. Howes, 218
 Piazza, Prof. M., Amodio, Dr. A., and Pane, Dr. G. : Distribution of the Intestinal Factor in Normal Mice which inactivates Murine Virus Hepatitis, 1009
 Pickford, Prof. R. W., *et al.* : Human Senses and Perception, review by Prof. O. L. Zangwill, 981
 Pierre, Dr. L. L. : Guanine Activity of the Symbionts and Fat Bodies of the Cockroach, *Leucophaea maderae*, 666
 Pietsch, Dr. Paul, and McCollister, S. B. : Replication and the Activation of Muscle Differentiation, 1170
 Pig Eggs : Failure of the Zora Reaction in Five (Prof. P. J. Dziuk and Z. Dickmann), 502
 Pig, Rhesus Monkey and Human Ovarian Oocytes : Maturation *in vitro* of Mouse, Sheep, Cow, (Dr. R. G. Edwards), 349
 Pigeon Habitats in London : Isolation of *Cryptococcus neoformans* from (Dr. H. S. Randhawa *et al.*), 80
 Pigeons : Polymorphism of Lactate Dehydrogenase Isozymes in (Prof. Wm. H. Zinkham *et al.*), 28
 Pigment : Chloroquine Resistance and the Nature of Malarial (Prof. I. W. Sherman *et al.*), 691
 Pigment in Leaf Galls of *Salix fragilis* L. : Red (G. Blunden and Dr. S. B. Challen), 388
 Pigments : Chemistry of Plant (Dr. J. B. Harborne), 1051
 Pigs in Biomedical Research : Use of (Dr. L. K. Bustad and R. O. McClellan), 531
 Pik, C., and Rattazzi, M. C. : Haemoglobin Polymorphism in the Cod (*Gadus morhua*) : a Single Peptide Difference, 489
 Pilet, Prof. P. E. : Action of Gibberellic Acid on Auxin Transport, 1344
 Pitocarpine : Preferential Release of Adrenaline from the Adrenal Medulla by Muscarine and (Prof. W. W. Douglas and Dr. A. M. Poinser), 1102
 Pine in relation to Weevil Attack : Leucoanthocyanins of White (F. S. Santamour, jun.), 407
 Pineal Extracts : Separation Method for Melatonin in (Dr. F. C. G. van de Veerdonk), 1324
 'Pink Spot' in the Urine of Schizophrenics (R. E. Bourdillon, Prof. C. A. Clarke, A. P. Ridges, Prof. P. M. Sheppard), (Dr. P. Harper) and (Dr. S. A. Leslie), 453
 Pintera, Jan. : Low Concentrations of Serum Haptoglobin in Mothers of Children with Haemolytic Disease of the Newborn, 694
 Pioneers of Pest Control : Two, Dr. W. E. Ripper and Dr. E. P. Jones, *Obituaries* (Dr. G. S. Hartley), 430
 Pipeline over the Alps : Cili, 435
 (Pisces : Centropomidae) from Lake Chad : Winter Scale Rings in *Lates niloticus* (A. J. Hopson), 1013
 (Pisces : Cichlidae) : Signa Value of the Genital Tassel in the Male *Tilapia macrochir* Blgr. (Dr. W. Wickler), 595
 Pits in Ice Crystals : Pairs of Spiral Etch (J. Muguruma), 180
 Pituitaries in the Eel : A Functional Relationship between Neurosecretory Fibres and (Sir Francis Knowles and L. Vollrath), 1343
 Pituitary Gland of the Cow : Introduction of a Permanent Electrode into the (B. Hynek *et al.*), 650
 Pituitary Glands : Isolation and Amino-acid Sequence of β -LPH from Sheep (Prof. Choh Hao Li *et al.*), 1093
 Pituitary *in vitro* : Direct Action of Thyroxine on the Trout (Dr. B. I. Baker), 1234
 Pituitary of the Eel : A Dual Neurosecretory Innervation of the Pars Distalis of the (Sir Francis Knowles and L. Vollrath), 1343
 Pituitary Transplants in the Teleost, 123
 Placental Ageing : Transplantation Experiments on (R. L. Simmons and J. Weintraub), 82
 Placental Protein with Immunological Similarity to Human Growth Hormone : Further Purification and Characterization of a (Dr. H. Friesen), 1214
 Planarian : its Significance in Biochemical Evolution : Arginine and Urea Biosynthesis in the Lard (Prof. J. W. Campbell), 1299
 Planet called Earth (Prof. George Gamow), review by Patrick Moore, 961
 Planet Mercury : Rotational Period of the (W. E. McGovern *et al.*), 375
 Planet Mercury : Rotational Period of the (Dr. G. Colombo), 575
 Planetary Surface Conditions : Lunar and (Dr. N. A. Weil), review by Dr. G. Fielder, 961
 Planets and Satellites : Tidal De-spin of (Dr. P. Goldreich), 375
 Planktonic Foraminiferal Biostratigraphy of the Neogene : Progress in the (Dr. F. T. Banner and Dr. W. H. Blow), 1164
 Planning a New Medical School, 634
 Planning : Political and Economic (P.E.P.), Annual Report, 229
 Plant Breeding : Genetic Principles and (Prof. W. Williams), review by Dr. K. Jones, 7
 Plant Breeding Station : Scottish, Director, 228
 Plant Cells : Pectic Polysaccharides in the Growth of (S. E. B. Gould *et al.*), 876
 Plant Cells : their Relation to Growth and Growth Induction : Direct and Indirect Effects of Radiation on (Dr. R. D. Holsten *et al.*), 850
 Plant-growth Regulators : Morphactins, a Novel Group of (G. Schneider *et al.*), 1013
 Plant Growth Substances in Earthworms demonstrated by Paper Chromatography and the Went Pea Test : Presence of (R. L. Nielson), 1113
 Plant Material : Rapid Method for Selenium Assay of (R. Duff and Prof. M. Chessin), 1001
 Plant Physiologists : American Society of, Awards and Officers, 231
 Plant Physiology : Encyclopedia of (edited by W. Ruhland), Vol. 15, Two Parts, review by Dr. C. R. Metcalfe, 619
 Plant Physiology in Canada (Prof. J. A. Webb), 27
 Plant Pigments : Chemistry of (Dr. J. B. Harborne), 1051
 Plant Roots : Mechanism of Uptake of Trace Elements by (G. Findenegg and Prof. E. Broda), 196
 Plant Tumour Virus in Arthropod Host : Microcrystal Formation. (E. Shikata and Dr. K. Maramorosch), 507
 Plant Viruses : Antiviral Action of Oxidized Spermine-Inactivation of (U. Bachrach *et al.*), 1095
 Plants : Influence of Hexadecane on Absorption of Carbon Dioxide by (Prof. J. M. Riedhart), 300
 Occurrence of Fructo-e-l-phosphate in Tissues of Higher (D. Graham and Dr. T. ap Rees), 88
 Origin of the Geoelectric Effect in (Prof. M. B. Wilkins and A. E. R. Woodcock), 990
 Photosynthetic Mechanisms of Green, review by Dr. E. A. C. MacRobbie, 212
 Physiological Basis of Salt Tolerance in (Dr. B. P. Stroganov), review by Dr. P. A. Thompson, 211
 Shade Tolerance in Flowering (Dr. J. P. Grime), 161
 Some Postulated Relationships among Autumn Phenomena in Woody (R. B. Van Huyssee *et al.*), 89
 Uptake by, of Radiostromium from Contaminated Soils (A. J. Andersen), 195
 Plants at Sub-atmospheric Oxygen-levels : Effects of Oxygen Pressure and Salts on Germination of Winter Rye : Experimentation with (Dr. S. M. Siegel, O. Daly and C. Giunarro), 1012
 Plants of Europe : Vascular, Flora Europaea (edited by T. G. Tutin *et al.*), review by Prof. J. A. Nanfeldt, 8
 Plaque-formation by Non-immune and X-irradiated Lymphoid Cells on Monolayers of Mouse Embryo Cells (G. Moller and E. Moller), 260
 Plaque Size of Western Equine Encephalitis Virus : Influence of Heavy Water (Deuterium Oxide) on (R. I. Carp and Dr. D. Kritchewsky), 93
 Plasma Cholinesterase : Proposed Physiological Function for (H. S. Funnell and Prof. W. T. Oliver), 689
 Plasma Co-factors in Adenosine Diphosphate-induced Aggregation of Human Platelets (Dr. D. Deykin, C. R. Pritzker and E. M. Scolnick), 296

- Plasma Diagnostics with Microwaves (Dr. M. A. Heald and C. B. Wharton), review by A. E. Dangor, 965
- Plasma - Experimental Observations on the Structure of Collisionless Shock Waves in a Magnetized (Dr. J. W. M. Paul et al.), 133
- Plasma Globulins : Formation of Complexes between Basic Proteins of Leucocytes and (Dr. W. B. Jolley and H. W. Allen), 390
- Plasma Growth-hormone Levels in Chronic Starvation in Man (Dr. V. Marks, N. Howorth and F. C. Greenwood), 686
- Plasma Instabilities in a Dipole Magnetic Field : Laboratory Observations of (Prof. R. G. Quinn), 376
- Plasma Interaction Problems : A Wave Mechanical Approach to the (Prof. V. C. Liu), 883
- Plasma : Mechanism of Intestinal Absorption : Transport of Methionine and Sodium Butyrate by Intracellular (S. L. Hart and Dr. J. A. Nissim), 145
- Plasma of the Positive Column on some External and Internal Parameters : Dependence of the Properties of Moving Striations in the (G. Lakatos and Dr. J. Bitó), 445
- Plasminogen Activator by the Isolated Perfused Dog Kidney . Release of (Dr. R. Hølemans et al.), 291
- Plasmodium berghiei* in Different Experimental Hosts : Primary Tissue Phase of (Prof. M. Yoeli et al.), 903
- Plasmodium berghiei*, Vincke and Lips, 1948 : Mepacrine- and Primaquine-resistant Strains of (Dr. W. Peters), 693
- Plasmodium knowlesi* : Immunity to Malaria : Antigenic Variation in Chronic Infections of (Dr. K. N. Brown and I. N. Brown), 1286
- Plasmodium vinckei* . Gametogenesis of (Dr. J. Bafort et al.), 1230
- Plate and Shell Theory : Introduction to (C. E. Turner), review by Prof. B. N. Cole, 1137
- Platelets : Plasma Co-factors in Adenosine Diphosphate-induced Aggregation of Human (Dr. D. Deykin et al.), 296
- Platinized Platinum and Palladized Palladium Cathodes : Diffusion or Hydride Component of Hydrogen Overpotential at (M. N. Hull and Dr. F. A. Lewis), 887
- Platinum(II) $C_{10}H_{16}O_4PtCl_2$: Structure of a Diolefin Complex of (D. Gibson et al.), 580
- Platinum and Palladized Palladium Cathodes : Diffusion or Hydride Component of Hydrogen Overpotential at Platinized (M. N. Hull and Dr. F. A. Lewis), 887
- Platinum/Halide Metal Interfaces : Adsorption and the Differential Capacitance of the Electrical Double-layer at (A. D. Graves and Dr. D. Inman), 481
- Plattner, Prof. P. A. (edited by) : Proceedings of the International Symposium on the Chemotherapy of Cancer, 1964, review by Dr. D. C. Roberts, 1032
- Pleistocene Glacial-marine Zones in North Atlantic Deep-sea Sediments (Dr. J. R. Conolly and Dr. M. Ewing), 135
- Plenk, Prof. H. P., et al. : Blood Group Secretion Factors in Bronchogenic Carcinoma, 299
- Plenum Press Handbooks of High Temperature Materials, Three Volumes (Dr. P. T. B. Shaffer, Prof. G. V. Samsonov and W. D. Wood, H. W. Deem and C. F. Lucks), review by Dr. E. H. Andrews, 517
- Plička, Dr. M. : Origin of Fossil 'Zoophycos', 579
- Plutonium in Serum Proteins following Intravenous Injection into Rats : Distribution of (G. Boocock and D. S. Poplewell), 282
- Pneumococcus* by a Hormone-like Cell Product : Control of the Competent State in (Dr. A. Tomasz), 155
- Pochin, Dr. E. E. : The Thyroid, review, 979
- Poisner, Dr. A. M., and Douglas, Prof. W. W. : Preferential Release of Adrenaline from the Adrenal Medulla by Muscarine and Pilocarpine, 1102
- Poisoning : Food, 630
- Poisson Distribution of Miniature End-plate Potentials and some Implications : Evidence for a (Dr. P. W. Gage and Dr. J. I. Hubbard), 395
- Pokorny, Dr. V. : Principles of Zoological Micropalaeontology, Volume 2, review by Dr. R. H. Bate, 974
- Polanyi, Prof. Michael : Science, Faith and Society, review, 985
- Polar Liquids : Sub-millimetre Wave Spectra of (Dr. G. W. Chantry and Dr. H. A. Gebbie), 378
- Polar Transport of Two Auxins : Promotion by Kinetin of the (Dr. C. C. McCready et al.), 1065
- Polarization and Hybridization (Dr. T. A. Claxton), 691
- Polarized OH Emission : Observations of (Dr. S. Weinreb et al.), 440
- Polenov, Dr. A. L., and Belenky, M. A. : Electron Microscope Observations of Neurosecretory Elements in the Neurointermediate Lobe in Skates, 94
- Poletti, N., and Morris, R. A. : Frequency Spectrum of Doppler-shifted Whistler-mode Signals, 479
- Policy : Rethinking Civil Service, 415
- Polish Industry : Scientific Research in the Development of, 120
- Political and Economic Planning, Annual Report, 229
- Pollen Grains in the Katrol Formation (Upper Jurassic) of Kutch, India : Occurrence of Gnetalean (Y. K. Mathur and Mrs. K. Mathur), 912
- Pollen Grains of the Euphorbiaceae : Warts (Dr. J. M. Matthews), 803
- Pollen Physiology and Fertilization (edited by Prof. H. F. Linskens), Symposium at Nijmegen, 1963, review by Dr. P. A. Thompson, 218
- Pollen Studies in New York State, 1262
- Pollock, Dr. J. R. A., and Stevens, Dr. R. (edited by) : Dictionary of Organic Compounds, Fourth Edition, Vols. 1 to 5 and Supplement, review by Dr. F. G. Mann, 417
- Pollock, Prof. M. R. : 1965 Nobel Prize for Medicine, 1250
- Pollock, Prof. M. R., et al. : A Suggested Phenotypic Classification and Terminology for Enzyme Mutants in Micro-organisms, 841
- Polonium-210 in Man (Dr. C. R. Hill), 423
- Polya, G. : Mathematical Discovery, Volume 2, review by Prof. T. A. A. Broadbent, 986
- Polyamide Layer Chromatography of DNP Amino-acids (Prof. K.-T. Wang and J. M.-K. Huang), 281
- Polyamide Layer Chromatography of Oestrogens (Y. C. Tung and Prof. Kung-Tsung Wang), 581
- Polycyclic Aromatic Hydrocarbons with the Ionization Potential : Correlation of the Third Excited State of (Dr. M. A. Slikin), 380
- Polyethylene Glycol in Investigations of Absorption of Fat : Use of (Dr. H. Vavřínková and Dr. A. Krondl), 293
- Polyethylene : Mechanism of Radical Decay in Irradiated (Dr. D. O. Geymer and Dr. C. D. Wagner), 72
- Polymerized Orosomucoid : Inhibition of Influenza Virus Haemagglutination by (P. H. Whitehead et al.), 915
- Polymers : Absence of Diffusion Control of Reaction Rate in the Thermal Decomposition of Amorphous (D. A. Smith), 1200
- Polymorphic Insect : Changes in Size of the Corpus Allatum in a (D. White), 807
- Polymorphism and Mitochondrial Activity in Sleeping Sickness Trypanosomes (Dr. K. Vickerman), 762
- Polymorphism in some Argid Sawflies (Hymenoptera, Symphyta) : Larval (Dr. M. F. Claridge and Dr. J. M. Edington), 301
- Polymorphism of Lactate Dehydrogenase Isozymes in Pigeons (Prof. Wm. H. Inkham, L. Kupchik, A. Blanco and H. Isensee), 284
- Polyoma Antibodies on Polyoma Tumour Cells : Distinction between the Effects of Antiviral and Anticellular (I. Hellstrom), 652
- Polypeptide Chain Configurations : Hexagonal-mesh Structures and Stable (Prof. R. E. Dickerson), 139
- Polypeptides as Permeability Factors : Interspecies Differences in Response to (Dr. J. Carr and Prof. D. L. Wilhelm), 653
- Polyphenylalanine Synthesis : Reticulocyte Ribosome Fraction with an Exceptional Capacity for (Dr. J. O. Bishop), 361
- Polyplody : Deoxyribonucleic Acid Synthesis in Cultured Human Cells and its Bearing on the Concepts of Endoreduplication and (Dr. U. Mittwoch et al.), 242
- Polyribonucleotides : Heterogeneous Binding of Acridine Orange by (Prof. R. F. Beers, jun., and G. Armilei), 466
- Polysaccharide Flocculant and Flocculated Kaolinite by Electron Microscopy . Examination of a (Dr. A. Audsley and A. Fursey), 753
- Polysaccharides in the Growth of Plant Cells : Pectic (S. E. B. Gould et al.), 876
- Pongolini, Gian-Franco, and Neuness, H. W. : Breeding a Pelagic Copepod, *Euterpina acutifrons* (Dana), in the Laboratory, 571
- Popović, D. A. : Rapidly Labelled Synthesis of Ribonucleic Acid in Ehrlich Ascites C-2 Tumour Cells treated with Actinomycin D, 49
- Poplewell, D. S., and Boocock, G. : Distribution of Plutonium in Serum Proteins following Intravenous Injection into Rats, 282
- Population Density : Relation of, to Sodium Availability and Sodium Selection by Microtine Rodents (G. D. Aumann and Prof. J. T. Emlen), 198
- Population : Elements of the Natural Movement of (Egon Viölrose), review by E. Grebenik, 1032
- Population of Tristan da Cunha : Hair Colour of the (Dr. E. Sunderland), 412
- Pore Radius : Errors in the Calculation of Surface Area and Average (Dr. D. Dollimore and Dr. G. R. Heal), 1092
- Porous Rock : Detonability of Nitroglycerin contained in (J. E. Hay and F. H. Scott), 1197
- Porsin, Dr. Michel, et al. : A New Bisalbuminaemic Family, 1222
- Portal Blood Supply in Liver Regeneration : Role of (Dr. R. Y. Thomson and A. M. Clarke), 392
- Portraits of Tasmanian Aborigines, 230
- Positive Column on some External and Internal Parameters : Dependence of the Properties of Moving Striations in the Plasma of the (G. Lakatos and Dr. J. Bitó), 445
- Posner, Prof. A. S., et al. : Intermediate States in the Precipitation of Hydroxyapatite, 365
- Postgate, Prof. J. R. : Appointed to Chair of Microbiology in the University of Sussex, 20
- Postgate, Prof. J. R. : Obituary of K. R. Butler, 831
- Potassium Ion on the Mammalian Neuromuscular Junction : Presynaptic Effects of (Dr. R. L. Parsons et al.), 590
- Potassium Perfusion into Dog Adrenals : Aldosterone Secretion : Difference in Direct Effects of Rubidium and (Prof. J. G. Hilton et al.), 1099
- Potassium Transport of L 1210 Leukaemia Cells : Role of Sialic Acid in (Dr. J. L. Glick and Dr. S. Githens III), 88
- Potassium Vapour : Ionization Times in Shock-heated (R. M. Hill and B. Capp), 176
- Potato Variety 'Majestic' : Chimerical Nature of the Entire-leaf Variant in the (Dr. H. W. Howard), 917
- Potential Changes in the Unanaesthetized Cerebral Cortex of the Cat : Correlation between Unit Activity and Slow (A. D. J. Robertson), 757
- Potential : Correlation of the Third Excited State of Polycyclic Aromatic Hydrocarbons with the Ionization (Dr. M. A. Slikin), 380
- Potential in Hyperammonaemic States : Acetohydroxamate : Bacterial Urease Inhibitor with Therapeutic (Dr. W. N. Fishbein et al.), 46
- Potential of Rat Retina . A New Property of the Early Receptor (Dr. G. B. Arden and H. Ikeda), 1100
- Potentials and some Implications : Evidence for a Poisson Distribution of Miniature Endplate (Dr. P. W. Gage and Dr. J. I. Hubbard), 395
- Potentials generated by Freezing Dilute Aqueous Solutions : Electric (H. C. Parreira and A. J. Eydt), 33
- Potentials in the Cat : Alcohol and Evoked (A. K. Schweigerdt et al.), 688
- Potter, Prof. P. E., and Pettijohn, Prof. F. J. : Atlas and Glossary of Primary Sedimentary Structures, review by J. R. L. Allen, 315
- Poulík, Prof. M. D., and Lauf, Dr. P. K. : Heterogeneity of Water-soluble Structural Components of Human Red Cell Membrane, 874
- Poultry Meat : Objective Evaluation of Quality in (Dr. A. W. Khan), 204
- Pounder, Prof. E. R. : Physics of Ice, review by Dr. J. Hallett, 986
- Pouring, Dr. A. A., and Buckle, Dr. E. R. : Effects of Seeding on the Condensation of Atmospheric Moisture in Nozzles, 367
- Power Travelling-wave Tubes (J. F. Gittins), review by Dr. P. A. Lindsay, 1140
- Power's Advisory Council on Research and Development : Minister of, 433
- Prange, jun., Prof. A. J., and Lipton, Prof. M. A. : Effects of Propylthiouracil and Thyroid Feeding on the Response of Mice to Injected Convulsant Barbiturate, 791
- Precipitation of Hydroxyapatite : Intermediate States in the (Prof. E. D. Eanes et al.), 365
- Precipitin Activity in the Sera of Patients with Deficiency of β -lipoproteins (Acanthocytosis) (Dr. R. F. Murray, jun., and Prof. B. S. Blumberg), 357
- Pregnancy in the Rat : Teratogenic Effects of Synthetic Compounds related to Trypan Blue : the Effect of 1,7-Diamino-8-naphthol-3,6-disulphonic Acid on (Dr. G. A. Christie), 1219
- Prehistory : Australian, 835 ; South African, 22
- Prell, Patricia A., et al. : Comparison of Salivary Amylase-levels in the Human Male and Female, 1211
- Prenatal Experience on Adult Behaviour in Rats : Effect of Foster-mothers' Strain and (Dr. J. M. Joffe), 815
- Prentice, Dr. J. E., and Reading, Dr. H. G. : Recent Finds in the Upper Carboniferous of South-west England and their Significance, 745
- Preparation of Cementite (J. Freil and Dr. A. K. Galwey), 183
- Prescott, D. M. (edited by) : Methods in Cell Physiology, review by Dr. J. H. Humphrey, 6
- Presence of Plant Growth Substances in Earthworms demonstrated by Paper Chromatography and the Went Pea Test (R. L. Nielson), 1113
- Preservation of Foods : Radiation, review by G. Birch, 973
- Presland, A. E. B., and Jakolska, A. : Microstructure of Fibrous Sulphur, 1088
- Pressure Distribution in the Tetrahedral Anvil Apparatus (J. Lees), 31
- Pressure on Flow Resistance : Ascent of Sap in Trees : Effect of Negative Absolute (Prof. B. Johnson and Prof. J. R. Dixon), 1347
- Pressure on Silica Powders, as revealed by Deuterium Oxide Exchange : An Infra-red Investigation of the Effect of (F. H. Hambleton et al.), 138
- Pressures and the Condensed Phase : Physics of High (edited by A. van Itterbeek), review by Dr. R. S. Bradley, 515
- Presumed Sensory Cells in Fish Epidermis (Dr. M. Whitear), 703
- Presynaptic Effects of Potassium Ion on the Mammalian Neuromuscular Junction (Dr. R. L. Parsons, Prof. W. W. Hofmann and Prof. G. A. Feigen), 590
- Pre-synaptic Inhibition in the Lateral Geniculate Body : Evidence for (A. Angel, F. Magni and P. Strata), 495

- R**ABBIT : Effect of a Fyrimidopyrimidine Derivative on Thrombus Formation in the (Dr. P. R. Emmons *et al.*), 255
Rabbit Tissue Glycosidases : Hydrolysis of Two 17- α -Oestradiol Glycosides by (K. S. Whittemore and Dr. D. S. Layne), 288
Rabbit : Tubular Site of Urate Secretion in the (Dr. J. V. Møller), 492
Rabbit's Ear : Sex Differences in the Rate of Tissue Regeneration in the (Dr. J. Joseph and Dr. M. Dyson), 599

- Rabina, S., et al.: Antiviral Action of Oxidized Spermine, Inactivation of Plant Viruses, 1095
- Rabson, Dr. Alan S., et al.: Mixed Infection of African Green Monkey Kidney Cells by Adenovirus 7 and SV 40, 1350
- Rachman, Dr. S., and Eysenck, Prof. H. J.: Causes and Cures of Neurosis, review by Dr. B. Semeonoff, 1027
- Radiation: A Potential Lightning Hazard?, Ionizing (P. J. Gillespie), 577
- Radiation Balance of Jupiter and Saturn (T. Owen and T. E. Walsh), 476
- Radiation Biology: Volume 1: Advances in (edited by L. G. Augenstein, R. Mason and H. Quastler), review by J. St. L. Philpot, 1032
- Radiation 'Cerebral Death' by Hypoxia: Modification of (Dr. E. A. Wright and J. Shewell), 904
- Radiation: Effects of Age and of Hypoxia: Life-shortening in Mice exposed to (Dr. P. J. Lindop and Prof. J. Rotblat), 1070
- Radiation-induced Sex Reversal in *Ecballium elaterium* (C. Gómez-Campo and M. Casas-Builla), 805
- Radiation Measurements: Stratospheric Transport of Volcanic Dust inferred from Solar (Dr. A. J. Dyer and B. B. Hicks), 131
- Radiation on Plant Cells: their Relation to Growth and Growth Induction: Direct and Indirect Effects of (Dr. R. D. Holsten, Dr. M. Sugii and Prof. F. C. Steward), 850
- Radiation Preservation of Foods: Proceedings of an International Conference, 1964, review by G. Birch, 973
- Radical Decay in Irradiated Polyethylene: Mechanism of (Dr. D. O. Geymer and Dr. C. D. Wagner), 72
- Radical State of X-irradiated Lyophilized Liver: Influence of Oxygen and Cysteine on the (M. Abe et al.), 499
- Radio Continuum Background at High Galactic Latitudes: Structure of the (Dr. M. J. S. Quigley and Dr. C. G. T. Haslam), 741
- Radio Emission at Centimetre and Decimetre Wavelengths: Basic Component of Solar (Dr. M. K. Das Gupta and D. Basu), 739
- Radio Emissions of the Planet in the Decimetre Region: Possible Contribution of Jupiter's Magnetospheric Tail to the (G. M. Gruber), 1271
- Radio Observations of Spiral Galaxies (Prof. B. Y. Mills and J. R. Glanfield), 10
- Radio Propagation: Ionospheric (Dr. K. Davies), review by Dr. H. Rishbeth, 419
- Radio Source 3C 273B: Structure of the Quasi-stellar (M. J. Rees and Dr. D. W. Sciama), 371
- Radio Source W49: OH Emission in the Direction of (B. Zuckerman et al.), 441
- Radio Sources: Scintillations on the Small Diameter (Prof. M. H. Cohen), 277
- Radio Structure of the Galactic Centre Region (Dr. D. Downes, Prof. A. Maxwell and Prof. M. L. Meeks), 1189
- Radioactive Elements in the Continental Crust (Dr. K. S. Heier), 479
- Radioactive Fallout, Soil, Plants, Foods, Man (edited by E. B. Fowler), review, 218
- Radioactive Fossil Fish from Thurso, Scotland: An Unusually (W. R. Diggles and J. Saxon), 400
- Radiobiology: 'Cell Killing' in (Dr. D. M. Robinson), 500
- Radiocarbon Dates on Bone: Validity of (Dr. M. A. Tamers and F. J. Pearson, jun.), 1053
- Radiochemistry: Nuclear and (Dr. G. Friedlander et al.), Second Edition, review by Dr. H. A. C. McKay, 105
- Radiological Protection, 1965-69: Officers and Members of the International Commission on, 936
- Radiolysis of Pyrimidine Compounds: Site of Attack in the (Prof. L. S. Myers, jun., J. F. Ward, W. T. Tsukamoto and D. E. Holmes), 1086
- Radiolysis of Sodium Tetrathionate Dihydrate (Prof. R. L. Eager and Dr. D. S. Mahadevappa), 781
- Radio-sensitivity as a Function of Age in Mice: Acute (Dr. J. F. Spalding et al.), 905
- Radiostromium by the Human Body: Effect of Sodium Alginate in inhibiting the Uptake of (R. Hesp and B. Ramsbottom), 1341
- Radiostromium from Contaminated Soils: Uptake by Plants of (A. J. Andersen), 195
- Raguin, Prof. E.: Geology of Granite, Second Edition, review by Dr. P. E. Brown, 1030
- Railway, Saudi Arabia: Reactivation of the Hijaz, 1262
- Rainbow Trout Serum: Electrophoretic Mobility of an Immune Globulin from (H. O. Hodgins et al.), 1106
- Rain-water during 1964: Fall-out in London (Dr. R. Wood et al.), 1083
- Raisingham, K. H., et al.: Determination of Nicotine in Smokers' Urine by Gas Chromatography, 788
- Raistrick, A. S., et al.: Reactivity of the α -Amino Group of Collagen, 1094
- Ramsbottom, B., and Hesp, R.: Effect of Sodium Alginate in inhibiting the Uptake of Radiostromium by the Human Body, 1341
- Randell, B., and Russell, L. L.: ALGOL 60 Implementation, review by I. M. Khabaza, 217
- Randhawa, Dr. H. S., Clayton, Dr. Y. M., and Riddell, Dr. R. W.: Isolation of *Cryptococcus neoformans* from Pigeon Habitats in London, 801
- Randle, Prof. P. J., and Denton, R. M.: Hormonal Control of Lipid Concentration in Rat Heart and Gastrocnemius, 488
- Random Packing of Spheres: A Numerical Model of (Dr. M. M. Levine and J. Chernick), 68
- Ranger VII: Structures inside a Lunar Crater photographed by (Captain V. G. Smalley and L. B. Ronca), 930
- Rao, J. J., et al.: Lack of Mutagenic Effect of Irradiated *Drosophila* Medium, 702
- Rao, K. Prabhakara: Record of the Bivalve *Gasteropod Berthelinia limax* (Kawaguti and Baba 1959) from the Indian Ocean, 404
- Rao, M. Rama: Water Balance and Evaporation Studies, 776
- Rao, M. S., et al.: Induction of Crossing-over in *Drosophila* Males by Means of Ovarian Extract, 203
- Lack of Mutagenic Effect of Irradiated *Drosophila* Medium, 702
- Rapid Detection and Titration of Antihuman Serum Albumin Antibody (Dr. M. S. Tobin), 1338
- Rapid Method for Selenium Assay of Plant Material (R. Duff and Prof. M. Chessin), 1001
- Rapid Mixing and Sampling Techniques in Biochemistry: Proceedings of the First International Colloquium, 1964, (edited by B. Chance, R. H. Eisenhardt, Q. H. Gibson and K. K. Longberg-Holm), review by Prof. S. Dagley, 823
- Rapid Screening Test for Transfer Factors in Drug-sensitive Enterobacteriaceae (Dr. E. S. Anderson), 1016
- Rapidly Labelled Synthesis of Ribonucleic Acid in Ehrlich Ascites C-2 Tumour Cells treated with Actinomycin D (D. A. Popović), 49
- Rapoport, S., Ababei, L., Wagenknecht, C., and Sarkar, S. R.: Enzyme Regulatory Mechanisms at the Level of Lactate-oxidoreductase in Erythrocytes and Ascites Tumour Cells, 185
- Rasool, Dr. S. I., et al.: Rotation Period of the Planet Mercury, 375
- Rat: Chemotopic Organization in the Bulbar Gustatory Relay of the (B. P. Halpern), 393
- Effect of 'Furosemid' on Chloride and Water Excretion in Single Nephrons of the Kidney of the (G. Malnic et al.), 80
- Effect of Infantile Treadmill Experience on Body-weight and Resistance to Exhaustion in the (R. A. Levitt and Prof. W. B. Webb), 1128
- Formation of Amines in the Gastric Mucosa of the (R. Håkanson), 793
- Nature of the Hepatomegalic Effect produced by Ethyl-chlorophenoxy-isobutyrate in the (Dr. R. Hess et al.), 856
- Source of Blood Aminoacyl Naphthylamidase in the (Dr. B. Monis), 587
- Teratogenic Effects of Synthetic Compounds related to Trypan Blue: the Effect of 1,7-Diamino-8-naphthol-3,6-disulphonic Acid on Pregnancy in the (Dr. G. A. Christie), 1219
- Rat Heart and Gastrocnemius: Hormonal Control of Lipid Concentration in (R. M. Denton and Prof. P. J. Randle), 488
- Rat induced by feeding Phenylalanine: Behavioural Deficit in the (Dr. R. C. Leaf et al.), 1021
- Rat Intestine *in vitro*: Accumulation and Incorporation of Amino-acid in (Dr. J. R. Bronk and Dr. D. S. Parsons), 785
- Rat Liver Cell: Nucleo-cytoplasmic Interactions in the (E. R. Godwin et al.), 246
- Rat Liver Mitochondria: Stoichiometry of Proton Translocation through the Respiratory Chain and Adenosine Triphosphatase Systems of (Dr. P. Mitchell and Dr. J. Moyle), 147
- Rat Muscles: Interferometric Estimation of Effects of Testosterone and Methandrostenolone on the Dry Mass of Various (Dr. W. Staubli and Dr. T. Okada), 562
- Rat Retina: A New Property of the Early Receptor Potential of (Dr. G. B. Arden and H. Ikeda), 1100
- Rat Serum by the Liver: Synthesis of α -Glycoprotein (Darcy) of (Prof. H. E. Weimer et al.), 1221
- Rat Slow Alpha-Globulin: Involvement of the Adrenal Cortex in the Appearance of (Prof. W. G. Heim and S. R. Ellenson), 1330
- Rat Thymus on Delayed Hypersensitivity Reactions of Neonatally Thymectomized Rats: Effect of a Lipid Fraction from (Prof. B. D. Janković et al.), 356
- Rat Tissues: Distribution of Collagenase in (J. F. Woods and Prof. G. Nichols, jun.), 1325
- Rate of Chromosome Duplication at the End of the Deoxyribonucleic Acid Synthetic Period in Human Blood Cells (Dr. C. W. Gilbert, S. Muldal and Dr. L. G. Lajtha), 159
- Rate of Tissue Regeneration in the Rabbit's Ear: Sex Differences in the (Dr. J. Joseph and Dr. M. Dyson), 599
- Rats: Acid Mucopolysaccharide and Hexosamine Metabolism in Dermal Connective Tissue of Adrenalectomized (Prof. J. Fabianek et al.), 1212
- Agar-gel Electrophoresis of Soluble Lens Proteins in Galactose-fed (E. Wittgenstein and Dr. K. W. Rowe, jun.), 386
- Distribution of Plutonium in Serum Proteins following Intravenous Injection into (G. Boocock and D. S. Popplewell), 282
- Effect of Foster-mothers' Strain and Pre-natal Experience on Adult Behaviour in (Dr. J. M. Joffe), 815
- Effect of a Lipid Fraction from Rat Thymus on Delayed Hypersensitivity Reactions of Neonatally Thymectomized (Prof. B. D. Janković et al.), 356
- Human Indigenous Microflora in Gnotobiotic (Dr. H. A. Dymysa et al.), 1236
- Rats fed Calcium Cyclamate: Feeding and Reproduction of (P. O. Nees and P. H. Derse), 81
- Rats on Diets containing Aldrin or Dieldrin: Metabolites in Urine of (P. R. Datta et al.), 289
- Rats resistant to Tumour Transplantation: Isoprecipitin in the Sera of (H. Hirai), 798
- Rats used in Parlow's Test: Diurnal Variations in Ovarian Ascorbic Acid Content of Pseudopregnant Immature (Dr. M. de la Lastra and Dr. H. Croxatto), 789
- Rattazzi, M. C., and Piki, C.: Haemoglobin Polymorphism in the Cod (*Gadus morhua*): a Single Peptide Difference, 489
- Rattee, Prof. I. D., Lewis, D. M., and Stevens, Dr. C. B.: Concentration Effects in Chromatographic Analysis, 269
- Raup, D., and Kummel, B. (edited by): Handbook of Palaeontological Techniques, review by Prof. D. T. Donovan, 968
- Rauschenbach, M. O., et al.: Bone Marrow Allografts in Thymectomized X-irradiated Dogs, 399
- Rawlins, F. I. G.: Beyond the Edge of Certainty: Essays in Contemporary Science and Philosophy, review, 314
- God Beyond Time, review, 985
- L'homme Face à Son Destin, review, 318
- Rays: Daily Variation of Cosmic (I. B. Mercer and Prof. B. G. Wilson), 477
- Reaction of Adenine-specific Antibodies with Denatured Deoxyribonucleic Acid (Dr. S. Estrada-Parra, A. Schmill and R.-M. Martínez), 1010
- Reaction of Human Lymphocytes in Culture to Components of the Medium, (G. J. Johnson and Prof. P. S. Russell), 343
- Reaction Rate in the Thermal Decomposition of Amorphous Polymers: Absence of Diffusion Control of (D. A. Smith), 1200
- Reaction Rates with Electron Capture Cross-sections: Correlation of Sodium Atom (K. R. Wilson and Prof. D. R. Herschbach), 182
- Reactions involving Purified Oxidases and Tissue Slices: Kinetic Parameters for Oxygen in (Dr. M. McCabe and Dr. D. A. Gilbert), 450
- Reactivity of the α -Amino Group of Collagen (J. H. Bowes, A. S. Raistrick and Dr. J. A. Moss), 1094
- Reactor: Laboratory Photochemical, 935
- Reactor Physics: European-American Committee on, 1259
- Reading, Dr. H. G., and Prentice, Dr. J. E.: Recent Finds in the Upper Carboniferous of South-west England and their Significance, 745
- Reading: Horticultural Science at the University of (Dr. G. P. Harris), 437
- Reading: University of, Appointments of Professors of Chemistry, 20, Chair of Geography, 628, Chair of Psychology, 228
- Reagents: Organic Complexing (D. D. Ferrin), review by Dr. T. S. West, 3
- Reaveley, D. A., et al.: 'Lipid A' Component from the Cell-walls of *Pseudomonas aeruginosa*, 586
- Reavey, T. C., and Johnson, jun., R. H.: Evaluation of Ion Exchange Cartridges for Field Sampling of Iodine-131 in Milk, 750
- Reber, Dr. K.: Inhibition of Haemolysis by 'Cremophor' in Conserved Blood, 195
- Recent Developments in Cosmology (Prof. F. Hoyle), 111
- Recent Finds in the Upper Carboniferous of South-west England and their Significance (Dr. H. G. Reading), (Dr. J. E. Prentice), 745
- Receptor Potential of Rat Retina: A New Property of the Early (Dr. G. B. Arden and H. Ikeda), 1100
- Receptors for Myxovirus Attachment: Nuclear Surface N-Acetyl Neuraminic Acid Terminating (Prof. P. I. Marcus et al.), 1122
- Re-classification of the Ethiopian Cainozoic Volcanic Succession (P. A. Mohr), 177
- Recognition by Holography: Character (Prof. D. Gabor), 422
- Recognition: Nutrition and its International (Sir Rudolph A. Peters), 117
- Recognition of Cervical Cell Nuclei: Computer (G. W. McMaster), 485
- Recoil Atoms in Solids: Evidence of Thermal and Hot Processes in the 'Hot-zone' produced by Copper-64 (K. Yoshihara and H. Ebihara), 482
- Reconditioning Concrete Surfacings, 834
- Reconstruction of Phase Objects by Holography (Prof. D. Gabor), (Prof. G. W. Stroke, D. Brumm, A. Funkhouser and A. Labeyrie), 1159
- Record of the Bivalve *Gasteropod Berthelinia limax* (Kawaguti and Baba 1959) from the Indian Ocean (K. P. Rao), 404
- Recruitment: Anglo-African Joint Research Schemes and, 333
- Recruitment to the Civil Service, 327, Sixth Report of Estimates, 1964-65, review, 311

- Rectifiers : Semiconductor Controlled (F. E. Gentry *et al.*), review by Prof. W. Fishwick, 719
- Red Cell and Leucocyte Pyruvate Kinase : Kinetic Differences between Human (J. O. Campos *et al.*), 194
- Red Cell Membrane : Heterogeneity of Water-soluble Structural Components of Human (Prof. M. D. Poulik and Dr. P. K. Lauf), 874
- Red Pigment in Leaf Galls of *Salix fragilis* L. (G. Blunden and Dr. S. B. Challen), 388
- Reddi, Dr. O. S., Reddy, G. M., Rao, J. J., Ebenezer, D. N., and Rao, M. S. : Lack of Mutagenic Effect of Irradiated *Drosophila* Medium, 702
- Reddi, O. S., Reddy, G. M., and Rao, M. S. : Induction of Crossing-over in *Drosophila* Males by Means of Ovarian Extract, 203
- Reddick, Dr. R. L., *et al.* : Release of Plasminogen Activator by the Isolated Perfused Dog Kidney, 291
- Reddy, G. M., *et al.* : Induction of Crossing-over in *Drosophila* Males by Means of Ovarian Extract, 203, Lack of Mutagenic Effect of Irradiated *Drosophila* Medium, 702
- Redshaw, Edward S., *et al.* : Respiratory Changes in the Cacao Seed Cotyledon Coincident with Seed Death, 1348
- Reduction of Diphenyl Picrylhydrazyl on Surfaces : Simultaneous Oxidation and (Prof. J. G. Aston *et al.*), 181
- Reed, W. D. C., Elliott, Dr. R. C., and Topps, Dr. J. H. : Phosphorus Excretion of Cattle fed on High-energy Diets, 953
- Re-entrant Hysteresis Loop and Multistable Ferrites (Dr. R. M. Glaister and Dr. E. W. Lee), 67
- Rees, Dr. D. A., *et al.* : Pectic Polysaccharides in the Growth of Plant Cells : Molecular Structural Factors and their Role in the Germination of White Mustard, 876
- Rees, Dr. K. R., *et al.* : Nucleo-cytoplasmic Interactions in the Rat Liver Cell, 246
- Rees, M. J., and Sciamia, Dr. D. W. : Structure of the Quasi-stellar Radio Source 3C 273B, 371
- Rees, W. J. : Freshwater Life of the British Isles, Third Edition, review, 988
- Reeves, Marjorie (edited by) : Eighteen Plus. Unity and Diversity in Higher Education, review by Sir James Cook, 313
- Refining : Advances in Petroleum Chemistry and, Vol. 10 (edited by J. J. McKetta, jun.), review by H. B. Milner, 969
- Reflex in Binocular Rivalry and Saccadic Suppression : Suppression of the Pupillary Light (M. Lorber *et al.*), 558
- Reflex Response of Normal Human Subjects : Relationship between the Electromyogram and the Force of the Isometric (A. M. Clarke), 551
- Reflexes in Man : Nerve Potential Recordings during electrically and mechanically evoked Monosynaptic (Dr. M. M. Gassel and Dr. E. Diamantopoulos), 1004
- Regeneration in the Rabbit's Ear : Sex Differences in the Rate of Tissue (Dr. J. Joseph and Dr. M. Dyson), 599
- Regeneration : Mechanisms of Neural (edited by M. Singer and J. P. Schade), review by Prof. K. Krnjević, 1248
- Regeneration : Role of Portal Blood Supply in Liver (Dr. R. Y. Thomson and A. M. Clarke), 392
- Regional Economic Development, 935
- Regional Geochemical Reconnaissance Applied to some Agricultural Problems in Co. Limerick, Eire (Prof. J. S. Webb and W. J. Atkinson), 1056
- Register of Scientific Research in the Netherlands (S. T. Groenman), 28
- Regression of a Phenotypic Value on the Values for the Parents and Grandparents (Dr. I. J. Good), 203
- Regulators : Morphactins, a Novel Group of Plant-growth (G. Schneider *et al.*), 1013
- Regulatory Mechanism in Bacteria : Example for New Type of (Dr. A. Tomasz), 155
- Regulatory Mechanisms at the Level of Lactate-oxidoreductase in Erythrocytes and Ascites Tumour Cells : Enzyme (S. Rapoport *et al.*), 185
- Regulatory Sequences in Messenger Ribonucleic Acid : Purine Rich (Prof. R. H. Doi), 552
- Reiser, A., and Frazier, V. : Ultra-violet Absorption Spectra of Aromatic Nitrenes and Dinirennes, 682
- Relation of Population Density to Sodium Availability and Sodium Selection by Microtine Rodents (Glenn D. Aumann and Prof. J. T. Emlen), 198
- Relationship between Galactans from Normal Bovine Lung and from *Mycoplasma mycoides* : Serological (Dr. M. Shifrine and R. N. Gourlay), 498
- Relationship between Neurosecretory Fibres and Pituicytes in the Eel : A Functional (Sir Francis Knowles and L. Vollrath), 1343
- Relationship between the Removal of the Frontal Ganglion and Protein Starvation in *Locusta migratoria* L. (Dr. K. U. Clarke and C. Gillott), 808
- Relationship of the Velocity of a Charged Particle to its Relative Biological Effectiveness (Dr. J. E. Turner) and (H. Hollister), 36
- Relationships among Autumn Phenomena in Woody Plants : Some Postulated (R. B. Van Huystee, C. J. Weiser and P. H. Li), 89
- Relationships between Constituents of Cones and Male Flowers of the Hop (*Humulus lupulus*, L.) (Dr. R. D. Hartley and Dr. R. A. Neve), 804
- Relative Biological Effectiveness (RBE) : Relationship of the Velocity of a Charged Particle to its (Dr. J. E. Turner) and (H. Hollister), 36
- Relative Growth in Arthropods and Man : Evolutionary Tendency of (Dr. R. Matsuda), 1228
- Relative Ionization Efficiencies of Hydrocarbon Molecules as a Function of Bombarding Electron Energy (J. A. Takacs and Prof. G. R. Freeman), 996
- Relative Similarities in One Dimension (Prof. J. W. Carmichael, Prof. R. S. Julius and Dr. P. M. D. Martin), 544
- Relativistic Objects : Pulsation Periods of General (J. P. Wright), 65
- Relativity : Special Theory of (Prof. David Bohm), review by Prof. C. W. Kilmister, 986
- Relativity : Stability of Rotating Massive Stars in General (B. Durney and I. W. Roxburgh), 1304
- Relaxation Effects in the Electrophoresis of Disperse Systems (J. N. Shaw and Dr. R. H. Ottewill), 681
- Relaxation from Tonic Contractions ('Catch') in an Invertebrate Muscle : Energy Requirements for (Dr. K. Minihan and Prof. R. E. Davies), 1327
- Release of Plasminogen Activator by the Isolated Perfused Dog Kidney (Dr. Rudolf Holemans, Dr. J. G. Johnston and Dr. R. L. Reddick), 291
- Release of Vascular Permeability Factor or Histamine by Specific Protease Inhibitor in Tissue Culture : Inhibition of Anaphylactic (Prof. H. Hayashi *et al.*), 1007
- Relictogobius kryzhanovskii and the Penetration of Mediterranean Gobies into the Black Sea (Dr. P. J. Miller), 474
- Removal of the Brain in Bats : Technique for the (A. M. Greenhall), 1014
- Removal of the Frontal Ganglion and Protein Starvation in *Locusta migratoria* L. : Relationship between the (Dr. K. U. Clarke and C. Gillott), 808
- Renatured $\alpha 1$ Sub-units of Collagen : Long-spacing Segments from (K. Kuhn *et al.*), 685
- Reovirus Particles : 'Ring-forms' of (Dr. F. J. Grose *et al.*), 606
- Replication : A New Model for Virus Ribonucleic Acid (Dr. F. Brown and Dr. S. J. Martin), 861
- Replication and the Activation of Muscle Differentiation (Dr. P. Pietsch and S. B. McCollister), 1170
- Reproduction of Rats fed Calcium Cyclamate : Feeding and (P. O. Nees and P. H. Derse), 81
- Reproductive Tract : Oxidative and Biosynthetic Utilization by Human Spermatozoa of a Metabolite of the Female (Prof. C. Turner), 1115
- Research : Biological Rhythm (Prof. A. Sollberger), review by Dr. A. D. Lees, 973
- Research : Seaweed (Dr. E. Conway and Dr. E. G. Young), 840
- Research : The Case for Applied, 1025
- Research : Use of Pigs in Biomedical (Dr. L. K. Bustad and R. O. McClellan), 531
- Research and Development in British Industry, 101
- Research and Development in the Building and Civil Engineering Industries, 629
- Research and Development in France : Industrial, 834
- Research and Development : Minister of Power's Advisory Council on, 433
- Research and Economic Growth in Belgium, 834
- Research and Management in East African Wildlife (Dr. J. Morton Boyd), 828
- Research and the Research Associations : Industrial, 1
- Research Council : Social Science, 629
- Research Council Support for University Science and Technology : Science, 117
- Research Frontiers in Fluid Dynamics (edited by Dr. R. J. Seeger and Dr. G. Temple), review by Prof. K. Stewartson, 967
- Research in Human Genetics and Studies of Twins, 230
- Research in New Zealand : Scientific, 233
- Research in New Zealand : Scientific and Industrial, 1156
- Research in Photography : Mitteilungen aus den Forschungslaboratorien der Agfa-Gevaert AG Levariksen-Munchen, Band IV (edited by Prof. E. Klein and R. Matejec), review by K. O. Ganguin, 720
- Research in the Development of Polish Industry : Scientific, 120
- Research in the Netherlands : Register of Scientific (S. T. Groenman), 28
- Research, Oslo, 1963 : Proceedings of the 3rd International Conference on Operational (edited by G. Kreweras and G. Morlat), review by S. J. Morrison, 1245
- Research Schemes and Recruitment : Anglo-African Joint, 333
- Resins in Ireland : Use of CIBA, 21
- Resistance to Exhaustion in the Rat : Effect of Infantile Treadmill Experiences on Body-weight and (R. A. Levitt and Prof. W. B. Webb), 1128
- Resistivity of Anodic Oxide Films on Valve Metals : A.C. (Dr. G. C. Wood and C. Pearson), 547
- Resolution of the Optical Isomers of DL Tryptophan, 5-Hydroxy-DL-tryptophan and 6-Hydroxy-DL-tryptophan by Paper and Thin-layer Chromatography (Dr. S. F. Contractor and J. Wragg), 71
- Resonance Frequency Shift and Internal Stress in Gamma-ray Irradiated Sodium Chlorate : Pure Quadrupole (Dr. H. Budak), 382
- Respiratory Chain and Adenosine Triphosphatase Systems of Rat Liver Mitochondria : Stoichiometry of Proton Translocation through the (Dr. P. Mitchell and Dr. J. Meyle), 147
- Respiratory Changes in the Cacao Seed Cotyledon coincident with Seed Death (I. A. Casas, E. S. Redshaw and M. L. Ibáñez), 1348
- Respiratory Disease : Association of Adenoviruses with Bovine (Dr. J. H. Darbyshire *et al.*), 307
- Respiratory System : Self-adaptive Control and the (Dr. I. P. Priban and Dr. W. F. Fincham), 339
- Response of Melanocytes of Dermis and Epidermis to Lightening Agents (Prof. J. McGuire and H. Möller), 493
- Response of Mice to Injected Convulsant Barbiturate : Effects of Propylthiouracil and Thyroid Feeding on the (Prof. A. J. Prange, jun., and Prof. M. A. Lipton), 791
- Response of Normal Human Subjects : Relationship between the Electromyogram and the Force of the Isometric Reflex (A. M. Clarke), 551
- Response to Polypeptides as Permeability Factors : Interspecies Differences in (Dr. J. Carr and Prof. D. L. Wilhelm), 653
- Responses of Superior Colliculus Neurons : Somesthetic and Visual (Dr. D. Jassik-Gerschenfeld), 678
- Responses to Alternating Current Stimulation : Squid Giant Axon : Repetitive (Prof. H. R. Hirsch), 1218
- Restoration of Roman Mosaics : Modern Technique in, 334
- Retention of Noradrenaline by the Spleen : Effect of Altered Perfusion Rates on the (Dr. D. M. Paton and Prof. C. N. Gillis), 391
- Rethinking Civil Service Policy, 415
- Reticulocyte Ribosome Fraction with an Exceptional Capacity for Polyphenylalanine Synthesis (Dr. J. O. Bishop), 361
- Reticulocytes maturing *in vivo* : Protein Synthesis in (Dr. P. T. Rowley), 244
- Reticulum of Lymph Node Follicles as shown by High-resolution Autoradiography : Ultrastructure of the Antigen-retaining (J. Mitchell and A. Abbot), 500
- Retina : A New Property of the Early Receptor Potential of Rat (Dr. G. B. Arden and H. Ikeda), 1100
- Return of Leucocytes to the Bone Marrow in Chronic Myelogenous Leukaemia (Dr. J. H. Moxley, Prof. S. Perry, Prof. G. H. Weiss and Dr. M. Zelen), 1281
- Reversal in *Ecballium elaterium* : Radiation-induced Sex (C. Gómez-Campo and M. Casas-Builla), 805
- Reversal of Adrenergic Vasoconstriction : Pronethalol-induced (Dr. M. G. Moreira and Dr. W. Osswald), 1006
- Reyner, J. H. : God Beyond Time, review by F. I. G. Rawlins, 985
- Rheology, 1963 : Proceedings of the Fourth International Congress on (edited by E. H. Lee and A. L. Copley), Four Parts, review by Dr. G. W. Scott Blair, 965
- Rhesus Monkey and Human Ovarian Oocytes : Maturation *in vitro* of Mouse, Sheep, Cow, Pig, (Dr. R. G. Edwards), 349
- Rhesus Monkeys : Social Companions and the Mother-Infant Relationship in (Y. Spencer-Booth *et al.*), 301
- Rheumatism Research : Laboratory Animals for, 1046
- Rhodesia : National Museums of Southern, 122
- Rhythm Research : Biological (Prof. A. Sollberger), review by Dr. A. D. Lees, 973
- Ribonucleic Acid and Production of Haemagglutinin : Influence of Heterologous Histone on the Synthesis of Cellular and Viral (A. G. Bukrinskaya *et al.*), 557
- Ribonucleic Acid by *Escherichia coli* during Inhibition by Azetidine-2-carboxylic Acid : Synthesis of (Dr. J. M. Midgley and Dr. D. G. Wild), 585
- Ribonucleic Acid in Ehrlich Ascites C-2 Tumour Cells treated with Actinomycin D : Rapidly Labelled Synthesis of (D. A. Popović), 49
- Ribonucleic Acid in Mammalian Ribosomes : Optical Rotatory Dispersion and Secondary Structure of (A. Blake and Dr. A. R. Peacocke), 1319
- RNA in Nucleated Erythrocytes : Stable Messenger (Dr. R. B. Scott and R. A. Malt), 497
- Ribonucleic Acid in Sub-apical Sections of *Avena* Coleoptile by Indolyl-3-acetic Acid : Stimulation of Synthesis of (Prof. T. H. Hamilton *et al.*), 1180
- RNA in Synchronized HeLa Cells : Synthesis of Ribosomal (Prof. M. D. Scharff and Dr. E. Robbins), 464
- t-RNA Methylating Enzymes during Insect Metamorphosis : Changes in the (Dr. B. S. Baliga *et al.*), 555
- Ribonucleic Acid : Purine Rich Regulatory Sequences in Messenger (Prof. R. H. Doi), 552
- Ribonucleic Acid Replication : A New Model for Virus (Dr. F. Brown and Dr. S. J. Martin), 861

- Ribonucleic Acid : Separation of High-molecular Deoxyribonucleic Acid and (Dr. O. Klammer), 1318
- RNA Synthesis : Correlation of the Binding to DNA Loops or to DNA Helices with the Effect on (Prof. J. H. Frentzer), 1093
- Ribonucleic Acid Synthesis Induced in Cultured Mammalian Cells by 7:12-Dimethylbenzanthracene : Differential Inhibition of Deoxyribonucleic Acid and (L. J. Alfred), 1339
- Ribonucleic Acids in the Zonal Ultracentrifuge : Fractionation of (Dr. J. R. B. Hastings *et al.*), 645
- Ribosomal RNA in Synchronized HeLa Cells : Synthesis of (Prof. M. D. Scharff and Dr. E. Robbins), 464
- Ribosome Fraction with an Exceptional Capacity for Polyphenylalanine Synthesis : Reticulocyte (Dr. J. O. Bishop), 361
- Ribosomes : Optical Rotatory Dispersion and Secondary Structure of Ribonucleic Acid in Mammalian (A. Blake and Dr. A. R. Peacocke), 1319
- Ricciuti, Dr. N. V., and Gibbs, Dr. C. L. : Heat Production in a Cardiac Contraction, 897
- Rice Fields : Selective Control of Weeds in, 1154
- Richards, B. G. : Thermistor Hygrometer for determining the Free Energy of Moisture in Unsaturated Soils, 608
- Richards, E. W. T. : Molecular Orbital Theory, review, 110
- Richards, L. A., *et al.* : Fall-out in London Rain-water during 1964, 1083
- Richardson, M., and Black, Dr. M. : Promotion of Germination in Light-requiring Seed by Chloramphenicol, 1114
- Richardson, N. G., *et al.* : Pectic Polysaccharides in the Growth of Plant Cells : Molecular Structural Factors and their Role in the Germination of White Mustard, 876
- Richmond, Sir Ian : *Obituary* by J. P. Gillam, 831
- Richmond, Dr. M. H., *et al.* : A Suggested Phenotypic Classification and Terminology for Enzyme Mutants in Micro-organisms, 841
- Ricketts, Dr. C. R., Cox, Dr. J. S. G., Fitzmaurice, C., and Moss, G. F. : The Iron Dextran Complex, 237
- Rickover, Vice-Admiral H. G. : A Humanistic Technology, 721
- Riddell, Dr. R. W., *et al.* : Isolation of *Cryptococcus neoformans* from Pigeon Habitats in London, 801
- Ridges, A. Pauline, *et al.* : 'Pink Spot' in the Urine of Schizophrenics, 453
- Ridgway, Dr. G. J., *et al.* : Electrophoretic Mobility of an Immune Globulin from Rainbow Trout Serum, 1106
- Ridley, Dr. Mark, and Phillips, Dr. I. : Relative Instability of Cephaloridine to Staphylococcal Penicillinase, 1076
- Riedhart, Prof. J. M. : Influence of Hexadecane on Absorption of Carbon Dioxide by Plants, 300
- Riess, W., *et al.* : Nature of the Hepatomegalic Effect produced by Ethyl-chlorophenoxyisobutyrate in the Rat, 856
- Rift System : Coincidence of Magnetic Disturbances with Local Earthquakes recorded from the Ethiopian (Prof. P. Gouin), 541
- Riha, Dr. I., *et al.* : A Localized Haemolysis in Gel Method for the Detection of Cells producing 7S Antibody, 858
- Riles, Mrs. Linda : A Lactate Dehydrogenase Variant in the Mouse, 814
- 'Ring-Forms' of Reovirus Particles (Dr. F. J. Grose, Prof. M. H. Bernstein and Dr. A. M. Lerner), 606
- Rings : Antimatter and Tree (V. S. Venkatavaran), 772
- Rings : Heterocyclic Compounds with Three- and Four-membered (edited by Dr. A. Weissberger), Parts 1 and 2, review by Dr. F. G. Mann, 2
- Rings in *Lates niloticus* (Pisces : Centropomidae) from Lake Chad : Winter Scale (A. J. Hopson), 1013
- Riordan, J. F., Wacker, Dr. W. E. C., and Vallee, Prof. B. L. : 'Buried' Tyrosyl Residues and the Activity of Trypsin, 1209
- Ripper, Dr. W. E. : *Obituary* by Dr. G. S. Hartley, 431
- Rohbeth, Dr. H. : Ionospheric Radio Propagation, review, 419
- Road to Peace, 513
- Robbins, Dr. E., and Scharff, Prof. M. D. : Synthesis of Ribosomal RNA in Synchronized HeLa Cells, 464
- Roberts, Dr. D. C. : Proceedings of the International Symposium on the Chemotherapy of Cancer, 1964, review, 1032
- Robertson, A. D. J. : Correlation between Unit Activity and Slow Potential Changes in the Unanaesthetized Cerebral Cortex of the Cat, 757
- Robertson, G. B., *et al.* : Structure of a Diolefin Complex of Platinum (II) $C_{10}H_{18}O_2PtCl_2$, 580
- Robin, Dr. G. de Q. : Antarctic Aeronomy and Ice Sheets, 439
- Robinson, Dr. B. J., *et al.* : Anomalous Intensity Ratios of the Interstellar Lines of OH in Absorption and Emission, 1193
- Robinson, Dr. B. J., and Koehler, J. A. : Hydrogen Content of Virgo Cluster Galaxies, 993
- Robinson, Dr. D. M. : 'Cell Killing' in Radiobiology, 500
- Robinson, Mr. H. G. R. : Appointed Deputy Chief Scientific Officer and Head of the Instruments and Electrical Engineering Department, Royal Aircraft Establishment, Farnborough, 19
- Robinson, Prof. Ivor, Schild, Prof. A., and Schucking, Prof. E. L. : Quasi-Stellar Sources and Gravitational Collapse, review by Sir Bernard Lovell, 963
- Robinson, Sir Robert : Biochemistry of Phenolic Compounds, review, 515
- Robson, Dr. Margaret : Protonation Equilibria and Alkaline Hydrolysis of Glycine Ethyl Ester, 265
- Robson, Dr. P. : Identification and Analysis of Surface-active Agents by Infrared and Chemical Methods, Two Volumes, review, 617
- Rock : Detonability of Nitroglycerin contained in Porous (J. E. Hay and F. H. Scott), 1197
- Rockefeller Institute 1901-1953 : A History of the (Dr. G. W. Corner), review by the Lord Adrian, 1244
- Rockets and Satellites : Dynamics of (edited by G. V. Groves), review by D. G. King-Hele, 516
- Rocks : Age Determinations of North American, 435
- Rocks and Minerals : Chemical Analyses of, 1261
- Rocks : Palaeomagnetism of the Isachsen Diabasic (A. Laroche *et al.*), 179
- Rodda, John C. : Forest Hydrology, 1157
- Roddy, W. T., *et al.* (edited by) : Chemistry and Technology of Leather, Vol. 4, review by Prof. A. G. Ward, 617
- Rodents : Relation of Population Density to Sodium Availability and Sodium Selection by Microtine (G. D. Aumann and Prof. J. T. Emlen), 198
- Rodgers, Dr. A. W., *et al.* : Common Features of the Optical Continua associated with Violent Cosmic Events, 1190
- Rods : Effect of Incubation Temperature on the Flagellation of Gram-negative (D. A. Orr and Dr. M. M. Taylor), 1017
- Rogers, A. E. E., *et al.* : Observations of Polarized OH Emission, 440
- Rohan, P., *et al.* : Thromboses in Large Veins and Pulmonary Embolisms induced by Catecholamines or Serotonin, 900
- Rohrl, H., *et al.* : Funktionentheorie, Fourth Edition, review by Dr. J. C. Burkill, 963
- Role of Portal Blood Supply in Liver Regeneration (Dr. R. Y. Thomson and A. M. Clarke), 392
- Role of Sialic Acid in Potassium Transport of L1210 Leukaemia Cells (Dr. J. Leslie Glick and Dr. S. Githens III), 88
- Role of the Gut in Synthesizing the Protein Component of Low-density (Beta) Lipoprotein (P. J. Scott and C. C. Winterbourn), 494
- Roll, Dr. H. U. : Physics of the Marine Atmosphere, International Geophysics Series, Vol. 7, review by Prof. J. Darbyshire, 419
- Rolsten, Dr. Robert F., Hopkins, Alan K., and Hunt, H. H. : Welding by High-velocity Particle Impact, 1273
- Romagnoli, A., *et al.* : Production of Carotenoid by a Green Alga, 1342
- Roman Mosaics : Modern Technique in Restoration of, 334
- Ronca, L. B., and Smalley, Captain V. G. : Structures inside a Lunar Crater photographed by *Ranger VII*, 930
- Roorkee, India, Central Building Research Institute, 629
- Root Caps : Cytoplasmic Organelles and Cell Growth in (Dr. B. E. Juniper and Dr. F. A. L. Clowes), 864
- Root Regeneration in Detached Shoots of Fruit-tree Rootstocks : Increase during Winter in Capacity for (Dr. B. H. Howard), 912
- Root Systems and Roots : Methods for the Examination of (Dr. J. J. Schuurman and Dr. M. A. J. Goedewaagen), review by W. D. Clayton, 319
- Roots : Mechanism of Uptake of Trace Elements by (G. Findenegg and Prof. E. Broda), 196
- Roots : Methods for the Examination of Root Systems and (Dr. J. J. Schuurman and Dr. M. A. J. Goedewaagen), review by W. D. Clayton, 319
- Rosi, D., Peruzzotti, G., Dennis, Dr. E. W., Berberian, Prof. D. A., Freele, H., and Archer, Dr. S. : A New, Active Metabolite of 'Miracid D', 1005
- Ross, Gordon, and White, Fred N. : Blood Flow in Turtles, 759
- Ross, Dr. J., and Burke, Dr. D. C. : Molecular Weight of Chick Interferon, 1297
- Ross, J. G. : Experimental Infections of Cattle with *Fasciola hepatica* : A Comparison of Low and High Infection Rates, 907
- Rotating Massive Stars in General Relativity : Stability of (B. Durney and I. W. Roxburgh), 1304
- Rotation Period of the Planet Mercury (W. E. McGovern, Dr. S. H. Gross and Dr. S. I. Rasool), 375
- Rotational Line Strengths of the Hydrogen Halides : Sub-millimetre Dispersion and (Dr. J. E. Chamberlain and Dr. H. A. Gebbie), 480
- Rotational Period of the Planet Mercury (Dr. G. Colombo), 575
- Rotatory Dispersion of a Chloroplast Preparation : Optical (Dr. Bacon Ke), 573
- Rotblat, Prof. J., and Lindop, Dr. P. J. : Life-shortening in Mice exposed to Radiation : Effects of Age and of Hypoxia, 1070
- Rothman, U., and Lidén, S. : Isolation of a Lymphoid-cell Protein with Relation to Delayed Hypersensitivity, 389
- Round, Dr. F. E. : Biology of the Algae, review by Dr. M. Parke and G. T. Boalch, 824
- Rous Sarcoma Virus (Bryan) : Subcutaneous Sarcomata in Hamsters Induced by (Prof. M. L. Eidinoff *et al.*), 336
- Rousseau, Dr. Alain, *et al.* : A New Bis-albuminaemic Family, 1222
- Rowe, jun., Dr. K. W., and Wittgenstein, E. : Agar-gel Electrophoresis of Soluble Lens Proteins in Galactose-fed Rats, 386
- Rowland, Dr. G. F., *et al.* : Nucleo-cytoplasmic Interactions in the Rat Liver Cell, 246
- Rowley, Dr. P. T. : Protein Synthesis in Reticulocytes maturing *in vivo*, 244
- Rowson, Dr. B., *et al.* : New Limits to the Angular Sizes of Some Quasars, 275
- Roxburgh, Ian W. : Angular Momenta of Eclipsing Binaries and the Fission Theory of their Origin, 65
- Roxburgh, I. W., and Durney, B. : Stability of Rotating Massive Stars in General Relativity, 1304
- Royal Hordes, Nomad Peoples of the Steppes (E. D. Phillips), review by M. C. Burkitt, 1138
- Royal Society of Edinburgh, 1260, Elections, 627
- Royal Society of London : Anniversary Address by Lord Florey, November 30th, 1965, 1141
- Medal Awards, 934, 1141 ; Royal, 832, James Smithson, 324
- Montague Napier Trust Fund, 1155
- Officers, 1044
- Research Professorship, 527
- Rubber Implant : Prolonged Administration of Atropine or Histamine in a Silicone (P. Bass *et al.*), 591
- Rubber in Engineering Practice (A. B. Davey and A. R. Payne), review by Dr. L. R. G. Treloar, 823
- Rubbo, Prof. Sydney D., and Gardner, Dr. J. F. : A Review of Sterilization and Disinfection as Applied to Medical, Industrial and Laboratory Practice, review by R. Hare, 1249
- Rubella Virus Complement-fixation Test (Dr. H. Stern), 200
- Rubidium and Potassium Perfusion into Dog Adrenals : Aldosterone Secretion : Differences in Direct Effects of (Prof. J. G. Hilton *et al.*), 1099
- Rubredoxin : Nature of the Non-haem Iron in Ferredoxin and (Dr. R. D. Gillard *et al.*), 769
- Rubus procerus* Muell. and *R. laciniatus* Willd. : 'Complement Fractionation' in a Natural Hybrid between (Dr. R. K. Bamm), 608
- Ruddle, Dr. F. H., and Young, R. : Inactivation of T-2 Bacteriophage by Sensitized Leucocytes *in vitro*, 1105
- Rudman, Dr. P. : Fine Structure of Wood, 55
- Ruffert, K., *et al.* : Fluorochrome Stains for Histological Diagnosis of Visceral Mycoses, 796
- Ruffie, J., *et al.* : I Antigen in Leukaemic Patients, 1329
- Ruhland, W. (edited by) : Encyclopedia of Plant Physiology, Vol. 15, Two Parts, review by Dr. C. R. Metcalfe, 619
- Ruminants : Possible Relationship between Trace Metals and Bloat in (P. J. Harris and Prof. F. Sebba), 869
- Rumsey, A. F., *et al.* : Stimulation of Synthesis of Ribonucleic Acid in Sub-apical Sections of *Avena* Coleoptile by Indolyl-3-acetic Acid, 1180
- Russell, Dr. Elizabeth S., *et al.* : Artificial Insemination of Dysphoric Mice with Mixtures of Spermatozoa, 1126
- Russell, G. B., and Bailey, Dr. R. W. : Disruption of Protozoa by Indole and Related Compounds, 1001
- Russell, L. J., and Randell, B. : ALGOL 60 Implementation, review by I. M. Khabaza, 217
- Russell, Prof. P. S., and Johnson, G. J. : Reaction of Human Lymphocytes in Culture to Components of the Medium, 343
- Russell, P. S., and Simmons, Dr. R. L. : Histocompatibility Antigens in Transplanted Mouse Eggs, 698
- Ruthenium Complexes : Homogeneous Hydrogenation and Hydroformylation using (D. Evans *et al.*), 1203
- Rutherford, Prof. D. E. : Introduction to Lattice Theory, review by Prof. T. A. A. Broadbent, 984
- Rutland, Dr. R. W. R., Guest, Dr. J. E., and Grasty, Dr. R. L. : Isotopic Ages and Andean Uplift, 677
- Rydhölm, Dr. S. A. : Pulping Processes, review by Dr. J. Grant, 719
- Rye : Effects of Oxygen Pressure and Salts on Germination of Winter (Dr. S. M. Siegel *et al.*), 1012
- Ryu, D. Y., *et al.* : Measurement of Unsteady State Growth Rates of Micro-organisms, 263

- SACCADIC Suppression:** Suppression of the Pupillary Light Reflex in Binocular Rivalry and (M. Lorber *et al.*), 558
- Sachs, Dr. L., *et al.*: Biological Systems at the Molecular Level, 1048
- Saggers, D. T., *et al.*: 2-Trifluoromethylbenzimidazoles: a New Class of Herbicidal Compounds, 1166
- Saidy, F., Morgan, W. A., and Wark, Dr. D. Q.: Determination of Cloud Altitudes from Gemini-Titan-5, 775
- Salb, Jesse M., *et al.*: Nuclear Surface N-Acetyl Neuraminic Acid Terminating Receptors for Myxovirus Attachment, 1122
- Salisbury, Prof. G. W., *et al.*: Measurements of Bovine Chromosomes, 97
- Salivary Amylase-levels in the Human Male and Female: Comparison of (Prof. R. L. McGeechin *et al.*), 1211
- Salivary Gland Chromosomes after Treatment with Carbon Dioxide: Puffing of (Prof. W. J. Burdette and R. Anderson), 409
- Salix fragilis* L.: Red Pigment in Leaf Galls of (G. Blunden and Dr. S. B. Challen), 388
- Salmon: Control of Furunculosis in Impounded Adult (A. C. Leaman), 1344
- Salmonella typhimurium*: Characterization of a Transfer Factor associated with Drug Resistance in (Dr. E. S. Anderson and Dr. M. J. Lewis), 843
- Salt Tolerance in Plants: Physiological Basis of (Dr. B. P. Stroganov), review by Dr. F. A. Thompson, 211
- Saltmarsh-Andrew, M. J., and Millin, Dr. D. J.: Nucleotide Peptides in Yeast, 468
- Salts: Oxidation of some 2,4-Dinitrophenylamines with Ceric (Miss S. Haworth and Dr. J. R. Holker), 184
- Salvinia auriculata* Aublet on the Congo River: Occurrence of (Dr. E. C. S. Little), 1111
- Sammons, Dr. H. G., *et al.*: Inhibition of Influenza Virus Haemagglutination by Polymerized Orosomucoid, 915
- Sample, Sheila D., and Djerassi, Prof. C.: Mass Spectrometric Fragmentation of Nitrophenylhydrazones, 1314
- Sampling of Iodine-131 in Milk: Evaluation of Ion Exchange Cartridges for Field (R. H. Johnson, jun., and T. C. Reavey), 750
- Samsonov, Prof. G. V.: Plenum Press Handbooks of High Temperature Materials, Vol. 2, Properties Index, review by Dr. E. H. Andrews, 517
- Sander, Dr. G.: Histologie und Mikroskopische Anatomie des Menschen, 5th German Edition, review, 109
- Sandor, Dr. Georges, Martin, Dr. L., Porsin, Dr. M., Rousseau, Dr. A., and Martin, Dr. R.: A New Bis-albuminemic Family, 1222
- Sandstrom, A. E.: Cosmic Ray Physics, review by Dr. J. J. Quenby, 315
- Santamour, jun., F. S.: Leucoanthocyanins of White Pine in relation to Weevil Attack, 407
- Sap in Trees: Effect of Negative Absolute Pressure on Flow Resistance: Ascent of (Prof. B. Johnson and Prof. J. R. Dixon), 1347
- Saporoschetz, Inna B., *et al.*: A Simple Bioassay for Antioxidants based on Protection of *Tetrahymena pyriformis* from the Photodynamic Toxicity of Benzo(a)pyrene, 655
- Sarcoma Virus (Bryan): Subcutaneous Sarcomata in Hamsters induced by Rous (Prof. M. L. Eidinoff *et al.*), 336
- Sarcomata in Hamsters induced by Rous Sarcoma Virus (Bryan): Subcutaneous (Prof. M. L. Eidinoff *et al.*), 336
- Sarcophaga bullata*: Differential Deoxyribonucleic Acid Replication in the Giant Foot-pad Cells of (Dr. J. M. Whitten), 1019
- Sargassum* Tannin, an Antibiotic which retards Fouling (Prof. J. McN. Sieburth and Prof. J. T. Conover), 52
- Sargent, W. L. W., *et al.*: Common Features of the Optical Continua associated with Violent Cosmic Events, 1190
- Sarkar, S. R., *et al.*: Enzyme Regulatory Mechanisms at the Level of Lactate-oxidoreductase in Erythrocytes and Ascites Tumour Cells, 185
- Saruhashi, K., *et al.*: Strontium-90 and Caesium-137 in Columbia River Plume, July 1964, 1084
- Satellites: Dynamics of Rockets and (edited by G. V. Groves), review by D. G. King-Hele, 516
- Tidal De-spin of Planets and (Dr. P. Goldreich), 375
- Visual Observation of (D. G. King-Hele), 1156
- Satkowski, J. A., and Smets, H. B. (edited by): Magnetohydrodynamic Electrical Power Generation, Symposium 1964, Four Volumes, review by Prof. M. W. Thring, 966
- Sato, Dr. Mitsuo: Structure of Interstratified (Mixed-layer) Minerals, 70
- Sato, Prof. M., Oinuma, K., and Kobayashi, K.: Interstratified Mineral of Illite and Montmorillonite, 179
- Saturn: Radiation Balance of Jupiter and (T. Owen and T. E. Walsh), 476
- Saudi Arabia: Reactivation of the Hijaz Railway, 1262
- Saure, L., and Hyvarinen, H.: Seasonal Changes in the Histological Structure of the Spinal Column of *Sorex araneus* (L.), 705
- (Sauria: Iguanidae): Interspecific Karyotypic Variation as a Systematic Character in the Genus *Anolis* (G. C. Gorman), 95
- Sawflies (Hymenoptera, Symphyta): Bilateral Asymmetry: A Larval Polymorphism in some Argid (Dr. M. F. Claridge and Dr. J. M. Edington), 301
- Sawyer, R.: Determination of pH, review, 318
- Saxon, J., and Diggle, W. R.: An Unusually Radioactive Fossil Fish from Thurso, Scotland, 400
- Scale Rings in *Lates niloticus* (Pisces: Centropomidae) from Lake Chad: Winter (A. J. Hopson), 1013
- Scattering in Sodium Chloride: Direct Ultramicroscopic Observation of Thermal (Dr. G. Heilmann), 884
- Schade, J. P., and Singer, M. (edited by): Mechanisms of Neural Regeneration, review by Prof. K. Krnjević, 1248
- Schapp, W.: Metaphysik der Naturwissenschaft, review, 988
- Schardin, Prof. H.: *Obituary* by G. H. Lunn, 832
- Scharff, Prof. M. D., and Robbins, Dr. E.: Synthesis of Ribosomal RNA in Synchronized HeLa Cells, 464
- Scherbaum, Prof. O. H., and Lee, Dr. Y. C.: Isolation of Macronuclei from the Ciliate *Tetrahymena pyriformis* GL, 1350
- Schild, Prof. Alfred, *et al.*: Quasi-stellar Sources and Gravitational Collapse, review by Sir Bernard Lovell, 963
- Schistocerca gregaria* (Forsk.) (Orthoptera: Acrididae): An Inhibitory Effect of Allatectomized Males and Females on the Sexual Maturation of Young Male Adults of (M. J. Norris and M. P. Pener), 1122
- Schizophrenia as a Genetic Morphism (Prof. A. Hoffer), 306
- Schizophrenics: 'Pink Spot' in the Urine of (R. E. Bourdillon *et al.*), 453
- Schjerve, Dr. Ole A., McCandless, R. G., and Munn, R. J.: Biology of the Cytoplasm, 247
- Schmidt, M., *et al.*: Prevention of Cholesterol-induced Cholelithiasis by Nacymycin, 1226
- Schmill, Amanda, *et al.*: Reaction of Adenine-specific Antibodies with Denatured Deoxyribonucleic Acid, 1010
- Schneider, Dr. C. H., and de Weck, Dr. A. L.: New Chemical Aspect of Penicillin Allergy: the Direct Reaction of Penicillin with ϵ -Amino-groups, 57
- Schneider, G., Erdmann, D., Lust, S., Mohr, G., and Niethammer, K.: Morphactins, a Novel Group of Plant-growth Regulators, 1013
- Schoenborn, Dr. B. P.: Binding of Xenon to Horse Haemoglobin, 760
- Schoental, Dr. R.: Induction of Intestinal Tumours by N-Ethyl-N-nitrosourethane, 300
- Scholarship: Emmie Clough 735
- Schonfield, Prof. D.: Memory Changes with Age, 918
- School: Planning a New Medical, 634
- Schools: Applied Science in the (T. A. Burdett), 1269
- Schrank, Prof. A. R., *et al.*: Stimulation of Synthesis of Ribonucleic Acid in Sub-apical Sections of *Avena* Coleoptile by Indolyl-3-acetic Acid, 1180
- Schrödinger, Erwin: My View of the World, review by Prof. C. K. Grant, 5
- Schrödinger's Logic: My View of the World, review by Prof. C. K. Grant, 5
- Schucking, Prof. E. L., *et al.*: Quasi-stellar Sources and Gravitational Collapse, review by Sir Bernard Lovell, 963
- Schumacher, W., *et al.* (rewritten by): Strasburger's Textbook of Botany, review by Dr. A. Allsopp, 618
- Schuurman, Dr. J. J., and Goetjewaagen, Dr. M. A. J.: Methods for the Examination of Root Systems and Roots, review by W. D. Clayton, 319
- Schwartz, Vera Galinovic, *et al.*: Nuclear Surface N-Acetyl Neuraminic Acid Terminating Receptors for Myxovirus Attachment, 1122
- Schweigerdt, A. K., Dravid, Dr. A. R., Stewart, A. H., and Himwich, Dr. H. E.: Alcohol and Evoked Potentials in the Cat, 688
- Sciama, Dr. D. W., and Rees, M. J.: Structure of the Quasi-stellar Radio Source 3C 273 B, 371
- Science and Economic Growth, 1260
- Science and Engineering at the Massachusetts Institute of Technology: Materials, 726
- Science and Mathematics Teachers: Guinness Awards for 631
- Science and Philanthropy: James Smithson: Pathfinder in (Dr. L. Carmichael), 321
- Science and Philosophy: Beyond the Edge of Certainty: Essays in Contemporary (edited by R. G. Colodny), review by F. I. G. Rawlins, 314
- Science and Technology: High-pressure, 328
- Science Citation Index 1964 Annual Cumulation: International Interdisciplinary Index to the Literature of Science and Technology in Eight Parts (edited by E. Garfield), review by C. W. Cleverdon, 717
- Science Congress: Eleventh Pacific, 328
- Science, Faith and Society (Prof. Michael Polanyi), review, 985
- Science in Belgium, 1045
- Science in France, 733
- Science in History (Prof. J. D. Bernal), Third Edition, review by R. Brightman, 986
- Science in Hungary (edited by Dr. T. Erdely-Grúz, and Dr. I. Trancsényi-Waldapfel), review by Prof. D. Gabor, 983
- Science in the Nineteenth Century (edited by René Taton), review by Dr. W. P. D. Wightman, 958
- Science in the Schools: Applied (T. A. Burdett), 1269
- Science Information and Documentation Services, World Guide to, issued by Unesco, 122
- Science, Inventiveness and Creativity, 921
- Science Papers of the Victorian Era, 935
- Science Research Council support for University Science and Technology, 117
- Science: Teaching in the History and Philosophy of (Dr. W. Mays), 937
- Sciences of India: National Institute of, 936
- Scientific and Industrial Research in New Zealand, 1156
- Scientific and Technological Information in Parliament, 209
- Scientific Civil Service: Organization of the, 1133
- Scientific Periodicals: Directory of Indian, 434
- Scientific Periodicals from the U.S.S.R., 328
- Scientific Research in New Zealand, 233
- Scientific Research in the Development of Polish Industry, 120
- Scientific Research in the Netherlands: A Register of (S. T. Groenman), 28
- Scientist: Education and Application of the Materials (Dr. G. F. Freeguard), 939
- Scientists: Early Seventeenth Century (edited by R. Harré), review by Dr. W. L. Sumner, 985
- Scientists in the 'New Deal', 1260
- Scintillation Counting: Use of Dimethyl Sulphoxide in Liquid (E. Wright and K. R. Millar), 889
- Scintillations on the Small Diameter Radio Sources (Prof. M. H. Cohen), 277
- Scintilligraphy: Use of Technetium-99m in Hepatic (Dr. O. Degrossi *et al.*), 86
- Scirites from the Speeton Clay: Holothurian (B. N. Fletcher), 281
- Scolnick, E. M., *et al.*: Plasma Co-factors in Adenosine Diphosphate-induced Aggregation of Human Platelets, 296
- Scotland: An Unusually Radioactive Fossil Fish from Thurso, (W. R. Diggle and J. Saxon), 400
- Scott, F. H., and Hay, J. E.: Detonability of Nitroglycerin contained in Porous Rock, 1197
- Scott, J. E.: Chemical Physiology of the Acid Mucopolysaccharides, 1270
- Scott, Dr. O. C. A.: *Obituary* of Dr. L. H. Gray, 226
- Scott, P. J., and Winterbottom, C. C.: Role of the Gut in Synthesizing the Protein Component of Low-density (Beta) Lipoprotein, 494
- Scott, Dr. R. B., and Malt, R. A.: Stable Messenger RNA in Nucleated Erythrocytes, 497
- Scott Blair, Dr. G. W.: Proceedings of the Fourth International Congress on Rheology, 1963, Four Parts, review, 965
- Scottish Hydroelectric Schemes, 1153
- Scottish Plant Breeding Station: Director, 228
- Screening Test for Substances inhibiting the Cancer Coagulative Factor (Dr. E. M. Glaser, P. Spink and Prof. R. A. Q. O'Meara), 1008
- Screening Test for Transfer Factors in Drug-sensitive Enterobacteriaceae: A Rapid (Dr. E. S. Anderson), 1016
- Scribbles: Form-similarity between Phosphores of Adults and pre-School Children's (R. Kellogg *et al.*), 1129
- Sea: Measurements of Spontaneous Bioluminescence in the (Dr. B. P. Boden *et al.*), 1078
- Sea-stars: Ancestry of (Dr. C. M. Philip) (Prof. H. B. Fell), 766
- Seals: Functional Significance of Sweat Glands and Sebaceous Glands in (J. K. Ling), 560
- Search for Mutation in Chemical Systems at Steady State (Dr. G. Allen), 258
- Searle, Prof. L., Rodgers, Dr. A. W., Sargent, W. L. W., and Oke, J. B.: Common Features of the Optical Continua associated with Violent Cosmic Events, 1190
- Seasonal Changes in the Histological Structure of the Spinal Column of *Sorex araneus* (L.) (L. Saure and H. Hyvarinen), 705
- Sea-water: Desalination of, 329
- Seaweed Research: Fifth International Symposium (Dr. E. Conway and Dr. E. G. Young), 840
- Sebaceous Glands in Seals: Functional Significance of Sweat Glands and (J. K. Ling), 560
- Sebba, Prof. F., and Harris, P. J.: Possible Relationship between Trace Metals and Bloat in Ruminant, 869
- Second International Conference on Protozoology (Prof. P. C. C. Garnham), 330
- Second Law: Carathéodory's Principle and the Kelvin Statement of the (J. Dunning-Davies), 576

- Second 'Structural' Locus determining Human Phosphoglucosomutase : Evidence for a (Dr. D. A. Hopkinson and Prof. H. Harris), 410
- Secondary Structure of Ribonucleic Acid in Mammalian Ribosomes : Optical Rotatory Dispersion and (A. Blake and Dr. A. R. Peacocke), 1319
- Secretan, L., et al. : Measured Velocities of Interplanetary Dust Particles, 673
- Secretion Factors in Bronchogenic Carcinoma : Blood Group (Prof. C. McNeil et al.), 299
- Secretion of Newly Synthesized Insulin *in vitro* (S. L. Howell, D. G. Parry and Dr. K. W. Taylor), 487
- Sedimentary Structures : Atlas and Glossary of Primary (Prof. F. J. Pettijohn and Prof. P. E. Potter), review by J. R. L. Allen, 315
- Sediments : Pleistocene Glacial-marine Zones in North Atlantic Deep-sea (Dr. J. R. Conolly and Dr. M. Ewing), 135
- Seeber, J. M., et al. : Use of Technetium-99m in Hepatic Scintigraphy, 86
- Seed-borne Fungi : Description of 77 Fungus Species (J. P. Malone and A. E. Muskett), Proceedings of the International Seed Testing Association, Vol. 29, No. 2, review by R. W. G. Dennis, 319
- Seed Death : Respiratory Changes in the Cacao Seed Cotyledon coincident with (I. A. Casas et al.), 1348
- Seeding : Effects of, on the Condensation of Atmospheric Moisture in Nozzles (Dr. E. R. Buckle and Dr. A. A. Pouring), 367
- Seedlings : Air Ion Effects on the Oxygen Consumption of Barley (S. Kotaka et al.), 1112
- Seeger, Dr. R. J., and Temple, Dr. G. (edited by) : Research Frontiers in Fluid Dynamics, review by Prof. K. Stewartson, 967
- Segal, Dr. Stanton, and Crawhall, J. C. : Sulphocysteine in the Urine of the Blotched Kenya Genet, 1320
- Segal, Dr. W. : Biosynthesis of 2-Aminoethanephosphonic Acid : a Phosphoramidic Acid Re-arrangement, 1284
- Segments from Renatured α 1 Sub-units of Collagen : Long-spacing (K. Kuhn et al.), 685
- Selsmity and other Properties of Geological Provinces (Prof. E. R. Kanasewich), 1275
- Seitz, F., and Turnbull, D. (edited by) : Solid State Physics : Advances in Research and Applications, Vol. 15, review by Prof. C. W. McCombie, 4
- Selby, F. W., et al. : Altered Electrophoretic Mobilities of some Erythrocytic Enzymes as a Function of their Age, 76
- Selection and Transmissible Disease : Natural (Prof. N. A. Barnicot), 535
- Selective Control of Weeds in Rice Fields, 1154
- Selective Effect of Particulate Insecticides on *Simulium* among Stream Fauna (Prof. W. E. Kershaw, T. R. Williams, S. Frost and Prof. H. B. N. Hynes), 199
- Selenium Assay of Plant Material : Rapid Method for (R. Duff and Prof. M. Chessin), 1001
- Self-adaptive Control and the Respiratory System, (Dr. I. P. Prihan and Dr. W. F. Fincham), 339
- Self-stimulation : Facilitation as a Function of Temporal Spacing of Stimuli in Intracranial (Prof. J. A. Deutsch and R. Collins), 592
- Selye, Prof. H., Tuchweber, B., and Rohan, P. : Thromboses in Large Veins and Pulmonary Embolisms induced by Catecholamines or Serotonin, 900
- Semeonoff, Dr. B. : Experiments in Behaviour Therapy, review, 1027
- Causes and Cures of Neurosis, review, 1027
- Semiconductors : Physique des : Comptes Rendus du 7e Congres International, review by P. T. Landsberg, 217
- Semiconductor Controlled Rectifiers, Principles and Applications of *p-n-p-n* Devices (F. E. Gentry, F. W. Gutzwiller, N. Holonyak, jun., and E. E. Von Zastrow), review by Prof. W. Fishwick, 719
- Sendai Virus Multiplication by Puromycin and Actinomycin D : Stimulation of (Dr. D. O. White and I. M. Cheyne), 813
- Senses and Perception : Human (Prof. G. M. Wyburn et al.), review by Prof. O. L. Zangwill, 981
- Sensitivity of *in vitro* Murine Leukaemia Cells to Fractionated X-rays and Fast Neutrons : Increased (Dr. W. L. Caldwell et al.), 168
- Sensitivity of *Serratia marcescens* with Relative Humidity : Variation of the X-ray (H. G. Morrison and Prof. D. V. Cormack), 91
- Sensitivity to Drugs : Micromethod for Measuring Microbial (Dr. W. Koch and Dr. D. Kaplan), 50
- Sensory Cells in Fish Epidermis : Presumed (Dr. M. Whitear), 703
- Separation Method for Melatonin in Pineal Extracts (Dr. F. C. G. van de Veerdonk), 1324
- Separation of Aerosol Particles : Ultrapurification by (Dr. S. Behrendt), 581
- Separation of Coumarins (R. S. Shah and Dr. S. L. Bafna), 76
- Separation of High-molecular Deoxyribonucleic Acid and Ribonucleic Acid (Dr. O. Klammerth), 1318
- Separation of Proteins by Electrophoresis on Acrylamide Gels : Taxonomic Investigation of Several Brassica Species using Serology and the (Dr. J. G. Vaughan et al.), 704
- Separations within Deoxyribonucleic Acid during Selective Transcription : Localized Strand (Prof. J. H. Frentzen), 894
- 'Sephadex' Gel for the Estimation of Molecular Weight of Humic Substances in Natural Water : Use of (E. T. Giessing), 1091
- Sera of Patients with Deficiency of β -Lipoproteins (Acanthocytosis) : Precipitin Activity in the (Dr. R. F. Murray, jun., and Prof. B. S. Blumberg), 357
- Sera of Rats resistant to Tumour Transplantation : Isoprecipitin in the (H. Hirai), 798
- Serological Relationship between Galactans from Normal Bovine Lung and from *Mycoplasma mycoides* (Dr. M. Shifrine and R. N. Gourlay), 498
- Serology and the Separation of Proteins by Electrophoresis on Acrylamide Gels : Taxonomic Investigation of Several Brassica Species using (Dr. J. G. Vaughan et al.), 704
- Seromucoid Fraction : Factors affecting the Amount and Composition of the Serum (Dr. A. J. Anderson), 491
- Serotonin (Prof. S. Garattini and Prof. L. Valzelli), review by Dr. G. B. West, 1247
- Serotonin after Head X-irradiation and its Significance in the Potentiation of Barbiturate Hypnosis : Regional Changes in Brain (Dr. V. Nair), 1293
- Serotonin Depletion by Dietary Leucine (Dr. A. Yuwiler and Dr. E. Geller), 83
- Serotonin in the Tissues and Venoms of Two Pit Vipers : Norepinephrine and (Prof. A. H. Anton and Prof. J. F. Gennaro, jun.), 1174
- Serotonin : Thromboses in Large Veins and Pulmonary Embolisms induced by Catecholamines or (Prof. H. Selye et al.), 900
- Serratia marcescens* with Relative Humidity : Variation of the X-ray Sensitivity of (H. G. Morrison and Prof. D. V. Cormack), 91
- Serratosa, J. M. : Use of Infra-red Spectroscopy to determine the Orientation of Pyridine sorbed on Montmorillonite, 679
- Serum Albumin Antibody : Rapid Detection and Titration of Antihuman (Dr. M. S. Tobin), 1338
- Serum by the Liver : Synthesis of α -Glycoprotein (Darcy) of Rat (Prof. H. E. Weimer et al.), 1221
- Serum : Electrophoretic Mobility of an Immune Globulin from Rainbow Trout (H. O. Hodgins et al.), 1106
- Serum γ -Globulins in Mice : Breakdown Rate of (Dr. D. E. H. Tee et al.), 251
- Serum Haaptoglobin in Mothers of Children with Haemolytic Disease of the Newborn : Low Concentrations of (J. Pintera), 694
- Serum Proteins following Intravenous Injection into Rats : Distribution of Plutonium in (G. Boocock and D. S. Popplewell), 282
- Serum Seromucoid Fraction : Factors affecting the Amount and Composition of the (Dr. A. J. Anderson), 491
- Serum Type Systems : Linkage in Man : the Inv and the Lp (Dr. T. Gedde-Dahl, jun., and Dr. K. Berg), 1126
- Services : Science Information and Documentation, 122
- Sessa, Dr. Grazia, et al. : Effect of Steroids and 'Triton X-100' on Glucose-filled Phospholipid/Cholesterol Structures, 649
- Seventeenth Century Scientists : Early (edited by R. Harré), review by Dr. W. L. Sumner, 985
- Severi, Prof. L. : Lung Tumours in Animals, 632
- Sex-dependent Factor in Aplastic Anaemia ? (Dr. D. E. Pegg and J. E. Burns), 1187
- Sex Differences in the Rate of Tissue Regeneration in the Rabbit's Ear (Dr. J. Joseph and Dr. M. Dyson), 599
- Sex Reversal in *Ecballium elaterium* : Radiation-induced (C. Gómez-Campo and M. Casas-Builla), 805
- Sexual Dimorphism in *Tilapia* (J. Chervinski), 703
- Sexual Maturation of the Insect *Pyrhacoris apterus* : Effect of Certain Types of Paper on (Prof. C. A. B. Clementson), 510
- Sexual Maturation of Young Male Adults of *Schistocerca gregaria* (Forsk.) (Orthoptera : Acrididae) : An Inhibitory Effect of Allatectomized Males and Females on the (M. J. Norris and M. P. Pener), 1122
- Shade Tolerance in Flowering Plants (Dr. J. P. Grime), 161
- Shaffer, Dr. P. T. B. : Plenum Press Handbooks of High Temperature Materials, Vol. 1, Materials Index, review by Dr. E. H. Andrews, 517
- Shah, R. S., and Bafna, Dr. S. L. : Separation of Coumarins, 76
- Shambat : University of Khartoum, Sudan : Silver Jubilee of the Faculty of Agriculture, (Prof. M. A. Nour), 620
- Shamberger, R. J., et al. : Tumour-promoting Agents in Unburned Cigarette Tobacco, 584
- Sham-thymectomized Mice : Time-course Studies on Antibody Response in Thymectomized and (Dr. N. R. Sinclair), 1104
- Shannon, J. S., et al. : Spectroscopy in Australia, 125
- Shapiro, I. M., et al. : Influence of Heterologous Histone on the Synthesis of Cellular and Viral Ribonucleic Acid and Production of Haemagglutinin, 557
- Sharp, John R. : Some Fundamentals of Information Retrieval, review by P. C. Williams, 822
- Shaw, J. N., and Ottewill, Dr. R. H. : Relaxation Effects in the Electrophoresis of Disperse Systems, 681
- Shearman, D. J., and Skipwith, Sir Patrick A. d'E. : Organic Matter in Recent and Ancient Limestones and its Role in their Diagenesis, 1310
- Sheep, Cow, Pig, Rhesus Monkey and Human Ovarian Oocytes : Maturation *in vitro* of Mouse, (Dr. R. G. Edwards), 349
- Sheep : Establishment of a Pure Infection of the Nematode *Ostertagia circumcincta* in the (J. W. Copland), 1229
- Sheep in New Zealand in Relation to Wear of Teeth : Ingestion of Soil by (W. B. Healy and T. G. Ludwig), 806
- Sheep Pituitary Glands : Isolation and Amino-acid Sequence of β -LPH from (Prof. Choh Hao Li et al.), 1093
- Sheffield, J., et al. : Experimental Observations on the Structure of Collisionless Shock Waves in a Magnetized Plasma, 133
- Shell Research Fellowship : New, 1263
- Shell Theory : Introduction to Plate and (C. E. Turner), review by Prof. B. N. Cole, 1137
- Shepard, Prof. T. H., Gordon, Dr. L. H., and Wollenweber, Dr. J. E. : Lactic Dehydrogenase Isoenzymes in Muscle from Patients with Duchenne Muscular Dystrophy, 1107
- Shepherd, Dr. Audrey M., and Clarke, Dr. A. J. : Zinc and Other Metallic Ions as Hatching Agents for the Beet Cyst Nematode, *Heterodera schachtii* Schm., 502
- Shepherd, Mr. J. A. : Spencer Wells, the Life and Work of a Victorian Surgeon, review by Sir Arthur MacNalty, 1135
- Shepley, Kate, Ambrose, Dr. E. J., and Kirby, Dr. K. S. : Alteration of Hamster Cells by Nucleic Acid *in vitro*, 1072
- Sheppard, jun., H., and Smith, Prof. E. D. : Quantitative Gas Chromatography of Amino-acids as Trimethylsilyl Derivatives, 878
- Sheppard, Prof. P. M., et al. : 'Pink Spot' in the Urine of Schizophrenics, 453
- Sherbet, Dr. G. V., et al. : Biological Systems at the Molecular Level, 1048
- Sherby, Prof. O. D., et al. : Dispersion-softened Zinc Alloys, 579
- Sherman, Prof. I. W., Mudd, J. B., and Trager, Prof. W. : Chloroquine Resistance and the Nature of Malarial Pigment, 691
- Shewell, Jennifer, and Wright, Dr. E. A. : Modification of Radiation 'Cerebral Death' by Hypoxia, 904
- Shifrine, Dr. M., and Gourlay, R. N. : Serological Relationship between Galactans from Normal Bovine Lung and from *Mycoplasma mycoides*, 498
- Shift and Internal Stress in Gamma-ray Irradiated Sodium Chlorate : Pure Quadrupole Resonance Frequency (Dr. H. Budak), 382
- Shift at Low Elevations : Effects of Pronounced Electron-content Variations on Doppler (H. Sorcher), 644
- Shift Working : The Arrangement of Hours on Night Work (Mrs. G. de la Mare and Dr. J. Walker), 1127
- Shih, Ling-yu, et al. : Characterization of Glucose-6-phosphate Dehydrogenase among Chinese, 1323
- Shikata, E., and Maramorosch, Dr. K. : Plant Tumour Virus in Arthropod Host : Microcrystal Formation, 507
- Shock-heated Potassium Vapour : Ionization Times in (R. M. Hill and B. Capp), 176
- Shock Waves in a Magnetized Plasma : Experimental Observations on the Structure of Collisionless (Dr. J. W. M. Paul et al.), 133
- Shope, R. E., and Toda, A. : Transmission of Guam and Orinoco Viruses by Naturally Infected Mosquitoes, 304
- Shriner, R. L., Fuson, R. C., and Curtin, D. Y. : Systematic Identification of Organic Compounds, Fifth Edition, review by Dr. T. Lewis, 1032
- Shrinking Aggregates as a Cause of Failure in Concrete, 1261
- Shulman, Dr. A., Laycock, G. M., and Henry, J. A. : Titration Curves depicting Central Stimulant, Depressant and Dual Stimulant-depressant Action of Glucaramide Homologues in Mice, 568
- Shurmer, C. R., and Williams, T. R. G. : Effect of Temperature on the Discontinuity in the S/N Fatigue Curve of a Stainless Steel, 379
- Shy, Prof. G. Milton, and Gonatas, Dr. N. K. : Virus-like Particles in Sub-acute Sclerosing Encephalitis, 1338
- Shyne, Prof. J. C., et al. : Dispersion-softened Zinc Alloys, 579
- Sialic Acid in Potassium Transport of L 1210 Leukaemia Cells : Role of (Dr. J. L. Glick and Dr. S. Githens III), 88
- Siddall, Dr. T. H., and Prohaska, Dr. C. A. : Slow Inversion at the Nitrogen Atom in Certain Amides, 582
- Sieburth, Prof. J. McN., and Conover, Prof. J. T. : *Sargassum* Tannin, an Antibiotic which retards Fouling, 52
- Siegel, Dr. S. M., Daly, O., and Giumarro, C. : Experimentation with Plants at Sub-atmospheric Oxygen-levels : Effects of Oxygen Pressure and Salts on Germination of Winter Rye, 1012

- Siegfried, Dr. Andre : Germs and Ideas : Routes of Epidemics and Ideologies, review by F. Hawking, 1249
- Signal Value of the Genital Tassel in the Male *Tilapia macrochir* Blgr. (Pisces : Cichlidae) (Dr. W. W. Wickler), 595
- Signals : Frequency Spectrum of Doppler-shifted Whistler-mode (R. A. Morris and N. Poletti), 479
- Sikov, Prof. M. R. : Conversion of the Inconvertible L-I Lymphosarcoma to Ascitic Form, 902
- Silica Powders, as revealed by Deuterium Oxide Exchange : An Infra-red Investigation of the Effect of Pressure on (F. H. Hambleton et al.), 138
- Silica-gel 'Aerosil' : Infra-red Spectra of Quaternary Ammonium Compounds adsorbed on (Dr. L. C. F. Blackman and R. Harrop), 777
- Silicon in Modified Aluminium-Silicon Eutectic : Interconnexion of (J. A. E. Bell and Prof. W. C. Winegard), 177
- Silicon Rubber Implant : Prolonged Administration of Atropine or Histamine in a (P. Bass et al.), 591
- Silicon to 60 kilobars : Fusion Curves of Zinc, Aluminium, Germanium and (J. Lees and B. H. J. Williamson), 278
- Silva, D. G., and Wilson, Dr. A. S. : Ultrastructure of the Phrenic Nerve, 707
- Sim, Dr. A. K., and Coulson, Dr. C. B. : Proteins of Wheat Flour, 583
- Simian Cell Cultures : Growth and Cytopathogenicity of H-viruses in Human and (Dr. H. Toolan and Dr. N. Lednicki), 812
- Similarities between the Cytoplasmic Organelles of Melanocytes and the Mitochondria of Hepatocytes : Functional (Dr. M. H. Van Woert et al.), 810
- Similarities in One Dimension : Relative (Prof. J. W. Carmichael et al.), 544
- Simmonds, Mr. N. W. : Appointment as Director of the Scottish Plant Breeding Station, 228
- Simmons, Dr. R. L., and Russell, P. S. : Histocompatibility Antigens in Transplanted Mouse Eggs, 698
- Simmons, R. L., and Weintraub, J. : Transplantation Experiments on Placental Ageing, 82
- Simon, Brian : Education and the Labour Movement 1870-1920, review by Prof. W. H. G. Armytage, 960
- Simplified Technique for Preparative Disc Electrophoresis (Prof. D. Sulitzanu and W. F. Goldman), 1120
- Simulium among Stream Fauna : Selective Effect of Particulate Insecticides on (Prof. W. E. Kershaw et al.), 199
- Simultaneous Oxidation and Reduction of Diphenyl Picrylhydrazyl on Surfaces : Evidence for Catalytic Activity of F-centres (Prof. J. G. Aston, Dr. D. N. Misra and K. M. Cresswell), 181
- Sinclair, Dr. N. R. : Time-course Studies on Antibody Response in Thymectomized and Sham-thymectomized Mice, 1104
- Singer, M., and Schade, J. P. (edited by) : Mechanisms of Neural Regeneration review by Prof. K. Krnjević, 1248
- Singing of Birds : Origin and Significance of the Power of Vocal Imitation : with Special Reference to the Antiphonal (Dr. W. H. Thorpe and M. E. V. North), 219
- Sinnatt, Dr. O. S. : *Obituary* by Group Capt. C. E. P. Suttle, 227
- Size Analysis in the Sub-sieve Range by Electronic Counter (Prof. C. C. Harris and Dr. A. Jowett), 175
- Size : International Paper, 528
- Size Measurement : Particle, 529
- Size of the Corpus Allatum in a Polymorphic Insect : Changes in (D. White), 807
- Skates : Electron Microscope Observations of Neurosecretory Elements in the Neurointermediate Lobe in (Dr. A. L. Polenov and M. A. Belenky), 94
- Skeen, J. B., and Sumner, Dr. M. E. : Measurement of Exchangeable Aluminium in Acid Soils, 712
- Skeletal Muscle : Enzymatic Adaptation to Contractile Activity in (J. Kendrick-Jones and Prof. S. V. Perry), 1068
- Skin Boats of North America : Bark Canoes and, 22
- Skin *in vitro* : Effects of Cyclic Adenosine Monophosphate and Melanocyte-stimulating Hormone on Frog (Dr. M. W. Bitensky and S. R. Burstein), 1282
- Skin-lightening Agent, Melatonin, in Amphibians : Enzymatic Synthesis of the (Dr. J. Axelrod et al.), 386
- Skipwith, Sir Patrick A. d'E., and Shearman, D. J. : Organic Matter in Recent and Ancient Limestones and its Role in their Diagenesis, 1310
- Skull recovered from a Late Glacial Deposit near Lake Simcoe, Ontario : A Well-preserved Grizzly Bear (R. L. Peterson), 1233
- Sky Brightness at 141 Mc/s (K. W. Yates and Dr. R. Wielebiński), 64
- Slee, O. B., et al. : New Limits to the Angular Sizes of Some Quasars, 275
- Sleeping Sickness Trypanosomes : Polymorphism and Mitochondrial Activity in (Dr. K. Vickerman), 762
- Slifkin, Dr. M. A. : Correlation of the Third Excited State of Polycyclic Aromatic Hydrocarbons with the Ionization Potential, 380
- Slow Alpha-Globulin : Involvement of the Adrenal Cortex in the Appearance of Rat (Prof. W. G. Heim and S. R. Ellenson), 1330
- Slow-bend Testing of Hydrated Zirconium (C. E. Coleman and Dr. D. Hardie), 69
- Slow Potential Changes in the Unanaesthetized Cerebral Cortex of the Cat : Correlation between Unit Activity and (A. D. J. Robertson), 757
- Small, Myra, et al. : A Simple Bioassay for Antioxidants based on Protection of *Tetrahymena pyriformis* from the Photodynamic Toxicity of Benzo(a)pyrene, 655
- Small Diameter Radio Sources : Scintillations on the (Prof. M. H. Cohen), 277
- Smalley, Captain V. G., and Ronca, L. B. : Structures inside a Lunar Crater photographed by *Ranger VII*, 930
- Smets, H. B., and Satkowski, J. A. (edited by) : Magnetohydrodynamic Electrical Power Generation, Symposium 1964, Four Volumes, review by Prof. M. W. Thring, 966
- Smith, Dr. A. M. : Experimental Pedology; Proceedings of 11th Easter School in Agricultural Science, 1964, review, 718
- Smith, Dr. B. C., and Thakur, L. : Boron-Nitrogen Bond Strengths in Borazoles, 74
- Smith, David E., and Hazard, J. B. (edited by) : The Thyroid, review by Dr. E. E. Pochlin, 979
- Smith, Derek A. : Absence of Diffusion Control of Reaction Rate in the Thermal Decomposition of Amorphous Polymers, 1200
- Smith, Prof. Edgar D., and Sheppard, Jun. H. : Quantitative Gas Chromatography of Amino-acids as Trimethylsilyl Derivatives, 878
- Smith, H., et al. : Crystalline Modifications of Ampicillin, 999
- Smith, Dr. J. A. B. : Cheese, Two Volumes, review, 975
- Smith, J. S., et al. : Milker's Nodule Virus Infections in Dorset and their Similarity to Orf, 505
- Smith, Dr. J. T., et al. : Interaction of Cephaloridine with Penicillinase-producing Gram-negative Bacteria, 235
- Smith, Dr. James W., and Frame, Dr. J. : Electron Microscope Observations on the Free Protein and the Protein-polysaccharide Complex of Bovine Synovial Fluid, 867
- Smith, P. E., et al. : Acid Phosphatases, 382
- Smith, R., and Howe, J. P. (edited by) : Beryllium Oxide, review by Dr. M. F. Lappert, 9
- Smith, R. L., et al. : Thalidomide as a Possible Biological Acylating Agent, 1208
- Smith-Rose, Dr. R. L. : U.S. Standard Frequency and Time Services, 25
- Smithson, James : Pathfinder in Science and Philanthropy (Dr. L. Carmichael), 321
- Smithsonian Bicentennial Celebrations : Scholarly Sessions at the, 324
- Smithsonian Institution, Washington, D.C., 320
- Smokers' Urine by Gas Chromatography : Determination of Nicotine In (Dr. N. L. McNiven et al.), 788
- Smooth Muscle Preparations : Effect of Gastrin on Isolated (Dr. A. Bennett), 170
- Snail *Otala (Helix) lactea* : a Specific Agglutinin in the (Prof. W. C. Boyd and R. Brown), 593
- Sneath, Dr. P. H. A., et al. : A Suggested Phenotypic Classification and Terminology for Enzyme Mutants in Micro-organisms, 841
- Snodgrass, J. M., et al. : Measurements of Spontaneous Bioluminescence in the Sea, 1078
- Social and Industrial Gerontology in Great Britain (C. E. Fleming), 623
- Social Companions and the Mother-Infant Relationship in Rhesus Monkeys, (Y. Spencer-Booth, Prof. R. A. Hinde and M. Bruce), 301. Addendum to Fig. 1, 836
- Social Science Research Council, Appointments, 629
- Society : Ecology and the Industrial (edited by G. T. Goodman et al.), review by Dr. A. Macfadyen, 618
- Society : Science, Faith and (Prof. M. Polanyi), review, 985
- Sodium Alginate in Inhibiting Uptake of Radiostrophium by the Human Body : Effect of (R. Hesp and B. Ramsbottom), 1341
- Sodium Alkyl Sulphates in Water : Rate of Micelle Formation of (P. F. Mijnliff and R. Dismarsch), 889
- Sodium Atom Reaction Rates : Correlation of, with Electron Capture Cross-sections (K. R. Wilson and Prof. D. R. Herschbach), 182
- Sodium Availability and Sodium Selection by Microtine Rodents : Relation of Population Density to (G. D. Aumann and Prof. J. T. Emlen), 198
- Sodium Butyrate by Intracellular Plasma : Mechanism of Intestinal Absorption : Transport of Methionine and (S. L. Hart and Dr. J. A. Nissim), 145
- Sodium Chlorate : Pure Quadrupole Resonance Frequency Shift and Internal Stress in Gamma-ray Irradiated (Dr. H. Budak), 382
- Sodium Chloride : Direct Ultramicroscopic Observation of Thermal Scattering in (Dr. G. Heilmann), 184
- Sodium Pump : Molecular Model for a (Dr. L. J. Opit and Dr. J. S. Charnock), 471
- Sodium Tetrathionate Dihydrate : Radiolysis of (Prof. R. L. Eager and Dr. D. S. Mahadevappa), 781
- Soicher, H. : Effects of Pronounced Electron-content Variations on Doppler Shift at Low Elevations, 644
- Soil : Effect of pH and Addition of Organic Materials on Denitrification Losses from (Dr. M. Ekpete and Dr. A. H. Cornfield), 1200
- Soil : Ingestion of, by Sheep in New Zealand in Relation to Wear of Teeth (W. B. Healy and T. G. Ludwig), 806
- Soil and Landscape at Archaeological Sites, 530
- Soil Catenas, 123
- Soil during Decomposition of Carbohydrates : Fixation of Metabolic Products in the (H. Sørensen), 97
- Soil Fabric Patterns : Photographic Reproduction of (D. Lafeber and M. Kurbanovic), 609
- Soil Micro-fauna, 435
- Soil Science : Experimental Pedology (edited by E. G. Hallsworth and D. V. Crawford), review by Dr. A. M. Smith, 718
- Soil Survey of Great Britain, 123
- Soils : Measurement of Exchangeable Aluminium in Acid (J. B. Skeen and Dr. M. E. Sumner), 712
- Organo-mineral Complex, n, 734
- Thermister Hygrometer for determining the Free Energy of Moisture in Unsaturated (B. G. Richards), 608
- Uptake by Plants of Radiostrophium from Contaminated (A. J. Andersen), 195
- Soils and Foundations, 1153
- Soils of the Mendip District of Somerset, 328
- Sokolovskaya, A. V., et al. : Initiation of Chain Reaction under an Ultrasonic Wave Effect, 945
- Solanum : Double Chromosome Reduction in a Tetraploid (G. E. Marks et al.), 359
- Solar Motion and Sunspot Comparison (Dr. R. M. Wood and Prof. K. D. Wood), 129
- Solar Nebula : Enrichment of Iron during Accretion in the (Prof. S. R. Taylor), 886
- Solar Radiation Measurements : Stratospheric Transport of Volcanic Dust inferred from (Dr. A. J. Dyer and B. B. Hicks), 131
- Solar Radio Emission at Centimetre and Decimetre Wavelengths : Basic Component of (Dr. M. K. Das Gupta and D. Basu), 739
- Solid Circuits and Microminiaturization (edited by G. W. A. Dummer) : Proceedings of Conference 1963, review by Dr. J. R. Tillman, 1249
- Solid State Physics : Advances in Research and Applications, Vol. 15 (edited by F. Seitz and D. Turnbull) review by Prof. C. W. McCombie, 4
- Solids : Evidence of Thermal and Hot Processes in the 'Hot-zone' produced by Copper-64 Recoil Atoms in (K. Yoshihara and H. Ebihara), 482
- Sollberger, Prof. A. : Biological Rhythm Research, review by Dr. A. D. Lees, 973
- Soltész, M. B., et al. : Vital Staining of the Granulated Juxtaglomerular Cells in the Mouse Kidney, 1331
- Soluble Lens Proteins in Galactose-fed Rats : Agar-gel Electrophoresis of (E. Wittgenstein and Dr. K. W. Rowe, jun.), 386
- Solution : Nomadic Behaviour of Yttrium Ions and their Structure in (R. C. Vickery), 678
- Solutions at pH 8.7 : Acrylamide-gel Electrophoresis of β -Lactoglobulins stored in (Dr. P. Akroyd), 488
- Solutions : Electric Potentials generated by Freezing Dilute Aqueous (H. C. Parreira and A. J. Eyd), 33
- Solway Firth Barrage, 629
- Somerset : Soils of the Mendip District of, 328
- Somesthetic and Visual Responses of Superior Colliculus Neurons (Dr. D. Jassik-Gerschenfeld), 898
- Sørensen, Henning : Fixation of Metabolic Products in the Soil during Decomposition of Carbohydrates, 97
- Sorex araneus (L.) : Seasonal Changes in the Histological Structure of the Spinal Column of (L. Saure and H. Hyvärinen), 705
- Source 3C 273B : Structure of the Quasi-stellar Radio (M. J. Rees and Dr. D. W. Sciama), 371
- Source W49 : OH Emission in the Direction of Radio (B. Zuckerman et al.), 441
- Source of Potential Immunologically Competent Cells in the Adult Mouse : Bone Marrow as the Major (Dr. M. L. Tian and Dr. L. J. Cole), 1223
- South African Prehistory, 22
- South Australian Museum, Annual Report, 1964, 1046
- South-East Asia Treaty Organization : Research Fellowships, 1263
- Southard, Janice L., Wolfe, H. G., and Russell, Dr. E. S. : Artificial Insemination of Dystrophic Mice with Mixtures of Spermatozoa, 1126
- Southern, H. N. : Physiological Mammalogy, Volumes 1 and 2, review, 1136
- Southern Rhodesia : National Museums of, 122
- South-west England : Recent Finds in the Upper Carboniferous of, and their Significance (Dr. H. G. Reading) ; (Dr. J. E. Prentice), 745
- Space Science : United Nations Review of, 21
- Spain : Meeting of Biochemists in (Dr. J. R. Villanueva), 632

- Spalding, Dr. J. F., Johnson, O. S., and Archuleta, R. F.: Acute Radio-sensitivity as a Function of Age in Mice, 905
- Spärck, Prof. R.: *Obituary* by Dr. T. Wolff, 625
- Speakman, F. P., and Makin, Dr. B.: Advances in Liquid Chromatography, 1087
- Special Theory of Relativity (Prof. David Bohm), review by Prof. C. W. Kilmister, 986
- Species of *Cissus*: Development of the Ovary in a (N. C. Nair), 402
- Species of Mouse Interferon induced by Statolon: Different Molecular (Prof. T. C. Merigan and Dr. W. J. Kleinschmidt), 567
- Specific Agglutinin in the Snail *Otala (Helix) lactea* (Prof. W. C. Boyd and R. Brown), 593
- Specimens: Simple Technique for embedding and supporting Delicate Biological (R. H. Harris), 199
- Spectra of Aromatic Nitrenes and Dinitrenes: Ultra-violet Absorption (A. Reiser and V. Frazer), 682
- Spectra of Minerals: Infra-red Emission (Prof. M. J. D. Low), 1089
- Spectra of Polar Liquids: Sub-millimetre Wave (Dr. G. W. Chantry and Dr. H. A. Gebbie), 378
- Spectra of Quaternary Ammonium Compounds adsorbed on Silica-gel 'Aerosil': Infra-red (Dr. L. C. F. Blackman and R. Harrop), 777
- Spectra of Some Square-planar Ions: Far-infra-red (Dr. D. M. Adams and D. M. Morris), 283
- Spectral Density Functions of Hydromagnetic Emissions at High Latitude (Dr. A. Egeland), 539
- Spectrometric Fragmentation of Nitrophenylhydrazones: Mass (Prof. C. Djerassi and S. D. Sample), 1314
- Spectroscopic and X-ray Techniques: Structure of Lipids by (Dr. D. Chapman), review by Dr. F. D. Gunstone, 1246
- Spectroscopy and Molecular Structure (Dr. G. W. King), review by Dr. R. F. Barrow, 214
- Spectroscopy: Flame (Dr. R. Mavrodineanu and Dr. H. Boiteux), review by Dr. D. T. Lewis, 5
- Spectroscopy in Australia (Dr. G. C. Barraclough, Dr. P. J. Black, D. J. David, Dr. A. D. E. Pullin and J. S. Shannon), 125
- Spectroscopy in the Analysis of Chromatograms: Ultra-violet (Dr. D. M. Abelson), 784
- Spectroscopy to determine the Orientation of Pyridine sorbed on Montmorillonite: Use of Infra-red (J. M. Serratos), 679
- Spectrum of Cu^{++} in Diamine Oxidase: Electron Spin Resonance (Prof. A. van Heuvelen), 888
- Spectrum of the CH_3O -radical: Electronic Absorption (J. F. Ogilvie), 1315
- Spectrum of Doppler-shifted Whistler-mode Signals: Frequency (R. A. Morris and N. Poletti), 479
- Speeton Clay: Holothurian Sclerites from the (B. N. Fletcher), 281
- Spencer-Booth, Y., Hinde, Prof. R. A., and Bruce, M.: Social Companions and the Mother-Infant Relationship in Rhesus Monkeys, 301
- Spencer Wells, the Life and Work of a Victorian Surgeon (Mr. J. A. Shepherd), review by Sir Arthur MacNalty, 1135
- Spermatozoa: Artificial Insemination of Dystrophic Mice with Mixtures of (Dr. J. L. Southard *et al.*), 1126
- Spermatozoa of a Metabolite of the Female Reproductive Tract: Oxidative and Biosynthetic Utilization by Human (Prof. C. Terner), 1115
- Spermatozoa Pairing in the Epididymis of American Forms: Marsupial (Dr. J. D. Biggers and Dr. E. D. Delamater), 402
- Spermine: Apparent Suppression of Mutation Rates in Bacteria by (Dr. H. G. Johnson and Dr. M. K. Bach), 408
- Spermine-Inactivation of Plant Viruses: Antiviral Action of Oxidized (U. Bachrach *et al.*), 1095
- Spheres: A Numerical Model of Random Packing of (Dr. M. M. Levine and J. Chernick), 68
- Spheres with Five-fold Symmetry: Dense Packing of Hard (B. G. Bagley), 674
- Spiers, Dr. A. S. D., and Baikie, Dr. A. G.: Chronic Granulocytic Leukaemia: Demonstration of the Philadelphia Chromosome in Cultures of Spleen Cells, 497
- Spinal Column of *Sorex araneus* (L.): Seasonal Changes in the Histological Structure of the (L. Saure and H. Hyvärinen), 705
- Spink, P., *et al.*: A Screening Test for Substances inhibiting the Cancer Coagulative Factor, 1008
- Spiral Etch Pits in Ice Crystals: Pairs of (J. Muguruma), 180
- Spiral Galaxies: Radio Observations of (Prof. B. Y. Mills and J. R. Glanfield), 10
- Spiral Structure of Human Chromosomes: Demonstration of the (Dr. Y. Ohnuki), 916
- Spleen Cells: Demonstration of the Philadelphia Chromosome in Cultures of (Dr. A. S. D. Spiers and Dr. A. G. Baikie), 497
- Spleen: Effect of Altered Perfusion Rates on the Retention of Noradrenaline by the (Dr. D. M. Paton and Prof. C. N. Gillis), 391
- Splenomegaly Systems of Graft-versus-host Assay in the Chick Embryo: Comparison of the Chorioallantoic Membrane and (L. W. Copleston and Dr. D. Michle), 53
- Spontaneous Bioluminescence in the Sea: Measurements of (Dr. B. P. Boden *et al.*), 1078
- Spontaneous Murine Tumours by the M-P Virus: Inhibition of Transplantable and (Dr. N. Molomut and Dr. M. Padnos), 948
- Spore Discharge in *Leptothorax konradi* (Dr. M. H. Zoberi), 405
- Spores of *Bacillus cereus* Strain T: Timing of Protein Synthesis during Germination and Outgrowth of (W. Steinberg *et al.*), 710
- Spores of *Penicillium roqueforti*: Activation of (R. C. Lawrence), 801
- Spotorno, G., *et al.*: Lysozymes characterized in Duck Egg-white: Isolation of a Histidine-less Lysozyme, 1204
- Sprayberry, Dr. B. A., Hall, Prof. W. C., and Miller, Dr. C. S.: Biogenesis of Ethylene in *Penicillium digitatum*, 1322
- Springell, P. H., and Leach, S. J.: Cysteine Content of Cattle Hair, 1326
- Square-planar Ions: Far-infra-red Spectra of some (Dr. D. M. Adams and D. M. Morris), 283
- Squid Giant Axon: Repetitive Responses to Alternating Current Stimulation (Prof. H. R. Hirsch), 1218
- Srinivasan, Prof. P. R., *et al.*: Changes in the t-RNA Methylating Enzymes during Insect Metamorphosis, 555
- Stability of Rotating Massive Stars in General Relativity (B. Durney and I. W. Roxburgh), 1304
- Stable Messenger RNA in Nucleated Erythrocytes (Dr. R. B. Scott and R. A. Malt), 497
- Stacey, Prof. M.: Methods in Carbohydrate Chemistry, Volumes 4 and 5, review, 971
- Oligosaccharides, review, 971
- Stahlmann, C., *et al.*: Effect of Trans-Solutes on the Fluxes of Chloride Ions across Artificial Membranes, 187
- Staining: Mixed Antiglobulin Immunofluorescence or Labelled Antigen Indirect Immunofluorescence (Prof. E. H. Beutner *et al.*), 353
- Staining of the Granulated Juxtaglomerular Cells in the Mouse Kidney: Vital (V. Szokoly, Sz. Gomba and M. B. Soltész), 1331
- Stainless Steel: Effect of Temperature on the Discontinuity in the S/N Fatigue Curve of a (T. R. G. Williams and C. R. Shurmer), 379
- Stainless Steel: Irradiation Embrittlement of Low-boron Type 304 (W. R. Martin *et al.*), 73
- Stains for Histological Diagnosis of Visceral Mycoses: Fluorochrome (G. Bader *et al.*), 796
- Stamp, Sir Dudley: Man's Place in the Island Ecosystem, A Symposium, review, 109
- Standard Frequency and Time Services: U.S. (Dr. R. L. Smith-Rose), 25
- Standards Division, National Physical Laboratory, Teddington, Retirement of Superintendent, 118
- Standards: U.S. National Bureau of, 119
- Standish, Dr. M. M., *et al.*: Cation Permeability of Phospholipid Model Membranes: Effect of Narcotics, 1295
- Stangroom, J. E., *et al.*: Nature of the Non-haem Iron in Ferredoxin and Rubredoxin, 769
- Staphylococcal Penicillinase: Relative Instability of Cephaloridine to (Dr. M. Ridley and Dr. I. Phillips), 1076
- Stark, Dr. L., *et al.*: Suppression of the Pupillary Light Reflex in Binocular Rivalry and Saccadic Suppression, 558
- Stark, W., and Forss, D. A.: n-Alkan-1-ols in Butter after Oxidative Deterioration, 190
- Stars in General Relativity: Stability of Rotating Massive (B. Durney and I. W. Roxburgh), 1304
- Starvation and Protein Depletion on Mercury Retention in Two Strains of Chickens: Effects of (D. V. Larkin, V. L. Miller, G. E. Bearse and Dr. C. M. Hamilton), 706
- Starvation in Man: Plasma Growth-hormone Levels in Chronic (Dr. V. Marks *et al.*), 686
- Starving Bacteria: Metabolism of Nucleic Acids and Protein In (B. Brdar *et al.*), 303
- State of Liquid-vapour Equilibrium with the same Constants for Both Phases: Equation of (Dr. I. Kuhn), 1315
- Statistical Inference (Prof. J. C. R. Li), Two Volumes, review by Prof. D. V. Lindley, 420
- Statistics and Experimental Design in Engineering and the Physical Sciences, Two Volumes (Prof. N. L. Johnson and Prof. F. C. Leone), review by N. E. Day, 214
- Statistics, Probability and (Prof. H. Freudenthal), review by Prof. D. J. Finney, 217
- Statolon: Different Molecular Species of Mouse Interferon induced by (Prof. T. C. Merigan and Dr. W. J. Kleinschmidt), 667
- Stäubli, Dr. W., and Okada, Dr. T.: Interferometric Estimation of Effects of Testosterone and Methandrostenolone on the Dry Mass of Various Rat Muscles, 562
- Stäubli, Dr. W., *et al.*: Nature of the Hepatomegalic Effect produced by Ethyl-chlorophenoxisobutyrate in the Rat, 856
- Staudinger, Prof. Hermann: *Obituary* by Prof. C. E. H. Bawn, 626
- Steady State: A Search for Mutation in Chemical Systems at (Dr. G. Allen), 258
- Steel, K. J. (the late), and Cowan, S. T.: Manual for the Identification of Medical Bacteria, review by Dr. D. Allan, 213
- Steel: Effect of Temperature on the Discontinuity in the S/N Fatigue Curve of a Stainless (T. R. G. Williams and C. R. Shurmer), 379
- Steel: Irradiation Embrittlement of Low-boron Type 304 Stainless (W. R. Martin *et al.*), 73
- Steele, J. W., *et al.*: Pectic Polysaccharides in the Growth of Plant Cells: Molecular Structural Factors and their Role in the Germination of White Mustard, 876
- Steels: Mechanism of Oxidation of Chromium Diffusion Coatings on Plain Carbon (Dr. I. A. Menzies and D. Mortimer), 1309
- Steenfot Nielsen, Mrs. M.: Introduction to the Flowering Plants of West Africa, review by F. N. Hepper, 976
- Stehbens, Dr. W. E., and Biscoe, Dr. T. J.: Electron Microscopic Observations on the Carotid Body, 708
- Stein, R. B., and Matthews, Dr. P. B. C.: Differences in Variability of Discharge Frequency between Primary and Secondary Muscle Spindle Afferent Endings of the Cat, 1217
- Steinberg, W., Halvorson, Prof. H. O., Keynan, A., and Weinberg, E.: Timing of Protein Synthesis during Germination and Outgrowth of Spores of *Bacillus cereus* Strain T, 710
- Steiner, Dr. H.: Oxidation of Hydrocarbons in the Liquid Phase, review, 420
- Steinfink, Prof. H., *et al.*: Structure of Calcium Oxalate Monohydrate, 1197
- Steinglass, M., *et al.*: Subcutaneous Sarcomata in Hamsters Induced by Rous Sarcoma Virus (Bryan), 336
- Stenhouse, Dr. D.: A General Theory for the Evolution of Intelligent Behaviour, 815
- Steppes: The Royal Hordes, Nomad Peoples of the (E. D. Phillips), review by M. C. Burkitt, 1138
- Sterility induced by Deuterium Oxide in Mice: Histological Investigations of the Mechanism of (A. M. Hughes and Dr. L. E. Glass), 1119
- Sterilization and Disinfection as Applied to Medical, Industrial and Laboratory Practice: A Review of (Prof. S. D. Rubbo and Dr. J. F. Gardner), review by R. Hare, 1249
- Stern, Dr. H.: Rubella Virus Complement-fixation Test, 200
- Steroid Complexes with p-Bromophenol: Androgenic (Dr. C. Eger and Dr. D. A. Norton), 997
- Steroids and 'Triton X-100': Effect of, on Glucose-filled Phospholipid/Cholesterol Structures (Dr. G. Weissmann, Dr. G. Sessa and Dr. S. Weissmann), 649
- Sterzl, Dr. J., and Riha, Dr. I.; Dresser, Dr. D. W., and Wortis, Dr. H. H.: A Localized Haemolysis in Gel Method for the Detection of Cells producing 7S Antibody, 858
- Stevens, Dr. C. B., *et al.*: Concentration Effects in Chromatographic Analysis, 269
- Stevens, Dr. R., and Pollock, Dr. J. R. A. (edited by): Dictionary of Organic Compounds, Fourth Edition, Five Volumes and Supplement, review by Dr. F. G. Mann, 417
- Steward, Prof. F. C., *et al.*: Direct and Indirect Effects of Radiation on Plant Cells: their Relation to Growth and Growth Induction, 850
- Stewart, Ann H., *et al.*: Alcohol and Evoked Potentials in the Cat, 688
- Stewart, Dr. D. J.: Formation of Protocatechuate from Quinic Acid by Fluorescent Pseudomonads, 200
- Stewart, Prof. F. H.: *Obituary* of Prof. Arthur Holmes, 525
- Stewart-Tull, D. E. S.: Occurrence of Dimorphic Forms of *Mycobacterium phlei*, 603
- Stewartson, Prof. K.: Research Frontiers in Fluid Dynamics, review, 967
- Stiller, D., *et al.*: Fluorochrome Stains for Histological Diagnosis of Visceral Mycoses, 796
- Stimulant, Depressant and Dual Stimulant-depressant Action of Glutaramide Homologues in Mice: Titration Curves depicting Central (Dr. A. Shulman *et al.*), 568
- Stimulation of Sendai Virus Multiplication by Puromycin and Actinomycin D (Dr. D. O. White and I. M. Cheyne), 813

- Stimulation of Synthesis of Ribonucleic Acid in Sub-apical Sections of *Avena* Coleoptile by Indolyl-3-acetic Acid (Prof. T. H. Hamilton, R. J. Moore, A. F. Rumsey, A. R. Means and Prof. A. R. Schrank), 1180
- Stimulant Properties of Compounds with Peripheral Muscarinic Properties: Central (G. B. Leslie), 1291
- Stimulation: Squid Giant Axon: Repetitive Responses to Alternating Current (Prof. H. R. Hirsch), 1218
- Stimuli in Intracranial Self-stimulation: Facilitation as a Function of Temporal Spacing of (Prof. J. A. Deutsch and R. Collins), 592
- Stinebring, Prof. W. R., and Youngner, Prof. J. S.: Interferon Appearance Stimulated by Endotoxin, Bacteria, or Viruses in Mice pretreated with *Escherichia coli* Endotoxin or infected with *Mycobacterium tuberculosis*, 456
- Stoerwald, G. S., et al.: Human Indigenous Microflora in Gnotobiotic Rats, 1236
- Stoichiometry of Proton Translocation through the Respiratory Chain and Adenosine Triphosphatase Systems of Rat Liver Mitochondria (Dr. P. Mitchell and Dr. J. Moyle), 147
- Stoller, A., and Collmann, R. D.: Virus Aetiology for Down's Syndrome (Mongolism), 903
- Storage of Books, 434
- Stored-energy Release at 200°C in Electron-irradiated Graphite: Mechanism of (Prof. E. W. J. Mitchell and M. R. Taylor), 638
- Stoss, Dr. B., and Herrmann, Dr. R.: Immunogenic Activity of Lipids of *M. tuberculosis*, 1224
- Stoydin, G., and Beydon, Dr. K. I.: Application of an Agar-Agar Diffusion Procedure to Pesticide Residue Analysis and to the Cholinesterase Screening of Candidate Pesticides, 748
- Strand Separations within Deoxyribonucleic Acid during Selective Transcription: Localized (Prof. J. H. Frentzen), 894
- Strasburger's Textbook of Botany (rewritten by R. Harder, W. Schumacher, F. Firbas and D. Von Denffer), New English Edition from 28th German Edition, review by Dr. A. Allsopp, 618
- Strata, P., et al.: Evidence for Pre-synaptic Inhibition in the Lateral Geniculate Body, 495
- Strathclyde, University of, Chair of Pharmaceutical Technology, 934
- Stratigraphy and Structure of the Eastern Aden Protectorate (Dr. Z. R. Beydoun), review by F. E. Wellings, 615
- Stratigraphy: Lower Palaeozoic, Les Temps Fossillifères (Prof. H. Termier and G. Termier), Vol. 1, review by Prof. A. Williams, 614
- Stratospheric Transport of Volcanic Dust inferred from Solar Radiation Measurements (Dr. A. J. Dyer and B. B. Hicks), 131
- Stream Fauna: Selective Effect of Particulate Insecticides on *Simulium* among (Prof. W. E. Kershaw et al.), 199
- Strebel, Dr. R., et al.: Epileptogenic Effect of Extradural and Intracranial Cobalt, 792
- Strengths in Borazoles: Boron-Nitrogen Bond (Dr. B. C. Smith and L. Thakur), 74
- Stress in Gamma-ray Irradiated Sodium Chlorate: Pure Quadrupole Resonance Frequency Shift and Internal (Dr. H. Budak), 382
- Stress: Thermal (edited by Dr. P. P. Benham and Dr. R. Hoyle), review by Dr. E. H. Andrews, 517
- Striations in the Plasma of the Positive Column on some External and Internal Parameters: Dependence of the Properties of Moving (G. Lakatos and Dr. J. Bitó), 445
- Strickholm, Dr. A., and Wallin, B. G.: Intracellular Chloride Activity of Crayfish Giant Axons, 790
- Stroeva, O. G., and Lopashov, G. V.: Development of the Eye, review by Dr. R. A. Weale, 979
- Strogonov, Dr. B. P.: Physiological Basis of Salt Tolerance in Plants, review by Dr. P. A. Thompson, 211
- Stroke, Prof. G. W., et al.: Reconstruction of Phase Objects by Holography, 1159
- Strontium-90 and Caesium-137 in Columbia River Plume, July 1964 (Prof. K. Park, M. J. George, Y. Miyake, K. Saruhashi, Y. Katsuragi and T. Kanazawa), 1084
- Strontium and Calcium: Effects of Dietary Levels of Phosphorus and Calcium on the Comparative Behaviour of (K. Kostial et al.), 1110
- Structural Basis of Neutron and Proton Magic Numbers in Atomic Nuclei (Prof. L. Pauling), 174
- 'Structural' Locus determining Human Phosphoglucomutase: Evidence for a Second (Dr. D. A. Hopkinson and Prof. H. Harris), 410
- Structure and Activity of Enzymes, Symposium 1964 (edited by T. W. Goodwin, J. I. Harris and B. S. Hartley), review by Dr. D. T. Elmore, 972
- Structure and Biological Activity using Thin-layer Chromatography: Simple Assessment of Partition Data for correlating (Dr. C. B. C. Boyce and Dr. B. V. Milborrow), 537
- Structure Antigénique de Tumeurs Expérimentales (Dr. C. Deckers), review by R. R. Dourmashkin, 1140
- Structure of a Dioxole Complex of Platinum(II) $C_{10}H_{14}O_2PtCl_4$ (D. Gibson, C. Oldham, Prof. J. Lewis), (D. Lawton, Prof. R. Mason and G. B. Robertson), 580
- Structure of Calcium Oxalate Monohydrate (Prof. H. J. Arnott, Dr. F. G. E. Pautard and Prof. H. Steinrück), 1197
- Structure of Collisionless Shock Waves in a Magnetized Plasma: Experimental Observations on the (Dr. J. W. M. Paul et al.), 133
- Structure of Condensed Tannins (L. L. Creasy and Dr. T. Swain), 151
- Structure of Interstratified (Mixed-layer) Minerals (Dr. M. Sato), 70
- Structure of Lipids by Spectroscopic and X-ray Techniques (Dr. D. Chapman), review by Dr. F. D. Gunstone, 1246
- Structure of Phosphobenzene (Dr. J. J. Daly and Dr. L. Maler), 383
- Structure of the Eastern Aden Protectorate: Stratigraphy and (Dr. Z. R. Beydoun), review by F. E. Wellings, 615
- Structure of the Quasi-stellar Radio Source 3C 273 B (M. J. Rees and Dr. D. W. Sciama), 371
- Structure of the Radio Continuum Background at High Galactic Latitudes (Dr. M. J. S. Quigley and Dr. C. G. T. Haslam), 741
- Structure of Wood: Fine (Dr. P. Rudman), 55
- Structured Media: Diffusive Transport Rates in (Prof. A. B. Metzner), 267
- Structures inside a Lunar Crater photographed by Ranger VII (Captain V. G. Smalley and L. B. Ronca), 930
- Structures resembling Crystalline Carbons at the Active Centre of Enzymes: Evidence for (Dr. E. D. Wachsmuth), 942
- Students in Britain: New Commonwealth, review by Lord Twining, 957
- Studiekring voor de Geschiedenis van de Landbouw, 1939-1964, review by G. E. Fussell, 1138
- Sub-acute Sclerosis Encephalitis: Virus-like Particles in (Dr. N. K. Gonatas and Prof. G. M. Shy), 1338
- Sub-apical Sections of *Avena* Coleoptile by Indolyl-3-acetic Acid: Stimulation of Synthesis of Ribonucleic Acid (Prof. T. H. Hamilton et al.), 1180
- Sub-atmospheric Oxygen-levels: Experimentation with Plants at (Dr. S. M. Siegel et al.), 1012
- Subcutaneous Sarcomata in Hamsters induced by Rous Sarcoma Virus (Bryan) (Prof. M. I. Eidinoff, B. Bates, M. Steinglass and Prof. J. R. Haddad), 336
- Sub-millimetre Dispersion and Rotational Line Strengths of the Hydrogen Halides (Dr. J. E. Chamberlain and Dr. H. A. Gebbie), 480
- Sub-millimetre Wave Spectra of Polar Liquids (Dr. G. W. Chantry and Dr. H. A. Gebbie), 378
- Sub-sieve Range by Electronic Counter: Size Analysis in the (Prof. C. C. Harris and Dr. A. Jowett), 125
- Succession: Re-classification of the Ethiopian Cenozoic Volcanic (P. A. Mohr), 177
- Sudan: University of Khartoum, Silver Jubilee of the Faculty of Agriculture, Shambat (Prof. M. A. Kaur), 620
- Sugii, Dr. M., et al.: Direct and Indirect Effects of Radiation on Plant Cells, their Relation to Growth and Growth Inhibition, 850
- Sulitzanu, Prof. D., and Goldsman, W. F.: Simplified Technique for Preparative Disc Electrophoresis, 123
- Sulphocysteine in the Urine of the Blotched Kenya Genet (J. C. Crawhall and Dr. S. Segal), 1320
- Sulphur: Microstructure of Fibrous (A. Jakolska and A. E. B. Presland), 1088
- Sumner, Dr. M. E., and Skeen, J. B.: Measurement of Exchangeable Aluminium in Acid Soils, 712
- Sumner, Dr. W. L.: Early Seventeenth Century Scientists, review, 985
- Sun: Ionosphere and X-ray Images of the (Dr. C. M. Minnis), 476
- Sunderland, Dr. E.: Hair Colour of the Population of Tristan da Cunha, 412
- Sunspot Comparison: Solar Motion and (Dr. R. M. Wood and Prof. K. D. Wood), 129
- Superfused Carotid Body: Some Effects of Drugs on the Isolated (Dr. T. J. Biscoe), 294
- Superior Colliculus Neurons: Somesthetic and Visual Responses of (Dr. D. Jassik-Gerschenfeld), 89E
- Support for University Science and Technology: Science Research Council, 117
- Supporting Delicate Biological Specimens: Simple Technique for embedding and (R. H. Harris), 199
- Suppression of Mutation Rates in Bacteria by Spermine: Apparent (Dr. H. G. Johnson and Dr. M. K. Each), 408
- Suppression of the Pupillary Light Reflex in Binocular Rivalry and Saccadic Suppression (M. Larber, Dr. B. L. Zuber and Dr. L. Stark), 558
- Surface Active Agents, 529
- Surface-active Agents by Infrared and Chemical Methods: Identification and Analysis of (Prof. D. Hummel), Two Volumes, review by Dr. P. Robson, 617
- Surface Area and Average Pore Radius: Errors in the Calculation of (Dr. D. Dollimore and Dr. G. R. Heal), 1092
- Surface Charges on Arc Track Oxide Layers (Dr. W. P. Jayasekara, Dr. A. E. Guile and Dr. K. A. Nayor), 774
- Surface Conditions: Lunar and Planetary (Dr. N. A. Weil), review by Dr. G. Fielder, 961
- Surfaces: Simultaneous Oxidation and Reduction of Diphenyl Picrylhydrazyl on (Prof. J. G. Aston et al.), 181
- Surfacings: Reconditioning Concrete, 834
- Surfactants: Ultrasonic Method of Determining the Critical Micelle Concentration of (J. Kuppusami and C. V. Suryanarayana), 780
- Surgeon: Spencer Wells, the Life and Work of a Victorian (Mr. J. A. Shepherd), review by Sir Arthur MacNalty, 1135
- Surgeon's Craft (Prof. H. Atkins), review by N. W. Nisbet, 7
- Survey of Great Britain: Soil, 123
- Surveys: Directorate of Overseas, 118
- Suryanarayana, C. V., and Kuppusami, J.: Ultrasonic Method of Determining the Critical Micelle Concentration of Surfactants, 780
- Sussex: University of, Chair of Microbiology, 20, Chair of Physics, 833
- Sutherland, Sir Gordon: Resignation from Directorship of the National Physical Laboratory, 732
- Suttle, Group Capt. C. E. P.: *Obituary* of Dr. O. S. Sinnatt, 227
- Suzuki, Yoshiaki, and Okamoto, S.: Chloramphenicol, Dihydrostreptomycin, and Kanamycin-inactivating Enzymes from Multiple Drug-resistant *Escherichia coli* carrying Episome 'R', 1301
- Swain, Dr. T., and Creasy, L. L.: Structure of Condensed Tannins, 151
- Swansea, University College of, Appointments, 936, Appointment of Professor of Industrial Engineering, 19
- Sweat Glands and Sebaceous Glands in Seals: Functional Significance of (J. K. Ling), 560
- Swinton, Dr. W. E.: Fossil Evidence for Human Evolution, Second Edition, review, 1140
- Syllabus der Pflanzenfamilien (A. Engler), Vol. 2, Angiosperms: Twelfth Edition (edited by Prof. H. Melchior), review by Prof. T. G. Tutin, 316
- Sylvester, J. C. (edited by): Antimicrobial Agents and Chemotherapy, 1964, review by Prof. E. P. Abraham, 980
- Symbionts and Fat Bodies of the Cockroach, *Leucophaea maderae*: Guanase Activity of the (Dr. L. L. Pierre), 666
- Symmetry: Dense Packing of Hard Spheres with Five-fold (B. G. Bagley), 674
- Symonds, Dr. J. L.: Formation of Hydroxyl Molecules in Interstellar Space, 1195
- Symphyla: Bilateral Asymmetry: a Larval Polymorphism in some Argid Sawflies (Hymenoptera), (Dr. M. F. Claridge and Dr. J. M. Edington), 301
- Synapse and Catecholamine Transport: Co-ordination Chemistry and Membrane Function with Particular Reference to the (Dr. J. W. Maas and Dr. R. W. Colburn), 41
- Synchronized HeLa Cells: Synthesis of Ribosomal RNA in (Prof. M. D. Scharff and Dr. E. Robbins), 464
- Syndrome: Autoradiographic Identification of the D(13-15) Chromosome responsible for Dr. Trisomic Man's (Dr. F. Giannelli), 669
- Cutaneous Elastin in Ehlers-Danlos (Dr. D. P. Varadi and Dr. D. A. Hall), 1224
- Hyperlipaemia following Viral Infection in the Chicken Embryo: A New (Dr. S. E. Grossberg and Prof. W. M. O'Leary), 954
- Syngeneic Thymus Grafts: Induction of Malignant Lymphomas by Urethane in Adult Mice bearing (Dr. L. Fiore-Donati et al.), 398
- Synovial Fluid: Electron Microscope Observations on the Free Protein and the Protein-polysaccharide Complex of Bovine (Dr. J. W. Smith and Dr. J. Frame), 867
- Synthesis of Cellular and Viral Ribonucleic Acid and Production of Haemagglutinin: Influence of Heterologous Histone on the (A. G. Bukrinskaya et al.), 557
- Synthesis of Condensed Phosphates: Photochemical (A. Beck and L. E. Orgel), 1000
- Synthesis of Ribonucleic Acid by *Escherichia coli* during Inhibition by Azetidine-2-carboxylic Acid (Dr. J. M. Midgley and Dr. D. G. Wild), 585
- Synthesis of Ribonucleic Acid in Ehrlich Ascites C-2 Tumour Cells treated with Actinomycin D: Rapidly Labelled (A. A. Popović), 49
- Synthesis of Ribonucleic Acid in Sub-apical Sections of *Avena* Coleoptile by Indolyl-3-acetic Acid: Stimulation of (Prof. T. H. Hamilton et al.), 1180
- Synthesis of Ribosomal RNA in Synchronized HeLa Cells (Prof. M. D. Scharff and Dr. E. Robbins), 464
- Synthesis of the α -Glycoprotein (Garcy) of Rat Serum by the Liver (Prof. H. E. Weimer, D. C. Benjamin and D. A. Darcy), 1221
- Synthesis of the Skin-lightening Agent, Melatonin, in Amphibians: Enzymatic (Dr. J. Axelrod et al.), 386
- Syrian Hamster: Lactic Dehydrogenase Relative to Chaetepithelioma Induction in the (Prof. F. T. Algard et al.), 567

INDEX

- Systematic Character in the Genus *Anolis* (Sauria: Iguanidae): Interspecific Karyotypic Variation as a (G. C. Gorman), 95
- Systems at the Molecular Level: Biological (Dr. B. Askonas *et al.*), 1048
- Synthesizing the Protein Component of Low-density (Beta) Lipoprotein: Role of the Gut in (P. J. Scott and C. C. Winterbourn), 494
- Synthetic Period in Human Blood Cells: Rate of Chromosome Duplication at the End of the Deoxyribonucleic Acid (Dr. C. W. Gilbert *et al.*), 159
- Szokoly, V., Gomba, Sz., and Soltész, M. B.: Vital Staining of the Granulated Juxtaglomerular Cells in the Mouse Kidney, 1331
- TACHYGLOSSUS ACULEATUS**: Immune System in a Monotreme: Studies on the Australian Echidna, (Dr. E. Diener and Dr. E. H. M. Ealey), 950
- Tactile-vision: Thermal and Texture Cues in the Discrimination of Black and White (C. N. French), 1352
- Takacs, J. A., and Freeman, Prof. G. R.: Relative Ionization Efficiencies of Hydrocarbon Molecules as a Function of Bombarding Electron Energy, 996
- Takenobu, M., *et al.*: Air Ion Effects on the Oxygen Consumption of Barley Seedlings, 1112
- Take-off in Anthophorid Bees, *Anthophora acraensis* Fab.: Periodic Aggregating and (Prof. L. K. H. Goma), 807
- Tamers, Dr. M. A., and Pearson, jun., F. J.: Validity of Radiocarbon Dates on Bone, 1053
- Tannenbaum, Dr. S. R., and Miller, Prof. S. A.: Urinary Metabolites in the α -Oxidation of 2,4-Dimethylheptanoic Acid, 452
- Tannin, an Antibiotic which retards Fouling: *Sargassum* (Prof. J. McN. Sieburth and Prof. J. T. Conover), 52
- Tannins: Structure of Condensed (L. L. Creasy and Dr. T. Swain), 151
- Tannock, G. A.: Nucleic Acid of Infectious Laryngotracheitis Virus, 711
- Tapered Quartz Tube in the Laser Near Field: Transmission through a (H. J. Caulfield), 773
- Tapes turgida*: Biphasic Dose-response Relationship with Acetylcholine on the Heart of the Mollusc (P. R. Carroll and G. B. Chesher), 1220
- Tasmanian Aborigines: Portraits of, 230
- Tassel in the Male *Tilapia macrochir* Blgr. (Pisces: Cichlidae): Signal Value of the Genital (Dr. W. W. Wickler), 595
- Taton, René (edited by): Science in the Nineteenth Century, review by Dr. W. P. D. Wightman, 958
- Tatton, J. O'G., *et al.*: Organochlorine Pesticides in the Atmospheric Environment, 1317
- Tavla, Maria, and Fujimori, E.: Interaction between Chlorophyll and Hydroquinone, 78
- Taxonomic Investigation of Several Brassica Species using Serology and the Separation of Proteins by Electrophoresis on Acrylamide Gels (Dr. J. G. Vaughan, A. Waite, Dr. D. Boulter and S. Walters), 704
- Taylor, Dr. C. A., and Lipson, Prof. H.: Optical Transforms: Their Preparation and Application to X-ray Diffraction Problems, review by Dr. C. W. Bunn, 1031
- Taylor, C. B. G.: International Directory of Isotopes, Third Edition, review, 987
- Taylor, Prof. D. B., Creese, R., Nedergaard, O. A., and Case, R.: Labelled Depolarizing Drugs in Normal and Denervated Muscle, 901
- Taylor, Prof. H. F. W.: Appointment as Professor of Inorganic Chemistry in the University of Aberdeen, 20
- Taylor, Prof. I. G.: Physics of the Ear, review, 1136
- Taylor, J. A. G., *et al.*: An Infra-red Investigation of the Effect of Pressure on Silica Powders, as revealed by Deuterium Oxide Exchange, 138
- Taylor, J. P., and Thompson, K. F.: Identical S Alleles in Different Botanical Varieties of *Brassica oleracea*, 306
- Taylor, Dr. K. W., *et al.*: Secretion of Newly Synthesized Insulin *in vitro*, 487
- Taylor, Dr. Margaret M., and Orr, D. A.: Effect of Incubation Temperature on the Flagellation of Gram-negative Rods, 1017
- Taylor, M. R., and Mitchell, Prof. E. W. J.: Mechanism of Stored-energy Release at 200°C in Electron-irradiated Graphite, 538
- Taylor, Dr. R. B.: Decay of Immunological Responsiveness after Thymectomy in Adult Life, 1334
- Les Anticorps de Transplantation, review, 988
- The Thymus in Immunobiology, review, 318
- Taylor, Prof. S. R.: Enrichment of Iron during Accretion in the Solar Nebula, 886
- The Wolf Creek Iron Meteorite, 944
- Taylor, Prof. S. R., and White, Dr. A. J. R.: Geochemistry of Andesites and the Growth of Continents, 271
- Taylor, Dr. T. K. F., and Little, Dr. K.: Intercellular Matrix of the Intervertebral Disk in Ageing and in Prolapse, 384
- Teachers: Guinness Awards for Science and Mathematics, 631
- Teaching in the History and Philosophy of Science (Dr. W. Mays), 937
- Technetium-99m in Hepatic Scintigraphy: Use of (Dr. O. Degross *et al.*), 86
- Technical Staff in the Civil Service: Professional and, 120
- Technics and the Nature of Man (Lewis Mumford), 923
- Technique for embedding and supporting Delicate Biological Specimens: Simple (R. H. Harris), 199
- Technique for Preparative Disc Electrophoresis: Simplified (Prof. D. Sulitzanu and W. F. Goldman), 1120
- Technique for the Removal of the Brain in Bats (A. M. Greenhall), 1014
- Technique in Restoration of Roman Mosaics: Modern, 334
- Technological Information in Parliament: Scientific and, 209
- Technology: High Pressure Science and, 328
- A Humanistic (Vice-Admiral H. G. Rickover), 721
- Materials Science and Engineering at the Massachusetts Institute of, 726
- Ministry of, 1152, Director of Building Research, 228, East Midlands Regional Office of, 833
- Science Research Council Support for University Science and, 117
- Transport and, 934
- Technology Conference: 'Clean Room', 231
- Technology of Leather: Chemistry and (edited by F. O'Flaherty *et al.*), Vol. 4, review by Prof. A. G. Ward, 617
- Tee, Dr. D. E. H., Watkins, Dr. J., and Wang, M.: Breakdown Rate of Serum 7S γ -Globulins in Mice, 251
- Tee, Dr. G. H., *et al.*: Milker's Nodule Virus Infections in Dorset and their Similarity to Orf, 505
- Teeth: Ingestion of Soil by Sheep in New Zealand in Relation to Wear of (W. B. Healy and T. G. Ludwig), 806
- Teleost: Pituitary Transplants in the, 123
- Television: Colour, 1153
- Temperature: Effect of, on the Discontinuity in the S/N Fatigue Curve of a Stainless Steel (T. R. G. Williams and C. R. Shurmer), 379
- Temperature, Effect of Incubation, on the Flagellation of Gram-negative Rods (D. A. Orr and Dr. M. M. Taylor), 1017
- Temple, Dr. G., and Seeger, Dr. R. J. (edited by): Research Frontiers in Fluid Dynamics, review by Prof. K. Stewartson, 967
- Temporal Spacing of Stimuli in Intracranial Self-stimulation: Facilitation as a Function of (Prof. J. A. Deutsch and R. Collins), 592
- Temps Fossilières: Les (Prof. H. Termier et G. Termier), Vol. 1, Paléozoïque Inférieur, review by Prof. A. Williams, 614
- Tension Wood: Induction of, with the Anti-auxin 2,3,5-Tri-iodobenzoic Acid (Prof. R. W. Kennedy and Prof. J. L. Farrar), 406
- Teratogenic Effects of Synthetic Compounds related to Trypan Blue: The Effect of 1,7-Diamino-8-naphthol-3,6-disulphonic Acid on Pregnancy in the Rat (Dr. G. A. Christie), 1219
- Ternary Complexes and Catecholamine Binding: Adenosine Triphosphate-Metal-Norepinephrine (Dr. R. W. Colburn and Dr. J. W. Maas), 37
- Termier, Geneviève, and Termier, Prof. H.: Les Temps Fossilières, Vol. 1, Paléozoïque Inférieur, review by Prof. A. Williams, 614
- Termier, Prof. H., and Termier, G.: Les Temps Fossilières, Vol. 1, Paléozoïque Inférieur, review by Prof. A. Williams, 614
- Terminating Receptors for Myxovirus Attachment: Nuclear Surface N-Acetyl Neuraminic Acid (Prof. P. I. Marcus *et al.*), 1122
- Terminology for Enzyme Mutants in Micro-organisms: A Suggested Phenotypic Classification and (Dr. J. F. Collins *et al.*), 841
- Terner, Prof. C.: Oxidative and Biosynthetic Utilization by Human Spermatozoa of a Metabolite of the Female Reproductive Tract, 1115
- Testosterone and Methandrostenolone on the Dry Mass of Various Rat Muscles: Interferometric Estimation of Effects of (Dr. W. Staubli and Dr. T. Okada), 562
- Tetracyclines to Bone Binding of (Dr. D. D. Perrin), 787
- Tetrahedral Anvil Apparatus: Combined Very High Pressure/High Temperature Calibration of the (J. Lees and B. H. J. Williamson), 278
- Tetrahedral Anvil Apparatus: Pressure Distribution in the (J. Lees), 31
- Tetrahymena pyriformis* from the Photodynamic Toxicity of Benzo(a)pyrene: A Simple Bioassay for Antioxidants based on Protection of (Dr. S. S. Epstein *et al.*), 655
- Tetrahymena pyriformis* GL: Isolation of Macronuclei from the Ciliate (Dr. Y. C. Lee and Prof. O. H. Scherbaum), 1350
- Tetraploid *Solanum*: Double Chromosome Reduction in a (G. E. Marks *et al.*), 359
- Textile and Apparel Technology Center of the National Bureau of Standards Institute, Appointment of Assistant Chief, 628
- Texture Cues in the Discrimination of Black and White: Tactile-vision: Thermal and (C. N. French), 1352
- Thakur, L., and Smith, Dr. B. C.: Boron-Nitrogen Bond Strengths in Borazoles, 74
- Thalassaemia: An *in vitro* Study: Globin Synthesis in (Dr. D. J. Weatherall *et al.*), 1061
- Thalassaemia: Apoferritin Synthesis in Human Erythroid Cells in (Dr. E. H. Eylar and Dr. G. Matiolli), 661
- Thalidomide as a Possible Biological Acylating Agent (S. Fabro, R. L. Smith and Prof. R. T. Williams), 1208
- Theoretical Structure: Comparison of Experimental Data of the Effective Ion Mass in the Upper Ionosphere with a (P. R. Arendt), 443
- Theory for the Evolution of Intelligent Behaviour: A General (Dr. D. Stenhouse), 815
- Theory of Crystal Dislocations (Prof. A. H. Cottrell), review by J. W. Christian, 110
- Theory of Ice Ages: Wilson's (J. T. Hollin), 12
- Theory of Magnetism (D. C. Mattis), review by Dr. C. A. Bates, 616
- Therapeutic Potential in Hyperammonaemic States: Acetohydroxamate: Bacterial Urease Inhibitor with (Dr. William N. Fishbein *et al.*), 46
- Therapy: Experiments in Behaviour (edited by Prof. H. J. Eysenck), review by Dr. B. Semeonoff, 1027
- "There is all Africa" (Dr. A. S. Thomas), 16
- Thermal and Hot Processes in the "Hot-zone" produced by Copper-64 Recoil Atoms in Solids: Evidence of (K. Yoshihara and H. Ebihara), 482
- Thermal and Texture Cues in the Discrimination of Black and White: Tactile-vision (C. N. French), 1352
- Thermal Comfort in Industry, 122
- Thermal Contraction in Twigs (I. J. McCracken and Prof. T. T. Kozlowski), 910
- Thermal Decomposition of Amorphous Polymers: Absence of Diffusion Control of Reaction Rate in the (D. A. Smith), 1200
- Thermal Scattering in Sodium Chloride: Direct Ultramicroscopic Observation of (Dr. G. Heilmann), 884
- Thermal Stress (edited by Dr. P. P. Benham and Dr. R. Hoyle), review by Dr. E. H. Andrews, 517
- Thermistor Hygrometer for determining the Free Energy of Moisture in Unsaturated Soils (B. G. Richards), 608
- Thermogravimetric Data: Tschebychev Approximation to the Equation of (Dr. E. L. Jones), 1306
- Thermoluminescence and Ultra-violet Phosphorescence of the Nucleic Acid Bases (Dr. R. J. Fleming and R. M. Kerr), 1198
- Thinking: Pathology of (Dr. B. V. Zeigarnik), review by Prof. R. C. Oldfield, 982
- Thin-layer Chromatography: Heterogeneity of Titan Yellow demonstrated by (R. J. Hall and L. R. Flynn), 1202
- Thin-layer Chromatography: Resolution of the Optical Isomers of DL-Tryptophan, 5-Hydroxy-DL-tryptophan and 6-Hydroxy-DL-tryptophan by Paper and (Dr. S. F. Contractor and J. Wragg), 71
- Thin-layer Chromatography: Simple Assessment of Partition Data for correlating Structure and Biological Activity using (Dr. C. B. C. Boyce and Dr. B. V. Milborrow), 537
- Thinning of the Human Cornea on Awakening (Dr. R. B. Mandell and Prof. I. Fatt), 292
- dl-Thioctic Acid and Other Disulphides: Charge Transfer Properties of (W. M. Moreau and Prof. K. Weiss), 1203
- Thiomore on Growth of B.H.K. Cells in the Hamster Cheek Pouch: Effect of β -Propiolactone and (S. A. Keeble and C. S. Heymann), 1125
- Third Dimension of Management (John J. Haywood), 928
- Third Excited State of Polycyclic Aromatic Hydrocarbons with the Ionization Potential: Correlation of the (Dr. M. A. Slikin), 380
- Thomas, Dr. A. S.: "There is all Africa", 16
- Thompson, Prof. H. W.: Award of Davy Medal of the Royal Society, 1142
- Thompson, K. F., and Taylor, J. P.: Identical S Alleles in Different Botanical Varieties of *Brassica oleracea*, 306
- Thompson, Prof. M. W.: Appointment to Chair of Physics in the University of Sussex, 833
- Thompson, Dr. P. A.: Physiological Basis of Salt Tolerance of Plants, review, 211, Pollen Physiology and Fertilization, review, 218
- Thomson, Dr. J., *et al.*: Organochlorine Pesticides in the Atmospheric Environment, 1317
- Thomson, Dr. R. Y., and Clarke, A. M.: Role of Portal Blood Supply in Liver Regeneration, 392
- Thorne, Dr. C. J. R.: Advances in Enzyme Regulation, Vol. 2, review, 319
- Thorpe, Dr. W. H., and North, M. E. W.: Origin and Significance of the Power of Vocal Imitation: with Special Reference to the Antiphonal Singing of Birds, 219

- Three Haemoglobins K : Woolwich, An Abnormal, Cameroon and Ibadan, Two Unusual Variants of Human Haemoglobin A (Dr. N. Allan), (D. Beale, D. Irvine and Dr. H. Lehmann), 658
- Threlfall, T. L., et al. : Metabolism of 5-Chloro-2-cyclohexyl-1-oxo-6-sulphamoyl isondoline, Chlorhexolone (M and B 8430), 286
- Thring, Prof. M. W. : Magnetohydrodynamic Electrical Power Generation, Symposium 1964, Four Volumes, review, 966
- Thromboses in Large Veins and Pulmonary Embolisms induced by Catecholamines or Serotonin (Prof. H. Selye, B. Tuchweber and P. Rohan), 900
- Thrombus Formation in the Rabbit : Effect of a Pyrimidopyrimidine Derivatives on (Dr. P. R. Emmons et al.), 255
- Thunderstorms : Some Characteristics of Tropical (Prof. S. V. C. Aiyá), 641
- Thurso, Scotland : An Unusually Radioactive Fossil Fish from (W. R. Diggie and J. Saxon), 400
- Thymectomized and Sham-thymectomized Mice : Time-course Studies on Antibody Response in (Dr. N. R. Sinclair), 1104
- Thymectomized Rats : Effect of a Lipid Fraction from Rat Thymus on Delayed Hypersensitivity Reactions of Neonatally (Prof. B. D. Janković et al.), 356
- Thymectomized X-irradiated Dogs : Bone Marrow Allografts in (I. L. Chertkov et al.), 399
- Thymectomy in Adult Life : Decay of Immunological Responsiveness after (R. B. Taylor), 1334
- Thymectomy in Adult Life on Immunological Competence : Delayed Effect of (Dr. D. Metcalf), 1336
- Thymectomy in Adult Mice on Immunological Responsiveness : Effect of (Dr. J. F. A. P. Miller), 1337
- Thymidine for Cell Transfer Experiments : Labelling of Mouse Thymocytes *in vivo* with Tritiated (Dr. K. Borum), 253
- Thymocytes *in vivo* with Tritiated Thymidine for Cell Transfer Experiments : Labelling of Mouse (Dr. K. Borum), 253
- Thymus and the Production of Antibody-plaque-forming Cells (Dr. J. F. A. P. Miller, P. M. de Burgh and G. A. Grant), 1332
- Thymus Grafts in Aged Mice : Multiple (Dr. D. Metcalf), 87
- Thymus Grafts : Induction of Malignant Lymphomas by Urethane in Adult Mice bearing Syngeneic (Dr. L. Fiore-Donati et al.), 398
- Thymus in Immunobiology (edited by Dr. A. Good and A. E. Gabrielsen), review by Dr. R. B. Taylor, 318
- Thymus in Mice with Myeloid Virus-induced Leukaemia : Lymphatic Neoplastic Transformation of (L. Chicco-Bianchi et al.), 697
- Thymus on Delayed Hypersensitivity Reactions of Neonatally Thymectomized Rats : Effect of a Lipid Fraction from Rat (Prof. B. D. Janković et al.), 356
- Thyroid (edited by J. B. Hazard and David E. Smith), review by Dr. E. E. Pochin, 979
- Thyroid Feeding on the Response of Mice to Injected Convulsant Barbiturate : Effects of Propylthiouracil and (Prof. A. J. Prange, jun., and Prof. M. A. Lipton), 791
- Thyroid *in vitro* : Biosynthesis of Triiodothyronine Sulphate by Beef (G. L. Cohn), 80
- Thyroxine on the Trout Pituitary *in vitro* : Direct Action of (Dr. B. I. Baker), 1234
- Tick, *Hyalomma dromedarii* (Ixodidae) : An Improved Method for obtaining, *in vitro* Uniform Cell Monolayer Sheets from Tissues of the (Dr. M. G. R. Varma and Miss W. Wallers), 602
- Tidal De-spin of Planets and Satellites (Dr. P. Goldreich), 375
- Tilapia macrochir* Blgr (Pisces : Cichlidae) : Signal Value of the Genital Tassel in the Male (Dr. W. W. Wickler), 595
- Tilapia* : Sexual Dimorphism in (J. Chervinski), 703
- Tillman, Dr. J. R. : Solid Circuits and Microminiaturization, review, 1249
- Timber as Building Material in Hot Climates, 1153
- Timbers : Tropical, 329
- Time-course Studies on Antibody Response in Thymectomized and Sham-thymectomized Mice (Dr. N. R. Sinclair), 1104
- Time : God Beyond (J. H. Reyner), review by F. I. G. Rawlins, 985
- Time : Psychology of (Prof. P. Fraisse), review by Dr. W. Gooddy, 822
- Time-scale : A Proposal on Nature's (Prof. N. G. McCormick), 334
- Time Services : U.S. Standard Frequency and (Dr. R. L. Smith-Rose), 25
- Timing of Protein Synthesis during Germination and Outgrowth of Spores of *Bacillus cereus* Strain T (W. Steinberg, Prof. H. P. Halvorson, A. Keynan and E. Weinberg), 710
- Timperman, G., et al. : Gametogenesis of *Plasmodium vinckei*, 1230
- Tissue Culture : Effect of Anaphylactic Release of Vascular Permeability Factor of Histamine by Specific Protease Inhibitor in (Prof. H. Hayashi et al.), 1007
- Tissue Slices : Kinetic Parameters for Oxygen in Reactions Involving Purified Oxidases and (Dr. M. McCabe and Dr. D. A. Gilbert), 450
- Tissues : Distribution of Collagenase in Rat (J. F. Woods and Prof. G. Nichols, jun.), 1325
- Tissues of Higher Plants : Occurrence of Fructose-1-phosphate in (D. Graham and Dr. T. ap Rees), 88
- Titan Yellow demonstrated by Thin-layer Chromatography : Heterogeneity of (R. J. Hall and L. R. Flynn), 1202
- Titration Curves depicting Central Stimulant, Depressant and Dual Stimulant-depressant Action of Glutaramide Homologues in Mice (Dr. A. Shulman, G. M. Laycock and J. A. Henry), 568
- Titration of Antihuman Serum Albumin Antibody : Rapid Detection and (Dr. M. S. Tobin), 1338
- Tizard (R. W. Clark), review, 110
- Tkocz, C., et al. : Long-spacing Segments from Renatured α 1 Sub-units of Collagen, 685
- Toad Oocytes : Emission of Feulgen-positive Particles during the *in vitro* Maturation of (Prof. J. Brachet), 596
- Tobacco and Transpirational Water Loss : Phosphorus-deficiency Symptoms in (Prof. A. Wallace and E. Frolich), 1231
- Tobacco : Tumour-promoting Agents in Unburned Cigarette (Dr. F. G. Bock et al.), 584
- Tobias, Prof. P. V. ; Holloway, jun., Prof. R. L. : Cranial Capacity of the Hominine from Olduvai Bed 1, 205
- Tobin, Dr. Mark S. : Rapid Detection and Titration of Antihuman Serum Albumin Antibody, 1338
- Toda, Amazonia, and Shope, R. E. : Transmission of Guamá and Oriboca Viruses by Naturally Infected Mosquitoes, 304
- Tolerance in Flowering Plants : Shade (Dr. J. P. Grime), 161
- Tomasz, Dr. A. : Control of the Competent State in *Pneumococcus* by a Hormone-like Cell Product : An Example for New Type of Regulatory Mechanism in Bacteria, 155
- Tonic Contractions ('Catch') in an Invertebrate Muscle : Energy Requirements for Relaxation from (Dr. K. Minihan and Prof. R. E. Davies), 1327
- Toolan, Dr. H., and Ledinko, Dr. N. : Growth and Cytopathogenicity of *H*-viruses in Human and Simian Cell Cultures, 812
- Topps, Dr. J. H., et al. : Phosphorus Excretion of Cattle fed on High-energy Diets, 953
- Torrens, I. McC., and Chadderton, Dr. L. T. : Correlated Atomic Collisions in Irradiated Crystal Lattices, 880
- Towards a University of the Air, 715
- Towe, Dr. A. L., et al. : Influence of the Cerebral Cortex on the Cuneate Nucleus of the Monkey, 1215
- Townsend's First Ionization Coefficient for Compressed Nitrogen (Dr. B. W. Ward), 994
- Toxic Decomposition Products of Tribromoethanol : Protective Effect of Oestrogens against the (Prof. T. Nicol et al.), 1098
- Toxicity of Benzo(a)pyrene : A Simple Bioassay for Antioxidants based on Protection of *Tetrahymena pyriformis* from the Photodynamic (Dr. S. S. Epstein et al.), 655
- Trace Elements by Plant Roots : Mechanism of Uptake of (G. Findenegg and Prof. E. Broda), 196
- Trace Metals and Bloat in Ruminants : Possible Relationship between (P. J. Harris and Prof. F. Sebba), 869
- Trager, Prof. W., et al. : Chloroquine Resistance and the Nature of Malarial Pigment, 691
- Training for Management in Industry, 613
- Transcription : Localized Strand Separations within Deoxyribonucleic Acid during Selective (Prof. J. H. Frenster), 894
- Transfer Experiments : Labelling of Mouse Thymocytes *in vivo* with Tritiated Thymidine for Cell (Dr. K. Borum), 253
- Transfer Factor associated with Drug Resistance in *Salmonella typhimurium* : Characterization of a (Dr. E. S. Anderson and Dr. M. J. Lewis), 843
- Transfer Factors in Drug-sensitive Enterobacteriaceae : A Rapid Screening Test for (Dr. E. S. Anderson), 1016
- Transformation of Thymus in Mice with Myeloid Virus-induced Leukemia : Lymphatic Neoplastic (L. Chicco-Bianchi et al.), 697
- Transforms : Optical (Dr. C. A. Taylor and Prof. H. Lipson), review by Dr. C. W. Bunn, 1031
- Translocation through the Respiratory Chain and Adenosine Triphosphatase Systems of Rat Liver Mitochondria : Stoichiometry of Proton (Dr. P. Mitchell and Dr. J. Moyle), 147
- Transmissible Disease : Natura. Selection and (Prof. N. A. Barnicot), 535
- Transmission of Guamá and Oriboca Viruses by Naturally Infected Mosquitoes (A. Toda and R. E. Shope), 304
- Transmission of Hop Mosaic Virus : Mechanical (Dr. J. T. Legg), 1017
- Transmission through a Tapered Quartz Tube in the Laser Near Field (H. J. Caulfield), 773
- Transphosphorylation of Adenosine Di- and Tri-phosphates in the Presence of Calcium Phosphate Precipitates (Dr. R. W. Burley), 683
- Transpirational Water Loss : Phosphorus-deficiency Symptoms in Tobacco and (Prof. A. Wallace and E. Frolich), 1231
- Transplantable and Spontaneous Murine Tumours by the M-P Virus : Inhibition of (Dr. N. Molomut and Dr. M. Padnos), 948
- Transplantation : Les Anticorps de (André Govaerts), review by R. B. Taylor, 988
- Transplantation Experiments on Placental Ageing (R. L. Simmons and J. Weintraub), 82
- Transplantation : Isoprecipitin in the Sera of Rats resistant to Tumour (H. Hirai), 798
- Transplanted Mouse Eggs : Histocompatibility Antigens in (Dr. R. L. Simmons and P. S. Russell), 698
- Transplants in the Teleost : Pituitary, 123
- Transport and Technology, 934
- Transport : Co-ordination Chemistry and Membrane Function with Particular Reference to the Synapse and Catecholamine (Dr. J. W. Maas and Dr. R. W. Colburn), 41
- Transport of L1210 Leukaemia Cells : Role of Sialic Acid in Potassium (Dr. J. L. Glick and Dr. S. Githens III), 88
- Transport of Methionine and Sodium Butyrate by Intracellular Plasma : Mechanism of Intestinal Absorption : (S. L. Hart and Dr. J. A. Nisim), 145
- Transport of Two Auxins : Promotion by Kinetin of the Polar (Dr. C. C. McCready et al.), 1065
- Transport of Volcanic Dust inferred from Solar Radiation Measurements : Stratospheric (Dr. A. J. Eyer and B. B. Hicks), 131
- Transport Rates in Structured Media : Diffusive (Prof. A. B. Metzner), 267
- Trans-Solutes on the Fluxes of Chloride Ions across Artificial Membranes : Effect of (S. Kitahara, Dr. E. Henz and C. Stahlmann), 187
- Travelling-wave Tubes : Power of F. Gittins), review by Dr. P. A. Lindsay, 1140
- Treadmill Experience on Body-weight and Resistance to Exhaustion in the Rat : Effect of Infantile (R. A. Levitt and Prof. V. B. Webb), 1128
- Trease, Prof. G. E. : Pharmacy in History, review by Dr. Frank Hartley, 982
- Treatise on Trigonometric Series : (Prof. N. K. Bary), Vols. 1 and 2, review by Prof. T. A. A. Broadbent, 106
- Tree Rings : Antimatter and (V. S. Venkatavaran), 772
- Trees : Effect of Negative Absolute Pressure on Flow Resistance : Ascent of Sap in (Prof. B. G. Johnson and Prof. J. R. Dixon), 1347
- Treloar, Dr. L. R. G. : Rubber in Engineering Practice, review, 823
- Trencsényi-Waldapfel, Dr. J., and Erdey-Grúz, Dr. T. (edited by) : Science in Hungary, review by Prof. E. Gabor, 983
- Trentelman, E., et al. : Blood Group Secretion Factors in Bronchogenic Carcinoma, 299
- Trexler, Prof. P. C., et al. : Human Indigenous Microflora in Gnotobiotic Rats, 1236
- Tribromoethanol : Protective Effect of Oestrogens against the Toxic Decomposition Products of (Prof. T. Nicol et al.), 1097
- Tridente, G., et al. : Induction of Malignant Lymphomas by Urethane in Adult Mice bearing Syngeneic Thymus Grafts, 398
- Lymphatic Neoplastic Transformation of Thymus in Mice with Myeloid Virus-induced Leukaemia, 697
- 2-Trifluoromethylbenzimidazoles : A New Class of Herbicidal Compounds (Dr. D. E. Burton, Dr. A. J. Lambie, J. C. L. Ludgate, Dr. G. T. Newbold, Dr. A. Percival and D. T. Sagers), 1166
- 2-Trifluoromethylbenzimidazoles as Uncouplers of Oxidative Phosphorylation : Activity of (Dr. O. T. G. Jones and W. A. Watson), 1169
- Trigonometric Series : Treatise on (Prof. N. K. Bary), Vols. 1 and 2, review by Prof. T. A. A. Broadbent, 106
- 2,3,5-Tri-iodobenzoic Acid : Induction of Tension Wood with the Anti-auxin (Prof. R. W. Kennedy and Prof. J. L. Farrar), 406
- Triiodothyronine Sulphate by Beef Thyroid *in vitro* : Biosynthesis of (G. L. Cohn), 80
- Trimethylsilyl Derivatives : Quantitative Gas Chromatography of Amino-acids as (Prof. E. D. Smith and F. Sheppard, jun.), 878
- Triolein Deiodination *in vitro* : Oleic Acid labelled with Iodine-131, and (L. J. Anglieri), 896
- Dr. Trisomic Patau's Syndrome : Autoradiographic Identification of the D(13-15) Chromosome responsible for (Dr. F. Giannelli), 669
- Tristan da Cunha : Hair Colour of the Population of (Dr. E. Sunderland), 412
- Tritiated Thymidine for Cell Transfer Experiments : Labelling of Mouse Thymocytes *in vivo* with (Dr. K. Borum), 253
- Tritiating Methylation : Quantitative Chromatographic Analysis of Organic Acids by (G. K. Koch and the late G. Jurriens), 1312
- Tritium : Labelling of Beta-lipoprotein with (T. Gosztonyi et al.), 381

- 'Triton X-100' on Glucose-filled Phospholipid/Cholesterol Structures : Effect of Steroids and (Dr. G. Weissmann *et al.*), 649
- Tropical High Forest : Production Ecology in, 22
- Tropical Thunderstorms . Some Characteristics of (Prof. S. V. C. Aiyar), 641
- Tropical Timbers, 329
- Trout Pituitary *in vitro* : Direct Action of Thyroxine on the (Dr. B. I. Baker), 1234
- Trout Serum . Electrophoretic Mobility of an Immune Globulin from Rainbow (H. O. Hodgins *et al.*), 1106
- Trypan Blue : the Effect of 1,7-Diamino-8-naphthol-3,6-disulphonic Acid on Pregnancy in the Rat : Teratogenic Effects of Synthetic Compounds related to (Dr. G. A. Christie), 1219
- Trypanosomes : Polymorphism and Mitochondrial Activity in Sleeping Sickness (Dr. K. Vickerman), 762
- Trypsin : 'Buried' Tyrosyl Residues and the Activity of (J. F. Riordan *et al.*), 120
- Tryptophan and Indole Uptake in *Escherichia coli* : Interference by the Carcinogenic 4-Nitro-quinoline N-oxide of (A. C. Zahalsky and Prof. S. L. Marcus), 296
- DL-Tryptophan, 5-Hydroxy-DL-tryptophan and 6-Hydroxy-DL-tryptophan by Paper and Thin-layer Chromatography : Resolution of the Optical Isomers of (Dr. S. F. Contractor and J. Wragg), 71
- Tsao, Dr. Y. C. *et al.* : Characterization of Glucose-6-phosphate Dehydrogenase among Chinese, 1323
- Tschebyshev Approximation to the Equation of Thermogravimetric Data (Dr. E. L. Jones), 1306
- Tsakamoto, W. T. *et al.* : Site of Attack in the Radiolysis of Pyrimidine Compounds, 1086
- Tubular Site of Urate Secretion in the Rabbit (Dr. J. V. Møller), 492
- Tuchweber, Beatriz, *et al.* : Thromboses in Large Veins and Pulmonary Embolisms induced by Catecholamines or Serotonin, 900
- Tuffs of County Mayo, Eire - Observations on the Mont Pelée Eruption of 1902 and their Significance to Ordovician Welded (Dr. J. McManus), 578
- Tumeurs Expérimentales : Structure Antigénique de (Dr. C. Deckers), review by R. R. Dourmashkin, 1140
- Tumour Cells by *in vitro* Contact with Cells containing Foreign H-2 Antigens : Allogeic Inhibition of (K. E. Hellstrom *et al.*), 458
- Tumour Cells : Distinction between the Effects of Antiviral and Anticellular Polyoma Antibodies on Polyoma (I. Hellstrom), 652
- Tumour Cells : Enzyme Regulatory Mechanisms at the Level of Lactate-oxidoreductase in Erythrocytes and Ascites (S. Rapoport *et al.*), 185
- Tumour Cells treated with Actinomycin D : Rapidly Labelled Synthesis of Ribonucleic Acid in Ehrlich Ascites C-2 (D. A. Popovici), 49
- Tumour-promoting Agents in Unburned Cigarette Tobacco (Dr. F. G. Bock, R. J. Shamberger and H. K. Myers), 584
- Tumour Transplantation : Isoprecipitation in the Sera of Rats resistant to (H. Hirai), 798
- Tumour Virus in Arthropod Host : Microcrystal Formation : Plant (E. Shikata and Dr. K. Maramorosch), 507
- Tumours : Androgen-Oestrogen-induced (Prof. F. T. Algard *et al.*), 567
- Tumours by N-Ethyl-N-nitrosourea : Induction of Intestinal (Dr. R. Schoental), 300
- Tumours by the M-P Virus : Inhibition of Transplantable and Spontaneous Murine (Dr. N. Molomot and Dr. M. Padnos), 948
- Tumours in Animals : Lung (Prof. L. Severi), 632
- Tumours in Ducks fed a Low Level of Toxic Groundnut Meal . Hepatic (R. B. A. Carnaghan), 308
- Tung, Y. C., and Wang, Prof. Kung-Tsung : Polyamide Layer Chromatography of Oestrogens, 581
- Tuplin, Prof. W. A. : Matrix Analysis of Vibration, review, 616
- Turbulent Flow in Open Channels : Laminar and (Dr. J. Allen), 1196
- Turnbull, D., and Seitz, F. (edited by) . Solid State Physics. Advances in Research and Applications, Vol. 15, review by Prof. C. W. McCombie, 4
- Turner, C. E. : Introduction to Plate and Shell Theory, review by Prof. B. N. Cole, 1137
- Turner, Dr. J. E., and Hollister, H. : Relationship of the Velocity of a Charged Particle to its Relative Biological Effectiveness, 36
- Turtle Eggs : Hatching Time of (Sister Marie Therese Dimond), 401
- Turtles : Blood Flow in (F. N. White and G. Ross), 759
- Tutin, T. G. *et al.* (edited by) : Flora Europaea, Vol. 1, review by Prof. J. A. Nannfeldt, 8
- Tutin, Prof. T. G. : Syllabus der Pflanzfamilien, Vol. 2, Angiosperms, Twelfth Edition (A. Engler), review, 316
- Tuttobello, L., *et al.* : Production of Carotenoid by a Green Alga, 1342
- Twigs : Thermal Contraction in (I. J. McCracken and Prof. T. T. Kozlowski), 910
- Twining, Lord . New Commonwealth Students in Britain, review, 957
- Twins : Research in Human Genetics and Studies of, 230
- Two Nearly Equal Quantities : Calculations with (Dr. J. H. Fremlin : Dr. F. T. Worrell), 1306
- Tyan, Dr. M. L., and Cole, Dr. L. J. : Bone Marrow as the Major Source of Potential Immunologically Competent Cells in the Adult Mouse, 1223
- Tyler, Dr. John E. : Colour of 'Pure' Water, 549
- Tyrosyl Residues and the Activity of Trypsin : 'Buried' (J. F. Riordan *et al.*), 1209
- Tyrrell, M. W., Demetopoulos, I. C., and Collinson, A. J. L. : Determination of the Total Alpha Activity of Drinking Water using a Nuclear Emulsion, 1074
- UGANDA** : Vegetation of, 1047
- Uible, H. E., *et al.* : Mammals of the World, Three Volumes, review by Dr. L. Harrison Matthews, 210
- Ullberg, S., *et al.* : Specific Uptake in Corpora Lutea of a Non-steroid Substance with Anti-gestagenic Properties, 461
- Ultracentrifuge : Fractionation of Ribonucleic Acids in the Zonal (Dr. J. R. B. Hastings *et al.*), 645
- Ultramicroscopic Observation of Thermal Scattering in Sodium Chloride : Direct (Dr. G. Heilmann), 884
- Ultra-purification by Separation of Aerosol Particles (Dr. S. Behrendt), 581
- Ultrasonic Method of Determining the Critical Micelle Concentration of Surfactants (J. Kuppasami and C. V. Suryanarayana), 780
- Ultrasonic Wave Effect : Initiation of Chain Reaction under an (Prof. I. E. Elpiner *et al.*), 945
- Ultrastructure of the Antigen-retaining Reticulum of Lymph Node Follicles as shown by High-resolution Autoradiography (J. Mitchell and A. Abbot), 500
- Ultrastructure of the Phrenic Nerve (Dr. A. S. Wilson and D. G. Silva), 707
- Ultra-violet Absorption Spectra of Aromatic Nitrenes and Dinitrenes (A. Reiser and V. Frazier), 682
- Ultra-violet and X-irradiated Homocysteine-thiolactone : Electron Spin Resonance Investigations of (Prof. R. Koch and Dr. H. Monig), 86
- Ultra-violet Irradiation on the Electrical Conductivity of Zinc Oxide Single Crystals : Effect of (Dr. R. B. Lal and Dr. G. M. Arnett), 1305
- Ultra-violet Phosphorescence of the Nucleic Acid Bases : Thermoluminescence and (Dr. R. J. Fleming and R. M. Kerr), 1198
- Ultra-violet Spectroscopy in the Analysis of Chromatograms (Dr. D. M. Abelson), 784
- Unanaesthetized Cerebral Cortex of the Cat : Correlation between Unit Activity and Slow Potential Changes in the (A. D. J. Robertson), 757
- Unburned Cigarette Tobacco : Tumour-promoting Agents in (Dr. F. G. Bock *et al.*), 584
- Uncouplers of Oxidative Phosphorylation : Activity of 2-Trifluoromethylbenzimidazoles as (Dr. O. T. G. Jones and W. A. Watson), 1169
- Undernutrition and Early Embryonic Mortality in Merino Ewes (T. N. Edey), 1232
- Unirradiated DNA in *Drosophila* : Mutagenic Effect of Irradiated and (A. H. Khan and Dr. T. Alderson), 700
- Unit Activity and Slow Potential Changes in the Unanaesthetized Cerebral Cortex of the Cat : Correlation between (A. D. J. Robertson), 757
- U.K. Atomic Energy Authority and Central Electricity Generating Board Co-operation Scheme, 120
- United Nations Review of Space Science, 21
- U.S. Medical Research Fellowship Analyses, Assistant to the Chief, 433
- U.S. National Academy of Sciences : Information Activities of the, 1260
- U.S. National Bureau of Standards : Retirement of Dr. W. J. Youden from the Applied Mathematics Division, 119
- Appointment of Dr. P. H. Verdier as a Physical Chemist at the Institute for Materials Research, 119
- U.S. Standard Frequency and Time Services (Dr. R. L. Smith-Rose), 25
- U.S.S.R. : Scientific Periodicals from the, 328
- University College of Swansea : Appointments, 936, Appointment of Professor of Industrial Engineering, 19
- University Entrance Requirements in Britain, 433
- University, Melbourne : La Trobe, 434
- University : The Modern (Prof. G. L. Brook), review by R. Brightman, 518
- University of Aberdeen : Appointment of Third Professor of Chemistry, 20
- Appointments, 436
- Chair of Genetics, 436, 833
- University of Belfast : Appointments, 836, 1047
- University of Birmingham : Appointments, 231, 436, 836
- University of Bristol : Appointments, 1047
- University of Cambridge : Fellowships at Churchill College, 936
- University of Edinburgh : Appointments, 936
- Chair of Mathematics, 19, 832
- University of Hull : Chair of Electronic Engineering, 732
- University of Khartoum, Sudan : Silver Jubilee of the Faculty of Agriculture, Shambat (Prof. M. A. Nour), 620
- University of Leeds : Appointments, 1047
- University of Leicester : Department of Museum Studies, 122
- University of London : Appointments, 735, 1047
- University of Manchester : Appointments, 436, 1047
- Chair of Hydrodynamics and Hydrology, 436
- University of Newcastle upon Tyne : Chair of Psychology, 21
- Chair of Zoology, 432
- University of Oxford : Chair of Metallurgy, 627
- Vice-Chancellor's Oration, 1155
- University of Reading : Appointments of Professors in the Department of Chemistry, 20
- Chair of Geography, 628
- Chair of Psychology, 228
- Horticultural Science at the (Dr. G. P. Harris), 437
- University of Scratchlyde : Chair of Pharmaceutical Technology, 934
- University of Sussex : Chair of Microbiology, 20
- Chair of Physics, 833
- University of the Air : Towards a, 715
- University of the Congo at Elisabethville : Official, 124
- University Science and Technology : Science Research Council Support for, 117
- Unsaturated Soils : Thermistor Hygrometer for determining the Free Energy of Moisture in (B. G. Richards), 608
- Unsteady State Growth Rates of Micro-organisms : Measurement of (Prof. R. I. Mateles *et al.*), 263
- Uplift : Isotopic Ages and Andean (Dr. R. W. R. Rutland *et al.*), 677
- Upmanis, Miss Rasma S., *et al.* : Primary Tissue Phase of *Plasmodium berghei* in Different Experimental Hosts, 903
- Upper Carboniferous of South-west England : Recent Finds in, and their Significance (Dr. H. G. Reading), (Dr. J. E. Prentice), 745
- (Upper Jurassic) of Kutch, India : Occurrence of Gnetalean Pollen Grains in the Katrol Formation (Y. K. Mathur and Mrs. K. Mathur), 912
- Uptake by Plants of Radiostrontium from Contaminated Soils : (A. J. Andersen), 195
- Uptake in Corpora Lutea of a Non-steroid Substance with Anti-gestagenic Properties : Specific (A. Hannngren, N. Eimer-Jensen and S. Ullberg), 461
- Uptake of Radiostrontium by the Human Body : Effect of Sodium Alginate in Inhibiting (R. Hesp and B. Ramsbottom), 1341
- Uptake of Trace Elements by Plant Roots : Mechanism of (G. Findenegg and Prof. E. Broda), 196
- Urate Secretion in the Rabbit : Tubular Site of (Dr. J. V. Møller), 492
- Urea Biosynthesis in the Land Planarian : its Significance in Biochemical Evolution : Arginine and (Prof. J. W. Campbell), 1299
- Urease Inhibitor with Therapeutic Potential in Hyperammonaemic States : Acetohydroxamate : Bacterial (Dr. W. N. Fishbein), 46
- Urethane in Adult Mice bearing Syngeneic Thymus Grafts : Induction of Malignant Lymphomas by (Dr. L. Fiore-Donati *et al.*), 398
- Uridine-5-³H in Human Lymphocytes cultured with Phytohaemagglutinin : Cytoplasmic Labelling with (Dr. G. C. B. Winter and Prof. J. M. Yoffey), 1018
- Urinary Metabolites in the ω -Oxidation of 2,4-Dimethylheptanoic Acid (Dr. S. R. Tannenbaum and Prof. S. A. Miller), 452
- Urine of Rats on Diets containing Aldrin or Dieldrin : Metabolites in (P. R. Datta *et al.*), 289
- Urine of Schizophrenics : 'Pink Spot' in the (R. E. Bourdillon *et al.*), 453
- Urine of the Blotched Kenya Genet : Sulphocysteine in the (J. C. Crawhall and Dr. S. Segal), 1320
- Urokinase : Molecular Weight of (R. A. Burges *et al.*), 894
- Use of CIBA Resins in Ireland, 21
- Use of Polyethylene Glycol in Investigations of Absorption of Fat (Dr. H. Vavřínková and Dr. A. Krondl), 293
- Use of Technetium-99m in Hepatic Scintigraphy (Dr. O. Degrossi, J. M. Seeber, H. Gotta, A. Olivar and A. Chwojnik), 86
- Uterine Muscle Strips : Electrical Activity in Human (Dr. T. B. MacLachlan *et al.*), 290
- Utilization by Human Spermatozoa of a Metabolite of the Female Reproductive Tract : Oxidative and Biosynthetic (Prof. C. Turner), 1115
- Utopia Reviewed, 229
- Utter, F. M., *et al.* : Electrophoretic Mobility of an Immune Globulin from Rainbow Trout Serum, 1106

- VALENTINE, D. H., et al. (edited by): *Flora Europaea*, Vol. I, review by Prof. J. A. Nannfeldt, 8
- Validity of Radiocarbon Dates on Bone (Dr. M. A. Tamers and F. J. Pearson, jun.), 1053
- Vallee, Prof. B. L., et al.: 'Buried' Tyrosyl Residues and the Activity of Trypsin, 1209
- Values for the Parents and Grandparents: Regression of a Phenotypic Value on the (Dr. I. J. Good), 203
- Valve Disease: Mitral, 631
- Valve Metals: A.C. Resistivity of Anodic Oxide Films on (Dr. G. C. Wood and C. Pearson), 547
- Valzelli, Prof. L., and Garattini, Prof. S.: Serotonin, review by Dr. G. B. West, 1247
- Van de Veerdonk, Dr. F. C. G.: Separation Method for Melatonin in Pineal Extracts, 1324
- Vanderberg, Dr. J., et al.: Primary Tissue Phase of *Plasmodium berghei* in Different Experimental Hosts, 903
- Van Gelder, R. G., and Mayer, W. V. (edited by): *Physiological Mammalogy*, Volumes 1 and 2, review by H. N. Southern, 1136
- Van Heuvelen, Prof. Alan: Electron Spin Resonance Spectrum of Cu^{++} in Diamine Oxidase, 888
- Van Huystee, R. B., Weiser, C. J., and Li, P. H.: Some Postulated Relationships among Autumn Phenomena in Woody Plants, 89
- Van Isterbeek, A. (edited by): Physics of High Pressures and the Condensed Phase, review by Dr. R. S. Bradley, 515
- Van Landingham, S. L.: Evidence for Microfossils in the Alais and Orgueil Carbonaceous Meteorites, 947
- Van Woert, Dr. M. H., Nicholson, A. R., and Cotzias, Dr. G. C.: Functional Similarities between the Cytoplasmic Organelles of Melanocytes and the Mitochondria of Hepatocytes, 810
- Vapour: Ionization Times in Shock-heated Potassium (R. M. Hill and B. Capp), 176
- Varadi, Dr. David P., and Hall, Dr. D. A.: Cutaneous Elastin in Ehlers-Danlos Syndrome, 1224
- Varcoe, J. S., et al.: Nucleo-cytoplasmic Interactions in the Rat Liver Cell, 246
- Variability of Discharge Frequency between Primary and Secondary Muscle Spindle Afferent Endings of the Cat: Differences In (R. B. Stein and Dr. P. B. C. Matthews), 1217
- Variant in the Mouse: A Lactate Dehydrogenase (Mrs. L. Riles), 814
- Variation according to Latitude of the Electron Content of the Ionosphere near the Auroral Zone (L. Liszka), 280
- Variation of Cosmic Rays: Daily (J. B. Mercer and Prof. B. G. Wilson), 477
- Variation of the X-ray Sensitivity of *Serratia marcescens* with Relative Humidity (H. G. Morrison and Prof. D. V. Cormack), 91
- Variations In Ovarian Ascorbic Acid Content of Pseudopregnant Immature Rats used in Parlow's Test: Diurnal (Dr. M. de la Lastra and Dr. H. Croxatto), 789
- Variations on Doppler Shift at Low Elevations: Effects of Pronounced Electron-content (H. Soicher), 644
- Varieties of *Brassica oleracea*: Identical S Alleles in Different Botanical (K. F. Thompson and J. P. Taylor), 306
- Varley, Prof. G. C.: Catalogue of Type Specimens of Microlepidoptera in the British Museum (Natural History) described by Edward Meyrick, review, 108
- Varma, Dr. M. G. R., and Wallers, Miss W.: An Improved Method for obtaining, in vitro, Uniform Cell Monolayer Sheets from Tissues of the Tick, *Hyalomma dromedarii* (Ixodidae), 602
- Vascular Permeability Factor or Histamine by Specific Protease Inhibitor in Tissue Culture: Inhibition of Anaphylactic Release of (Prof. H. Hayashi et al.), 1007
- Vascular Plants of Europe: *Flora Europaea* (edited by T. G. Tutin et al.), review by Prof. J. A. Nannfeldt, 8
- Vasodepression: Pronethalol-induced Reversal of Adrenergic (Dr. M. G. Moreira and Dr. W. Osswald), 1006
- Vaughan, G. A., and Grant, D. W.: Determination of p.p.m. Concentrations of Carbon Disulphide in Benzene by Electron Capture Gas Chromatography, 75
- Vaughan, Dr. J. G., Waite, A., Boulter, Dr. D., and Walters, S.: Taxonomic Investigation of Several *Brassica* Species using Serology and the Separation of Proteins by Electrophoresis on Acrylamide Gels, 704
- Vavřínková, Dr. H., and Krondl, Dr. A.: Use of Polyethylene Glycol in Investigations of Absorption of Fat, 293
- Vegetable Extracts: Methylcholanthrene Carcinogenesis influenced by (V. Vendég), 598
- Vegetation of Nebraska: Native (Prof. J. E. Weaver), review by Dr. D. R. Hunt, 1249
- Vegetation of Uganda, 1047
- Veins and Pulmonary Embolisms induced by Catecholamines or Serotonin: Thromboses in Large (Prof. H. Selye et al.), 900
- Velocities of Interplanetary Dust Particles: Measured (C. S. Nilsson et al.), 673
- Velocity Dispersion in some Non-associated Organic Liquids: Acoustic (Dr. S. E. A. Hakim and W. J. Comley), 1082
- Velocity of a Charged Particle to its Relative Biological Effectiveness: Relationship of the (Dr. J. E. Turner) and (H. Hollister), 36
- Vendég, V.: Methylcholanthrene Carcinogenesis influenced by Vegetable Extracts, 598
- Venkatavaradan, V. S.: Antimatter and Tree Rings, 772
- Venoms of Two Pit Vipers: Norepinephrine and Serotonin in the Tissues and (Prof. A. H. Anton and Prof. J. F. Gennaro, jun.), 1174
- Verdier, Dr. P. H.: Appointment as a Physical Chemist in Institute for Materials Research of the U.S. National Bureau of Standards, 119
- Vernon, Dr. C. A., Gaudie, J., Hanson, J. M., Humphreys, J. M., Smith, P. E., Laurence, A. J., and Banks, Dr. B. E. C.: Acid Phosphatases, 382
- Vernon, Prof. Magdalen D.: Retirement from the Chair of Psychology in the University of Reading, 228
- Vernon-Roberts, Dr. B., et al.: Protective Effect of Oestrogens against the Toxic Decomposition Products of Tribromoethanol, 1098
- Vértes, Dr. L., and Kretzoi, Dr. M.: Lower Palaeolithic Hominid and Pebble-industry in Hungary, 205
- Verticillium albo-atrum* Reinke et Berthold: Phytotoxin by (Dr. G. F. Pegg), 1228
- Veterinary Non-proprietary Names, 630
- Vibration (Prof. R. E. D. Bishop), review by K. R. Weiss, 964
- Vibration: Matrix Analysis of (Prof. R. E. D. Bishop et al.), review by Prof. W. A. Tuplin, 616
- Vibrations (Prof. D. K. Magnus), review by K. R. Weiss, 964
- Vicinity of the Cetus Arc: Filamentary Nebulosity in the (Dr. J. Meaburn), 575
- Vickerman, Dr. Keith: Polymorphism and Mitochondrial Activity in Sleeping Sickness Trypanosomes, 762
- Vickery, Dr. R. C.: Nomadic Behaviour of Yttrium Ions and their Structure in Solution, 678
- Victorian Era: Science Papers of the, 935
- Vielrose, Egon: Elements of the Natural Movement of Population, review by E. Grebenik, 1032
- Villanueva, Dr. J. R.: Meeting of Biochemists in Spain, 632
- Vincke, I. H., et al.: Gametogenesis of *Plasmodium vincke*, 1230
- Vipers: Norepinephrine and Serotonin in the Tissues and Venoms of Two Pit (Prof. A. H. Anton and Prof. J. F. Gennaro, jun.), 1174
- Viral Infection in the Chicken Embryo: A New Syndrome: Hyperlipaemia following (Dr. S. E. Grossberg and Prof. W. M. O'Leary), 954
- Virgo Cluster Galaxies: Hydrogen Content of (Dr. B. J. Robinson and J. A. Koehler), 993
- Virus: Influence of Heavy Water (Deuterium Oxide) on Plaque Size of Western Equine Encephalitis (R. J. Carp and Dr. D. Kritchewsky), 93
- Inhibition of Transplantable and Spontaneous Murine Tumours by the M-P (Dr. N. Molomut and Dr. M. Padnos), 948
- Mechanical Transmission of Hop Mosaic (Dr. J. T. Legg), 1017
- Nucleic Acid of Infectious Laryngotracheitis (G. A. Tannock), 711
- Propagation and Properties of Hepatitis (Dr. Z. F. Ch. Kachani), 605
- Virus Aetiology for Down's Syndrome (Mongolism) (A. Stoller and R. D. Collmann), 903
- Virus (Bryan): Subcutaneous Sarcomata in Hamsters induced by Rous Sarcoma (Prof. M. L. Eidinoff et al.), 336
- Virus Complement-fixation Test: Rubella (Dr. H. Stern), 200
- Virus Haemagglutination by Polymerized Orosomucoid: Inhibition of Influenza (P. H. Whitehead et al.), 915
- Virus Hepatitis: Distribution of the Intestinal Factor of Normal Mice which inactivates Murine (Prof. M. Piazza et al.), 1009
- Virus in Arthropod Host: Microcrystal Formation: Plant Tumour (E. Shikata and Dr. K. Maramorosch), 507
- Virus-induced Leukaemia: Lymphatic Neoplastic Transformation of Thymus in Mice with Myeloid (L. Chicco-Bianchi et al.), 697
- Virus Infections in Dorset and their Similarity to Orf: Milker's Nodule (Dr. J. Nagington et al.), 505
- Virus Inhibitors: Derivatives of 2-Aminoethyl-guanidine and of a Eugenol as (S. Pácsa et al.), 409
- Virus-like Particles in Sub-acute Sclerosing Encephalitis (Dr. N. K. Gonatas and Prof. G. M. Shy), 1338
- Virus Multiplication by Puromycin and Actinomycin D: Stimulation of Sendai (Dr. D. O. White and I. M. Cheyne), 813
- Virus Ribonucleic Acid Replication: A New Model for (Dr. F. Brown and Dr. S. J. Martin), 861
- Viruses: Antiviral Action of Oxidized Spermine-Inactivation of Plant (U. Bachrach et al.), 1095
- Viruses: Transmission of Guanā and Oriboca, by Naturally Infected Mosquitoes (A. Toda and R. E. Shope), 304
- H-viruses in Human and Simian Cell Cultures: Growth and Cytopathogenicity of (Dr. H. Toolan and Dr. N. Ledinko), 812
- Viruses in Mice pretreated with *Escherichia coli* Endotoxin or infected with *Mycobacterium tuberculosis*: Interferon Appearance stimulated by Endotoxin, Bacteria, or (Prof. J. S. Youngner and Prof. W. R. Stenebrink), 456
- Visceral Mycoses: Fluorochrome Stains for Histological Diagnosis of (G. Bader et al.), 796
- Visit to the Lower Amazon (I. R. Bishop and Prof. G. E. H. Foxon), 114
- Visual Acuity: Monocular versus Binocular (Dr. F. W. Campbell and Dr. D. G. Green), 191
- Visual Observation of Satellites (D. G. King-Hele), 1156
- Visual Responses of Superior Colliculus Neurones: Somesthetic and (Dr. D. Jassik-Gerschenfeld), 898
- Vital Staining of the Granulated Juxtaglomerular Cells in the Mouse Kidney (V. Szokoly et al.), 1331
- Vocal Imitation: With Special Reference to the Antiphonal Singing of Birds: Origin and Significance of the Power of (Dr. W. H. Thorpe and M. E. W. North), 219
- Vojvodic, S., et al.: Effects of Dietary Levels of Phosphorus and Calcium on the Comparative Behaviour of Strontium and Calcium, 1110
- Volcanic Dust inferred from Solar Radiation Measurements: Stratospheric Transport of (Dr. A. J. Cyer and B. B. Hicks), 131
- Volcanic Succession: Re-classification of the Ethiopian Cainozoic (P. A. Mohr), 177
- Volcanological Research in Britain, 435
- Volleying: Upper Frequency Limits detectable in the Auditory System: Neural (Capt. J. C. Boudreau), 1257
- Vollrath, L., and Knowles, Sir Francis: A Dual Neurosecretory Innervation of the Pars Distalis of the Pituitary of the Eel, 1343
- A Functional Relationship between Neurosecretory Fibres and Pituitocytes in the Eel, 1343
- Von Denffer, D., et al. (rewritten by): Strasburger's Textbook of Botany, review by Dr. A. Allsopp, 618
- Von Zastrow, E. E., et al.: Semiconductor Controlled Rectifiers, review by Prof. W. Fishwick, 719
- WACHSMUTH, Dr. E. D.: Evidence for Structures resembling Crystalline Carbons at the Active Centre of Enzymes, 942
- Wacker, Dr. W. E. C., et al.: 'Buried' Tyrosyl Residues and the Activity of Trypsin, 1209
- Waddington, Prof. C. H.: Heredity and the Nature of Man, review, 959
- Wadsworth, Dr. G. R.: Fish as Food, Vols. 3 and 4, Processing, review, 317
- Wagenknecht, C., et al.: Enzyme Regulatory Mechanisms at the Level of Lactate-oxidoreductase in Erythrocytes and Ascites Tumour Cells, 185
- Wagner, Dr. C. D., and Geymer, Dr. D. O.: Mechanism of Radical Decay in Irradiated Polyethylene, 72
- Waite, A., et al.: Taxonomic Investigation of Several *Brassica* Species using Serology and the Separation of Proteins by Electrophoresis on Acrylamide Gels, 704
- Walters, S., et al.: Taxonomic Investigation of Several *Brassica* Species using Serology and the Separation of Proteins by Electrophoresis on Acrylamide Gels, 704
- Wakeley, H. G., and Kashin, P.: An Insect 'Bitometer', 462
- Waksman, S. A.: The Brilliant and Tragic Life of W. M. H. Haffkine; Bacteriologist, review by Dr. H. I. Jhala, 418
- Walker, E. P., et al.: Mammals of the World, Three Volumes, review by Dr. L. Harrison Matthews, 210
- Walker, Dr. J., and de la Mare, Mrs. G.: Shift Working: the Arrangement of Hours on Night Work, 1127
- Walker, Dr. P. M. B., and McLaren, Dr. A.: Fractionation of Mouse Deoxyribonucleic Acid on Hydroxyapatite, 1175
- Wallace, Prof. A., and Frolich, E.: Phosphorus-deficiency Symptoms in Tobacco and Transpirational Water Loss, 1231
- Walters, Miss Wendy, and Varra, Dr. M. G. R.: An Improved Method for obtaining, in vitro, Uniform Cell Monolayer Sheets from Tissues of the Tick, *Hyalomma dromedarii* (Ixodidae), 602

- Wallin, B. G., and Strickholm, Dr. A. : Intracellular Chloride Activity of Crayfish Giant Axons, 790
- Walsh, T. E., and Owen, T. : Radiation Balance of Jupiter and Saturn, 476
- Walter, Dr. Harry, Selby, F. W., and Francisco, J. R. : Altered Electrophoretic Mobilities of some Erythrocytic Enzymes as a Function of their Age, 76
- Walter and Eliza Hall Institute of Medical Research, Melbourne, Directorship, 729
- Walters, S. M., et al. (edited by) : *Flora Europaea*, Vol. 1, review by Prof. J. A. Nannfeldt, 8
- Wang, Prof. Kung-Tsung, and Tung, Y. C. : Polyamide Layer Chromatography of Oestrogens, 581
- Wang, Prof. Kung-Tsung, and Huang, J. Mei-Kuo : Polyamide Layer Chromatography of DNP Aminoacids, 281
- Wang, Mildred, et al. : Breakdown Rate of Serum γ 5 γ -Globulins in Mice, 251
- Wanless, R. K., et al. : Palaeomagnetism of the Isachsen Diabasic Rocks, 179
- Ward, Dr. A. : Distribution of Deoxyribonucleic Acid along Human Chromosomes, 486
- Ward, Dr. A., and McMaster, G. W. : Pattern Measurement, 428
- Ward, Prof. A. G. : Chemistry and Technology of Leather, Vol. 4, review, 617
- Ward, Dr. B. W. : Townsend's First Ionization Coefficient for Compressed Nitrogen, 994
- Ward, J. F., et al. : Site of Attack in the Radiolysis of Pyrimidine Compounds, 1086
- Wark, Dr. D. Q., et al. : Determination of Cloud Altitudes from *Gemini-Titan-5*, 775
- Warnick, F., et al. : Mammals of the World, Three Volumes, review by Dr. L. Harrison Matthews, 210
- Warren, E. W., et al. : Anticoccidial Activity of Nicotinamide Antagonists, 397
- Warted Pollen Grains of the Epacridaceae (Dr J. M. Matthews), 803
- Washington, D. C. : Smithsonian Institution, 320
- Water Affairs in Zambia, 120
- Water Balance and Evaporation Studies (M. R. Rao), 776
- Water : Colour of 'Pure' (Dr J. E. Tyler), 549
- Water Excretion in Single Nephrons of the Kidney of the Rat : Effect of 'Furosemid' on Chloride and (G. Malnic et al.), 80
- Water Loss : Phosphorus-deficiency Symptoms in Tobacco and Transpirational (Prof. A. Wallace and E. Frolich), 1231
- Water-soluble Structural Components of Human Red Cell Membrane : Heterogeneity of (Prof. M. D. Poulik and Dr. P. K. Lauf), 874
- Water : Use of 'Sephadex' Gel for the Estimation of Molecular Weight of Humic Substances in Natural (E. T. Gjessing), 1091
- Water using a Nuclear Emulsion : Determination of the Total Alpha Activity of Drinking (M. W. Tyrrell et al.), 1074
- Watkins, Dr. J., et al. : Breakdown of Serum γ 5 γ -Globulins in Mice, 251
- Watson, P. A., and Asquith, Dr. R. S. : Changes in Amino-nitrogen Content of Solutions of γ -Keratin from Wool Keratin, 786
- Watson, W. A., and Jones, Dr. O. T. G. : Activity of 2-Trifluoromethylbenzimidazoles as Uncouplers of Oxidative Phosphorylation, 1169
- Watts, J. O., et al. : Metabolites in Urine of Rats on Diets containing Aldrin or Dieldrin, 289
- Wave Mechanical Approach to the Plasma Interaction Problems (Prof. V. C. Liu), 883
- Wave Spectra of Polar Liquids : Sub-millimetre (Dr. G. W. Chantry and Dr. H. A. Gebbie), 378
- Wavelengths : Basic Component of Solar Radio Emission at Centimetre and Decimetre (Dr. M. K. Das Gupta and D. Basu), 739
- S Waves : Damping of (Sir Harold Jeffreys), 675
- Waves in a Magnetized Plasma : Experimental Observations on the Structure of Collisionless Shock (Dr. J. W. M. Paul et al.), 133
- Weale, Dr. R. A. : Development of the Eye, review, 979
- Wear of Teeth : Ingestion of Soil by Sheep in New Zealand in Relation to (W. B. Healy and T. G. Ludwig), 806
- Weardale Granite : Age of the (F. J. Fitch and Dr. J. A. Miller), 743
- Weatherall, Dr. D. J., Clegg, Dr. J. B., and Naughton, Dr. M. A. : Globin Synthesis in Thalassemia : an *in vitro* Study, 1061
- Weatherall, R. : Malaria and the Opening-up of Central Africa, 1267
- Weaver, Prof. H., Williams, Dr. D. R. W., Dieter, Dr. N. H., and Lum, W. T. : Observations of a Strong Unidentified Microwave Line and of Emission from the OH Molecule, 29
- Weaver, Prof. J. E. : Native Vegetation of Nebraska, review by Dr. D. R. Hunt, 1249
- Weaver, Dr. Warren : Elected to receive the Pacific Science Center Foundation Arches of Science Award, 326
- Webb, C. G., et al. : Evidence for the Release of Individual Amino-acids from the Resting Human Forearm, 588
- Webb, D. A., et al., (edited by) : *Flora Europaea*, Vol. 1, review by Prof. J. A. Nannfeldt, 8
- Webb, Prof. J. A. : Plant Physiology in Canada, 27
- Webb, Prof. John S., and Atkinson, W. J. : Regional Geochemical Reconnaissance applied to Some Agricultural Problems in Co. Limerick, Eire, 1056
- Webb, Prof. W. B., and Levitt, R. A. : Effect of Infantile Treadmill Experience on Body-weight and Resistance to Exhaustion in the Rat, 1128
- Webber, Prof. M. M., and Hintz, Dr. B. : Antichymic Antibody Localization in the Mouse, 797
- Weber, George, (edited by) : Advances in Enzyme Regulation, Vol. 2, review by Dr. C. J. R. Thorne, 319
- Webster, W. S., et al. : Deoxyribonucleic Acid Synthesis in Cultured Human Cells and its Bearing on the Concepts of Endoreduplication and Polyploidy, 242
- Weedon, Dr. D. S. : Corona Structures in the Basic Igneous Masses of East Aberdeenshire, 885
- Weeds in Rice Fields : Selective Control of, 1154
- Weevil Attack : Leucoanthocyanins of White Pine in relation to (F. S. Santamour, jun.), 407
- Weil, Dr. N. A. : Lunar and Planetary Surface Conditions, review by Dr. G. Fielder, 961
- Welmer, Prof. H. E., Benjamin, D. C., and Darcy, D. A. : Synthesis of α -Glycoprotein (Darcy) of Rat Serum by the Liver, 1221
- Weinberg, E., et al. : Timing of Protein Synthesis during Germination and Outgrowth of Spores of *Bacillus cereus* Strain T, 710
- Weiner, Dr. J. S., et al. : A Hundred Years of Anthropology, Third Edition, revised, review by Prof. M. Fortes, 215
- Weinreb, Dr. S., Meeks, Prof. M. L., Carter, J. C., Barrett, Prof. A. H., and Rogers, A. E. : Observations of Polarized OH Emission, 440
- Weintraub, J., and Simmons, R. L. : Transplantation Experiments on Placental Ageing, 82
- Weintroub, S. : Commercial Nuclear Power Stations in Britain, 25
- Institute of Physics and the Physical Society, 232
- Weir, J. R., et al. : Irradiation Embrittlement of Low-boron Type 304 Stainless Steel, 73
- Weiser, C. J., et al. : Some Postulated Relationships among Autumn Phenomena in Woody Plants, 89
- Wells, Prof. George H., et al. : Return of Leucocytes to the Bone Marrow in Chronic Myelogenous Leukaemia, 1281
- Weiss, Prof. H. S., Beckman, D. L., and Wright, R. A. : Delayed Mortality in Adult Chickens exposed to 1 Atmosphere Oxygen, 1003
- Weiss, Prof. Karl, and Moreau, W. M. : Charge Transfer Properties of *dl*-Thioctic Acid and Other Disulphides, 1203
- Weiss, K. R. : Vibrations (Prof. D. K. Magnus), review, 964
- Vibration (Prof. R. E. D. Bishop), review, 964
- Weiss, Dr. R. A. : Adaptive Growth, review, 107
- Weissberger, Dr. Arnold, (edited by) : Heterocyclic Compounds with Three- and Four-membered Rings, Parts 1 and 2, review by Dr. F. G. Mann, 2
- Weissmann, Dr. G., Sessa, Dr. G., and Weissmann, Dr. S. : Effect of Steroids and 'Triton X-100' on Glucose-filled Phospholipid/Cholesterol Structures, 649
- Weissmann, Dr. S., et al. : Effect of Steroids and 'Triton X-100' on Glucose-filled Phospholipid/Cholesterol Structures, 649
- Welding by High-velocity Particle Impact (Dr. R. F. Rolsten, A. K. Hopkins and H. H. Hunt), 1273
- Wellcome Laboratories of Tropical Medicine : New, 23
- Wellings, F. E. : Stratigraphy and Structure of the Eastern Aden Protectorate, review, 615
- Wells, Spencer : the Life and Work of a Victorian Surgeon (Mr. J. A. Shepherd) review by Sir Arthur MacNalty, 1135
- Welsh College of Advanced Technology, Cardiff, Appointment of Dr. J. D. Geddes as Professor of Civil Engineering, 1152
- Wendell-Smith, Prof. C. P., Blunt, Prof. M. J., and Hertz, Dr. L. : Possible Role of Neuroglia, 600
- Went Pea Test : Presence of Plant Growth Substances in Earthworms demonstrated by Paper Chromatography and the (R. L. Nielson), 1113
- Wernick, P. R., et al. : Effects of Hydrocortisone Succinate on the Complement System, 755
- Wertz, Dr. Richard K., O'Connor, C. C., Rabson, Dr. A. S., and O'Connor, G. T. : Mixed Infection of African Green Monkey Kidney Cells by Adenovirus 7 and SV 40, 1350
- West, Dr. G. B. : Serotonin, review, 1247
- West, Prof. J. C. : Electrical Machinery and Control, review, 9
- West, Dr. T. S. : Organic Complexing Reagents, review, 3
- West Africa : Introduction to the Flowering Plants of (Mts. M. Steentoft Nielsen), review by F. N. Hepper, 976
- Western Equine Encephalitis Virus : Influence of Heavy Water (Deuterium Oxide) on Plaque Size of (R. I. Carp and Dr. D. K. Ritchevsky), 93
- Weston, Dr. J. C. : Appointment as Director of Building Research, Ministry of Technology, 228
- Wharton, C. B., and Heald, Dr. M. A. : Plasma Diagnostics with Microwaves, review by A. E. Dangor, 965
- Wheat (Dr. R. F. Peterson), review by Dr. G. D. H. Bell, 976
- Wheat Flour : Proteins of (Dr. C. B. Coulson and Dr. A. K. Sim), 583
- Wheat Research Institute, New Zealand D S I.R., Director, 327
- Wheel-running Preferences of *Peromyscus* (Prof. J. L. Kavanau and Prof. D. H. Brant), 597
- Whistler, Prof. Roy L. (edited by) : Methods in Carbohydrate Chemistry, Vol. 4, Starch, and Vol. 5, General Polysaccharides, review by Prof. M. Stacey, 971
- Whistler-Mode Signals : Frequency Spectrum of Doppler-shifted (R. A. Morris and N. Poletti), 479
- White, Dr. A. J. R., and Taylor, Prof. S. R. : Geochemistry of Andesites and the Growth of Continents, 271
- White, Dinah : Changes in Size of the Corpus Allatum in a Polymorphic Insect, 807
- White, Dr. D. O., and Cheyne, I. M. : Stimulation of Sendai Virus Multiplication by Puromycin and Actinomycin D, 813
- White, Fred N., and Ross, Gordon : Blood Flow in Turtles, 759
- White, Prof. R. G., and Humphrey, J. H. : Immunology for Students of Medicine, Second Edition, review by Dr. P. D'Arcy Hart, 1028
- White Diamond : Laser-induced Damage in Natural (P. Whiteman and Dr. G. W. Wilson), 66
- White Pine in relation to Weevil Attack : Leucoanthocyanins of (F. S. Santamour, jun.), 407
- Whitehead, Dr. Mary : Presumed Sensory Cells in Fish Epidermis, 703
- Whitehead, P. H., Flewett, T. H., Foster, J. R., and Sammons, Dr. H. G. : Inhibition of Influenza Virus Haemagglutination by Polymerized Orosomucoid, 915
- Whiteman, P., and Wilson, Dr. G. W. : Laser-induced Damage in Natural White Diamond, 66
- Whittemore, Katharine S., and Layne, Dr. D. S. : Hydrolysis of Two 17 α -Oestradiol Glycosides by Rabbit Tissue Glycosidases, 288
- Whitten, Dr. Joan M. : Differential Deoxyribonucleic Acid Replication in the Giant Foot-pad Cells of *Sarcophaga bullata*, 1019
- Whyte, R. O. : Grassland and Fodder Resources in India, Revised Edition, review by Dr. W. Davies, 821
- Wickler, W. : Mimicry and the Evolution of Animal Communication, 519
- Wickler, Dr. W. : Signal Value of the Genital Tassel in the Male *Tilapia macrochir* Blgr (Pisces : Cichlidae), 595
- Wielebinski, Dr. R., and Yates, K. W. : Sky Brightness at 14.1 Mc/s, 64
- Wiggins, Mr. W. D. C. : Appointment as Director of Overseas Surveys and Surveys Adviser, Ministry of Overseas Development, 118
- Wigglesworth, Sir Vincent : The Juvenile Hormone, 522
- Wightman, Dr. W. P. D. : Ethology and the 'Baconian' Method, 918
- Science in the Nineteenth Century, review, 958
- Wild, Dr. D. G., and Midgley, Dr. J. M. : Synthesis of Ribonucleic Acid by *Escherichia coli* during Inhibition by Azetidine-2-carboxylic Acid, 585
- Wild Animals in Africa : Meat from, 122
- Wildfowl Trust, 733
- Wildlife : Research and Management in East African (Dr. J. Morton Boyd), 828
- Wiley, J. N., et al. : Prolonged Administration of Atropine or Histamine in a Silicone Rubber Implant, 591
- Wilhelm, Prof. D. L., and Carr, Dr. J. : Interspecies Differences in Response to Polypeptides as Permeability Factors, 653
- Wilkins, Prof. M. B., and Woodcock, A. E. R. : Origin of the Geoelectric Effect in Plants, 990
- Wilkinson, Prof. D. H. : Award of the Hughes Medal of the Royal Society, 1142
- Wilkinson, Prof. G., et al. : Homogeneous Hydrogenation and Hydroformylation using Ruthenium Complexes, 1203
- Williams, Prof. A. : Les Temps Fossilières, Vol. 1, Palaeozoïque Inférieur, review, 614
- Williams, Dr. D. R. W., et al. : Observations of a Strong Unidentified Microwave Line and of Emission from the OH Molecule, 29
- Williams, I. P., and Baines, Dr. M. J. : Growth of Interstellar Grains, 1191
- Williams, P. C. : Some Fundamentals of Information Retrieval, review, 822
- Williams, P. N. : *Obituary* of Prof. T. P. Hilditch, 730
- Williams, Prof. R. T., et al. : Thalidomide as a Possible Biological Acylating Agent, 1208
- Williams, Dr. T. I. (revised by) : A Hundred Years of Chemistry (Prof. A. Findlay), Third Edition, review by Dr. R. G. Partington, 969
- Williams, T. R., et al. : Selective Effect of Particulate Insecticides on *Simulium* among Stream Fauna, 199

- Williams, T. R. G., and Shurmer, C. R.: Effect of Temperature on the Discontinuity in the S/N Fatigue Curve of a Stainless Steel, 379
- Williams, Prof. Watkin: Genetical Principles and Plant Breeding, Botanical Monographs, Vol. 5, review by Dr. K. Jones, 7
- Williamson, B. H. J., and Lees, J.: Combined Very High Pressure/High Temperature Calibration of the Tetrahedral Anvil Apparatus, Fusion Curves of Zinc, Aluminium, Germanium and Silicon to 60 kilobars, 278
- Wilson, Dr. A. S., and Silva, D. G.: Ultrastructure of the Phrenic Nerve, 707
- Wilson, Prof. B. G., and Mercer, J. B.: Daily Variation of Cosmic Rays, 477
- Wilson, Dr. G. W., and Whiteman, P.: Laser-Induced Damage in Natural White Diamond, 66
- Wilson, K. R., and Herschbach, Prof. D. R.: Correlation of Sodium Atom Reaction Rates with Electron Capture Cross-sections, 182
- Wilson's Theory of Ice Ages (J. T. Hollin), 12
- Winegard, Prof. W. C., and Bell, J. A. E.: Interconnexion of Silicon in Modified Aluminium-Silicon Eutectic, 177
- Winter, Dr. G. C. B., and Yoffey, Prof. J. M.: Cytoplasmic Labelling with Uridine-5-³H in Human Lymphocytes cultured with Phytohaemagglutinin, 1018
- Winter Rye: Effects of Oxygen Pressure and Salts on Germination of (Dr. S. M. Siegel *et al.*), 1012
- Winter Scale Rings in *Lates niloticus* (Pisces: Centropomidae) from Lake Chad (A. J. Hopson), 1013
- Winterbourn, C. C., and Scott, P. J.: Role of the Gut in Synthesizing the Protein Component of Low-density (Beta) Lipoprotein, 494
- Wittgenstein, Eva, and Rowe, jun., Dr. K. W.: Agar-gel Electrophoresis of Soluble Lens Proteins in Galactose-fed Rats, 386
- Wodzicki, Dr. K. A.: Retirement as Director of the Animal Ecology Division of the New Zealand Department of Scientific and Industrial Research, 326
- Wolf Creek Iron Meteorite (Prof. S. R. Taylor), 944
- Wolfe, H. Glenn, *et al.*: Artificial Insemination of Dystrophic Mice with Mixtures of Spermatozoa, 1126
- Wolff, Dr. Torben: *Obituary* of Prof. R. Sparck, 625
- Wollenweber, Dr. J. E., *et al.*: Lactic Dehydrogenase Isoenzymes in Muscle from Patients with Duchenne Muscular Dystrophy, 1107
- Women: Fasting Levels of Growth Hormone in Men and (Dr. C. Faiman), 687
- Wong, Paul W. K., Shih, Ling-yu, Hsia, Prof. D. Y.-Y., and Tsao, Dr. Y. C.: Characterization of Glucose-6-phosphate Dehydrogenase among Chinese, 1323
- Wood, Dr. G. C., and Ferguson, J. M.: Metal Concentration Profiles in Oxide Scales on Nickel-10.9 per cent Cobalt Alloy, 369
- Wood, Dr. G. C., and Pearson, C.: A.C. Resistivity of Anodic Oxide Films on Valve Metals, 547
- Wood, J. A.: Metal Grains in Chondritic Meteorites, 1085
- Wood, Prof. K. D., and Wood, Dr. R. M.: Solar Motion and Sunspot Comparison, 129
- Wood, Dr. R., Kerr, J. R. W., and Richards, L. A.: Fall-out in London Rain-water during 1964, 1083
- Wood, Dr. R. M., and Wood, Prof. K. D.: Solar Motion and Sunspot Comparison, 129
- Wood, W. D., Deem, H. W., and Lucks, C. F.: Plenum Press Handbooks of High Temperature Materials, Vol. 3, Thermal Radiative Properties, review by Dr. E. H. Andrews, 517
- Wood: Fine Structure of (Dr. P. Rudman), 55
- Wood: Induction of Tension, with the Anti-auxin 2,3,5-Tri-iodobenzoic acid (Prof. R. W. Kennedy and Prof. J. L. Farrar), 406
- Wood Pulp: Manufacture of, review by Dr. J. Grant, 719
- Woodcock, A. E. R., and Wilkins, Prof. M. B.: Origin of the Geoelectric Effect in Plants, 990
- Woods, John F., and Nichols, jun., Prof. G.: Distribution of Collagenase in Rat Tissues, 1325
- Woods Hole Oceanographic Institution, Promotions, 327
- Woods: Indian, Vol. 2: Linaceae to Moringaceae (edited by S. S. Ghosh *et al.*), review by Dr. C. R. Metcalfe, 212
- Woody Plants: Some Postulated Relationships among Autumn Phenomena in (R. B. Van Huystee *et al.*), 89
- Wool Keratin: Changes in Amino-nitrogen Content of Solutions of γ -keratose from (Dr. R. S. Asquith and P. A. Watson), 786
- Woolwich, an Abnormal, Cameroon and Ibadan, Two Unusual Variants of Human Haemoglobin A. Three Haemoglobins K: (Dr. N. Allan *et al.*), 658
- Work: Shift Working: the Arrangement of Hours on Night (Mrs. G. de la Mare and Dr. J. Walker), 1127
- Working Environment: Ergonomics: Man in his (K. F. H. Murrell), review by C. E. Fleming, 106
- Working: the Arrangement of Hours on Night Work: Shift (Mrs. G. de la Mare and Dr. J. Walker), 1127
- World Health Organization, 1964, 437
- World: Mammals of the (E. P. Walker *et al.*), Three Volumes, review by Dr. L. Harrison Matthews, 210
- World Review of Nutrition and Diets, Vol. 5, (edited by G. H. Bourne), review by Dr. A. M. Copping, 421
- Worrell, Dr. Francis T.: Fremlin, Dr. J. H.: Calculations with Two Nearly Equal Quantities, 1306
- Worthington, Dr. E. B.: The International Biological Programme, 223
- Worthington, Dr. E. B., and McConnell, Dr. R. L.: Man-made Lakes, 1039
- Wortis, Dr. H. H., *et al.*: A Localized Haemolysis in Gel Method for the Detection of Cells producing 7S Antibody, 858
- Wound Infection: Glove Damage as a Cause of, 836
- Wragg, Josephine, and Contractor, Dr. S. F.: Resolution of the Optical Isomers of DL-Tryptophan, 5-Hydroxy-DL-tryptophan and 6-Hydroxy-DL-tryptophan by Paper and Thin-layer Chromatography, 71
- Wright, Evan, and Millar, K. R.: Use of Dimethyl Sulphoxide in Liquid Scintillation Counting, 889
- Wright, Dr. E. A., and Shewell, J.: Modification of Radiation 'Cerebral Death' by Hypoxia, 904
- Wright, James P.: Pulsation Periods of General Relativistic Objects, 65
- Wright, P. F., *et al.*: Mammals of the World, Three Volumes, review by Dr. L. Harrison Matthews, 210
- Wright, R. A., *et al.*: Delayed Mortality in Adult Chickens exposed to 1 Atmosphere Oxygen, 1003
- Wyard, Dr. S. J., and Elliott, Dr. J. P.: Electron Spin Resonance in X-irradiated Oriented Deoxyribonucleic Acid, 483
- Wyburn, Prof. G. M. (edited by), Pickford, Prof. R. W., and Hirst, Prof. R. J.: Human Senses and Perception, review by Prof. O. L. Zangwill, 981
- X-irradiated Lymphoid Cells or Monolayers of Mouse Embryo Cells: Plaque-formation by Non-immune and (G. Moller and E. Moller), 260
- X-irradiated Lyophilized Liver: Influence of Oxygen and Cysteine on the Radical State of (M. Abe *et al.*), 499
- X-irradiated Oriented Deoxyribonucleic Acid: Electron Spin Resonance in (Dr. J. P. Elliott and Dr. S. J. Wyard), 483
- X-irradiation and its Significance in the Potentiation of Barbiturate Hypnosis: Regional Changes in Brain Serotonin after Head (Dr. V. Nair), 1293
- X-ray Images of the Sun: Ionosphere, and (Dr. C. M. Minnis), 476
- X-ray Sensitivity of *Serratia marcescens* with Relative Humidity: Variation of the (H. G. Morrison and Prof. D. V. Cormack), 91
- X-ray Studies on Highly Pure Magnesium Oxide (Dr. R. A. Brown), 481
- X-ray Survival of HeLa Cells *in vitro* after Treatment with 'Methotrexate': A Reduced Oxygen Enhancement Ratio for (Dr. R. J. Berry), 1108
- X-ray Techniques: Structure of Lipids by Spectroscopic and (Dr. D. Chapman), review by Dr. F. D. Gunstone, 1246
- X-rays and Fast Neutrons: Increased Sensitivity of *in vitro* Murine Leukaemia Cells to Fractionated (Dr. W. L. Caldwell *et al.*), 168
- XXX Male: Aggressive Behaviour, Mental Sub-normality and the (P. A. Jacobs *et al.*), 1351
- YANPOL'SKII, A. R., and Lyus-ernik, L. A. (edited by): Mathematical Analysis: Functions, Limits, Series and Continued Fractions, review by Prof. T. A. A. Broadbent, 1140
- Yasuda, T., *et al.*: Measurement of Unsteady State Growth Rates of Micro-organisms, 263
- Yates, K. W., and Wielebinski, Dr. R.: Sky Brightness at 14.1 Mc/s, 64
- Yavalkar, V. D., *et al.*: Effect of Hydrolysed Glucose Cycloacetate in the Prevention of Fatty Infiltration in the Liver, 1001
- Yeast Form of *Paracoccidioides brasiliensis*: Glycogen in (Dr. F. Kanetsuna and Dr. L. M. Carbonell), 626
- Yeast: Formation of Hydroxamates during Proteolysis by an Enzyme System from (Dr. D. E. Briggs and Dr. D. J. Millin), 287
- Yeast: Nucleotide Peptides in (Dr. D. J. Millin and M. J. Saltmarsh-Andrew), 468
- Yero, I. L., *et al.*: NMN-Pyrophosphorylase Activity in Ascites Cell Extracts, 347
- Yoeli, Prof. M., Vanderberg, C. J., Upmanis, Miss R. S., and Most, Prof. H.: Primary Tissue Phase of *Plasmodium berghei* in Different Experimental Hosts, 903
- Yoffey, Prof. J. M., and Winter, Dr. G. C. B.: Cytoplasmic Labelling with Uridine-5-³H in Human Lymphocytes cultured with Phytohaemagglutinin, 1018
- Yoshihara, K., and Ebihara, H.: Evidence of Thermal and Hot Processes in the 'Hot-zone' produced by Copper-64 Recoil Atoms in Solids, 482
- Yoshinaga, M., *et al.*: Inhibition of Anaphylactic Release of Vascular Permeability Factor or Histamine by Specific Protease Inhibitor in Tissue Culture, 1007
- Youden, Dr. W. J.: Retirement from the Applied Mathematics Division at the U.S. National Bureau of Standards, 119
- Young, Dr. E. G., and Conway, Dr. E.: Seaweed Research, 840
- Young, Prof. F. G., and Martia, T. E.: An *in vitro* Action of Human Growth Hormone in the Presence of Actinomycin D, 684
- Young, Robert, and Ruddle, Dr. F. H.: Inactivation of T-2 Bacteriophage by Sensitized Leucocytes *in vitro*, 1105
- Youngner, Prof. J. S., and Snebring, Prof. W. R.: Interferon Appearance stimulated by Endotoxin, Bacteria, or Viruses in Mice pretreated with *Escherichia coli* Endotoxin or Infected with *Mycobacterium tuberculosis*, 456
- Yttrium Ions and their Structure in Solution: Nomadic Behaviour of (Dr. R. C. Vickery), 678
- Yuwiler, Dr. A., and Geller, Dr. E.: Serotonin Depletion by Dietary Leucine, 83
- ZACHARIAS, Bengt, and Holme, Prof. T.: Differences in the Antigenic Pattern of *Azotobacter* grown on Different Nitrogen Sources, 1235
- Zahalsky, A. C., and Marcus, Prof. S. L.: Interference by the Carcinogenic 4-nitro-quinoline N-Oxide of Tryptophan and Indole Uptake in *Escherichia coli*, 296
- Zaimis, Prof. Eleanor: Handbuch der Experimentellen Pharmakologie, Band 17: Ions Alkalino-Terreaux, Two Volumes in French, review, 720
- Zambia: Water Affairs in, 121
- Zangwill, Prof. O. L.: Human Senses and Perception, review, 981
- Zeigarnik, Dr. B. V.: Pathology of Thinking, review by Prof. R. C. Oldfield, 982
- Zelen, Dr. Marvin: Return of Leucocytes to the Bone Marrow in Chronic Myelogenous Leukaemia, 1281
- Zelotes: Cation Sites in Synthetic (Dr. T. I. Barry and L. A. Lay), 1312
- Zimmermann, B., *et al.*: Long-spacing Segments from Renatured α 1 Sub-units of Collagen, 685
- Zinc Alloys: Dispersion-softened (W. H. McCarthy *et al.*), 579
- Zinc, Aluminium, Germanium and Silicon to 60 kilobars: Fusion Curves of (J. Lees and B. H. J. Williamson), 278
- Zinc and Other Metallic Ions as Hatching Agents for the Beet Cyst Nematode, *Heterodera schachtii* Schn. (Dr. A. J. Clarke and Dr. A. M. Shepherd), 502
- Zinc Oxide Single Crystals: Effect of Ultra-violet Irradiation on the Electrical Conductivity of (Dr. R. B. Lal and Dr. G. M. Arnett), 1305
- Zinkham, Prof. Wm. H., KupcLyk, L., Blanco, A., and Isensee, H.: Polymorphism of Lactate Dehydrogenase Isozymes in Pigeons, 284
- Zirconium: Slow-bend Testing of Hydrozid (C. E. Coleman and Dr. D. Hardie), 69
- Zoberl, Dr. M. H.: Spore Discharge in *Lepiota konradii*, 405
- Zona Reaction in Five Pig Eggs: Failure of the (Prof. P. J. Dziuk and Z. Dickmann), 502
- Zonal Ultracentrifuge: Fractionation of Ribonucleic Acids in the (Dr. J. R. B. Hastings *et al.*), 645
- Zones in North Atlantic Deep-Sea Sediments: Pleistocene Glacial-Marine (Dr. J. R. Conolly and Dr. M. Ewing), 135
- Zoological Micropalaeontology: Principles of, Vol. 2 (Dr. V. Pokorny), review by Dr. R. H. Bate, 974
- Zoological Nomenclature: International Commission on, 1154
- Zoological Society of London, 1046
- 'Zoophycos': Origin of Fossil (Dr. M. Plička), 579
- Zuber, Dr. B. L., *et al.*: Suppression of the Papillary Light Reflex in Binocular Rivalry and Saccadic Suppression, 558
- Zuckerman, B., Willey, Prof. A. E., and Penfield, H.: OH Emission in the Direction of Radio Source W49, 441
- Zuffante, S. M., *et al.*: Afatoxin: Effect on Cultured Heteroplod Human Embryonic Lung Cells, 345
- Zweig, G., and Avron, Dr. M.: Dependence of Photophosphorylation by Isolated Chloroplasts on the Oxidation-Reduction State of N-Methylphenazinium Methyl Sulphate (Phenazine Methosulphate), 190
- XANTHINE Dehydrogenase by 4-Hydroxypyrazolo (3,4-d) pyrimidine: Inhibition *in vivo* of (E. C. Keller, jun., and Dr. E. Glassman), 202
- Xenon to Horse Haemoglobin: Binding of (Dr. B. P. Schoenborn), 760
- X-irradiated Dogs: Bone Marrow Allografts in Thymectomized (I. L. Chertkov *et al.*), 399
- X-irradiated Homocysteine-thiolactone: Electron Spin Resonance Investigations of Ultra-violet and (Prof. R. Koch and Dr. H. Monig), 86

Printed in Great Britain by FISHER, KNIGHT & CO LTD, The Gainsborough Press, St. Albans,
and published by MACMILLAN (JOURNALS), LTD, at 4 Little Essex Street, London, WC 2,
and ST MARTIN'S PRESS, INC, 175 Fifth Avenue, New York 10010, N.Y., U.S.A.
Saturday, May 21, 1966

NATURE

No. 5005

SATURDAY, OCTOBER 2, 1965

Vol. 208

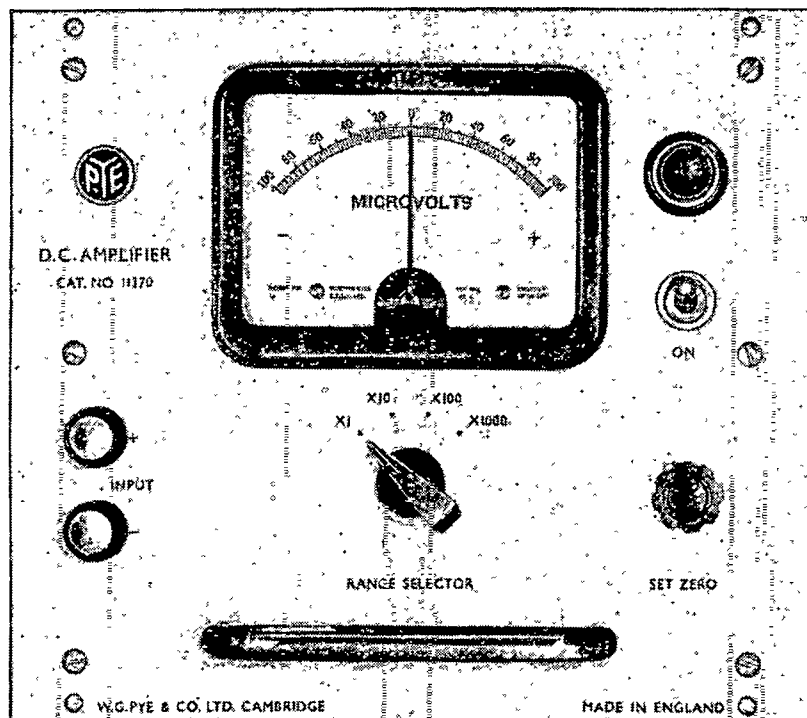
Published by
MACMILLAN (JOURNALS) LTD.

CONTENTS

	<i>Page</i>
INDUSTRIAL RESEARCH AND THE RESEARCH ASSOCIATIONS	1
CHEMISTRY OF THE SMALLER ORGANIC HETEROCYCLIC SYSTEMS. By Dr. F. G. Mann, F.R.S.	2
ANALYTICAL CHEMISTRY. By Dr. T. S. West	3
SOLID-STATE PHYSICS. By Prof. C. W. McCombie	4
SCHRÖDINGER'S LOGIC. By Prof. C. K. Grant	5
SPECTROSCOPIST'S VADE-MECUM. By Dr. D. T. Lewis, C.B.	5
THE CHROMAFFIN CELL. By Dr. B. A. Callingham	6
TECHNIQUES IN CELL METABOLISM RESEARCH. By Dr. J. H. Humphrey	6
SURGERY AND SCIENCE. By N. W. Nisbet	7
PRINCIPLES APPLIED. By Dr. K. Jones	7
VASCULAR PLANTS OF EUROPE. By Prof. J. A. Nannfeldt	8
 RADIO OBSERVATIONS OF SPIRAL GALAXIES. By Prof. B. Y. Mills, F.R.S., and J. R. Glanfield	10
WILSON'S THEORY OF ICE AGES. By John T. Hollin	12
"THERE IS ALL AFRICA." By Dr. A. S. Thomas	16
 NEWS AND VIEWS	19
 FUEL POLICY FOR BRITAIN	24
U.S. STANDARD FREQUENCY AND TIME SERVICES. By Dr. R. L. Smith-Rose, C.B.E.	25
COMMERCIAL NUCLEAR POWER STATIONS IN BRITAIN. By S. Weintraub	25
PHYSICS EDUCATION. By Dr. J. M. A. Lenihan	26
PLANT PHYSIOLOGY IN CANADA. By Prof. J. A. Webb	27
A REGISTER OF SCIENTIFIC RESEARCH IN THE NETHERLANDS. By S. T. Groenman	28
OBSERVATIONS OF A STRONG UNIDENTIFIED MICROWAVE LINE AND OF EMISSION FROM THE OH MOLECULE. By Prof. Harold Weaver, Dr. David R. W. Williams, Dr. N. H. Dieter and W. T. Lum	29
PRESSURE DISTRIBUTION IN THE TETRAHEDRAL ANVIL APPARATUS. By J. Lees	31
ELECTRIC POTENTIALS GENERATED BY FREEZING DILUTE AQUEOUS SOLUTIONS. By H. C. Patreira and A. J. Eydtt	33
RELATIONSHIP OF THE VELOCITY OF A CHARGED PARTICLE TO ITS RELATIVE BIOLOGICAL EFFECTIVENESS. By Dr. J. E. Turner and Hal Hollister	36
ADENOSINE TRIPHOSPHATE-METAL-NOREPINEPHRINE TERNARY COMPLEXES AND CATECHOL- AMINE BINDING. By Dr. Robert W. Colburn and Dr. James W. Maas	37
CO-ORDINATION CHEMISTRY AND MEMBRANE FUNCTION WITH PARTICULAR REFERENCE TO THE SYNAPSE AND CATECHOLAMINE TRANSPORT. By Dr. James W. Maas and Dr. Robert W. Colburn	41
ACETOHYDROXAMATE: BACTERIAL UREASE INHIBITOR WITH THERAPEUTIC POTENTIAL IN HYPERAMMONAEMIC STATES. By Dr. William N. Fishbein, Paul P. Carbone and H. Donald Hochstein	46
RAPIDLY LABELLED SYNTHESIS OF RIBONUCLEIC ACID IN EHRlich ASCITES C-2 TUMOUR CELLS TREATED WITH ACTINOMYCIN D. By Dušan A. Popović	49
MICROMETHOD FOR MEASURING MICROBIAL SENSITIVITY TO DRUGS. By Dr. Walter Koch and Dr. Deborah Kaplan	50
<i>Sargassum</i> TANNIN, AN ANTIBIOTIC WHICH RETARDS FOULING. By Prof. John McN. Sieburth and Prof. John T. Conover	52
COMPARISON OF THE CHORIOALLANTOIC MEMBRANE AND SPLENOMEGALY SYSTEMS OF GRAFT- VERSUS-HOST ASSAY IN THE CHICK EMBRYO. By L. W. Coppleston and Dr. Donald Michie	53
FINE STRUCTURE OF WOOD. By Dr. P. Rudman	55
A NEW CHEMICAL ASPECT OF PENICILLIN ALLERGY: THE DIRECT REACTION OF PENICILLIN WITH ϵ -AMINO-GROUPS. By Dr. C. H. Schneider and Dr. A. L. de Weck	57
OSMOTROPOTAXIS IN THE HONEYBEE. By Dr. H. Martin	59

(Continued overleaf)

Pye precision d.c. amplifiers



The Pye d.c. Amplifier, Cat. No. 11370, continuously monitors small voltage signals, such as those produced by many forms of transducer or detects the null point in various types of electrical measuring circuits and bridge networks. Its advantages are its freedom from vibration effects, constant response independent of bridge and circuit resistance and its capability to withstand overloads by virtue of the natural saturation of its actual amplifying circuit. Employing a contact modulator and special filter circuits it has been designed to operate without either input terminal grounded under all normal operating conditions. Its panel meter is scaled $100\mu\text{V}$ - 0 - $100\mu\text{V}$ in divisions of $2\mu\text{V}$ and range multipliers of $\times 1$, $\times 10$, $\times 100$ and $\times 1000$ are provided. Its gain is exceptionally stable and zero drift is less than $1\mu\text{V}$ per day. Response time of the panel meter is less than 1 second. Input resistance is 3500 ohms. An output of $\pm 10\text{V}$ at currents up to 5mA is provided for continuous recording and controlling. Primarily developed for the technologist this instrument is being used increasingly by research workers who require maximum stability and reliability.

W. G. Pye & Co. Ltd., P.O. Box 60, York St., Cambridge
Tel: Cambridge 58866 Grams: Pye Cambridge Telex: 81215

Pye is an acknowledged leader in the design of electronic measuring instruments which have faultless performance and exceptional reliability. In addition to various specialised d.c. amplifiers for chromatography, which measure currents as low as $1 \times 10^{-12}\text{A}$, are the null detectors/amplifiers listed below. Please write for details outlining your requirements.

d.c. Amplifier & Microvoltmeter, Cat. No. 11340/S.

A unique system of amplification which employs an oil-damped taut-suspension galvanometer modulator. Meter scaled $10\mu\text{V}$ - 0 - $10\mu\text{V}$ with range multipliers of $\times 1$, $\times 10$, $\times 100$, $\times 1000$. Input resistance is 50 ohms. Very low drift rate and noise level. Output 1mA f.s.d.

d.c. Nanoammeter, Cat. No. 11360.

A dual-purpose instrument similar to 11340/S but with ranges of $10\mu\text{V}$ - 0 - $10\mu\text{V}$ and $0.01\mu\text{A}$ - 0 - $0.01\mu\text{A}$ with multipliers of $\times 1$, $\times 10$, $\times 100$, $\times 1000$ on voltage and current. Very low drift rate and noise level 10^{-17} watts. Input resistance 1000 ohms. Output 1mA f.s.d.

Galvanometer Preamplifier, Cat. No. 11330.

Designed for fast response, highly sensitive null detection in d.c. bridge and potentiometer circuits. This robust, oil damped instrument gives an amplification of about 125 times the input current. Sensitivity with Scalamp Galvanometer, Cat. No. 7903/S, approx. $10000\text{mm}/\mu\text{A}$.

SCIENTIFIC  INSTRUMENTS

LETTERS TO THE EDITOR

RADIO ASTRONOMY		PHARMACOLOGY		
Sky Brightness at 14.1 Mc/s.—K. W. Yates and Dr. R. Wielebinski	64	Serotonin Depletion by Dietary Leucine.—Dr. Arthur Yuwiler and Dr. Edward Geller	83	
ASTROPHYSICS		IMMUNOLOGY		
Pulsation Periods of General Relativistic Objects.—James P. Wright	65	Mathematical Model for the Process of Aggregation in Immune Agglutination.—Tao Kuang Ming, Dr. Harold S. Goodman and Barry Brown	84	
Angular Momenta of Eclipsing Binaries and the Fission Theory of their Origin.—Ian W. Roxburgh	65	RADIOBIOLOGY		
PHYSICS		BIOLOGY		
Laser-induced Damage in Natural White Diamond.—P. Whiteman and Dr. G. W. Wilson	66	Electron Spin Resonance Investigations of Ultra-violet and X-irradiated Homocysteine-thiolactone.—Prof. R. Koch and Dr. H. Mönig	86	
Re-entrant Hysteresis Loop and Multistable Ferrites.—Dr. R. M. Glaister and Dr. E. W. Lee	67	Use of Technetium-99m in Hepatic Scintillography.—Dr. O. Degrossi, J. Martinez Seeber, H. Gotta, A. Olvari and A. Chwojnik	86	
A Numerical Model of Random Packing of Spheres.—Dr. M. M. Levine and J. Chernick	68	MICROBIOLOGY		
METALLURGY		VIROLOGY		
Slow-bend Testing of Hydrided Zirconium.—C. E. Coleman and Dr. D. Hardie	69	Multiple Thymus Grafts in Aged Mice.—Dr. Donald Metcalf	87	
MINERALOGY		CYTOLOGY		
Structure of Interstratified (Mixed-layer) Minerals.—Dr. Mitsuo Sato	70	Role of Sialic Acid in Potassium Transport of L1210 Leukaemia Cells.—Dr. J. Leslie Glick and Dr. Sherwood Githens III	88	
CHEMISTRY		GENETICS		
Resolution of the Optical Isomers of DL-Tryptophan, 5-Hydroxy-DL-tryptophan and 6-Hydroxy-DL-tryptophan by Paper and Thin-layer Chromatography.—Dr. S. F. Contractor and Josephine Wragg	71	Occurrence of Fructose-1-phosphate in Tissues of Higher Plants.—D. Graham and Dr. T. ap Rees	88	
Mechanism of Radical Decay in Irradiated Polyethylene.—Dr. D. O. Geymer and Dr. C. D. Wagner	72	Some Postulated Relationships among Autumn Phenomena in Woody Plants.—R. B. Van Huystee, C. J. Weiser and P. H. Li	89	
Irradiation Embrittlement of Low-boron Type 304 Stainless Steel.—W. R. Martin, J. R. Weir, R. E. McDonald and J. C. Franklin	73	Freeze-fixation, a New Method for Electron Microscopy.—Prof. O. Nilsson	90	
Boron-Nitrogen Bond Strengths in Borazoles.—Dr. B. C. Smith and Lambodar Thakur	74	SOIL SCIENCE		
Determination of p.p.m. Concentrations of Carbon Disulphide in Benzene by Electron Capture Gas Chromatography.—D. W. Grant and G. A. Vaughan	75	Fixation of Metabolic Products in the Soil during Decomposition of Carbohydrates.—Henning Sørensen		97
Separation of Coumarins.—R. S. Shah and Dr. S. L. Bafna	76			
BIOCHEMISTRY				
Altered Electrophoretic Mobilities of some Erythrocytic Enzymes as a Function of their Age.—Dr. Harry Walter, Fred W. Selby and Juanita R. Francisco	76			
Deterioration of Stored Inulin Solutions.—S. Nilwarangkur and Dr. G. M. Berlyne	77			
Interaction between Chlorophyll and Hydroquinone.—Eiji Fujimori and Maria Tavlá	78			
Biosynthesis of Triiodothyronine Sulphate by Beef Thyroid <i>in vitro</i> .—George L. Cohn	80			
PHYSIOLOGY				
Effect of 'Furosemid' on Chloride and Water Excretion in Single Nephrons of the Kidney of the Rat.—Gerhard Malnic, Francisco Lacaz Vieira and Hiroko Enokibara	80			
Feeding and Reproduction of Rats fed Calcium Cyclamate.—P. O. Nees and P. H. Derse	81			
Carbon Dioxide Production of Whole Blood <i>in vitro</i> .—L. H. Capel, E. C. Fletcher and Prof. J. F. Nunn	82			
Transplantation Experiments on Placental Ageing.—Richard L. Simmons and Joel Weintraub	82			

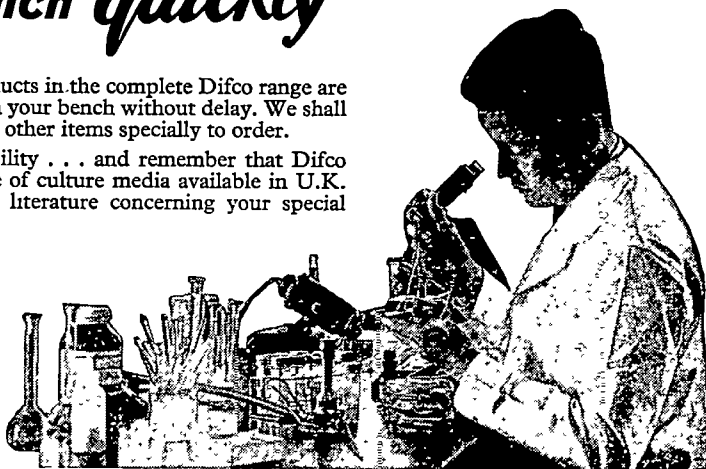


*Microbiological
reagents
and media*

**delivered
to your bench quickly**

Hundreds of different products in the complete Difco range are kept in stock ready to be on your bench without delay. We shall always be pleased to obtain other items specially to order.

Speed, convenience, reliability . . . and remember that Difco offer the only *complete* line of culture media available in U.K. Please send for the latest literature concerning your special interests



BAIRD & TATLOCK (LONDON) LTD., CHADWELL HEATH, ESSEX, ENGLAND
Branches in London, Manchester, Birmingham and Glasgow
Member of the Derbyshire Stone Group.

TAS/DO.13A



DIAMOND WHEEL TUBE AND ROD CUT-OFF MACHINE

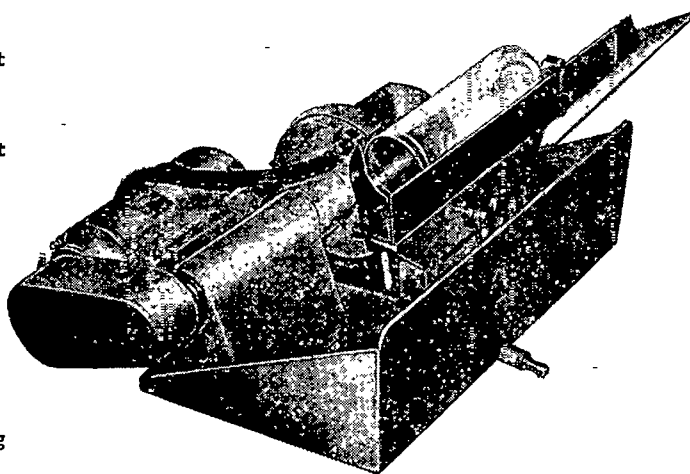
Cuts Glass, Refractories, Ceramics and Semiconductors

CAPACITY

With Large Angle Rest
Tubes from 2" dia.
to 4" dia.

With Small Angle Rest
Tubes from 0-2" dia.
Rods from 0-1 1/4" dia.

Angle Rests
Interchangeable
Mounting bench or stand
Finish grey waterproof
stove enamel
Drive flat endless belt
Adjustable stop on angle
rest for repetition cutting



Model TCO.104

SPINDLE

High speed vibration free
taper roller bearings
Grease lubrication

MOTOR

Totally enclosed fan
Cooled continuous rating

COOLANT

Main water or soluble
oil
Soluble oil pump and tank
unit a separate extra

DIAMOND WHEEL

Standard wheel 6" dia.
× 1 mm.
4" dia. × .015" can be
fitted for small Rods and
Tubes

CUTROCK ENGINEERING CO. LTD.

Phone Finchley 5978-9

35 BALLARDS LANE, LONDON, N.3

INDUSTRIAL RESEARCH AND THE RESEARCH ASSOCIATIONS

LAST February, in his Jubilee Memorial Lecture to the Society of Chemical Industry on the organization of basic research for the British chemical industry, Prof. J. W. Mitchell made some passing reference to the research associations, as well as to sponsored research and to the research institutes as supplementing the research effort within industry itself. The last two, he suggested, had four important functions: first, they could bring firms together in a joint project of mutual interest, though possibly not of immediate commercial significance, and probably at a smaller investment than would be required if the firms undertook it individually. This is obviously something in the nature of a small and temporary research association.

Next, they can supplement the firm's own industrial research effort at a time of crisis by undertaking sponsored confidential work. This represents in effect a temporary expansion of the firm's own research laboratories. Thirdly, Prof. Mitchell envisages such institutes as undertaking unsponsored basic research to acquire new knowledge of value to the industry as a whole, this function representing rather that of a central laboratory and perhaps more particularly the type of basic research in which some research associations seek to engage. Fourthly, he places the educational function arising out of the opportunities provided for graduates to extend their experience in research.

Prof. Mitchell's comments on the critical size of a research laboratory, which have previously been discussed (*Nature*, 207, 113; 1965), are highly pertinent here, as they are also in relation to his much briefer remarks on the work of research associations. The inability of most of the smaller firms to provide the financial resources required to establish laboratories of at least this critical size is one of the fundamental reasons for establishing a research association. Moreover, contrary to the customary protestation of the smaller firm, it is to them rather than to the larger firms of the industry that the benefits of co-operative research mainly accrue. Prof. Mitchell does not believe that sufficient basic research is as yet being undertaken in Britain to meet the needs of the medium-sized and smaller firms. Instead, he suggests that the more successful research associations have worked to improve services provided essentially on a non-competitive basis and have also undertaken research on improving and characterizing well-established products and developing equipment for standardization and control. He recognizes that difficulties arise over the granting of rights for developing and manufacturing new products based on discoveries or inventions made by a research association, but he does not refer to the inherent advantage that the industrial research laboratory inside a firm necessarily possesses over one outside through the closer contact with the production side or the customers' needs.

About the same time as Prof. Mitchell's lecture was delivered, the Association of Scientific Workers submitted to the Ministry of Technology a memorandum on industrial research associations. In this memorandum the Association argues that research associations should be a principal means for putting ideas across to industry and for making a scientific and technological impact on the industries served by the associations. A research association, it suggests, would serve its industrial members best if it consistently looked to the future, and to a fairly long-

term future. The associations should also accept a considerable responsibility for disseminating information, particularly to the smaller firms which are less likely to spend money and effort on an internal information service. This function is regarded as including the use by management of the information already available.

The memorandum admits that there are wide differences in the effectiveness with which different research associations fulfil their role, and these differences are attributed at least in part to the methods by which the research associations are financed—this affecting in turn the amount of money available. Recognizing also that industries which are lagging technically tend to lose interest in research and become even more backward, the memorandum suggests that amalgamation of appropriate research associations might give a firmer basis for development and that it would be worth while examining industries without co-operative research and co-ordinating the research efforts of companies undertaking substantial research in the same field. This latter somewhat naïve suggestion is not discussed further, but it is proposed that all research associations should operate within a national plan and be linked with the economic development council structure.

The present methods of finance are then criticized in some detail, especially for their failure to provide sufficient assurance of a reasonable level of income over a period of, say, five years: this is regarded as quite impossible if a research association has to depend on voluntary subscriptions. The position is made worse by the instability of prices and recurrent mergers and the like. The memorandum criticizes the progressive stiffening of grant terms by the Department of Scientific and Industrial Research, and it makes the valid point that too much effort has to be spent by directors of research in securing funds for research. Earmarked grants and civil research contracts are regarded as tending to increase financial strain and should not be looked on as a substitute for improved contributions from the Government to the regular programme.

As a consequence of this financial situation, the facilities for research in most associations compare poorly with those of Government stations. Total expenditure per qualified scientist and engineer in the research associations is put at much lower than the estimated average of £8,000 for all manufacturing industries in 1960; that for the research stations of the Department of Scientific and Industrial Research (now the Science Research Council) exceeds this figure. The consequent poor salary scales and prospects compared with the Scientific Civil Service make it difficult to attract and retain competent staff, and on the whole staff relations generally need improvement. The Association of Scientific Workers contends that an assured, adequate and independent income would immediately improve such relations. The unsatisfactory financial situation, of course, also hinders the acquisition of modern scientific equipment.

The system of finance is intimately linked with the method of control, and the memorandum maintains that the methods practised by the heads of businesses who dominate the Council of Research Associations are not always appropriate to the needs of technical departments. Moreover, financial and general control is largely a part-time interest of members of these Councils, leading to

haphazard and meretricious decisions, which may be based on narrow financial considerations. As a result, levels of expenditure may fall below the minimum necessary for efficiency and effectiveness, and voluntary levies agreed with the Board of Trade may not necessarily be fixed to ensure a sufficient level of expenditure. The memorandum also asserts that representations of the Department of Scientific and Industrial Research on the Councils and the Official Visitors are ineffective in influencing policy in proportion to the Government stake in the research associations.

Seven proposals are advanced in the memorandum to improve this position. First, a compulsory levy of 0.2 per cent of net output of the industries concerned is suggested in order to double the present total income of £10 million. Within this concept of a statutory levy, the Government contribution could be increased where necessary to pound for pound with the industrial contribution. Specifically, the appropriate Government agency should examine the need for establishing a research association, with a compulsory levy, for certain sections of the chemical industry. Possible amalgamations should be carefully considered and, in consultation with industry, the Minister of Technology should examine the programmes of all research associations. One function of the research associations should be to advise the Government in connexion with placing civil research and development contracts with industry. Establishment of an effective advisory service, using the most advanced techniques, is also recommended to ensure the dissemination of knowledge and techniques and their application to industry, and all research associations should participate in this service.

To some extent this last-mentioned recommendation anticipated the establishment of the Office of Scientific and Technological Information, but its effectiveness presupposes the existence within the small firm of those at the top capable of appreciating the significance of new knowledge and possessing the authority to take appropriate action. In most small-scale industry this condition is not fulfilled, and here, like so many other reports and memoranda, the memorandum is not sufficiently ruthless. There is too much disposition in Britain to protect the inefficient and it is this practice above all that must be ended. The research associations will never serve Britain effectively if they are used to feather bed the inefficient and backward.

Also early this year Dr. F. N. Woodward, scientific counsellor to the Organization for Economic Co-operation and Development, submitted to the Organization a survey of the different types of research association in member countries with particular reference to their organization, co-ordination, methods of operation and resultant benefit to the industries they serve. This report has now been published under the title *Structure of Industrial Research Associations**, but, although at some points the report supports some of the recommendations of the Association of Scientific Workers, there is such wide variation in the extent and character of Government participation from country to country that generalization is extremely difficult. Very little light is, in fact, thrown on the deeper aspects of co-operative research and the conditions in which it functions most successfully. By and large, the survey adds surprisingly little to the assessment of the research association movement in Britain contained in a series of articles in *Research* and since published as a

supplement to Volume 15 in 1962, or even, for that matter, to Prof. R. S. Edwards's earlier book, *Co-operative Industrial Research*, or the report of the Federation of British Industries on *Industrial Research in Manufacturing Industry, 1959-60*.

The factual data in Dr. Woodward's report show that the industrial research association has developed to a much larger extent in the United Kingdom than elsewhere in Western Europe: the income in Britain of some £10 million in 1962 was more than double that in Germany, which comes next, though it is less than that expended in France on the rather different industrial research centres, which amounted to some £16.7 million in 1961. The idea, moreover, differs significantly in structure and working philosophy from country to country, and its development from the British prototype has been influenced to a marked degree by historical background and local requirements. None the less, the disadvantages and shortcomings of the research association scheme, as revealed by Dr. Woodward's enquiry, coincide with observations in the memorandum from the Association of Scientific Workers. This is notably so in the criticism of the excessive proportion of time which a director of research is required to spend on raising money, of the slender resources of most of the associations and their inherent disadvantage as against the industrial research laboratory of a private firm.

In addition, the research associations in general are regarded as not possessing a good public image either in the industries which they serve or in the scientific world from which they must attract their staff. This is unlikely to be assisted by the development of contract research. It is at this point that there is need for more fundamental and independent thinking about the precise functions of the research associations even in those fields in which co-operative effort, so far from being at a disadvantage compared with private enterprise, is the most economic as well as the most potentially fruitful method. It should be recognized that while a certain minimum scale of effort is essential, that scale should not be secured by the use of public money to support the inefficient or reluctant, and that there are circumstances in which a Government research station may be a more effective and prudent answer than an amalgamation of research associations or a solution involving the coercion of reluctant firms. There is warning enough of the dangers of precipitate action in Dr. Woodward's survey to suggest a cautious approach to some at least of the proposals of the Association of Scientific Workers. Certainly, the hard thinking and further enquiry necessary as to the merits of private or public enterprise should be kept free from any party political prejudice.

CHEMISTRY OF THE SMALLER ORGANIC HETEROCYCLIC SYSTEMS

Heterocyclic Compounds with Three- and Four-membered Rings

Edited by Arnold Weissberger. Part 1: Pp. xi+1-646. Part 2: Pp. xii+647-1174. (The Chemistry of Heterocyclic Compounds: a Series of Monographs, Vol. 19, Parts 1 and 2.) (New York and London: Interscience Publishers, a Division of John Wiley and Sons, Inc., 1964.) 488s. the two parts.

THESE two books, each separately bound and of considerable size, form Parts 1 and 2 of Volume 19 in the series *The Chemistry of Heterocyclic Compounds*

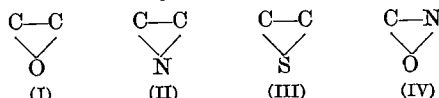
* *Structure of Industrial Research Associations*. By F. N. Woodward. Pp. 55. (Paris: Organization for Economic Co-operation and Development, London: H.M.S.O., 1965.)

which, under the able editorship of Dr. A. Weissberger, have been appearing steadily and (in general) with increasing size since 1950. Some of the more recent volumes have appeared in two or more parts, and the number of actual books in the series is now twenty-five.

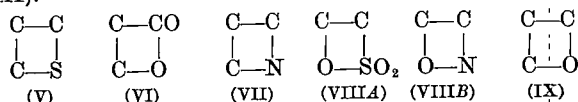
Part 1 deals with four types of three-membered ring systems and Part 2 with six types of four-membered systems: the pages of the two Parts are numbered as one volume, and Part 2 has an author and a subject index covering both Parts.

In view of the number of volumes which have preceded Volume 19, it might cause some surprise that these smaller and simpler systems have hitherto escaped attention in this series. Dr. Weissberger in the preface points out that heterocyclic chemistry has two closely related aspects, namely, the investigation of different derivatives of the respective cyclic systems, and that of the reactions in which the heterocyclic systems themselves undergo change. The latter aspect, he notes, is particularly important with heterocyclic systems having less than five atoms in the ring, some of which belong to the most reactive compounds in organic chemistry. This high reactivity undoubtedly underlies the recent wide extension of the study and the technical application of most of these compounds: it also probably explains the comparative slowness of earlier development, for less reactive and more stable systems attracted greater attention.

The contents of the two Parts can be briefly indicated. Part 1 contains chapters devoted individually to the systems listed as ethylene oxides (I); aziridines (II);



ethylene sulphides (III) and oxaziranes (IV). Part 2 contains the systems thietanes (V); β -lactones (VI), trimethyleneimines (VII), and a brief note on β -sultones (VIII A) and 1,2-oxazetene (VIII B), and finally oxetanes (IX).



An interesting point of nomenclature arises here. The International Union of Pure and Applied Chemistry (IUPAC) has recommended specific names for almost all heterocyclic systems up to the 10-membered units, and these names are now incorporated in the recent second edition of the *Ring Index* and its two supplementary volumes. Some of these names are far from self-explanatory and differ markedly from the older accepted names. It is interesting to note the extent to which they are accepted and used by the authors of Parts 1 and 2.

Dr. André Rosowsky entitles Chapter 1, on the compounds having the reduced system (I), "Ethylene Oxides", and points out that the IUPAC name is 'oxirane', and states: "in deference to common usage the present chapter will retain the terms 'apoxide' and 'ethylene oxide'". Dr. Fanta in Chapter 2 uses the systematic name 'aziridines' for compounds having the reduced system (II). Dr. Reynolds and Dr. Fields (Chapter 3) give the title "Ethylene Sulphides" to compounds having the system (III) and state: "The name thiirane is in accord with good nomenclature, but it has been slow to find a place in the literature". Dr. Emmons adopts the systematic name 'oxaziranes' throughout Chapter 4 for compounds having the system (IV). Dr. Etienne and his collaborators in Chapter 5 use the term 'thietane' for compounds having the reduced ring (V), but abandon it for the corresponding sulphonium salts (p. 650). Chapter 6 he entitles " β -Lactones", and states: "Nor does the (systematic) designation 2-oxetanone . . . seem to have been adopted". Dr. Moore gives the name "Trimethyleneimines" for Chapter 7 on compounds having the reduced

system (VII), but in the text refers to them throughout as "azetidines". Systematic names are used for the remaining three systems. This summary will indicate the degree of confusion which still exists in the nomenclature in use for even simple heterocyclic systems.

These two books contain such a wealth of information that a true review, in the sense of a critical assessment of the merits and demerits of the work presented, cannot be briefly achieved. The general nature of the treatment of each class, and of the information presented, can best be shown by reference to Dr. Rosowsky's excellent exposition of the ethylene oxides in Chapter 1, which, running to 523 pages finishing with 2,040 references, is almost a book in itself. It contains the sections: (1) "Physical Properties" (that is, molecular geometry; energetics; infra-red, ultra-violet and nuclear magnetic spectroscopy; theoretical models); (2) "Occurrence of Epoxides in Nature"; (3) "Synthesis of Epoxides"; (4) "Chemical Reactions of Epoxides"; (5) "Analytical Methods"; (6) "References": sections (3) and (4) are presented in considerable detail. This chapter incidentally reveals the contribution made by the French chemists, from the early work of Wurtz to the later work of Tiffeneau and others.

The remaining chapters follow much the same pattern, and the complete volume forms a very thorough and highly valuable record of chemistry of these smaller ring systems.

It is noteworthy that the synthesis of oxaziranes (Chapter 4) was first achieved in 1956-57 by three different research groups, and that in Chapter 8 only one example of a 1,2-oxazetidine, prepared by Staudinger and Jelagen in 1911, can be recorded. Our knowledge of the chemistry of such systems must still be incomplete.

One might perhaps question the space devoted in Chapter 3 to the "Table of Physical Properties" (6½ pages), for only the boiling (or melting) points are recorded for certain compounds: similarly in Chapter 6, Table 2 (18 pages) and Table 3 (10 pages) giving the physical properties of aliphatic and aromatic β -lactones, respectively, also provide very limited information, and certain pages of the Table contain only three of the more complex derivatives. The sections in this Chapter on the preparation of β -lactones have been largely anticipated by the recent Volume 7, Part 2, of *Methoden der Organischen Chemie*, by Houben-Weyl (*Nature*, 204, 1122; 1964): the latter is, however, strictly limited to synthetic methods, whereas Chapter 6 of the present work gives a full treatment of the whole subject.

It should be mentioned that the preface concludes with an expression of deep gratitude from Dr. Etienne and his colleagues for the faultless translation of their Chapters 5 and 6 from the French by the late Mrs. Weissberger, to whose help in his life and work Dr. Weissberger pays an eloquent and moving tribute.

F. G. MANN

ANALYTICAL CHEMISTRY

Organic Complexing Reagents

Structure, Behavior, and Application to Inorganic Analysis. By D. D. Perrin. (Chemical Analysis: a Series of Monographs on Analytical Chemistry and Its Applications, Vol. 18.) Pp. xi + 335. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, Inc., 1964.) 90s.

THE doyen of analytical chemistry, Fritz Feigl, has remarked that the story of modern analytical chemistry is the story of co-ordination chemistry. This remark has made a profound impression on many a young research worker, as has S. E. Q. Ashley's more recent comment that the chemistry is going out of analytical chemistry. These observations apparently point in two directions, but in truth they do not really conflict. While

an ever-increasing proportion of analyses is being carried out instrumentally rather than by balance, beaker and burette, the instrumental hardware, except in a few cases such as mass spectrometry, relies on chemical reactions. These reactions are largely based on complexation and are usually further backed up by refined modern separation techniques which again rely almost exclusively on complexation. The comments may, therefore, be regarded as complementary.

Complex formation between aquo-metal ion complexes and neutral or negatively charged ligands extensively modifies the normal (aqueous) chemical reactions of most ions and many characteristic physical properties usually associated with them. Thus many metal ions become central in anionic complexes and hence respond differently to sorption processes such as ion exchange and ion association reactions. Many become bound in uncharged complexes and precipitate from solution or can be persuaded to partition preferentially into water immiscible solvents. Some even become capable of being volatilized or distilled. In most cases changes are brought about in absorption characteristics in one or more regions of the electromagnetic spectrum. Not infrequently unusual valency states are preferentially stabilized by complexation, and the changes in oxidation potential which accompany such reactions open out avenues of examination by electrochemical techniques. Complex formation even extends a profound influence on to flame plasma techniques such as atomic absorption spectroscopy and flame spectrophotometry. In these cases, the supply of cations bound as neutral chelate or ion-associated complexes and dissolved in organic solvents can give considerable enhancement of sensitivity by purely mechanical surface tension/viscosity effects and can produce new reactions due to shifts from (normal) endothermic to exothermic dissociation-decomposition mechanisms. Many interionic interferences in flame techniques are also simply eliminated by virtue of complex formation. Similarly many apparently intractable techniques can be profoundly indebted to complex formation for their success. Thus, radioactivation analysis relies heavily on the solvent extraction of chelate complexes from aqueous solution to achieve the separation-concentration processes on which the radiocounting procedures depend.

Most modern texts on analytical chemistry are, of course, concerned with instrumental analysis and are at pains to emphasize the basic physical chemistry, the physics and even the electronics underlying these procedures. Few of them pay much attention to the chemistry involved. *Organic Complexing Reagents* is concerned with just this facet and takes the same pains to set the scene behind the chemical reactions in terms of modern theories of inorganic chemistry. In my opinion it succeeds admirably in this and the result is stimulating and worthwhile. The only book which has previously been written on this topic is Feigl's *Specific, Selective and Sensitive Reactions*, and although the latter is now sixteen years old, it is still one of the most fascinating and important of all books on analytical chemistry. This new book is written from the same point of view of basic chemical reactivity and is the first real development since the original publication. Unquestionably this is an important book of unusual interest and should be found by most analytical chemists and by many others to be a sparkling jewel of chemical interest set against the grey background of instrumental methodology on the bookshelf. It is equally certain that it will bid fair to become one of the most thumbed volumes on the shelf because of the wide-ranging nature of its interests.

The text itself is well arranged. A short chapter on chemical bonding set in terms of valence bond, molecular orbital and ligand-field theory follows a brief introductory survey of the phenomena of complex formation between metal ions and organic ligands, and sets the scene for subsequent chapters. This treatment is concise and en-

tirely adequate for those who have had some experience of modern theories, but those who are not familiar with them may well have to supplement their knowledge by further reading from the recommended list of books in order to make full use of the discussions which follow in subsequent chapters. The stereochemical configuration and stability of metal complexes are considered in terms of polarizability and covalent character and the thermodynamic nature of the formation reaction, etc. Numerous examples of ligand-field effects are cited in the transition series. The effects of complexation on oxidation potential are discussed in relation to factors which determine the magnitude of these potentials, ligand-field stabilization energies and so on, with examples drawn chiefly from among iron and copper complexes and cyanide complexes in the transition series.

A résumé of factors which govern rates of formation and dissociation introduces a chapter of a more applied nature on the effects of complexation on the concentration of cations in aqueous media and which is, of course, principally concerned with compleximetric titration, with masking and with demasking. Surface phenomena associated with complex formation are related to analytical techniques such as ion-exchange, chromatography, use of adsorption indicators and, somewhat illogically in this context, polarography. The chapter on visible and ultraviolet absorption spectra includes some useful notes on types of spectra and considers re-emission phenomena in relation to fluorescence and chemiluminescence. It also incorporates an interesting survey of the design of ligands in relation to absorption spectrophotometry. The two chapters on the solubility of complexes and extraction into organic solvents give an excellent coverage of their subject-matter and relate gravimetric and separation procedures to the nature of the reagent reacting with the metal cation. The reaction of inorganic anions with organic reagents and with metal complexes is considered from a variety of angles in a short but thought-provoking section. The final three chapters deal with procedures for detecting and determining inorganic neutral molecules, the general analytical properties of the elements and with some speculative matter on seeking new organic reagents for use in inorganic analysis. The book ends with a full subject index; there is no author index.

This, to my mind, is one of the most important books to have been written on inorganic analytical chemistry within recent years.

T. S. WEST

SOLID-STATE PHYSICS

Solid State Physics

Advances in Research and Applications, Vol. 15. Edited by Frederick Seitz and David Turnbull. Pp. xvi + 505. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1963.) 118s.

THE series of volumes entitled *Solid State Physics* has come to be of considerable importance to solid-state physicists. For most people active in this field there are several articles, scattered through the volumes, to which they and their research students return again and again. The articles are, for the most part, closely written and demand quite intensive study if they are to yield a substantial portion of their content. Most will offer something to the generally informed physicist who reads quickly through them, but this is not their main function. One wonders in some cases whether this class of reader, and even the experts and prospective experts (not to mention reviewers), might be helped by a somewhat more emphatic distinction between the main points to be made in the article and subsidiary elaboration. The articles fall little short of small books, and a suitably discreet modification of the 'on a first reading' formula might not be inappropriate.

Volume 15 of the series resembles previous ones in being occupied mainly by essentially theoretical articles in which the properties of well-defined models are worked out and compared, more or less thoroughly, with experiment. The longest article, that by R. W. James on the "Dynamical Theory of X-Ray Diffraction", falls very clearly into this category. It will prove of great importance to workers in electron diffraction as well as to those concerned with X-rays. In treating the "Elementary Theory of the Optical Properties of Solids", F. Stern combines a discussion of phenomenological electromagnetic properties, including the wave-length dependent dielectric constant, with treatments of the free electron gas, electrons and holes in semiconductors and optical modes in ionic crystals: the application of dispersion relations is considered. L. J. Sham and J. M. Ziman discuss carefully the basic concepts of "Electron-Phonon Interaction" and the resulting electron scattering in solids. The considerations which they present resolve a number of questions and doubts on some fundamental points, and lead to clear definitions of the matrix elements which appear in the theory: topics treated include deformation potential, pseudopotential and screening effects. In discussing "Spin Temperature and Nuclear Relaxation in Solids", L. C. Hebel impresses one further with the rich interplay of theoretical and experimental activity (theorist and experimenter often being the same person), which has characterized work in nuclear magnetic resonance.

The first article in the volume, that by G. Borelius on "Changes in Energy Content, Volume, and Resistivity with Temperature in Simple Solids and Liquids", is in a different category. Here the experimental results dominate, and such theoretical discussion as there is frequently emphasizes the apparent inadequacies of present-day theory. It is good to see this approach represented.

C. W. McCOMBIE

SCHRÖDINGER'S LOGIC

My View of the World

By Erwin Schrodinger. Translated from the German by Cecily Hastings. Pp. viii + 110. (Cambridge: At the University Press, 1964.) 18s. net; 3.50 dollars.

THIS book consists of two long essays, hitherto unpublished. Although separated by a long period—the first was published in 1925, and the second in 1960—there are close links between them in respect of subject-matter and treatment. There are also affinities with the reflexions with which Schrodinger concluded his book *What is Life?* (1944), although this book is not mentioned in the present work.

In these pages Schrodinger puts forward a philosophical and indeed metaphysical view of the world, of human experience and of human nature. He rightly does not attempt to derive these conclusions from theories in the natural sciences but adopts a starting point which is philosophical—that is to say, one in which the sciences themselves are located in the wider context of human experience of the world and of moral relationships. Schrodinger accepts the validity of Kant's criticism of speculative metaphysics but argues, in my view correctly, that to some degree metaphysics, in the sense of pre-suppositions of enquiry, is indispensable. The particular problem which concerns him is the basic one of the relation between experience and reality. He points out that the commonsense view that independent observers, or 'selves', have similar experiences of the same external world is not self-authenticating; here Schrodinger is clearly under the influence of his mentors, Mach and Avenarius. There is no doubt that Schrödinger is right in saying that this is a metaphysical point of view in the sense explained. It is with less plausibility that he seeks to supplant it with an alternative and indeed inverted metaphysical doctrine which he derives from the Vedanta.

Instead of thinking of different selves experiencing a common world, he invites us to think of a multiplicity of selves sharing a common consciousness. According to this idea there is no ultimate distinction between different minds or indeed between consciousness and its object. To a Westerner, at any event, this is a paradoxical view which lacks inherent plausibility; unfortunately Schrodinger does not offer convincing arguments in its support.

Another difficulty is that Schrödinger appears to think that it goes some way to solving a problem the genuineness of which is very much open to doubt. "If she who is now your mother had cohabited with someone else and had a son by him, and your father had done likewise, would you have come to be? Or were you living in them, and in your father's father . . . thousands of years ago? And even if this is so, why are you not your brother, why is your brother not you, why are you not one of your distant cousins?" To ponder why I am not my brother, or where or what I would be if my parents had not met, is to induce a kind of intellectual vertigo for which the cure is not the Vedanta, but rather a closer attention to the logic of our language. The reason why I am I and not someone else, or that this pencil is this pencil and not another pencil, is to be found in the logical principle of identity—that is, a tautology. Schrodinger is not the first, and will not be the last, to find in logic a source of wonderment, and in metaphysics an apparent resolution of it—but what precisely is the 'problem', as distinct from the wondering?

C. K. GRANT

SPECTROSCOPIST'S VADE-MECUM

Flame Spectroscopy

By Radu Mavrodineanu and Henri Boiteux. (Wiley Series in Pure and Applied Spectroscopy.) Pp. xiv + 721. (New York and London: John Wiley and Sons, Inc., 1965.) 315s.

FLAME Spectroscopy, by Drs. R. Mavrodineanu and H. Boiteux, is probably one of the most detailed and comprehensive works on flame emission spectroscopy and atomic absorption spectrophotometry which has ever been written. It may be regarded as a modernized edition of the original *L'Analyse Spectrale Quantitative par la Flamme*, published in France in 1954.

The authors deliberately avoid the discussion of practical laboratory methods for the analysis of specific elements or compounds, although most extensive references are given to published papers of this type.

The present publication has a far more generic approach to the theoretical and instrumental aspects of flame spectroscopy, the descriptive text being embellished with photographic illustrations and diagrammatic representations of the highest quality and most commendable clarity. The coverage given to spectrographic instrumentation is truly international, the products of the world's most well-known instrument makers being given detailed attention. The construction, performance and general physical parameters of these spectrometers, etc., are concisely listed for rapid comparative reference.

The present work is presented in three parts comprising twenty-two chapters. The first part deals in a most exhaustive fashion with the fundamental principles of flame production; inflammability, burning velocity, temperature contours, etc., are but a few of the topics lucidly described. In addition to the less common flames of cyanogen-oxygen or hydrogen-perchloryl fluoride, much attention is directed to plasma jet, atomic hydrogen and high-frequency flame systems.

Numerous types of burners and atomizers are clearly described, the range covered extending from the apparatus of Beckman (1899) and Lundegårdh (1936) to the ultrasonic device of Peskin and Raco (1963). Much valuable detail is also given regarding the properties of materials

suitable for the construction of these flame spectroscopic accessories.

Sensing elements of the photographic plate and photo-sensitive type receive detailed treatment. Interfering phenomena are listed; the advantages of the absorption method for resonance radiation investigations is noted and many allied topics of vital spectroscopic interest are clearly presented. Part 2 deals with the fundamental theoretical treatment of atomic and molecular spectroscopy; modern ideas of atomic structure are related to values of emission frequencies. Parity, selection rules, coupling, isoelectronic sequences and hyperfine structure are just some of the numerous topics discussed. The band spectra of molecules receive similar detailed treatment.

Subsequent chapters contain extensive data from spectral examinations of atoms and molecules excited in acetylene flames. The wave-length tables are accompanied by photographic reproductions of typical spectra and provide a reference work of the greatest value and utility to the practising analyst.

The publication may appear expensive (315s.) but it is a massive, well-produced work of the greatest value and authority. It will provide a powerful teaching and experimental aid and contains much that is new and scientifically helpful. Since it must prove a well-thumbed vade-mecum, the publishers have thoughtfully provided a durable additional cover-case which will help to protect it from the buffetings of constant use. D. T. LEWIS

THE CHROMAFFIN CELL

The Natural History of the Chromaffin Cell

By Prof. Rex E. Coupland. Pp. xi+279. (London: Longmans, Green and Co., Ltd., 1965.) 50s. net.

EVER since Vulpian in 1856 noticed that sections or extracts of the adrenal medulla could produce colours on oxidation, the significance of chromaffin tissue and the adrenal medulla in particular has interested a great number of investigators. So important did the adrenal medulla appear to be that for a time the vital functions of the adrenal cortex were completely ignored. Interest appears to have declined in recent years due to the counter-attractions of the postganglionic sympathetic nerve-endings.

The Natural History of the Chromaffin Cell has appeared at a most opportune moment to describe what has already been achieved and to point out the possibilities for further useful work on the study of the chromaffin cell. Until now it has been very difficult to obtain an overall picture of the published work on this subject since so many different specialists have been involved. Prof. Coupland has succeeded in combining the anatomical, histological and physiological data in a way that makes this book a fine reference source and at the same time a pleasure to read. He restricts his scope to a consideration of the chromaffin cell defined as "an element developed from the neuro-ectoderm, innervated by pre-ganglionic sympathetic nerve fibres capable of synthesizing and secreting catecholamines and storing them in sufficient quantities to give a positive chromaffin reaction with aqueous solutions of potassium dichromate or chromate". This, of course, excludes the enterochromaffin cells, mast cells and dopamine-storing cells. Without these a coherent account is possible. To include them would only confuse the story.

The first chapter is concerned with the histology, histochemistry and ultrastructure in which the various methods of preparation and staining are critically evaluated. Emphasis is placed on the histochemical methods available for the visualization of the catecholamines, but staining techniques for other cell constituents are also included, together with a section on the electron microscopy of chromaffin tissue. The embryological and foetal

development is then described in which the evidence bearing on the origin of the chromaffin cells from the neuroectoderm is explored, including the recent evidence that some of the cells may arise from the neural tube. The chapter on the post-natal fate of the extra-medullary chromaffin tissue gives Prof. Coupland a chance to point out the problems associated with the use of human material and also to describe his own work in some detail. He also fully reviews the literature available on the innervation and blood supply to chromaffin tissue. In the chapter on the synthesis, storage and secretion of catecholamines, a fair balance is maintained between the apparently conflicting evidence concerning the actions of reserpine on the innervated and denervated adrenal gland. A whole book could be written about the metabolism of catecholamines; the author does well to cover the subject within a reasonable space without the chapter degenerating into a list of references. The chapter concerned with functions of chromaffin cells in the body also, surprisingly, contains a section on the metabolic fate of the released catecholamines. There is a brief survey of the occurrence and characteristics of tumours of chromaffin tissue. The book ends with a chapter on the comparative anatomy of chromaffin tissue throughout the many orders of vertebrates and invertebrates. These final two chapters must be included for the book to be complete and will serve to widen the knowledge of many.

At the end of each chapter there is a comprehensive list of references with titles. This removes the need for the numbering of references and cross-indexing, but an author index would have been useful. The subject index is adequate for a book of this size. There is the usual quota of minor misprints which detract little from the overall quality of the book, and even *N-methylnoradrenaline* is readily translated as *N-methyladrenaline* by a quick look at the list of references.

Prof. Coupland is to be congratulated on the excellence of this monograph and the publishers for the quality of production at, for these times, a reasonable price.

B. A. CALLINGHAM

TECHNIQUES IN CELL METABOLISM RESEARCH

Methods in Cell Physiology

Vol. 1. Edited by David M. Prescott. Pp. xiii+465. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1964.) 118s.

ALTHOUGH much of the progress and orientation of modern biochemical research on cell metabolism and its control has derived from investigations on bacteria, there are many problems relating to the regulation of cellular processes for the study of which larger unicellular organisms are more suitable. Such are the control of mitosis, nucleolar function, nuclear-cytoplasmic relationships, and pinocytosis, as well as problems relating to the control of special forms of differentiation, such as encystment. When cells are large enough to permit microsurgery, such as the transplantation of nuclei, or when synchronous mitosis or synchronous encystment can be induced, the opportunities for investigating cell regulation at a genetic or a biochemical level are enormously improved. Although various organisms with these properties are known, and have been extensively studied in the laboratory, many research workers who might feel inclined to use them for their own purposes, but are not already familiar with them, are liable to be put off trying to do so because the techniques for handling such organisms are unfamiliar and their difficulty hard to evaluate. With Volume 1 of *Methods in Cell Physiology* in their hands such doubts and hesitations should vanish.

This excellent volume contains a series of chapters devoted to the techniques for growing and handling cells

of different kinds, with discussion of their advantages and limitations for particular purposes. Each chapter is written from extensive practical experience, and gives details not only of how to grow these cells but also, where necessary, of how to stain them and how to make the special tools for handling them, such as those required for microsurgery. Cells suitable for synchronous culture include *Chlorella*, *Acetabularia*, *Euglena*, *Tetrahymena*, and mitotic synchrony can be induced in *Physarum polycephalum* by coalescence of the microplasmidia. Cells suitable for microsurgery of various kinds are *Stentor*, *Amoeba* and *Euplotes*, and grasshopper neuroblasts. A separate chapter is devoted to methods for studying pinocytosis, especially in amoebae.

There are also five useful chapters on autoradiography which alone would make this volume worth purchasing. These also are full of practical details for obtaining the best results in quantitative autoradiography, high-resolution autoradiography, and autoradiography of water-soluble materials and of mammalian chromosomes. A most valuable feature is the discussion of the theoretical and the practical limitations of the techniques advocated. Finally—and appropriately—J. E. Edström contributes a chapter on micro-extraction and micro-electrophoresis for determining and analysing nucleic acids in isolated cells which makes even this *tour de force* seem possible for other mortals. The editor, D. M. Prescott, is to be congratulated on the production of a thoroughly useful volume.

J. H. HUMPHREY

SURGERY AND SCIENCE

The Surgeon's Craft

By Prof. Hedley Atkins. Pp. x+201. (Manchester: Manchester University Press, 1965.) 15s. net.

THIS readable little book traces the development of the surgeon's craft from prehistoric times to the present day and is presented in a form readily understandable by the non-medical reader. It should therefore appeal to scientists engaged in the physical, chemical and biological sciences on which surgery has so extensively drawn to make many of the achievements described possible. Here in short compass is recorded how surgeons operate on the heart and other vital organs with impunity; that these achievements are of comparatively recent origin is shown by the startling statement that well into the nineteenth century surgery was "bloody, brutal and dangerous"! Asepsis and anaesthesia were soon to alter that fearsome state of affairs. The distinction which the author draws between the surgeon and his more intellectually inclined physician colleague is probably fair, but he makes it clear that, especially in university departments of surgery and in the Royal Colleges, more and more younger men are undertaking basic research, both biochemical and biological, and thus bridging the gap between the laboratory and the wards and operating theatre.

The thorough, exacting and protracted training of the surgeon, and what kind of man he is, are described. This should give the non-medical scientist the chance of comparing this with his own training and attributes. Culmination for the surgeon is reached on appointment to a consultant post, usually in his late thirties, and no doubt the question as to why the surgical training takes so long will be asked. The answer would seem to be the immense responsibility in dealing with human patients, and this is reflected in the financial awards which will be of great interest to other scientists.

The author sees a glowing future for surgery, despite the ultimate object of surgery being the abolition of surgery. By the combined attack of many scientific disciplines it is to be hoped that a disease such as cancer will one day be conquered and so make the mutilating operations needed for its complete extirpation a thing of

the past. Truly then would the ultimate object of surgery be achieved. It is interesting to speculate what would be the effect on the surgeon's craft of the elimination of cancer, rheumatoid arthritis and cardiac and renal diseases. The solution of the homograft problem in transplantation would undoubtedly bring other outstanding problems into the surgical arena, and future advances must of necessity bring a profound change of outlook and approach for the surgeon.

Thus although *The Surgeon's Craft* still presents surgery in the main as a 'craft', the reliance of modern surgery on many scientific disciplines is obvious and should be a pointer to the training and occupation of the surgeon of the future. Prof. Atkins skilfully blends the clinical aspects of surgery with its scientific foundations. The proceeds of the sale of this book have been generously donated for the preservation of Down House as a memorial to Charles Darwin, from whom modern surgery drew much inspiration.

N. W. NISBET

PRINCIPLES APPLIED

Genetical Principles and Plant Breeding

By Prof. Watkin Williams. (Botanical Monographs, Vol. 5.) Pp. x+504. (Oxford: Blackwell Scientific Publications, 1964.) 70s.

THE title of this book suggests a similarity of content with several others written on the theme of plant breeding in recent years, but it differs from them in at least two important respects. First, it is directed towards a student audience, principally to those who are studying in the general sphere of agriculture, and secondly, it consistently relates the pure and applied aspects of genetics without burdening the text with either crop botany or the intricacies of the routine methods of crop improvement. It is tailor-made for course requirements and aims to provide an understanding of the genetical principles governing the lives of flowering plants, and of the methods which lead to their improvement.

The first five chapters deal with such basic considerations as the genetic components, the organization of the genes, the chromosome complement, mutation, and the role of the cytoplasm in heredity. Basic Mendelism and descriptions of mitosis and meiosis are not included as a knowledge of these is presumed. Genetic systems governing sex determination and breeding behaviour occupy three chapters with especial emphasis given to incompatibility systems. The following two chapters are concerned with plant populations, their adaptation to their environment, and the influence of selection. The genetic basis of disease resistance forms the subject of the penultimate chapter and, finally, there is a description of special techniques such as chromosome substitution and addition, the utilization of haploids and the production of polyploids. The text is liberally supplied with line drawings, tables and photographs and the bibliography is adequate.

Genetical Principles and Plant Breeding is attractively written with a degree of lucidity which will make it popular with many. It covers the broad subject adequately and with the authority which we may expect from Prof. Williams's wide experience of practical plant breeding and genetics. It is refreshing to see attention directed to chimeras and plastid mutation though equally it is disappointing that *Xenia* receives no mention.

Although this book seems remarkably free from factual errors, like others which have gone before, it perpetuates a few fallacies which are now in danger of becoming regarded as facts. For example, emphasis is placed on the incomplete meiotic pairing of autopolyploids with its detrimental effect on fertility, and the relative natural rarity of such plants is attributed to these causes. What is here overlooked is the complete pairing and high fertility of many autopolyploids and the ready response to selection, either natural or artificial, of those which are irregu-

lar. To then presume that infertility is the explanation for the lack of success of autopolyploids is to ignore the fact that many allopolyploids have also to overcome, and do, a barrier of sterility even more extreme than that of autos, albeit at the diploid level.

The success of this book will ultimately be judged by generations of students, but already it seems likely to be assured. The contribution which it makes can scarcely be said to startle or surprise, rather will it interest and satisfy, both the students for whom it is intended and others of more advanced levels of learning. K. JONES

VASCULAR PLANTS OF EUROPE

Flora Europaea

Edited by T. G. Tutin, V. H. Heywood, N. A. Burges, D. H. Valentine, S. M. Walters and D. A. Webb, with the assistance of P. W. Ball and A. O. Chater. Vol. 1: Lycopodiaceae to Plantanaceae. Pp. xxxii+464+5 maps. (Cambridge: At the University Press, 1964.) 84s.

THE publication of this volume (the first of four) means a most remarkable event to all biologists. To the layman and perhaps also to numerous biologists it may be a great surprise that no such work existed earlier. It is true that at the beginning of scientific botanical taxonomy the number of known species was so small that it was possible to publish works enumerating and describing them all. In his *Species Plantarum* (first ed., 1753), Linnaeus listed about 6,000 species in all, about 5,400 phanerogams and about 600 cryptogams. He believed the flora (and the fauna) of the tropics to be rather uniform and estimated the total number of plant species (of all groups) to be about 10,000. How wrong he was! The number grew rapidly and steadily through expeditions to various parts of the world. The microscope opened new worlds among the cryptogams. It became impossible to embrace the whole world in one *Flora*, and also other kinds of specialization became necessary. Besides phanerogamists (and pteridologists) there came bryologists, phycologists, mycologists and lichenologists. It has nowadays almost become a convention that a *Flora* without any specifying attribute means one treating only phanerogams or vascular plants (that is, phanerogams + pteridophytes). The *Flora Europaea* is no exception, for it is no complete *Flora* but one restricted to vascular plants.

Even the relatively poor phanerogamic flora of Europe exceeds numerically the 10,000 species which Linnaeus thought to represent the final and total sum of all plant species. The total number of known species of phanerogams alone is to-day judged to approach 250,000. Their final number cannot be estimated with any certainty as yet. In recent years the number of new species proposed seems to be on the decline, which may indicate that the stock of unknown species is now becoming exhausted. As to the European phanerogamic flora there remain certainly rather few species to be detected as new to science (or as new to Europe).

Why have we not had a comprehensive *Flora* of European vascular plants much earlier? The main reason is that floristic (and faunistic) studies have in the past mostly been restricted geographically by political or even provincial boundaries. A positive merit of such restricted areas is that the making of inventories can be more thorough and lead to relative completeness within reasonable time. But there are also negative merits. The limits of distribution of plants (and animals) do not follow administrative boundaries. Even where there has been no 'iron curtain' scientific contacts and field studies across the boundaries have been much too scarce, and so it may happen that the same plant is identified differently in different countries and, vice versa, that different species pass under the same name in different countries. Moreover, the full amplitude of intraspecific variation and the sterility bar-

riers of a species cannot be elucidated without following it throughout its whole area.

Most European countries possess *Floras* of their own, often modern and good ones. There are also *Floras* of a more regional character, for example, those treating two or more of the Nordic countries. The most pretentious *Synopsis der Mitteleuropäischen Flora*, started in 1896 by Ascherson and Graebner, has remained an unfinished work, but Hegi's magnificent *Illustrierte Flora von Mitteleuropa*, begun in 1908, runs now into its second edition. The eastern part of Europe is included in the gigantic *Flora URSS* (30 volumes, 1934-64).

In the 1940's the German W. Rothmaler projected a *Flora Europaea*, which was to cover not only Europe but also the Caucasus, Transjordan and the parts of North Africa with Mediterranean flora. Post-war-time conditions were, however, too unfavourable for the realization of his project. On British initiative the question was discussed at the eighth International Botanical Congress (Paris, 1954) and shortly afterwards a committee was formed in Britain with T. G. Tutin (Leicester) as the chairman and V. H. Heywood (Liverpool) as the secretary. With enthusiasm they went to work. They gained support from all parts of Europe and financial aid from various sources, especially the D.S.I.R. Editors for the different families, regional advisers for the different countries and contributors were appointed. It was soon realized that the inclusion of North Africa would mean a disproportionately large amount of work, and so Europe was taken in its traditional sense. It seems queer, however, that Novaya Zemlya is excluded.

The editors' intention has been to produce a concise and complete *Flora* in the shortest possible time instead of aiming at the solution of all problems by meticulous monographic studies. The first volume was ready in 1964, and it is planned to complete the *Flora* over the next eight years.

The first volume treats the pteridophytes, the gymnosperms and part of the dicotyledons (Salicales—part of Rosales). Among the larger families may be mentioned Caryophyllaceae, Ranunculaceae, Cruciferae and Saxifragaceae. The largest genera are *Silene* (166 species), *Ranunculus* (131 spp.), *Saxifraga* (123 spp.) and *Dianthus* (121 spp.). It follows from the aim of the work that the treatment cannot be uniform. This seems to be the case especially with the higher taxa. The delimitation of families and genera is sometimes hypermodern but mostly conservative; the generic concept is sometimes wide but mostly rather narrow. *Huperzia*, *Lepidotis* and *Diphasium* are thus segregated from *Lycopodium*, *Consolida* from *Delphinium*, *Hepatica* and *Pulsatilla* from *Anemone*, and *Jovibarba* from *Sempervivum*. The species concept is, on the whole, moderately narrow. Sub-species are included but as a rule not taxa of lower rank. In some cases rather local races (especially some from the British Isles) have been treated as sub-species; in numerous other cases such are discussed shortly without being given a definite taxonomic rank. The descriptions of the taxa of all ranks are clear and concise. There are keys not only to genera, species and sub-species but also to the families, which is a most unusual feature. I have not as yet tried to use the keys, but they look reliable. I have looked up all the Nordic plants and found no serious errors or omissions but a few debatable points and several interesting suggestions which deserve careful consideration. For less-known parts of Europe the volume contains a wealth of new information. In all parts of Europe it will certainly stimulate further research along different lines, as it gives an excellent review of the present state of our knowledge and makes the results of previous studies easily available. Last but not least, it will be the indispensable tool for everybody wishing to name a European plant from outside his own country. The next three volumes are eagerly awaited. All engaged in the project are to be congratulated on this first volume.

J. A. NANNFELDT

Modern Trends in Immunology, I

Edited by Prof. Robert Cruickshank. Pp. vii+263. (London: Butterworth and Co. (Publishers), Ltd., 1963.) 65s.

THE rapid developments in immunology to-day undoubtedly call for a 'modern trend' series to supplement the classical text-book treatment of the subject. The horizons of immunology are expanding all the more quickly because of the removal of barriers between different disciplines and the exchange of techniques.

This book is indeed a good start to a series of such modern trends. Although much of the basic knowledge is reviewed, for completeness, the modern views are put into perspective and recent technical developments are well covered. The chapters on immunity have nothing new to add when dealing with natural or acquired bacterial immunity and it is surprising to see so much space allotted to such text-book information. The chapters on acquired viral and protozoal immunity, however, bring the subject up to date. The chapter on immunization "follows a course more or less as empirical as the subject itself" (to quote the author), and it is unfortunate that the reporting of the part played by maternal antibody is not up to date. In this chapter there is much overlap with that on acquired bacterial immunity. Hypersensitivity is well presented and, as the author says, the different disciplines now engaged in this subject may well result in the resolution of many of the problems in the next few years. The chapters on auto-immunity and transplantation immunology are most informative and do much to bring the book into the modern trend. Here again it is noteworthy that the authors comment that "the fusion of transplantation with other immunological disciplines also involves a complementary process".

This book is most useful to the post-graduate student and we hope it is the first of an interesting and informative series.

F. T. PERKINS

Electrical Machinery and Control

By Prof. Irving L. Kosow. (Prentice-Hall Series in Engineering Technology.) Pp. xx+707. (Englewood Cliffs, N.J., and London: Prentice-Hall, Inc., 1964.) 94s.

ONE of the major difficulties that impose unnecessary restrictions for the control engineer designing a complex system is that the specialist designers of the components that go to make up the system are motivated by different criteria. Thus, the electrical machine designer is concerned with iron and copper losses and with stray load losses. While these are the keystones of good design for large power machines when used independently, they are of secondary importance to such factors as controllability, torque to inertia ratio and power gain when used as a component part of a control mechanism.

It is therefore with great expectation that one approaches a new work combining both control and machinery. The connexion is, however, never made; control is represented by a final short chapter on servo-mechanisms so elementary and trivial as to be useless. Not once in the whole 700 pages are the dynamic characteristics of machines mentioned. The steady-state approach is used throughout and so is of little use to the control engineer. The addition of the term 'Control' to the title of the book is thus misleading.

As a book on machine principles it is also very doubtful if it will arouse much interest. It is written in an elementary manner with no attempt at analysis and a lack of modern fundamental unifying concepts. As an example one could quote the treatment of the force on a conductor carrying current in a magnetic field. The simple law is quoted but there is no discussion on the physical implications. There is no treatment of current embedded in slots in iron and the distribution of forces on the iron, and little evidence for the student that in modern machines

the field around a conductor is negligible and that the tooth forces produce the drive.

It is also extremely naïve in its approach. After stating that synchronous induction motors are only made in smaller sizes, it says "Because of the salient pole rotor the motor pulls into synchronism quite easily . . .". This is not true; it is the difficulty of the synchronizing phenomenon (which is a transient dynamic problem and therefore ignored) which prevents large-scale machines of this type from being constructed.

Each chapter concludes with a number of problems and the answers given separately. A set of trigonometry tables is provided as an appendix.

JOHN C. WEST

Beryllium Oxide

Edited by R. Smith and J. P. Howe. (Proceedings of the First International Conference on Beryllium Oxide, Sydney, Australia, October 21-25, 1963.) (Reprinted from *Journal of Nuclear Materials*, 14, 1964.) Pp. 498. (Amsterdam: North-Holland Publishing Company, 1964.) 200s.

THE first International Conference on Beryllium Oxide, attended by scientists from six countries, was held at Sydney in October 1963. The conference was sponsored and organized by the Australian Atomic Energy Commission to review and discuss recent advances in knowledge of the behaviour of beryllium oxide, particularly from the point of view of its possible use in nuclear reactor systems, as a moderator material.

The present volume, which is reprinted from the *Journal of Nuclear Materials*, is a collection of the fifty-five papers presented at that Conference, together with an account of the discussion which they provided. The papers are divided into seven sections. These deal with: (i) utilization of beryllium oxide in nuclear reactors; (ii) radiation effects in beryllium oxide; (iii) structure and physical properties of beryllium oxide; (iv) sinterability and sintering of beryllium oxide powders; (v) fabrication and mechanical properties of beryllium oxide; (vi) corrosion of beryllium oxide by water vapour; (vii) beryllium oxide-based dispersion fuels.

The book is attractively produced.

M. F. LAPPERT

European Brewery Convention

Report of the Barley Committee: Trials, 1963. Pp. 184. (London: The Institute of Brewing, 1965.) 15s.

THIS consists very largely of tabulated results of growth and malting trials of about twelve comparatively new hybrid barley varieties in fifteen Western European countries. The data, mostly arranged country by country but with a broad introductory survey, cover a much wider range than might be anticipated. Thus, they include summaries of trial lay-out, field observations, meteorological conditions as well as the crops themselves and their analytical examination. The objects behind this massive effort are not specifically or solely concerned with those qualities, such as disease resistance, yield, earliness in ripening and production of straw, which might first come to mind. These considerations are all economically important but are in the present context secondary to the main one of malting quality. It is clearly unlikely that any one variety will be equally desirable under a wide range of climatic and agricultural conditions. The continuing experience afforded by these trials, nevertheless, must be generally valuable, not least in respect of the economic conditions brought about by the European Common Market. The scientific value of this *Report*, however, encompasses more than the results themselves or the clear way they are presented; it presents what is probably a unique picture of the inevitable complexity involved in selecting improved varieties of even a single crop when many slight or scarcely appreciated variables may be brought into play.

A. H. COOK

RADIO OBSERVATIONS OF SPIRAL GALAXIES

By PROF. B. Y. MILLS, F.R.S., and J. R. GLANFIELD

Cornell-Sydney University Astronomy Centre,
University of Sydney

AN opportunity to investigate some of the brighter southern galaxies has been afforded by the recent completion of the east-west arm of the cross-type radio telescope at the Molonglo Radio Observatory¹. While a thoroughgoing investigation requires the resolution of the whole instrument, it is found that the east-west arm alone, operating as a simple total power radiometer, provides quite useful information. It has a beam-width measuring 1.5' E.-W. by 4° N.-S. at a frequency of 408 Mc/s and is limited in sensitivity by confusion effects at a flux density of about 0.5–1 flux unit (10^{-26} W m⁻² (c/s)⁻¹). Several observational programmes are being carried out while work proceeds on the completion of the north-south arm.

We here report observations of the three brightest southern spiral galaxies, *NGC 253*, *NGC 4945* and *NGC 5236*. These were first observed by Mills at a frequency of 85 Mc/s with a beam-width of 49' arc². It appeared that their integrated emission is very similar to spiral galaxies observed in the northern hemisphere by Hanbury Brown and Hazard³, but the resolution was not adequate to resolve the galaxies and examine their structure. More recent observations by Mathewson and Rome⁴ gave surprising results. Using the Parkes 210-ft. reflector at a frequency of 1,410 Mc/s, at which the beam-width is 14' arc, they found that none of the foregoing galaxies was resolved, although their optical sizes are such that a clearly visible widening of the radio telescope response was expected. They concluded that the model of radio emission comprising a very extensive radio corona, as proposed by Hanbury Brown and Hazard, was not universal, and that in the case of these three galaxies the emission was concentrated close to the nucleus. However, the earlier observations of the similarity in the integrated fluxes was confirmed; it appeared that the total radio emission is closely proportional to the optical emission, irrespective of its concentration to the nucleus.

The Observations

Our new observations are represented by the drift scans shown in Fig. 1. In these, the aerial is pointed at the declination of a galaxy and scanned across it in an east-west direction by the rotation of the Earth. Sidereal time markers are shown at minute and half-minute intervals. Also, under the galaxy records, the east-west extent of the optical image of the main body of the galaxy is shown.

In all cases there exists an extended component of emission roughly co-extensive with the visible galaxy. In addition, both *NGC 253* and *NGC 4945* show the presence of a small bright source close to the centre of the extended component. It is unresolved ($< 0.5'$ arc) in the case of *NGC 253* and of the order of $0.75'$ wide in the case of *NGC 4945*.

Scans were also made at declinations 2° north and south of the galaxy positions. The results were consistent with an origin of the emission entirely within the galaxies, except for the concentration immediately following *NGC 4945*, which may be an unconnected point source at a slightly different declination. For the purposes of analysis it will be assumed to be unrelated to the galaxy, and its contribution to the flux subtracted.

Before discussing our new results in more detail it is necessary to enquire why Mathewson and Rome were

unable to detect the extended components. They quoted angular sizes $\leq 5'$ arc, whereas inspection of the records in Fig. 1 shows that the overall east-west extension varies between about 8' arc for *NGC 5236* to approximately 18' arc for *NGC 253*. Probably their results arose as the combination of several factors. In the case of *NGC 5236* the size is not much larger than their lower limit, so that the failure to observe an extension is probably not significant. Although the extended component is larger in the case of *NGC 4945*, it is quite understandable that the strong central component would dominate the picture. However, the size and integrated emission of the extended component of *NGC 253* would appear to be easily observable. It seems likely that it was not observed because of a rather fundamental difficulty. The resolution of a radio telescope may be controlled by changing the observing frequency, but, if a radio source is completely resolved,

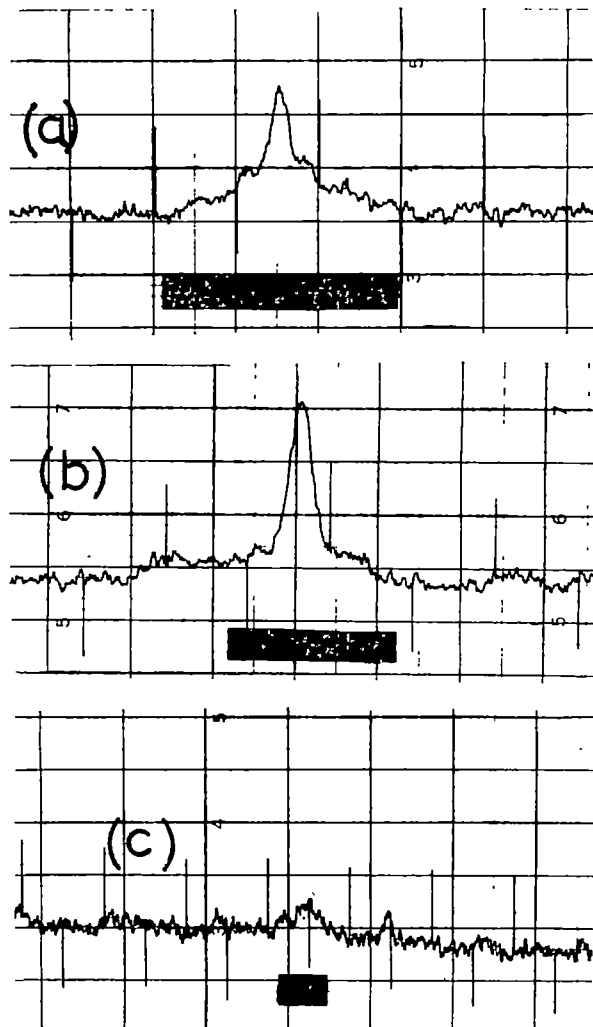


Fig. 1. Transit observations of the three galaxies (a) *NGC 253*, (b) *NGC 4945*, (c) *NGC 5236*. Minute and half-minute time markers are shown and in each case the east-west extent and position of the main body of the galaxy is indicated by the black line immediately below the chart record. The receiver output time constant is 0.8 sec

the received power is proportional to $\lambda^{2+\alpha}$, whereas the received power of an unresolved source is proportional to λ^α , where α lies in the range 0.5–0.7 for the corona of a normal galaxy. Thus, if one increases the resolution by decreasing the wave-length, it becomes increasingly difficult to observe the extended sources which are resolved. This effect is seen in its most acute form in the corona of the Milky Way which, using present techniques, is unobservable at centimetre wave-lengths.

Physical Properties of the Galaxies

Our knowledge of the physical properties of the galaxies comes essentially from the work of de Vaucouleurs. Most information is available about *NGC* 4945, for which values of distance modulus, total absolute magnitude and apparent magnitude are given, together with other photometric data which are not at present relevant⁸. Equivalent data are also presented for *NGC* 253 but not in such detailed form and probably with lower accuracy⁸. Little information is available about *NGC* 5236, and to compare it with the others we have supplemented de Vaucouleurs's data⁷ with *The Hubble Atlas of Galaxies*⁸. To obtain a distance scale, we have assumed that the absolute magnitude is the same as *NGC* 253. This leads to a distance modulus close to that of *NGC* 4945, consistent with the suggestion of de Vaucouleurs that they belong to a single physical grouping of galaxies⁸.

All the galaxies are late type spirals, the earliest being *NGC* 5236, which Sandage denotes as *Sc/SBb*, and the latest being *NGC* 4945, which de Vaucouleurs denotes as *SB(s)cd*. Their basic properties obtained as here are summarized in Table 1: the magnitudes have been corrected for internal, as well as galactic, absorption.

Calibration of the Equipment

The instrument has only recently been put into operation and calibration programmes are at present being undertaken to place the measurements of flux density and position on a suitable absolute standard. For the present observations the right ascensions have been taken directly from the transit times measured on the uncalibrated instrument. Checks which have been made with lunar occultation sources of accurately known position suggest that errors do not exceed 10" arc. The declination is defined with an accuracy better than 0.25°.

The flux density scale is not yet in such a satisfactory state, and for these observations our procedure was to check the calibration at intervals by observations of convenient sources in the catalogue of Bolton, Gardner and Mackey⁹, which gives fluxes of radio sources at 408 Mc/s based on the scale of Conway, Kellerman and Long¹⁰. The accumulated uncertainties appear quite large and our relative fluxes are thought to be accurate only to about ± 15 per cent: the uncertainty in the absolute calibration may also be of this order. This degree of accuracy is considered adequate for the observations recorded here.

Analysis of the Observations

NGC 253. The right ascension of the central source is 00h 45m 05s (epoch 1950.0). The position of the galaxy is given as 00h 45.1m (1950) (ref. 3), so that our observations are consistent with a point source placed at the nucleus of the galaxy. The flux density of the source is 3.5 flux units.

Table 1. PROPERTIES OF THE GALAXIES

Galaxy	Corrected (mpg)	Corrected (Mpg)	Distance (pc $\times 10^3$)	Radius in plane of galaxy (pc $\times 10^3$)
<i>NGC</i> 253	6.5	-20.5	2.5	8
<i>NGC</i> 4945	7.55	-20.35	3.8	8
<i>NGC</i> 5236	7.1	-20.5	3.4	5

After making allowance for the smoothing effect of the aerial beam, the east-west extent of the extended component is approximately 18' arc; this corresponds to an angle of 19.5' arc measured along the major axis at position angle 67°. The integrated flux is 20 flux units.

It is interesting to enquire what spatial distribution of emission would reproduce the observed telescope response. As we have no information about the north-south distribution we have assumed the emission is distributed in a series of uniform spheroidal shells of differing emissivity, centred on the galaxy, and have determined the radial distribution of emissivity along the major axis which best fits our observed galactic profile. A more refined analysis should be possible with the completed instrument.

Using a fan beam aerial which does not resolve the source in a north-south direction, it is easy to show that the radial distribution of emission in a spherically symmetric source is given by:

$$E_\theta \propto \frac{1}{\theta} \frac{dT_\theta}{d\theta}$$

where T_θ is the aerial temperature at the angle θ from the centre of the distribution. With a spheroidal source of unknown ellipticity the same expression may be used to determine approximately the emissivity in the galactic plane at an east-west angle θ from the nucleus. If the ellipticity is large or the major axis nearly east-west, the errors will be small and probably negligible compared with uncertainties introduced by random noise and unresolved sources. It is worth pointing out that the foregoing equation implies that a line scan of an infinitesimally thin spherical shell produces a rectangular response.

The observed profile can be fitted quite well by a three-component model as follows: (1) an unresolved nuclear source with a radius less than 200 pc. and a total emission of 2.6×10^{21} W(c/s)⁻¹; (2) a less-concentrated component of uniform emissivity, radius 2.5 kpc and total emission 5.5×10^{21} W(c/s)⁻¹; (3) an extended component of uniform emissivity, radius 7 kpc and total emission 9×10^{21} W(c/s)⁻¹.

Using the same distance scale, the overall radius of the visible galaxy is approximately 8 kpc. A bright central structure can be observed extending out to about 3 kpc, perhaps corresponding to our second radio component. The nucleus is not evident on photographs available to us.

NGC 4945. The right ascension of the central source is 13h 02m 33s (epoch 1950.0), and its flux density is 9 flux units. The position of the galaxy is given as 13h 02.4m (ref. 7). The galaxy is observed edge on and the relatively heavy obscuration hides the nucleus so that the optical position must be judged from the rather irregular brightness distribution. The agreement is sufficiently good to be sure that we are observing the galaxy.

We have analysed the extended component in the same way as before, but this time find that a single ellipsoid of uniform emissivity is adequate to fit the observation. The total flux density is roughly 10 flux units, with some additional uncertainty because of the small source close to the galaxy.

Thus, after allowing for a position angle of 42°, the observations may be represented by the following two-component model: (1) a central, presumably nuclear, source with a radius of the order of 400 pc and total emission 15×10^{21} W(c/s)⁻¹; (2) an extended component of uniform emissivity with a radius of 8 kpc and a total emission of 17×10^{21} W(c/s)⁻¹.

The radius of the visible galaxy is also about 8 kpc so that the second component here corresponds to the third component in *NGC* 253, both having the same extent as the visible galaxy. An intermediate component may be present in *NGC* 4945, but it is not separately distinguishable.

NGC 5236. This galaxy is in a rather confused region of several radio sources. Nevertheless, a small extended

source at the galaxy position is quite clear. There is no obvious nuclear component so that it is difficult to quote an accurate position. The centroid of the whole source is approximately 13h 34m 11s, which agrees well with the galaxy position of 13h 34.3m (ref. 7). The integrated flux density of the whole galaxy is 5 flux units and its overall extent, after allowing for the aerial resolution, approximately 8' arc. In this example the galaxy is seen face on so that there is no need to allow for the position angle of the major axis.

Because of the poor signal-to-noise ratio and the presence of confusing sources, an attempt to separate different components is not justified. Although there does appear to be evidence of fine structure, a simple model comprising an ellipsoid of uniform emissivity fits the observations reasonably well. The total emission is 6×10^{21} W(c/s)⁻¹ and the radius of the distribution approximately 4 kpc. As this is close to the radius of the visible galaxy, the component appears to correspond with the extended components in the other galaxies.

Discussion

We have found that all three galaxies examined have extended components of radio emission co-extensive with the visible galaxy and of the same order of total emission. The ratio of optical emission from the whole galaxy to the radio emission of this component is also roughly the same in all cases. Additional more concentrated components exist in two of the galaxies, but not in any fixed ratio.

Apparently similar components have also been recognized in the Milky Way, where we have a nuclear source, a disk component with the greater part of the emission confined within a radius of about 6 kpc and, finally, a corona of approximately uniform emissivity extending to about 12 kpc in the galactic plane¹¹. The average overall extent of the Milky Way itself, including the Perseus arm, must be about 13 kpc.

It appears to be very reasonable to identify the Milky Way corona with the extended components found in the other galaxies: the disk and nuclear components at the distance of these galaxies would be too weak to be detectable. Both NGC 4945 and NGC 253 have, by contrast, very strong nuclear components. In order to avoid postulating an additional galactic component, it also seems reasonable to identify, tentatively, the intermediate extended component of NGC 253 with the disk component of the Milky Way. Although this component is relatively much stronger in NGC 253, it appears to be related to the visible structure of the galaxy as in the Milky Way.

In Table 2, we accept these suggested identifications and compare the galaxies directly. Values for the disk and corona of the Milky Way have been taken from the 85 Mc/s results¹¹, assuming an emission proportional to $v^{-0.6}$ and a distance to the nucleus of 10 kpc. The emission

Table 2. RADIO PROPERTIES AT 408 Mc/s

	NGC 253	NGC 4945	NGC 5236	Milky Way
Nucleus: emission W(c/s) ⁻¹ $\times 10^{21}$	2.6	15	Undetected	0.04
Radius pc	<200	~400	—	200
Disk: emission W(c/s) ⁻¹ $\times 10^{21}$	6.5	Undetected	Undetected	0.5
Radius pc $\times 10^3$	2.5	—	—	6–10
Corona: emission W(c/s) ⁻¹ $\times 10^{21}$	9	17	6	7
Radius pc $\times 10^3$	7	8	4	12
Visible galaxy				
Radius pc $\times 10^3$	8	8	5	13
Radio index M_{408} -Mpg				
(a) Corona	+2.4	+1.5	+2.7	+2.6*
(b) Whole galaxy	+1.7	+0.8	+2.7	+2.5*

* Assuming the Milky Way has the same absolute magnitude as NGC 253, that is, $M_{pg} = -20.5$.

of the nucleus itself has been obtained directly at 408 Mc/s using the present instrument.

Although these results make a satisfactorily coherent picture, it appears that the only other spiral galaxy to be studied in detail, the Andromeda Nebula, has much more extensive radio components, although the total emission is similar. Observations at 408 Mc/s using the Jodrell Bank 250-ft. reflector¹² may be compared directly with ours if we assume a distance of 8×10^6 pc to the galaxy⁸. After allowing for the beam-width, the results appear to indicate a coronal component of total emission 10×10^{21} W(c/s)⁻¹ extending to a radius of about 40 kpc along the major axis, and a disk component of emission 0.6×10^{21} W(c/s)⁻¹: the extent of this component is uncertain as it was not completely resolved, but it is probably of the order of 12 kpc along the major axis. No nuclear component was detected. The main body of the galaxy, however, has a radius of only about 14 kpc.

Thus, if this interpretation of the observations is correct, it appears that the distribution of coronal radio emission in the Andromeda Nebula is not similar to the distribution in the other spiral galaxies. Clearly, it will be necessary to observe many more galaxies with high resolution to establish the pattern more reliably.

This work was supported by a grant from the U.S. National Science Foundation.

¹ Mills, B. Y., Aitchison, R. E., Little, A. G., and McAdam, W. B., *Proc. Inst. Radio Eng. Austral.*, 24, 156 (1963).

² Mills, B. Y., *Austral. J. Phys.*, 8, 368 (1955).

³ Hanbury Brown, R., and Hazard, C., *Mon. Not. Roy. Astro. Soc.*, 122, 479 (1961).

⁴ Mathewson, D. S., and Rome, J. M., *Observatory*, 83, 20 (1963).

⁵ de Vaucouleurs, G., *Astrophys. J.*, 139, 899 (1964).

⁶ de Vaucouleurs, G., *Astrophys. J.*, 130, 718 (1959).

⁷ de Vaucouleurs, G., and de Vaucouleurs, A., *Reference Catalogue of Bright Galaxies* (Univ. Texas Press, Austin, 1964).

⁸ Sandage, A., *The Hubble Atlas of Galaxies* (Carnegie Inst. Washington, 1961).

⁹ Bolton, J. G., Gardner, F. F., and Mackey, M. B., *Austral. J. Phys.*, 17, 340 (1964).

¹⁰ Conway, R. G., Kellerman, K. I., and Long, R. J., *Mon. Not. Roy. Astro. Soc.*, 125, 261 (1963).

¹¹ Mills, B. Y., *Paris, Symp. Radio Astronomy*, edit. by Bracewell, 431 (Stanford Univ. Press, 1959).

¹² Large, M. I., Mathewson, D. S., and Haslam, C. G. T., *Nature*, 183, 1250 (1959).

WILSON'S THEORY OF ICE AGES

By JOHN T. HOLLIN

Department of Geology, Princeton University, New Jersey

A PAPER by A. T. Wilson¹ suggests that the Antarctic ice sheet is unstable: that it builds up until it begins to melt at the base, then 'surges' rapidly outwards to form a large floating ice shelf in the Southern Ocean. Wilson suggests that, in the Tertiary, shelves formed in this way periodically displaced enough sea-water to account for the cyclothems of that time; and that in the Quaternary, helped by some longer-term reduction of temperature, they periodically cooled the oceans enough to cause ice ages in the northern hemisphere. A paper

by me² points out that, if these shelves did cause ice ages, traces should exist of (otherwise anomalous) rapid but temporary rises of sea-level, of 30–100 ft., just before each ice age. This present article discusses the available evidence for and against such rises. Up to now, most of the evidence seems favourable to Wilson's theory.

'Surges' and 'catastrophic advances' in glaciers have received increasing attention recently. More than forty such events have been reported from Alaska and north-west Canada alone³. The largest surge reported so far is

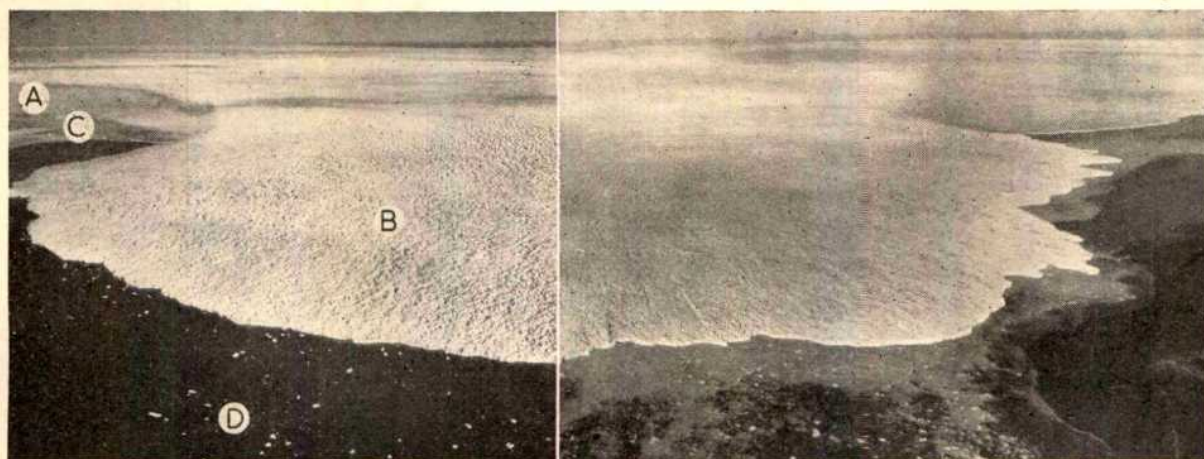


Fig. 1. Bråsvellbreen, looking northwards. Only the western and eastern edges (30 km apart) are shown. A (and to the right), 'normal' ice cap; B, Bråsvellbreen; C, moraines and rocks; D, Barents Sea. (Photographs by Norsk Polarinstittutt, 1938)

probably that of the Bråsvellbreen (a sector of a roughly circular ice cap in Spitsbergen), which advanced up to 21 km on a front of 30 km, some time between 1935 and 1938 (ref. 4) (Fig. 1). The fastest surge measured so far is probably that of the Kutiah Glacier¹, at 110 m/day, although hearsay surges include one so fast that it overtook two villagers fleeing from it⁵. The cause of surges is unknown. In Robin's theory, which Wilson quotes, surges occur when the continuing build-up of the glacier allows geothermal and frictional heat to bring the base to the melting-point. Once melting begins, large glaciers may find it difficult to expel the large amounts of water they generate and, in Weertman's theory⁶, it is the resulting basal water layer which accounts for the high speed of the surges. Now, without further work on these and other theories, it is really impossible to say whether or not surges should occur in large ice caps and ice sheets, and I have personally been somewhat sceptical about such surges^{7,8}. However, so as to test Wilson's theory, in order to know what to look for in the geological record of sea-level changes, it is worth while trying to estimate how large and how fast his surges would have to be. My estimate that they would have to involve sea-level rises of 30–100 ft. was based mainly on the assumption that surges smaller than this would have too short a period to fit the time-scale of ice ages, and on the fact that deglaciations larger than this are almost impossible to imagine glaciologically, at least without moving Antarctica northwards. To illustrate this, we can refer to some of the known facts from Antarctica⁷. The current average thickness of the ice sheet there is roughly 2,300 m. Suppose that this builds up to 2,400 m. Suppose that a surge emerges from either Wilkes Land or the Filchner area, captures the drainage⁹ from half the ice sheet, and lowers that half by 50 per cent. Such a surge, spread out to the typical ice shelf thickness of 200 m, would reach 55° S and, as Wilson points out, would appreciably increase the Earth's albedo. The central thickness of the ice sheet is roughly 4,000 m. The central accumulation rate is roughly 3 g/cm² yr, so that a central lowering by 50 per cent would take approximately 70,000 yr to build up again. Very roughly, this would be the period of the surge. The surge just described would be in the middle of my estimated range: ignoring isostatic effects, it would raise sea-level by 65 ft. On the speed of the surges, if they are in fact a cause of ice ages, then they must certainly be rapid to overwhelm the great capacity of the Southern Ocean for breaking up and melting Antarctic ice¹⁰. It is interesting, therefore, to realize that if the ice in the centre of the ice sheet began to surge with the velocity of 110 m/day quoted above, it would in fact reach the edge of the ice sheet in only 40 yr.

These estimates of the size and speed of Wilson's surges are incorporated in Fig. 2. Fig. 2 (1) shows the conventional view of sea-level change during ice ages such as the Weichsel (Würm, Wisconsin), with the Antarctic ice sheet remaining roughly constant in volume. Fig. 2 (2) shows the sea-level change required by Wilson's theory in Tertiary time: rapid rises accompanying the surges from Antarctica, and slow falls accompanying the build-ups there. Fig. 2 (3) shows the change required for Quaternary time: a rise accompanying each surge, a fall as both the Antarctic and northern ice sheets build up, the end of the ice age and a levelling out as the northern ice sheets disappear, and a subsequent small fall accompanying the final build-up in Antarctica. Although these estimates are very speculative glaciologically, they form a very specific test geologically. The chances seem small that rapid rises of sea-level of 30–100 ft., repeated in several interglacials at precisely the break of climate, could be caused by anything other than Antarctic surges.

As might be expected, the most detailed evidence for and against the sea-level changes of Fig. 2 (3) comes from the uppermost, most-studied part of the Quaternary, especially from north-west Europe. In this area, sea-level movements are recorded by changes in the land- or sea-shell content of deposits, and the date of the movements is recorded by the pollen content. Each of the last few interglacials in north-west Europe can be divided now into several pollen zones, and this permits very accurate dating. Within north-west Europe, the best areas for an investigation of this type appear to be southern England and northern France, where the horizontality of old, high-level marine features suggests that these areas are essentially stable. Not so many deposits have been examined there as in Holland and Germany, but in those areas there has been considerable warping, and it is difficult to reconstruct the old elevations involved. Fig. 3, therefore, is an attempted reconstruction of sea-levels during the last two interglacials in southern England. (For the Hoxnian interglacial, it has been necessary to

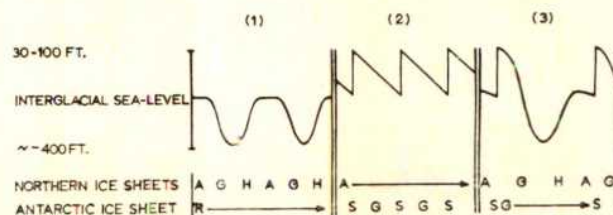


Fig. 2. Three patterns of sea-level change. The time and elevation scales are compressed in the ice ages. A, absent; G, growing; H, shrinking; R, roughly constant in volume; S, surging

use some data from eastern England, and in this particular case the possibility of warping is discussed below.) Sea-level positions suggested by field studies are plotted, and an attempt is made to connect them with a curve of the Fig. 2 (3) type. How well this curve fits the data is discussed in the following four paragraphs. For those interested, the pollen zones and the sites of Fig. 3 are discussed more fully in papers by West¹¹ (note that, in ref. 11, the legends of the key Figs. 12 and 13 should be interchanged).

In Fig. 2 (3), the sea-level just before a surge changes only slowly, whereas after a surge it changes rather quickly, because not only the Antarctic but also the northern ice sheets are now building up. Therefore, marine benches are more likely to be cut at a primary, pre-surge level than at a secondary, post-surge one. Accordingly, to agree with those authors who argue that a marine bench between 5 and 30 ft. O.D. in south-west England and north-west France must belong to the Hoxnian interglacial^{11,12}, I have attributed elevations in the middle of this range to the primary Hoxnian level in Fig. 3. What is striking is that the pollen data from the Hoxnian show that late in the interglacial, at the end of the main warm zone 2, apparently just as the climate broke, a remarkable transgression occurred in eastern England. The transgression has been recorded at 9 ft. at Clacton¹³, at 21 ft. in the Nar Valley (where marine deposits continue to at least 65 ft.)¹⁴, and at 80 ft. at Kirmington¹⁵. The record of freshwater sedimentation at Southelmham¹⁶, at roughly 80 ft., ends at the end of zone 2, and would fit a transgression at that elevation at that time. It seems reasonable to assume that the upper limit of the transgression was the 100-ft. 'Tyrrhenian' beach and 'Boyn Hill' terrace level traditionally assigned to the Hoxnian in southern England and elsewhere. The fact that the transgression occurred at all these different elevations at the same time suggests that it was unusually rapid. This rapidity makes it very unlikely that it was due to downwarping. For example, let us, to avoid the effects of tilting, focus our attention on the one small area of the Nar Valley¹⁴. The 21-ft. difference in elevation between the uppermost zone 3 marine sediments at Summer End and the uppermost freshwater sediments at Horse Fen suggests that at least that amount of transgression must almost certainly have occurred in that relatively short zone. It is difficult to suggest what could have caused a downwarping at this rate, which has obviously not been maintained since. That the transgression at Clacton was the result of a eustatic rise of sea-level rather than of downwarping has already been argued, by Oakley and Leakey¹⁷. They claim that, for the Clacton area, the rise to the Boyn Hill level is represented by the gravels at 85 ft. at Upper Dovercourt.

In the last, Ipswichian (Eemian, Riss-Würm, Sangamon) interglacial, early in the main warm zone *f*, the sea is known to have risen above -5 ft., at Selsey¹⁸. Presumably this was the end of the post-Riss rise, because appreciably later in zone *f* the sea was still at -2 ft., at Stone¹⁸. By the end of zone *f*, river flood deposits were being laid down at -3 ft. at Trafalgar Square¹⁹, which suggests that the sea had by now fallen a little below that level. Now, exactly when the Ipswichian climate began to deteriorate is difficult to say, but it was almost

certainly in zone *g*²⁰. What is striking is that in that zone a transgression occurred in north-west Europe. The transgression is very well documented in north-west Germany^{21,22} and, elsewhere, it seems to have formed the Eemian 'Portlandia Sea' of the Baltic²³ and the 'marine intercalation' of Holland²⁴. The German pollen data in particular favour a transgression in the first half of zone *g*. At about this time in England, a remarkable aggradation occurred in the Thames estuary where, at 23 ft. at Ilford²⁵, pollen-bearing sediments were covered by the well-known Thames 'brickearth' or silt. Because of the low percentage of *Carpinus* in the uppermost sediments at Ilford, West *et al.* attribute them to the end of zone *f*. However, because low percentages of *Carpinus* can sometimes occur in zone *g*²⁰, and because, otherwise, the percentages of *Pinus*, *Quercus* and *Corylus* in the uppermost sediments are quite typical of that zone, it seems quite likely that all these north-west European pollen dates reflect the same event. At Ilford, the aggradation of the brickearth continued to 42 ft., and at other places in the estuary to 50 ft. Since the gradient of the Thames and its terraces in this area is less than 3 in./mile (ref. 26), it seems quite possible that the aggradation of the brickearth was in fact a response to a rise of the sea to the 50- or 60-ft. 'Taplow', 'Main Monastirian' level traditionally assigned to this interglacial. That the Main Monastirian level may indeed have been merely a secondary one (from which the sea immediately began to drop to its Würm level) is suggested by the fact that it is only rarely a bench level, but is chiefly a beach and river terrace level²⁷. Similarly, in North America, although the coastal Cape May formation, assigned now to the last interglacial²⁸, was attributed by Salisbury and Knapp to a sea reaching 50 ft. or so, they remarked that only faint terraces rather than coastal cliffs were to be found at that level²⁹. Of course, as well as the 55-ft. Main Monastirian, a 25-ft. 'Late Monastirian' level²⁶ is often assigned to the last interglacial. This 'double' level is a problem for any theory of ice ages, but, according to Fig. 3, the Late Monastirian level might represent the brief reoccupation of the primary Hoxnian level by the regressing Hoxnian and Ipswichian seas. The Ipswichian regression across O.D. in zone *i* was recorded in northern France¹⁸.

The Ipswichian interglacial provides the only evidence I have been able to find that is plainly incompatible with the reconstruction in Fig. 3. In eastern England, the Histon Road, Cambridge, deposit³⁰ lies at 15-42 ft. O.D., but shows terrestrial as opposed to marine sedimentation between zones *g* and *i*. Now, because this is the only deposit of its kind so far, it is possible that it is deceptive in some way. One possibility is that the unconformity 500 cm below the top of the deposit, that is, at 25 ft. O.D., does represent a marine interval. The sediments below 500 cm contain high percentages of *Quercus* and *Corylus*, and could quite easily belong to the first part of zone *g*. Then, as Sparks and West point out, a true zone *h* is missing in the deposit, and it is possible that sedimentation above 500 cm did not begin again until zone *i*. If so, the unconformity would fit Fig. 3 exactly. Other East Anglian evidence, such as the 55-ft. Ipswichian terrace at Cambridge³¹, certainly fits Fig. 3. However, although the data from Histon Road are insufficient by themselves to disprove the reconstruction of Fig. 3, they do emphasize one of its chief weaknesses: the shortage of really high quality evidence for an Ipswichian sea-level above O.D. As we have seen, the north European data, the Thames evidence and the Monastirian tradition do favour such a level. However, evidence that would really favour Wilson's theory would be aggradation deposits similar to the Ilford ones in other estuaries, or, better, zone *h* marine deposits in the 20-40 ft. range. Because a late Ipswichian sea-level rise would have been short-lived, and the marine extension shallow, and because any deposits in it would afterwards have been

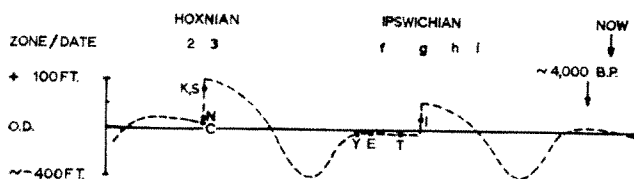


Fig. 3. Sea-level changes suggested for England. The time and elevation scales are compressed in the ice ages. The dots refer to pollen studies: C, Clacton; N, Nar Valley; K, Kirmington; S, Southelmham; T, Trafalgar Square; I, Ilford.

leached, frost-heaved and eroded for the whole of the Weichsel, such deposits, if they exist, may be hard to find. On the other hand, if they cannot be found, and if more deposits such as the Histon Road one are discovered, then these latter would be strong evidence against Wilson's theory, at least as it applies to the latest ice age.

In the current interglacial, since the northern ice sheets disappeared, Wilson's theory requires that sea-level should be falling slightly, as the Antarctic build-up continues. It is possible, of course, that a fall is being offset by continuing glacial retreat in the Canadian archipelago or Greenland, or even by minor surges from Greenland or Antarctica. However, there are many papers which do document a falling sea-level over the past few thousand years^{32,33}. On the other hand, a probably larger set of papers describes a rising sea-level³⁴. At present, the difference between these results cannot be explained, although it may be due, for example, to local land movements³⁵. In this situation, an indication of the world-wide average movement of sea-level might be given by the change, if any, in the Earth's rate of rotation. Analysing the change suggested by the data from ancient eclipses, Munk and MacDonald³⁶ conclude that there may have been a fall of sea-level of 2 m over the past 2,000 years, and this of course would favour Wilson's theory. A fall of 2 m in 2,000 years would be equivalent to a continuing 25 per cent surplus of accumulation over ablation in Antarctica (assuming that the area of the ice sheet is 1/30 that of the oceans, and the average accumulation on it 14 g/cm² yr)⁷. This is larger than the 5 per cent surplus which, ideally, is all that would be required to replace the late Ipswichian surge of Fig. 3 over the roughly 100,000 yr since it occurred. However, the figure of 5 per cent would be bigger if there have been minor surges since the late Ipswichian one. (Such surges could have been one cause of Weichsel 'stadial' advances.) Glaciologically, even a surplus of 25 per cent cannot really be distinguished yet in Antarctica, because our knowledge of ablation rates there is so very limited⁸. The known activity of the ice sheet at the edges of Antarctica suggests that if any build-up is occurring it must be concentrated in the centre of the continent. Evidence for or against this may come in a few years from Franco-Soviet and other strain measurements in that area: evidence that the ice is stretching at less than the rate required to dispose of the snow accumulation would support Wilson's theory.

So far, I have said little about the speed of the sea-level rises. Obviously, our first problem is to find out whether they really occur or not. There are two reasons, however, for saying a little more about their speed. First, Wilson's theory implies that the current interglacial will end with a new surge, perhaps suddenly, some time between now and say A.D. 50,000. Since, from Fig. 3, the sea might rise then by 75 ft. or so, the speed of the rise might have important human implications. Second, chiefly for the purpose of drawing Fig. 2, I mentioned a possible surge time of 40 yr. However, a sea-level rise much faster than this might have left some very unconventional deposits, and unless this is appreciated, these deposits may remain unrecognized. To illustrate this point, let us look, for example, at the brickearth, laid down during the late Ipswichian aggradation in the Thames estuary. The brickearth consists chiefly of silts, which are locally 30 ft. thick but which, unlike the alluvium of the current interglacial, are quite devoid of layers of vegetation, developed during intervals of sub-aerial exposure. These silts are famous for their numerous, curiously intact remains of large mammals. Many geologists have argued that the silts must have accumulated, and the mammals drowned, during floods much greater than those known to-day³⁸. The brickearth is extremely patchy and, indeed, the alluviation of the whole Thames estuary, from below -3 ft. to above 50 ft.,

would represent an enormous amount of deposition for the end of an interglacial, when if anything the Thames should have been incising rather than aggrading. Also, despite their situation, the shells in the brickearth are overwhelmingly freshwater ones, and only rarely estuarine ones. So long as Wilson's theory is being discussed, the possibility ought to be borne in mind that in fact the brickearth never was a normal alluvium, but was merely the soil and fauna of zone *g*, re-worked by a very rapid rise of sea-level, and swept into the embayments of the marine extension. Only after this diluvium had settled would conventional estuarine and marine deposits have begun to accumulate above it and, as we saw two paragraphs ago, such deposits may be hard to find. (The same possibility ought to be borne in mind for the brickearth at Stutton^{11,37}, where the evidence is otherwise incompatible, though not decisively so, with the curve of Fig. 3. Note that the data from nearby Bobbitshole³⁸ fit Fig. 3.)

Even if these late interglacial transgressions do prove to have been very rapid, determining their precise velocity may be difficult, except in one eventuality. As opposed to the Kutiah velocity in the second paragraph, the hearsay velocity (presumably several km/h) yields a surge time of only 1 week. Now a surge in only 1 week or so would involve a very rapid redistribution of ocean water, and the question arises whether or not the sea-level rises might have occurred in the form of waves or 'tsunamis', such as sometimes run several miles inland when they break on the coasts of the present-day Pacific Ocean³⁹. This is a possibility that can be tested, as follows. Let us look this time at the Hoxnian interglacial, for which a secondary, post-surge coastline of 100 ft. was suggested. If waves occurred, sediment-pollen studies some way inland from that contour should detect some disturbance at the time of the surge. The waves would be unlikely to have left any marine traces, such as shells, but they might have left a layer of debris, such as gravel or tree trunks. So far, I have been able to find only one investigation from such a situation, from Kilbeg⁴⁰ in southern Ireland. The profile there does show an unconformity covering the break of the Hoxnian climate, but the gap covers too long a time to be good evidence for waves. However, if further investigations in such situations can be found or made, it should be possible to complete this test.

If all large glaciers are indeed susceptible to surges, either because of the instability discussed above or, though this seems less likely, because of some external cause, then this raises many interesting questions. One of the most interesting must be whether or not surges of the Gondwanaland ice sheet may account for the Carboniferous cyclothems, especially for the rapid submergence of the coal beds. However, although these questions do deserve our attention, it looks as though the most rigorous progress in the matter of surges will be made by concentrating on Wilson's theory of Quaternary ice ages, particularly on events late in the last interglacial. As was mentioned earlier, the sea-level test for Wilson's theory is a very specific one, and it is certainly intriguing to find these traces of late interglacial transgressions with exactly the required timing, amplitude and speed. One explanation which might be advanced for them would be that they were merely continuations of the main post-glacial transgressions, and due either to a normal (as opposed to surge) deglaciation of Antarctica by the continuing warmth of the interglacial⁷, or else due to local downwarping. Another explanation would be that they were merely recoveries from a regression due to a cold spell in the middle of the interglacial. Up to now, Fig. 3 and the foregoing discussion are strongly against these explanations, and are favourable to the Wilsonian explanation. There is clearly a need for more data, but there seems little doubt that further work along these lines will complete the test of Wilson's theory.

I thank G. Denton, D. Fullerton, B. C. McDonald, Dr. J. F. Nye, Dr. R. G. West, Prof. S. Judson and Prof. H. H. Hess for their advice.

- ¹ Wilson, A. T., *Nature*, **201**, 147 (1964).
- ² Hollin, J. T., *Nature*, **202**, 1099 (1964).
- ³ Post, A. S., *Science*, **148**, 366 (1965).
- ⁴ Glen, A. R., *Geog. J.*, **88**, 206 (1941).
- ⁵ Mason, K., *Geog. J.*, **85**, 24 (1935).
- ⁶ Weertman, J., *Intern. Union Geod. and Geophys.*, Pub. 58, 31 (Intern. Assoc. Sci. Hydrol., 1962).
- ⁷ Hollin, J. T., *J. Glac.*, **4**, 173 (1962).
- ⁸ Hollin, J. T., *J. Glac.*, **4**, 312 (1962).
- ⁹ Weertman, J., *Intern. Union Geod. Geophys.*, Pub. 61, 245 (Intern. Assoc. Sci. Hydrol., 1963).
- ¹⁰ Maximov, I. V., *Information Bull. Soviet Antarctic Expedition*, No. 25, 38 (1961).
- ¹¹ West, R. G., *Proc. Geol. Assoc.*, **74**, 147 (1963).
- ¹² Orme, A. R., *Irish Geog.*, **4**, 279 (1962).
- ¹³ Pike, K., and Godwin, H., *Quart. J. Geol. Soc. London*, **108**, 261 (1953).
- ¹⁴ Stevens, L. A., *Quart. J. Geol. Soc. London*, **115**, 291 (1960).
- ¹⁵ Watts, W. A., *Proc. Yorks. Geol. Soc.*, **32**, 145 (1959).
- ¹⁶ West, R. G., *Trans. Norfolk and Norwich Nat. Soc.*, **19**, 365 (1961).
- ¹⁷ Oakley, K. P., and Leakey, M., *Proc. Prehist. Soc.*, **3**, 217 (1937).
- ¹⁸ West, R. G., and Sparks, B. W., *Phil. Trans. Roy. Soc. B*, **243**, 95 (1960).
- ¹⁹ Franks, J. W., *New Phytol.*, **59**, 145 (1960).
- ²⁰ Jessen, K., and Milthers, V., *Danm. Geol. Unders.*, II Raekke, No. 48 (1928).
- ²¹ Sindowski, K. H., *Z. Deutsch. Geol. Gesell.*, **112**, 527 (1960).
- ²² Sindowski, K. H., *Z. Deutsch. Geol. Gesell.*, **115**, 163 (1963).
- ²³ Woldstedt, P., *Das Eiszeitalter*, 2 (Stuttgart, 1958).
- ²⁴ Van Der Helde, S., *Geol. en Mijnbouw*, **19**, 272 (1957).
- ²⁵ West, R. G., Lambert, C. A., and Sparks, B. W., *Phil. Trans. Roy. Soc. B*, **247**, 185 (1964).
- ²⁶ Zeuner, F. E., *The Pleistocene Period* (London, 1959).
- ²⁷ Bull, A. J., *Proc. Geol. Assoc.*, **53**, 1 (1942).
- ²⁸ Richards, H. G., *Trans. Amer. Phil. Soc.*, N.S., **52**, Part 3 (1962).
- ²⁹ Salisbury, R. D., and Knapp, G. N., *New Jersey State Geologist Final Rep. Ser.*, **8** (Trenton, 1917).
- ³⁰ Sparks, B. W., and West, R. G., *Eiszeitalter und Gegenwart*, **10**, 123 (1959).
- ³¹ Sparks, B. W., *Geol. Mag.*, **101**, 334 (1964).
- ³² Schoffeld, J. C., and Thompson, H. R., *N.Z. J. Geol. Geophys.*, **7**, 359 (1964).
- ³³ van Andel, T. H., and Laborel, J., *Science*, **145**, 580 (1964).
- ³⁴ Shepard, F. P., *Science*, **143**, 574 (1964).
- ³⁵ Munk, W. H., and MacDonald, G. J. F., *The Rotation of the Earth* (London, 1960).
- ³⁶ Whitaker, W., *The Geology of London and of Part of the Thames Valley*, 1 (Mem. Geol. Surv. Eng. and Wales, 1889).
- ³⁷ Sparks, B. W., and West, R. G., *Proc. Geol. Assoc.*, **74**, 419 (1963).
- ³⁸ West, R. G., *Phil. Trans. Roy. Soc. B*, **241**, 1 (1957).
- ³⁹ Wheeler, W. H., *A Practical Manual of Tides and Waves* (London, 1906).
- ⁴⁰ Watts, W. A., *Proc. Roy. Irish Acad.*, **60**, B, 79 (1959).

"THERE IS ALL AFRICA"

By DR. A. S. THOMAS

EVER since the first explorers penetrated the centre of Africa, Europeans have desired to bring peace and prosperity to the continent; the most striking proof of that desire has been the devotion and self-sacrifice of the missionaries. But there was often a bland assumption that European methods of clothing, farming and political organization could be applied without modification near the equator. In courses of training for work in the tropics there was much emphasis on primitive agriculture—unchanging and resistant to change.

But, as a farmer's son who had done much hard work, some of which seemed unnecessary, I found myself thinking how sensible the Ghana farmers were to grow their cocoa crops with a minimum of work. Their mixtures of food crops looked untidy after European monoculture, but many of these crops had been introduced to Africa, some in the far past, some recently—bananas and cocoyams from Asia, cassava, other cocoyams, maize and sweet potatoes from America—and their culture must have involved great changes.

The Krobo farmers explained that their farms were in strips across the valleys because that was the only fair way of dividing the land—there was one kind of soil on top of the hills, one on the sides and one in the valleys. That important point had not been mentioned in any of the lectures on soil science to which I had listened, though the same sense of values had led to division of much of southern England into long strips of parishes. My appointment in Ghana lasted only for one tour; money was short in 1930 and there was considered to be no need for research on cocoa; six years later a Ghana farmer took specimens of swollen shoot to the Division of Agriculture and asked why his cocoa was dying.

On arrival in East Africa, I found that the soil chemists were studying soils in series or catenas. Most of Africa is a vast peneplain of old land surfaces dissected by water-courses; there are few level areas except the plains of dark clay which are under water for part of the year, dry and deeply cracked for another part; even these plains show slight differences in levels; parts which are submerged for much of the year must be considered in conjunction with the higher ground on which wild and domestic animals graze during the rains.

People are taught about Africa from small-scale maps which cannot show the undulating surface or the variety of vegetation associated with it; then some people go to Africa expecting to see vast stretches of virgin forest, their false ideas having been reinforced by travel films—"And so we set off through the African jungle", taken in a place where there is no jungle, only the coffee bushes shown on the screen. Even when African forests are tall, they may be quite young; a patch in the Botanic Gardens, Entebbe, Uganda, is so fine that it has been used as the setting for a "Tarzan" film; but a photograph shows that fifty years ago it was a grove of raphia palms without any trees.

Many theories have been postulated about tropical forests on the assumption that the areas examined were climax communities when, in reality, they were unstable young phases of forest growth. Even the woodland areas have been represented as climax, with maximum energy flow; the exposed strata on any river bank of such an area will show that there has been no stability; the layers of water-borne and wind-borne deposits prove there has been no stable state, only a sequence of changes.

Philosophical phrases such as "Man in harmony with his environment" would not mean much to an African peasant whose blood is invaded with malaria parasites, whose farm is invaded by elephants, monkeys and wild pigs. When you have recurrent dysentery and malaria you come to regard your body, not as an individual, but as an ecosystem; one man, who had a tapeworm as well, used to say: "We have enjoyed our meal".

Conservationists talk and write of man as a destroyer, not mentioning the way in which man builds up the soil of his settlements. There are many proofs of this building-up: the depth of deposits in old European cities, the huge mounds which cover ancient cultures in the desert areas of Africa and Asia, and the deep layers of broken pottery on some hill tops in the wetter parts of Africa.

Furthermore, the soils of settlements are richer than those of the country around them. Saltpetre used to be extracted in India from the soils of places where there were dense populations of men and domestic stock, and a residue of ashes from wood fires—conditions which can be matched in Africa. Africans select former village and

cattle kraal sites for the cultivation of crops like bananas and tobacco which demand high fertility. The banana is a most useful indicator plant in the wetter parts of Africa, for it will flourish and persist only on rich soils like those of the western slopes of Mount Elgon which have an agricultural population of several hundreds, even more than a thousand, per square mile.

If a house of wood and grass is deserted, it soon rots away under an equatorial climate; no trace will remain above ground, and the only clue will be in the soil—the local accumulation of bases, especially of lime, which is easily detected by tests for soil acidity. Some crops may benefit from the presence of bases; others, such as the tea plant, will die out in patterns to show the plans of former villages, proved by abundance of potsherds.

Certain trees often indicate former settlements; in the drier parts of Africa, the baobab, *Adansonia digitata*, is largely confined to past and present settlements; in the woodland areas, groups of very large shea butter trees, *Butyrospermum parkii*, are to be seen in the villages, or where the villages have been.

Chlorophora excelsa, a magnificent tree of the forest zone, is associated with the spirits of ancestors both in East and West Africa; the belief seems founded on the fact that the tree needs rich soil and protection from fire, conditions usually only found on farms. Soil tests near many *Chlorophora* trees on farms, in forests and woodlands, always showed the soil to be more alkaline near the tree than at a distance from it, where the soil often was too poor for the tree to grow. Yet this tree has been used to denote plant associations without mention of its restricted growth or of the unsuitability of the soil for its growth. It is restricted to settlements, just as in many grasslands the shrubs are restricted to the termite mounds, the soil of which is less acid than the rest of the land.

Diversity of soil is a usual feature in Africa; soils on the hills are always different from soils in the valleys, and the incidence of human settlements and termite mounds is likely to give diversity to the soils in any single zone; hills not very far apart may show distinct differences in their soils and in the way in which they are used¹. "In Ghana, soil series rarely cover a sufficient area in individual expanses to make it practical to map them on any but very large-scale maps"². Yet there are still being produced maps which show generalized arbitrary classifications; it is most interesting to consider places which one knows well, to compare the different classifications by people who may or may not have visited the area and to think how untrue these classifications represent the actual vegetation.

There are many difficulties in African ecology. More than twenty years ago I discussed the difficulty of learning the names of the plants with another man and found that he, like me, after two years in Africa had felt that he would never be able to learn the names; but that, again like me, after three years suddenly found that he knew enough names to make ecological investigations without taking a large number of specimens for determination in the herbarium. We were both quite intelligent, both very hard-working; and I refuse to believe that much good work could be done by those who write descriptions after a few weeks in Africa, looking at plants they had not seen before.

Furthermore, as Buxton showed, the aspect of any small piece of African vegetation alters completely with the seasons³; the eye selects those species which are in flower at the season when they are viewed. So I made it a rule never to publish an account of any patch of vegetation unless I had visited it at different seasons and had described it without reference to previous notes. Yet many of the papers describe large areas after very short visits; if work is done carefully and factually, it must be confined to a small plot; and there is no justification for extending such a description over a region. Yet

once a description is in print, it is regarded as factual and is reproduced in the text-books.

The ease and rapidity of modern travel only add to the unreliability of some accounts founded on observations from a motor-car. A road may seem to pass through dense forest when in reality there is only a screen of trees hiding the farms and villages; but in open country a road may pass through a string of farms although the land away from the road is not cultivated. An expert whom I was escorting through Uganda once stopped the car and said: "You have told me that *Cynodon dactylon* is not important in the natural grassland of this region; we have been through a belt of it, fifty miles wide". I tactlessly suggested that the belt was fifty miles long and ten feet wide, as he could see if he got out of the car; he did not, and he did not speak to me for the rest of the day as he was so angry that neither I nor the grasses conformed to his ideas.

In the old days, when aeroplanes flew low and slow, ecology could be done from them, but that is seldom possible from modern jet airliners. But air photographs are a most valuable means of survey; they show intricate patterns of fields, fallows, grasslands, woodlands and forests which give a truthful picture of the countryside, quite different from the generalized subjective accounts so often printed. The camera is objective.

One thing which horrifies visitors to Africa is the intensity of the fires which sweep through woodlands and grasslands. But Africa has been burnt over for many hundred thousand years; some fires are started by lightning and, long before there was any farming, fires were used in hunting and collecting honey. On my first visit to Mount Kadam, my Karamojong porters were astonished at my anger when they burnt a patch of forest to get a pound or so of honey, and they thought me fussy when I made them extinguish every spark. They looked on fire as part of the climate, and so do many African plants; a large living collection of Uganda grasses dwindled under cultivation, in spite of careful weeding; but when, in desperation, I set fire to them, they responded with normal vigorous growth.

There has been much outcry about the destruction of organic matter, but agricultural scientists are now agreed that burning is the only feasible way for the African farmer to get rid of rubbish before planting his crops. The burning of grassland has been condemned, but many people are coming to realize that it is the only practicable way to clear the mass of dry inedible stems and to produce flushes of young leaves for livestock during the long dry seasons. The wild animals, especially the smaller antelopes, also benefit from this burning; they dare not venture into tall unburnt grass, for fear of predators, and often may be seen grazing close to the cattle; the films usually show them on short young grass but do not show the cattle. Furthermore, in controlled experiments it has been found that the soils of grasslands burnt every year may contain more organic matter than do the soils of unburnt forests on similar ground.

Where there are cattle in Africa, there is usually erosion. It is not the treading of cattle which has attracted attention, but the grazing; yet in reality the treading is the main cause of erosion, for it makes the surface soil hard and as impermeable as concrete, so that the water rushes off and the lower layers remain dry as in a desert. Goats have caused much erosion in Mediterranean countries, but not in tropical Africa; when cattle are driven away from a place through the spread of tsetse fly, and only sheep and goats can be kept, the grass cover will improve; sheep and goats graze more closely than cattle, but they tread more lightly.

Agriculture in temperate regions is changing rapidly, and so is the attitude to change; it is welcomed. The outlook towards African agriculture is changing; there used to be elaborate statistical experiments to give proof of small differences of yields per acre, under conditions

where it was labour and not land which was the limiting factor; many panaceas have been recommended during the past thirty years—green manure crops, contour ploughing and mixed farming are some of them. But an increasing number of agricultural scientists are now demonstrating the wisdom of African farmers, of mixed planting to cover the soil and of giving a minimum of cultivation. We used to be taught that cultivation was necessary; my belief in it was shattered by an old Muganda's explanation of the vigour of a magnificent spreading coffee tree outside his house: "I sweep the ground every day".

Ecology is changing; as Raup showed in his address to the Jubilee Meeting of the British Ecological Society, Clementian ideas of plant succession have been much modified or discarded in America⁴. The influence of these ideas, alas, has extended to Africa. "Ecological studies in the tropics and sub-tropics have been dogged by attempts to fit the observed facts into a framework of classification tracing back to the ideas evolved in temperate Europe and North America, usually those of the schools of Cowles-Clements-Tansley or the Zurich-Montpellier group. The results are often worse than useless; they are misleading, and he who wants a knowledge of the ecological units present in a region has to disentangle factual descriptions from abstract discussion of successional relationships."⁵

The travellers who went to Africa in the nineteenth century had no abstract ecological concepts; they described what they saw, and their writings seem factual, all except those of Stanley, who was a journalist and wrote for effect; if he had known those concepts, he would have used them even more than do some journalists at the present day. Truth of the other accounts has been checked by careful examination on the ground and has shown some astonishing changes; for example, there is no doubt that one hundred years ago there were in Uganda far more people and far fewer trees than at present.

There have been many modern maps of Africa which were compiled by people much influenced by theories and philosophies, based on concepts which cannot be grasped by those who doubt their validity. Even the word 'ecosystem' can have no precise meaning in Africa, where mammals migrate for hundreds of miles and birds for thousands of miles; no region is free from influence outside it; there has been much outcry about the advance of the Sahara, but little mention of the great benefit from the vast quantities of base-rich desert dust brought down by the harmattan wind.

Changes are rapid in Africa. I recently took back to Africa some of the photographs which I had made about thirty years before; they proved changes which I had not expected. Cameras have no ecological concepts, they record facts; and the facts, not the concepts, will be useful in the future.

There is a great danger that, with increasing education, much of the traditional lore of African peoples will be lost, that Africans will place too much trust in the written word. It took me years to work out that the only feasible explanation for localized patches of reddened soil was that they were sites of settlements; an African assistant said: "You are quite right, sir; our people have always known that", and gave instances which I had not noticed. There continue to be many theories about the origin of lateritic ironstone from natural causes, but little attention to the frequency of worked stones, even of grindstones, in it.

Tribal history may be more significant than any survey in the explanation of existing vegetation. There were many wars between Ashanti and the Ivory Coast, so the forest on the borders is young, growing over former farmland. The Mabira Forest by the River Nile, on the borders of Buganda and Busoga, is tall, but it also is young; soil tests for a tea planter indicated a house site

in an unlikely spot; a fortnight later, the headman who was clearing the land greeted me: "You said truly, sir; when we dug where you said the house had been, we found this, and I kept it to show you". It was a hand-made hoe, the most treasured possession of a farmer and buried in the floor of his house; he had been killed or enslaved, the house had disappeared; only the hoe was left, sealed in the clay; and it was not even rusted.

Much of Africa has been photographed from the air to give a true picture of the general aspect. If this photography is combined with objective recording along transects and not by subjective surveys, then a factual and more detailed description is obtained. Specimens can be correctly identified by reference to herbaria like Kew, where floras of the African regions are being compiled. There is need for some agreed system of recording, so that the results for one country can be compared with those of another; and there is need for simple descriptive words like woodland, instead of imported vernacular terms like savanna, so incorrectly used sometimes in Africa.

Africans are skilled at recognizing plants from their leaves, which must be done in plant recording, for there is no season when all species will be in flower or fruit. Moreover, Africans must not be subjected to ecological theories before they do this recording; so many concepts have been enunciated, accepted, disproved and discarded during the short history of the subject that those at present in fashion are unlikely to last. As I had no training in ecology, I was not exposed to the full impact of the obsolete ideas in vogue when I was a student; but I had much training in soil science and was so indoctrinated with soil erosion that I had been years in Africa before I clearly understood that the grey soils in the valleys were poorer than the red soils on the hills—a pattern which is common in East and West Africa.

Tribal history and legends should be recorded and investigated before they are lost; they may be of more significance than some philosophies, for they do have some connexion with fact. Their examination will facilitate true knowledge of the past, the present and the possible future; for example, the Hamitic people and their cattle moved across Uganda from the north-east about four hundred years ago, which would not be possible at the present time; there must have been open grassland then, with fewer trees, tsetse flies and trypanosomes than there are now. More history and less philosophy are needed for progress in ecological studies in Africa (and, for that matter, in Britain as well). Furthermore, it is far better to leave blank spaces in maps than to fill them in with generalized inferences.

The spectacular increases in yields of cocoa which have followed scientific use of insecticides and fertilizers show that production in Africa need not be limited by 'natural' resources, as is assumed by those whose proposals are limited by ideas of 'conservation'. If there was less abstract discussion, if agriculture was left to the agricultural scientists and farmers, if forestry was left to the foresters, then the conservationists could devote their attention to the problems of wild life, so urgent in Africa. There is more than enough for them to do if the term 'conservation' was altered to 'wild life management' as in America; for the state of some Nature reserves is alarming—the destruction of grass by hippopotamus in the Queen Elizabeth National Park of Uganda and the extermination of trees by elephants in the Murchison Falls National Park.

Africans have adopted from other continents the things which they liked, such as bananas for food, cocoa for cash and football for recreation; they have rejected the things which they did not like, such as some of the labour-wasting methods of farming which have been advocated. Some African practices may seem strange to new-comers, but there is a reason, or there has been a reason, for them. It must not be expected that the

evolution of cultural change will be exactly the same in Africa as in other continents. Those who go to Africa must consider it their duty to learn before they start to teach. "There is all Africa, and her prodigies in us."

¹ Thomas, A. S., *J. Ecol.*, **33**, 10, 153 (1946).

² Brammer, H., *Agriculture and Land Use in Ghana*, edit. by Willis, J. B., 89 (Oxford Univ. Press, 1962).

³ Buxton, P. A., *J. Ecol.*, **23**, 134 (1935).

⁴ Raup, H. M., *British Ecol. Soc. Jubilee Symp.*, 19 (1964).

⁵ Coldrake, J. E., "Some Concepts and Methods in Subtropical Pasture Research", in *Bull.* **47**, *Com. Bur. Pastures and Field Crops* (1964).

NEWS and VIEWS

Deputy Chief Scientific Adviser, Ministry of Defence:
Prof. A. H. Cottrell, F.R.S.

ON June 30, Prof. A. H. Cottrell left the Department of Metallurgy at Cambridge to take up the post of Deputy Chief Scientific Adviser to the Ministry of Defence. This represents the beginning of a new phase in the career of an outstanding scientist who has already made great contributions in the academic and public sectors of science. He was professor of physical metallurgy at the University of Birmingham from 1949 until 1955 and was then appointed deputy head of the Metallurgy Division of the Atomic Energy Research Establishment at Harwell. From there he went to Cambridge in 1958 as Goldsmiths' professor of metallurgy. The interchanges in his career between academic and applied science environments are representative of Prof. Cottrell's attitude to his work. Although he is a first-rate theoretician, he nevertheless has a deep-rooted belief in the importance of applied science. This is revealed in much of his scientific writing, in which theories are developed not as an end in themselves, but as a means of solving the problems which arise when real materials are used in practice. His gift of picking out the essential features of a problem makes his papers and books stimulating reading for first-year undergraduates and research workers alike, and the same quality is evident in his lectures. He was elected a Fellow of the Royal Society in 1955 and, although still only in his middle forties, he has been honoured by many scientific bodies and universities at home and abroad. While at Cambridge he devoted himself wholeheartedly to both teaching and research, and the results will continue to benefit the Department of Metallurgy for many years to come. He will be greatly missed, but Cambridge's loss is the nation's gain since it would indeed be difficult to imagine anyone better equipped to deal with the responsibilities of his new post.

Ministry of Aviation Appointments:

Mr. H. G. R. Robinson, O.B.E.

MR. H. G. R. Robinson has been promoted to deputy chief scientific officer and appointed head of the Instruments and Electrical Engineering Department at the Royal Aircraft Establishment, Farnborough. Mr. Robinson joined the Guided Weapons Department of the Royal Aircraft Establishment in 1948 after graduating with honours in electrical engineering at the Imperial College of Science and Technology. After a year of postgraduate study at California Institute of Technology in 1952, he rejoined the Guided Weapons Department where he was engaged in theoretical and free flight investigation of kinetic heating at very high speeds. This work led, in 1955, to the design of the *Black Knight* re-entry test vehicle, with which Mr. Robinson was directly concerned. In 1960 he became head of the Satellite Launcher Division of Space Department, Royal Aircraft Establishment, and continued in this Department until his present appointment. His main concern in the Space Department was the British technical contribution to the European Launcher Development Organization (E.L.D.O.) launcher *Europa I*, and development of *Black Knight*, for space applications. Mr. Robinson is a Whitworth Scholar, and was awarded the Bronze Medal of the Royal Aeronautical Society in 1960 for furtherance of ballistic missile techniques.

Mr. R. R. Duddy

MR. R. R. Duddy has been promoted to deputy chief scientific officer and appointed director of the *Concord* Project in the Ministry of Aviation. Mr. Duddy was educated at Judd School, Tonbridge, and took a first-class honours degree in mechanical engineering at King's College, London. He first joined the Royal Aircraft Establishment in July 1939 as a vacation student in the Aerodynamics Department, and from 1954 until 1959 he was head of the Flight Division of the Department. He then spent two years with the Defence Research Staff in Washington and returned in 1961 to become head of the Naval Air Department of the Royal Aircraft Establishment at Bedford.

Mathematics in the University of Edinburgh:

Prof. F. F. Bonsall

PROF. F. F. BONSALL, at present professor of pure mathematics in the University of Newcastle upon Tyne, has been appointed to the McLaurin chair of mathematics in the University of Edinburgh as from October 1. Prof. Bonsall was educated at Merton College, Oxford. In 1947, after an undergraduate career which had been interrupted by six years of wartime service in the Royal Engineers, he graduated with first-class honours, and was appointed to a lectureship in mathematics in the University of Edinburgh. He joined the Department of Mathematics in Newcastle as a lecturer in 1948, was promoted to a readership in 1956, and has held the chair of pure mathematics at Newcastle since 1959. Prof. Bonsall has made substantial original contributions in functional analysis. His early work in this field dealt with some of the more algebraic aspects of the theory of Banach algebras. He then became interested in partially ordered vector spaces, a subject on which he is a leading authority. Generalizing a result of Krein and Rutman, he proved that various classes of positive operators possess positive eigenvectors. His work in this area also includes the study of order ideals, related extension theorems for linear functionals, and numerous applications in other branches of analysis. His recent researches deal with the classification of certain semi-algebras of functions, the structure of locally compact semi-algebras, and the further investigation of the spectral properties of positive operators by means of semi-algebra theory.

Industrial Engineering in the University College of Swansea:

Dr. T. O. Jeffries

DR. T. O. JEFFRIES, who has recently been appointed professor of industrial engineering in the University College of Swansea, was educated originally as a physicist, having gained an honours degree in physics from New College, Oxford, in 1947, with a special subject in nuclear physics in 1948. After this he was for three years Faraday Fellow at St. John's College, Oxford, during which time, in addition to his tutorial and lecture duties, he was concerned with the borderline between physics and engineering, participating in the design and construction of the Clarendon Laboratory 400-keV and 1-MeV Cockcroft and Walton accelerators, and completely designing and constructing a smaller 100-keV accelerator to study the D-D reaction. From the Clarendon, Dr. Jeffries moved

to the University of Birmingham as lecturer in physics, where among other work he designed and constructed a propane bubble chamber for use with the 100-MeV proton synchrotron. In 1956 he joined the Atomic Power Division of the English Electric Co., where he worked successively as physicist, mathematician and finally as head of the control and instrumentation group, a department of about 140 engineers, scientists, draughtsmen and technicians. While with the English Electric Co., Dr. Jeffries was responsible for designing and building the *Saturn* analogue computer and the smaller *Mars* computer, as well as for the design and construction of a *Monte Carlo* analogue computer which he used for the shielding calculations on nuclear reactors. During this period, Dr. Jeffries became extremely interested in the application of computers to design problems and to the use of data processing techniques to assist with the design of circuits and in the production of wiring and cabling diagrams.

Inorganic Chemistry in the University of Aberdeen :

Prof. H. F. W. Taylor

DR. H. F. W. TAYLOR took up, on October 1, his appointment as the third professor in the Department of Chemistry of the University of Aberdeen, with special responsibility for inorganic chemistry. Taylor, who hails from Nottingham, studied at University College there, graduating B.Sc. (London) with first-class honours in chemistry; he worked with the late Prof. J. M. Gulland and Dr. (now Prof.) D. O. Jordan on the physical chemistry of nucleic acids—work for which he was awarded a Ph.D. (London) in 1947. On leaving Nottingham he carried out research at Bedford College under Dr. (now Prof.) R. M. Barrer (hydrothermal chemistry of aluminosilicates) and at Birkbeck College under Prof. J. D. Bernal (chemical and crystallographic aspects of the hydration of Portland cement and related topics). For these and later contributions to the chemistry and crystallography of silicates Taylor was awarded the degree of D.Sc. by the University of London in 1957. When appointed lecturer at Aberdeen in 1953 (senior lecturer, 1963) Dr. Taylor continued his studies into the wide field of silicate chemistry especially by the use of X-ray techniques, and he has built up, assisted by Dr. F. Glasser, an active and enthusiastic research team. Investigations are being carried out along two main lines, calcium silicate hydrates and the chemistry of cement hydration—work which gained for him the Mineralogical Society of America Award for 1959, and also on topotactic reactions. His work in these fields is of international repute and he has been invited to take part in and has helped to organize conferences, etc., in Britain, the United States and elsewhere. He is the author of some eighty papers, editor and part author of *Chemistry and Cements*, and co-author with L. Heller of *Crystallographic Data for Calcium Silicates*. Following in the tradition of the Scottish universities, Dr. Taylor has taken, and will continue to take, a great interest in the teaching of the first-year classes. He has found this a challenging problem in both content and method and has introduced, into the practical course, programmed learning, multiple choice testing and the use of the computer for certain types of marking.

Chemistry in the University of Reading

THE years 1965 and 1966 form an important period in the development of the Department of Chemistry in the University of Reading. Now established in one of the largest of the new buildings in Whiteknights Park, the Department will at the end of the session 1965–66 lose the services of its present head, Prof. E. A. Guggenheim, who was appointed professor of chemistry in 1946, having been a member of the Department since 1933. An appointment to a second chair in the Department has already been made; Prof. D. Bryce-Smith, professor of organic chemistry, took up that post in June.

The appointment is now announced of professors in physical and in inorganic chemistry. Dr. G. W. Fowles, who will become professor of inorganic chemistry from January 1, 1966, and Dr. H. M. Frey, who will become professor of physical chemistry from October 1, 1966, are both at present on the staff of the University of Southampton.

Prof. G. W. Fowles

DURING 1943–44 and 1945–46, Dr. Fowles was an analyst in an industrial laboratory, spending the intervening period in H.M. Forces. He entered the University of Bristol in 1946, and in 1949 obtained the degree of B.Sc. with first-class honours in chemistry. He then continued with research and gained his Ph.D. in 1952. Dr. Fowles was awarded the D.Sc. degree in 1964. He was appointed to the Department of Chemistry in the University of Southampton in 1952, where he is now reader in inorganic chemistry. He was Canadian Research Council–Nuffield Foundation visiting professor for 1959 at Laval University, Quebec. He has twice visited the United States, for a Gordon Research Conference and a meeting of the American Chemical Society; and he has spoken at the seventh (1962) and eighth (1964) International Conferences on Co-ordination Compounds. During 1962–65 he was local representative and a member of the Council of the Chemical Society. His research has been concerned mainly with the chemistry of transition metals of the first part of the Periodic Table, although he is also actively working on organometallic compounds of other metals. His present research group is principally concerned with the spectroscopic and magnetic properties of co-ordination compounds of elements of the titanium, vanadium, and chromium sub-groups. He has published numerous papers, and, with E. Cartmell, a book, *Valency and Molecular Structure* (Butterworths, 1956 and 1961).

Prof. H. M. Frey

DR. FREY, after National Service in the Royal Air Force, entered Balliol College, Oxford, in 1949, and in 1951 was University Gibbs scholar in chemistry. He obtained first-class honours in chemistry in 1953, and then became War Memorial student and Imperial Chemical Industries Research Fellow, gaining his D.Phil. in 1955. During 1955–57 he held a Commonwealth Fund Fellowship at the University of California at Berkeley and at Harvard University. In 1957 he was appointed lecturer in the University of Southampton, and has been senior lecturer since 1963. His principal research has been concerned with the elucidation of the features and mechanism of inter- and intra-molecular energy transfer, particularly in relation to unimolecular reactions. Virtually all this work has been in the gas phase, though at present some investigations are being extended to the liquid phase. Much of his recent work has been on highly vibrationally excited molecules formed by methylene addition reactions, or by the photochemical decomposition of diazocompounds and diazirines.

Microbiology in the University of Sussex :

Prof. J. R. Postgate

DR. J. R. POSTGATE, who has been appointed to a chair of microbiology in the University of Sussex, was educated at Kingsbury County School and Balliol College, Oxford, which he entered as a Williams exhibitioner in 1941. He graduated in chemistry with honours in 1945, and found his interest in microbiology when taking his Part II in the field of bacterial growth kinetics under Prof. (now Sir) Cyril Hinshelwood. This interest was developed by a year's study of microbial chemistry under the combined tutorship of the late Prof. D. D. Woods and Prof. A. G. Ogston, and his transformation from chemist to biologist was complete when he took his degree of Ph.D. on bacterial drug resistance under Prof. Woods in 1948. He then joined K. R. Butlin's group

at the Chemical Research Laboratory, Teddington, where he carried out his well-known work on sulphate-reducing bacteria and associated problems in economic microbiology. This work was notable for the discovery of cytochrome c_3 , the first cytochrome to be found in an anaerobic organism, and for developing techniques for handling and studying sulphate-reducing bacteria. This very successful phase of Dr. Postgate's work was abruptly closed by the disbandment of K. R. Sutlin's group in 1959, and Dr. Postgate then undertook and published research on the death of bacteria in conditions of freezing and starvation at the Microbiological Research Establishment, Porton. There he remained until 1963, with an intermission as visiting professor of microbiology in the University of Illinois during 1962-63. In 1963 he joined the Agricultural Research Council to assist Prof. J. Chatt to establish a new Unit of Nitrogen Fixation, now located at the University of Sussex. He will continue to be assistant director of the Unit and his chair is a personal one for the period of his association with the Unit.

Psychology in the University of Newcastle upon Tyne: Prof. J. Brown

DR. J. BROWN has been appointed to the chair of psychology in the University of Newcastle upon Tyne with effect from August 1, 1966. Dr. Brown was born in August 1925. He went to Emmanuel College, Cambridge, graduating B.A. in 1950 after reading the Moral Sciences Tripos. He gained his Ph.D. degree in 1955, and spent three years on research at Cambridge after graduating and then spent one year as a temporary assistant lecturer in the University of Bristol. In 1954 he went to Birkbeck College as assistant lecturer, becoming lecturer in 1956. For the year 1965-66 he has been appointed visiting associate professor in the University of California (Berkeley). His main interests are in perception, learning and remembering, especially problems of immediate memory.

United Nations Review of Space Science

THE United Nations Committee on the Peaceful Uses of Outer Space was formed in 1959, and has served a useful function as the only international governmental body concerned solely with the subject. In 1963 the Committee decided to sponsor a publication describing the activities of the various international and national organizations concerned with space, and this report has now been published by the United Nations under the title *Space Activities and Resources* (Pp. v+172. (A/AC.105/26.) New York: United Nations, 1965. 2 dollars). The report covers first the international governmental organizations which are in some way concerned with space, such as the International Telecommunication Union and the World Meteorological Organization. The next sections describe regional organizations, such as the European Space Research Organization, and non-governmental bodies such as the Committee on Space Research (COSPAR) of the International Council of Scientific Unions. The remainder of the report, about two-thirds of the whole, reviews national space activities. Although the United States and the U.S.S.R. have the largest contributions, there are reports from a total of 37 countries, ranging from Argentina to Yugoslavia, and covering the period up to early 1964. This publication will be welcomed as a work of reference by anyone wishing to sort out the tangled inter-relations between the many international organizations which have decided that space is a subject they cannot afford to ignore. It also provides a useful review of national space activities, especially those of the smaller countries, which are little publicized.

Molecular Pharmacology

THE first reaction of a scientific reader to a new journal is one of alarm: he has already to look at too many of them

and his absorptive capacity is limited. Moreover, the title of the recently issued *Molecular Pharmacology* is really a tautology. Pharmacology is the science of the examination of the interaction of organic and inorganic molecules with living matter. It follows that all pharmacology is molecular. However, much can be said in defence of this new journal (1, No. 1, July, 1965. Edited by Avram Goldstein. Pp. 1-112. New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1965. Subscription rates: Vol. 1, 1965, 3 issues—International subscribers, 11 dollars; Private subscribers certifying that the journal is for personal use only, 5 dollars). It is being launched under the auspices of the American Society for Pharmacology and Experimental Therapeutics, which is also responsible for the well-known *Journal of Pharmacology and Experimental Therapeutics*, a publication that carries much material of immediate interest also to the physiologist and clinician. The need may have been felt for a paper that appeals more to the reader whose interests lie mainly in the theoretical aspects of pharmacology. A glance at the first issue shows that it is mainly the development of modern biochemistry (and biophysics) that has made this new venture necessary. The study of enzymes, of proteins in general, of nucleic acids, of cell particles and of cell membranes makes up the main content, and there is also material that deals with transport, with receptor theory and with the genetic code. *Molecular Pharmacology* carries the sub-title "An International Journal". The members of the advisory board and of the editorial board include scientists from many lands, well known to all workers in this field. The editor is Dr. Avram Goldstein, of Stanford University Medical School. We wish him success in this new enterprise.

Use of CIBA Resins in Ireland

THE growth potential in the use of synthetic resins and ancillary products in rapidly expanding industries both in Ulster and in the Republic of Ireland has been recognized, since the beginning of 1965, by the establishment by Ciba (A.R.L.), Ltd., Duxford, Cambridge, of an office and distributing depot in Belfast. Some details of actual developments involving the use of the well-known 'Aerolite' and 'Araldite' products in blockboard and paint manufactures, for example, are given in a recent issue of *Technical Notes*, entitled "Ciba Resins in Ireland" (Pp. 8. Ciba (A.R.L.), Ltd., Duxford, Cambridge. June 1965). In Northern Ireland there has been, in recent years, a notable expansion in the building industry, especially concerning prefabricated units. One example is the manufacture of wood chipboard, involving the use of large quantities of 'Aerolite' resin each week, at the factory of the Ulster Chipboard Company at Coleraine, County Londonderry; in this case the raw material is forest thinnings from timber felled from three of the largest forests in Northern Ireland; the process consists of coating the wood chips with resin, pressing the boards, then sanding on both sides to specified thickness. Other firms using 'Aerolite' adhesive are in large-scale production of such items as panel doors, flush doors, casement windows, garage doors, staircases, school and church furniture. In Eire, similar activities and modern trends in prefabricated timbers are briefly described. In the factories of T. and C. Martin, Ltd., Dublin, a finger-jointing machine produces continuous lengths of jointed timber by gluing selected raw timber of relatively short length with 'Aerodux 500' resorcinol resin adhesive, clamping and pressing, then cutting automatically to the desired length; timbers jointed in this way are eminently suitable as laminated members for structural engineering. Among paint manufacturers, Syntheses, Ltd., Dublin, use 'Araldite' epoxy resin formulations in their production of high-duty paints demanding good mechanical and chemical resistant properties. A further example of this is quoted in the case of Kosangas, Ltd., Dublin, suppliers of

R342

gases in steel bottles—containers which are subject to hard use; they are successfully coated with 'Araldite'-based paint, after shot-blasting, applied by electrostatic spray. These Ciba resins are being increasingly used by other industries in Ireland and such uses are planned for description in forthcoming issues of *Technical Notes*.

Iron Ore Production and Research at Broken Hill, Australia

THE well-known *B.H.P. Technical Bulletin*, produced periodically by the Broken Hill Proprietary Co., Ltd., Australia, is always a source of valuable technical information on the iron ore industry, arising from operational and research activities within this great organization, and the issue published in April 1965 is no exception to this dictum (No. 22: 9, No. 1; April 1965). An important contribution is a series of articles describing a new ore agglomeration laboratory built at the Company's Central Research Laboratories at Shortland, New South Wales. A general description of the new premises is first given by L. C. Bogan, who describes the laboratory layout, technological capabilities and limitations of the pilot plants installed, programmes scheduled, and services available; the latter includes chemical analysis by direct-reading spectrophotometry, petrology, and X-ray diffraction and X-ray spectroscopy. "Comminution and Green Pellet Production" is the title of a second article, by D. H. Harraway, in which he discusses the several pilot plant operations involved in production of green pellet agglomerates, comminution of the ore and mixing it with additives and water to form the ore pellets. A third article, "The Experimental Pellet Heat-Hardening Unit", by C. W. Brock, describes how, having produced the green pellets, it is necessary to heat-treat them "... to form a strong, approximately spherical aggregate capable of withstanding weathering and the high handling stresses which may be expected in stockpiling, transportation and blast furnace charging". Further contributions to this *Bulletin* include an account of "The Development of Koolan Island", by M. T. Phillips; this is one of the islands of the Yampi group off the north-west coast of Australia, where production of iron ore commenced in 1964. Petrological research is represented by an interesting paper by Sylvia Whitehead entitled "Petrological Notes on the Koolyanobbing Iron Ore Formation", the location being the Koolyanobbing range of hills situated about 35 miles north-east of Southern Cross, Western Australia. An important haematite ore-producing region in South Australia is the Middleback Ranges, and K. L. Ashworth describes this and other prospects in his article on "Geology of Iron Duke, South Australia", situated approximately 36 miles west of Whyalla, on the eastern Eyre peninsula. Finally, there is a paper by J. A. Gregory and S. Wolski on "Experimental Assessment of Factors Controlling the Production of High-Basicity Sinter".

South African Prehistory

AN interesting pamphlet entitled *Bowmen, Spears and Shields in Southern Rhodesian Rock Art*, concerned with the types of shield depicted by the artists and the distribution of the bowmen and the spears, has recently been published (*Cimbebasia—SWA Research*, No. 10. Pp. 8. Windhoek, S.W.A.: State Museum, 1964). The author, C. K. Cooke, has personally visited more than a thousand rock shelter and cave sites and has, of course, drawn on the works of other authorities. The important fact which emerges is that the shields figured are of definitely Bantu type, and this would suggest that many of the painted rock shelters must be A.D. in date. It has been the custom to ascribe a great antiquity to the rock paintings of Southern Rhodesia and South Africa. However, it has constantly been forgotten that paintings in open rock shelters could scarcely have survived serious climatic changes, such as have occurred in the distant past

—conditions which permitted much precipitation and subsequent evaporation of water vapour on the rock shelter walls. It is therefore very interesting that research into the types of shield figured in this late phase of Southern Rhodesian rock art points to a comparatively late date for its creation. The late C. van Riet Lowe once remarked that the celebrated "White Lady" of Brandberg could as well have been painted in A.D. 1500 as in 1500 B.C. The June issue of the *South African Journal of Science* contains an interesting article by R. R. Inskeep. Not far from Port Elizabeth a series of springs on the hillside behind the homestead on the Amanzi Estates has deposited thick sediments which contain many lower palaeolithic implements. The author has undertaken some considerable excavation and gives us geological sections and a determined stratigraphy. The implements are almost all of the core type, though some worked flakes do occur. Hand-axes, cleavers and choppers have been collected, and the author dates the finds to an early stage in the Old Stone Age. Some samples of pollen were noted, and tentative identifications indicate the presence of members of the Cyperaceae, Gramineae, Compositae and Leguminosae (including an acacia) families. Mr. Inskeep is to be congratulated on a useful piece of work.

Bark Canoes and Skin Boats of North America

LIKE many traditional crafts, the building of bark canoes is dying out and much information about it has already been lost. E. T. Adney, who died in 1950, was a skilled artist, who devoted a good deal of his life to the study of these canoes and acquired practical experience of building them. He left a lot of models, now in the Mariner's Museum at Newport News, Virginia, as well as numerous papers "in a highly chaotic state". H. I. Chappelle, who is curator of transportation in the Museum of History and Technology, a constituent of the U.S. National Museum, has carried out the arduous task of compiling the relevant information from these papers and the models (Smithsonian Institution, Washington. Museum of History and Technology. Bulletin No. 230: *The Bark Canoes and Skin Boats of North America*. Pp. xiv + 242. Washington, D.C.: Government Printing Office, 1964. 3.25 dollars). He has added a chapter on Arctic skin boats and one on temporary craft—canoes of bark other than birch, moose-hide boats and the coracle-like bull boats. There is an appendix on rolling kayaks by J. D. Heath. Information derived from Adney makes up the main part of the book. It consists of three chapters on early history, materials and tools, and form and methods of construction, followed by three chapters treating in detail the canoes of the Eastern Maritime Region, Central Canada, and North-West Canada, of which the first is the fullest. The work is fully illustrated with photographs from many sources and excellent drawings, mostly redrawn from Adney's by Chappelle. Amid a wealth of detailed information a fact of general interest emerges. There is a fundamental difference between bark canoes and Eskimo skin boats, namely that the frame of the bark canoe (and this includes 'temporary' Indian skin boats) is built up within the cover and would collapse without it, whereas the Eskimo boats have a rigid frame over which the skin cover is afterwards stretched. As is well known, bark canoes are so suitable for their purpose that they were at once adopted by Europeans on arrival, and used on a large scale in the fur trade. This suggests that this valuable publication may find a practical use in addition to its great historical importance.

Production Ecology in Tropical High Forest

NOT much work has been carried out on production ecology in tropical high forest although it is estimated that 38 per cent of the forests of the world belong to this type. However, a very useful investigation has been carried out in the southern Ivory Coast (*Production*

Brute, Pertes par Respiration et Production Nette dans la Forêt Ombrophile Tropicale. Par D. Müller et Jørgen Nielsen. Pp. 69-160, illus. *Det. Forstlige Forsøgsvaesen*. Denmark, 1965). Field measurements were made in 1955, 1957 and 1960, in two sample plots measuring 30 m × 30 m and 40 m × 40 m. Altogether there were 209 trees of more than 3 cm diameter breast height but 58 per cent belonged to 5 of the 45 species represented. All the trees in the smaller plot were felled after the 1955 measurements, care being taken first to clean a sufficient area so that the entire crowns fell on places where it was possible to collect all the leaves from the sample trees. In the larger plot basal areas, form factors, volumes and current annual increments were calculated from diameter and height measurements. Other data provided statistics for the branch percentage, loss of dry matter by respiration, surface area and weight of leaves, leaf-fall and photosynthetic efficiency. Special attention is given to the occurrence of 'light' leaves and 'shade' leaves which is a characteristic shared by deciduous trees of temperate and tropical forests, but dominants in the latter tend to have 'light' leaves only and it is suggested that this is why their crowns are usually small. In this particular example the net production of dry matter came to 9.0 metric tons per hectare per annum (roots, stems and branches) but the gross production was 52.5 metric tons per hectare per annum. It is interesting to note that although the production of dry matter is only very slightly less than for an even-aged beech forest in Denmark, the gross production is more than twice. Indeed, it is a characteristic of lowland tropical high forest that there is a large gross production and a great loss of dry matter by respiration in the stem, branches and leaves. This is a most welcome contribution to the subject of production ecology and the authors have provided useful data. Perhaps the exercise also demonstrates the difficulty of taking an adequate sample in tropical high forest and yet keeping the work within the realm of practical possibility. For example, familiar dominants of the tropical high forest of West Africa are absent from the tree lists given. In the smaller plot, the second largest tree by diameter (63.6 cm) and in height (27 m) is *Turraecanthus africanus*, which is not a typical emergent of this forest. The tallest tree recorded from both plots is 50.2 m and only two others reach 40 m or more.

Elementary Particle Physics

FOLLOWING the review of *Group Theoretical Concepts and Methods in Elementary Particle Physics* (*Nature*, 207, 118; 1965), Messrs. Gordon and Breach, Science Publishers, Inc., have written to the Editor, stating that two prices are available for this book. There is a professional edition published at 14.50 dollars, and a reference edition published at 21.50 dollars.

Announcements

THE Wellcome Laboratories of Tropical Medicine which since 1934 have been housed in the Wellcome Building, Euston Road, London, have moved to new laboratories on the site of the Wellcome Research Laboratories at Langley Court, Beckenham, Kent.

WITH the July 1965 issue, the *Bulletin of the Libraries of Commerce, Science and Technology* of the Sheffield City Libraries takes a new style and arrangement (No. 1, July 1965. Pp. 18. Sheffield: Sheffield City Libraries, 1965). While continuing to notify accessions, with the exception of some pamphlets and miscellaneous material, the *Bulletin* will now announce any developments within the libraries affecting readers and users of their services. The present issue includes a list of directories and also a list of recent metallurgical reports.

OPEN days will be held at the Tropical Products Institute during October 14-15. Further information can be

obtained from the Director, Tropical Products Institute, 56-62 Gray's Inn Road, London, W.C.1.

A CONFERENCE on "Technology and Society" will be held in Bath during October 14-16. Further information can be obtained from Mr. J. H. Lamble, Bristol College of Science and Technology, Ashley Down, Bristol 7.

THE third INTERKAMA Exhibition and Congress will be held in Düsseldorf during October 13-19. Further information can be obtained from Nordwestdeutsche Ausstellungs- und Messe-Gesellschaft m.b.H., 4 Düsseldorf 10, Postfach 10203.

THE Arthur Stanley Eddington Memorial Lecture for 1965, entitled "The Brain and the Unity of Conscious Experience", will be delivered by Sir John Eccles in Cambridge on October 15. Further information can be obtained from W. B. Harland, Sedgwick Museum, Cambridge.

A SYMPOSIUM on "The Future Pattern of Research in Instrument Technology", arranged by the Tees-Side Section of the Society of Instrument Technology, will be held at Middlesbrough on October 14. Further information can be obtained from Mr. G. F. Shute, 10 Mayberry Grove, Linthorpe, Middlesbrough, Yorkshire.

THE ninth conference on "Analytical Chemistry in Nuclear Technology", sponsored by the Analytical Chemistry Division of the Oak Ridge National Laboratory, will be held in Gatlinburg, Tennessee, during October 12-14. Further information can be obtained from C. D. Susano, Oak Ridge National Laboratory, P.O. Box X, Oak Ridge, Tennessee 37831.

AN international conference on "Dynamic Stability of Structures", sponsored jointly by the U.S. Air Force Office of Scientific Research and Northwestern University, will be held in Evanston, Illinois, during October 18-20. Further information can be obtained from Prof. George Herrmann, Technological Institute, Northwestern University, Evanston, Illinois.

THE annual meeting of the Conference on Electrical Insulation of the National Academy of Sciences-National Research Council will be held at Buck Hill Falls, Pennsylvania, during October 25-27. Further information can be obtained from Mr. D. W. Thornhill, Conference on Electrical Insulation, National Academy of Sciences, 2101 Constitution Avenue, N.W., Washington, D.C.

CORRIGENDUM. In the article entitled "Environmental Determination of Insular Variation in Bird Species Abundance in the Gulf of Guinea" by Prof. T. H. Hamilton and N. E. Armstrong, which appeared on p. 148 of the July 10, 1965, issue of *Nature*, the following corrections should be made: (1) Second paragraph, line after equation (4) should read: "where $z \neq 1$ " (not ' $x \neq 1$ '). (2) Third paragraph, thirteenth line, should read: "estimate the radius . . . as 7.25 miles" (not '. . . as 7 . . .'). (3) Third paragraph, seventeenth line, should read:

$$". . . \pi r \sqrt{r^2 + h^2}" \text{ (not } '\pi r \sqrt{r^2 - h^2} \text{').}$$

CORRIGENDUM. In the communication entitled "Radiochemical Determination of the Endogenous and Exogenous Respiration of Bacterial Spores" by H. Desser and Prof. E. Broda, which appeared on p. 1270 of the June 19, 1965, issue of *Nature*, line 33 should read ". . . The specific activities were up to 6.6×10^3 ". Also, H. Desser and Prof. E. Broda thank Prof. H. O. Halvorson, Madison, for the gift of *Bacillus cereus* and for advice on its cultivation.

ERRATUM. In the communication entitled "Benzene-sulphonylcarbamates: New Herbicides" by Helen J. Cottrell and Dr. B. J. Heywood, which was published on p. 655 of the August 7, 1965, issue of *Nature*, the first reference should read as follows:

¹ Carpenter, K., Heywood, B. J., Parnell, E. W., Boesch, R., and Metivier, J., Brit. Pat. Appln. 32407/62, and Brit. Pat. Appln. 8805/63.

FUEL POLICY FOR BRITAIN

ON January 22, 1965, Mr. F. Lee, the Minister of Power, told the House of Commons that he was undertaking a general review of fuel policy and had already initiated discussions with the chairman of the nationalized fuel industries. To assist consultation on a wider basis he was setting up an Energy Advisory Council. This Council would consider and advise him about the energy situation and outlook, and about plans and policies of the fuel and power industries in relation to national objectives for economic growth. At the end of June he told the British Electrical Power Convention at Brighton that he expected the results of this review of fuel policy to be ready soon. At the same time, he asked the industry to increase capacity to the point where reasonable, but not excessive margins, were secured. Moreover, he asked the industry to do this for the lowest practical demands on real resources and a very substantial use of self-generated funds, absorbing wherever possible the increases in operating costs so that prices to consumers remained stable. Concerning co-operation between the nationalized fuel and power industries in research and development, Mr. Lee said in the House of Commons on July 13 that he had already approached the industries; the National Coal Board and the Central Electricity Generating Board had for some time had a research liaison committee. The Gas Council had now joined with them to form a joint Liaison Committee for research and development so that their scientific potential might be used to the fullest advantage. The primary, though not the exclusive, objective was to improve the technology of utilizing coal. Mr. Lee said that the research programmes of the three industries were extensive, but he was bringing them into closer co-operation.

In view of all this a Broadsheet, *Questions of Fuel Policy*, issued by Political and Economic Planning*, is of some special interest. The Broadsheet starts by comparing the terms of reference of the Energy Advisory Council with the instructions given to the Ridley Committee on National Policy for the Use of Fuel and Power Resources when it was established in 1951. It points out that any recommendations the new Council may be able to agree on as to national policy are likely to represent, at the best, the highest common factor of agreement between existing vested interests. The Ridley Committee, on the other hand, was an independent group of public men, economists and technologists, examining the fuel industries rather than representing them, and was more likely to be free to formulate fresh policy in a way that the new Energy Advisory Council could not be expected to do. This in itself makes the independent review in this Broadsheet of the greater interest, although it must be admitted that in practice the advice of the Ridley Committee was not taken very seriously.

The Broadsheet notes that in 1964 Britain used primary energy equivalent to about 285 million tons of coal. Of this total about 187 million tons was actually coal; petroleum products accounted for about 93 million tons of coal equivalent, of which some 30 million tons was road- or air-transport fuel or used in refineries. Of the remaining general fuel used, coal accounted for nearly three-quarters compared with nearly 90 per cent in 1951. In absolute terms, coal consumption in 1964 was 20 million tons less than in 1951. Over the same period, the consumption of oil as a general fuel rose about five times to some 55 million tons. So far as total fuel consumption is concerned, the forecasts of the Ridley Committee in 1952 for fuel consumption during the

period 1959-63 were fairly well confirmed (coal providing however, only some 70 per cent of the total instead of 95 per cent, as forecast). Apart from the switch to oil there has been a further decline in the direct use of coal and more and more of the final consumption of this fuel is now in the form of electricity.

On the framework of present-day policy, P.E.P. points out that imports of coal are subject to licensing and in practice no licences are granted; moreover, import licences have not been granted for any increased imports of Russian oil. Since 1961 a fairly high excise duty of almost £2 a ton has been imposed on fuel oil and other oil products sold in the general energy market, while some restraint has at times been placed on the development of electricity generation from oil. Nuclear energy constitutes another factor and the Government has also abolished the customs duty on methane, which has slightly improved the economics of the scheme for importing liquefied natural gas from Algeria.

Considering new factors in the forecasts for the consumption of fuel, the Broadsheet points out that the forecasts and investment programmes of the major fuel industries represent, even over the short term, a higher total fuel consumption than is generally forecast—as has been pointed out by the National Economic Development Council. Forecasting demand for fuels is fairly hazardous in the commercial or technical sense beyond the 7-10 year period affected by decisions regarding investment that must be taken in the next 2-3 years. Petroleum exploration in the North Sea is particularly hazardous, and however welcome might be the impact of North Sea gas to upset established forecasts, it may not be an economic proposition. A more practical factor in present calculations is the firmer offer of cheap nuclear power. The rapid downward revision of nuclear costs could mean a much higher rate of commissioning of nuclear plant in the construction programmes of the supply industry by 1975 than has so far been envisaged, and in consequence an earlier levelling off in the demand for coal by power stations.

A working group of P.E.P. which is considering the energy situation and the kind of national fuel policy most suitable for Britain in the 'sixties expects to publish a full-scale report by the autumn of 1965. In the meantime, the Broadsheet indicates some of the problems which must face those responsible for formulating policy in this field. First, it points out there is the question of whether fiscal policies, such as the levying of a fuel tax on oil used in power but not on oil used in gas manufacture, should be altered so as to favour gas less. Alternatively, should gas be assisted to take over more of the demand for space heating and electricity discouraged in this sector of the domestic market? Again, there is the question of whether Ministerial policy should attempt to settle the constantly vexed arguments between the two industries and their cross-accusations about the special financial incentives alleged to be offered to local authorities and other housing developers. Again, is the right balance between amenity and efficiency being struck at present in the lengthy procedure of licensing, public enquiry and the like required for the construction of electricity production and transmission equipment? Some might also be disposed to ask whether the present structure of Government is such as to enable amenity issues to be raised at a sufficiently early stage for full effect to be given to their arguments before the electricity authorities are committed to particular schemes. Another important question is whether research and development are adequately and properly organized in these industries,

* *Planning*, 31, No. 489 (June 1965): *Questions of Fuel Policy*. Pp. 183-218 (London: Political and Economic Planning, 1965) 6s.

and in particular whether the balance of activity and initiative in nuclear development between the Central Electricity Generating Board and the Atomic Energy Authority is correct. Here again questions of Government structure may also arise, particularly in respect of the position of the Ministry of Technology.

Other questions suggested for consideration in the Broadsheet are the structural division of electricity supply for England and Wales and for Scotland, and whether there is scope for forms of combined technical development between the industries, and possibly coal or oil also, that are being ignored at present because of the structural separation and competition. The Broadsheet also questions whether limits should be put to commercially competitive promotion between the nationalized fuel industries, and how far they should be obliged to keep their investment policies with the Government's forecasts of solid and other smokeless fuel requirements arising from application of the Clean Air Policy. The principles to be followed in permitting these nationalized industries to diversify their activities in the fields outside those in which they now operate also require definition. The Broadsheet hints plainly that the consumer is not at present adequately informed of the price trends in the various fuels. The present structure of the Ministry of Power also requires consideration in the context of

how far it conduces to a sensible co-ordination of national energy policy and to objective assessments of conflicts of policy between the nationalized fuel industries.

It should also be remembered that some of the technological changes that could occur in the supply of energy would bring with them new questions about the structure of the British fuel industries. For example, if large-scale natural gas-fields were in fact found in the North Sea, the present gas industry, with its virtual monopoly and its commitment to manufacture gas at lower calorific values, might not be the ideal instrument to introduce the natural gas into the British market. Possibly a measure of competition should be introduced when gas supply becomes more and more based on the end-products of oil refining. Nor should it be without due consideration whether the economies of scale in supply or in transmission are as compelling, or whether monopoly is as appropriate a form of organization for gas as for electricity supply. The Broadsheet offers no answers to these problems, but it does suggest that urgent public consideration should be given to them and particularly to the determination of the right order of priorities. This is especially so in regard to what protection the fuel industry should receive, particularly British coal, and in what form.

U.S. STANDARD FREQUENCY AND TIME SERVICES

A REVISED (1965) publication of the United States National Bureau of Standards* gives a detailed and illustrated description of the services provided by the Standard Frequency Stations now operating in Washington, Hawaii and Colorado. The first is the well-known station, *WWV*, at Greenbelt, Maryland, which has been in operation since 1933, and now provides services of radio and audio frequencies, musical pitch, time signals and the departure of these from astronomical time; propagation forecasts and geophysical alerts are also issued from *WWV*. An additional service was started from Hawaii in 1948; but this is confined to radio frequencies, time signals and their corrections. In 1963, two low-frequency transmissions were added from stations at Fort Collins, Colorado. One, *WWVB*, broadcasts continuously on the standard frequency of 60 kc/s, and provides time signals and corrections, while the other, *WWVL*, broadcasts continuously on the standard frequency of 20 kc/s. The frequencies of all these services are held stable in general to a few parts in 10^{11} , and they are ultimately referred to the resonance frequency of caesium, which is measured in terms of ephemeris time.

All the carrier and modulation frequencies at *WWV* and *WWVB* are derived from precision quartz oscillators of

* United States Department of Commerce: National Bureau of Standards. Miscellaneous Publication 238: *Standard Frequencies and Time Services of the National Bureau of Standards*. Pp. 8. (Washington, D.C.: Government Printing Office, 1965.) 15 cents.

high stability. These are offset from the standard by a small but precisely known amount to reduce departure between the time signals as broadcast and astronomical time, U.T.2. Although the latter is subject to unpredictable changes readily noted at this level of precision, it is expected that the present offset—150 parts in 10^{10} —established in 1964, will remain in effect for the present calendar year (1965). For the benefit of users who wish to make direct comparisons of absolute frequencies, *WWVB* has been transmitting on 60 kc/s with no offset since January 1, 1965.

A comprehensive description, with diagrams, of all these services is given in the report, together with details of the propagation forecasts and geophysical alerts which are disseminated at hourly intervals. The forecast announcement tells users, in a simple code form, the condition of the ionosphere at the regular time of issue, and the quality of radio services due to propagation conditions to be expected during the next 6 h. A series of letter symbols is used to indicate the current geophysical conditions and give notice of any outstanding solar or geophysical events which are expected or which have occurred in the preceding 24 h.

The revised issue of this publication will be welcomed by all users of frequencies and time signals, as well as by research workers in the fields of radio and the geophysical and solar sciences.

R. L. SMITH-ROSE

COMMERCIAL NUCLEAR POWER STATIONS IN BRITAIN

THE principal contents of the July number of the *Journal of the British Nuclear Energy Society* (4, No. 3; 1965) consists of the texts of the six papers contributed by members of the Central Electricity Generating Board and the South of Scotland Electricity Board and presented to the symposium on the performance of commercial nuclear power stations in the United Kingdom, which was held at the University of Leicester on June 30.

H. M. Carruthers, in his discussion of the evolution of magnox station design, points out that, in the nine years since the first Calder Hall reactor went critical, some

thirty-five reactors of this basic type have been built, of which twenty-six are in Great Britain. The reactors during this period have developed from a small unit of about 40 MW(e) output to one of nearly 600 MW(e) with at the same time reduced capital and generating costs. The main technical improvements in layout, shielding, refuelling methods, pressure circuit technology and fuel element design since the start of the commercial magnox programme are described in some detail.

The commissioning of a nuclear power station requires not only the setting of the plant to work safely and

reliably, but also at the minimum cost computed over the total life of the station. The history of the commissioning of the Berkeley and Bradwell stations is reviewed by C. D. Heath and D. J. Silverlead, and in their assessment they direct attention to the need for greater flexibility in the timing of the various tests and measurements, improvements in the planning of construction and plant installation, and some simpler form of commissioning organization.

Two papers deal with the performance of reactor cores and materials and of power station plant, and a third with power station maintenance. Of the nine civil nuclear stations, Berkeley and Bradwell have had more than two years' full-power operation and Hunterston one year. Serious defects have been experienced at all three stations and the extra fuel costs have amounted to about six and a half million pounds. The major defects were in the fuelling routes, turbines and boilers. Experience suggests that more reliable operation in the future will occur. With

regard to maintenance, it is emphasized that health physics requirements are an important factor in maintenance planning and that future stations should be better planned and equipped for the maintenance of radioactive equipment.

The final paper in the symposium, by P. H. G. Holbrook and A. C. Horne, deals with some of the special problems, not necessarily technical, which arise in the operation of a nuclear power station. The provisions of the Nuclear Installations Licensing and Insurance Act, 1959, the Radioactive Substances Act, 1960, and the Generating Board's safety regulations have to be enforced and accordingly appropriate inspectorates and organizations have to be established. The increased automation of future stations, with centralized control and computational operation, may lead to a change in the pattern of station organization with economies in technical man-power and less risk to personnel.

S. WEINTROUB

PHYSICS EDUCATION

IDEALISM and iconoclasm were offered in equal proportions by speakers at the International Conference on the Education of Professional Physicists, held in London during July 15-21. The meeting was arranged by the Institute of Physics and the Physical Society for the International Union of Pure and Applied Physics, and attracted participants from twenty-five countries.

Lord Beeching (Imperial Chemical Industries, Ltd.), in a forthright introductory speech, deplored the attitude of university departments which, while disclaiming interest in vocational training, were in fact preparing students exclusively for the vocation of fundamental research. It was, he asserted, not in the public interest that academic research should be presented as the best career for physicists at a time when the industrial, economic and political problems of the community were increasingly dependent on the widespread use of science and technology. Though the Government had great responsibilities in the deployment of scientific and technical resources, only a handful of Junior Ministers had any scientific training. The cause of this deficiency was in the attitude which asked: "Why should we train a good physicist, only to have him wasted as a politician?"

Students of physics must learn that they were not going to be employed exclusively in the narrow practice of their specialty, Lord Beeching continued. Many graduates would find careers in which knowledge of physics was only a small part of the requirement. His own company employed 290 physicists, of whom half held posts not necessarily filled by physicists and about 10 per cent were doing work for which scientific qualifications were not essential. The object of the Government and the universities should be to create an apparent surplus of scientists, many of whom would move into management, administration and politics.

Most of the subsequent contributors were concerned with the scope and content of the undergraduate curriculum, but there was lively discussion also on the extent to which industry's needs were fulfilled by the products of the universities.

A discussion of the young physicist's training and progress in industry, offered by G. S. Bosworth (English Electric Co., Ltd.), was strongly criticized by A. B. Pippard (Cavendish Laboratory, Cambridge) as showing how second- or third-class people could be turned into useful members of an industrial team, while offering nothing to attract the first-class scientists who were badly needed in British industry but were repelled by the Philistine attitude of prospective employers.

Extreme differences of opinion such as this were infrequent. It was generally recognized that the primary

purpose of a university was the advancement of learning and that an industrial concern would fail unless it was conducted to make a profit. Efforts to secure closer collaboration between the university and the industrial community in undergraduate and postgraduate work were reported from several parts of the world; it was agreed that physicists were somewhat better prepared for a practical career in countries such as Holland, where industrial scientists and engineers had a substantial role in academic research and teaching.

Discussion of the content of the curriculum embraced three main topics: (a) mathematics, (b) laboratory work, and (c) lectures. Commenting on the importance of adequate mathematical training, M. Y. Bernard (Conservatoire National des Arts et Métiers, Paris) proposed, not entirely in jest, that special courses should be provided for university teachers of physics, who might otherwise be embarrassed by the superior mathematical ability of their students. The consensus of opinion in France, he reported, called for three kinds of instruction. Professional mathematicians should be responsible for about 25 per cent of the undergraduate's course, dealing with topics where a rigidly logical approach was valid. A physicist should have about 40 per cent of the available time, to cover inductive methods and the development of general laws. The rest of the course should be devoted to exercise classes.

More radical views were asserted by Bernard Friedman (University of California), who condemned the "pernicious influence of sound old-fashioned courses in classical mathematical physics". Up to the end of the nineteenth century, he said, important problems giving insight into the physical world were tackled and solved by the use of special functions, separation of variables and often complicated techniques of integration. These methods were now out of date, partly because of the increasing mathematical sophistication of modern physics and partly because of the rapid advance in electronic computing. When numerical results were required, the computer would generally give more accurate results with less effort. If, on the other hand, a qualitative approach was attempted, mathematical techniques of a more abstract kind were now available.

To understand the mathematical content of present-day physics, the student must be trained in linear algebra, probability and Markoff processes, functional analysis, group representations and Lie algebras. Time does not allow the prospective physicist to follow the standard mathematical courses on these topics, but a briefer curriculum must not be allowed to degenerate into a collection of recipes for solving standard types of problem. Emphasis

should be put on the teaching of abstract concepts such as linear spaces, linear operators, convergence in metric spaces and group invariances and symmetries, even if the immediate practical applications were not fully described.

Comments on practical work were remarkable for the diversity of ideas revealed. S. C. Brown (Massachusetts Institute of Technology) claimed that the main object was to teach what a physicist does. Systematic experiments and laboratory reports should therefore be abandoned in favour of project work, closely linked with the research programme of the department. It was suggested that only the 2,000 physics students at Massachusetts Institute of Technology could ever enjoy such lavish attention, but Brown reported that most of the forward-looking universities in the United States used the same method. Some speakers thought that practical physics could be taught in lectures, though manipulative skill could only be acquired by experience at the bench. E. Mendoza (University of Wales) and F. H. Read (University of Manchester) favoured seminars in which a class (of up to fifty students) conducted one experiment under the guidance of a lecturer.

However great the importance of mathematical and practical instruction, the core of the undergraduate cur-

riculum is in the systematic lectures by which the principles and applications of the subject are uncovered. The burdens laid on the undergraduate's knowledge and on his maturity of understanding are augmented every year. A. B. Pippard showed, from a study of examination papers, how to-day's student is expected to know just as much about electricity, thermodynamics and other long-established divisions of physics as his counterpart of 1940—and in addition, to be conversant with many newer topics of a substantial kind. His solution was to reduce the first-degree course to two years and to provide a more comprehensive training for a proportion of the student body.

The conference passed a number of resolutions of an unexciting character, but its real significance was based on divergence rather than unanimity. Physicists are produced with evident success in educational systems sufficiently varied to include strict Government control of priorities and syllabuses in the U.S.S.R., unfettered academic enterprise in the United States and the amalgam of tradition and innovation characteristic of both the older and newer British universities. The competence and enthusiasm of the finished products encourage the belief that the confusion over aims and methods is superficial.

J. M. A. LENIHAN

PLANT PHYSIOLOGY IN CANADA

THE annual scientific and business meeting of the Canadian Society of Plant Physiologists—La Société Canadienne de Physiologie Végétale—was held at the University of New Brunswick, Fredericton, during June 2-5.

The programme commenced with a symposium entitled "Problems in Marine Algal Physiology". J. S. Craigie (N.R.C., Halifax) discussed the problems of isolating and identifying marine algal excretory products from sea-water. Compounds excreted by several brown and red algae were identified as condensed polyphenolic substances with distinct growth-promoting properties. A brominated benzyl alcohol derivative excreted by *Polysiphonia lanosa* was isolated and crystallized. A pure crystalline extracellular chitin excreted by the diatom *Thalassiosira fluviatilis* was isolated and characterized and was claimed to be the first recorded evidence for the occurrence of poly-*N*-acetyl glucosamine in the algae. L. Provasoli (Haskins Laboratories, New York) discussed the nutritional requirements of marine algae grown aseptically. Unlike unicellular algae, seaweeds grown autotrophically require a range of vitamins in specific concentrations and ratios for normal morphological development. The growth of *Ulva* and *Monostroma* in aseptic culture was maintained only in the presence of plant hormones, phenolic compounds, and by the addition of supernatants containing active substances from a variety of red and brown seaweeds and from several unicellular algae grown aseptically on artificial media. These unidentified active substances are ethanol extractable and heat resistant and their ubiquitous production in the sea underlies the significance of external metabolites in the water environment. R. T. Wilce (Amherst, Massachusetts) discussed algal survival in the Arctic Sea. Reproductive activity at the end of the winter period and an abundant spring and summer growth were found to depths of 30 metres. The greatest concentration of algal biomass occurred at 15-20 metres, where extremely low light intensities were recorded. Survival under these conditions requires either the possession of a highly efficient photosynthetic apparatus or the facility to exist heterotrophically. Evidence for the heterotrophic growth of pigmented algae in deep lakes and unlit caves suggested

that these arctic populations also thrived by the heterotrophic utilization of organics from the sea.

Several scientific sessions followed the symposium. Continuing their work on plant photorespiration, G. Krotkov *et al.* (Queers, Kingston) showed a distinction in spruce tissue between photo- and dark-respiration with the latter inhibited by light. W. Turner and R. G. S. Bidwell (Toronto) presented evidence for the enhancement of assimilation of carbon dioxide by leaf blades with application of IAA. E. R. Waygood *et al.* (Manitoba), working with detached wheat leaves, showed that photo-phosphorylation could be both restored and enhanced by benzimidazole treatment and that the synthetic pathway of NAD and NADP from ¹⁴C-carbonyl-nicotinic acid was essentially the same as that reported for animal tissues and micro-organisms. The permanent bleaching of *Euglena gracilis* by nitrofurans derivatives was discussed by D. R. McCalla (McMaster, Hamilton). Other mutagens and radio-mimetic agents were used as bleaching agents, but only *N*-methyl-*N*-nitroso guanidine proved non-lethal. It was suggested that these bleaching agents damaged chloroplast DNA. Continued interest in the biosynthesis of aromatic compounds and phenolase enzyme systems was reported by A. C. Neish *et al.* from the Atlantic Regional Laboratory (N.R.C., Halifax). In particular the biosynthesis of the cinnamic acid derivatives as the C₆-C₃ intermediates in lignin formation was discussed. Other biochemical contributions of interest dealt with the *in vitro* synthesis of glycine and the metabolic utilization of ¹⁴C-ethanol (E. A. Cossins *et al.*, Alberta). N. E. Good (Michigan) discussed the preparation of several new H ion buffer systems. They were mostly substituted derivatives of *tris*(hydroxymethyl)-amino methane covering the pH range from 6 to 8 and were claimed to be superior to both *tris* and phosphate buffers.

Several contributions described studies of the structure and the effects of environment on plant cells by electron microscope and light microscope. A. J. Mia and G. Setterfield (Carleton, Ottawa) reported the synthesis of cell wall material by apposition in *Rauwolfia* sclereids using ³H-glucose pulse labelling. In a session on translocation in the higher plants, contributions from the Biosciences Division (N.R.C., Ottawa) described the experimental

control of ^{14}C -sugar movement by changing the node temperature (J. A. Webb and P. R. Gorham), and discussed the distinct lack of evidence demonstrating sugar conduction in the sieve tubes (D. C. Mortimer and M. Suzuki). D. S. Fensom and D. C. Spanner (London, England) reported their measurements of microelectrode potentials in the conducting tissue of *Nymphoides* and *Heracleum* and calculated that the electro-osmotic efficiency was sufficient to maintain sugar flow through partially blocked sieve plate pores.

In a final session on plant growth substances, several contributions were concerned with the influence of exogenously supplied gibberellins and IAA on plant development. Three reports discussed the interesting problems of IAA conjugation in plant tissues. E. Schneider and F. Wightman (Carleton, Ottawa) showed IAA-2- ^{14}C to be metabolized mainly through conjugation to indoleacetyl aspartic acid, indoleacetyl glucose and 2-OH indoleacetylglucose in barley seedlings over a 24-h period. A. Winter and K. V. Thimann (Harvard University), however, could find no evidence for conjugate compounds over a 2-h period in *Avena* coleoptiles and con-

sidered fed ^{14}C -IAA to be physically bound to a protein fraction. N. A. Andreae (Department of Agriculture London) showed that conjugate products were formed with IAA inactivation, when pea roots were pretreated with an excess of IAA and NAA. An adaptive period of 2-4 h was necessary before the conjugate products were discerned. 2,4-D was not conjugated and remained inhibitory regardless of the pretreatment time. J. I. Toohey and C. D. Nelson (Queens, Kingston) reported two new herbicides derived from a soil bacterium inhabiting old pastures. The compounds were toxic to algae and higher plants but not to insects, fish or mammals. They were isolated and identified as phenazine-1-carboxylic acid (most toxic to higher plants) and the 2-OH derivative (most toxic to the algae).

Officers of the society elected for 1965-66 were: *President*, Dr. G. H. N. Towers (University of British Columbia); *Vice-president*, Dr. D. Simminovitch (Department of Agriculture, Ottawa); *Secretary-treasurer*, Dr. D. Canvin (Queens, Kingston); *Eastern director*, Dr. A. R. A. Taylor (University of New Brunswick); *Western director*, Dr. M. S. Spencer (University of Alberta). J. A. WEBB

A REGISTER OF SCIENTIFIC RESEARCH IN THE NETHERLANDS

IN 1964 the Netherlands Organization for the Advancement of Pure Research (Z.W.O.) began a register of scientific research in the Netherlands. The results of this work are to be published in the form of a periodical guide giving details of current scientific investigations. The purpose of this guide is to foster contact between research scientists. It supplies both Dutch and foreign scientists with information on the research being carried out in the Netherlands. With the view of foreign readership it appears in English.

For recent scientific information the research scientist must depend either on personal contacts or on articles in journals, reports, etc. In the case of leading scientists such as university professors working in small and highly specialized fields, personal contact often provides sufficient communication; however, this is much less true of their staff. As regards research scientists in larger and above all multi-disciplinary fields, personal contact usually satisfies only a small part of the need for communication.

So far as journals, reports, etc., are concerned, not only does it take a long time until the original publications are reported in abstracting journals, but also there is a considerable interval before the results of a research project are published. Moreover, the research scientists concerned may have information available long before then. It may also be remarked that a great deal of research is never published, for a variety of reasons, for example in the event of discontinuance of the project; valuable information may be lost in this way.

The marked expansion of research at the universities makes it still more desirable that the need for recent scientific information be satisfied. The guide contributes towards this by presenting research scientists with a basis on which further contacts can be established.

The guide has been inspired by the British publication *Scientific Research in British Universities and Colleges*, published by the Department of Scientific and Industrial Research and the British Council; this gives concise information on scientific research in Great Britain*.

At first there was some doubt about the value of this publication in Britain, but in the course of the years its utility has been clearly proved. Consequently, Z.W.O. decided to build up experience by starting in one field and

submitting the result to scientists for discussion; physics was chosen as this first field.

In principle the guide only mentions research projects by or under the direct control of professors, lecturers and scientific staff.

Taking into account the purpose of the guide, preference was given to a main grouping by subject rather than by university department. As research scientists are best served by a classification that they already know, it was decided to follow the leading abstracting journal for each field of science. As the register is extended to more fields the disadvantage will have to be accepted of a certain lack of balance as compared with a general classification, such as the Universal Decimal Classification, which was devised as a whole.

For physics, *Physics Abstracts*, published by the Institution of Electrical Engineers, London, has been chosen. The research scientist was asked to classify his research project under one of the eighteen headings of *Physics Abstracts*. In order to reduce discrepancies to a minimum, a copy of the classification followed by this journal was attached to the questionnaire.

In the guide the research projects are arranged under the afore-mentioned headings of *Physics Abstracts*. Within these groups there is a sub-division according to the institution where the research is being carried out. Besides a name index and a subject index, a list of co-operating research institutes, with their addresses, is given.

Before questionnaires were sent out, the arrangement was submitted to the chairmen of the physics departments of the various universities. The idea of this was to obtain their criticism in good time, so that allowance could be made for it in the questionnaire; most of them also proved ready to call on the co-operation of the professors and lecturers to be covered by the survey.

The questionnaire was kept simple so that filling it in would not take up too much of the scientist's time. Only those questions were asked which are directly connected with the three questions which the guide tries to answer, namely: (1) What kind of research is being done? (2) Who is doing it? (3) Where is it being done?

To every professor and lecturer of physics were sent 5-10 questionnaires on which to report the relevant research; in most cases they were sent direct, though in

* Now published by the Department of Education and Science and the British Council (*Nature*, 207, 691; 1965).

a few cases they went to a contact person for distribution to all the research scientists at a certain institution.

Although for practical reasons the first survey had to be confined to the universities, a small number of other bodies doing important physical research were also included in this first trial. Owing to the fact that investigations in borderline fields, such as physical chemistry and biophysics, are likewise done in non-physical laboratories, complete coverage was not entirely possible even within the universities; it will, however, be approached as the registration progresses.

For rapid processing of the data received—particularly with the view of future expansion to more fields—mechanization is desirable. In the case of the first guide the procedure was as follows: The copy is typed in the order in which it is received on a 'Flexowriter' which produces a punched tape as well as a typescript. After the copy has been completed the punched tape is read by a computer. This arranges the data for text and indexes in the desired order and records these on a new punched tape. The latter is then run through a 'Flexowriter'

which types the manuscript for the printer without manual intervention. The pages are made up and reproduced in photo-offset.

In the preparation of the guide, Z.W.O. has received the assistance of the Statistics Department TNO for the mechanical processing of the copy and of the State Printing Department for printing. The title of the first publication, which has just appeared, is *Current Research in the Netherlands, Physics, 1965* (Pp. 66. The Hague: Netherlands Organization for the Advancement of Pure Research (Z.W.O.), 1965). The guide for physics covers about 500 current research projects on which about 750 scientists are engaged. Institutions and research scientists can obtain this publication free of charge from the Dutch Embassy in their country, or direct from the Netherlands Organization for the Advancement of Pure Research (Z.W.O.), P.O. Box 2138, The Hague.

For the following issue of the register the choice has fallen on biology; this will be registered in co-operation with the Royal Netherlands Academy of Sciences. Other sciences will follow.

S. T. GROENMAN

OBSERVATIONS OF A STRONG UNIDENTIFIED MICROWAVE LINE AND OF EMISSION FROM THE OH MOLECULE

By PROF. HAROLD WEAVER, DR. DAVID R. W. WILLIAMS, DR. N. H. DIETER and W. T. LUM

Radio Astronomy Laboratory, University of California, Berkeley

AN investigation of the galactic distribution of the OH molecule as shown by the microwave lines arising from transitions between the hyperfine levels of the ground state has led us to the following general conclusions: (1) The OH molecule is sharply confined to the galactic plane. In the plane, however, we detect OH in absorption in virtually every source brighter than a specifiable limit. (2) The OH molecule is in emission in some sources. (3) A strong unidentified emission line is present at frequency 1,665 Mc/s in a number of sources.

This discussion deals with the last two of these topics. We shall consider the galactic distribution of OH elsewhere.

Our observations were made with the 85-ft. antenna and the 100-channel switched-frequency double-comparison receiver and digital data system recently installed at the Hat Creek Radio Observatory. This system, which will be described elsewhere in detail by one of us¹, provides a spectrum consisting of 100 contiguous spectral elements which are either 10 kc/s or 2 kc/s wide. The band-width of the spectral elements is fixed by interchangeable crystal filters. The receiver includes a tunable parametric amplifier having a band-width of about 20 Mc/s. The total system noise temperature (including instrumental effects such as spill-over and the like) is approximately 130° K.

An 'observation', as the term will be used in this discussion, consists of the difference between the spectrum as observed for a specified source (integration time 1,600 sec) and the spectrum of a comparison region (integration time 1,600 sec) near the source but sufficiently far away so that the source is out of the antenna beam.

Observations of a strong unidentified microwave emission line. The Orion Nebula has been thoroughly searched for OH at 1,667 Mc/s by Robinson, Gardner, van Damme and Bolton². They found no line and set an upper limit of 0.005 to the optical depth of OH in the direction of Orion. We concur with Robinson *et al.* that there is no observable line at 1,667 Mc/s. However, our observations of Ori A at 1,665 Mc/s show several emission components

with a maximum antenna temperature of 3° K when the spectral resolution is 10 kc/s (Fig. 1).

W3, a bright extended H II region which we have also observed, shows, at 1,367 Mc/s, an absorption component of OH with a depth of approximately 1° K (Fig. 1). At 1,665 Mc/s we find, as would be predictable, the second strongest OH microwave line arising from the ground state. The depth of the 1,665 line, again as predicted, is about 0.5° K. At 1,612 Mc/s there is no absorption feature visible (Fig. 1) in the one observation we were able to make before this investigation ended. Our single observation is inadequate to show the expected OH feature at 1,612 Mc/s (predicted depth ~0.1° K), but it is adequate to demonstrate that there are no large anomalies at 1,612 Mc/s. The outstanding feature of these three spectral regions is the intense emission feature (maximum antenna temperature 8.2° K for 10-kc/s spectral resolution)

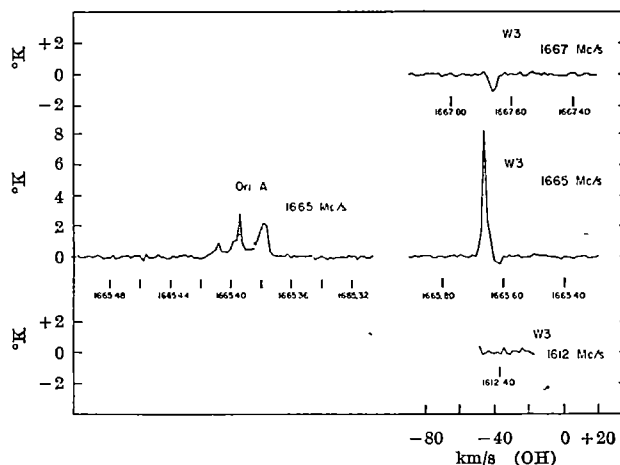


Fig. 1. Spectra of Ori A and W3 with a resolution of 10 kc/s (1.8 km/sec). Frequency and velocity scales are with respect to the local standard of rest. The frequencies in the 1,667 Mc/s spectrum of W3 should be decreased by 0.10 Mc/s. Those in Ori A should read 1,665.80, .60, .40, .20, .00. The ordinate is antenna temperature

upward in frequency from the 1,665 line of OH. In Fig. 2 we exhibit this strong emission line in *W3* (and the OH absorption feature downward in frequency from it) with spectral resolution 2 kc/s. With this high spectral resolution we observe a maximum antenna temperature of 31° K.

There is no known identification of the strong emission line at 1,665 Mc/s shown in Fig. 1. In what follows, for brevity in writing and to emphasize the surprising nature of the observation just presented, we shall speak of this unidentified line as arising from 'mysterium'.

We have observed 'mysterium' in two other thermal radio sources also: *W51* and *W75*. In each of these we find OH weakly in absorption and of normal intensity ratio for the lines at frequencies 1,667/1,665 Mc/s. Additionally, for each of the sources *W51* and *W75* we find a weak emission feature at 1,665 Mc/s that has no counterpart at 1,667 Mc/s. In general, in behaviour, these sources resemble the more extreme case *W3*.

Observations of OH in emission. In two sources, *W49* and *NGC 6334*, we find OH in emission. Spectra of these objects at 1,667 Mc/s and 1,665 Mc/s, spectral resolution 2 kc/s, are illustrated in Fig. 3.

W49, reported as a point source by Westerhout³, is a heavily obscured H II region recently investigated by Akabane and Kerr⁴. On the basis of their hydrogen absorption observations they point out that *W49* is at a probable distance of 15 kpc, and that its intrinsic energy output at 1,420 Mc/s is 300 times as great as that of the Orion Nebula and one-fourth as great as that of 30 Doradus.

NGC 6334 has been less well investigated than *W49*. The *NGC* object is one of a compact group of emission nebulae which are probably optically visible parts of one object which is crossed by dense variable obscuration. Photographs of *NGC 6334* have been published by Duncan⁵. Clark, Radhakrishnan, and Wilson⁶ report a hydrogen absorption feature at +27 km/sec and infer a distance to *NGC 6334* > 0.8 kpc. It thus appears that in the 1 Gc/s range the intrinsic energy output of *NGC 6334* is greater than that of Orion but less than that of *W49*.

The spectra of *W49* and *NGC 6334* are complex, consisting of numerous narrow emission maxima, some of these (as exemplified in the 1,667 line illustrated in Fig. 3) being as sharp as 5 kc/s (1 km/sec) at half-intensity. In the spectrum of *NGC 6334* there is present OH absorption as well as OH emission.

These spectra are unusual not only because they show OH emission; they are also unusual because the intensity

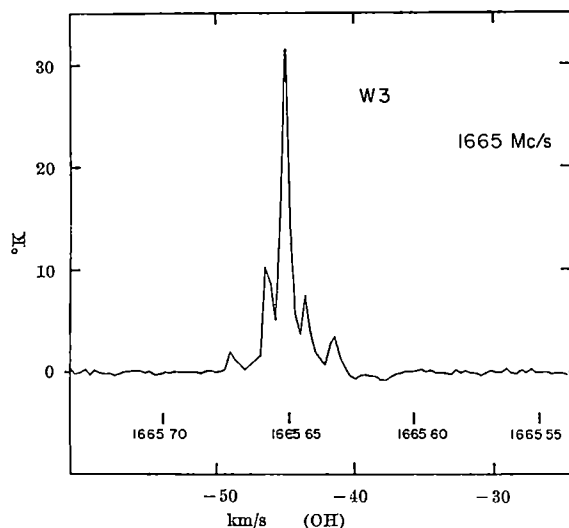
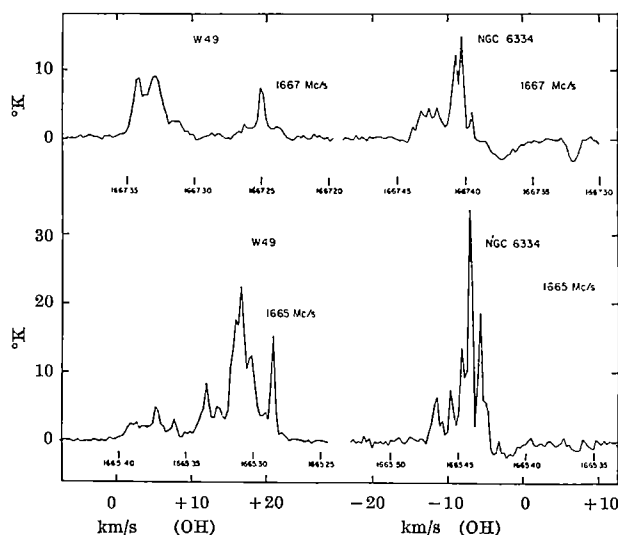


Fig. 2. Spectrum of *W3* at 1,665 Mc/s with a resolution of 2 kc/s (0.4 km/sec)



The narrowness of the features is the more surprising because the emission appears to be associated with the central regions of bright diffuse nebulae, where mass motions are typically 5 or more km/s, and temperatures are 10^4 °K. We do not know the atomic or molecular weight of 'mysterium'. We can, however, assume various weights, attribute the entire width (0.4 km/sec) to thermal broadening and compute an upper limit to the kinetic temperature of 'mysterium'. If the weight of 'mysterium' is equal to the atomic weight of He, $T_{kin} \leq 14^\circ$ K; if it is equal to OH, $T_{kin} \leq 50^\circ$ K; if it is equal to Ca, $T_{kin} \leq 140^\circ$ K. The kinetic temperature of 'mysterium' must be very low; turbulence must be negligible.

The extraordinary sharpness of the 'mysterium' features indicates that the transition probability for the 1,665 'mysterium' transition must be quite low and that the principal energy-level involved must either be the ground state or have a long half-life.

Indications are that the rest frequency of the 'mysterium' line is higher than the 1,665.402 Mc/s line of OH.

In those sources in which the two lines can be conveniently compared, the 'mysterium' line appears at a slightly higher frequency than the 1,665 Mc/s of OH in the same source.

We thank Mr. David Lesh and the staff at the Hat Creek Observatory for their assistance in making the observations and Mr. A. F. Setteducati for his help in making the reductions.

The 100-channel receiver was constructed with the aid of a grant from the U.S. National Science Foundation. The Hat Creek Observatory is operated under contract Nonr 222(66) with the U.S. Office of Naval Research.

¹ Williams, David R. W. (in preparation).

² Robinson, B. J., Gardner, F. F., van Damme, K. J., and Bolton, J. G., *Nature*, **202**, 989 (1964).

³ Westerhout, G., *Bull. Astro. Inst. Netherlands*, **14**, 215 (1958).

⁴ Akabane, K., and Kerr, F. J., *Austral. J. Phys.*, **18**, 91 (1965).

⁵ Duncan, J. C., *Pub. Amer. Astro. Soc.*, **10**, 48 (1940).

⁶ Clark, B. G., Radhakrishnae, V., and Wilson, R. W., *Astrophys. J.*, **135**, 151 (1962).

PRESSURE DISTRIBUTION IN THE TETRAHEDRAL ANVIL APPARATUS

By J. LEES

Standard Telecommunication Laboratories, Ltd., Harlow, Essex

PRESSURES above 20 kbars cause most conventional fluids to solidify at room temperature, so that apparatus for generating pressures in this range relies mainly on solid pressure transmitting media. The use of pyrophyllite, a natural aluminium silicate, is widespread, particularly in apparatus of the 'belt' or 'anvil' types^{1,2} where its high shear strength at pressure allows the formation of pressure-retaining gaskets (see, for example, Bundy, ref. 3). At the same time, its low compressibility allows pressure generation within the test cell. Since the shear strength is high, the stress system within the test cell at pressure would be expected to be neither hydrostatic nor uniform, and this is especially the case in anvil apparatus where relatively large movements of material occur during gasket formation⁴. Such variations in stress could be large, and so introduce uncertainties in pressure measurement in physical and chemical experiments at ultra-high pressure.

Stress inhomogeneities have been demonstrated in both anvil and cylindrical ultra-high-pressure apparatus, by observation of the 'smearing out' effect^{5,6} produced on the normally rapid I→II resistance transition in bismuth at 25.4 kbars (ref. 7). To minimize this effect, pressure calibration specimens are usually surrounded by a 'pressure equalizing' sleeve made from a low shear strength material, typically silver chloride. Pressure within such a sleeve is expected to be reasonably hydrostatic and closely related to the mean hydrostatic stress in the surrounding pyrophyllite. By using sleeved calibration specimens at various positions within the cell (following the work of Deaton and Graf⁸), the variation of this stress has been determined for an N.B.S.-type tetrahedral apparatus⁹, at pressures around the 25.4-kbar bismuth transition.

The apparatus used had steel anvils of 3.81 cm edge, to En26 specification, with a hardness of 530 d.p.n.; these were coated with jeweller's rouge prior to each experiment. Pyrophyllite for the tetrahedra was obtained under the trade name of 'Alsil': we have found this particular grade to be of excellent quality, machining easily, and quite free of the foreign inclusions sometimes encountered. The calibration samples in these experiments consisted of bismuth wires 1 mm diameter × 5 mm long. These were mounted in silver chloride sleeves of 3 mm outside diameter. Electrical contact was made

directly to the anvil faces by suitably shaped soft copper plugs; by using all four anvils, the resistance of three samples could be followed simultaneously as the anvil load was increased.

Two sets of experiments were performed. In the first, the samples were located as in Fig. 1a: No. 1 was at the centroid; No. 2 with its centre on the line joining the centroid to a mid-point (half-way along an altitude) of a face; No. 3 on the normal from the centroid to an edge.

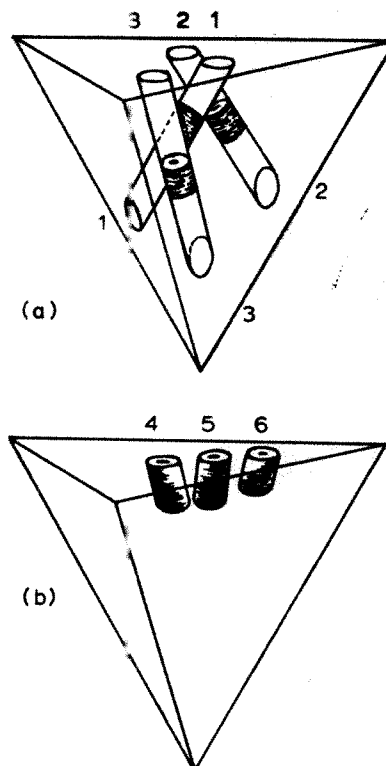


Fig. 1. Sample positions in tetrahedron. a, No. 1, at centroid; No. 2, between centroid and mid-point of face; No. 3, between centroid and edge. b, No. 4, at face centroid; No. 5, at mid-point of altitude; No. 6, towards apex (see text). The arrangement of Nos. 5 and 6 corresponds to deformed tetrahedron.

Distances from the centres of samples No. 1 to No. 2 and No. 1 to No. 3 were 6 mm and 7 mm respectively. Fig. 2 shows the loads at which the 25.4 kbar transition was observed to begin at each of the sample positions, and for various degrees of tetrahedron oversize. (The variables P and V are, respectively, the transition load divided by the load necessary to produce the transition pressure over an area equal to an anvil end-face, and the precompression tetrahedron volume divided by the tetrahedron volume defined by the anvil end faces.) Maximum variation in load in repeated experiments is estimated at ± 4 per cent: I believe that this is mainly due to small differences in the mechanical properties of pyrophyllite in a given batch of tetrahedra.

It will be seen that the differing values of P in Fig. 2 imply quite appreciable pressure gradients in all cases; also that the optimum value of V (based on the minimum load to reach the transition) is much the same for all three specimen positions. Values of P and V at the minimum for sample 1 are 1.00 and 1.95, agreeing well with the corresponding results of Houck and Hutton⁶ for $\frac{1}{2}$ -in. and 1-in. tetrahedral units with carbide anvils.

In the second set of three experiments 4.76-cm tetrahedra ($V=1.95$) were used. Samples were arranged on an altitude of a face as shown in Fig. 1b. Sample No. 4 was at the face centroid, No. 5 half-way along the altitude from the vertex to the base, and No. 6 towards the vertex the same distance from No. 5 as the latter was from No. 4, that is ~ 5.5 mm. Reasonably consistent transition loads on increasing pressure were found for samples Nos. 4 and 5, typical values of P being 1.07 and 1.13 respectively. Values of P for No. 6, however, varied considerably, two samples giving 1.27 and 1.36 respectively; the third had not transformed at $P=1.4$ which was the maximum load applied in these experiments.

If the two sets of experiments on 4.76-cm tetrahedra are now combined, a rough picture emerges of pressure falling off fairly slowly from the centroid towards the faces, and rather more rapidly towards the edges. It is difficult to relate the values of P at each sample to corresponding pressures at each of the other samples, since we do not have a load/pressure relation for each sample position. However, an approximate relation may be derived by assuming it is of the same general shape normally observed¹⁰, and differs only by a proportional factor in load (determined by the value of P) at each sample position. This assumption is probably not very greatly in error since the actual pressure range involved around 25.4 kbars is small. Pressures derived on this basis when sample 6 is at 25.4 kbars (the minimum observed value of P of 1.27 is used) are shown in Fig. 3 displayed with a cross-section of the deformed tetrahedron. It should be noted that the actual physical displacement of the

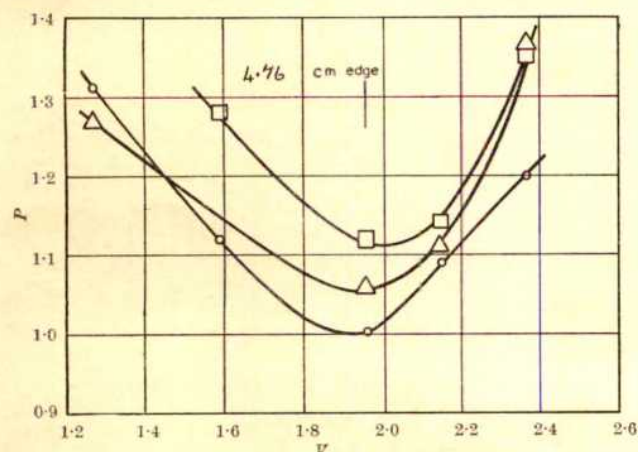


Fig. 2. Loads to produce B.I.U. 25.4-kbar resistance transition at three sample positions for various tetrahedron sizes. \circ , Sample No. 1; Δ , sample No. 2; \square , sample No. 3.

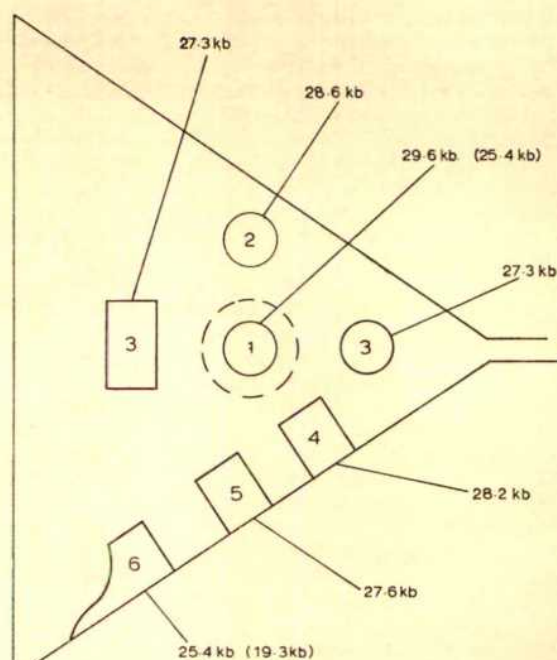


Fig. 3. Sample positions on a mid-section of the deformed tetrahedron, with corresponding pressures on loading (figures in parentheses for sample Nos. 1 and 6 are for unloading).

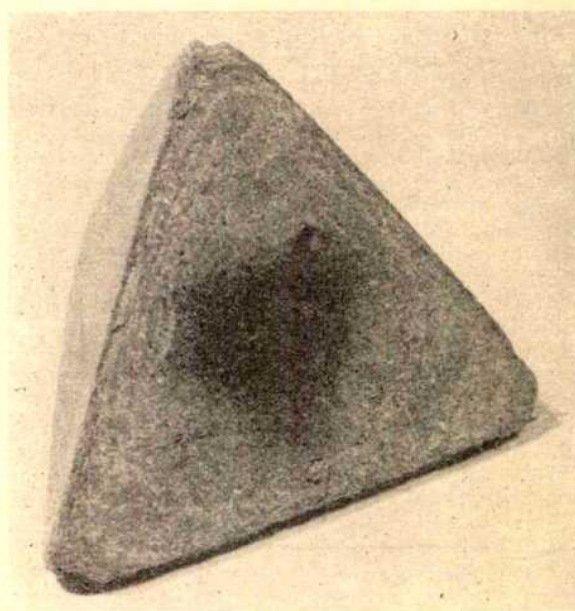


Fig. 4. Tetrahedron after compression, showing dark area on face

samples due to compression of the tetrahedron was found to be small, except in the case of sample No. 6 as shown. This would be expected if, as seems very reasonable, the flow pattern of pyrophyllite during compression is similar to that previously derived for a 1.90-cm edge tetrahedral apparatus⁴.

The reverse transition loads on unloading were also determined, and with the exception of sample No. 1, were found to be somewhat unrepeatable. Generally, however, pressure gradients were greater during unloading. Thus, for sample No. 6, the minimum value of P for the II \rightarrow I transition obtained was 1.10, compared with 0.72 for sample No. 1; the corresponding pressures are shown in Fig. 3 in parenthesis. It will be noted that, on loading, the pressure difference between No. 1 and No. 6 corre-

sponds to 14 per cent of the pressure at No. 1; on unloading the minimum difference is increased to 24 per cent.

Fig. 3 shows that in the experiments with 4.76-cm tetrahedra (a) there is a small but definite pressure gradient in the central regions with a larger gradient towards the edges and apices, and (b) gradients appear to be increased on unloading. Maximum pressure is generated at the tetrahedron centroid, thus confirming the suspected intensification effect noted by Houck and Hutton⁶; a similar effect has been observed in a cylindrical high-pressure apparatus by Tydings and Giardini⁵. Both groups of research workers ascribed the effect to possible 'cones' of undeformed pyrophyllite which act as anvil (or piston) extensions of small area, so increasing pressure in the central regions. Further evidence for 'cones' of pyrophyllite is provided by flow patterns in deformed tetrahedra⁴, and by the appearance of tetrahedron after compression. The dark marks (Fig. 4) on each face are due to material which has not sheared during compression: these marks very probably represent the bases of such cones. Actual pressure differences inferred for the central regions of the tetrahedron are ~7 per cent between centroid and face. These would account for the changes previously observed in the load/pressure relation when different lengths of calibration sample are used⁴.

The present results thus show gradients considerably greater in the central regions than the 1 per cent found by Deaton and Graf in their experiments at various pressures with a 1.98-cm edge tetrahedral apparatus.

This could be associated with the relatively smaller pressure-sensing samples in the experiments described here (the dotted line in Fig. 3 shows Deaton and Graf's sample size on the same scale), but it could also be due to the differing absolute tetrahedron size or even to the use of steel anvils. Another possibility lies in variations in the properties of pyrophyllite: small differences which are found in the grain of machined surfaces, and in the machining properties, could well point to such an effect. Generally the pressure variations observed in the particular experiments described here indicate the need for caution in pressure calibration work, even when a pressure calibrating sample is included with the specimen under investigation: this is particularly so when the calibration sample is situated some distance away from the specimen.

I thank my colleagues K. Ashcroft and C. H. L. Goodman for discussions; also D. G. Ingham, who carried out many of the experiments.

¹ Hall, H. T., *Rev. Sci. Instr.*, **29**, 287 (1958).

² Hall, H. T., *Rev. Sci. Instr.*, **31**, 125 (1960).

³ Bundy, F. P., in *Modern Very High Pressure Techniques*, edit. by Wentorf, R. H., 1 (London, Butterworths, 1962).

⁴ Ashcroft, K., and Lees, J., *Nature*, **198**, 957 (1963).

⁵ Tydings, J. E., and Giardini, A. A., in *High Pressure Measurements*, edit. by Giardini, A. A., and Lloyd, E. C., 200 (Washington, Butterworths, 1963).

⁶ Houck, J. C., and Hutton, U. O., in *High Pressure Measurement*, edit. by Giardini, A. A., and Lloyd, E. C., 221 (Washington, Butterworths, 1963).

⁷ Kennedy, G. C., *J. Geophys. Res.*, **67**, 851 (1962).

⁸ Deaton, B. C., and Graf, R. B., *Rev. Sci. Instr.*, **34**, 45 (1963).

⁹ Lloyd, E. C., Hutton, U. O., and Johnson, D. P., *J. Res. Nat. Bur. Stand.*, **63C**, 59 (1959).

¹⁰ Lees, J., *Nature*, **203**, 965 (1964).

ELECTRIC POTENTIALS GENERATED BY FREEZING DILUTE AQUEOUS SOLUTIONS

By H. C. PARREIRA* and A. J. EYDT

Stanley-Thompson Laboratories, School of Engineering and Applied Science, Columbia University, New York

THE purpose of this article is to describe an investigation of the large electric potentials which occur when very dilute solutions freeze under the influence of a temperature gradient. This phenomenon is identical to the thermoelectric effect discovered by Costa Ribeiro¹. Since the discovery of electrical potentials associated with freezing of aqueous solutions of inorganic compounds was first announced, little systematic investigation has been reported².

When water is frozen under the influence of a temperature gradient, if certain precautions are taken, a potential can be measured between the ice and the water phases. It was established by the work of previous investigators²⁻⁴ that the freezing potential does not depend on the temperature of freezing alone; it depends on the rate of freezing and also on the concentration of the impurities present in the system. Most of the work published on the influence of electrolytes on freezing potentials of aqueous solutions deals with concentrations above 10^{-5} M. The few data available for solutions below that concentration show practically no electrical effects.

It was found in the course of the present work that the concentration range between 10^{-7} and 10^{-5} M presents potential values which are even larger (but of opposite sign) than those reported for concentrations above 10^{-5} M. These findings are at variance with previous works published on the influence of electrolytes⁵. The treatment of the conductivity water is probably one of the causes of this disagreement.

The phenomenon set up by the temperature gradient imposed on the system is easy to reproduce if the many parameters involved are carefully controlled. Devices

used in earlier work³ for that specific purpose probably failed to control accurately the experimental parameters on which the electrical phenomena are dependent, thus the failure to reproduce the potentials.

The results reported here were obtained with an apparatus which ensured good temperature control. The main part of the apparatus is composed of a freezing cell which consists essentially of a cup (about 1 cm³) made of a 'Teflon' sleeve that fits snugly in a brass cylinder. The top of the brass cylinder (the bottom of the freezing cell cavity) is covered with a gold plate to prevent direct contact between the brass cylinder and the solution. The cell is depicted in Fig. 1. An alumel-'Chromel' thermocouple is placed just under the gold plate, ensuring accurate temperature measurements. The cell is next placed in a cylindrical copper well (diam. 1.6 cm, depth 2 cm). The copper well is part of a thermoelectric heat pump and can be cooled down to -30° C with the loaded cell in place; the cooling unit is represented in Fig. 2. The temperature in the well (and cell) is dependent on the current flowing through the thermoelectric modulus, and the power unit supplying the modulus current is fitted with a current read out. The temperature at the bottom of the copper well is also determined with the help of a thermocouple attached to the bottom of the well; the power unit read out is then used for temperature control. The freezing potentials were measured with the help of a Keithley electrometer model (610 amp) the output of which was fed into a Varian recorder. A potential divider built with precision resistors had to be made because the potentials often exceeded the useful range of the electrometer.

A platinum wire immersed in the water phase was connected to the electrometer; the ice phase was grounded

* Present address: Central Research Laboratories, Interchemical Corporation, Clifton, New Jersey, U.S.A.

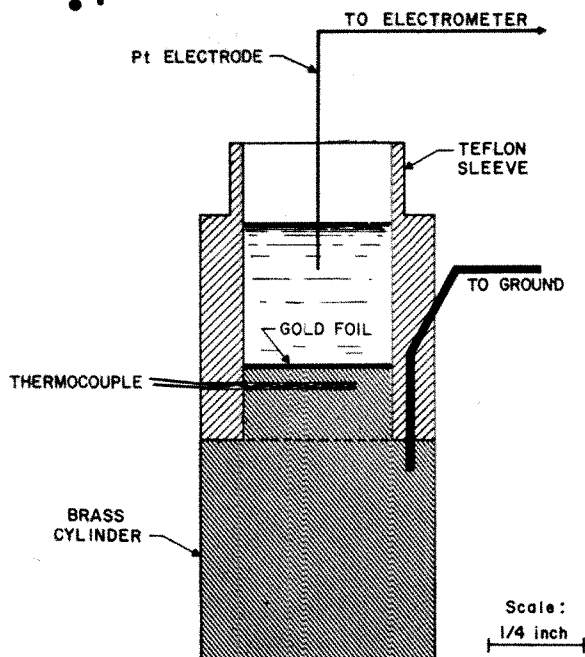


Fig. 1. Freezing cell

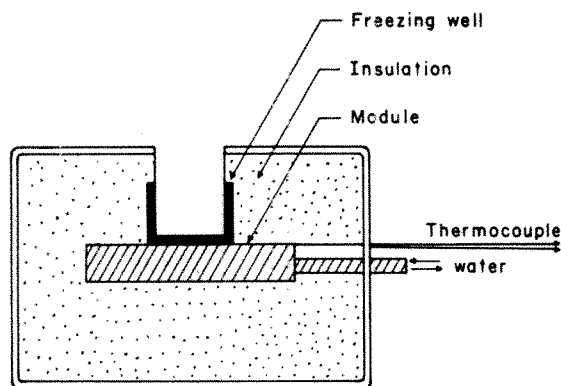


Fig. 2. Freezing well

through the bottom of the cell (Fig. 1). In this article the maximum potential values are given, that is, the highest potential value attained in the potential versus time curve. The time of occurrence of the maximum potential seems to depend on concentration, the other parameters being kept constant. For example, for 2×10^{-4} M solution of ammonium chloride the maximum potential value was observed to occur 2 min after the beginning of the experiment, whereas pure water developed the maximum value nearly 10 min after the solution was placed in the cell. The time corresponding to the maximum potential appears to increase with the decreasing concentration of ammonium chloride; no trend was observed, however, for other electrolytes such as hydrochloric acid and sodium chloride. In all cases the freezing potential value drops practically to zero at the moment of complete solidification of the water phase. In the device used in the study described here complete freezing occurs between 14 and 16 min after starting the experiment. The results reported here were obtained under the following experimental conditions: Room temperature, $24 \pm 1^\circ \text{C}$; brass cylinder, $-25 \pm 1^\circ \text{C}$; initial volume of liquid phase, 0.8 cm^3 ; height of cell, 1.5 cm ; cross-section, 0.64 cm^2 . The solution was pipetted into the cell once the temperature of the brass cylinder reached -25°C . The platinum electrode was lowered into the water phase. The potential, which appeared immediately after the solution was placed in the cup, was then measured. The

details of the construction of the whole experimental apparatus were given elsewhere⁶.

The effect of temperature on the freezing potentials of water and dilute aqueous solutions of electrolytes was examined earlier³.

The effect of the addition of both organic and inorganic substances has been investigated and is briefly reported here. No effort was made to eliminate carbon dioxide, although fresh solutions were made up with recently distilled water. Bidistilled water from an all-Pyrex[®] water still was used (conductivity $1.2 \times 10^{-6} \text{ mho cm}^{-1}$). This water gave freezing potentials of $+75 \text{ V}$ (mean of 18 independent determinations). The freezing potential dropped to $+40 \text{ V}$ for once-distilled water (conductivity $1.8 \times 10^{-6} \text{ mho cm}^{-1}$) and decreased to zero for distilled water of conductivity around $4 \times 10^{-6} \text{ mho cm}^{-1}$. Generally no relationship could be found between the water conductivity and the freezing potential; however, the E_m versus conductivity plot for sodium chloride gave an excellent straight line up to 10^{-5} M (conductivity range: 1×10^{-6} – $3 \times 10^{-6} \text{ mho cm}^{-1}$). The E_m for dilute solutions was plotted against the logarithm for the molar concentration. Very dilute solutions of organic and inorganic substances showed a similar behaviour except solutions containing the NH_4^+ ion. The freezing potential for aqueous solutions drops to zero in the concentration range between 10^{-7} and 10^{-5} M . The potential often reverses its sign, in the case of many inorganic salt solutions, beyond $c \approx 10^{-5} \text{ M}$ and drops to zero at a concentration around 10^{-3} M ; however, the magnitude of the maximum potential is much smaller than that observed for the potentials measured in the dilute region (10^{-7} – 10^{-5} M). The freezing potential of solutions of inorganic acids drops to zero at $c = 10^{-3} \text{ M}$ and remains at zero beyond that concentration value. All organic substances investigated (sucrose, glucose, urea, etc.) also cause a drop in the potential, which is eliminated altogether at concentrations around 10^{-3} M ; however, the potential sign is never reversed. The E_m versus $\log c$ plots for ammonium chloride and sodium chloride are presented in Fig. 3. Two regions can be observed in the sodium chloride curve: (a) a very dilute region I showing large potentials and a positive ice phase; (b) a region II where the ice is negatively charged with respect to the water phase but never shows the large potential differences which occur in region I. Between the two regions there is a narrow concentration range where the potential shows an erratic behaviour and very low values. The behaviour of ammonium chloride is quite different, as is shown in Fig. 3. A maximum is found at $c = 2.5 \times 10^{-6} \text{ M}$ for which $E_m = +115 \text{ V}$; the potential, however, drops to zero at $c \sim 10^{-3} \text{ M}$. The chlorides of Ca^{++} , Ba^{++} , Sr^{++} and Mg^{++} also reverse the sign of the freezing potential,

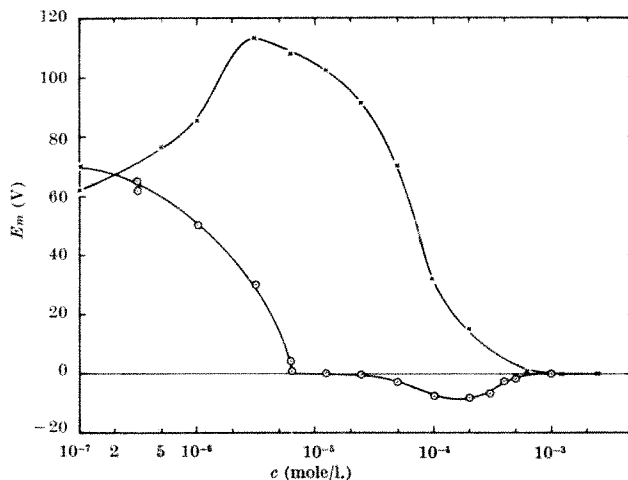


Fig. 3. Influence of electrolytes on freezing potential of water, E_m versus $\log c$; block temperature -25°C . \circ , NaCl; \times , NH_4Cl

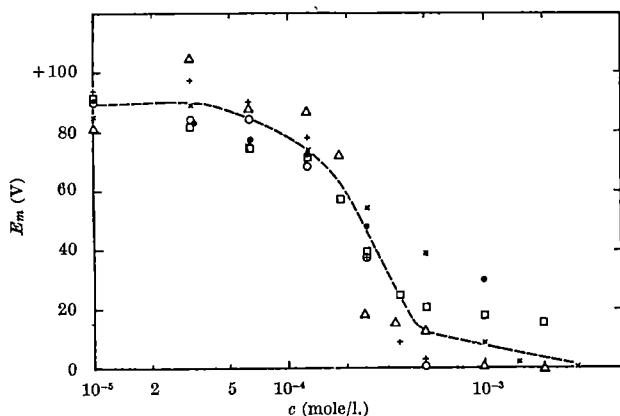


Fig. 4. Influence of aliphatic alcohols on freezing potentials of water E_m versus $\log c$; block temperature -25°C . \times , Methanol; \bullet , ethanol, \circ , propanol; $+$, butanol; Δ , pentanol; \square , hexanol

but do not show potential values as high as those produced by metallic uni-univalent electrolytes. The chlorides of Fe^{+++} , Al^{+++} and Cr^{+++} show only a region I; beyond 2×10^{-6} M the freezing potential is zero. The 'charge reversal' appears to be a property of uni-univalent and uni-divalent metallic salts, with the exception of those containing the NH_4^+ ion.

The large freezing potentials obtained for conductivity water are in agreement with the findings of Workman and Reynolds³ but are at variance with the results obtained by Lodge *et al.*⁵, who could not measure any potential for conductivity water passed through an ionic exchange resin column. The influence of a series of organic compounds such as sugars, glycols, alcohols, ketones and urea has also been investigated. The behaviour of aliphatic alcohols from methanol to hexanol is remarkably similar, as is shown in Fig. 4.

A theoretical interpretation of the electrical phenomena which appear during the course of a phase transition is not easy^{1,8}. If all macroscopic parameters (temperature gradient, rate of freezing, orientation of the crystallographic axis of the ice formed) are kept invariant, then it is possible to ascribe the phenomenon to the following processes: (1) proton conductance; (2) dipole orientation of water molecules at the ice/water interface; (3) ionic entrapment in the ice lattice; (4) ionic diffusion.

The proton conduction process, whether taking place by quantum mechanical tunnel effect, rotation of water molecules or any other rate-determining mechanism, could be the predominant factor in the production of the freezing current, although process IV may be at play when ionic impurities are present (Fig. 3). In fact the tunnel effect has been suggested (from theoretical considerations) by others as a possible rate-determining mechanism for the proton gumping process^{9,10}.

Mechanism (2) may be largely responsible for the existence of the high potential values between the ice and the water phases in region I, although mechanism (1) may be operating throughout the whole concentration range investigated. Furthermore, because it is impossible to rule out completely the presence of ionic entrapment in the ice lattice, it is also possible that mechanisms (3) and (4) are also at play even at very low ionic concentrations (region I); their contribution, however, would be small in region I. The process of ionic entrapment alone does not seem to explain clearly the presence of a freezing current, as it does not explain the large potentials in conductivity water. It is possible that all processes are at play in region II, although dipole contribution would be smaller than the others. In region I the proton conduction mechanism may originate the freezing current, whereas the dipole orientation contribution at the ice/water interface may build up the large freezing potentials.

The following results were obtained for a 6.25×10^{-6} M ammonium chloride solution: $E_m = +110$ V (maximum

potential, ice positive), $i_m = 1.0 \times 10^{-9}$ amp (maximum current) and $q = 5.7 \times 10^{-7}$ coulombs (1,700 e.s.u. c.g.s., total charge transferred per gram of water). The value given here for the charge is the maximum value obtained in the concentration range between 10^{-6} and 2×10^{-3} M. The values obtained by other investigators³ (for the same substance and same concentration range) exceed by orders of magnitude the values reported here, a fact which could not be explained at the time. The use of dipole orientation to explain the appearance of the phase transition potentials is in agreement with the findings of Costa Ribeiro¹, who suggested that the ice/water interface is the site of the electrical phenomena reported here. This view was also the basis of the theoretical treatment of B. Gross⁸, confirmed experimentally by Gill and Alfrey¹¹, Cobb¹² and by ourselves⁷. Moreover, because of chemical bond requirements between the molecules at the interface and those present in the liquid bulk phase, it is to be expected that the preferential dipole orientation of the water molecules may extend to a considerable depth. This interfacial region is thick enough to accommodate a number of layers of water dipoles. The dipole orientation occurs at both the air/water and the ice/water interface. However, the contribution of the dipoles at the air/water interface is much smaller than that of the several dipole layers at the ice/water interface because of the more random nature of the air/water interface. No surface orientation is likely to occur inside the ice phase as a consequence of the loss in configurational entropy of the ice lattice. Experiments carried out in these laboratories indicate that the dipole orientation contributions at both interfaces are being determined while the measurements of freezing potentials are made. Assuming that the surface phase (in the water) near the ice can accommodate a few layers of oriented dipoles (say, 5 layers) and that there are $c. 10^{15}$ dipoles per sq. cm, a value of 22 V is calculated; an array of 15–20 dipole layers would account completely for the large potential values obtained for conductivity water. Fletcher¹³, using a thermodynamic approach and elementary electrostatics, found a value of 30 V for the potential in the ice. In conclusion it seems possible that the electric potentials caused by the freezing of conductivity water and very dilute solutions are largely due to orientation of water dipoles at the ice/water interface. The predominant processes occurring in region II could be, in the decreasing order of importance: proton conduction, ionic entrapment and ionic diffusion. In region II the dipole orientation contribution would be small. The elimination of the freezing potential by organic molecules could be explained, at least qualitatively, if one accepts the presence of the proton conductance mechanism and the surface orientation of the water dipoles. The organic molecules behave as a proton trap¹⁴, thereby eliminating the freezing current, and also as a disorganizing agent of the dipole arrays at the interface causing the complete annihilation of the freezing potential.

We thank Prof. Jack H. Schulman for his advice. This work was supported by the Geophysics Branch, U.S. Office of Naval Research, contract ONR 4259(03).

¹ Costa Ribeiro, J., thesis, Universidade do Brasil (1945).

² Dinger, J. E., and Gunn, R., *Terr. Mag. Atmos. Elect.*, **51**, 477 (1946).

³ Workman, E. J., and Reynolds, S. E., *Phys. Rev.*, **78**, 254 (1950).

⁴ Gross, G. W., in *Forms of Water in Biological Systems* (N.Y. Acad. Sci., in the press).

⁵ Lodge, J. P., Baker, M. L., and Pierrard, J. M., *J. Chem. Phys.*, **24**, 716 (1956).

⁶ Schulman, J. H., and Parrera, H. C., *Electrical Phenomena at the Ice/Water Interface*, Rep. No. 1, ONR Contract No. 4259(03) (June 1964).

⁷ Parrera, H. C., and Eyd, A. J. (in preparation).

⁸ Gross, B., *Phys. Rev.*, **94**, 545 (1954).

⁹ Conway, B. E., and Bockris, J. O'M., *J. Chem. Phys.*, **28**, 354 (1958).

¹⁰ Conway, B. E., Bockris, J. O'M., and Linton, H., *J. Chem. Phys.*, **24**, 834 (1956).

¹¹ Gill, E. W. B., and Alfrey, S. A., *Nature*, **169**, 203 (1952).

¹² Cobb, A. W., unpublished report, New Mexico Inst. Mine and Tech., Socorro (1964).

¹³ Fletcher, N. H., *Phil. Mag.*, **7**, 255 (1962).

¹⁴ Heinmetz, F., in *Forms of Water in Biological Systems* (N.Y. Acad. Sci., in the press).

RELATIONSHIP OF THE VELOCITY OF A CHARGED PARTICLE TO ITS RELATIVE BIOLOGICAL EFFECTIVENESS

By DR. J. E. TURNER

Health Physics Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

AND

HAL HOLLISTER

Division of Biology and Medicine, U.S. Atomic Energy Commission, Washington, D.C.

THE relative biological effectiveness (RBE) of an incident charged particle is often discussed in terms of its rate of linear energy transfer (LET). When most of the energy transferred is absorbed locally in a small target (for example, a single cell), RBE is described as a function of LET_∞ , which is the stopping power $-dE/dx$ of the target for the particles¹. Because stopping power depends only on the two properties, charge and velocity, of the incident radiation², RBE thus described for a particle is a function of its charge and velocity. In what follows, the 'LET dependence' of RBE will imply a dependence on both these quantities. We shall restrict the discussion to small targets in which the spatial dose distribution is uniform.

The ratio of RBE values of two radiations, RBE_1 and RBE_2 , is given in terms of absorbed doses, D_1 and D_2 (energy absorbed per unit mass), by the relation:

$$\frac{RBE_1}{RBE_2} = \frac{D_2}{D_1} \quad (1)$$

In comparing the two radiations, the absorbed doses are adjusted experimentally to produce the same degree of biological effect, or end-point; other factors in the experiment, such as dose rate and species, are held constant. When a mono-energetic beam of charged particles passes normally through the face of a thin slice of tissue, the absorbed dose in the tissue can be expressed by writing $D = \phi(-dE/dx)\Delta x/100\Delta m$. Here ϕ denotes the flux of particles in cm^{-2} , $-dE/dx$ is the stopping power of tissue for the radiation in ergs/cm, Δx is the thickness of the tissue slice in cm and Δm its mass in grams, and the factor 100 converts ergs/gram into rads (1 rad = 100 ergs/g). The ratio of RBE values of two kinds of charged particles, therefore, is given by:

$$\frac{RBE_1}{RBE_2} = \frac{\phi_2}{\phi_1} \cdot \frac{(dE/dx)_2}{(dE/dx)_1} \quad (2)$$

In recent years a number of investigations have extended and generalized dosimetric concepts to cover a wider range of phenomena than before³. One group of investigations has led to a quantitative description of RBE in terms of the physical properties of radiation for certain biological end-points⁴. This theory, which appears to describe the RBE of neutrons, photons, electrons and singly charged ions, and to give reasonable results for α -particles, implies that RBE depends only on the velocity of a charged particle rather than LET⁵. The theory thus implies that the RBE of a charged particle is independent of the particle's charge. This finding differs from the conventional description of RBE¹.

The present article attempts to clarify the question of charge dependence of RBE by the application of general principles of collision theory. Although the interactions and mechanisms that lead from an initial physical event between radiation and matter to biological damage are doubtlessly complex, the way in which charge enters the picture is given explicitly in the formalism of quantum mechanics. (The same is also true, of course, in classical mechanics.) In particular, the formalism of successive Born approximations gives an expansion of exact scattering amplitudes in powers of charge. This permits making

a definite choice between velocity dependence and LET dependence on theoretical grounds within well-defined limits known from quantum mechanics. As the following analysis shows, the validity of the limits of charge independence of RBE appears to be identical to the limits of the validity of the first Born approximation in collision theory.

Non-relativistically, an incident particle having a charge $+ze$ interacts with an atom consisting of electrons of charge $-e$ and a nucleus of charge $+Ze$ by means of the Coulomb energy operator:

$$V = -\frac{zZe^2}{|\vec{r} - \vec{R}|} + \sum_{j=1}^Z \frac{ze^2}{|\vec{r} - \vec{r}_j|} \quad (3)$$

Here \vec{r} denotes the position vector of the incident particle, \vec{R} that of the nucleus, and \vec{r}_j that of the j th electron. The sum is taken over all electrons ($j = 1, \dots, Z$) in the atom. The transition probability, w_{no} , for excitation of the atom from one state o to another state n with the simultaneous transfer of momentum (and energy) from the incident particle is, in the first Born approximation, proportional to the square of the matrix element of V taken between the initial- and final-state wave functions, ψ_o and ψ_n , of the system, atom-plus-particle. The operator $1/|\vec{r} - \vec{R}|$ has zero matrix element between different (orthogonal) states depending on \vec{r}_j , and so only the second term in equation (3) contributes to w_{no} . The transition probability is proportional to the square of the charge of incident particle:

$$w_{no} \sim |\langle \psi_n | V | \psi_o \rangle|^2 \sim z^2 e^4 |\langle \psi_n | \sum_j \frac{1}{|\vec{r} - \vec{r}_j|} | \psi_o \rangle|^2 \quad (4)$$

where the symbol $\langle || \rangle$ denotes the matrix element for the operators and wave functions shown. The total cross-section σ_i for the interaction of a single incident particle per cm^2 with one atom of type i per cm^3 is calculated by integrating w_{no} over all possible scattering directions and then taking the proper average over initial states and sum over final states of the system. The charge dependence shown in equation (4) is not affected by these operations and so we may write:

$$\sigma_i = z^2 e^4 f(v) \quad (5)$$

where $f(v)$ is a function depending on the velocity, but not the charge, of the incident particle.

The stopping power of the medium is closely related to σ_i . It is calculated by first multiplying w_{no} by the excitation energy of the transition from state o to state n and then carrying out the foregoing operations, after which the result is weighted by the number of atoms N_i of type i per cm^3 in the medium and summed over i . Explicit evaluation of the stopping power² gives an equation of the form:

$$-\frac{dE}{dx} = z^2 e^4 g(v) \quad (6)$$

where $g(v)$ is, for a given stopping medium, a universal function of velocity v for all charged particles. (The function $g(v)$ is given explicitly, for example, by equation (38) of ref. 2.) The expressions (5) and (6) also hold relativistically in the first Born approximation. The inclusion of the second and higher approximations destroys the homogeneity of these expressions in the incident particle's charge ze .

In order to relate the discussion to RBE, a connexion has to be made between the occurrence of a physical collision on a microscopic scale and the occurrence of observable biological damage. We assume that the amount of damage done to a biological system is related to the number of primary quantum transitions or 'hits' which occur between the incident radiation and the target. In keeping with the definition of RBE, we shall assume only that the same extent of damage is produced by two charged radiations (other conditions being the same) when the number of primary quantum transitions per unit volume in the systems is the same. (Since we are considering small targets with uniform dose distribution, this approach is equivalent to relating biological damage to the total number of primary transitions in the system. The volume of the system would then appear explicitly in this description, but would cancel later from both sides of equation (7).) This assumption does not imply a quantitative relationship between degree of damage and number of transitions, but states merely the biological equivalence of two radiations producing the same end-point. This criterion for damage need hold only statistically, because the number of primary quantum transitions leading to observable damage is large.

Since the number of primary transitions per unit volume in a system due to a flux ϕ is $\phi \sum_i N_i \sigma_i$, we describe equal biological damage from two radiations by writing:

$$\phi_1 \sum_i N_i \sigma_{i1} = \phi_2 \sum_i N_i \sigma_{i2} \quad (7)$$

where σ_{i1} and σ_{i2} are the values of σ_i for the two radiations. The resulting flux ratio, when substituted into equation (2) for RBE, gives:

$$\frac{\text{RBE}_1}{\text{RBE}_2} = \frac{\sum_i N_i \sigma_{i1}}{\sum_i N_i \sigma_{i2}} \cdot \frac{(dE/dx)_2}{(dE/dx)_1} \quad (8)$$

The charge dependence of this ratio can be written at once from equations (5) and (6):

$$\frac{\text{RBE}_1}{\text{RBE}_2} = \frac{z_1^2 e^4 \sum_i N_i f(v_1)}{z_2^2 e^4 \sum_i N_i f(v_2)} \cdot \frac{z_2^2 e^4 g(v_2)}{z_1^2 e^4 g(v_1)} = F(v_1, v_2) \quad (9)$$

The explicit dependence on the charges of the incident particles cancels, and it follows that the ratio of RBE values can be expressed as a function $F(v_1, v_2)$ depending only on the velocities of the particles. This is the principal result of the work described here.

While it is true that a highly charged ion causes more damage (that is, a larger number of primary quantum

transitions) than a singly charged one at the same velocity (lower LET), it does not follow that more damage results per unit absorbed dose. Whereas the stopping power of an ion is proportional to z^2 , the flux needed to produce a given number of transitions is proportional to $1/z^2$. The magnitude of the charge acts as a coupling constant for the strength or intensity of the interaction between radiation and matter. Its effect cancels when different radiations are compared on the basis of their absorbed doses, as equation (2) for RBE specifies.

The foregoing results also hold relativistically in the first Born approximation, which coincides with the limits of accuracy of Bethe's original stopping power theory⁶ including various corrections². Physically this means that the theory is valid so long as the speed of the incident particle is much greater than the speed of atomic electrons. For light elements, which are the chief constituent of soft tissue, the first Born approximation, particularly when it includes shell corrections, is known experimentally to give an adequate description of charged particle slowing down for radiation of practical interest in radiobiology. For protons in water, for example, the theory is adequate down to energies of less than 1 MeV (ref. 7). Direct experimental evidence for the possible failure of the first Born approximation appears to have been found only recently, in connexion with the Σ^+ hyperon mass anomaly⁸. The second Born approximation introduces a difference in the stopping powers of positive and negative particles that are otherwise identical. The observed difference is of the order of a few per cent.

In summary, it appears that the validity of charge independence for RBE is equivalent to the validity of the first Born approximation in quantum mechanics. Within the framework of this approximation, the concept of 'LET' dependence for RBE should be replaced by the more precise 'velocity' dependence. Higher-order approximations are needed to describe particles at lower speeds⁹, and charge cannot be factored out and cancelled from the resulting expressions. Experiment indicates, however, that corrections resulting from inclusion of higher-order approximations may amount to only a few per cent.

This work was sponsored by the U.S. Atomic Energy Commission in part under contract with Union Carbide Corporation.

¹ Rep. RBE Committee to Intern. Commissions on Radiological Protection and on Radiological Units and Measurements, *Health Phys.*, 9, 357 (1963).

² Compare, for example, Fano, U., *Ann. Rev. Nucl. Sci.*, 13 (Annual Reviews, Inc., Palo Alto, Calif., 1963).

³ For example, Rossi, H. H., *Rad. Res.*, 10, 522 (1959). Rossi, H. H., Blavati, M. H., and Gross, W., 15, 431 (1961). Hurst, G. S., and Ritchie, R. H., *Health Phys.*, 8, 117 (1962).

⁴ Turner, J. E., and Hollister, H., *Health Phys.*, 8, 523 (1962). Turner, J. E., *Health Phys.* (in the press).

⁵ Hollister, H., and Turner, J. E., submitted for publication in *Health Phys.* This work was summarized in Paper No. 114, Ann. Meeting Health Phys. Soc., Los Angeles, California, June 14-17, 1965.

⁶ Bethe, H. A., *Handbuch Phys.*, 24/1, 491 (Springer, Berlin, 1933).

⁷ Whaling, W., *Encyclopedic Phys.*, 34/2, 193 (Springer, Berlin, 1958).

⁸ Barkas, W. H., Dyer, J. N., and Heckman, H. H., *Phys. Rev. Letters*, 11, 26 (1963).

⁹ For heavy charged particles, however, the first Born approximation is justified when the incident particle moves much slower than the atomic electrons. Compare with Henneberg, W., *Z. Phys.*, 86, 592 (1933).

ADENOSINE TRIPHOSPHATE-METAL-NOREPINEPHRINE TERNARY COMPLEXES AND CATECHOLAMINE BINDING

By DR. ROBERT W. COLBURN and DR. JAMES W. MAAS

Section on Psychosomatic Medicine, Adult Psychiatry Branch, National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland

CATECHOLS and ethanolamines have been shown to form relatively stable chelate bonds¹⁻³, and metals have frequently been associated with catecholamine activity⁴⁻⁸ so that a reasonable approach to the study of

catecholamine binding would seem to be from the point of view of co-ordination chemistry. With the catecholamines a wide variety of chelate species are possible and the different structures would have varied

electrostatic charges and solubilities. Some of these species have recently been elucidated by Walaas *et al.*⁹ in work on Cu(II) chelates of norepinephrine (NE) and epinephrine in connexion with ceruloplasmin. In anaerobic systems the Cu(II) NE chelate was found stable whereas under aerobic conditions oxidation occurred at a moderate rate, which was in agreement with the findings of Chaix *et al.*¹⁰.

In this article results are presented which suggest that co-ordination complexes may be of major importance in the understanding of the biological phenomenon of binding of the catecholamines. This possibility was independently derived by Carlsson *et al.*⁶, but set aside when by paper chromatographic methods sufficient quantities of metals were not found in adrenal medullary particles.

In the work recorded here the levels of metals were determined in sub-cellular fractions of brain known to have high concentrations of the catecholamines. Adult male mice were killed by decapitation, the brains quickly removed, the cerebellum and cerebral mantle dissected away and the remaining brain tissue (the medulla, pons, mesencephalon, diencephalon, hippocampi, and pyriform cortices) was homogenized in 0.32 M sucrose; the technique of Gray and Whittaker¹¹ was used to obtain synaptosomes, and the method of DeRobertis *et al.*¹² was used to obtain synaptic vesicles. In the sub-cellular fractionation the method was slightly modified in that the sucrose (Baker and Adamson reagent grade) was washed with glass-distilled water on a Buchner funnel, dried, dissolved with water to 1.2 molar, and passed over a washed 'Dowex 50' column in the hydrogen form. Succinic acid dehydrogenase (SDH)¹³ and NE levels¹⁴ were determined for the myelin, synaptosome, and mitochondrial fractions to determine the degree of separation of the mitochondria from the synaptosomes. The metals quantitatively measured were magnesium, iron, copper and zinc. For the metal analyses the sub-cellular fractions were dried under vacuum, ashed at 600°C, dissolved in N hydrochloric acid, and measured against standards in an atomic absorption spectrometer (Perkin-Elmer model 303). From the results of these analyses, shown in Fig. 1, it may be seen that in general a good share of the metals in brain are present in the nerve endings. Moreover, while the metals present in this fraction tend to be in proportions similar to the proportions found in brain, copper seems to be concentrated several-fold relative to the

other metals. These findings indicate that the metals magnesium, copper, iron and zinc are present in synaptosomes in sufficient quantities for chelation to be of biological significance.

Metal levels for the synaptic vesicles were determined, in addition, as described above. In this case the metals were again found present in good yield and the level of copper was again noted to be increased relative to the amounts of other metals (Table 1). These findings suggest that metals are present in structures associated with NE rather than with unrelated proteins in the synaptosomes.

Table 1. METAL VALUES FOR SYNAPTIC VESICLES ARE EXPRESSED AS μg PER SYNAPTIC VESICLES OBTAINED FROM 1 g WHOLE BRAIN \pm S.E.M.

Metal	Whole mouse brain ($\mu\text{g/g}$)	Synaptic vesicles ($\mu\text{g/g}$)
Mg	116 \pm 1.7	14.05 \pm 0.29
Fe	51.7 \pm 3.9	1.7 \pm 0.56
Zn	18.7 \pm 0.8	1.05 \pm 0.05
Cu	10.7 \pm 0.5	2.0 \pm 0.16

In summary, both quantities and intracellular location of metals support the hypothesis that co-ordination between metal and NE in the synapse is of biological significance. It therefore seemed of interest to test the effects of a chelating agent on the uptake of exogenous NE. The test materials used were synaptic vesicles rather than synaptosomes and the chelating agent used was ethylenediamine hydrochloride. The control system contained ethylamine hydrochloride at twice equimolar concentration to standardize for ionic effects^{4,15} and possible reactions between amines and quinones even to a limited degree¹⁶. The results obtained in this experiment are shown in Table 2 and the data clearly indicate that the uptake of the exogenous NE was inhibited by this chelating agent. These findings represent additional support, therefore, for chelation as a mechanism for the binding of catecholamines.

Table 2

No addition	0.6 μM <i>d,l</i> -NE	0.6 μM <i>d,l</i> -NE plus 3.2 μM ethylamine hydrochloride	0.6 μM <i>d,l</i> -NE plus 1.6 μM ethylenediamine hydrochloride
14.4*	39.6 \pm 1.4	34.0 \pm 1.1	17.6 \pm 4.0

μg NE in synaptic vesicles after incubation at 0°C for 30 min

Synaptic vesicles were prepared from 4 g whole mouse brain, divided into 4 equal aliquots, and brought to 1 ml. with 0.32 M sucrose + additions as noted. No. = 5 each value. Values are expressed as μg NE \pm S.E.M.

* To obtain this value the NE released from synaptic vesicles during the incubation (3.4 \pm 0.2 μg) was subtracted from the endogenous level without incubation (17.8 \pm 0.8 μg).

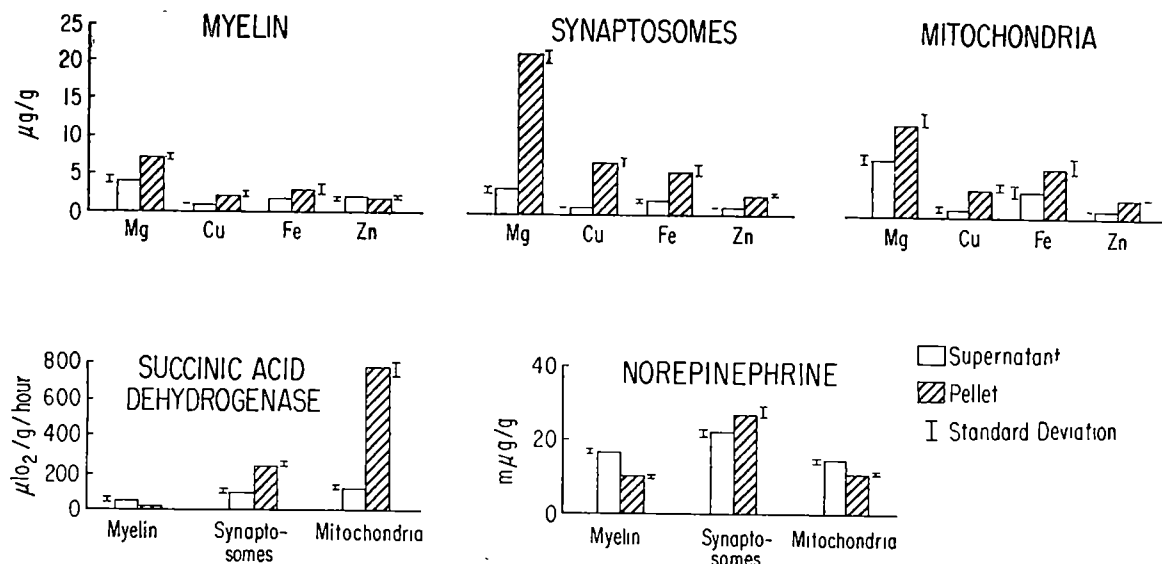


Fig. 1. Metal, SDH and NE levels in sub-cellular fractions of dissected mouse brain. NE was determined by a modification of the method of Anton and Sayre (ref. 14). Metal values are expressed as μg sub-cellular fraction obtained from 1 g of original tissue. SDH and NE values as μl O_2 /sub-cellular fraction from 1 g original tissue/h and μg /sub-cellular fraction from 1 g original tissue respectively.

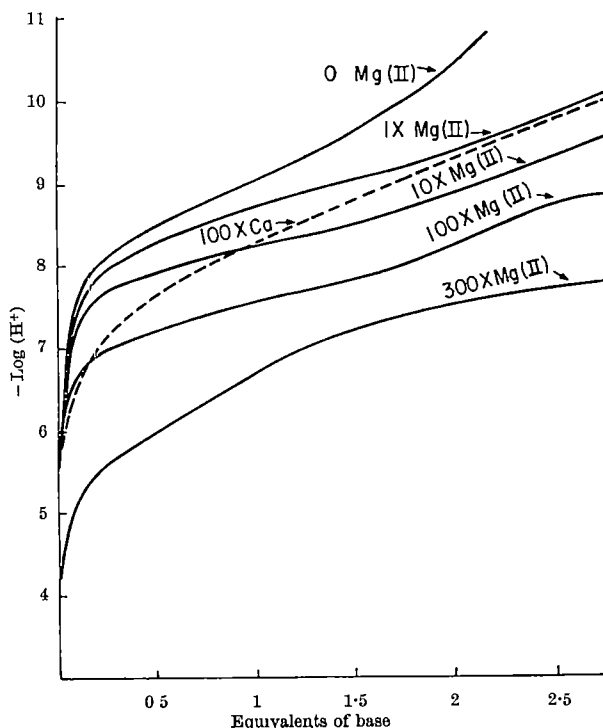


Fig. 2. Potentiometric titration of NE (0.1 mmoles in 10 ml. aqueous solution of 0.1 M KCl, 0.02 M NaCl) with increasing molar ratios of Mg(II) and Ca(II) with carbonate-free 0.1 N KOH

To study the co-ordination chemistry of the metals, magnesium, copper and iron with NE, the method used was potentiometry. This technique was thought best for this survey since it is generally applicable with this class of compounds and since the degree of chelation may be estimated directly from the titration curves. When NE was titrated with and without magnesium in increasing molar ratios it was found that with equimolar magnesium the chelation was negligible at physiological pH, but that with increased molar ratios of magnesium the chelation increased to levels easily of biological significance (Fig. 2). The titration curves for NE with Ca(II) at a molar ratio of 100, shown in addition in Fig. 2, indicate that NE forms somewhat less stable chelates with Ca(II) than with Mg(II). It would be of interest to determine stability constants for NE from the above titration curves, but since chelation may occur at either the catechol moiety or ethanalamine side-chains, additional data are required for such calculations.

For the titration of NE with Cu(II) and Fe(II) it was found necessary to use an organic solvent since the chelates formed were insoluble in water over the pH range examined. The solvent found suitable was water: propanol:butanol 2:3:2, and the electrodes were standardized with *tris* buffer in this solvent. The titration curves obtained in this case with NE and Cu(II) and Fe(II) indicate that at physiological pH, NE essentially completely chelates the metal present even at equimolar metal concentrations (Fig. 3).

In addition it seemed of interest to titrate ATP plus metals with and without NE present, since ATP has recently been found concentrated in synaptosomes¹⁷ and since ATP has been associated with NE storage in many studies^{4,6}. A reasonable hypothesis in this case seemed to be that ATP, metal, and NE might form an ATP-metal-NE ternary complex and that such complexes might be of particular importance in the reaction mechanisms of catecholamines. During the titration of the samples containing Cu(II) and Fe(II) with NE present, it was found that additional protons were released through the pH range 6–8 (Fig. 4). Since NE normally releases

protons only at pH values > 7.5, this, and the formation of an intense blue colour in the case of Fe(II), indicated that a good proportion of the NE was chelated at pH 7.4. The question remaining was whether NE displaced the ATP or chelated with additional co-ordination positions of the metal. It was therefore of interest to note that no precipitate was observed in the samples containing either iron or copper with NE and ATP, whereas a precipitate forms when NE alone is in solution with copper or iron (see foregoing). This finding, in conjunction

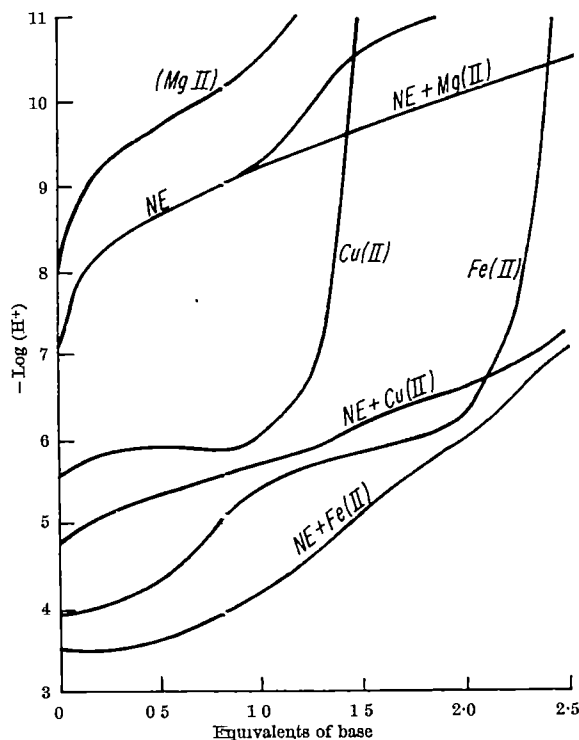


Fig. 3. Potentiometric titration of NE in the presence of Mg(II), Fe(II) and Cu(II) (equimolar, 0.05-mmoles in 100 ml. of solvent) with carbonate-free 0.1 N KOH. Solvent system, water: propanol: butanol, 2:3:2

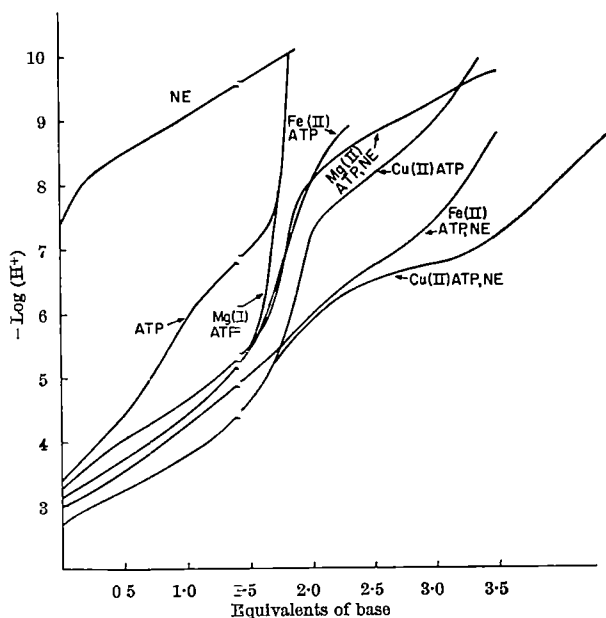


Fig. 4. Potentiometric titration of NE in the presence of various combinations of ATP, Mg(II), Cu(II) and Fe(II) (equimolar, 0.1 mmoles in 10 ml. aqueous solution of KCl, 0.02 M NaCl) with carbonate-free 0.1 N KOH

ATP-NE complex. A unifying concept was then constructed with metal or metal-ATP complexes representing the intravesicular binding sites for catecholamines. The way in which such a hypothesis might explain a variety of reported experimental findings was outlined.

¹ Timberlake, C. F., *J. Chem. Soc.*, 4987 (1957).

² Flannery, E. J., Ke, B., Grieb, M. W., and Trivich, D., *J. Amer. Chem. Soc.*, 77, 2996 (1955).

³ Sillen, L. G., and Martell, A. E., *Stability Constants* (Chemical Society Spec. Pub. 17, London, 1964).

⁴ Kirshner, N., *J. Biol. Chem.*, 237, 2311 (1962).

⁵ Stjärne, L., von Euler, U. S., and Lishajko, F., *Biochem. Pharm.*, 13, 809 (1964).

⁶ Carlsson, A., Hillarp, N. A., and Waldeck, B., *Acta Physiol. Scand.*, Supp. 59, 215 (1963).

⁷ Senoh, S., Tokuyama, Y., and Witkok, B., *J. Amer. Chem. Soc.*, 84, 1719 (1962).

⁸ Nagatsu, T., Levitt, M., and Udenfriend, S., *J. Biol. Chem.*, 239, 2910 (1964).

⁹ Walaas, E., Walaas, O., Haavaldsen, S., and Pedersen, Bjorn, *Arch. Biochem. Biophys.*, 100, 97 (1963).

¹⁰ Chaix, P., and Pallaget, C., *Biochim. Biophys. Acta*, 10, 462 (1959).

¹¹ Gray, E. G., and Whittaker, V. P., *J. Anat.*, 96, 76 (1962).

¹² DeRobertis, E., Delores, G. R., Salganicoff, L., DeIrald, A. P., and Zieher, L. M., *J. Neurochem.*, 10, 225 (1963).

¹³ Umbreit, W. W., Burris, R. H., and Stauffer, J. F., *Monometric Techniques* (Burgess, Minneapolis, Minn., 1957).

¹⁴ Anton, A. H., and Sayre, D. F., *J. Pharmacol. Exp. Therapeut.*, 138, 360 (1962).

¹⁵ Mirkin, B. L., Glarman, N. J., and Freedman, D. X., *Biochem. Pharmacol.*, 13, 1027 (1964).

¹⁶ Weil-Malherbe, H., *Biochim. Biophys. Acta*, 40, 351 (1960).

¹⁷ Nyman, M., and Whittaker, V. P., *Biochem. J.*, 87, 248 (1963).

¹⁸ Cohn, M., and Hughes, T. R., *J. Biol. Chem.*, 237, 176 (1962).

¹⁹ Ingraham, L. L., *Biochemical Mechanisms* (John Wiley and Sons, 1962).

²⁰ Musacchio, J. M., Kopin, I. J., and Weise, V. K., *Nature*, 205, 606 (1960).

²¹ Snyder, S. (in the press).

²² Axelrod, J., in *The Chemical Chemistry of Monoamines* (Elsevier, Amsterdam, 1963).

²³ Von Euler, U. S., and Lishajko, F., *J. Neurochem.*, 10, 145 (1963).

²⁴ Hillarp, N. A., *Acta Physiol. Scand.*, 47, 271 (1959).

CO-ORDINATION CHEMISTRY AND MEMBRANE FUNCTION WITH PARTICULAR REFERENCE TO THE SYNAPSE AND CATECHOLAMINE TRANSPORT

By DR JAMES W. MAAS and DR. ROBERT W. COLBURN

Section on Psychosomatic Medicine, Adult Psychiatry Branch, National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland

IN an earlier investigation¹ results were presented which indicated that a fraction of pinched-off nerve ending particles (synaptosomes) and synaptic vesicles from brain tissue contained appreciable quantities of copper, iron, and magnesium. The significance of this finding, viewed from the perspective of co-ordination chemistry, for the mechanism of binding of norepinephrine (NE) was discussed. It was also noted that these metals were not freely soluble but tended to be associated with the particulate fraction. This finding suggested that these metals might be co-ordinating with membrane constituents and hence be importantly related to membrane structure and function. Data and theory bearing on this possibility are presented and discussed in this article. In addition, the manner in which such co-ordination structures might be related to the binding and transport of NE is further explored.

Phosphatidyl-L-serine (PS) was obtained from Nutritional Biochemical Corporation, phosphatidyl inositol (PI or fraction I of Folch) and adenosine triphosphate (ATP) as the disodium salt from Sigma Chemical Co. The phospholipids used are constituents of cell membranes in general and have been reported to be present in the membranes of synaptosomes and synaptic vesicles². Sub-cellular fractionation of brain tissue was done as follows: Male rats of approximately 90 days of age were killed, the brains quickly removed, the cerebellum and cerebral mantle dissected away and the remaining brain tissue homogenized in 0.32 M sucrose cleaned of metals as previously noted¹. The technique of Whittaker^{3,4} was used to prepare a fraction of synaptosomes. Evidence that this fraction was composed of synaptosomes was obtained by a spot check of succinic acid dehydrogenase activity (SDH)⁵ and NE content⁶ of the myelin, synaptosome, and mitochondrial fractions. The SDH activity in μ 10₂/g original tissue/h was 18, 60 and 793 respectively. NE values in μ g/g original tissue were found to be myelin: 7.5; synaptosomes, 52; and mitochondria, 41.5. It is assumed that the NE levels for the mitochondrial fraction represent a contamination by synaptosomes. Lipid extracts of pellets of synaptosomes were prepared by the method of Folch, Lees, and Sloane-Stanley⁷. An upper phase non-lipid fraction was also obtained by this method. This fraction is hereafter referred to as 'upper phase extract of synaptosomes'. The fractions were evaporated to dryness and resuspended

in water. Titrations and metal determinations were carried out as previously noted¹. The solubility characteristics of various metal complexes in aqueous-ether phospholipid dispersions were obtained as follows: The combination of compounds to be examined were added in noted amounts to glass-distilled water and then the particular metal being used was added and the total volume brought to 1 ml. The pH was then adjusted to 7.3 with sodium hydroxide. This aqueous phase was then overlain with 3 c.c. of diethyl ether, the tubes capped, shaken for 15 min, and centrifuged at 2,500 r.p.m. for 15 min. At this point the colour of the ether phase was noted and 0.4 c.c. of the ether phase was removed for metal assay. If NE values were to be determined, tritiated *d,l*-NE (New England Nuclear Corp.) was mixed in tracer amounts with cold *d,l*-NE in noted quantities. C.p.m. were determined from both aqueous and ether phases using a Packard liquid scintillation spectrometer, and partition coefficients calculated as:

$$\frac{\text{c.p.m./c.c. ether phase}}{\text{c.p.m./c.c. aqueous phase}}$$

The results obtained may be summarized as follows. When phosphatidyl-L-serine is titrated in the presence of Mg(II), Cu(II) or Fe(III) the curves obtained indicate co-ordination of these metals with the phospholipid (Fig. 1). Moreover, evidence for complex formations between metal and phosphatidyl-L-serine altering the physical characteristics of the components is indicated in Table 1, in which it is noted that the metal content of the ether phase increases. If ATP is added to the phosphatidyl-L-serine metal complex it is found that: (a) precipitation does not occur; (b) the metal is not found in the ether phase (Table 1). The ATP effect might occur owing to the formation of co-ordination complexes between metal and ATP with no metal being available for the phospholipid. However, titration data as shown in Fig. 1, taken in conjunction with solubility characteristics (Table 1), suggest that a ternary metal-ATP-PS co-ordination complex forms. More explicitly, when PS is added to Fe(III) ATP solutions, Cu(II) ATP solutions or additional protons are released. This could occur owing to the formation of a ternary complex or the displacement of ATP by the phospholipid. If the latter were the case,

however, due to alterations in solubility, one would expect to find significant quantities of Fe(III) in the ether phase (Table 1). That this does not occur supports the argument for ternary complex formation between Fe(III), ATP and phospholipids. Moreover, in titrations (not shown) of metal, PS, and ATP in aqueous solutions (0.1 N KCl, 0.05 N NaCl) a precipitate did not form whereas precipitation did occur if only metal and the phospholipid were present. Owing to the physical characteristics of Mg(II), evidence for ternary complex formation from Mg(II) titration data is less firm than in the case of Fe(III) and Cu (II). However, if magnesium were present in greater than equimolar concentrations, as might occur in biological systems, then by the law of mass action increased complex formation would occur, as was shown for the case of Mg (II) and NE in an earlier report¹. The increased solubility of the metal-phosphatidyl-1-serine-ATP complex in water and decreased solubility in ether is thought to be a function of the quadrivalent ATP. The presence of a ternary complex is not too surprising as ATP is known to form a chelate structure $M(ATP)^{8-12}$ and the serine group could easily form a 5-membered chelate ring utilizing the remaining co-ordination positions of the metal. In terms of overall electrostatic charge, alterations in solubility may be understood.

Table 1

Metal	5.00 μ M phosphatidyl-1-serine		5.00 μ M phosphatidyl inoside		No phospholipid
	Without ATP	6.25 μ M ATP	Without ATP	6.25 μ M ATP	
6.25 μ M $CuCl_2$	2.32 \pm 0.64	0.016 \pm 0.002	1.66 \pm 0.021	0.071 \pm 0.005	0
6.25 μ M $FeCl_3$	0.68 \pm 0.32	0.025 \pm 0.002	0.88 \pm 0.76	0	0
6.25 μ M $MgCl_2$	0.59 \pm 0.18	0.03 \pm 0	1.16 \pm 0.29	0.087 \pm 0.005	0

Values refer to μ M metal/3 ml. of ether \pm S.E.M. The No. for all metals + phospholipid is 3 and for all metals + phospholipid + ATP is 2.

As can be seen from Table 1, in the presence of phosphatidyl inoside metal is found in the ether phase in increased amounts. Titration results (Fig. 2) suggest the presence of a metal-phosphatidyl inoside co-ordination complex. It is also noted that, as before, metal incorporation into the ether phase is essentially reduced to zero in the presence of ATP. Evidence from titration data (Fig. 2) and solubility characteristics (Table 1) suggest the formation of a ternary co-ordination structure between metal, ATP, and phosphatidyl inoside. The dissociation characteristics of phosphatidyl inoside are such that the titration data with this phospholipid more clearly suggest

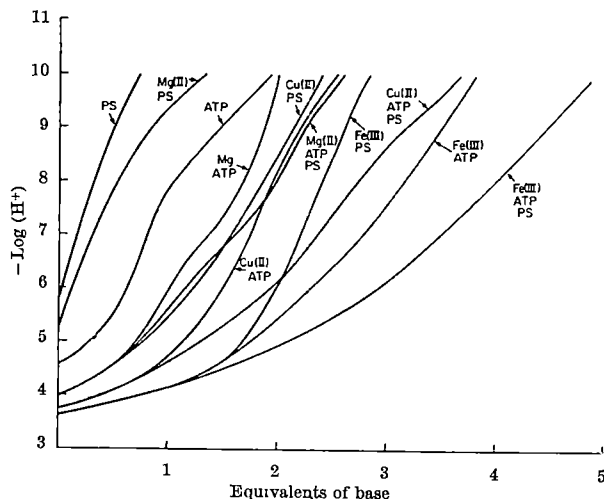


Fig. 1. Potentiometric titration of phosphatidyl-1-serine in the presence of various combinations of ATP, Mg(II) and Cu(II) (equimolar, 0.01 mmoles in 100 ml water isopropanol-*n*-butanol, 2:3:2) with carbonate-free 0.1 N KOH. Samples containing Fe(III) were equimolar with the other components at 0.01 mmoles

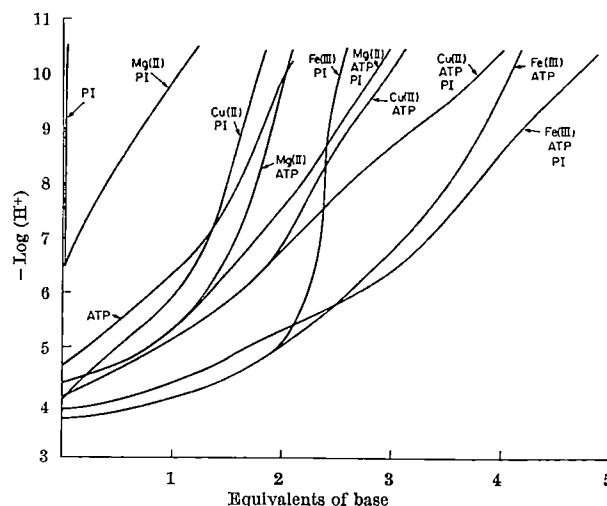


Fig. 2. Potentiometric titration of phosphatidyl inoside in the presence of various combinations of ATP, Mg(II), Cu(II) and Fe(III) (equimolar, 0.01 mmoles in 100 ml water isopropanol-*n*-butanol, 2:3:2) with carbonate-free 0.1 N KOH

ternary complex formation for Cu(II) and Mg(II) as well as Fe(III).

Although the co-ordination structures of these phospholipid-metal complexes are not clear it does seem that the structures are sufficiently stable in the presence of water to alter the solubility characteristics of the metal-phospholipid complex.

The two phospholipids thus far mentioned were chosen because of differences in their polar groups as well as being naturally occurring membrane constituents. It is not felt, however, that the change in solubility characteristics induced by metal complexes need be limited to these two. Any molecule which can co-ordinate stably with metal with an alteration in overall electronic structure would be expected to exhibit the characteristics shown by the two phospholipids examined. This phenomenon is well known, namely, precipitation of amino-acids by Cu(II), the inner complex salt of Werner¹³.

To be certain that the results and theory constructed from them would be applicable to the central nervous system, lipids were extracted from a pellet of the synaptic fraction of an ultra-centrifugate of dissected brain. As a control the upper phase as defined by Folch, Lees and Sloane-Stanley⁷, which contains predominantly water-soluble material, was used. The ability of this lipid extract to increase the incorporation of metals into the ether phase and a prevention of this by the presence of ATP are shown in Table 2. This was not found with upper phase material, which is taken as suggesting that lipids from biological material, synapses, will co-ordinate with metals and alter their electronic structure. Further, in the light of preceding data it seems reasonable to assume that ternary complexes form between synaptic lipids, metal, and ATP.

It is of interest that Folch, Lees, and Sloane-Stanley⁷ find that $MgCl_2$ 0.003 N, $CaCl_2$ 0.003 N, $NaCl$ 0.05 N, KCl 0.05 N are able to alter the distribution of lipids between the upper and lower phases, that is, the metals increase the yield of lipids in the lower, chloroform phase. Wolman and Wiener¹⁴ report that when myelins

Table 2

	Lipid extract of synaptosomes Without ATP	6.25 μ M ATP	Upper phase extract of synaptosomes	No. extract
6.25 μ M $CuCl_2$	1.17 \pm 0.126	0.012 \pm 0.001	0	0
6.25 μ M $FeCl_3$	0.61 \pm 0.022	0.035	0.023 \pm 0.0071	0
6.25 μ M $MgCl_2$	0.086 \pm 0.013	0.025 \pm 0.005	0	0

Values are expressed as μ M metal/3 c.c. ether/mg extract (dry weight) \pm S.E.M.; No.=4 (approximately 1 mg dry wt of lipid extract was used per tube, approximately 4 mg dry wt. of 'upper phase' extract was used per tube) 11 mg of the lipid extract of synaptosome before addition of metals was found to contain 0.005 μ M Cu, 0.0048 μ M Fe, and 0.037 μ M Mg.

are homogenized in 0.5 M calcium chloride an aqueous extraction yields only small amounts of lipid. They interpret this as indicating that the Ca^{++} has induced a transition of phospholipids to a water-in-oil pattern (Fig. 3). West¹⁵ also notes the ability of metals to induce this transition. Others have suggested a role for membrane phospholipids in ion transport^{16,17}. These investigators, however, have used the concept of electrostatic, attractive forces to explain the effect of metals on polar lipids. It is suggested here that the use of the theory and facts of co-ordination chemistry is more appropriate for the understanding of interactions between membrane phospholipids and metals as this body of knowledge: (a) aids in the understanding of differences in behaviour of various cations; (b) allows the introduction of steric considerations; (c) suggests that relatively stable complex formation (such as chelation) may occur between membranes and metals with an alteration in their solubility characteristics; (d) raises the possibility of ternary complex formation between a membrane constituent, metal, and biologically active molecules, namely, ATP amino-acids, metal activated enzymes. This latter point is of particular importance because of the well-known relationship between ATP enzymes and the maintenance of membrane function.

The results so far presented may be interpreted as follows. Phospholipids alone dispersed in aqueous systems are oriented with their polar heads towards the aqueous phase (in either lamellar sheets or globular arrays). In the presence of metals co-ordination complexes between the metal and phospholipids form which alter the electronic structure of the complex in such a way that a portion of the phospholipid dispersion assumes a water-in-oil configuration (Fig. 3) and hence the increase in ether solubility of the metal-phospholipid complex. If one adds ATP, however, the situation is quite different. Owing to either the formation of ternary complexes or the functional removal of metal by ATP the phospholipid dispersions are shifted towards a predominantly oil-in-water pattern. In this system metals and ATP have a role as important modulators of the configurational array assumed by phospholipids in aqueous systems.

The significance of this for biological membranes is suggested to be as follows. Metal-ATP-phospholipid complexes can be seen as forming lamellar sheets in agreement with the most generally accepted model depicting the cellular membrane¹⁸⁻²² as composed of two unimolecular lipoprotein leaflets having the phospholipid molecules so oriented that the hydrocarbon tails point

toward each other. However, if the ATP is removed, by ATPase, for example, the electronic structure of the metal-phospholipid complex will favour the development of a quite different configuration. Replacement of the ATP, by a metabolic source, will lead to a resumption of the usually depicted lamellar configuration. In the understanding of membrane function in nerve, some mechanism, whereby rapid reversible alterations in membrane structure occur, is necessary. How such a mechanism might operate in a bio-logical system, such as a synapse, is presented in Fig. 4. If such a model is correct, the following can be predicted.

(1) Whether on the inner or outer surface of the cellular membrane, metals will tend to induce configurations of the water-in-oil type, that is, to produce a mosaic of water-filled channels or pores lined with charges. The dimensions of these pores may easily²³ be of sufficient size to permit the passage of ions and small molecules across the membranes. Larger molecules which can co-ordinate with the sterically available metals may cross the membrane in an exchange reaction.

(2) The pores in this model have fixed charges and varying dimensions of a type dependent on the particular phospholipid and metal involved. They could thus assume the properties of permselective membranes²⁴. In this respect it is of interest that copper converts the frog skin membrane into a structure which becomes selectively impermeable to chloride ions²⁵.

(3) It is generally agreed that ATP is quite important in membrane function and is known to be present in the *milieu interieur* of the cell and synapse; for example, Nyman and Whittaker²⁶ have shown that a stable ATP fraction which is probably only about 10 per cent of that found *in vivo* is present in synapses in a molar concentration some 200 times greater than serotonin. From our results it is suggested that complexes between ATP-metal-phospholipids would tend to maintain the lamellar arrangement, and as the metabolic source of ATP is in the interior of the cell this will lead to different structural configurations between the inner and outer surfaces of the membrane, that is, there are many less water-in-oil configurations on the inner surface. This would mean that in the presence of ATP the energy barrier to passage of small ions such as sodium or potassium and larger molecules in either direction across the internal interface would be relatively large, that is, selective ion concentrations and hence potential gradients would tend to be maintained as well as there being a barrier to the passage of polar molecules.

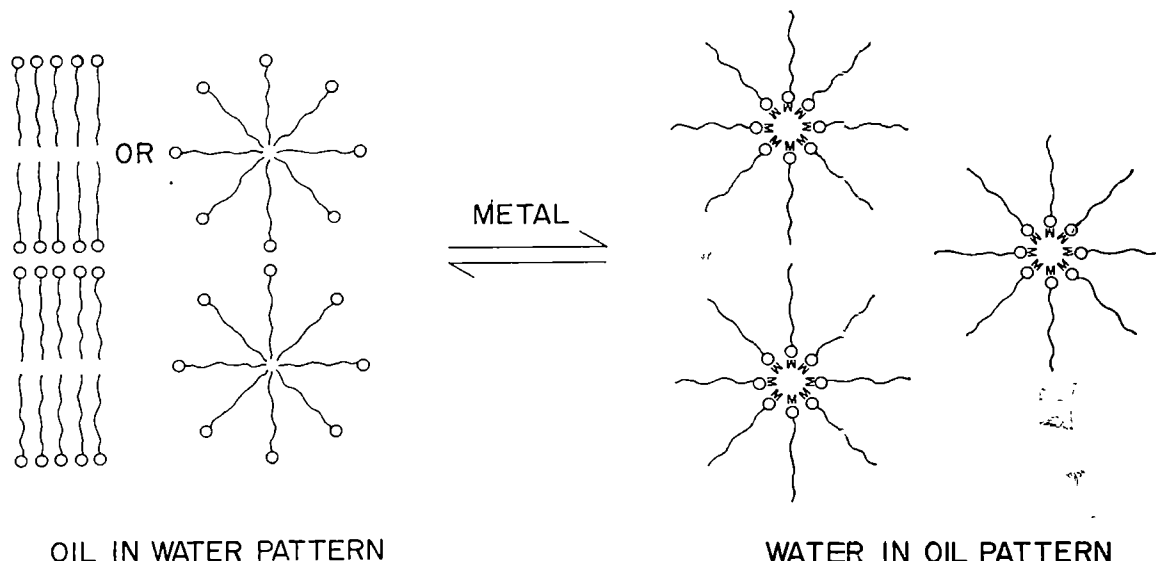


Fig. 3. Schematic representation of oil-in-water and water-in-oil patterns

(4) Removal of ATP from the ATP-metal phospholipid complex as by ATPase, which may be intimately associated with membrane function in nerve²⁷, would result in the internal surface of the membrane spontaneously assuming a water-in-oil configuration, that is, one would now have a mosaic of charged pores both on the inner and outer surfaces of the membrane with consequent fluxes of water and ions across the membrane in both directions. Two especially interesting features predicted from this are: (a) the loss of the membrane potential due to Na^+ and K^+ exchange; (b) a change in membrane resistance.

(5) Replacement of ATP on the metal-phospholipid groups would result in a resumption of the resting structure, that is, one without pores on the inner surface and an energy barrier to movement of ions and larger molecules across it. Without a steady supply of ATP this resumption would not occur and the membrane would progressively, functionally deteriorate.

(6) Some miscellaneous features of the model to be noted are as follows: Luzzati and Husson²⁸ have shown that the phospholipids extracted from brain, at body temperature, are not in a coagel but a liquid crystalline state, that is, at 37° the hydrocarbon tails have melted. As such the energy barrier of the hydrocarbon tails to transitions between the two configurations would be less than with a coagel. Furthermore, they have found by X-ray diffraction data that such water-in-oil to oil-in-water transitions can spontaneously occur in phospholipid-aqueous dispersions depending on concentration. As such, counter forces in either direction could be quite determinant as to the configuration assumed.

It is of interest that Baker, Hodgkins and Meves²⁸ have raised the possibility of there being fixed negative charges on the inner surface of the membrane which they relate to ionized phospholipid and carboxyl groups. Quadrivalent

ATP might significantly contribute to such fixed negative groups.

Other, less metabolically active, molecules might also function as ATP in the maintenance of the oil-in-water configuration and would tend to stabilize the membrane in this form. Attention has been focused on ATP because of the importance of its production and dephosphorylation for normal membrane function.

Data and theory bearing on a mechanism whereby a neurotransmitter, norepinephrine, might be released from the pre-synaptic terminal are now presented. In the preceding article it was noted that metals found in the synaptosomes and synaptic vesicles tended to remain with the particulate fractions and not enter the supernatant. Further, evidence for the formation of metal-NE chelate structures and their importance in storage pools within synaptic vesicles and synaptosomes was discussed. In the light of results presented in the earlier portion of this article, it seems possible that ternary complexes might form between membrane phospholipids, metals, and NE or even quaternary complexes with ATP where the co-ordination number of the metal is 6 or more.

Titration curves (Fig. 5) suggest that such ternary complexes do occur between phosphatidyl-1-serine, metals and *d,l*-norepinephrine.

Solubility characteristics of metals and NE in the presence of two phospholipids are presented in Table 3. It is noted that in the presence of metals and phospholipids the quantities of metal in the ether phase and the partition coefficients of NE are markedly altered. A NE-metal-phospholipid complex is thus seen to have altered solubility characteristics such that a polar NE molecule becomes more soluble in an organic solvent. Phrased differently, the energy barrier to the movement of NE into an organic solvent is reduced by complexing. This might be of significance in understanding a mechanism

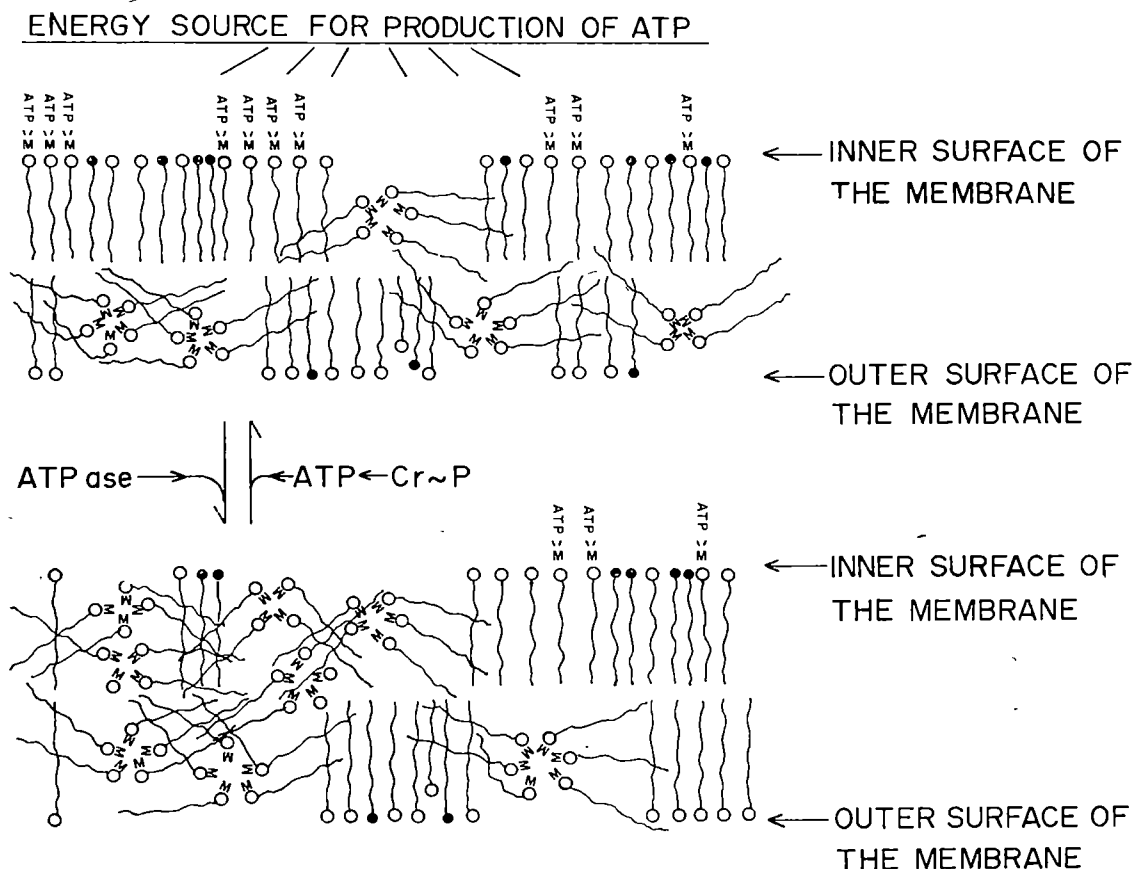


Fig. 4. Schematic representation of suggested cell membrane transitions as outlined in the text. (The letter *M* represents metals. Filled black circles indicate membrane components which form relatively unstable co-ordination complexes)

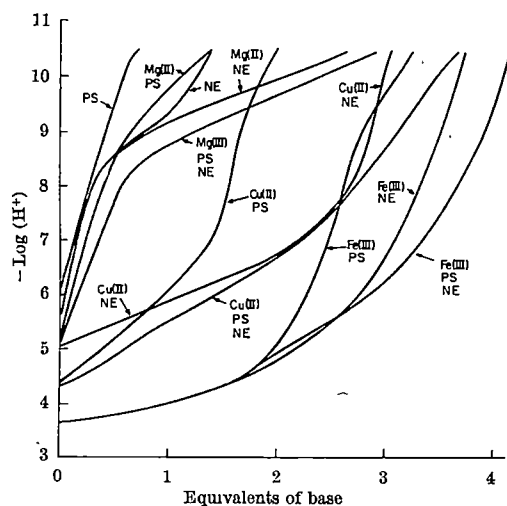


Fig 5. Potentiometric titration of phosphatidyl-1-serine in the presence of NE, Mg(II), Cu(II), and Fe(III) (equimolar, 0.01 mmoles in 100 ml. water: isopropanol: *n*-butanol, 2:3:2) with carbonate-free 0.1 N KOH

whereby a polar molecule such as NE moves across the potential energy barrier of the lipid membrane.

In Table 4 data on the effects of salt concentrations on metal concentrations in the ether phase and NE partition coefficients are presented. It is noted that there is a marked effect in general but that this is independent of the ion species, that is, sodium and potassium do not

cause differential effects. The changes noted are felt to be a function of the salts increasing the polarity of the aqueous phase with a consequent accentuation of solubility characteristics. This raises the possibility that in biological systems the solubility changes reported throughout this article would be accentuated.

In Table 5 it is noted that increasing concentrations of ATP progressively diminish the amount of metals found in ether as well as decreasing the partition coefficients of NE. Possible co-ordination complexes by which this might occur are several, but the important point is that the presence of ATP could serve as an important modulator of the amount of NE which is able to surmount the energy barrier represented by the membrane.

Finally, similar effects of lipid extracts of synaptosomes and upper phase extracts are presented in Table 6. It is noted again that the presence of the lipids increases both partition coefficients and metal content of the ether phase and that this is inhibited by ATP.

From these results the following hypothesis regarding synaptic membrane function and NE is suggested. In the resting membrane the presence of ATP increases the energy barrier to the movement of NE across the internal surface of the membrane. If ATP is removed, as by an ATPase, either (a) unfilled co-ordination positions on the metal-phospholipid become available to NE, or (b) the removal of ATP from a quaternary complex occurs and a ternary phospholipid-metal-NE complex remains. In either case, the electronic structure of this ternary complex is such that its solubility in the aqueous phase is decreased, whereas in the lipid phase (the cell membrane)

Table 3
Metal content is expressed as $\mu\text{M}/3$ c.c. ether \pm S.E.M. (No.=3 to 5; N.D. means not done)

Metal concentration		No phospholipid; no NE	5.00 μM phosphatidyl-1- serine + 6.25 μM NE	5.00 μM phosphatidyl inositol + 6.25 μM NE	6.25 μM NE
0	NE partition coefficient	—	0.0037 \pm 0.0003	0.007 \pm 0.0001	0.0103 \pm 0.0016
	Metal content	0	—	—	—
6.25 μM CuCl_2	NE partition coefficient	—	0.11 \pm 0.030	0.151 \pm 0.041	< 0.001
	Metal content	0	1.6 \pm 0.161	2.27 \pm 0.276	< 0.0009
6.25 μM MgCl_2	NE partition coefficient	—	0.035 \pm 0.004	N.D.	0.0127 \pm 0.0001
	Metal content	0	0.879 \pm 0.030	N.D.	0.0264 \pm 0.0072

Table 4

	NaCl = 0 KCl = 0		NaCl = 100 m molar KCl = 20 m molar		NaCl = 100 m molar KCl = 100 m molar		NaCl = 20 m molar KCl = 100 m molar	
	Partition coefficient (NE)	Mg ($\mu\text{M}/3$ ml.)	Partition coefficient (NE)	Mg ($\mu\text{M}/3$ ml.)	Partition coefficient (NE)	Mg ($\mu\text{M}/3$ ml.)	Partition coefficient (NE)	Mg ($\mu\text{M}/3$ ml.)
NE 6.25 μM (No. = 4)	0.0111 \pm 0.0017	0.014 \pm 0.022	0.0137 \pm 0.0024	0	0.0138 \pm 0.0024	0.012 \pm 0.03	0.014 \pm 0.0009	0.031 \pm 0.013
NE 6.25 μM MgCl_2 6.25 μM (No. = 4)	0.0159 \pm 0.0001	0.021 \pm 0.002	0.0135 \pm 0.0024	0.023 \pm 0.002	0.015 \pm 0.0002	0.021 \pm 0.003	0.0106 \pm 0.0003	0.023 \pm 0.003
NE 6.25 μM PS 5.0 μM (No. = 2)	0.0036 \pm 0.0007	0.033 \pm 0.011 \pm 0.0036	0.0098 \pm 0.001	0.021 \pm 0.025 \pm 0.0084	0.01 \pm 0.0007	0.009 \pm 0.005	0.0764 \pm 0.0071	0
NE 6.25 μM MgCl_2 6.25 μM PS 5.0 μM (No. = 6)	0.041 \pm 0.002	0.762 \pm 0.033 \pm 0.011	0.064 \pm 0.0033	0.906 \pm 0.063	0.0653 \pm 0.002	0.828 \pm 0.057	0.0596 \pm 0.0037	0.849 \pm 0.027

Table 5
Magnesium content is expressed as $\mu\text{M}/3$ ml. ether \pm S.E.M. The No. in all cases is 5

5.0 μM phosphatidyl-1-serine + 6.25 μM NE + 6.25 μM MgCl_2		ATP 0	ATP 1.25 μM	ATP 2.5 μM	ATP 5.0 μM	ATP 10.0 μM
	Partition coefficient NE	0.042 \pm 0.002	0.0351 \pm 0.0018	0.0251 \pm 0.0035	0.0107 \pm 0.002	0.0036 \pm 0.0005
	Magnesium content	0.639 \pm 0.051	0.537 \pm 0.018	0.411 \pm 0.09	0.156 \pm 0.036	0.021 \pm 0.0033

its solubility is increased. The net result is an increase in movement of NE across the cell membrane. The mechanism of movement of the NE molecule is not clear, that is, it might occur simply as an exchange reaction or be a function of the development of a water-in-oil pattern which sterically would place the NE molecule in the membrane pore as outlined in an earlier part of this article. Displacement of NE by ATP (from a metabolic source *in vivo* or an exogenous source *in vitro*) would bring about a resumption of the usual resting structure. Note that by this mechanism the membrane ATP will tend to prevent loss of NE from the vesicle and that a steady supply of ATP is necessary to prevent the vesicle from functionally deteriorating.

Table 6

		Lipid extract of synaptosomes + 6.25 μ M NE		Upper phase synaptosomes + 6.25 μ M NE
		Without ATP	6.25 μ M ATP	
6.25 μ M CuCl ₂	Partition coeffi- cient NE	0.046 \pm 0.017	0.003 \pm 0.001	All values < 0.001
	Copper content	0.320 \pm 0.113	0.0155 \pm 0.0027	0
No copper	Partition coeffi- cient NE	0.0027 \pm 0.0014	—	All values < 0.001

Metal content is expressed as μ M Cu/3 c.c. ether/mg extract (dry wt.) \pm S.E.M. with No. = 5 or 6. Approximately 1 mg (dry wt.) of lipid extract was used per tube; approximately 4 mg (dry wt.) of upper phase extract was used/tube.

The report²⁸ that cold NE can be taken up against a gradient by granules known to be rich in this amine can be satisfactorily explained by the presence of metals within the granules¹. In addition, reports^{29,30} suggest magnesium and ATP are necessary for uptake of NE or E by chromaffin granules. The great bulk of the data reported by these investigators involved rate curves for the appearance of radioisotopically labelled catecholamines within the granules. As such, any factor causing an increase in endogenous pool size would lead to a less rapid exit of the isotopically labelled molecule and hence higher c.p.m. As noted before¹, we feel that there is a true uptake, against a gradient, owing to the presence of metals within the granules, but that in addition the presence of magnesium and ATP diminishes the rate of

outflow of NE from the interior of the vesicle for reasons which follow from the suggested role of ATP and metal in membrane function and NE transport. The combination of true uptake against a gradient due to metal within the vesicle and diminished outflow due to magnesium-ATP then leads to greater amounts of radioactivity being found within the granule.

- ¹ Colburn, R. W., and Maas, J. W. (preceding article).
- ² Elmhberg, jun., J., Whittaker, V. P., and Dawson, R. M. C., *Biochem. J.*, **92**, 91 (1964).
- ³ Victor, V. P., *Biochem. J.*, **72**, 694 (1959).
- ⁴ Gray, E. G., and Whittaker, V. P., *J. Anat.*, **96**, 79 (1962).
- ⁵ Umbreit, W. W., Burris, R. H., and Stauffer, J. F., *Monometric Techniques* (Burgess, Minneapolis, Minn., 1957).
- ⁶ Anton, A. H., and Sayre, D. F., *J. Pharmacol. Exp. Therapeut.*, **138**, 360 (1962).
- ⁷ Folch, J., Lees, M., and Sloane-Stanley, G. H., *J. Biol. Chem.*, **226**, 497 (1957).
- ⁸ Cohn, M., and Hughes, T. R. F., *J. Biol. Chem.*, **237**, 176 (1962).
- ⁹ Nanniny, L. B., *J. Phys. Chem.*, **61**, 1144 (1958).
- ¹⁰ Boer, R. M., in *The Enzymes*, 2 (Academic Press, N.Y., 1960).
- ¹¹ Walaas, E., *Acta Chem. Scand.*, **12**, 528 (1958).
- ¹² Martell, A. E., and Schwarzenbach, G., *Helv. Chem. Acta*, **39**, 653 (1956).
- ¹³ Graddon, D. P., *An Introduction to Co-ordination Chemistry*, International Series of Monographs on Inorganic Chemistry, 90 (Pergamon Press, 1961).
- ¹⁴ Wolman, M., and Wiener, H., *Nature*, **200**, 486 (1963).
- ¹⁵ West, E. S., *Textbook of Biophysical Chemistry*, third ed. (Macmillan Co., N.Y., 1963).
- ¹⁶ Schulman, J. H., and Rosano, H. L., in *Retardation of Evaporation by Monolayers. Transport Processes* (Academic Press, N.Y. and London, 1962).
- ¹⁷ Wooley, D. W., and Campbell, N. E., *Biochem. Biophys. Acta*, **40**, 543 (1960).
- ¹⁸ Davson, H., and Danielli, J. F., *The Permeability of Natural Membranes*, 365 (Cambridge Univ. Press, Cambridge, 1952).
- ¹⁹ Danielli, J. F., *Symp. Sec. Exp. Biol.*, **6**, 1 (1952).
- ²⁰ Danielli, J. F., *Surface Chemistry and Cell Membranes in Surface Phenom. in Chem. and Biol.*, 246 (Pergamon Press, London, 1958).
- ²¹ Robertson, J. D., *J. Biophys. Biochem. Cytol.*, **4**, 349 (1958).
- ²² Robertson, J. D., *Prog. Biophys.*, **10**, 343 (1960).
- ²³ Luzzati, V., and Husson, F., *J. Cell Biol.*, **12**, 207 (1962).
- ²⁴ Sollner, K., *Svensk Kemisk Tidskrift*, 6-7 (1958).
- ²⁵ Koefoed-Johnson, V., and Ussing, H. H., *Acta Physiol. Scand.*, **42**, 298 (1958).
- ²⁶ Nyman, M., and Whittaker, V. P., *Biochem. J.*, **87**, 248 (1963).
- ²⁷ Skou, J. E., *Biochem. Biophys. Acta*, **42**, 6 (1960).
- ²⁸ Baker, P. F., Hodgkins, A. L., and Meves, H., *J. Physiol.*, **170**, 541 (1964).
- ²⁹ Carlsson, A., Hillarp, N. A., and Waldeck, B., *Acta Physiol. Scand.*, **59**, Supp. 215 (1963).
- ³⁰ Kirshner, N., *J. Biol. Chem.*, **237**, 7 (1962).

ACETOHYDROXAMATE: BACTERIAL UREASE INHIBITOR WITH THERAPEUTIC POTENTIAL IN HYPERAMMONAEMIC STATES

By DR. WILLIAM N. FISHBEIN and PAUL P. CARBONE

Medicine Branch, National Cancer Institute

AND

H. DONALD HOCHSTEIN

Clinical Pathology Department, Clinical Center, National Institutes of Health, Bethesda, Maryland

DURING investigations demonstrating that the anti-leukaemic agent, hydroxyurea, is both a substrate and an inhibitor of jackbean urease¹⁻³, acetohydroxamic acid (AHA) was used as an analogue of hydroxyurea. AHA was found to be an irreversible, non-competitive urease inhibitor with a rate constant of $10^3 \text{ M}^{-1} \text{ min}^{-1}$ at 25° C, that is, 1 mmole AHA produced complete urease inactivation in 3-4 min⁴. Since the compound has little toxicity for animals⁴ and has been shown to exert no inhibitory effects on a variety of other enzyme systems⁵, it was examined for possible clinical application in treating hyperammonaemic states by reducing the contribution to blood ammonia by intestinal urease activity. The need for such an agent has been expressed elsewhere^{6,7}. The work described here establishes the inhibitory effect

of AHA on bacterial urease *in vitro* and *in vivo*, and reports the effect of the compound in a single patient with liver disease.

Following preparation and purification of AHA⁸, a preliminary assessment of excretion and acute toxicity was made. Intraperitoneal administration of a single dose of 900 mg/kg to each of three adult rats resulted in no gross clinical disturbances over a one-month observation period, and the full dose was recovered (98, 100 and 110 per cent) in the urine excreted during the first 24 h, as determined by ferric chloride assay². Although this assay method is not definitive (it would not, for example, discriminate AHA from other hydroxamic acids that might occur as biological derivatives), the results suggest that AHA is rapidly and, in contrast

hydroxyurea⁸, completely excreted. Five mmoles HA (375 µg/ml.) in trypticase soy broth produced no growth inhibition in tetracycline-sensitive strains (0.2–5 µg/ml.) of *Staphylococcus aureus*, β-haemolytic *Streptococcus* Group A, *Escherichia coli*, *Klebsiella* sp., *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Brucella* sp., and *ordetella* sp. Unlike hydroxyurea, which regularly inhibits the growth of *Pseudomonas* organisms⁹, AHA appears to be devoid of antibacterial activity despite a very similar chemical structure.

Urease inhibition by AHA was evaluated for the same organisms (excepting the *Streptococcus* and *E. coli*) in standard bacteriological media¹⁰, which contain a highly buffered solution of urea at a concentration approximating that of urine. Gradation of the colour change of the phenol red indicator produced by the alkaline ammonium carbonate liberated from urea serves as a convenient index of the degree of urea decomposition. In all six species, AHA, at concentrations of 1 mmole or less, significantly inhibited the ureolytic activity. Fig. 1 illustrates the data for *Klebsiella* sp. and *Proteus mirabilis*.

In one experiment a duplicate set of cultures was used, omitting the indicator; aliquots were acidified, Seitz-filtered, diluted and analysed directly for urea¹¹. The inhibition is more impressively demonstrated by this method, and the correlation of urea decline with indicator changes is evident (Fig. 1). Since saturating levels of urea are present in these experiments, the non-competitive nature of AHA inhibition is further substantiated, and suggests that the action of the drug would not be compromised by even the high urea concentrations of urine. The ultimate appearance of ureolytic activity in the media containing AHA may simply reflect the unchecked bacterial growth.

AHA inhibition of bacterial urease activity *in vivo* was assessed in adult rats by the use of ¹⁴C-urea¹². Twenty-five µc. of the isotope was injected subcutaneously immediately after intraperitoneal injection of AHA. The animal was placed at once in a metabolic chamber¹³ and

Table 1. PERCENTAGE OF ¹⁴C-UREA EXPIRED AT ¹⁴CO₂ IN RATS GIVEN A 25-µc. DOSE SUBCUTANEOUSLY IMMEDIATELY AFTER INTRAPERITONEAL ADMINISTRATION OF AHA

The figures in parentheses denote percentage inhibition during the individual collection periods relative to the control values

Expt. sequence	Rat wt. (g)	AHA (mg/kg IP)	Cumulative % administered ¹⁴ C-urea expired as ¹⁴ CO ₂ (h)			
			1	2	3	4
Controls:						
4	295	0	2.36	6.7	10.3	12.6
6	230	Normal saline		6.6		
1	240	0			9.0	
Treated:						
2	260	100			4.7 (51)	
5	307	50	0.53 (69.5)	1.9 (72.5)	3.2 (63)	4.2 (58)
3	220	20			2.7 (72)	
7	280	5		4.26 (36)		
8	260	10		3.6 (45)		
		Tetra-cycline				

the expired carbon dioxide was collected in 4 N NaOH, aliquots of which were counted directly in a liquid scintillation spectrometer by the two-phase anthracene method of Steinberg¹⁴. The radioactivity of expired carbon dioxide amounted to about 3 per cent of the administered dose of urea per h in the control animals (Table 1); it was reproducible, and was consistent with the values obtained by others in mice^{15–17}. AHA, at doses of 20–50 mg/kg, resulted in a 70 per cent inhibition of urea decomposition during the first 3 h. In the experiment involving hourly collections, the inhibition seems to begin to decline in the third and fourth hours. The lesser degree of inhibition occurring when a large dose of tetracycline is substituted for AHA may be reasonably presumed to be due to the different modes of action of the two drugs. AHA directly inhibits urease without any demonstrable concomitant antibacterial activity (which, incidentally, suggests that under the particular conditions involved, urease is functioning as a 'scavenger' enzyme rather than in any essential metabolic sequence). On the other hand, the antibiotics, although capable of completely suppressing *in vivo* urease activity, do so not by any direct action on the enzyme, but by suppressing bacterial growth^{17,18}. The short time-interval used in these experiments, therefore, does not permit the indirect action of tetracycline to reach maximum effect. Interestingly, complete suppression of ureolysis by AHA was not attained even at high dose levels. While the reason for this is not clear, it may possibly represent persistent gastric urease activity owing to failure of the enzyme-inhibitor complex to form under acid conditions⁵.

Although urine collections over these brief periods are subject to considerable error, urinary radioactivity in these experiments approximated 13 per cent of the injected dose per hour. The fraction of acid-labile radioactivity was within the error of determination for both the ¹⁴C-urea reagent (1 per cent) and all urine specimens (5 per cent).

While AHA seems clearly to be an effective inhibitor of bacterial urease, a more critical question concerns the feasibility of using this agent as a drug to lower elevated blood ammonia-levels. Since the compound is not generally available, and chronic toxicity examinations will probably consume large quantities of it, a preliminary trial of the compound was made in a single patient to assist in deciding whether a full-scale investigation should be undertaken¹⁹. The patient, a 60-year-old male with chronic myelogenous leukaemia and leukaemic infiltration of the liver, had received an end-to-side portocaval shunt nine months before to relieve jaundice and hepatic coma consequent to portal vein thrombosis. At the time of this investigation, his haematological status was stable, despite elevated platelet and white cell counts; his liver functions were entirely normal save for elevated blood ammonia-levels. The patient was maintained at bed-rest

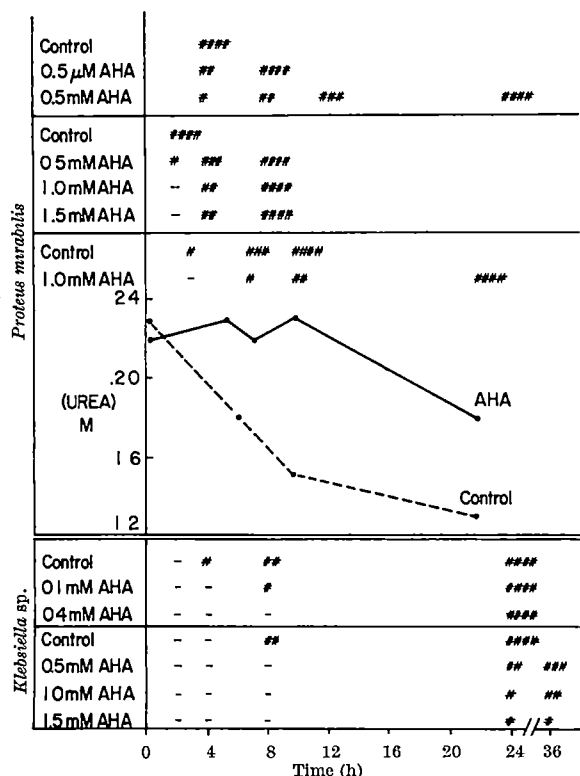


Fig. 1. Inhibition of bacterial ureolytic activity by AHA in urea broth cultures. The colour changes of phenol red indicated produced by alkaline ammonium carbonate liberated from urea are graded 1 to 4+. Direct measurement of urea was also performed. See text for details

and fasting until 2 p.m. each examination day and received the drug orally in capsule form, with half of each dose being enteric-coated. Antecubital vein blood was withdrawn without stasis via an indwelling Cournand needle, and blood ammonia determinations were performed immediately, in duplicate, by the method of Seligson and Hirahara²⁰. The results are shown in Fig. 2; the decline and return of blood ammonia-level following the larger dose of AHA, while certainly suggestive, are not definitive because of the wide physiological and/or measurement fluctuation apparent during the control day. It would seem unlikely that the absence of a striking decline in ammonia-level is due to inactivity of oral AHA in man, since the drug, in this instance, is acting, not on host enzymes, but on those of bacteria in the alimentary canal. It seems more reasonable to presume that, at least in this particular case, bacterial urease is not playing a decisive role in maintaining elevated blood ammonia-levels. While it is clear that urea nitrogen can be used in anabolic processes^{21,22} and that the administration of urease and/or urea can produce ammonia intoxication^{7,23,24}, the fraction of total blood ammonia-levels in man attributable to urease, as compared to other bacterial and host ammonia-generating enzyme systems, has never been evaluated. A combination investigation using ¹⁴C-urea, a specific urease inhibitor such as AHA, and antibiotics might provide an answer to this question. The limiting factor in such an approach, at present, is the variability in blood ammonia determinations²⁵. In the patient examined, for example, if the maximum decline in blood ammonia on day five was taken literally as due to AHA, then, assuming 70 per cent urease inhibition at this dose, the fraction of the stable blood ammonia contributed by this 'exogenous' enzyme would be an impressive 35 per cent. Yet this degree of decline in blood ammonia is still not a conclusive change.

No symptoms attended the administration of AHA to this patient, and no effects on the haemogram or hepatorenal function tests were observed during a five-day hospital observation period. Since the toxic effects of chemically related compounds occur and subside quickly in relation to drug administration and withdrawal^{26,27}, more extensive trials of AHA in man would appear practicable.

The present data indicate that further investigation of AHA to define its clinical potential is warranted. Since urease inhibition is a general feature of hydroxamic acids⁵, it is, of course, possible that another of these compounds may ultimately be found to be more suitable. AHA was selected by us because of information available regarding its specificity and low toxicity. Like AHA, hydroxyurea is also an irreversible urease inhibitor, but, unlike AHA, a residual fraction of enzyme activity does persist in its presence³. Hydroxyurea is decomposed by urease with

the liberation of ammonia, while AHA is inactive as substrate^{1,2}. Hydroxyurea produces extensive chromosomal breakage in cultures of hamster and mouse embryo cells²⁸ and of normal human leucocytes²⁹, while AHA is inactive in this regard²⁹. The problem of megaloblastosis attending hydroxyurea treatment^{30,31} may yet turn up, on extensive toxicity investigations with AHA, but on the basis of available knowledge, the latter compound would seem to offer a definite superiority as a urease inhibitor in man.

AHA appears to possess most of the characteristic desirable in an ideal anti-urease drug. (a) Its inhibition is specific, complete, and irreversible and occurs via complex formation rather than by oxidative phenomena^{3,4}. (b) It can be expected to be free of allergic danger attending the use of urease antibodies⁶. (c) Its action would complement, rather than mimic, that of antibiotics. In addition to the most evident applicable clinical situation, hepatic failure, it is possible that hyperammonaemia may play a part in the acute morbidity of certain septic caemias, consequent to the urease activity present in many bacterial pathogens³² in combination with hepatic anoxaemia. If so, a urease inhibitor might be of value in the initial critical phase of treatment, until antibiotic control of bacterial growth has been attained.

- ¹ Fishbein, W. N., and Winter, T. S., *Fed. Proc.*, **23**, 385 (1964).
- ² Fishbein, W. N., Winter, T. S., and Davidson, J. D., *J. Biol. Chem.* (in the press).
- ³ Fishbein, W. N., and Carbone, P. P., *J. Biol. Chem.* (in the press).
- ⁴ Unpublished data from several laboratories suggest that the toxicity of AHA for animals is several times lower than that of hydroxyurea, which itself has an *LD₅₀* for mice of 7.5 g/kg.
- ⁵ Kobashi, K., Hase, J., and Uehara, K., *Biochim. Biophys. Acta*, **65**, 380 (1962).
- ⁶ Thomson, A., and Visek, W. J., *Amer. J. Med.*, **35**, 804 (1963).
- ⁷ Haefely, W., Hürlimann, A., and Staub, H., *Med. Klin. (Munich)*, **57**, 696 (1962).
- ⁸ Davidson, J. D., and Winter, T. S., *Cancer Chemother. Rep.*, **27**, 97 (1963).
- ⁹ Fishbein, W. N., and Carbone, P. P. (unpublished data).
- ¹⁰ Gale, G. R., Kendall, S. M., McLain, H. H., and DuBois, S., *Cancer Res.*, **24**, 1012 (1964).
- ¹¹ Rustigian, R., and Stuart, C. A., *Proc. Soc. Exp. Biol. and Med.*, **47**, 108 (1941).
- ¹² Christensen, W. B., *J. Bact.*, **52**, 461 (1946). For *Proteus mirabilis* the broth preparation of Rustigian and Stuart was used directly for the other, less active, urease-producing bacteria, the less highly buffered preparations of Christensen was used, omitting the agar.
- ¹³ Fearon, W. R., *Biochem. J.*, **33**, 902 (1939).
- ¹⁴ 0.5 mc. crystalline ¹⁴C-urea, 7.17 µc/µmole, sealed under nitrogen in a glass ampoule, was obtained from Nuclear-Chicago Corporation and diluted in sterile 0.9 per cent saline at the time of use.
- ¹⁵ Skipper, H. E., White, jun., L., Bryan, C. E., and Hutchinson, O. S., *J. Biol. Chem.*, **173**, 371 (1948). 0.1 ml. benzyl alcohol was added to the urine chamber as a preservative.
- ¹⁶ Steinberg, D., *Analyt. Biochem.*, **1**, 23 (1960). Vials containing 3 ml. solution and 0.1 ml. 10 per cent 'Triton RG-5' per g of anthracene counted with an absolute efficiency of 10 per cent in the Packard 'Tri-Carb' liquid scintillation spectrometer. All samples exceeded a counting rate of 3,000/min and duplicates always checked within 4 per cent.
- ¹⁷ Leifer, E., Roth, L. J., and Hempelman, L. H., *Science*, **108**, 748 (1948). Obtained 14 per cent in the first 3 h.
- ¹⁸ Hastings, A. B., Langham, W. H., Carter, R. E., and Roth, L. J., *Nuclear Science Abstr.*, **4**, 806 (1950). Obtained 10 per cent per hour.
- ¹⁹ Dintzis, R. Z., and Hastings, A. B., *Proc. U.S. Nat. Acad. Sci.*, **39**, 571 (1953). Obtained 4 per cent in the first hour and 8 per cent in 3 h.
- ²⁰ Kornberg, H. L., Davies, R. E., and Wood, D. R., *Biochem. J.*, **56**, 355; 363 (1954).
- ²¹ Approval for this study was obtained from the Food and Drug Administration as project IND 1905.
- ²² Seligson, D., and Hirahara, K., *J. Lab. Clin. Med.*, **49**, 962 (1957). We are indebted to Dr. E. C. Cottlove, Chief, Clinical Chemistry Service, Clinical Pathology Department, Clinical Center, National Institutes of Health, for these determinations.
- ²³ Rose, W. C., and Dekker, E. E., *J. Biol. Chem.*, **223**, 107 (1956).
- ²⁴ Giordano, C., *J. Lab. Clin. Med.*, **62**, 231 (1963).
- ²⁵ Tauber, H., and Kleiner, I. S., *J. Biol. Chem.*, **92**, 177 (1931).
- ²⁶ Barnett, G. D., and Addis, T., *J. Biol. Chem.*, **30**, 41 (1917).
- ²⁷ Phillips, G. B., Schwartz, R., Gabuzda, jun., G. J., and Davidson, C. S., *New Eng. J. Med.*, **247**, 239 (1952).
- ²⁸ Moore, E. W., Strohmeier, G. W., and Chalmers, T. C., *Amer. J. Med.*, **35**, 350 (1963).
- ²⁹ Fishbein, W. N., Carbone, P. P., Freireich, E. J., Misra, D., and Frei, E., *III, Clin. Pharmacol. Ther.*, **5**, 574 (1964).
- ³⁰ White, F. R., *Cancer Chemother. Rep.*, **23**, 81 (1962).
- ³¹ Borenfreund, E., Krim, M., and Bendich, A., *J. Nat. Cancer Inst.*, **32**, 667 (1964).
- ³² Oppenheim, J., and Fishbein, W. N. (in preparation).
- ³³ Thurman, W. G., Bloedow, G., Howe, C. D., Levin, W. C., Davis, P., Lane, M., Sullivan, M. P., and Griffith, K. M., *Cancer Chemother. Rep.*, **29**, 103 (1963).
- ³⁴ Rosenthal, F., Wislicki, L., and Kollek, L., *Klin. Wschr.*, **7**, 972 (1928).
- ³⁵ Seneca, H., Latimer, J. K., and Peer, P., *Arch. Path.*, **74**, 19 (1962).

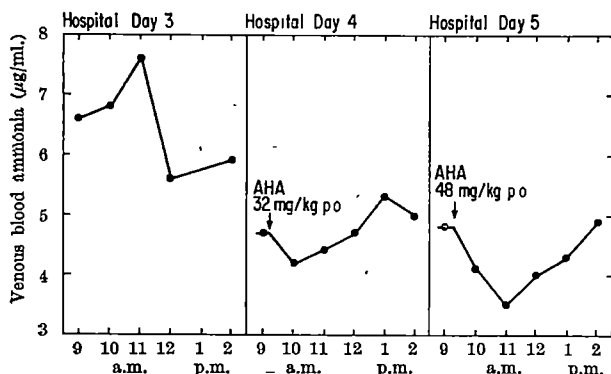


Fig. 2. Venous blood ammonia-levels in a human patient during a control day and following two oral (dose) trials of AHA. The patient was maintained fasting and at bed-rest during each study period. Normal venous values with this method are 0.7-2.0 µg NH₃/ml. blood

RAPIDLY LABELLED SYNTHESIS OF RIBONUCLEIC ACID IN EHRlich ASCITES C-2 TUMOUR CELLS TREATED WITH ACTINOMYCIN D

By DUŠAN A. POPOVIĆ

Institute for Medical Research, Novi Sad, Yugoslavia

ACTINOMYCIN D markedly inhibits DNA-dependent RNA polymerase, and inhibits DNA-primed RNA synthesis *in vitro* and *in vivo*^{1,2}. This biological activity is correlated with its ability to form complexes with DNA-guanin residues³. There are more types of DNA-actinomycin D binding sites *in vitro*⁴, and the synthesis of ribosomal and s-RNA *in vivo* is more sensitive⁵ than the synthesis of DNA-like RNA⁶. A rapid turnover of messenger RNA⁷ was originally thought to be a characteristic of this class of material, and may be defined, so far as is known at present, as a sequence-determining template in ribosomes for catalytic function in protein synthesis⁸. But in mammalian cells it is not clear how the transfer messenger RNA of the labelled nuclear RNA is related to the stable cytoplasmic RNA⁹. When cells were incubated for 10 min with ¹⁴C-adenine, then transferred to a non-radioactive medium containing actinomycin D, the pulse-labelled nuclear RNA underwent breakdown to acid-soluble end-products in the nucleus, no transfer of the pulse-labelled RNA from nucleus to cytoplasm could be detected¹⁰⁻¹³. On the other hand, when the cells were exposed to ¹⁴C-adenine for 20 min and the radioactive precursor remained in the medium, after adding actinomycin D, experiments demonstrated the transfer of the labelled nuclear RNA to the cytoplasm¹⁴. The radioactivity may continue to be incorporated into both nuclear and cytoplasmic RNA at the highest rate 3-4 h after addition of the actinomycin D (ref. 14). In our work we found the same difference between 'normal' pulse-labelled RNA and the 'second' pulse-labelled RNA when the cells were treated for 4-5 h with actinomycin D. In contrast to the 'normal' pulse-labelled, the 'second' pulse-labelled RNA passed quickly into the cytoplasmic compartment, and also exhibited other differences.

Ehrlich ascites mouse tumour cells C-2 were grown as described previously¹⁵. A suspension of cells (500 mg dry weight) in Oerle medium (95 per cent O₂-5 per cent CO₂ at 37° C) were exposed for 10 min to uracil 2-¹⁴C, 1 μC./ml. After labelling, the cells were washed with physiological saline and re-incubated in non-radioactive Oerle medium (5 μg/ml.) actinomycin D. Cells were taken after 15 min, and at intervals thereafter for 5 h. The respective fractions were denatured by acidification to 0.5 N PCA, washed once more with 0.4 N PCA, and neutralized with 1 N KOH using phenol red as an internal indicator. RNA was isolated with 10 per cent NaCl and subsequent ethanolic precipitation¹⁷. Following alkaline digestion with 0.4 N KOH for 17 h at 37° C the resulting ribonucleotides were separated by column procedure on 'Dowex-1' HCOO⁻ x-10 200-400 mesh¹⁸. The specific activities of RNA pyrimidines were determined in peak portions (0.1-0.2 μM) in a Nuclear Chicago assembly operating with a thin-window counter. The specific activities were expressed as c.p.m./μmole, based on the extinction coefficients given by Cohn¹⁹. The total error of the measurements by the parallel samples never exceeds ± 5 per cent.

During the 5 h more than 90 per cent of the radioactive pyrimidines is released from the total RNA to the acid-soluble end-products¹⁰⁻¹³. Residual radioactivity seems to come from heterogeneous RNA in a stable form. In this system we have studied various nucleic acids after 'normal' pulse-labelling and 'second' pulse-labelling 4-5 h later. Aliquots of cells were enucleated by controlled stirring in a solution of 'Tween 80' (ref. 20) and the nuclear nucleic acids were separated with 1 M NaCl (ref. 21). These cytoplasmic fractions, 1 M NaCl nuclear

extractable, and 1 M NaCl residual nuclear RNA, were treated for the specific activity of pyrimidines as described previously.

After the first pulse-labelling the earliest labelled RNA is extractable from nuclear RNAs with 1 M NaCl. In the

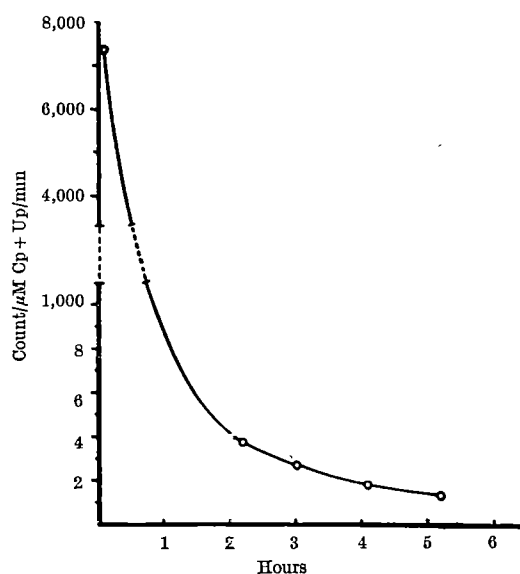


Fig. 1. Ehrlich ascites tumour cells C-2 were exposed to ¹⁴C-uracil for 10 min. They were then washed and transferred at zero time to non-radioactive medium containing actinomycin D at a concentration of 5 μg/ml. Specific activities of total RNA pyrimidines (Cp + Up) were determined for 6 h.

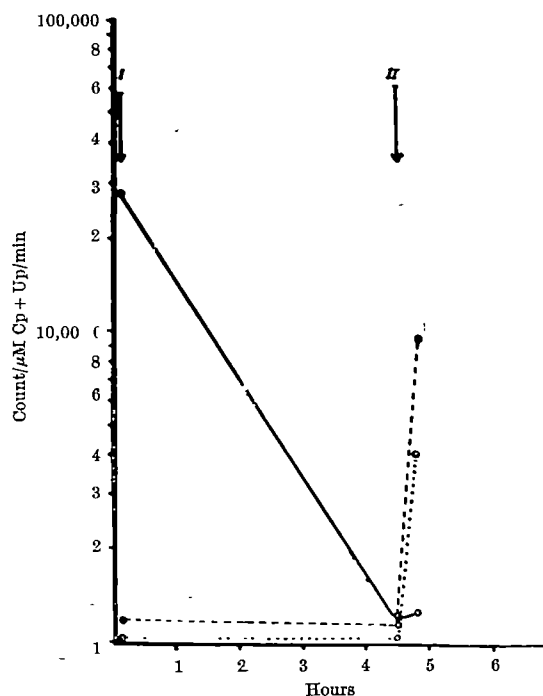


Fig. 2. Ehrlich ascites tumour cells C-2 were exposed to ¹⁴C-uracil for 10 min. They were then washed and transferred at zero time to non-radioactive medium containing actinomycin D at a concentration of 5 μg/ml. for 4-5 h. The cells were washed and re-incubated for 10 min in ¹⁴C-uracil. Specific activities of Cp + Up were determined for . . . cytoplasmic RNA, — nuclear 1 M NaCl extractable RNA, - - - nuclear 1 M NaCl non-extractable RNA.

nuclear residual and cytoplasmic RNAs no radioactivity was determined. After the second pulse-labelling (DNA primed and dependent RNA synthesis was abolished with actinomycin D and 'normal' pulse-labelled RNA was degraded to an acid-soluble end-product), both the residual nuclear and cytoplasmic RNA were labelled. No radioactivity was found in the nuclear 1 M NaCl-extractable RNA.

Our results suggest the possible role of the 'normal' pulse-labelled RNA on the other biosynthetic routes. There is some difference between 'normal' pulse-labelled and 'second' pulse-labelled RNAs: 'normal' 10-min-labelled RNA is nuclear 1 M NaCl-extractable; 'second' 10-min-labelled RNA is not, and a possible interpretation is that it more quickly passed into the cytoplasmic compartment.

The 'second' pulse-labelled RNA seems to be of chromosomal origin; the nuclei treated with 'Tween 80' have no nucleoli²². These results point to an RNA-dependent biosynthetic route in Ehrlich ascites cells²³.

The present investigations demonstrate that there are more 'pulse'-labelled RNAs and demonstrate the transfer of messenger RNA from the nucleus to the cytoplasm.

I thank Prof. H. Harris for his advice, and Merc Sharpe and Dohme, Inc., for the gift of actinomycin D.

- ¹ Goldberg, I. H., and Rabinowitz, M., *Science*, **136**, 315 (1962).
- ² Hurwitz, J., *J. Biol. Chem.*, **234**, 2351 (1959).
- ³ Hamilton, L. D., Fuller, W., and Reich, E., *Nature*, **198**, 538 (1963).
- ⁴ Hamilton, L. D., Fuller, W., Reich, E., *Nature*, **198**, 538 (1963).
- ⁵ Cavallieri, L. F., and Nemchin, R. G., *Biochim. Biophys. Acta*, **87**, 641 (1964).
- ⁶ Franklin, R. M., *Biochim. Biophys. Acta*, **72**, 555 (1963).
- ⁷ Harel, L., Harel, J., Boer, A., Imbenotte, J., and Capreni, N., *Biochim. Biophys. Acta*, **87**, 212 (1964).
- ⁸ Jacob, F., and Monod, J., *J. Mol. Biol.*, **3**, 318 (1961).
- ⁹ Lipmann, F., in *Progress in Nucleic Acid Research*, edit by Davidso J. N., and Cohn, W. E., 135 (Academic Press, 1963).
- ¹⁰ Harris, H., *Nature*, **202**, 249 (1964).
- ¹¹ Harris, H., *Nature*, **198**, 184 (1963).
- ¹² Levy, H. B., *Proc. Soc. Exp. Biol. and Med.*, **113**, 886 (1963).
- ¹³ Paul, J., and Struters, M. G., *Biochim. Biophys. Res. Comm.*, **11**, 135 (1963).
- ¹⁴ Liebermann, I., Abrams, R., and Ove, P., *J. Biol. Chem.*, **238**, 2141 (1964).
- ¹⁵ Girard, M., Penman, S., and Darnell, J. E., *Proc. U.S. Nat. Acad. Sci.*, **5**, 205 (1964).
- ¹⁶ Popović, D. (in preparation).
- ¹⁷ Harris, H., *Nature*, **202**, 1301 (1964).
- ¹⁸ Scholtisek, C., *Biochim. Ztsch.*, **332**, 458 (1960).
- ¹⁹ Petrovic, S., and Jankovic, V., *Bull. Inst. "Boris Kidric"*, **1**, 3282 (1962).
- ²⁰ Cohn, W. E., in *Methods in Enzymology*, V. III, 224 (Academic Press, 1957).
- ²¹ Fisher, H. W., and Harris, H., *Proc. Roy. Soc., B*, **156**, 521 (1962).
- ²² Wang, T., *Biochim. Biophys. Acta*, **68**, 52 (1963).
- ²³ La Cour, L. F., *Exp. Cell Res.*, **34**, 239 (1964).
- ²⁴ Burdon, R. H., and Smellie, R. M., *Biochim. Biophys. Acta*, **61**, 633 (1962).

MICROMETHOD FOR MEASURING MICROBIAL SENSITIVITY TO DRUGS

By DR. WALTER KOCH and DR. DEBORAH KAPLAN

Department of Microbiology and Public Health, Chicago Medical School, Chicago, Illinois

THREE methods are in use for testing sensitivity to drugs: (a) the accurate but laborious tube dilution method; (b) a method in which drugs are incorporated into ditch plates; (c) a method in which disks are impregnated with drugs. The last method, for its simplicity, is commonly used in clinical laboratories. In this method, the disk is placed on the surface of a sown, solid medium. The drug diffuses, and the presence of a zone, in which growth is inhibited, and the diameter of this zone, are considered to be indicative of the sensitivity of the organism towards the drug. However, it is difficult to relate the diameter of such a zone to results obtained in tube dilution tests. The diameter of a zone also represents a measure of the diffusibility of the drug. Penicillin, for example, has a much higher diffusibility than polymyxin, for which much higher disk contents are used¹. Another shortcoming of the disk method is that no estimate of combined activity can be given. The zone reading indicates inhibition of organisms by the drug which diffuses through agar most rapidly².

In order to obtain a faster and more uniform diffusion, impregnated paper disks were placed at the bottom of 10-mm × 75-mm test tubes, and 0.5-ml. Baltimore Biological Laboratory soy agar was poured on them. Hereby the path which the drug had to travel was kept extremely short. After 1 h the agar was inoculated with 0.05 ml. of an 18-h-old culture of *Staphylococcus aureus*, type 81, standardized to contain 10⁶ organisms/ml. (ref. 3). The results, however, were not consistent. When pouring the agar over the disk, a slightly raised agar rim forms. This rim apparently contains less drug than the plane surface of the agar. Colonies often developed on the rim, while growth was absent from the plane part of the agar.

Further experiments were carried out with streptomycin disks, containing 2, 5 and 10 µg, cut into halves and quarters. It was found that the degree of elution depends on the size of the exposed area. For example, one half of a 2 µg disk did not inhibit growth, while another half, cut into two quarters, did inhibit growth; one-half of a 5 µg disk did not inhibit growth, while growth was inhibited by a whole 2 µg disk.

We then went over to flat-bottomed, cylindrical 'Lucite' cups, which could stand in a closed Petri dish. Their

inner height was 8 mm, and their inner diameter 7 mm. Drugs were dissolved in distilled water, and the same volume of different drug concentrations dispensed into these cups. The cups were then dried *in vacuo* over 'Drierite', and stored in the refrigerator. Into the dried cups 0.3 ml. agar was dispensed and, after 1 h, they were inoculated.

Parallel experiments were carried out in tubes containing 10 ml. broth. Agreement was good for streptomycin. Whenever in the 10-ml. broth tubes growth was slight, in agar cups, at similar concentrations of streptomycin only a few colonies appeared on the surface.

Unexplained inconsistencies occurred with penicillin. We suspected a loss of penicillin while drying. In order to avoid this loss, different substances were added to penicillin before drying. The following substances proved not to interfere with penicillin activity: glycerol, egg albumen, and nutrient broth. Despite these additions, the differences in apparent penicillin resistance between reconstituted cups and 10-ml. broth tubes remained.

At this time it was found that, in tube dilution tests, using 10 ml. total volume of broth, inoculum size influences the results in penicillin-resistant strains⁴. In order to test whether size of inoculum is the only cause of the discrepancies, the dried penicillin was reconstituted not in agar but in 0.2 ml. nutrient broth, which already contained the inoculum. It was confirmed that inoculum size also influences the results in 0.2 ml. total volume of broth. However, results were not the same when tests were carried out in different broth volumes with the same size of inoculum.

Tests were carried out in which inoculum size (10³, 10⁴, 10⁵ and 10⁶) as well as total broth volume (0.2, 1, 2.5, 5 and 10 ml.) were varied. The following resistant strains were tested for penicillin sensitivity: *Staphylococcus*

Table 1. MEAN INHIBITION DOSES OF PENICILLIN PER ML. FOR DIFFERENT INOCULUM SIZES AND FOR DIFFERENT TOTAL VOLUMES OF BROTH

Total volume of broth (ml.)	Strain 81			
	10 ³	Total inoculum 10 ⁴	10 ⁵	10 ⁶
0.2	1.13	3.75	100	1,250
1.0	1.33	2.17	68	1,166
2.5	1.90	3.07	21	300
5.0	1.70	2.20	9	110
10.0	1.20	1.37	4	50

Table 2. THE THREE RECOMMENDED DOSES PER ML. FOR PENICILLIN (A), (B), (C), STREPTOMYCIN (E), (F), (G), AND COMBINATIONS THEREOF (J), (K), (L) Inhibiting dose of penicillin (D) and streptomycin (H). Total inoculum: 10^8 . Total volume of broth: 0.2 ml. Plus sign indicates growth; minus sign absence of growth. Penicillin in units, streptomycin in μ g

Strain	Penicillin				(E) 2	Streptomycin		(H)	Penicillin + streptomycin		
	(A) 1	(B) 3.16	(C) 10	(D)		(F) 6.32	(G) 20		(J) 1+2	(K) 3.16+6.32	(L) 10+20
80	+	+	+	113	+	+	+	913	+	+	+
81	+	+	+	108	+	+	+	8.1	+	+	+
MG	+	+	+	47	+	+	+	175	+	+	+
45162	+	+	+	100	+	+	+	750	+	+	+
46422	+	+	+	23	+	+	+	6,125	+	+	+
65126	+	+	+	100	+	+	+	542	+	+	+

aureus 80, 81, MG: and three 'Oxford' strains: 45162, 46422 and 65126. As all these strains gave similar results, 81 is chosen to represent the group. Results are summarized in Table 1; figures listed are averages of at least five experiments.

From Table 1 it can be seen that the volume of broth does not play a relevant part with small (10^3 and 10^4) inocula; with larger (10^5 and 10^6) inocula, results become dependent on total volume of broth. By choosing an inoculum of 10^5 micro-organisms for a total volume of 0.2-ml. broth, the results, in all instances tested, are rendered comparable to those obtained in 10-ml. broth in the tube dilution method.

In contrast to the foregoing, penicillin-sensitive strains tested (*Staphylococcus epidermidis*, *aureus*, 52A, 79, and three 'Oxford' strains: 46407, 46426 and 46479) show little dependence on broth volume.

The single 'Lucite' cups were now replaced by a plastic slab, which fits into a Petri dish. Twenty-five cavities were drilled into the slab. Eight radii of three cavities each radiate from a central cavity, which serves as control (Figs. 1a and b).

Into these cavities 0.05-ml. drug solutions were dried *in vacuo* over 'Drierite'. Different drugs, in different concentrations, singly or in combination, with or without glycerol, were dried into the cavities. Glycerol, in a concentration of 0.5 per cent of the fluid to be dried, facilitates reconstitution, and imparts to the dried drugs in the cavities a glossy sheen.

The dried slabs were tested immediately after drying, and after varying storage times; after two months' storage the same results were obtained.

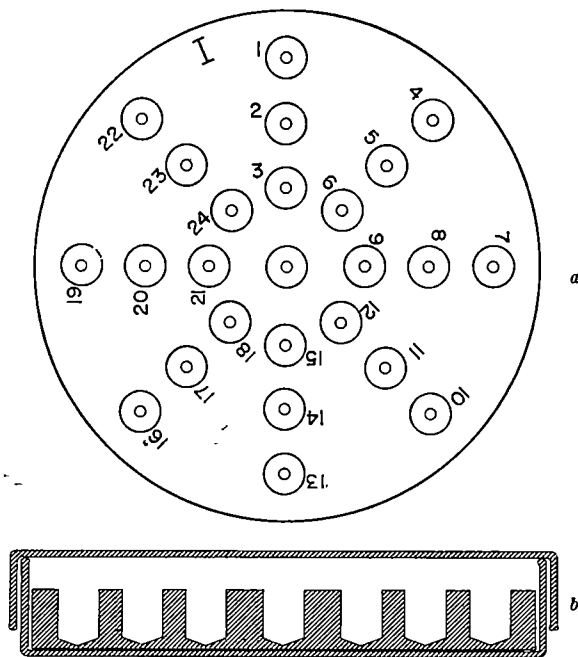


Fig. 1. a, Plan of slab. Twenty-four cavities are arranged in 8 radii. Each radius is formed by three cavities. The cavity in the centre accommodates the growth control. The small circles within each cavity are filled in, where growth occurs. b, Slab in Petri dish, cut through cavity 7 to cavity 19. Growth settles in the tip of the cavity

Slabs with dried drugs were subjected to shaking for 2 h in an inverted position; no loss in potency was observed.

For practical purposes, only three concentrations of a drug, or combinations of drugs, are needed to arrive at a valid estimate. The three concentrations are accommodated in the three cavities of a radius. One cavity contains the maximum attainable concentration of the drug in the blood; penicillin, for example, 10 units/ml. The third cavity contains one-tenth of this concentration; penicillin, for example, 1 unit/ml. The intermediate cavity of the radius contains the geometric mean, namely: $\sqrt{10 \times 1} = 3.16$ units/ml.

An organism which grows in the presence of the highest concentration of the drug is considered resistant. An organism which grows in the presence of the intermediate concentration is considered moderately sensitive. An organism which grows in the presence of the lowest concentration only is considered sensitive. If no growth occurs at any concentration the organism is considered highly sensitive.

For combinations of drugs also, the highest concentrations are the maximum levels attainable in blood; for example, penicillin 10 units/ml. plus streptomycin 20 μ g/ml. The lowest concentrations of drugs are one-tenth of the highest; penicillin 1 unit/ml. plus streptomycin 2 μ g/ml. The intermediate concentrations are the geometric means: penicillin 3.16 units/ml. plus streptomycin 6.32 μ g/ml. The interpretation of results is the same as for single drugs.

Table 2 illustrates results obtained from several strains.

From Table 2 it can be seen that *Staphylococcus* type 81 is penicillin-resistant (inhibitive dose 108 units/ml.), and moderately sensitive to streptomycin (inhibitive dose 8.1 μ g/ml.). This organism, however, is sensitive to a combination of penicillin and streptomycin (inhibitive doses: penicillin 2 units/ml. plus streptomycin 4 μ g/ml.). *Staphylococcus* 46422 is penicillin-resistant (inhibitive dose 22.5 units/ml.), streptomycin highly resistant (6,125 μ g/ml.), and is moderately sensitive to the combination of both (inhibitive doses: penicillin 10 units/ml. plus streptomycin 20 μ g/ml.).

On the other hand, *Staphylococcus* 65126, which is penicillin-resistant (inhibitive dose 100 units/ml.), and streptomycin-resistant (inhibitive dose 542 μ g/ml.), remains resistant to the combination of both drugs.

In the proposed method all one has to do with the prepared slab is to adjust an 18-h-old culture of micro-organisms in broth so as to contain 5×10^5 bacteria/ml., and to dispense 0.2 ml. into each cavity, starting with the cavity in the centre, the control. Results are clearly read with the naked eye; even the slightest growth can be detected. A sediment mostly forms in the tip of the cavity. Results are comparable with those from the tube dilution method in 10 ml. An estimate of combined drug activity is obtained.

We thank Dr. R. L. Vollum, director, Bacteriology Laboratory, Radcliffe Infirmary, Oxford, for kindly sending us the strains.

¹ Expert Committee on Antibiotics, WHO Org. Tech. Rep., Ser. No. 210 (1961).

² Report on Antibiogram Sensitivity Test Trial organized by the Bacteriology Committee of the Association of Clinical Pathologists (*J. Clin. Path.*, 18, 1, 1965).

³ Koch, W., and Kaplan, D., *Nature*, 203, 896 (1964).

⁴ Kaplan, D., and Koch, W., *Nature*, 203, 1091 (1964).

Sargassum TANNIN, AN ANTIBIOTIC WHICH RETARDS FOULING

By PROF. JOHN McN. SIEBURTH and PROF. JOHN T. CONOVER

Graduate School of Oceanography, University of Rhode Island, Kingston, Rhode Island

THE pelagic gulfweeds, *Sargassum natans* and *S. fluitans*, which belong to the Phaeophyta, have branch tips which are virtually free of an epibiota when they are at the centre of their distribution in the Sargasso Sea gyre. These tissues possess antibacterial activity¹. During filter-pad antibacterial assays the colour of active young tissues turned from yellow-green to black on incubation, while older and inactive preparations remained brownish in colour. This observation brought to mind the black pigment phycophaein formed by the oxidation of phaeophyte tannins² and the use of *Sargassum ring-goldianum* extracts in the tanning industry³. Extracts of actively growing branch tips were astringent, precipitated gelatin, and blackened ferric chloride solutions. These properties are characteristic of tannins, further suggesting that tannins were present in appreciable quantities in these tissues. Further observations were made during R.V. *Trident* cruise 10 (September 7-20, 1963) to the Sargasso Sea.

From samples of fouled weed a sensitive *Pseudomonas* strain was isolated and used as a test organism. This organism was more sensitive and had a shorter incubation period than the *Vibrio* isolate used previously¹. Only in material south-east of Bermuda in the Sargasso Sea gyre was there marked antibacterial activity and the presence of tannin-like substances in the branch tips. A comparison was made of the antibacterial activity of tannic acid (U.S.P., 'Mallinckrodt') and that of *S. natans* extracts (Fig. 1). Inhibition by tannic acid was detectable at concentrations greater than 0.01 per cent and was linear above 0.1 per cent. The more active *S. natans* preparations showed inhibition equal to that of a 0.2 per cent tannic acid solution. Although the inhibitory tannic acid and algal extracts were acidic, a hydrochloric acid solution diluted to pH 3.85 failed to inhibit the test organism. Inhibition by tannic acid and the phaeophyte tannin may be due to their protein precipitating activity.

Since tannins by definition precipitate protein, an attempt was made to observe the effect of adding extracts of young branch tips to the epifauna of fouled weeds. Hydroids (such as *Plumaria* and *Olytia*) were ideal test organisms as their tentacles remained active even after being poked by a glass rod or being hit by drops of sea-water. Initial observations with drops of a 1:4 w/v suspension in 25 ml. of sea-water 'froze' the active tentacles, which became deformed and exuded strings of

precipitated protein. The results with hydroids were so marked that a series of observations with different dilutions were made on a number of members of the epifauna; the results are summarized in Table 1. Dilutions of 1:125 affected most of the fouling organisms, while the hydroids were sensitive to dilutions as great as 1:1,000. Tannic acid solutions had a similar spectrum of activity with 0.0008 per cent solutions immobilizing 50 per cent of the hydroids. The tissue content of the 'tannins' appears to be sufficiently high to retard fouling.

Table 1. IMMOBILIZATION OF EPIFAUNA ON OLDER EPIBIOTA-ENRICHED GULF WEEDS BY SEA-WATER EXTRACTS OF YOUNG EPIBIOTA-FREE BRANCH TIPS OF *Sargassum natans*.

Test organisms*	Dilution of <i>S. natans</i> slurry in sea-water				
	1:125	1:250	1:500	1:1,000	1:2,000
Hydroids					
<i>Clytia noliiformis</i>	+	+	+	+	-
Other spp.	+	+	+	+	-
Bacteria ^a	+	± ^d	± ^d	+	-
Worms					
Planaried	+	-	-	-	-
Round (nematode)	+	-	-	-	-
Nereid	- ^e	-	-	-	-
Round (others)	-	-	-	-	-
Spirorbis	-	-	-	-	-
Pycnogonids	+	-	-	-	-
Copepods	+	-	-	-	-

* Observations on a number of active members of the community.

^a Immobilization accompanied by loss of cell structure and precipitation.

^b Complete loss of motility.

^c An enrichment culture predominantly of *Vibrio* spp.

^d Temporary inactivity only.

^e Violent motions.

Several attempts were made to concentrate and purify the 'tannins'. Only young branch tips free of fouling were suitable. Extraction was made difficult by the marked decrease in antibacterial activity following collection. Even when the tissue was preserved by freezing in packed plastic bags the activity was minimal after two to three weeks. The most successful extraction was conducted aboard ship on fresh material. Young branch tips triturated in a Waring blender with sufficient distilled water to make a slurry were extracted at 50° C for 15 min, and coarse-filtered through a clean towel to yield an opalescent suspension. Most of the chlorophyll was removed by repeated ether extraction. A red-brown fraction was brought into an iso-amyl alcohol phase with sodium sulphate. The iso-amyl alcohol fraction was evaporated to dryness in a rotary evaporator, and the residue was extracted with acetone. The acetone-soluble fraction was also evaporated to dryness, and the residue was taken up in a minimal amount of water to yield a deep amber-coloured liquid. This procedure extracted half the antibacterial activity as shown by assays while reducing the volume a hundred-fold. The dried extract had the solubility characteristics of *Sargassum* tannin², darkened a ferric chloride solution, precipitated gelatine, and was highly astringent. The insolubility of the active material in ethyl ether separates this material from the ether-extractable antibacterial, sarganin, reported for *S. natans*⁴.

The presence of tannic substances in brown algae is associated with refractile, acidic vesicles called physodes, fucosan vesicles or metachromatin granules^{2,5}. The excretion of phenolic substances from the physodes of living algae has been recently reported⁶. Accumulation of these tannins was greatest in the distal tips of the thalli. Antibacterial activity in extracts of epibiont-free branch tips of *S. natans* from the Sargasso Sea was observed in early October 1962 and September 1963 (R.V. *Trident* cruises 1 and 10), as well as in week-old frozen material brought back from other summer and autumn cruises. In contrast, observations made in January 1964 (R.V. *Trident* cruise 14) indicated that even the branch tips were fouled by

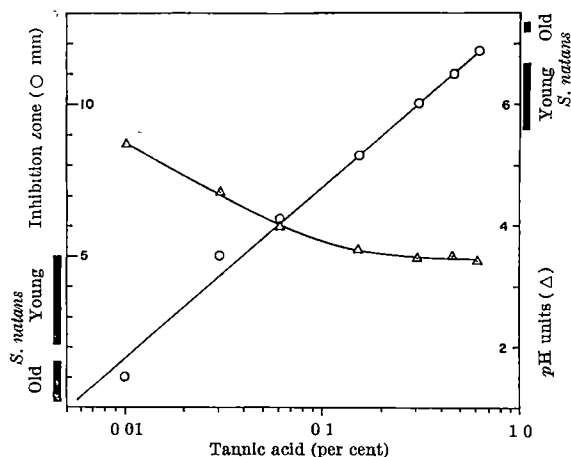


Fig. 1. Comparison of antibacterial activity and pH of tannic acid solutions with those obtained from young and old tissue suspensions (1:4 w/v) of *Sargassum natans*. A hydrochloric acid control of pH 3.85 did not inhibit the pseudomonad test organism.

hydroids and that antibacterial activity was not detectable. Both dried and preserved specimens were examined critically for physodes, which occur as yellow-refractive spherules 1 μ in diameter. The active distal tissue of algae collected during cruise 10 possessed 40–68 physodes per cell in contrast to 2–12 physodes from similar but inactive tissues from cruise 14. The presence of physodes appears to be coupled with antifouling in brown algae. This is further substantiated by reports on the absence of physodes in the cells of host brown algae adjacent to the invading filaments of parasitic epiphytes⁷. The antibiotic activity of marine algae in temperate zones has been shown to vary seasonally, peaks in activity occurring during active growth and being dependent on the boreal or tropical nature of the plant⁸. A drop of 3° C in the temperature of surface waters between cruises 10 and 14 was apparently sufficient to alter the physiology and antifouling activities of these plants. These observations on the seasonal change in the number of physodes per cell, in addition to those on tannin activity and antifouling in branch tips, further indicate the excretory function of these vesicles.

The recent work of Craigie and McLachlan⁹ has shown that a number of phaeophycean algae produce yellow, ultra-violet-absorbing materials which appear to be flavonols or catechin-type tannins. These substances were found to be toxic to unicellular algae at concentrations of 0.025–0.15 per cent. Concentrations of natural tannins similar to those inhibitory for our pseudomonad were also inhibitory for cellulolytic but not saprophytic bacteria⁹. The phaeophyte tannins should be included among the known algal antimicrobials¹⁰.

These observations indicated that tissues of algae containing tannins equal in activity to 0.1–0.8 per cent tannic acid (dry weight) retard fouling in *S. natans* and that unprotected tissues such as the tentacles of hydroids are sensitive to tannic acid solutions as dilute as 0.0008 per cent. Since fouling is one of the outstanding economic problems facing marine biology, an attempt was made to see if tannic acid *in vitro* would also retard fouling. Tannic acid (U.S.P., 'Mallinckrodt') dissolved in acetone at a 15 per cent (w/v) concentration. This solution was added to varnish and lacquers in equal volumes. Control preparations were diluted with acetone alone. Similar

preparations were made with paints using the cheaper grade of 'fluffy' tannic acid (N.F., 'Mallinckrodt'), but these were prone to peeling. Test panels were immersed both in Narragansett Bay, Rhode Island, and in Biscayne Bay, Florida. Although the tannic acid preparations were inferior to commercial antifouling paints, they did inhibit fouling by both barnacles and algae under both temperate and tropical conditions.

The antifouling properties of tannins were claimed as early as 1881 and paints containing tanner's bark, poke-root bark, pine bark, kelp and mangrove tree extracts have been granted U.S. Letters Patents as antifouling compositions of matter¹¹. Although tannins may be effective inhibitors to bacterial, algal and animal fouling organisms in young and actively growing tissues of at least the brown algae, their economic application is undoubtedly limited.

I thank colleagues and students, S. P. Meyers, Institute of Marine Science, Miami, Florida, and James H. Ware, International Minerals and Chemical Corporation, Skokie, Illinois, for their assistance. This work was supported in part by a grant from the U.S. Public Health Service (WP-00100-02) and a contract with the U.S. Office of Naval Research (Ncr 396-08).

Note added in proof. Subsequent investigations have shown that tannins from *Ralfsia verrucosa* affect the survival of planktonic animals in tide-pools (*Proc. Fifth Intern. Seaweed Symp.*, Halifax, August 1965).

- ¹ Conover, J. T., with Steiwarth, J. McN., *Botanica Marina*, 6, 147 (1964)
- ² Ogino, C., in *Physiology and Biochemistry of Algae*, edit. by Lewin, R. A., 437 (Academic Press, New York, 1962).
- ³ Noguchi, E., *Bull. Jap. Soc. Sci. Fisheries*, 12, 52 (1943).
- ⁴ Martinez Nadal, N. G., Rodriguez, L. V., and Casillas, C., in *Antimicrobial Agents and Chemotherapy*, edit. by Sylvester, J. C., 68 (Braun-Brumfield, Inc., Ann Arbor, 1964).
- ⁵ Haug, A., and Larsen, B., *Acta Chem. Scand.*, 12, 650 (1958)
- ⁶ Craigie, J. S., and McLachlan, J., *Canad. J. Bot.*, 42, 23 (1964); 42, 204 (1964).
- ⁷ Fritsch, F. E., in *Structure and Reproduction of the Algae*, 2, 548 (1945).
- ⁸ Pratt, R., Mantner, H., Gardner, G. M., Sha, Y., and Dufrenoy, J., *J. Amer. Pharm. Assoc.*, 43, 24 (1954). Vacca, D. D., and Walsh, R. A., *ibid.*, 43, 24 (1954). Chester, G. G. C., and Stott, J. A., in *Proc. Second Intern. Seaweed Symp.*, 49 (Trondheim, 1955). Roose, H., *Kiel. Meeresforsch.*, 13, 41 (1957). Almodovar, L. R., *Botanica Marina*, 6, 143 (1964)
- ⁹ Hems, Y., Tagari, H., and Volcani, B., *Appl. Microbiol.*, 12, 204 (1964)
- ¹⁰ Sieburth, J. McN., *Devel. Indust. Microbiol.*, 5, 124 (1964).
- ¹¹ Jones, E., U.S. Letters Patent No. 251,054 (1881). Dennys, N. B., *ibid.*, No. 444,665 (1891). Matthews, J., *ibid.*, No. 521,382 (1894). Childerson, T. J., *ibid.*, No. 552,418 (1895). Burnham, G. L., *ibid.*, No. 575,800 (1897). Cross, J. R., *ibid.*, No. 2,159,550 (1939).

COMPARISON OF THE CHORIOALLANTOIC MEMBRANE AND SPLENO-MEGALY SYSTEMS OF GRAFT-VERSUS-HOST ASSAY IN THE CHICK EMBRYO

By L. W. COPPLESON* and DR. DONALD MICHIE†

Medical Research Council, Research Group on Clinical and Experimental Problems of Transplantation, Department of Surgical Science, University of Edinburgh

THE chick embryo can be used as the arena of graft-versus-host reactions in two ways that are convenient to measure. Intravenous injection of immunologically competent cells results in an enlarged spleen which may be weighed¹. If a suspension of adult chick leucocytes is placed on the dropped chorioallantoic membrane (CAM) of a developing chick embryo, small white foci appear which may be counted²⁻⁴. This article describes the biometrical properties of the two systems as assays of immunological activity, and compares their efficiencies. The materials and methods used have been described elsewhere^{5,6}.

Assay systems

The essential feature of an assay is the expression of the concentration of some biologically active principle in a test preparation in terms of a standard preparation.

* Present address: McIndoe Memorial Research Unit, Blond Laboratories, Queen Victoria Hospital, East Grinstead, Sussex.

† Present address: Experimental Programming Unit, University of Edinburgh.

Estimates obtained therefore take the form of a relative potency. In illustration, we present a single assay more fully, in which the CAM reaction was used.

The material to be assayed consisted of frozen adult chick buffy coat cells⁶. The cell concentration of the test suspension was 4/3 times greater than that of the standard (unfrozen) suspension in order that the same part of the dose-response curve should be used as nearly as possible. The test cells, suspended in a solution containing 15 per cent dimethylsulphoxide and 30 per cent chick plasma, were frozen to -195° C and kept there for 0.5 h. The standard and test were each applied at three dose-levels, 1 in 1, 1 in 3 and 1 in 9; making 6 batches (that is, a 6-point assay⁷). A seventh batch of saline-treated eggs was included as a check for non-specific lesions, which occurred sufficiently rarely to be ignored. Six eggs were assigned to each batch.

The estimation of relative potency (relative immunological reactivity per cell) is illustrated in Fig. 1, using data from the first week of a series of seven replicates.

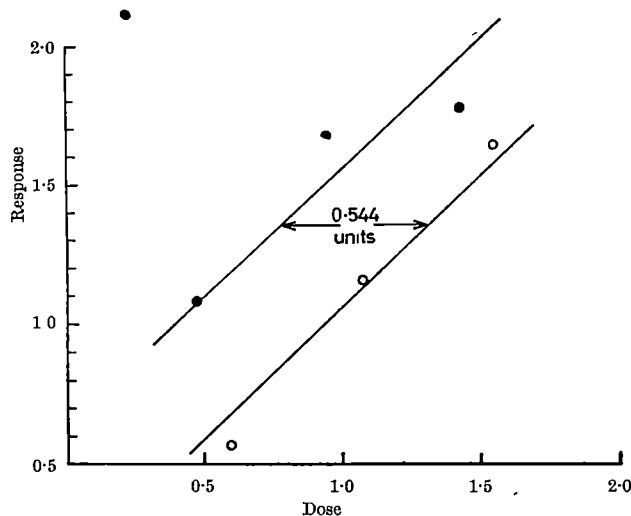


Fig 1 Illustrative assay of the immunological potency of a suspension of adult chick leucocytes using the CAM reaction. Serial three-fold dilutions were used, all doses are expressed as log cell concentration. The response is expressed as $\log(\text{No. of foci} + \frac{1}{2})$. ●, Standard; ○, test

The two parallel lines are the calculated lines of best fit, and the relative potency can be read off by taking the antilog of 0.544 which is 3.5 approximately, that is, the standard preparation has 3.5 times the potency of the test. Actually it is conventional to express the test in terms of the standard, so that we would say that the relative potency is $1.0/3.5$, that is, 0.29.

Parameters of the CAM system

Choice of interval. Inoculations were made on day 12 to conform with the practice of the Burnet group^{2,3,8}. Day 13 was tried but produced no appreciable difference from day 12. The membranes were collected 4 days after inoculation. Examination of day 5 and isolated trials of longer periods revealed such problems in the identification and counting of individual foci that the counts were considered less reliable than those obtained with a 4-day interval.

Numerical expression of response. In the CAM response, as has been shown elsewhere⁵, the scatter around the mean is grossly dependent on the mean itself, and hence on dose-level. Logarithmic transformation was found adequately to correct this feature. Since the counts occasionally contained zero values and the logarithm of zero is an infinite negative quantity, the transformation used was $\log_{10}(\text{No. of foci} + \frac{1}{2})$ rather than $\log_{10}(\text{No. of foci})$.

Parameters of the splenomegaly system

Choice of interval. Day 13 was chosen for intravenous injections as being technically extremely convenient and also because Solomon and Tucker's results⁹ indicate that maximum reactivity is developed at about this stage. Intervals of 3, 4 and 5 days were tested in two batches of Stirling eggs and one of inbred eggs (C-Line, Poultry Research Centre, Edinburgh). Using as the criterion of efficiency the ratio of the observed spleen enlargement to its standard deviation, the indicated order of preference in each case was 4 days, 5 days, 3 days. Four days was chosen as the interval for all subsequent work.

Numerical expression of response. The classical index of splenomegaly used by Simonsen was the ratio of the spleen weight in treated animals (mice) to that in litter-mate controls. In more recent work it has been customary to work with the logarithm of this ratio. In the chick the need for controlling inter-litter variation does not arise, so that the single measure $\log_{10}(\text{spleen weight})$ has been used throughout. Before standardizing on this measure one other possibility was investigated, namely, $\log_{10}(\text{spleen weight of treated embryos} - \text{mean spleen weight$

of untreated embryos). The expression inside the brackets measures the extra tissue present in the organ as a consequence of treatment. This transformation, while resulting in an improved slope, caused a deterioration in variance sufficient to counterbalance the gain.

Comparison of the CAM and splenomegaly systems

It was first pointed out by Gaddum¹⁰ that an objective measure of the efficiency of an assay system can be calculated from the dose-response slope, b , and the within-batch standard deviation, s . The quantity s/b is usually regarded as a convenient inverse measure of efficiency, having the property that its square, s^2/b^2 , is directly proportional to the number of animals which need to be used to attain a given precision in the estimate of relative potency¹¹. In an examination of 45 parallel-line assays for various drugs, Bliss and Cattell¹² found values of s/b ranging from 0.03 to 0.50.

Table 1. RELATIVE EFFICIENCY OF CAM AND SPLEEN ASSAYS IN STIRLING (S) AND $RIR \times LS$ MATERIAL

Stirling (S)		$RIR \times LS$	
CAM assays*	Spleen assays*	CAM assays†	Spleen assays
0.58 (17)	0.37 (3)	0.30 (5)	0.47 (4)

Figures represent the mean of s/b which is an inverse measure of the efficiency of the assay. Figures in parentheses denote the number of replicate assays performed.

* Includes data from ref. 6.

† From ref. 5.

In Table 1 we have set out some measures of efficiency obtained from 29 separate assays. It appears that in the Stirling embryos the splenomegaly system is more efficient whereas in the $RIR \times LS$ cross-breds the CAM system is more efficient. This interaction between genotype and test system is a real effect. If we compare the $S - (RIR \times LS)$ difference on the CAM test with that obtained on the splenomegaly test, the difference is significant at the 1 per cent level.

On the grounds of efficiency alone there is thus little to choose between the two methods of assay, the most important consideration being to fit the choice of recipient material to the assay system. Given suitable strain combinations, the choice between the two systems becomes primarily a matter of technical convenience. In the spleen assay it takes longer to administer the doses, but the response is less tedious and more objective to read. Therefore, for simple relative potency estimations the splenomegaly system is to be preferred. In the CAM system, on the other hand, inoculations are quick and easy, but the response is laborious and difficult to read. Consequently, the circumstances under which the CAM system is likely to be preferred are those in which it is desired to take advantage of the 'one focus, one competent cell' relation postulated by Burnet and supported by our own investigations⁶.

We thank Prof. M. F. A. Woodruff, Department of Surgical Science, University of Edinburgh, and Prof. M. Simonsen, Blond Laboratories, Queen Victoria Hospital, East Grinstead, for encouragement and provision of laboratory facilities.

¹ Simonsen, M., *Acta Path. Microbiol. Scand.*, **40**, 480 (1957).

² Boyer, G., *Nature*, **185**, 327 (1960).

³ Burnet, F. M., and Boyer, G., *Nature*, **186**, 175 (1960).

⁴ Burnet, F. M., and Boyer, G., *J. Path. Bact.*, **81**, 141 (1961).

⁵ Coppleson, L. W., and Michie, D., *Proc. Roy. Soc. B* (in the press).

⁶ Coppleson, L. W., and Woodruff, M. F. A. (in preparation).

⁷ Finney, D. J., *Statistical Method in Biological Assay*, second ed (Charles Griffin and Co., Ltd, London, 1964).

⁸ Szenteg, A., Warner, N. L., Burnet, F. M., and Lind, P. E., *Brit. J. Exp. Path.*, **43**, 129 (1962).

⁹ Solomon, J. B., and Tucker, D. F., *J. Embryol. Exp. Morph.*, **11**, 119 (1963).

¹⁰ Gaddum, J. H., *M.R.C. Special Rep. Ser.*, No. 183 (1933).

¹¹ See discussion by Biggers, J. D., McLaren, A., and Michie, D., *Nature*, **190**, 891 (1961).

¹² Bliss, C. I., and Cattell, McK., *Ann. Rev. Physiol.*, **5**, 479 (1943).

FINE STRUCTURE OF WOOD

By DR. P. RUDMAN*

Leverhulme Visiting Fellow, Astbury Department of Biophysics, University of Leeds

ALTHOUGH the advent of electron microscopy, and in particular the replica and shadowing techniques, has revealed the microfibrillar orientation in the lamellae of the primary wall (P) and the secondary wall layers (S_1 , S_2 and S_3), it has contributed little to our knowledge of cell wall capillaries or of intermicrofibrillar spaces. For general physiological reasons it is believed that cell wall capillaries exist; but until recently this belief was based on indirect evidence such as the weight/weight uptake of liquids by wood, or the encrustation of tracheid fragments with iodine¹ and the X-ray scattering of untreated and gold impregnated wood before and after delignification². More recently it was shown that electron diffraction diagrams of *Pseudotsuga menziesii* treated with inorganic solutions were consistent with cell wall penetration; the walls stained with dithio-oxamide throughout (but patchily), the middle lamella being heavily stained³⁻⁶. It was noted that the resulting complex was dichroic and similar dichroism has been found by other workers using different inorganic solutions in other softwood species^{7,8}. Such findings have been taken to indicate³⁻⁶ that the complexes are oriented parallel to the microfibrils of the cellulose fraction in these walls.

It has been found by Wardrop and Davies⁹ that when *Pinus radiata* is stained with congo red the layers S_1 and S_2 stain strongly, whereas the S_3 stained less strongly with the middle lamella barely staining. The authors suggested that if it were accepted that the degree of staining was indicative of the presence of capillaries of a particular minimum size, the observation would indicate that the capillaries in the true middle lamella were smaller than those of other regions; furthermore, the number of capillaries into which the dye could penetrate was greater in the layers S_1 and S_3 than in the layer S_2 . Optical microscopy and low-magnification electron microscopy have shown that copper-treated *P. radiata* contains tracheids in which the copper is in or is external to the layer S_1 (ref. 9); no fine structure was revealed in this work. Mention must be made of the pioneering work of Stamm¹⁰, whose investigations on flow demonstrated the existence of pores in the cell walls of wood cells. Similar work¹¹ based on electrical conductivity has recently confirmed that pores other than those in the pit membrane must traverse the cell wall completely.

Only one electron micrograph has been published^{5,6} which illustrates that a cell wall capillary may be filled or partly filled with an inorganic salt; in this micrograph, a few isodiametric deposits of a copper-chrome-arsenic wood-preserved may be seen in the cell wall of a tracheid of *P. menziesii*.

Other plant fibres are known to contain intermicrofibrillar spaces; but again it cannot be said that these are true capillaries because of the techniques adopted to reveal them. Wardrop⁹ has shown that in sections of fragments of delignified flax fibres treated with gold chloride solutions it was possible to see an irregular deposition of the salt. It was assumed that surface crystallization had commenced and that aggregates had formed in the 'grooves' between adjacent microfibrils. More recently, macerated ramie fibres treated with inorganic salts solutions have yielded excellent electron micro-

graphs showing oriented deposition of metal in the cell wall¹².

A study of the cell wall capillaries and pit membranes in wood has now been undertaken because of its wider implications in the field of wood-preservation. It was assumed that interstices could occur between and parallel to the microfibril, and that interstices in adjacent lamella would sometimes be linked, probably randomly, so that in effect a capillary system across and along the cell walls could exist. In theory it was thought that such a capillary system would allow the passage of polar liquids (but probably not non-polar liquids) from cell lumen to middle lamella, and, if this were also permeable, to the cell wall of the adjacent cell. Cutting of ultra-thin sections parallel to the microfibrillar orientation in metal-containing wood should then reveal the capillaries as elongated electron-dense zones, with more isodiametric zones indicating interstices linking lamellae.

Sapwood samples (3 in. long. \times 1 in. tang. \times 1 in. rad.) from two species of the hardwood genus *Eucalyptus* were placed under vacuum to remove air and then pressure-treated at 70 lb./in.² with aqueous solutions, either 10 per cent aqueous silver nitrate followed by 10 per cent aqueous hydrazine hydrate, or with a 10 per cent aqueous copper-chrome-arsenic wood-preserved ('Tanalith C'). After air drying, thick sections (30 μ) were cut at a small angle to the cell axis and portions of these embedded in methacrylate, 'Araldite' or 'Epon'. Thin sections (500 Å) were cut and examined in the electron microscope. Fig. 1 illustrates silver deposition in the capillaries in the S_2 and S_3 layers of a fibre tracheid from *E. obliqua* sapwood sections cut approximately tangentially to the S_2 microfibrillar direction. From this and similar electron micrographs it has been found that the average diameter of the deposits, and therefore approximately of the capillaries, is about 300–350 Å, no corrections being made for deformation due to crystal expansion during its growth. This same electron micrograph also shows limited evidence of the orientation in the S_3 layer.

The appearance of the tracheid wall of *E. maculata* sapwood impregnated with 'Tanalith C' (Fig. 2) and silver (Fig. 3) is somewhat different. Here, capillaries appear much smaller (approx. 100 Å) though, again, they can be seen to be oriented in relation to the microfibrils, but only certain areas are readily penetrated by the aqueous solutions. In Figs. 1, 2 and 3 small isodiametric electron-dense zones are also to be seen, indicating capillaries connecting adjacent lamellae.

Because of the importance of pits in any examination of liquid flow in wood, the structure of these has also been investigated. No evidence of large pit membrane pores has been found in *E. obliqua* and *E. maculata*; but the membranes exhibit a random pattern of interstices filled with the appropriate inorganic constituent (Fig. 4). In a few instances the pit membranes have been found to be torn (presumably during the initial sectioning) so that two thin membranes appear to be present. These findings are consistent with the belief that the membrane is composed of two primary walls without lignification and without a middle lamella.

No evidence has been found to indicate that the middle lamella is preferentially penetrated such as has been found previously^{5,9} with softwoods. However, other workers⁸ found that no copper was present in the middle lamella when sections of *P. radiata* were cut from copper-

* Permanent address: C.S.I.R.O. Division of Forest Products, South Melbourne, Australia.

† Note added in proof. See also Preston in *Cellular Ultrastructure of Woody Plants*, ed. Côté (Syracuse Univ. Press, 1965).



Fig. 1



Fig. 2

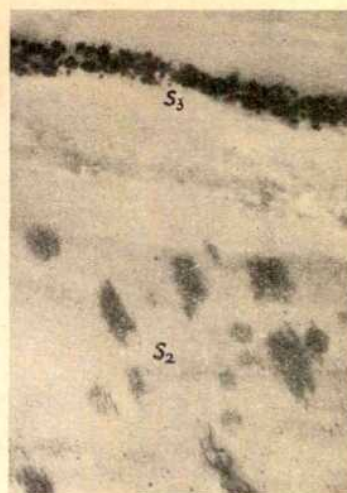


Fig. 3

Fig. 1. Electron micrograph of a fibre tracheid of *E. obliqua* containing silver in the cell wall capillaries (unshadowed longitudinal section $\times 23,000$)

Fig. 2. Electron micrograph showing the deposition of a copper-chrome-arsenic wood-preservative in the tracheid wall capillaries of *E. maculata* (unshadowed longitudinal section, $\times 15,000$)

Fig. 3. Electron micrograph of *E. maculata* showing silver deposited in the tracheid wall capillaries (unshadowed longitudinal section, $\times 23,900$)



Fig. 4. Electron micrograph of *E. maculata* in which the structure of the pit membrane of a vested pit is illustrated by the deposition of silver (unshadowed longitudinal section, $\times 10,860$)

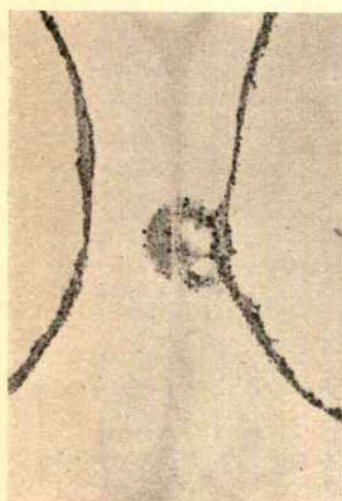


Fig. 5. Electron micrograph of *E. maculata* showing silver deposited in the end wall of a vertical parenchyma cell (unshadowed longitudinal section, $\times 5,700$)

treated blocks, but could be detected if sections were soaked in copper solutions.

Electron microscopy carried out in the work recorded here has revealed that after pressure impregnation with inorganic solutions the cell lumina are lined with inorganic salts, and it is usual to find that the S_3 layer has been

penetrated fairly frequently. An examination of the figures will show that the S_3 layer is quite an open network, with large capillaries. The rest of the wall is much less frequently penetrated and this could be due, for example, to hydrophobic cytoplasmic debris blocking the capillaries in the S_3 layer. The rather unusual silver deposition in a parenchyma cell of *E. maculata* which is shown in Fig. 5 provides an interesting illustration of relative penetrabilities of the various cell wall layers. It will be seen that, once the initial barrier is penetrated, movement through capillaries occurs and liquids may cross the middle lamella. Significantly, and in general agreement with the findings based on indirect staining investigations⁸, many of the capillaries in the middle lamella are smaller than those in other regions of the cell wall.

This work has illustrated that the cell walls in eucalyptus contain fine capillaries up to 450 Å in diameter, that these may be traversed by aqueous solutions which gain access from the cell lumen, that the middle lamella offers no resistance to flow and that direct access to the middle lamella from the pit chamber (Fig. 4) is not possible. Though the pit membrane contains no large pores, aqueous solutions can pass through it.

When this work is more fully reported elsewhere and the practical implications for the wood-preserving industry discussed, photomicrographic evidence will be presented to illustrate that the pits in eucalypts form the major pathway by which aqueous solutions travel from cell to cell, with the cell wall capillaries serving mainly to distribute the solution to the walls.

¹ Bailey, I. W., and Vestal, M. R., *J. Arnold Arboretum*, **18**, 185 (1937).

² Wardrop, A. B., *Biochim. Biophys. Acta*, **13**, 306 (1954).

³ Belford, D. S., Preston, R. D., Cook, C. D., and Nevard, E. H., *Nature*, **180**, 1081 (1957).

⁴ Belford, D. S., Myers, A., and Preston, R. D., *Nature*, **181**, 1516 (1958).

⁵ Belford, D. S., Preston, R. D., Cook, C. D., and Nevard, E. H., *J. App. Chem.*, **9**, 192 (1959).

⁶ Preston, R. D., *Proc. B.W.P.A. Ann. Convention*, 31 (1959).

⁷ Schulze, B., and Theden, G., *Holzforchung*, **4**, 79 (1950).

⁸ Wardrop, A. B., and Davies, G. W., *Holzforchung*, **15**, 129 (1961).

⁹ Bland, D. E., *Nature*, **200**, 267 (1963).

¹⁰ Stamm, A. J., *For. Prod. J.*, **13**, 503 (1963).

¹¹ Stone, J. E., *Tappi*, **40**, 539 (1957).

¹² Frey-Wyssling, A., and Mitrakos, K., *J. Ultrastructure Res.*, **3**, 228 (1959).

A NEW CHEMICAL ASPECT OF PENICILLIN ALLERGY: THE DIRECT REACTION OF PENICILLIN WITH ϵ -AMINO-GROUPS

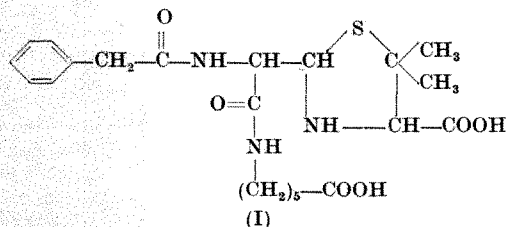
By DR. C. H. SCHNEIDER and DR. A. L. DE WECK

Allergy Research Unit, Department of Dermatology, University of Berne, Inselspital, Berne, Switzerland

IT seems well established that the penicilloyl group bound to carrier protein structures *in vivo* is a main antigenic determinant in penicillin allergy¹⁻³. On the other hand, the chemical basis for the formation of these penicilloyl protein conjugates has not yet been satisfactorily elucidated. Earlier views on the formation of the penicilloyl haptenic structure have held that a highly reactive degradation product of penicillin, penicillenic acid, might be the only form in which penicillin reacts irreversibly with proteins. Penicillenic acid is, in fact, spontaneously formed from penicillin in neutral solution and is able to react with free amino-groups, thereby forming a penicilloyl-amide⁴. The possibility that penicillins could react directly with ϵ -amino-groups by an opening of the β -lactam ring has been neglected mainly because an experimental demonstration of the reaction between penicillins and such amino-groups on proteins, peptides or low molecular weight amines in neutral aqueous solution was lacking⁵.

Two facts have been known for some time to throw some doubt on the concept of penicillenic acid being a main intermediate in the formation of the penicilloyl haptenic structure: (a) There is no correlation between the *in vitro* rate of formation of penicillenic acid from various penicillins bearing different acyl side-chains and the immunogenicity of these penicillins; (b) 6-aminopenicillanic acid (6-APA), being structurally incapable of rearrangement to a penicillenic acid intermediate, was shown to be a strong inducer of the allergic state in rabbits⁶.

In experiments initiated by these considerations, the incubation of penicillins with amines in neutral aqueous solution was examined. It was established that penicillins do react with amines under such conditions to yield penicilloyl-amides. In this article we report in a preliminary form some of our results together with findings obtained with dinitrophenyl-6-aminopenicillanic acid, demonstrating that the direct reaction of the β -lactam of penicillins with amines is an important general route to the penicilloyl-amide antigenic determinant. (A full account of this work will be published in *Helv. Chim. Acta.*)



Benzylpenicillin (0.5 M) was incubated at 37° with ϵ -aminocaproic acid (molar ratio, 1 : 4.25) in 0.5 M phosphate buffer pH 7.4, and also in 0.1 M sodium bicarbonate at pH 7.4. Additions of small amounts of sodium hydroxide kept the pH at the desired value. After one day a viscous gum was precipitated from the reaction mixture by acidification. From the precipitate, crystalline benzylpenicilloyl- ϵ -aminocaproic acid (I) in the form of its dibenzylammonium salt was obtained. The products obtained in the two runs agreed in properties (m.p. 110°–113° and 111.5°–113°. Corrected capillary melting points are reported; $[\alpha]_D^{25} = +69.7^\circ$ and $+69.2^\circ$ ($c=1$, water); penamaldade assay⁷: $E_m = 24,000$) with an

authentic sample of benzylpenicilloyl- ϵ -aminocaproic acid dibenzylammonium salt prepared in alkaline solution. Mixed melting points were not depressed. The yields were 20–30 per cent. In addition, benzylpenicillin was similarly incubated at physiological pH with poly-L-lysine of mean molecular weight about 1,000, the molar ratio of penicillin to ϵ -amino-groups being 1 : 0.4. Passage of the incubation mixture through a 'Sephadex G-25' column neatly separated the polylysine material (peak I) from the residual material (peaks II and III) (Fig. 1). Penicilloyl groups attached to the polylysine were measured by a modified penamaldade assay⁷. In accordance with the behaviour expected for a benzylpenicilloyl polylysine⁷, the material from the column (peak I) specifically precipitated antisera from rabbits which had been immunized with benzylpenicilloyl-bovine γ -globulin.

Other penicillins were incubated for 4 h at 37° with polylysine in 0.05 M phosphate buffer pH 7.4 at a concentration of 75 mM, the molar ratio of penicillin to ϵ -amino-groups being 1 : 1. Polylysine material was isolated from the incubation mixture through 'Sephadex G-25' gel-filtration and the amount of penicilloyl-amide on the polylysine was established by penamaldade analysis. That no other material developing under the conditions of the incubation and detectable by penamaldade assay, was carried with the polylysine peak, was verified in each case by a run in which polylysine was added only at the end of the incubation period. The polylysine peaks

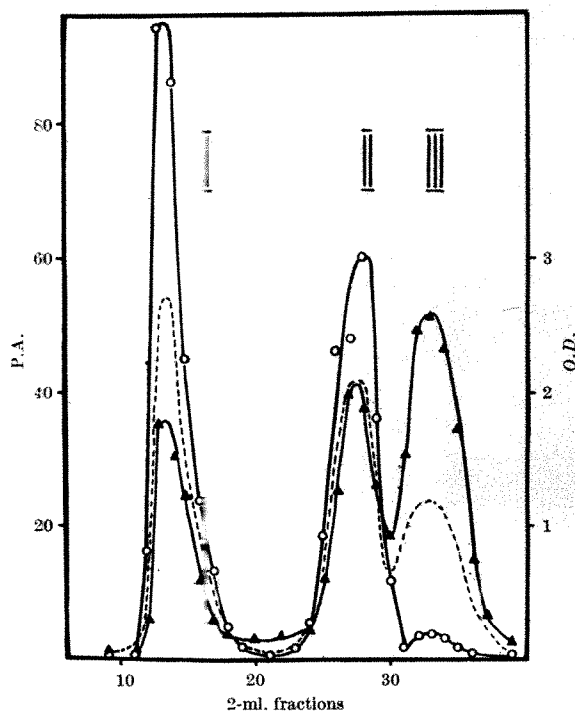


Fig. 1. Gel-filtration of 1 ml. of a solution of benzylpenicillin (250 mg) and polylysine (40 mg) in 4.0 ml. of McIlvaine's citrate-phosphate buffer incubated for 24 h at 37°, pH 7.4. The column (1.8 cm \times 30 cm) containing 'Sephadex G-25' was equilibrated and eluted with McIlvaine's citrate-phosphate buffer. Analysis of eluate: \circ , penamaldade assay (P.A.) as increase in optical density at 282 m μ after HgCl₂ treatment; \blacktriangle , optical density at 257 m μ ; ---, Folin-Lowry colour (ref. 8) as optical density at 750 m μ . Optical measurements were made in 1 cm cells

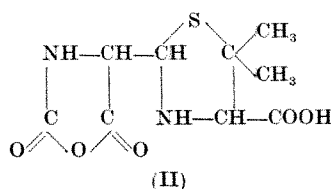
Table 1. AMINOLYSIS OF PENICILLINS AND DEGRADATION TO PENICILLINIC ACID

Penicillins	Penicilloyl groups on polylysine after incubation of 300 μ moles of penicillins with excess polylysine for 4 h at pH 7.4, at 37° μ moles*	Degradation to penicillenic acid at 37°, from (ref. 6)	
		Mole parts per thousand per min at pH 4.0	Mole parts per thousand per h at pH 7.4
Benzylpenicillin	19.3	2.0	0.26
Phenoxymethylpenicillin	21.1	0.06	0.008
Allylthiomethylpenicillin	22.7	0.5	0.06
Phenoxyethylpenicillin	22.6	0.08	0.01
Dimethoxyphenylpenicillin	12.3	2.5	0.29

* Calculated from the penamaldate assay with a mean molar extinction of 24,000.

obtained in these control runs did not contain significant amounts of material detectable by penamaldate analysis. The results are shown in Table 1.

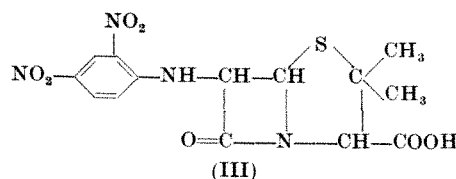
These experiments demonstrate the formation of the penicilloyl-amide bond in aqueous solutions at physiological pH. They make it unlikely that the route to penicilloyl-amides proceeds in general through penicillenic acid as a main intermediate, since no correlation between conversion to penicillenic acid of the various penicillins and the formation of penicilloyl polylysine is found (Table 1).



However, the use of a penicillin-like molecule unable to undergo the penicillenic acid rearrangement, but capable of acylating amines in neutral aqueous solution and of inducing the formation of specific antibodies *in vivo*, is required in order to demonstrate unambiguously that the direct acylation of amino-groups by the penicillin molecule through opening of the β -lactam ring, without involvement of a penicillenic acid type intermediate, occurs and constitutes an important route *in vivo* to the penicilloyl-amide antigenic determinant. 6-APA would seem to be a good choice in this respect. However, this compound is peculiar as it reacts easily with carbon dioxide, thereby yielding 8-hydroxypenicillanic acid⁹⁻¹¹. The reaction probably involves a CO₂-6-APA adduct (II) (ref. 9) as an intermediate which conceivably could react with amines. Furthermore, 6-APA is capable of intermolecular acylation by reaction of the amino-group of one 6-APA molecule with the β -lactam ring of another. On repetition of this process, polymers of 6-APA are formed for which evidence has been reported^{12,13}. Since the dimer penicillanyl-aminopenicillanic acid as well as the higher polymers are, in fact, penicillins, they are in principle capable of the penicillenic acid rearrangement. Therefore, the occurrence of a reaction between 6-APA and amines at neutrality could be considered as proof of a direct reaction of the β -lactam with amines only if the reaction products were identified as penicillanyl amides, or if additional information indicated that the contribution of the polymerization under the conditions used is negligible. On the other hand, the first step of the 6-APA polymerization, the *N*-acylation of a 6-APA molecule by a second one, obviously cannot proceed via a penicillenic acid intermediate and constitutes a demonstration of the direct attack of an amino-group on the β -lactam. However, the equivalence in this respect of the reacting amino-group of 6-APA and ϵ -amino-groups could not be taken for granted.

We have obtained preliminary evidence that 6-APA reacts with polylysine in neutral aqueous solution in a nitrogen atmosphere free of carbon dioxide to yield a conjugate which specifically precipitated a rabbit anti-benzylpenicilloyl antiserum. The reaction was followed

by penamaldate assay. It seemed diminished in sodium bicarbonate solution. We have further observed that, in the absence of polylysine and carbon dioxide, a considerable increase of material showing a penamaldate assay results on incubation of 6-APA alone in neutral solution. This could be due to hydrolysis but in part also to polymerization of 6-APA.



In view of the complications connected with 6-APA work, we proceeded with dinitrophenyl-6-aminopenicillanic acid (DNP-APA) (III). This substituted 6-APA contains all the features of penicillins required for the demonstration of a direct aminolysis of the β -lactam since a penicillenic acid rearrangement cannot occur and potential side-reactions of 6-APA are avoided by the blocking of the 6-amino-group. The compound was prepared by reacting 6-APA with dinitrofluorobenzene in a mixture of acetone and sodium bicarbonate solution. The DNP-APA could be well crystallized as the free acid from ether and from acetone-water (m.p. 164°. Analysis, calc. for C₁₄H₁₄O₇N₂S: C, 43.98; H, 3.69; N, 14.65; found: C, 44.23; H, 3.90; N, 14.58; neutralization equivalent, calc. 382.3; found: 383).

This compound (2.5 g) was incubated with ϵ -aminocaproic acid (3.8 g) in 0.1 M phosphate buffer (8 ml.) at 37° for one day. The incubation solution was kept at pH 7.2 with the aid of a pH-stat, delivering 1 N sodium hydroxide to the reaction vessel. Dinitrophenyl-6-aminopenicillanyl- ϵ -aminocaproic acid was isolated in the form of its crystalline dibenzylammonium salt in nearly 40 per cent yield (m.p. 135°–137°. Analysis, calc. for C₂₄H₄₅O₉N₇S: C, 56.10; H, 6.23; N, 13.47; found: C, 55.84; H, 6.71; N, 12.93; penamaldate assay: $E_m = 25,000$). Manipulations were performed in subdued light, since the desired product as well as DNP-APA itself are light-sensitive in neutral solution.

The formation of dinitrophenyl-6-aminopenicillanyl- ϵ -aminocaproic acid from DNP-APA and ϵ -aminocaproic acid in 0.05 M phosphate buffer at pH 7.4 was compared to the formation of benzylpenicillanyl- ϵ -aminocaproic acid from benzylpenicillin and ϵ -aminocaproic acid under the same conditions. To this end, solutions (0.36 M) of DNP-APA and benzylpenicillin in 0.05 M phosphate buffer were incubated in a pH-stat at pH 7.4 at 37° alone and in the presence of 1.45 M ϵ -aminocaproic acid. The opening of the β -lactam ring was followed by penamaldate assay¹⁴. Figs. 2 and 3 show the very similar rates of

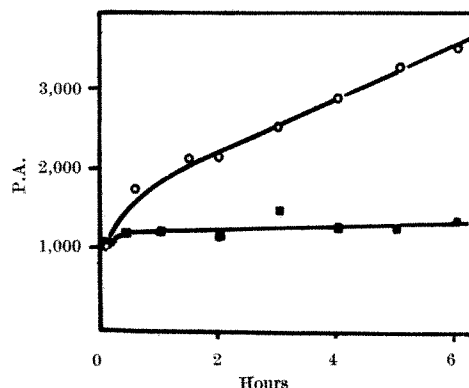


Fig. 2. Incubation of DNP-APA at pH 7.4, at 37°. ■, Alone; ○, in the presence of ϵ -aminocaproic acid. Penamaldate assay (P.A.) as increase in optical density at 282 m μ after HgCl₂ treatment (1 = 1 cm)

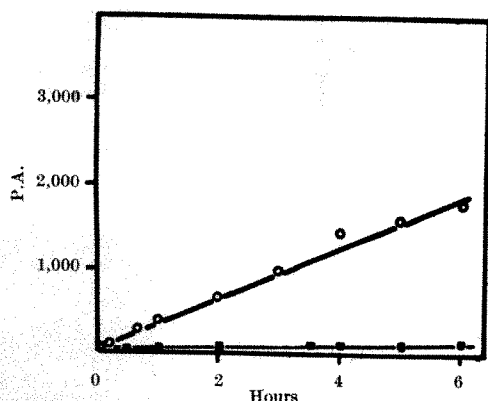


Fig. 3. Incubation of benzylpenicillin at pH 7.4 at 37°. ■, Alone; ○, in the presence of ϵ -aminocaproic acid. Penamaldate assay (P.A.) as increase in optical density at 282 m μ after HgCl₂ treatment (1 = 1 cm)

formation of the dinitrophenyl-6-aminopenicillanyl group and the benzylpenicilloyl group in the presence of ϵ -aminocaproic acid. The rate of hydrolysis of both DNP-APA and benzylpenicillin appears negligible under the conditions used. This result indicates similar rates of aminolysis for DNP-APA and benzylpenicillin and implies that the contribution of the penicillenic acid route to the aminolysis of benzylpenicillin could only be small under the *in vitro* conditions used.

The immunogenicity of DNP-APA was tested in rabbits. Animals were injected with DNP-APA and complete Freund's adjuvant (Difco) according to a procedure described previously¹. Antibodies specific for the dinitrophenyl-6-aminopenicillanyl determinant were demonstrated in the rabbit sera by haemagglutination¹. For the haemagglutination reaction, rabbit antisera absorbed with normal sheep erythrocytes were incubated with sheep erythrocytes previously treated with DNP-APA or benzylpenicillin in 0.1 M veronal buffer at pH 9.0. The haemagglutination titres of anti-DNP-APA sera with DNP-APA treated cells were comparable to those obtained with anti-benzylpenicillin rabbit sera and benzylpenicillin treated cells. The haemagglutination reaction was specifically inhibited by dinitrophenyl-6-aminopenicillanyl- ϵ -aminocaproic acid and by benzylpenicilloyl- ϵ -aminocaproic acid. The former compound was a more effective inhibitor. Only 1×10^{-4} M dinitrophenyl-6-aminopeni-

cillanyl- ϵ -aminocaproic acid was required in order to achieve the same degree of haemagglutination inhibition as obtained with 6×10^{-3} M benzylpenicilloyl- ϵ -aminocaproic acid.

The experiments described here demonstrate the reaction of penicillins with ϵ -amino-groups at physiological pH. It is shown that the resulting penicilloyl-amides are formed by direct penicilloylation without necessarily involving a penicillenic acid intermediate. Even in the case of benzylpenicillin, the contribution of the penicillenic acid route appears small under certain *in vitro* conditions. DNP-APA, which has no possibility of reacting via a penicillenic acid intermediate, was shown to be immunogenic in rabbits. It is therefore suggested that the direct penicilloylation of carrier protein structures is a general route to the penicilloyl antigenic determinant *in vivo*.

While this article was being prepared, the paper by Batchelor, Dewdney and Gazzard¹⁵ came to our notice. It is remarkable that some of the lines of thought were quite similar in the two laboratories.

This work was supported in part by grants of the U.S. Public Health Service, of the Swiss National Foundation for Scientific Research and of the Emil Barrell Foundation.

I thank Mrs. E. Stäuble for assistance.

¹ de Weck, A. L., *Intern. Arch. Allergy*, **21**, 20 (1962).

² Parker, C. W., Shapiro, J., Kern, M., and Eisen, H. N., *J. Exp. Med.*, **115**, 821 (1962).

³ Levine, B. B., and Ovary, E., *J. Exp. Med.*, **114**, 875 (1961).

⁴ Levine, B. B., *Arch. Biochem. Biophys.*, **93**, 50 (1961).

⁵ Wintersteiner, O., Stavely, H. E., Dutcher, J. D., and Spielman, M. A., in *The Chemistry of Penicillin*, edit. by Clarke, H. T., Johnson, J. R., and Robinson, R., 207 (Princeton Univ. Press, 1949).

⁶ de Weck, A. L., *Intern. Arch. Allergy*, **21**, 38 (1962).

⁷ Parker, C. W., de Weck, A. L., Kern, M., and Eisen, H. N., *J. Exp. Med.*, **115**, 803 (1962).

⁸ Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. Biol. Chem.*, **193**, 265 (1951).

⁹ Johnson, D. A., and Hardcastle, G. A., *J. Amer. Chem. Soc.*, **83**, 3534 (1961).

¹⁰ Ballio, A., Chain, E. B., Dentice di Accadia, F., Mauri, M., Rauer, R., Schlesinger, M. J., and Schlesinger, S., *Nature*, **191**, 909 (1961).

¹¹ Batchelor, F. R., Gazzard, L., and Naylor, J. H. C., *Nature*, **191**, 910 (1961).

¹² Grant, N. H., Clark, D. E., and Albura, H. E., *J. Amer. Chem. Soc.*, **84**, 876 (1962).

¹³ Batchelor, F. R., Cole, M., Gazzard, D., and Robinson, G. N., *Nature*, **195**, 954 (1962).

¹⁴ Woodward, R. B., Neuberger, A., and Trenner, N. R., in *The Chemistry of Penicillin*, edit. by Clarke, H. T., Johnson, J. R., and Robinson, R., 428 (Princeton Univ. Press, 1949).

¹⁵ Batchelor, F. R., Dewdney, J. M., and Gazzard, D., *Nature*, **206**, 362 (1965).

OSMOTROPOTAXIS IN THE HONEY-BEE

By DR. H. MARTIN

Zoological Institute, University of Frankfurt

OLFACTORY orientation in insects can follow two different paths: (a) Sampling of the scented area with movable antennae in temporal sequence makes it possible to perceive differences in the concentration of the scent and to make adjustments in a klinotactic orientation. This alone can be carried out with a single sensory organ on one side. (b) Simultaneous spatial perception of the scent gradient by both antennae: the animals succeed in orienting themselves tropotactically to the scent source. That two sense organs lie symmetrically on the body must be assumed (theoretically receptor can replace the word sense organ¹).

Many authors believe that osmotropotactic orientation can be shown in one-antenna animals. One-antenna animals move in circles toward the antenna side in a scent current, in a different scent area, and also in a scent gradient, *Geotrupes silvaticus* Panz. and *Geotrupes vernalis* L.², *Calliphora erythrocephala*³, *Leptinotarsa decemlineata* Say⁴, meal-worm beetle⁵, *Drosophila melanogaster*^{6,9},

Habrobracon juglandis Ashmead⁷, *Ips curvidens*⁸, and silkworm¹⁰.

On the other hand, experiments have been performed with one-antenna animals as proof of a klinotactic olfactory orientation. The animals with one antenna still show a fully developed olfactory orientation; sometimes they show only a slight deviation towards the antenna side. Attaining the goal results from klinotaxis, and for this one antenna suffices for *Rhodnius prolixus*¹¹, *Nemeritis canescens*¹² and *Geotrupes silvaticus* Panz¹³.

Other authors discuss olfactory orientation as quite a different mechanism. The scent acts only as a stimulant. Actual finding of the objective is only possible when an air current gives the animal the chance to fly rheotactically against the scent gradient^{6,14-17}.

For the first time we have succeeded in showing osmotropotactic orientation in a scent area for bees^{18,19}. The way it works, its quantitative effect, and its interaction with osmoklinotaxis are described in this article.

Normal and experimental bees trained to a scented food source had to show in an appropriate experimental situation that they were able to distinguish between the scentless and the scent-filled branches of a Y-tube (Figs. 1a and b). At the moment of the decision they had the choice of running into one or the other of the tube branches in order to reach the food. The experimental set-up was free of air currents. The Y-tube was placed at an angle of 5° – 6° to the horizontal plane. This guaranteed a flow of scent molecules (rate of flow: 0.3–1.5 cm/sec). Observation was through a ruby glass plate.

(1) *Bees with one movable antenna and with fixed antennae.* With the scent source on one side (0.002 ml. oil of anise), normal bees run straight to the scented box (Fig. 2). 2.5 sec were required to run a distance of 11.5 cm; 87.0 per cent positive choices were made. If one antenna is cut off the orientation towards the scent is scarcely impaired (78.8 per cent positive runs). Only occasionally a slight 'zig-zagging' can be observed. A tendency to move sideways^{5,9,10} does not occur: time per run, 2.9 sec. If both antennae were fixed in their normal position

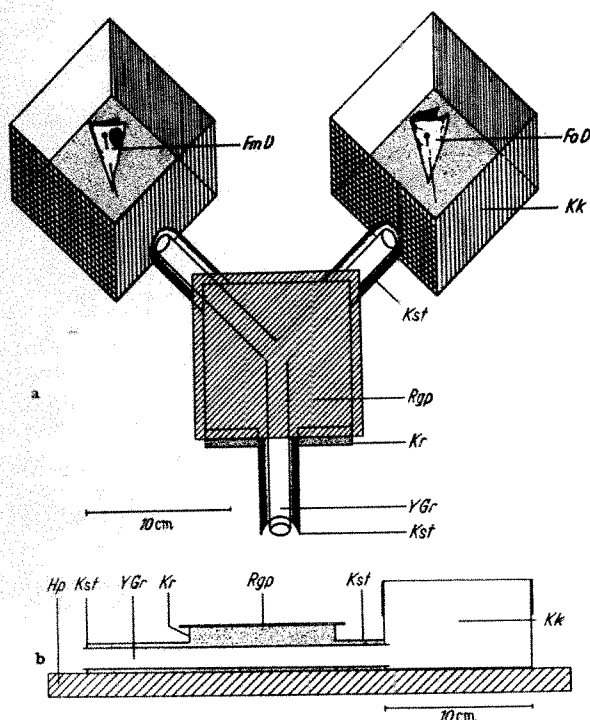


Fig. 1. a, Experimental arrangement for the Y-tube tests; FmD, filter paper with scent; FoD, filter paper without scent; Kk, cardboard box with lid removed; Kr, framework of the cardboard box (support for the ruby glass plate); Kst, box top (dark box); Rgp, ruby glass plate; YGr, Y-glass tube. b, Cross-section: Hp, fibreglass; Kk, cardboard box with lid; YGr, Y-glass tube; Kr, framework of the cardboard box; Kst, box top; Rgp, ruby glass plate

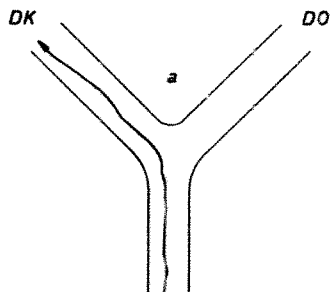


Fig. 2. Normal bee in the Y-tube (No. 157): without zig-zag movements the bee runs in a straight line towards the scented box. DK, scented box; DO, box without scent

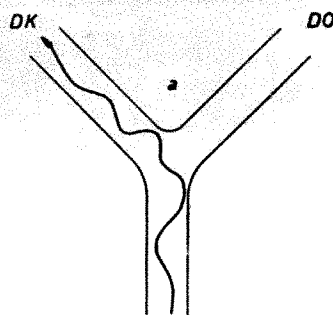


Fig. 3. Run of bee No. 108. Right antenna amputated, left antenna fixed on the morphologically correct side of the body. Zig-zagging run as indicator of klinotactic orientation

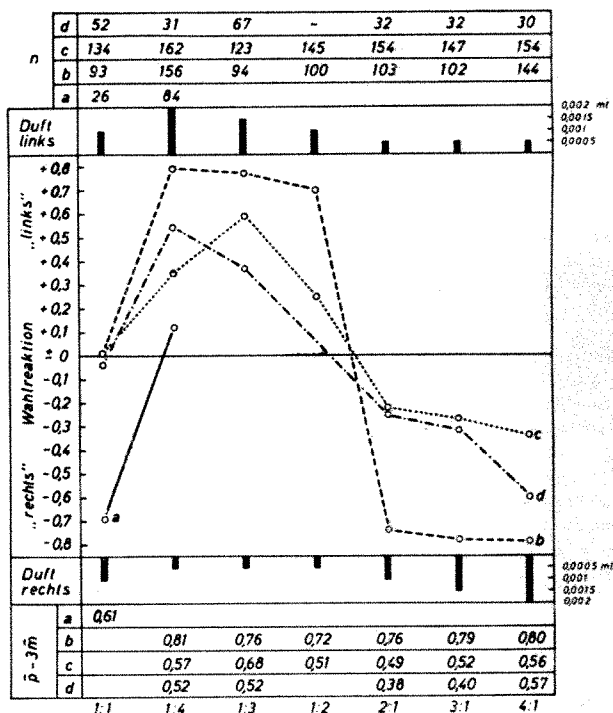


Fig. 4. Alternative choice of differently handled bees when different quantities of scent are offered simultaneously from the left and from the right. a, Bees with one fixed antenna (left antenna cut off); b, normal bees; c, bees with one movable antenna (right antenna cut off); d, bees with both antennae fixed in normal position

(distance between the tips of the antennae, 7–8 mm), then still 83 per cent positive choices were recorded. The run itself is a straight line; once in a while a slight zig-zagging appears: time per run, 3.4 sec.

Bees which have one antenna fixed and the other cut off tend to show a bias toward the intact side. The results in finding the objective vary from one condition to another: if the scent is given on the antenna side, then 76.9 per cent of these bees run into the tube branch with the scent. On the contrary, if the scent is offered on the amputated side, only 39.9 per cent of these bees run into the scented branch. The run to the scent source is a distinct zig-zag run (Fig. 3). Furthermore, the runs are appreciably slower (4.6 sec running time). One can show that the side tendency, which is frequently explained as an osmotropotactic reaction, is superimposed by a klinotactic orientation component. There is, therefore, a tendency toward the intact side. If scent is offered simultaneously and at different concentrations in the left and right tube branches, then normal bees, bees with fixed antennae, and bees with one movable antenna all choose the higher concentration (Fig. 4). The different scent ratios,

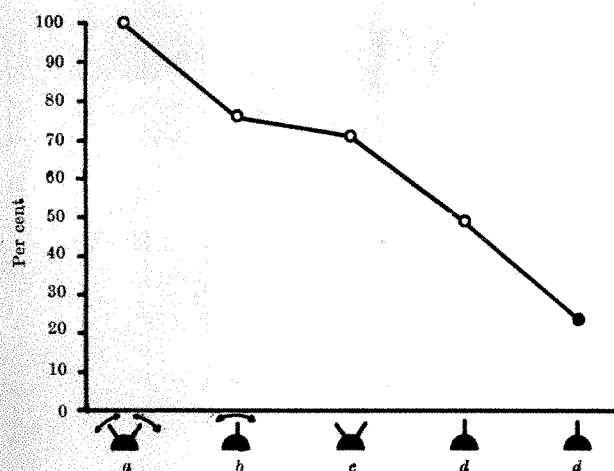


Fig. 5. 'Absolute' value in orientation (absolute orientation performance coupled with the time necessary finding the goal). The 'absolute' value in orientation for normal bees set at 100 per cent. *a*, Normal bees; *b*, bees with one movable antenna (the second antenna is cut off); *c*, bees with both antennae fixed in normal position; *d*, bees with one fixed antenna (the second antenna is cut off). ●, Bees with one fixed antenna: scent is offered on the side without an antenna. ○, bees with one fixed antenna: scent offered on the antenna side

however, are analysed with varying success by the different bee groups (Fig. 4). Bees with one fixed antenna and the other antenna cut off compensate for the side tendency only, if the scent ratio is 1:4 and when the greater concentration is given on the side without antenna (Fig. 4, curve *a*).

This method allows one not only to separate the side tendency and its connected klinotactic orientation component from one another, it also gives the first hint of the integration process in the central nervous system. The scent gradient between the left and the right side must be 1:4, so that a difference in the information from two sides will be recorded in the central nervous system. This is effective for cases in which the klinotactic component is experimentally removed from the orientation act and only the tropotactic adjustment is allowed (experiments on bees with both antennae fixed in normal position). The same holds also for the information from one side when the tropotactic component is eliminated and only klinotactic adjustment is allowed (experiments on bees with one antenna free). By a combination of tropotactic and klinotactic adjustment the central nervous system can detect smaller concentration differences (experiments on normal bees being about a ratio of 1:2).

Olfactory perception of the attracting scent therefore consists of two components: perception of the concentration gradient simultaneously in space and successive perception in time. The simultaneous component demands paired, symmetrically arranged receptors—at least two; they do not need to be movable. The successive component requires movement of the sense organ (theoretically one receptor would be enough) or movement of the animal. Normal bees use both these components in coupled ways. Bees with one antenna can only use successive perception of the concentration for orientation. The net performance in scent orientation (calculated in percentages) is almost equal for normal bees (87.0 per cent), for bees with one movable antenna (78.8 per cent), and for bees with fixed antennae (83.8 per cent). However, the individual groups approach the goal with different velocity ($P < 0.0027$). The net performance in scent orientation coupled with this time factor gives the 'absolute' value of orientation (Fig. 5). It shows us quite clearly the true grade of performance in olfactory orientation.

(2) *Crossing the antennae.* The antennae of bees which were trained to odorants are crossed at the scapus and fixed into this position (Fig. 6). By this crossing, the

instinctive, natural side-correct evaluation of information is no longer given. Bees with crossed, fixed antennae turn spontaneously and without hesitation at the point of decision into the scentless tube branch. This 'changing of polarity' of the antennae causes the olfactory information in the central nervous system to be recorded as reversed. It also causes differences to be sent out side-reversed. This way brings about the 'spontaneous turning' into the tube branch without scent. Their behaviour shows that they perceive the scent gradient spatially simultaneously between the antennae.

If the tips of the symmetrically crossed antennae are placed closer together, step by step along the median line, then a change from osmotropotaxis to klinotaxis occurs when the distance between the tips of the antennae is 2.0 mm (Table 1). This reaction is quite definite. If the distance is smaller than 2.0 mm, then the bees turn into the tube branch with the scent and make a zig-zag run to the scented box. This means, therefore, that simultaneous-spatial distinctions are no longer made, and only temporal sequences can be evaluated. Quite astonishingly, in antenna positions I-V, almost the same number of 'side-reversed correct' choices are made (see Table 1). The change from one orientation type to the other does not happen gradually, but rather suddenly. With asymmetrical crossing of the antennae (Fig. 7) the 'effective' boundary distance totals 2.7 mm.

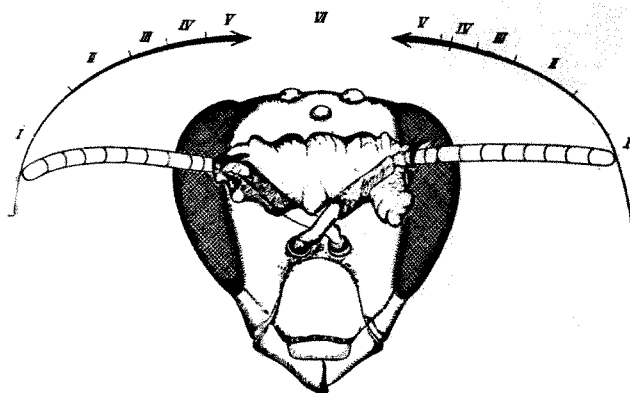


Fig. 6. Bee head with symmetrically crossed and fixed antennae. The sections I, II, III, IV, V and VI give the possible position of the antennae during the experimental series. If the antennae are in position VI, then the bees orient themselves klinotactically; in all the other positions, osmotropotactically

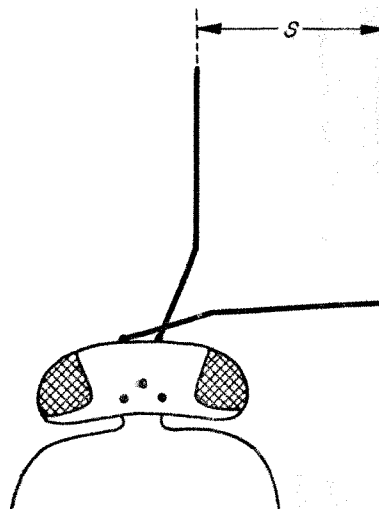


Fig. 7. Schematic representation of a bee head with asymmetrically crossed antennae. Both antennae are fixed on one morphological side of the body. *s*, 'Effective' distance between the antennae tips

Table 1. CHOICES FOR THE BRANCHES OF THE Y-TUBE FROM BEES THE ANTENNAE OF WHICH ARE GLUED AND SWITCHED TO THE OPPOSITE SIDE AT VARIOUS DIFFERENT ANGLES

Position of the crossed antennae	Scent from the right (0.002 ml. anise oil)					
	I	II	III	IV	V	VI
'Correct', side-reversed choices (%)	79.9	76.6	72.4	77.5	74.3	23.4
Total number of choices	194	205	181	111	202	124
Distance between the antenna tips in mm	9-7	7-5	5-4	4-3	3-2	2-0

Osmoklinotactic orientation behaviour appears also when one antenna is fixed and the second antenna is left free, provided that the 'median distance' of the fixed antenna (Fig. 8) is smaller than 2.5 mm. The movable antenna can compensate for the false report of the crossed, fixed antenna only so long as the 'median distance' of the second antenna does not exceed 2.5 mm (Fig. 9).

(3) *Quantitative effect of osmotropotaxis.* How great must the difference of concentration between the left and the right antenna be in order to be perceived osmotropotactically?

Fine glass capillaries of a certain weight and diameter are slipped over the antennae and are glued on the scapus (Fig. 10). (The bees are previously trained to the scent.)

Liquid paraffin with scent of different concentrations is placed in the capillaries to produce different scent

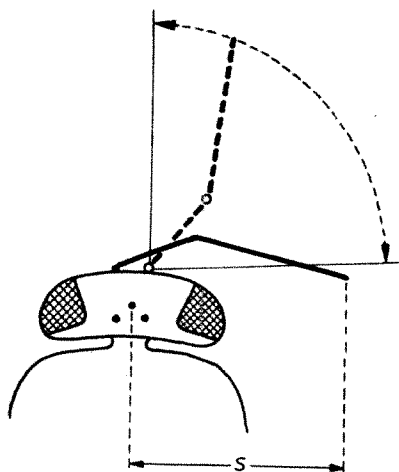


Fig. 8. Schematic representation of a bee head with one fixed and one movable antenna. Left antenna fixed on the right side of the head. Right antenna movable. *s*, Median distance (equals the projected, perpendicular distance in the horizontal plane between the stinocellus and the tip of the fixed antenna)

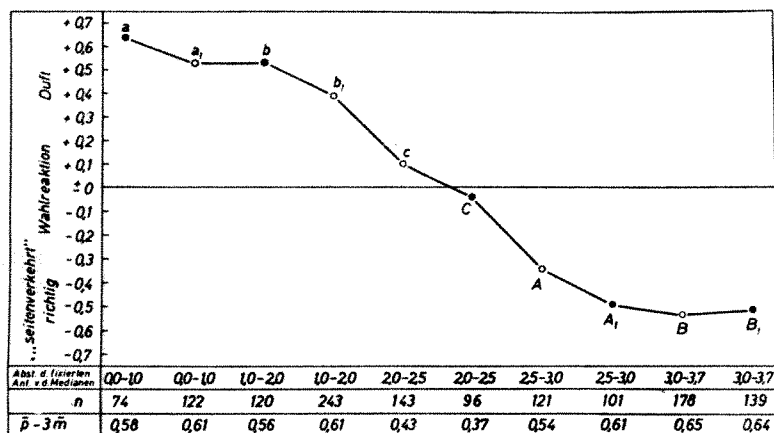


Fig. 9. Reaction curve of bees with one antenna fixed on the opposite side (with different-sized median distances) while the other antenna remains movable. Scent from one side (0.002 ml. oil of anise). Distance of the fixed antenna from the median line is given in mm. ●, Values for those which received the scent on the side without antenna; ○, values for those which received the scent on the antenna side

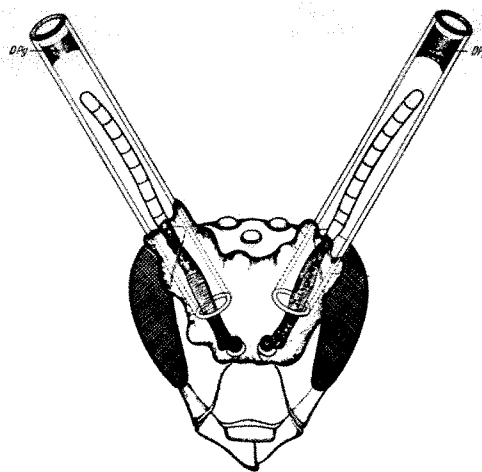


Fig. 10. Bee head with glass tubes slipped over the antennae. The scapus-head joint is blocked. *DPg*, Mixture of liquid paraffin with scent

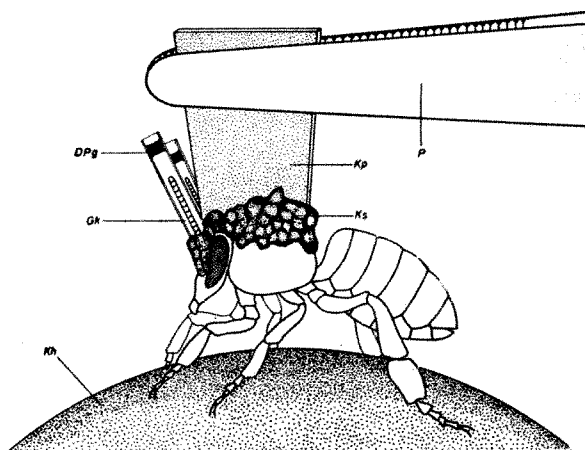


Fig. 11. Experimental bee stuck on to the cork hemisphere. Only a part of the cork hemisphere, the 'running area', is illustrated. *DPg*, Mixture of liquid paraffin with scent; *Gk*, glass capillaries; *Kh*, cork half-sphere; *Kp*, pieces of cardboard; *Ks*, glue; *P*, pincers

recognition reactions by the two antennae. The insect in the experiment is placed on a movable cork hemisphere (diameter, 4 cm) and is held down by means of a small plate on its thorax (Fig. 11). Direction and strength of the reaction are read off by means of a pointer and a scale (Fig. 12). So long as a difference between scent concentrations in the right and the left capillaries can be perceived, a directed run towards the more strongly scented side occurs. That means that the hemisphere turns exactly opposite to the direction in which the animal would turn if it were not glued down. Therefore, if the higher concentration is found in the right capillary, then the animal tries to compensate for the difference of concentration by turning to the right; that results in the cork hemisphere being turned to the left.

The lower concentration, 'initial' concentration, is held constant in all experiments. The 'test' concentration is varied for each test (a turn of 90° is given the absolute value of 1).

With high initial concentrations (1/10,000) the osmotropotactic ability to discriminate different scent concentrations is of the ratio 1 : 10 (Fig. 13). The change to

osmoklinotaxis occurs gradually at this concentration level (Fig. 13, curve *a*). With low initial concentrations (1/100,000), the ability to analyse different scent concentrations is at a ratio of 1:2.5 (Fig. 14). Here the change to klinotactic orientation occurs suddenly (Fig. 13, curve *a*). Therefore the osmotropotactic threshold is not an absolute value, but rather it is a variable depending on the initial concentration. This is biologically significant for the evaluation of different scent zones through which a bee moves when it is approaching a scent source. It is especially shown in these experiments that the olfactory perception of scent differences can operate basically without anemotaxis. In Nature, of course, anemotaxis works along with osmotropotaxis.

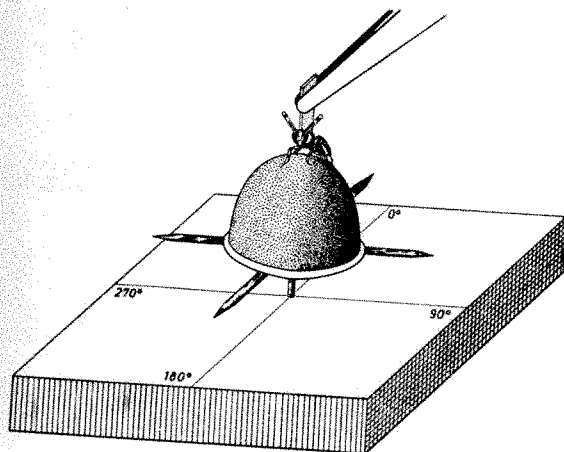


Fig. 12. Representation of the experimental arrangement for determining the osmotropotactic threshold in the scent field. The cork hemisphere (3.1 g weight) can be turned almost without resistance. With the help of the attached pointer, the direction and the strength of the turning tendency per unit of time can be read off. A turn of 90° of the cork hemisphere—the direction does not matter—gives the relative value of 1

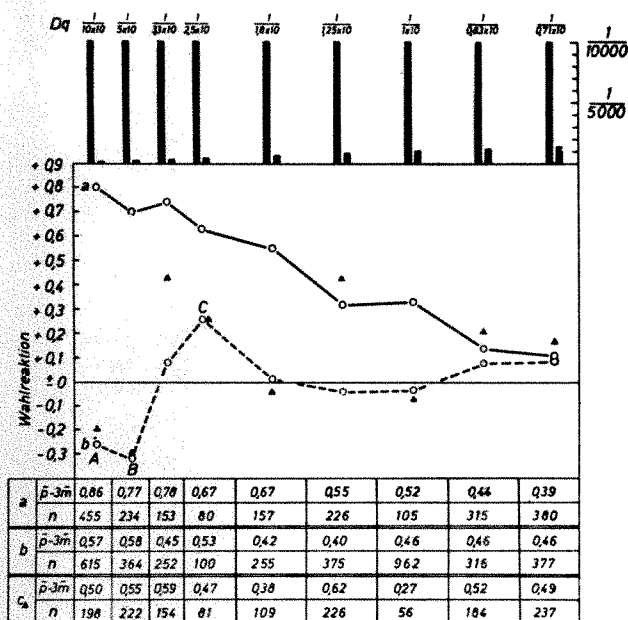


Fig. 13. The determination of the osmotropotactic threshold with high initial concentration (1/10,000). Scent oil: methylheptenone. The black columns give the concentration ratios in both capillaries: *a*, values from 0-3 min calculated from the beginning of the experiment; *b*, corresponding values in the 5-10 min period; *c*, Δ , values of 3-5 min. In the 3-5 min period the osmotropotactic reaction ends. The osmotropotactic threshold with high initial concentrations is at a concentration ratio of 1:10 between both antennae. *Dq*, Scent gradient quotient

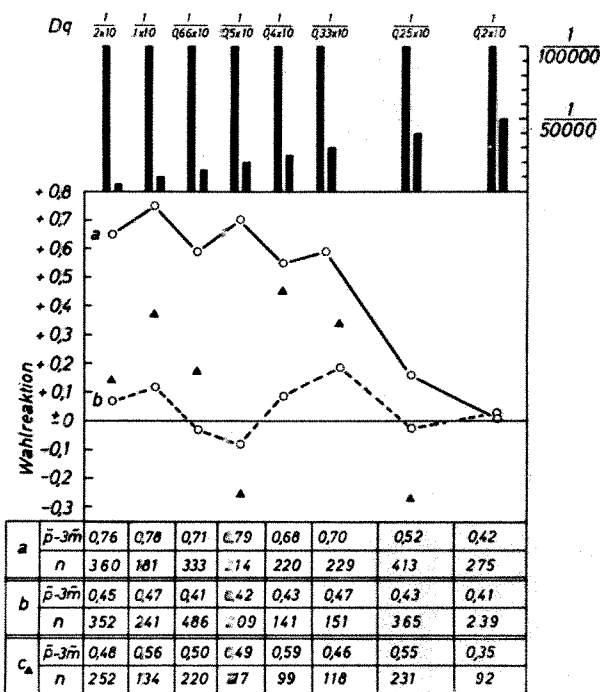


Fig. 14. Determination of the osmotropotactic threshold with low initial concentration (1/100,000). Scent oil: methylheptenone. The osmotropotactic threshold with lower initial concentrations is at a concentration ratio of 1:2.5 between the antennae. The remaining explanation is the same as for Fig. 13

Noted also was that the determination of thresholds can be carried out very well on the basis of the osmotropotaxis (Table 2).

Table 2. DETERMINATION OF THE LEVEL OF METHYLHEPTENONE AND BROMSTYROL

For both substances the threshold is at a concentration of 1:200,000

Concentration	Methylheptenone		Bromstyrol	
	Scent choices (%)	0-5 min after releasing the scent (No.)	Scent choices (%)	0-5 min after releasing the scent (No.)
1:50,000	83.0	697	90.5	154
1:100,000	88.5	637	85.5	513
1:200,000	73.4	672	71.0	888
1:300,000	50.8	850	58.1	931

These experiments have demonstrated that osmotropotaxis together with osmoklinotaxis are of vital importance in the rich mosaic of orientation behaviour of bees.

I thank Springer-Verlag (Heidelberg) for permission to reproduce the illustrations which were published in *Naturwissenschaften* (50; 1963) and *Z. Vergl. Physiol.* (48; 1964). This work was supported by Deutsche Forschungsgemeinschaft and the Rockefeller Foundation.

- Lindauer, M., *Fortschritte Zool.*, **16**, 57 (1963).
- Warnke, G., *Z. Vergl. Physiol.*, **14**, 121 (1931).
- Hartung, E., *Z. Vergl. Physiol.*, **22**, 119 (1935).
- Schanz, M., *Z. Vergl. Physiol.*, **35**, 353 (1953).
- Valentine, J. M., *J. Exp. Zool.*, **58** (1931).
- Flügge, Ch., *Z. Vergl. Physiol.*, **20**, 463 (1934).
- Murr-Danielczik, L., *Z. Vergl. Physiol.*, **11**, 210 (1930).
- Hierholzer, O., *Z. Tierpsychol.*, **7**, 588 (1950).
- Barrow, W. M., *J. Exp. Zool.*, **4**, 515 (1907).
- Kellogg, V. L., *Biol. Bull.*, **12**, 152 (1907).
- Wigglesworth, V. E., and Gillat, J. D., *J. Exp. Biol.*, **11**, 120 (1934).
- Stein-Beling, J. v., *Biol. Zbl.*, **54**, 147 (1953).
- Danzer, A., *Z. Vergl. Physiol.*, **39**, 76 (1956).
- Schwinck, I., *Z. Vergl. Physiol.*, **37**, 19 (1954).
- Schwinck, I., *Z. Vergl. Physiol.*, **37**, 439 (1955).
- Steiner, G., *Naturwiss.*, **40**, 514 (1953).
- Steiner, G., *Naturwiss.*, **41**, 287 (1954).
- Lindauer, M., and Martin, H., *Naturwiss.*, **50** (1963).
- Martin, H., *Z. Vergl. Physiol.*, **48**, 481 (1964).

LETTERS TO THE EDITOR

RADIO ASTRONOMY

Sky Brightness at 14.1 Mc/s

KNOWLEDGE of the intensity-frequency relation of extraterrestrial radio emission has been obtained from scaled aerial experiments at frequencies higher than 20 Mc/s (refs. 1 and 2) and from ground-based or satellite-borne radiometers operating below 10 Mc/s (refs. 3, 4 and 5). The low-frequency observations, whether ground-based or satellite-borne, are affected by the ionosphere. The consequence of these ionospheric effects has resulted in difficulties being experienced when reconciling the observations in frequency regions below 10 Mc/s with those above 20 Mc/s.

It has now been possible to extend the scaled aerial measurements of sky temperature down to a frequency of 14.1 Mc/s. The present sunspot minimum resulted in low values of the ionospheric critical frequencies, allowing unhampered night-time observations at this frequency. The frequency of the present observations is close to those used in satellite experiments of Hartz⁴ and Smith⁵, which provide good values of relative intensities below 10 Mc/s. No highly reliable absolute values of sky temperature were provided in these experiments. By joining the satellite results with our present 14 Mc/s results and our previous scaled aerial measurements, a curve of the sky brightness-frequency dependence is obtained for the frequency region 1–100 Mc/s.

The 14.1 Mc/s observations were made with a low-resolution aerial directed at the zenith ($\delta = -34^\circ$) which contains, at different times, the galactic centre, the disk and the south galactic pole. At 0800 and 1700 h R.A. 60 per cent of the radiation received originated from galactic latitudes $-20^\circ < b < 20^\circ$, while no radiation was received from this belt between 2130 and 0400 h R.A. The south galactic pole was in the centre of the beam at 0040 h R.A.

The aerial used was scaled from those previously used by us at frequencies 20, 30 and 85 Mc/s. An additional result was obtained at the frequency of 48.5 Mc/s.

The aerial consisted of a single half wave-length folded dipole suspended 0.25λ above a continuous mesh screen a wave-length square in area. The receiver was a switched radiometer using a B40B communications receiver preceded by a low-noise pre-amplifier of 75 Ω characteristic impedance. Calibration, which consisted of rematching the aerial and replacing it by a noise generator, was performed each hour. The noise generator used consisted of two CV2398 noise diodes in parallel, working into a 75 Ω load. The maximum temperature available from this generator was 80,000° K. This is well below the sky brightness temperature at 14.1 Mc/s. It was thus necessary either to amplify the noise signal or else attenuate the sky signal. Both methods were tried and found satisfactory. In practice, a 9 dB calibrated attenuator was inserted between the aerial and the receiver. The accuracy of the noise calibration was checked by comparing two different generators. The two systems reproduced calibrations to within 2 per cent.

Ionospheric effects are serious obstacles to reliable absolute measurements at this frequency. The two separate effects that need to be considered are absorption in the F layers and the 'iris' effect. All observations were carried out at night, thus avoiding D layer absorption. Absorption in the F layers has been theoretically investigated by Ellis⁶, using a model based on thermal loss by collision of electrons with ions and neutral atoms. The theoretical results obtained agreed with extinction curves

determined by Mitra and Shain⁷ and Steiger⁸ at 18 Mc/s. From our own results and ionospheric data from Camden, 15 km distant, it was possible to plot an extinction curve for each hour of R.A. At least five calibrated measurements were available for each hour spaced over 6 h local time. During the observing period, December 1964 to July 1965, the night-time values of f_oF_2 were almost always less than 4 Mc/s. This limited the extent of the extinction curves. For $f_oF_2 = 4$ Mc/s, absorption was observed to be less than 0.1 dB, agreeing with theory. The 'iris' effect produces total reflexion of galactic emission for zenith angles:

$$\chi \geq \cos^{-1} \frac{f_o F_2}{14.1 \text{ Mc/s}}$$

At such angles the Earth temperature of 300° K is reflected into the aerial beam. A calculation was made of the attenuation due to the iris effect and it was found to be negligible for f_oF_2 , less than 6 Mc/s.

In addition to correcting for the ionosphere an allowance was made for losses in the aerial system which amounted to 0.1 dB in the matching transformer and 0.1 dB in the balun. The mesh reflecting screen was assumed to be perfect.

Fig. 1 shows the observed sky temperatures as a function of right ascension for $\delta = -34^\circ$, after the foregoing corrections had been made. We note that the minimum occurs at 0400 with $T_B = 110,000^\circ$ K. At 0040 h R.A., when the south galactic pole is in the main beam, $T_B = 125,300^\circ$ K. Because of the low resolution of the aerial, temperatures observed when there is a sharp gradient of sky temperature in the main beam have little meaning and thus we have omitted results from 1600 h to 2000 h R.A. Overall accuracy is better than ± 7 per cent.

Fig. 2 shows the present flux measurement for the south polar region, together with other scaled aerial values and the two satellite observations. Absorption in ionized hydrogen regions is a minimum in the direction of the galactic poles, and thus Fig. 2 gives a good estimate of the synchrotron emission spectrum. We note that at 16 Mc/s the temperature spectral index β , ($T \propto \lambda^\beta$), has fallen from its high frequency value of 2.65 to 2.55 ± 0.15 .

Some caution needs to be observed when interpreting these results, since the observed sky temperatures include an isotropic extragalactic component superimposed on the galactic emission. The spectrum of this extragalactic component is thought to be steeper and thus would dominate at low frequencies. Using the scaled aerial results it should be possible to separate the galactic and extragalactic components if the two components

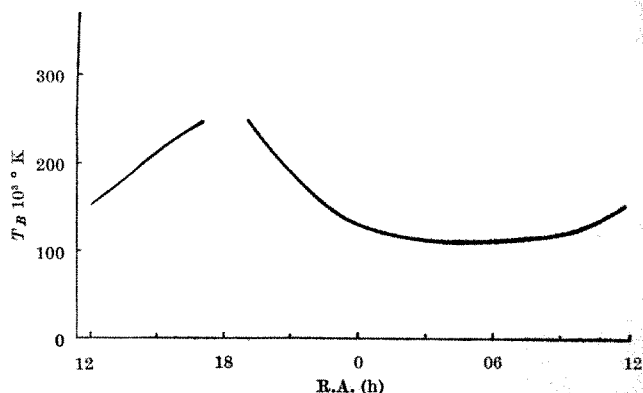


Fig. 1. Observed sky temperatures at 14.1 Mc/s, $\delta = -34^\circ$

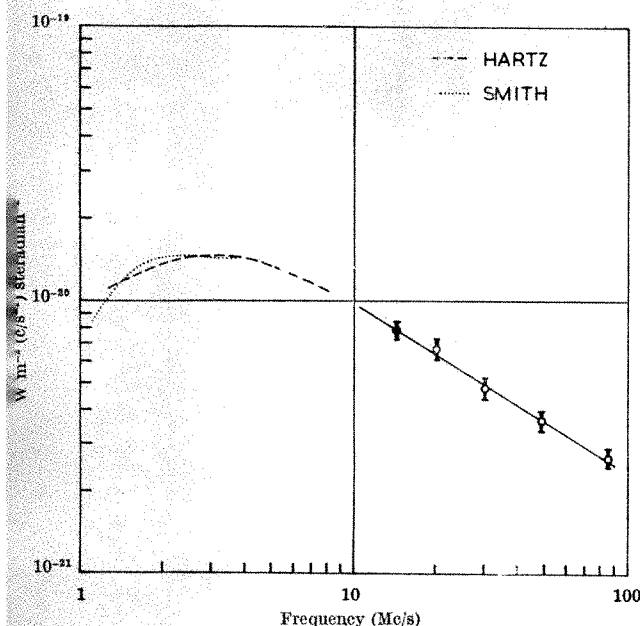


Fig. 2. Brightness-frequency relation. Scaled aerial results above 10 Mc/s, satellite results below 10 Mc/s

have differing spectra. The separation will be discussed by us elsewhere.

We are now making a new series of observations with scaled aeriels of medium resolution.

K. W. YATES
R. WIELEBINSKI

Cornell-Sydney University Astronomy Centre,
School of Electrical Engineering,
University of Sydney, Australia.

- ¹ Turtle, A. J., Pugh, J. F., Kenderdine, S., and Pauliny-Toth, I. I. K., *Mon. Not. Roy. Astro. Soc.*, **124**, 297 (1962).
² Wielebinski, R., and Yates, K. W., *Nature*, **205**, 581 (1965).
³ Ellis, G. R. A., *Nature*, **196**, 1079 (1962).
⁴ Hartz, T. R., *Nature*, **203**, 173 (1964).
⁵ Smith, F. G., *Ann. d'Astrophysique*, **27**, 819 (1964).
⁶ Ellis, G. R. A., *Austral. J. Phys.*, **16**, 411 (1963).
⁷ Mitra, A. P., and Shain, C. A., *J. Astro. Terr. Phys.*, **4**, 204 (1953).
⁸ Steiger, H., *J. Geophys. Res.*, **66**, 51 (1961).

ASTROPHYSICS

Pulsation Periods of General Relativistic Objects

THE pulsation period of the lowest radial mode for a spherical homogeneous object in Newtonian theory is¹ (if $\Gamma_1 = \text{const}$):

$$\tau = 2\pi[4\pi G\rho(\Gamma_1 - 4/3)]^{-1/2}$$

and if $\Gamma_1 - 4/3 > 0$, the period may vary over a wide range depending on the density.

However, a spherically symmetric homogeneous object in the post-Newtonian approximation has a period of²:

$$\tau = 2\pi c \left\{ 4\pi G\varepsilon(\Gamma_1 - 4/3) \left[1 - (GM/c^2 R) \left(\frac{10}{7} \Gamma_1 - 1 \right) \right] \right\}^{-1/2}$$

where ε is the total energy density. From this equation it is easily seen that there will be a minimum period even if $\Gamma_1 - 4/3$ is always positive and finite. Substituting:

$$\varepsilon = 3Mc^2/4\pi R^3$$

we obtain:

$$\tau = 2\pi \left\{ GMR^{-3}(3\Gamma_1 - 4) \left[1 - (GM/c^2 R) \left(\frac{10}{7} \Gamma_1 - 1 \right) \right] \right\}^{-1/2}$$

A very massive star (superstar) has a Γ_1 which is a function of mass and is approximately 4/3. The quantity $\Gamma_1 - 4/3 = \alpha(M/M_\odot)^{-1/2}$ (refs. 3 and 4). The minimum period will therefore occur at a radius of:

$$R = 76 \times GM/[63(\Gamma_1 - 4/3)c^2]$$

and:

$$\begin{aligned} \tau_{\min} &= (304 \times \pi GM/63c^3) (76/189)^{1/2} (\Gamma_1 - 4/3)^{-2} \\ &= 4.73 \times 10^{-5} \alpha^{-2} (M/M_\odot)^2 \text{ sec} \end{aligned}$$

or:

$$\tau_{\min} = 1.50 \times 10^{-12} \alpha^{-2} (M/M_\odot)^2 \text{ yr}$$

A superstar of $10^6 M_\odot$ would therefore have a period of (for $\alpha = 1.4$) 0.77 yr. If quasi-stellar sources are very large massive objects with fluctuations of the order of years, then one would not expect them to be much larger than $10^6 M_\odot$ because of the quadratic mass dependence of the periods. W. A. Fowler has recently pointed out that rotation may enable one to have smaller periods and therefore smaller radii.⁵

This general relativistic effect of a minimum period also manifests itself in neutron stars² and white dwarfs, although in these cases the models are not homogeneous and Γ_1 is not constant.

I thank A. D. Code for his advice.

JAMES P. WRIGHT

Smithsonian Astrophysical Observatory
and
Harvard College Observatory,
Cambridge, Massachusetts.

¹ Rosseland, S., *The Pulsation Theory of Variable Stars* (Clarendon Press, Oxford, 1949).

² Tsuruta, S., Wright, J. P., and Cameron, A. G. W., *Nature*, **206**, 1137 (1965).

³ Chandrasekhar, S., *An Introduction to Stellar Structure* (Dover, New York, 1937).

⁴ Fowler, W. A., and Hoyle, F., *Astrophys. J., Supp.*, No. 91, 201-320 (1964).

⁵ Fowler, W. A., Lecture Notes of 1965 Varenna Summer School on High Energy Astrophysics. Fowler, W. A., has also calculated the periods of massive stars.

Angular Momenta of Eclipsing Binaries and the Fission Theory of their Origin

IN a new development of the fission theory of the origin of close binary stars^{1,2} I showed that rotational instability would occur during the pre-main sequence contraction of rotating stars with no internal magnetic field. The theory predicted the observed mass range for contact binaries of W-Ursae Majoris type with satisfactory accuracy, and also gave the observed variation of angular momentum with mass for these systems. I now wish to show that the theory also predicts the observed relation between angular momentum and mass for all the close binary systems.

The rotational instability which gave rise to the formation of binary systems was due to the different behaviour of convective and radiative regions of stars. When the star is fully convective, as it is during early stages of contraction³, it rotates uniformly, and as the angular velocity increases the star spins off mass at the equator, and the effect of rotation is small over the bulk of the star. When the star begins to develop a radiative core, the rotation is no longer uniform as each element of the radiative core contracts, conserving its angular momentum. The parameter that measures the effect of rotation is:

$$\alpha = \frac{\Omega^2}{2\pi G \rho_c} \quad (1)$$

where Ω is the angular velocity and ρ_c the density at the centre of the star. When the star was fully convective, then with uniform rotation and centrifugal force balancing gravity at the surface, $\alpha = 0.04$ (ref. 4). With the development of the radiative core α varies like $\rho_c^{1/3}$ and so it will reach the critical value for instability, 0.187 (ref. 5), when ρ_c has increased by a factor 100.

The increase in ρ_c is due to two effects: the changing degree in central condensation due to the transition from convective energy transport, $\rho_c/\bar{\rho} = 6$, to radiative energy transport, $\rho_c/\bar{\rho} \approx 24$ for massive stars and 54 for small stars, and the increase in the mean density $\bar{\rho}$ due to the decrease in radius. As the readjustment in internal

structure takes place during a small change in radius, the central density will have increased to its critical value when the radius of the star has decreased by a factor of 2.3 for small stars and 3 for large stars.

The angular momentum of the star at the onset of rotational instability can be estimated. With centrifugal force balancing gravity, at the end of the fully convective stage the angular momentum is:

$$H_H = K G^{1/2} M^{3/2} R_H^{1/2} \quad (2)$$

where R_H is the radius of the star and $K^{1/2} R_H$ its radius of gyration. For a fully convective star $K \approx 0.13$ (ref. 4). During the fully convective phase it is known that the star has an approximately constant surface temperature $\approx 4,000^\circ \text{K}$. The radius R_H at which convection begins to die away can then be computed by equating the energy carried by radiation to the release of internal energy during the collapse of a fully convective star. This gives:

$$\lim_{r \rightarrow 0} \left[-\frac{4ac T^3}{3 \kappa \rho} \frac{dT}{dr} \right] = \frac{7 LP_c}{6 GM^2} R \quad (3)$$

where T is the temperature, κ the opacity, P_c the value of the pressure at the centre, a the radiation constant, c the velocity of light, L the luminosity $= \pi a c R^2 T_e^4$ (where $T_e \approx 4,000^\circ \text{K}$). With the opacity κ known as a function of pressure and density, and the temperature and pressure known in terms of the mass and radius for a fully convective star⁶ (a polytrope $n=1.5$) this is an equation to determine R_H , and hence from equation (2) H_H is known. Knowing the decrease in radius to the onset of instability we can get a lower bound on the angular momentum at the onset of instability, H_I , by neglecting the growing radiative core; an upper bound is obtained by assuming all the star is radiative. The actual value H_I must be somewhere between these limits, and we take the arithmetic mean^{1,2}. Since the decrease in radius to the onset of instability is at most by a factor of 3, and as the angular momentum varies like $R^{1/2}$ (equation 2) the lower bound on H_I is only less than H_H by at most 0.23 in the logarithm.

In Fig. 1 the predicted relation between angular momentum and mass of rotating stars at the onset of fissional instability is plotted, and also the observational values for all the close binary systems with spectroscopically determined mass ratios that are given in Kopal and Shapley's catalogue⁷, except for the few that are too massive to fit on our diagram. The agreement is very satisfactory.

The alternative theory of binary star formation, the capture theory, is unable to explain the observed correlation between mass and angular momentum, since the capture process is a chance phenomenon due to the close approach of two bodies in the presence of a third. There is no reason to suppose that this would give anything

other than a random distribution of masses and angular moments. The observed relation between mass and angular momentum lends support to the fission theory, and it is very satisfactory that the observations and predictions agree so well.

IAN W. ROXBURGH

High Altitude Observatory,
Boulder, Colorado,
and
King's College,
University of London.

¹ Roxburgh, I. W., *Astrophys. J.*, **70**, 330 (1965).

² Roxburgh, I. W., *Astrophys. J.* (in the press).

³ Hayashi, C., *Pub. Astro. Soc. Japan*, **13**, 450 (1961).

⁴ Monaghan, J., and Roxburgh, I., *Mon. Not. Roy. Astro. Soc.* (in the press).

⁵ Jeans, J., *Astronomy and Cosmogony* (Camb. Univ. Press, 1929).

⁶ Chandrasekhar, S., *Stellar Structure* (Chicago Univ. Press, 1939).

⁷ Kopal, Z., and Shapley, M., *Jodrell Bank Annals* (1956).

PHYSICS

Laser-induced Damage in Natural White Diamond

A PREVIOUS report¹ has described laser-induced damage in optically transparent materials such as glass, alkali halides, CaF_2 , MgO , sapphire and quartz; we wish to report the observation of this effect in natural white diamond. Intense, 0.3 joule, 10 MW pulses of 6943 Å radiation from a kryptocyanine Q-switched ruby were focused with a 5-cm focal-length lens on to the polished surfaces of the specimens. The dimensions of the specimens were very much smaller than the focal length of lens, thus ensuring that the power density of 10^9 W/cm^2 incident on the surface was constant throughout the specimen. The damage on the front surface was characterized by a seared mark apparently consisting of black carbon (Fig. 1), which indicated that a high surface temperature was attained. The rear surface damage was characterized by extensive fractures and cracking (Fig. 2). Because of the difficulty in sectioning diamond, it was not possible to determine the depth of the physical damage; however, as this was not observed on the front surface of the specimens it seems apparent that it is characteristic of the rear surface only. This is consistent with the observed damage in other optically transparent materials¹.

It has been proposed¹ that this damage is a result of acoustic phonons which travel in the forward direction through the material. These phonons are generated by stimulated Brillouin scattering of the laser light, and this process is similar to Raman laser action with the molecular vibration replaced by an acoustic wave. Other

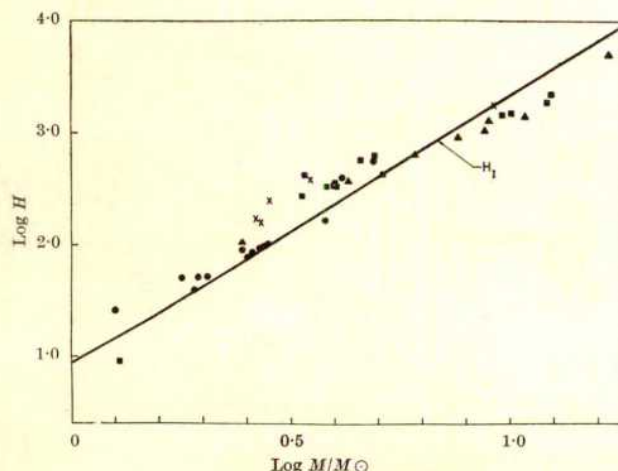


Fig. 1. Angular momentum of close binaries

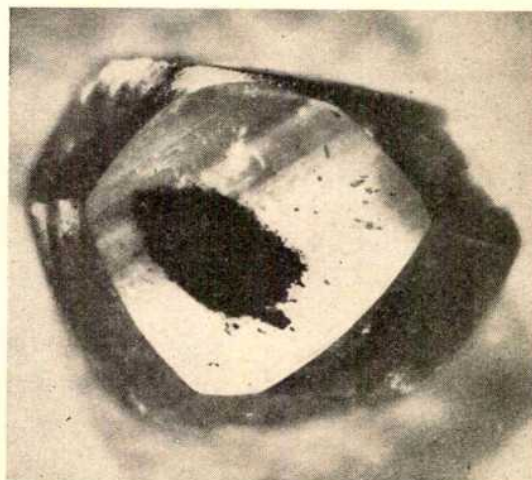
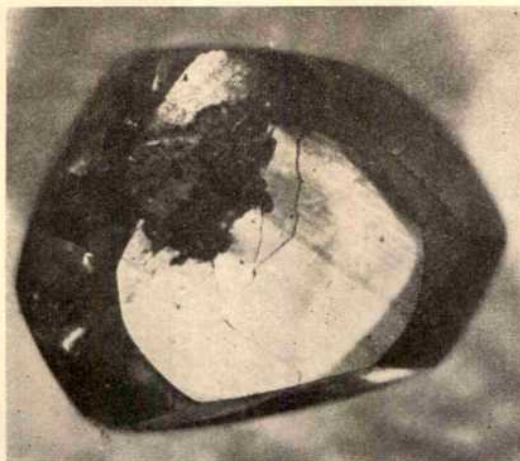


Fig. 1. Front surface ($\times 16$)

Fig. 2. Rear surface ($\times 16$)

damage mechanisms, such as direct heating from optical absorption and the interaction of light with material boundaries, were eliminated by detailed experiment. The acoustic phonon damage mechanism is supported by direct measurements of the frequency of the scattered radiation from the stimulated Raman process for quartz and sapphire².

The frequency of the acoustic phonon wave f is² $f = \frac{2f_1 v n}{c}$ where f_1 is the frequency of the incident light which is about 4×10^{14} c/s for ruby light, v is the velocity of sound in the material of refractive index n , and c is the velocity of light. For diamond, n is 2.4, and from measurements of the compressibility³ v is calculated as 1.8×10^6 cm/sec, whence f is about 120 Gc/s. This compares with 60 Gc/s for sapphire and 12 Gc/s for glass. The power in the acoustic wave is found from the ratio of the phonon to photon energies and is about 10^{-4} of the incident power or about 100 kW/cm² in these experiments.

These experiments were performed in an attempt to use a pulsed solid-state laser for the drilling of small holes in diamond. The results which we obtained show that the desired holes were not obtained because of the nature of damage mechanism.

We thank the directors of International Research and Development Co., Ltd., and the manager of the NELAS Division of the International Research and Development Co., Ltd., and Thermal Syndicate, Ltd., for their support of this work.

P. WHITEMAN
G. W. WILSON

International Research and Development
Co., Ltd.,
Newcastle upon Tyne, 6.

¹ Giuliano, C. R., *App. Phys. Letters*, **7**, 5, 137 (1964).

² Chiao, R. Y., and Townes, C. H., *Phys. Rev. Letters*, **12**, 21, 592 (1964).

³ *American Institute of Physics Handbook*, second ed., 3 (McGraw-Hill, 1963)

Re-entrant Hysteresis Loop and Multistable Ferrites

It is well known that certain ferrites, especially CoFe ferrite¹, become magnetically anisotropic when heated in a magnetic field. This treatment results in an increased remanence ratio (I_{rem}/I_{sat}) and a square hysteresis loop. The explanation in terms of an ionic diffusion ordering process has been well developed². It is also well known³ that annealing in a unidirectional field results in a loop which appears displaced along the H -axis, but no adequate explanation has been given for the phenomenon in these materials. Glaister and Viney⁴ have reported re-entrant

hysteresis loops in these materials, and offered a tentative explanation for this phenomenon.

We have now prepared ferrite toroids with multistable remanent states in addition to re-entrant loops, and propose an explanation both for these effects and for the asymmetry resulting from a unidirectional field anneal. The material comprises $\text{Co}_{0.15}^{2+}\text{Fe}_{0.85}^{3+}\text{Fe}_2\text{O}_4$ and is sintered under conditions that result in slight oxidation of Fe^{2+} content; this deficiency yields cation vacancies and thus greatly increased mobility⁵ for the Co^{2+} ions responsible for anisotropy. On cooling, these ferrites show constricted or 'perminvar' loops. The samples are annealed in alternating fields of amplitude just sufficient to achieve technical saturation at 200° C and slowly cooled with the field applied. A very square re-entrant loop may then be observed (Fig. 1a) with a display system in which the magnetizing field is sensitive to the rate of change of flux⁴.

The flux reversal (switching) time of a square loop ferrite is equal to the quotient of the average distance traversed by the domain walls and the wall velocity. Thus the number of domain walls that participate in flux reversal is proportional to the reciprocal product of the domain wall velocity and the switching time (or wall mobility and switching constant). An estimate of this wall mobility using the treatment of Menyuk and Goodenough⁶ ($3,400 \text{ cm sec}^{-1} \text{ Oe}^{-1}$) and measurement of the switching constant ($500 \mu\text{s Oe}$) for a toroid with 4-mm wall section suggests that it is unlikely that more than one wall is involved in the flux reversal. Pulse measurements confirm that the rate of change of flux reversal is almost constant so that the comparison is valid.

Although samples with pronounced re-entrance are bistable and with slowly varying fields switch directly from one near-saturation state to the other, it is possible by applying single-shot pulses of about 2 μs duration to bring the domain wall to a central position giving zero net remanence. If the sample is then annealed in this state⁷ at 200° C a loop of the form shown in Fig. 2a is obtained; the zero-magnetization state has become stabilized and a field of 26 Oe in excess of the wall coercive field (20 Oe) is required to free the domain wall. Such cores have three stable states. Evidently the component of the field within the Bloch domain wall directed along the toroid axis has resulted in localized anisotropy with an easy axis perpendicular to the general circumferential direction.

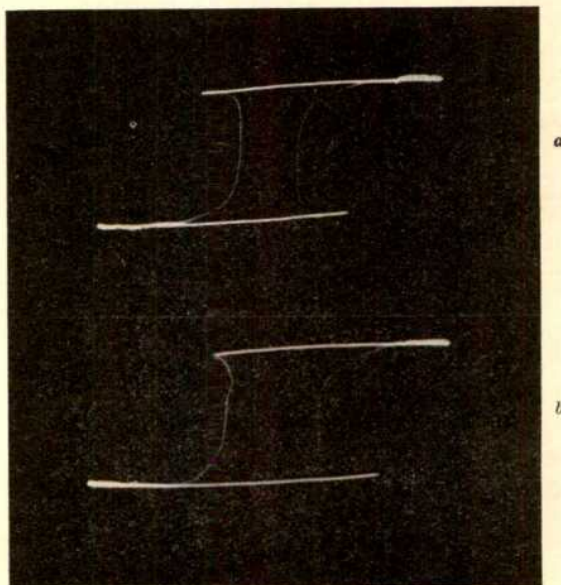


Fig. 1. Cobalt-ferrous ferrite. (a) Annealed with alternating field giving re-entrant loop; (b) annealed with unidirectional field giving loop re-entrant in one sense only. Applied 50 c/s field 35 Oe pk

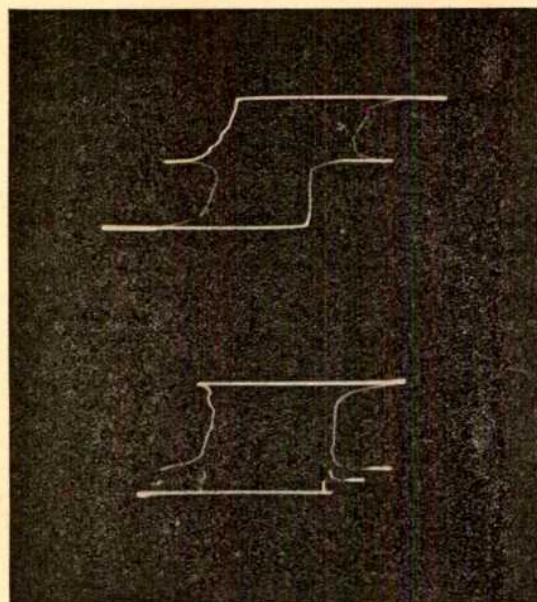


Fig. 2. Cobalt-ferrous ferrite. (a) Annealed in zero net remanence state giving tristable loop, and (b) annealed in intermediate remanence state. Applied 50 c/s field 17 Oe pk

By annealing a wall in an off-centre position, that is, with remanent magnetization between zero and full remanence, the relative position of the spikes on the two sides of the loop indicates whether the domain wall always moves in the same direction or oscillates to and fro. With the core geometry used (height greater than radial width) the spikes occur at the same flux-level (Fig. 2b) so that the wall evidently oscillates to and fro. Since cylindrical walls tend to move radially outwards, the field being strongest at the inner edge of the toroid, the movement is probably axial. Fig. 2b shows the loop resulting from the accidental annealing of two walls near one surface.

The same explanation holds for re-entrance; the wall is evidently not eliminated in the 'saturation' state and merely comes to rest near the sample surface. This is confirmed by the fact that excessive annealing fields which would tend to drive the wall out of the sample are found to reduce the re-entrance. The re-entrance of a partially annealed sample may be quite effectively increased by alternately switching from one remanent state to the other by the application of short pulses of alternate polarity (for example, 1-ms pulses with a pulse repetition frequency of 50 c/s). Thus annealing takes place substantially in the absence of an externally applied field. Further, when a unidirectional field is used for annealing, the loop is re-entrant on one side only (Fig. 1b). This would explain the asymmetry observed by previous workers using conventional display systems, for example, a ballistic galvanometer.

We thank G. W. Edwards for help with the experimental work and J. R. Halliwell for carrying out pulse measurements.

R. M. GLAISTER

Standard Telecommunication Laboratories, Ltd.,
Harlow, Essex.

E. W. LEE

Department of Physics, University of Sheffield.

¹ Wijn, H. P. J., Van der Heide, H., and Fast, J. F., *Proc. Inst. Elec. Eng.*, **104**, B, 412 (1957).

² Slonczewski, J. C., in *Magnetism*, edit. by Rado, G. T., and Suhl, H., **1**, 205 (Academic Press, New York, 1963).

³ Smit, J., and Wijn, H. P. J., in *Ferrites*, 312 (Cleaver-Hume Press, 1959).

⁴ Glaister, R. M., and Viney, I. V. F., *Proc. Intermag. Cong.*, 1965, Paper 4.1 (Inst. Elec. Electron Eng., 1965).

⁵ Iida, S., and Inoue, T., *J. Phys. Soc. (Japan)*, **17**, Supplement B-1, 281 (1962).

⁶ Menyuk, N., and Goodenough, J. B., *J. App. Phys.*, **26**, 8 (1955).

⁷ Glaister, R. M., and Lee, E. W., patent application 17043/65.

A Numerical Model of Random Packing of Spheres

A NUMERICAL approach using a digital computer has been used to construct a simple model of the unit cell in a randomly packed bed of identical hard spheres. The cell is built up by introducing spheres tangent to a central one at random positions around it. Each added sphere is accepted and its position recorded only if it does not overlap with any of the ones previously accepted. When there is no room for additional spheres, the cluster is complete and the volume of the unit cell is found. This unit is defined as the polyhedron formed by the perpendicular bisectors of the lines connecting the centre of the original sphere with its neighbours. There is some ambiguity in defining a unit cell in a random packing. The prescription used here has the justification that it is correct for the regular lattices with one sphere per unit cell such as the simple cubic, face centred cubic, and hexagonal close-packed arrangements.

Number of neighbours and cell volumes. An ensemble of about 3,000 of the clusters already described here was constructed.

Table 1. PACKING STATISTICS

<i>n</i>	Probability of <i>n</i> neighbours		Average cell volume for <i>n</i> nearest neighbours
	Touching	Within 1.35 diam.	
6	0.0054	0.0000	1.0349
7	0.0970	0.0026	0.9670
8	0.3878	0.1332	0.8924
9	0.3931	0.4316	0.8255
10	0.1137	0.3622	0.7688
11	0.0030	0.0638	0.7451
12	0.0000	0.0065	—
	$\bar{n} = 8.52$	9.37	$\bar{v} = 0.860$
	$\sqrt{\bar{n}^2 - \bar{n}^2}$	0.84	$\sqrt{\bar{v}^2 - \bar{v}^2}$

Table 1 gives the calculated frequency distributions of numbers of neighbours. In addition the average cell volume for each configuration is given, based on a unit ball diameter. Since just one ball is enclosed in the unit cell as defined here, the average solid fraction is given by:

$$f = (4/3)\pi(1/2)^3/0.860 = 0.609.$$

With the definition of the unit cell used here the proper solid fractions are obtained for the regular lattices as already mentioned.

Since packing densities between 0.61 and 0.64 have been found¹ depending on how well the bed of spheres has been shaken down, it appears that this model is describing a loose packing. This appears to be reasonable in view of the fact that no shaking down is allowed for in the process by which the ensemble of cells was constructed here.

In an experiment Bernal and Mason² found the mean number of contact points to be 8.5 counting only points in what they called total contact in a random close-packed assembly. Although this agrees superficially with the results obtained here, their distributions of numbers of contact points for both tight and loose packings were not the same as the present one. Fig. 1 shows the frequencies of various numbers of neighbours. Indeed, they observed cases with as few as four contact points. It is difficult to make a direct comparison with Bernal and Mason's results since their 'total contacts' include not only neighbours which touch the central ball but also neighbours somewhat farther out.

An attempt was made to include second neighbours in the cluster. This was done by filling in all the additional spheres that could rest without any overlapping on the stable positions determined by the nearest neighbours already found as described above. These positions were filled in random order.

Fig. 2 shows the density of ball centres as a function of distance from the centre of the cluster, both as found

here and as measured by Scott¹. The peak describing the first neighbours determined in the present calculation, which should actually be a delta function of area 8.52, is broadened in the plot to match the class width used by Scott in his measurement. It is clear from the plot that the second neighbours obtained by the process described here do not exhaust all the possibilities of nearby spheres.

Angular distribution. The angular distribution of neighbours around any given neighbour taken as a pole is shown in Fig. 3. Distributions are given for neighbours within 1.35 ball diameters as calculated here and as measured by Scott and Mader⁴. There is qualitative agreement between calculation and measurement, but the detailed shapes are not the same.

Method of calculation. The process of building up the cluster by introducing spheres at random positions around the central sphere becomes very inefficient when the cluster is nearly complete. This is because the remaining regions where neighbours can fit into the cluster become extremely narrow when the cluster is nearly complete. To speed up the calculation the actual computational scheme was modified as follows. After 250 possible neighbours were examined it was ascertained analytically whether and where there were gaps into which additional spheres could be fitted as neighbours. Each such gap was filled by inserting a ball at the centre of the gap. This introduced no discernible error since

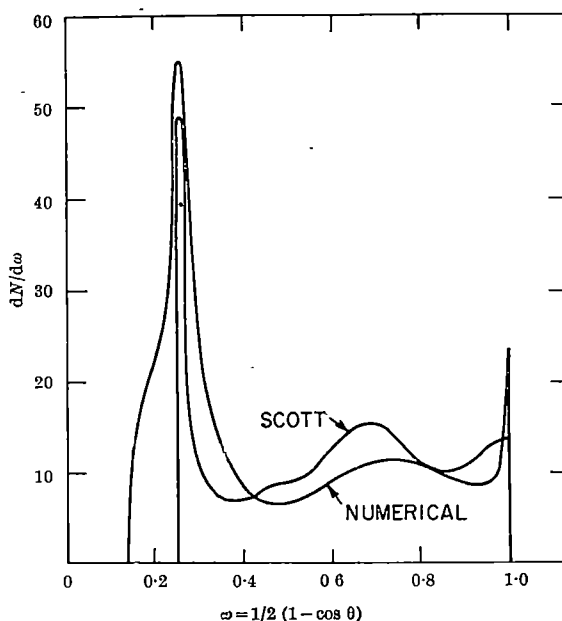


Fig. 3. Angular distribution of neighbours

after 250 tries the latitude in placement of any additional sphere or spheres is extremely small.

This work was carried out under the auspices of the U.S. Atomic Energy Commission.

M. M. LEVINE
J. CHERNICK

Brookhaven National Laboratory,
Upton, New York.

¹ Scott, G. D., *Nature*, **188**, 908 (1960).

² Bernal, J. D., and Mason, L., *Nature*, **188**, 910 (1960).

³ Scott, G. D., *Nature*, **194**, 956 (1962).

⁴ Scott, G. D., and Mader, D. L., *Nature*, **201**, 382 (1964).

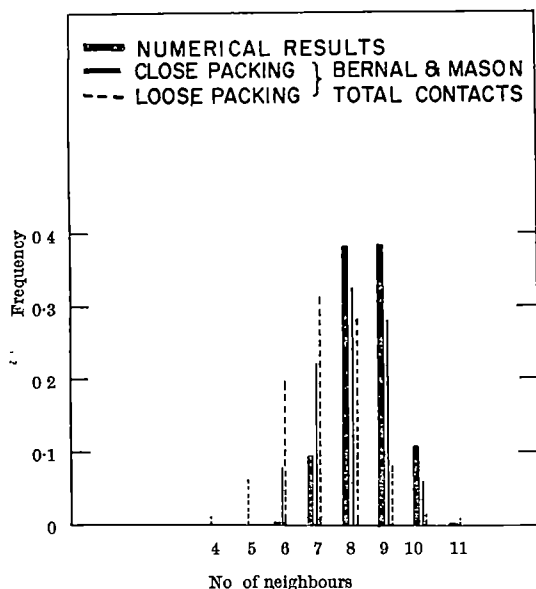


Fig. 1. Frequency distribution of nearest neighbours

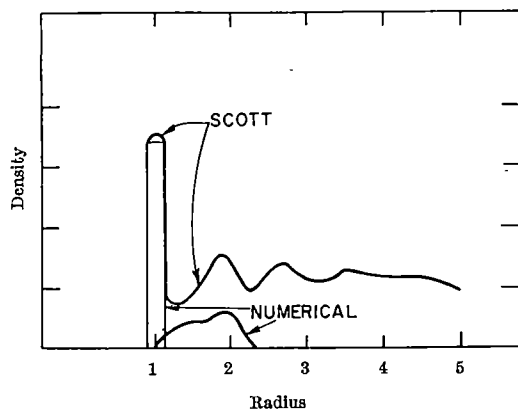


Fig. 2. Density of centres versus radial distance

METALLURGY

Slow-bend Testing of Hydrided Zirconium

THE embrittlement of zirconium by hydrogen is generally reflected by a decrease in the energy absorbed in fracture in an impact test, with a notched or unnotched specimen. Only in certain circumstances is a simultaneous reduction in ductility observed in a tensile test, and therefore the embrittlement has generally been considered attributable to high strain rates. However, some recent work we have carried out indicates that it is the actual mode of stressing—by bending—rather than the strain rate which promotes brittle fracture of hydrided zirconium in an impact test. Afterwards, slow-bend tests, both notched and unnotched, have proved useful in elucidating several of the factors which contribute to brittle fracture of zirconium. The relative unimportance of strain rate has been demonstrated by the occurrence of a brittle crack in a notched slow-bend test in which the bending to promote fracture took 48 h.

The slow-bend test also readily distinguishes between low-energy ductile failures and brittle cracks whereas the normal impact test does not. The three basic types of deformation curve obtained in slow-bend testing of hydrided zirconium are shown in Fig. 1, the deformation being taken simply as the movement of the straining head on the machine. The completely brittle specimen is typified by A which shows an elastic region I, and a plastic region II which varies slightly in extent. Once started, the crack is self-propagating, at a speed in the region of 25 m/sec, and needs no further deformation.

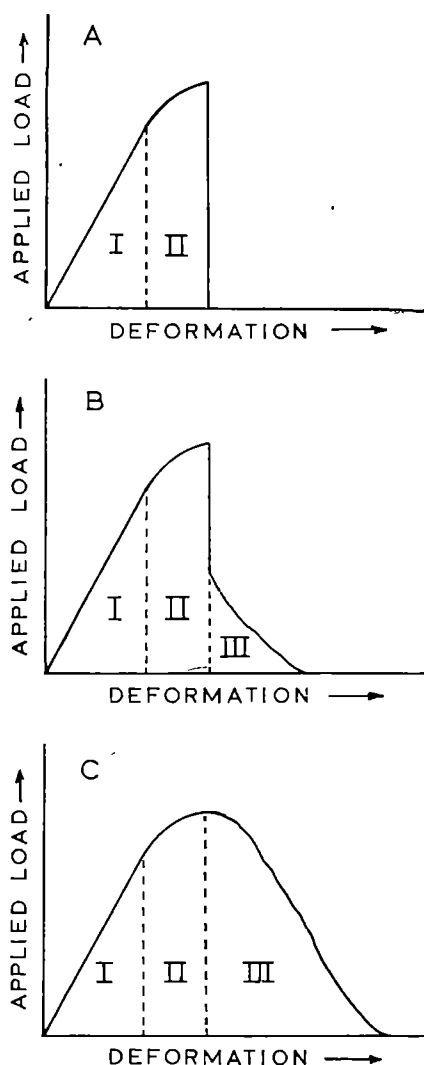


Fig. 1. Typical load-deformation curves for slow-bend tests on hydrided zirconium: A, Brittle fracture; B, low-energy ductile failure; C, high-energy ductile failure

On the other hand, a ductile specimen, as typified by C, requires continuous application of load throughout to promote propagation of a crack through the specimen, and therefore the rate of propagation is dependent on the rate of application of load. An additional region III in the curve represents the energy required for propagation.

Certain specimens give an intermediate type of curve, such as B, but the actual cracking is still ductile and requires a certain amount of further bending to cause propagation, but is preceded by a 'yield drop', during which 'ears' of plastic flow suddenly extend right across the sides of the specimen from the root of the notch, which has the effect of reducing the area of region III. Since the area under the deformation curve represents the energy required to fracture the specimen, as measured in a standard impact test, the usual impact test would not readily distinguish between a brittle specimen and a ductile one having a deformation curve of type B with an appreciable 'yield drop'.

It is interesting to note that the general shape of the load-deformation curves is similar to that of the load-time curves dynamically recorded by Fearnough and Hoy¹ during the Charpy impact testing of steels. Load-deformation curves for slow-bend tests are, however, much more readily obtained than load-time curves in a high-speed test.

Using a standard Hounsfield slow-bend specimen in the usual slow-bend jig, but attaching the latter to a hard beam testing machine, the effect of various variables on the hydrogen embrittlement of zirconium has been studied more effectively than could be accomplished by standard impact tests. The results indicate the importance of the 'yield drop' and its dependence on temperature, hydrogen content and inter-hydride spacing. The latter is a very important factor which may vary considerably with grain size and heat treatment even with a constant hydrogen content.

C. E. COLEMAN
D. HARDIE

Department of Metallurgy,
University of Newcastle upon Tyne.

¹ Fearnough, G. D., and Hoy, C. J., *J. Iron and Steel Inst.*, 202, 912 (1964).

MINERALOGY

Structure of Interstratified (Mixed-layer) Minerals

MANY papers dealing with the structure of interstratified minerals such as illite-montmorillonite, chlorite-montmorillonite, chlorite-vermiculite, etc., have been published up to the present. In describing the structure of these minerals, the terms 'random' and 'regular' have been often used, but their definition is not very clear, since the term 'regular' has been applied mostly to '1:1 regular structure', whereas the term 'random' has been used for structures other than regular. It is necessary to investigate in detail the structure of interstratified minerals.

Suppose two kinds of layers, A and B, their number, N_A and N_B ($N_A + N_B = N$), and the potential energy between two layers, $-\varphi_{AA}$, $-\varphi_{AB}$, $-\varphi_{BA}$, $-\varphi_{BB}$, assuming the nearest-neighbour interactions, that are equivalent to Reichweite $g=1$. Then the total interaction energy (E) in a one-dimensional polar structure can be expressed by statistical mechanics as follows:

$$E = -\varphi_{AA}N_A - \varphi_{BB}N_B + \{\varphi_{AA} + \varphi_{BB} - (\varphi_{AB} + \varphi_{BA})\} \cdot N_{AB}$$

N_{AB} : the number of A-B pairs.

In the foregoing expression, $\varphi_{AA} + \varphi_{BB} < \varphi_{AB} + \varphi_{BA}$ is a condition necessary for the formation of the interstratified structure. For definite values of N_A and N_B , E is determined by the value of N_{AB} . This suggests that the stability of the structure increases energetically in proportion to the number of A-B pairs. We define here W_S as the probability of finding S layer at any position and P_{ST} as the probability of finding T layer succeeding S layer.

Then N_{AB} is shown as $N_{AB} = W_{AB} \cdot N = W_A \cdot P_{AB} \cdot N$, where W_{AB} is the probability of finding an A-B pair at any position.

Thus we can calculate the maximum value of W_{AB} for two cases, $g=0$ and $g=1$.

(a) For $g=0$ (random), which is a special case of $g=1$, the following relation holds: $P_{AB} = P_{BB} = W_B$, $P_{BA} = P_{AA} = W_A$.

In this case, W_{AB} equals $W_A \cdot W_B$, and this is the only one result given for $g=0$.

(b) For $g=1$, the maximum value of W_{AB} can be mathematically shown as follows:

$$(1) W_A = W_B \quad W_{AB} = 2 W_A \cdot W_B > W_A \cdot W_B \quad (g=0)$$

$$(2) W_A \neq W_B \quad (W_A > W_B) \quad W_{AB} = W_B > W_A \cdot W_B$$

In case (1), the relation of $P_{AA} = P_{BB} = 0$, $P_{BA} = P_{AB} = 1$ is held. This implies a 1:1 regular interstratified structure, ABABABAB. . . . In case (2), the relation

of $P_{BB}=0$, $P_{BA}=1$ is held. This implies a structure having no B - B pairs, being neither 'regular' nor 'random'. In this case two arrangements, $ABAAA$ and $ABAAABA$, are equivalent in the total interaction energy, having equal numbers of A - B pairs, so it is expected that all such possible arrangements would occur with equal probability for $g=1$.

Next we put $P_{AA}=\alpha$, $P_{AB}=1-\alpha$, $P_{BB}=\beta$, $P_{BA}=1-\beta$, then an equation $\beta=K\alpha+(1-K)$, where $K=W_A/W_B$, can be derived from the relation $\sum W_S P_{ST}=W_T$. Using this

equation all the interstratified structures consisting of two kinds of layers can be plotted on the graph shown in Fig. 1.

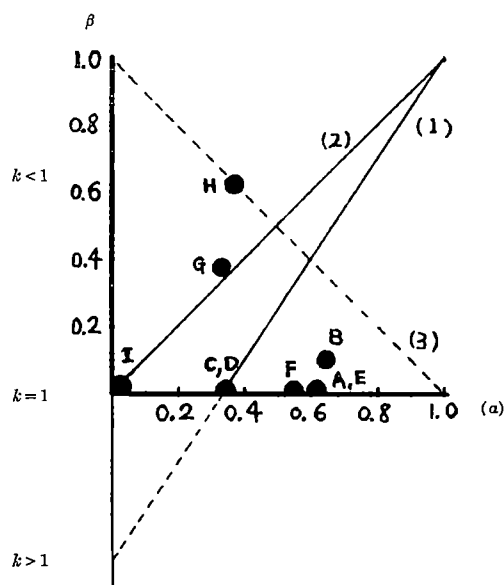


Fig. 1. The interstratified structure and some examples of illite-montmorillonite interstratified minerals examined

For example, the structure consisting of $W_A=0.6$, $W_B=0.4$, that is, $K=3/2$, is plotted on line (1), and $W_A=0.5$, $W_B=0.5$, on line (2). The random structures are on the diagonal dotted line (3). From the above view of interaction energy, it is expected that many interstratified minerals are distributed along or near the axes of co-ordinates.

In order to confirm this hypothesis, it is necessary to determine the values of W_A , W_B , α and β in a given specimen. Observed (00 ζ) reflexions of X-rays, where ζ is not an integer, were compared with mathematical intensity distribution due to one-dimensionally disordered structure. The equation used, which was presented by Kakinoki and Komura^{1,2}, is as follows:

$$I(\xi\eta\zeta) = N \bar{V}^2 + \sum_{m=1}^{N-1} (N-m) \text{Spur } \mathbf{V} \mathbf{F} \mathbf{Q}^m + \text{conj.}$$

\mathbf{V} is a matrix of layer-form factors V_S , \mathbf{F} is a matrix of W_S , \mathbf{Q} a matrix of P_{ST} multiplied by phase shift. N is the total number of layers. I programmed the equation for an electronic computer 'HIPAC 103', and the calculation for various combinations of W_A , W_B , α and β of illite-montmorillonite, chlorite-montmorillonite, etc., is now in progress. Results of some of the illite-montmorillonite interstratified minerals examined are plotted in Fig. 1.

(A) and (B) are from Kamisunagawa in Hokkaido³, (C) Honami mine in Nagano Pref.⁴, and (D) Kamikita mine in Aomori Pref.

(E), (F), (G) and (H) are the specimens investigated by MacEwan⁵ using the Fourier transform method. (I) is the 1:1 regular structures reported by other investigators^{4,6,7}. The result seems to support the foregoing view, and sug-

gests that it is necessary to treat the interstratified structures for $g \geq 1$, not $g=0$ (random).

I thank Prof. T. Sudo for his advice. MITSUO SATO*
Geological and Mineralogical Institute,
Faculty of Science,
Tokyo University of Education,
Otsuka Bunkyo-ku, Tokyo, Japan.

*Present address: Laboratory of Applied Mineral Chemistry, Department of Technology, Gumma University, Kiryu City, Gumma Prefecture, Japan.

¹ Kakinoki, J., and Komura, T., *J. Phys. Soc. Japan*, **7**, 30 (1952).

² Kakinoki, J., and Komura, T., *J. Phys. Soc. Japan*, **9**, 189, 177 (1954).

³ Kobayashi, K., and Oinuma, K., *J. Geol. Soc. Japan*, **779**, 506 (1960).

⁴ Sudo, T., Hayashi, H., and Shimoda, S., *Clay and Clay Minerals*, **8**, 378 (1962).

⁵ MacEwan, D. M. C., *Clay and Clay Minerals*, **2**, 166 (1955).

⁶ Brindley, G. W., *Amer. Min.*, **41**, 91 (1956).

⁷ Heystek, H., *Miner. Mag.*, **28**, 400 (1954).

CHEMISTRY

Resolution of the Optical Isomers of DL-Tryptophan, 5-Hydroxy-DL-tryptophan and 6-Hydroxy-DL-tryptophan by Paper and Thin-layer Chromatography

It is known that the optical isomers of many amino-acids can be metabolized by different pathways although it is often difficult to obtain suitable preparations for investigation. Several methods, both biological and chemical, are available for obtaining individual isomers in a pure state^{1,2}, but many of these tend to be practicable only if a large amount of the racemic form is available; yields obtained by classical chemical separation methods are low, while biological procedures usually lead to the destruction of one of the isomers. Separation has been achieved, however, by paper chromatography³⁻⁶. This approach seemed to be best suited to our requirement, the isolation of pure optical isomers of ¹⁴C-5-hydroxy-DL-tryptophan, a comparatively expensive compound. During our investigation, we noted that 5-hydroxy-DL-tryptophan (DL-5HTP), 6-hydroxy-DL-tryptophan (DL-6HTP) and DL-tryptophan (DL-TP) separated into two diffuse but distinct spots when chromatographed on Whatman No. 1 paper with butanol-pyridine-water (1:1:1, by vol.) as solvent system. The possibility of improving this separation by thin-layer chromatography was therefore explored.

Thin-layer plates (20 cm x 20 cm) were coated with a mixture obtained by homogenizing cellulose powder CC41 (Whatman, Ltd.) and water (1:2:3, w/v) for 30 sec at maximum speed in an M.S.E. blade homogenizer. A thin layer, 0.3 or 0.4 mm in thickness, was obtained by using a Camag applicator. Plates were air-dried overnight at room temperature, or oven-dried at 105° for 10 min. The compounds, dissolved in methanol or water, were applied to the thin layer and developed in the same solvent system as that used for paper chromatography. The solvent front was allowed to run for a distance of 10 cm which normally took about 1 h at room temperature. Plates were dried in a current of air at room temperature and sprayed with Ehrlich's reagent. The lowest concentration of DL-5HTP detected by this reagent was 0.05-0.1 µg. Table 1 shows the separation of isomers of the three amino-acids obtained by this technique.

It will be seen that the D-isomers of all three compounds have a greater R_F value than the corresponding L-isomers. D-5HTP used as a chromatographic marker was obtained by the action of L-amino-acid oxidase on DL-5HTP (ref. 7). L-5HTP was identified by incubation with guinea-pig kidney L-aromatic amino-acid decarboxylase⁸, followed by chromatography of the reaction products, which showed a disappearance of the L-5HTP and the

Table 1. R_F VALUES OF THE OPTICAL ISOMERS OF DL-TP, DL-5HTP, AND DL-6HTP CHROMATOGRAPHED ON CELLULOSE THIN LAYER IN BUTANOL-PYRIDINE-WATER (1:1:1) SOLVENT SYSTEM

	DL-TP	DL-5HTP	DL-6HTP
L-Isomer	0.52	0.47	0.36
D-Isomer	0.52	0.54	0.41

appearance of another spot, identified as 5-hydroxy-tryptamine. 6HTP was obtained presumably in the L-form from the hydroxylation of L-TP by *Chromobacterium violaceum*⁹.

To separate the isomers of DL-5HTP on a preparative scale, the compound was dissolved in methanol-0.1 N hydrochloric acid (5:1, by vol.) at a concentration of 25 mg/ml. and applied as a fine streak, 15 cm long, to a thin-layer plate. Using an applicator¹⁰, it is possible to apply several streaks on the same position after drying between each application. The application of two streaks was generally convenient, as this allowed the separation of about 1 mg of DL-5HTP. The chromatogram was developed in the solvent and, after drying, was sprayed with a 0.1 N solution of methanolic hydrochloric acid. The plate was viewed under ultra-violet light at 254 mμ (Camag 'Universal UV' lamp) and the outline of the two isomers delineated by their pink fluorescence. The CC41 powder corresponding to these areas was sucked into two tubes, each representing a different isomer. The powder was extracted with methanol and extracts were re-streaked and run on separate chromatograms. On viewing the two chromatograms under ultra-violet light, any contaminating isomer was seen to be well separated. Isomers were extracted with methanol as before and chromatographed, when each ran as a single spot.

When up to 1 mg of DL-5HTP is chromatographed, we have found that there is a certain amount of overlapping of the two isomers. By selecting the area of each isomer in such a way as to exclude this area of overlap, it is possible to obtain a fairly pure sample of each isomer in approximately 50 per cent yield. The area containing the mixed isomers could be kept separate and pooled with similar areas of other chromatograms. The pooled isomers could then be re-chromatographed to increase the overall recovery of the individual isomers.

Equilibration of the chromatogram for periods of up to one hour did not result in a better separation of the isomers; separation is, however, slightly improved if chromatography is carried out at 4°; this is presumably due to the increased developing time of the chromatogram.

We now hope to use this technique for separating the D- and L-isomers of ¹⁴C-5-hydroxy-DL-tryptophan for metabolic investigations.

We thank Dr. R. A. Ellis, of Sandoz, Ltd., for a gift of 6-hydroxy-DL-tryptophan.

This work was supported by grants from the Medical Research Council and the Peel Trust to each of us. We also thank Dr. M. Sandler for his advice.

S. F. CONTRACTOR
JOSEPHINE WRAGG

Bernhard Baron Memorial Research Laboratories
and Institute of Obstetrics and Gynaecology,
Queen Charlotte's Maternity Hospital,
London.

¹ Dunn, M. S., and Rockland, L. B., *Adv. Protein Chem.*, **3**, 296 (1942).

² Greenstein, J. P., *Adv. Protein Chem.*, **9**, 121 (1954).

³ Block, R. J., Durrum, E. L., and Zweig, G., *A Manual of Paper Chromatography and Paper Electrophoresis*, 141 (Academic Press, New York, 1958).

⁴ Losse, G., and Jeschkeit, H., *Pharmazie*, **15**, 164 (1960).

⁵ Greenstein, J. P., and Winitz, M., *Chemistry of the Amino Acids*, 750 (John Wiley, New York, 1961).

⁶ De Ligny, C. L., Nriboen, H., De Vilder, J. J. M., and Van Willigen, J. H. G., *Recueil*, **82**, 213 (1963).

⁷ Parikh, J. R., Greenstein, J. P., Winitz, M., and Birnbaum, S. N., *J. Amer. Chem. Soc.*, **80**, 953 (1958).

⁸ Clark, T., Weissbach, H., and Udenfriend, S., *J. Biol. Chem.*, **210**, 139 (1954).

⁹ Contractor, S. F., Sandler, M., and Wragg, J., *Life Sci.*, **3**, 999 (1964).

¹⁰ Contractor, S. F., *J. Chromatog.* (in the press).

Mechanism of Radical Decay in Irradiated Polyethylene

INVESTIGATIONS of the decay of free radicals in irradiated polyethylene demonstrate that the sec-alkyl radicals, formed with a *G*-value of about 3, decay at a significant rate at room temperature to leave a much smaller number

of allyl-type radicals that decay at a very much slower rate.

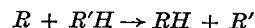
Although there is no direct proof that the cross-linking process involves combination of these free radicals, the correspondence in *G*-value between radical formation and cross-linking, and the fact that cross-linking results from ultra-violet irradiation in the presence of sensitizers¹, constitute strong evidence that cross-linking is a radical-combination process.

There has been much discussion in the literature as to whether the alkyl radicals decay by first-order or second-order kinetics. Lawton, Balwit and Powell² believed the decay to fit four first-order reactions with different decay constants. Cracco, Arvia and Dole³ fit their data with two first-order decay constants. Charlesby, Libby and Ormerod⁴, and Ormerod⁵, found their data to be consistent with a single decay constant of second order.

There is fairly general agreement that a very fast decay occurs in the initial stages, caused by a non-random distribution of radicals occasioned by their presence as near-neighbours. Since the initial deposition of energy in spurs would produce this distribution, it is not really necessary for this argument to postulate invariable production of radicals as near-neighbour pairs².

Since radical decay occurs even in the crystal⁷, where physical diffusion of the polymeric radicals and even significant segmental motion are prevented, it is necessary to postulate that either radicals are produced in very close proximity, or there is a chemical mode of radical migration. If the radicals are in groups in very close proximity, rate of decay should be first order, but there should be a continuous gradation of first-order rate constants, corresponding to near neighbours, more distant neighbours, and still more distant neighbour pairs reacting by limited segmental motion. If the radicals are not all in such close groups, a chemical mechanism for mobility must be present and the reaction will be second order after the initial combination of nearest neighbours.

Dole, Keeling and Rose⁸ first proposed that radical sites could migrate by what might be formulated as a succession of radical abstraction reactions:



in which the alkyl radicals are of the secondary type. Koritskii *et al.*⁹ supported this hypothesis. It is of interest to see whether known reaction rates for this reaction could provide a free radical diffusion constant that would give a second-order rate constant for radical decay that is of the right magnitude.

With each abstraction reaction the radical site moves an estimated 2.3 Å (average distance between hydrogens in the system). According to three-dimensional random walk theory, a radical which makes *n* jumps each of length *l* in random directions will travel a mean distance *r* from its starting point, given by:

$$r^2 = l^2 n$$

In particle theory the relationship of the diffusion constant to this mean square distance per unit time is given by:

$$D = \frac{1}{6} \frac{dr^2}{dt}$$

and we see no reason that this cannot be applied here as representing the constant for diffusion of the radical sites.

The relationship of a second-order rate constant to the diffusion constants of the reactants in a diffusion-controlled reaction is given by:

$$K = 4\pi r_0(D_1 + D_2)$$

where *r*₀ is the distance at which reaction will occur. In this instance, *K* is the diffusion-controlled rate constant for radical combination in this system.

From these expressions:

$$K = \frac{4\pi}{3} r_0 l^2 \frac{dn}{dt}$$

The reaction frequency, dn/dt , is the number of hydrogen abstractions per second undergone by each radical. This equals $K_a C_H$, where K_a is the rate constant for the abstraction reaction and C_H is the concentration of hydrogen atoms attached to secondary carbon atoms. The latter is equal to l^{-3} . We also assume r_0 is also approximately equal to l , the average distance between hydrogen atoms. From this:

$$K = \frac{4}{3} \pi K_a = 4.2 K_a$$

The diffusion-controlled rate constant for radical combination K can be calculated if we can determine the value of K_a . The rate constant K_a is not known for condensed phase and large molecules, and can only be estimated indirectly.

For gas phase, James and Steacie¹⁰ have determined the rate constant for abstractions of secondary hydrogens from *n*-heptane by ethyl radicals. If we consider the secondary hydrogens as one reactant, the rate constant at 25° is 120×10^{-23} c.c. molecule⁻¹ sec⁻¹.

No direct data are available on rate constants for such abstraction by secondary alkyl radicals. However, Kerr and Trotman-Dickenson¹¹ have tabulated such data for abstractions from aldehydes, and show that the rate constants for abstraction by isopropyl and *n*-propyl radicals are very nearly the same. Hoey and LeRoy¹² and Wijnen and Steacie¹³ provide data from which it can be concluded that the ratio of rate constants for the reactions $iPr \cdot + H_2 \rightarrow iPrH + H \cdot$ and $Et \cdot + H_2 \rightarrow EtH + H \cdot$ is about 0.2. On this basis we estimate the rate constant for abstraction of secondary hydrogens by secondary alkyl radicals to be roughly 40×10^{-23} c.c. molecule⁻¹ sec⁻¹.

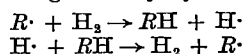
If we can assume that this rate constant is applicable to segments of the polymer molecule in the amorphous phase above the glass transition temperature, we have as a diffusion-controlled rate constant for radical combination at 25°:

$$K = 4.2 K_a = 170 \times 10^{-23} \text{ c.c. radical}^{-1} \text{ sec}^{-1}$$

The second-order rate constants for free radical disappearance in irradiated polyethylene measured by Charlesby *et al.*⁵ have values ranging from 1×10^{-23} c.c. radical⁻¹ sec⁻¹ to 200×10^{-23} c.c. radical⁻¹ sec⁻¹. The higher value, obtained with high-pressure polyethylene, is in reasonable agreement with that calculated above. The low values were obtained with high-density, rather crystalline polyethylene. For these, the chains are held relatively immobile, and the gas-phase rate constant would be inapplicable on both steric and energetic grounds.

While the foregoing considerations seem of value for the bulk of the decay, the importance of the initial spur reaction must still be considered. The question involves the fraction of radicals that must react before the remainder can be assumed to have random distribution. Ohnishi's¹⁴ results have special significance here. He found that irradiation of linear polyethylene at -196° gave a steady-state radical concentration of 2.6×10^{-4} moles/g. At this concentration, the average separation of radicals is 18 Å, or, assuming grouping of four to a spur, the average separation of spurs is 30 Å. With this spacing, added ionization and excitation destroys as many radicals as it creates. Thus, at 50 Mrads, at which 90 per cent of the steady-state value is reached, spur overlap is already very serious, and the validity of any influence of non-random distribution is questionable at such high doses.

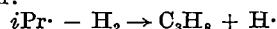
There has been much speculation about the role of hydrogen in enhancing mobility by the reactions:



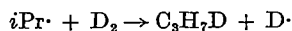
Dole and Cracco¹⁵ demonstrated its occurrence at a rate paralleling the rate of decay of radicals; they found the exchange ceased when the alkyl radicals had disappeared. However, they calculated that an average of only one or two exchanges of this type occurred per alkyl radical. It

is of interest to calculate the rate expected from gas-phase rate constants.

Hoey and LeRoy¹² determined the activation energy for the reaction:



to be 12.5 kcal/mole. This is 1.2 kcal/mole larger than the similar quantity for the reaction of ethyl radical. Wijnen and Steacie¹³ determined the activation energy for ethyl + deuterium to be 13.3 kcal. If we, therefore, take 14.5 kcal/mole for the activation energy for the reaction:



and a steric factor of 10^{-3} , we obtain a rate constant at room temperature of about 1.3×10^{-23} c.c. molecule⁻¹ sec⁻¹. In the system of Dole and Cracco, assuming radicals in chain segments have a similar rate constant for reaction with D_2 and using their value for the concentration of deuterium in the solid, we calculate the frequency of reaction of a radical with D_2 should be:

$$\begin{aligned} 1.3(10^{-23})(C_{D_2}) &= 1.3(10^{-23})(1.2)(10^{-17}) = \\ &1.6 \times 10^{-6} \text{ reactions/sec} \end{aligned}$$

and a radical should react on the average once in 170 h. This is about a factor of ten longer than observed; but the agreement is reasonable for the approximations involved.

This reaction is about a million times slower than the abstraction from alkanes, which occurs about thirty times per second with each radical. Thus, it is difficult to understand how the presence of hydrogen enhances radical combination by a significant extent. Yet Smith and Jacobs⁴, Cracco, Arvia and Dole³, and especially Ormerod⁶, noted that presence of an atmosphere of hydrogen enhances the rate constant for radical combination by a factor of about ten. Thus, there is still no adequate explanation for this effect.

D. O. GREYMER
C. D. WAGNER

Shell Development Co.,
Emeryville, California.

- ¹ Charlesby, A., Grace, C. S., and Pilkington, F. B., *Proc. Roy. Soc.*, A, **26**, 205 (1962).
- ² Lawton, E. J., Balwit, J. S., and Powell, R. S., *J. Chem. Phys.*, **33**, 395 (1960).
- ³ Cracco, F., Arvia, A. J., and Dole, M., *J. Chem. Phys.*, **37**, 2449 (1962).
- ⁴ Smith, W. V., and Jacobs, B. E., *J. Chem. Phys.*, **37**, 141 (1962).
- ⁵ Charlesby, A., Libby, D., and Ormerod, M. G., *Proc. Roy. Soc.*, A, **262**, 207 (1961).
- ⁶ Ormerod, M. G., *Polymer*, **4**, 451 (1963).
- ⁷ Salovey, R., and Yager, W. A., *J. Polym. Sci.*, A, **2**, 219 (1964).
- ⁸ Dole, M., Keelling, C. D., and Rose, D. G., *J. Amer. Chem. Soc.*, **76**, 4304 (1954).
- ⁹ Koritski, A. T., Molin, N. Yu., Shamsher, V. N., Bulen, N. Ya., and Voevodskii, V. V., *Vysokomolekul. Soedin.*, **1**, 1182 (1959).
- ¹⁰ James, D. G. L., and Steacie, E. W. R., *Proc. Roy. Soc.*, A, **244**, 289 (1958).
- ¹¹ Kerr, J. A., and Trotman-Dickenson, A. F., *Progress in Reaction Kinetics*, **V**, 1, 117 (Pergamon Press, New York and London, 1961).
- ¹² Hoey, G. E., and LeRoy, D. J., *Canad. J. Chem.*, **33**, 580 (1955).
- ¹³ Wijnen, M. H. J., and Steacie, E. W. R., *J. Chem. Phys.*, **20**, 205 (1952).
- ¹⁴ Ohnishi, S., *Bull. Chem. Soc. Japan*, **35**, 254 (1962).
- ¹⁵ Dole, M., and Cracco, F., *J. Phys. Chem.*, **66**, 193 (1962).

Irradiation Embrittlement of Low-boron Type 304 Stainless Steel

THERE is a growing amount of literature¹⁻⁷ that describes the magnitude and characteristics of the irradiation-induced embrittlement of alloys at high temperatures. These data appear to be consistent with the hypothesis that the grain boundary embrittlement is due to the helium generated by the $^{10}\text{B}(n, \alpha)$ (ref. 7) Li thermal neutron capture reaction^{1,8,9}.

This communication describes the effect of very small concentrations of boron on the irradiation embrittlement of 'type 304' stainless steel irradiated to a high exposure in a thermal neutron test reactor. These steels generally contain more than 1 p.p.m. B and it was necessary to electron-beam re-melt a commercial stainless steel to reduce the concentration of this element to very low values.

Five charges of approximately 650 g were prepared by cutting 1/4-in.-diameter rod into 2-in. lengths. The charges were melted using an 18-kW power-level and melting times of 4, 8, 12, 16 and 20 min. The resulting 'pancakes' were 4 in. in diameter and 7/16 in. thick. The chemistry of these pancakes is representative of the austenitic stainless steel class.

The boron concentration was determined using the spark source mass spectrograph of the Mattauch-Herzog geometry, which utilizes a radio frequency spark to produce ionization of the sample. The resulting ions are focused according to energy and direction and collected on an Ilford 'Q II' photographic plate. Elements are identified by position on the plate and concentrations are estimated from the density of the line as compared with an internal standard. In all samples of this series ^{53}Cr was used as an internal standard. Chromium-53 is a minor isotope and was free of any interference from other constituents of the sample. The samples were sparked so that a maximum exposure of 0.3 microcoulomb was obtained. For samples suspected, or known, to have high boron content, a 0.1-microcoulomb exposure was obtained. Detection limit varied from 5×10^{-9} to 8×10^{-8} atom fraction depending on exposure, plate and sample. In no case was the boron concentration lower than the detection limit for that sample. Results are summarized in Table 1. These data are believed to be accurate to within ± 50 per cent.

Table 1. BORON CONCENTRATION BY WEIGHT

Alloy	Re-melting time in furnace (min)	Natural boron (p p m.)	Detection limit (p p m.)
18141BC	0	3 90	0 016
EBM706	8	0 130	0 003
EBM707	12	0 015	0 003
EBM709	16	0 107	0 003
EBM710	20	0 023	0 001

After the various heats had been analysed, the electron-beam melted alloys and the commercial heat (in the as-received condition) were machined into subsize tensile specimens which were then irradiated in the core of the Oak Ridge Research Reactor at a temperature of 700° C. The samples were then removed from the reactor and tension-tested in a hot cell using an Instron machine. The post-irradiation ductilities, as given by the true uniform strain and total elongation, are listed in Table 2 for deformation at 704° and 842° C.

Table 2. EMBRITTLEMENT OF STAINLESS STEEL IRRADIATED AT 700° C

Tensile test temperature (° C)	Natural boron concentration (p p m.)	Ductility*			
		True uniform strain† Irradiated (%)	Unirradiated (%)	Total elongation Irradiated (%)	Unirradiated (%)
704	0 015	13.7		19.1	
	0 023	12.7	30.9	23.3	56.2
	0 107	13.1		21.6	
	0 130	12.7	19.6	19.6	60.4
	3.9	11.2	18	13.1	40.2
842	0 015	3.1		6.1	
	0 023	2.9		6.1	
	0 107	2.9	13.8	5.6	46.6
	0 130	3.3	18.2	6.1	57.2
	3.9	1.5	16.0	3.0	43.0

* Irradiated at B-8 position of the Oak Ridge Research Reactor to a neutron dose of 4.5×10^{20} neutrons/cm² (thermal) and 3.5×10^{20} neutrons/cm² ($E > 1$ MeV).

† Specimens strained at a rate of 0.2 per cent/min

Only small variations are found in the ductilities of all the electron-beam melted heats, although an order of magnitude exists between the concentrations of boron. The largest difference in ductility exists between the commercial alloy and the electron-beam re-melted alloys. The concentrations of helium were calculated for the ^{10}B reaction for each of the alloys and are given in Table 3. If one considers only the (n, α) reactions due to thermal neutrons, these data suggest that helium is not responsible for the embrittlement or that the embrittlement is quite independent of helium concentrations in the range of $0.13 - 1.1 \times 10^{-7}$ atom fraction. However, if one computes the concentration of helium due to fast neutron (n, α) reactions for all the elements present in stainless steel, then these data appear more compatible

Table 3. APPROXIMATE HELIUM CONCENTRATION FROM ALL (n, α) REACTION:

Alloy No.	Natural boron concentration (p p m.)	Helium concentration (atom fraction)		Total $\times 10^{-6}$
		Due to ^{10}B and thermal neutrons $\times 10^{-3}$	Due to fast neutron reactions $\times 10^{-4}$	
EBM707	0 015	0 013	0.3	0 313
EBM710	0 023	0 020	0.3	0 320
EBM709	0 107	0 091	0.3	0.391
EBM706	0 130	0 110	0.3	0 410
18141BC	3.9	3.3	0.3	3.6

with an irradiation embrittlement mechanism based on helium. The concentration of helium due to the (n, α) reactions with fast neutrons is also given in Table 3; these data were taken from the work of Bush, Motteff and Weir¹⁰. Considering the helium from all sources, the lack of variation in ductility of the electron-beam melted heats is more easily explained since the difference in total helium concentration is less than a factor of 2. This suggests that the irradiation of these electron-beam melted alloys in a more thermal neutron environment or to a lower fast neutron dose would result in a greater variation in the ductilities.

It is concluded that removing the ^{10}B from the alloy is not the ultimate solution for improving the ductility of irradiated alloys because, even in thermal neutron reactors, (n, α) reactions with fast neutrons are important, particularly for materials containing low boron.

We thank G. E. Angel for help in the preparation of the alloys, J. W. Woods for irradiation of the alloys, and K. W. Boling for the mechanical property tests. We also thank W. D. Harman and E. B. Griffen, of the Special Mass Spectrometry Laboratory, for their help. This work was supported by the U.S. Atomic Energy Commission under contract with Union Carbide Corporation.

W. R. MARTIN

J. R. WEIR

R. E. McDONALD

Oak Ridge National Laboratory,

J. C. FRANKLIN

Laboratory Division, Y-12 Plant,

Oak Ridge, Tennessee.

¹ Hinkle, N. E., *Amer. Soc. Testing Mater. Spec. Tech. Pub.*, **341**, 344 (1963).

² Phil, P. C. L., and Harries, D. R., paper presented at the Amer. Soc. Testing and Materials Symp., "Flow and Fracture of Metals and Alloys in Nuclear Environment", Chicago, June 21-26, 1964 (to be published).

³ Martin, W. R., and Weir, J. R., paper presented at the Amer. Soc. Testing and Materials Symp., "Flow and Fracture of Metals and Alloys in Nuclear Environment", Chicago, June 21-26, 1964 (to be published).

⁴ Hughes, A. N., and Caley, J. R., *J. Nucl. Mater.*, **10**, 60 (1963).

⁵ Fennell, J., and Roberts, A. C., *J. Nucl. Mater.*, **12**, 107 (1964).

⁶ Martin, W. R., and Weir, J. R., *Nuclear Applications* (in the press).

⁷ Comprelli, F. A., Beaudreau, B. C., Busboom, H. J., and Spalaris, C. N., *Trans. Amer. Nucl. Soc.*, **7**, 455 (1964).

⁸ Barnes, R. S., *Some Mechanisms of Radiation Induced Mechanical Property Changes*, AERE Rep.-R-4655 (United Kingdom Atomic Energy Authority, July, 1964).

⁹ Motteff, J., Robertshaw, F. C., and Kingsbury, F. D., *Effect of Neutron Irradiation on the Stress-rupture Properties of High Temperature Precipitation Hardening Alloys* (General Electric GE-TM 64-6-21, May 1964).

¹⁰ Bush, H., Motteff, J., and Weir, J. R., Paper presented at American Nuclear Society Topical Meeting on "Fast Reactor Technology", Detroit, April 26-28, 1965 (to be published).

Boron-Nitrogen Bond Strengths in Borazoles

THE bond order of boron-nitrogen compounds and the aromatic character of borazoles have been subjects of interest for many years (see, for example, ref. 1). It is shown here that bond energy terms and bond length measurements give apparently conflicting information about the relative strengths of some boron-nitrogen bonds.

The heats of hydrolysis in water at 25° C of *N*-trimethylborazole, borazole and *B*-trichloroborazole are -0.7 ± 0.1 , -19.7 ± 0.3 , and -113.6 ± 0.2 kcal/mole respectively. The value for *B*-trichloroborazole agrees with the previously reported² value of -113.8 ± 0.7 kcal/mole. The standard heats of formation of the compounds are -232.3 ± 0.1 , -204.9 ± 0.3 , and -256.1 ± 0.2 kcal/mole respectively. This value for borazole differs significantly from -131.1 ± 3.2 kcal/

Table 1 BORON-NITROGEN BONDS

Compound	<i>E</i> (kcal/mole)	<i>r</i> (Å)	Ref.
B ₃ H ₃ N ₃ Me ₃	121.4	1.42 ± 0.02*	6
B ₃ H ₃ N ₃ H ₃	117.0	1.47 ± 0.07*	7
		1.44 ± 0.02*	8
B ₃ Cl ₃ N ₃ H ₃	106.5†	1.41 ± 0.02*	6
		1.413 ± 0.01†	9
		1.415†	10

* Electron diffraction

† X-ray diffraction

‡ Compare with ref. 5.

mole deduced from the heat of combustion³. The boron-nitrogen bond energy terms, calculated from the standard heats of formation, the heats of vaporization or sublimation⁴, the heats of atomization of the elements⁵, and bond energy terms characteristic of other bonds in the molecules⁵, decrease in the order:



Bond energy terms, *E*, and internuclear distances, *r*, of the boron-nitrogen bonds in the three compounds are compared in Table 1. The differences between the bond length measurements are small compared with the experimental errors, but both series of measurements indicate that the boron-nitrogen bond order is greater in *N*-trimethylborazole than in borazole. The thermochemical data suggest that the bond order is least in the case of *B*-trichloroborazole. This is in qualitative agreement with the original interpretation of the ultra-violet spectra¹¹ and with the relative values of the diamagnetic anisotropy of borazole and *B*-trichloroborazole¹².

B. C. SMITH
LAMBODAR THAKUR

Department of Chemistry,
Birkbeck College,
University of London,
Malet Street, London, W.C.1.

¹ Sheldon, J. C., and Smith, B. C., *Quart. Rev. Chem. Soc.*, **14**, 200 (1960)

² Van Artsdalen, E. R., and Dworkin, A. S., *J. Amer. Chem. Soc.*, **74**, 3401 (1952)

³ Kilday, M. V., Johnson, W. H., and Prosen, E. J., *J. Res. Nat. Bur. Stand.*, **65**, A, 101 (1961)

⁴ Schlesinger, H. I., Ritter, M., and Burg, A. B., *J. Amer. Chem. Soc.*, **60**, 1296 (1938). Hough, W. V., Schaeffer, G. W., Dzurus, M., and Stewart, A. C., *J. Amer. Chem. Soc.*, **77**, 864 (1955). Brown, C. A., and Laubengayer, A. W., *J. Amer. Chem. Soc.*, **77**, 3699 (1955).

⁵ Cottrell, T. L., *The Strength of Chemical Bonds*, second ed. (Butterworths, London, 1958)

⁶ Coffin, K. P., and Bauer, S. H., *J. Phys. Chem.*, **59**, 193 (1955).

⁷ Stock, A., and Wierl, R., *Z. Anorg. Chem.*, **203**, 228 (1931).

⁸ Bauer, S. H., *J. Amer. Chem. Soc.*, **60**, 524 (1938).

⁹ Coursen, D. L., and Hoard, J. L., *J. Amer. Chem. Soc.*, **74**, 1742 (1952)

¹⁰ Lonsdale, K., *Nature*, **184**, 1060 (1959).

¹¹ Rector, C. W., Schaeffer, G. W., and Platt, J., *J. Chem. Phys.*, **17**, 460 (1949).

¹² Lonsdale, K., and Toor, E. W., *Acta Cryst.*, **12**, 1048 (1959). Watanabe, H., Ito, K., and Kubo, M., *J. Amer. Chem. Soc.*, **82**, 3294 (1960).

Determination of p.p.m. Concentrations of Carbon Disulphide in Benzene by Electron Capture Gas Chromatography

THE highly specific response of the Lovelock electron capture detector toward certain functional groups has been exploited in various ways, notably in the detection of trace amounts of halogenated compounds¹.

In this laboratory the electron capture detector has been found to be extremely sensitive to carbon disulphide, and the presence of low concentrations of this compound is readily detected in hydrocarbon solvents. In the case of benzene, the S.T.P.T.C. standard colorimetric method² already exists, but the use of electron capture gas-liquid chromatography would appear to offer the advantages of increased analysis speed and improved accuracy.

A 10-ft. gas-liquid chromatography column is used to separate the CS₂ from other compounds. This is packed with 5 per cent w/w trixylenyl phosphate on 60/72 B.S.S. 'Celite' and operated at 70° C with nitrogen as carrier gas. The electron capture detector is of conventional construction with an internal volume of 1 ml. Tritium is used in the cell as a source of electrons and the cell is operated at a constant potential difference of 60 volts. A conven-

tional electrometer amplifier is connected to the detector and the output is measured on a 1-mV high-speed recorder after appropriate attenuation. A typical chromatogram of a 10-μl. sample of benzene containing 1.0 p.p.m. by weight sulphur as CS₂ is shown in Fig. 1.

Fig. 2 shows the relationship between CS₂ peak height and concentration. The graphs were obtained from standard solutions of CS₂ in benzene prepared by the S.T.P.T.C. procedure (ref. 2). A sample volume of 10 μl. was used for the range 0.1–1.0 p.p.m. and a volume of 1.0 μl. for the range 1.0–10.0 p.p.m., precision microsyringes being used in each case.

The results are linear over both ranges, although the lower concentration range shows a slight deviation from the origin. The lowest concentration of CS₂ used in the calibration, namely 0.1 p.p.m., corresponds to a peak height of 0.5 cm and this was clearly seen against the background noise. The minimum limit detectable would be about 0.05 p.p.m. which would correspond to a signal-to-noise ratio of 2:1.

Parallel calibrations were also made of the S.T.P.T.C. standard method. This involves measuring the optical

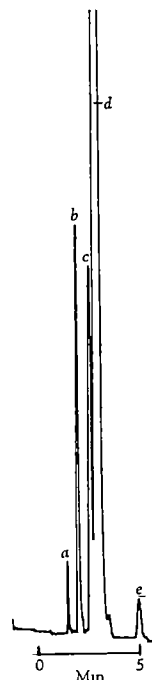


Fig. 1. Electron capture chromatogram of benzene containing 1.0 p.p.m. CS₂. a, Paraffins; b, CS₂; c, cyclohexane; d, benzene; e, toluene

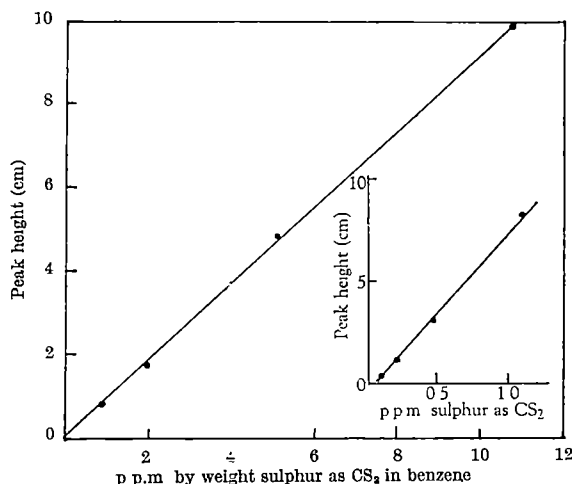


Fig. 2. Relationship between CS₂ peak height and electron capture response

density of the colour formed, after the addition of copper acetate and diethylamine, by means of a photoelectric absorptiometer. The graphs showed considerable deviations from linearity outside the range 0.5–5.0 p.p.m. CS_2 .

Table 1 shows a comparison of the two methods when used for the analysis of three benzene samples.

Table 1. SULPHUR AS CARBON DISULPHIDE (P.P.M. BY WEIGHT)
S.T.P.T.C. standard method G.L.C. method

Sample 1	6.3	6.4
Sample 2	0.4	<0.1
Sample 3	0.3	<0.1

A good agreement is obtained in the case of sample 1 having a relatively high CS_2 content.

The gas-liquid chromatography (G.L.C.) method is probably more accurate than the S.T.P.T.C. method with samples 2 and 3, since these results were based on the absence of any CS_2 peak, in contrast with a calibration sample containing 0.1 p.p.m. of this compound.

Table 2

Injection number	1	2	3	4	5	6	7	8	9	10
Peak ht. (cm)	10.50	10.76	11.21	10.95	10.65	10.95	10.75	11.15	11.35	11.15
Mean peak height	= 10.94 cm									
Standard deviation	= 0.274 cm									
Per cent standard deviation	= 2.5									

Table 2 gives the results of ten repeat injections of a 10- μ l. sample of benzene containing 1.0 p.p.m. sulphur as CS_2 .

The results indicate a relatively high degree of repeatability for this type of analysis.

D. W. GRANT
G. A. VAUGHAN

The Coal Tar Research Association,
Oxford Road, Gomersal, Leeds.

¹ Goodwin, E. S., Gaudin, R., and Reynolds, J. G., *Analyst*, **86**, 697 (1961)

² *Standard Methods for Testing Tar and its Products*, fifth ed., edit. by P. V. Watkins, 248 (1962).

Separation of Coumarins

By sorption on ion exchange resins¹⁻³ it is possible to separate some coumarins in dilute solution. A column of the strongly basic anion exchange resin, 'Amberlite IRA-400' (-40, +60 mesh; Rohm and Haas Co.), in the chloride form (bed length, 50 cm; bed volume, 25.8 c.c.) has been used for this purpose.

The coumarins studied were of three groups. Group A includes coumarin (I), 3-Me(I), 7-Me(I), 3,4-di Me(I), 6,7-di MeO-4-Me(I) and 7,8-di MeO(I); group B includes 7-OH(I) and 8-OH(I); group C includes 6,7-di OH-4-Me(I) and 7,8-di OH-4-Me(I).

In a run, 50 ml. 5.636×10^{-4} M solution of one of the above coumarins in 10 per cent methanol (by volume) was passed through the column at 5 ml./min and the sorbed solute on the column was eluted. The effluent was collected in samples and estimated for the solute content by ultra-violet absorption⁴. Group A compounds were eluted by 10 per cent methanol. Group B compounds were not eluted by 10 per cent methanol, but were slowly eluted by N/10 HCl in 10 per cent methanol. Group C compounds were not eluted by 10 per cent methanol or by N/10 HCl in 10 per cent methanol, but were eluted by N/100 HCl in methanol. It is therefore possible to separate binary and ternary mixtures containing not more than one compound from each group.

R. S. SHAH
S. L. BAFNA

Chemistry Department,
M.S. University of Baroda,
Baroda-2, India.

¹ Wheaton, R. M., and Bauman, W. C., *Ann. N.Y. Acad. Sci.*, **57**, 159 (1953).

² Skelly, N. E., *Anal. Chem.*, **33**, 271 (1961).

³ Patel, D. J., and Bafna, S. L., *Ind. Eng. Chem. (Product Research and Development)*, **4**, 1 (1965).

⁴ Shah, R. S., and Bafna, S. L., *Ind. J. Chem.*, **1**, 400 (1963).

BIOCHEMISTRY

Altered Electrophoretic Mobilities of some Erythrocytic Enzymes as a Function of their Age

A NUMBER of recent reports^{1,2} have indicated that the *in vitro* storage of human red blood cells leads to an increased electrophoretic mobility of some enzymes (for example, glucose-6-phosphate dehydrogenase, acid phosphatases) when compared with the mobility of the same enzyme obtained from fresh blood. This finding parallels that previously reported for haemoglobin derived from fresh or stored red cells³.

The availability of several methods for the separation of young and old red blood cells or of their lysates from a red blood cell population⁴⁻⁶ prompted us to investigate the electrophoretic behaviour of some enzymes derived from erythrocytes of different ages. This communication presents results of preliminary experiments indicating an altered electrophoretic mobility of some erythrocytic enzymes as a function of the age of the red cell from which they are derived.

It was also found that this altered electrophoretic behaviour is not shared by all erythrocytic enzymes, nor even necessarily by enzymes having the same substrate specificities but originating in different aged erythrocytes of other species. The implications of these experiments are briefly discussed.

100 ml. of human blood, collected in EDTA or acid-citrate-dextrose solutions, were washed six times with isotonic saline, great care being taken to remove the buffy white blood cell layer. The washed red cells were then subjected to the serial osmotic haemolysis procedure of Simon and Topper⁴ in which lysates from young and old erythrocytes are obtained separately. The smallest possible lysing volumes were used so as to have a high enzyme content per unit volume. Lysates were centrifuged at high speed (13,000g for 10 min) to remove the stroma. The haemolysates corresponding to the reticulocytes were not examined.

Control experiments on the behaviour of white blood cells in the serial osmotic haemolysis procedure indicated that the contribution of enzymes by residual, contaminating white blood cells in the red blood cells fractionated was small and most probably did not affect the results described here. Further experiments, however, using other separation procedures for young and old erythrocytes⁵ are in progress to confirm our results by another, independent method.

Glucose-6-phosphate dehydrogenase (G-6-PD) and glutamic-oxaloacetic transaminase (GOT) activities were assayed by standard spectrophotometric methods⁷⁻⁹ on the lysates of the youngest and older human erythrocytes. One unit of enzyme is defined as that amount required for a $\Delta O.D._{340} \text{ m}\mu$ of 0.001/min./ml. of lysate. For comparative purposes samples of blood were stored at 4°–5° C for up to four weeks. The latter samples were washed with saline after storage, lysed and enzyme activities determined as indicated above.

Starch-gel electrophoresis of G-6-PD was carried out according to the method of Fildes and Parr¹ except that, at these authors' suggestion, a 0.01 M phosphate buffer at pH 7.5 was substituted for the *tris* buffer described in their paper. GOT was electrophoresed following the method of Schwartz *et al.*¹⁰, but using a 0.01 M phosphate buffer at pH 7.3.

In all the electrophoretic runs the number of enzyme units of each lysate applied was identical to that of the fraction to which it was compared. About 10 units (as defined here) were applied for both G-6-PD and GOT.

Fildes and Parr¹ reported that G-6-PD has an increased electrophoretic mobility when the red blood cells from which it is derived have been stored. We have repeated these experiments and confirm their findings. The

difference in electrophoretic mobilities between fresh and stored G-6-PD is of the order of 10 per cent, although in some cases the difference has been appreciably greater. Our examination of GOT, obtained from fresh and stored red cells, revealed a smaller alteration in the electrophoretic mobility of this enzyme. These results, together with the described increase in electrophoretic mobilities of erythrocyte acid phosphatases², lead to the question of whether all erythrocytic enzymes manifest this behaviour. Examination of the electrophoretic behaviour of lactic acid dehydrogenase (LDH) isozymes¹¹ reveals that there is no alteration in the electrophoretic mobilities of these isozymes as a function of red blood cell storage *in vitro*. Thus, apparently, only certain enzymes display this behaviour.

Visualization after electrophoresis of G-6-PD and GOT derived from young or old erythrocytes reveals that the alteration in mobilities of these enzymes is not solely a function of their storage *in vitro*. These enzymes show a similar alteration in electrophoretic mobility on ageing *in vivo* (Figs. 1A and B). This may be analogous to results reported for haemoglobin in which haemoglobin A₂ (the old haemoglobin) displays an increased electrophoretic mobility¹² when compared with its younger counterpart (Hb A).

A number of suggestions have been made as to the possible cause of this increased mobility of haemoglobin on *in vivo* or *in vitro* ageing, one of which is that a mixed disulphide with glutathione is formed¹³ as a consequence of the diminished enzyme activities (for example, glutathione reductase) in older red cells. A number of other possibilities have also been discussed¹⁴ in view of the fact that a mixed disulphide formed between haemoglobin and glutathione *in vitro* has some properties that differ from those of Hb A₂ (ref. 3). In any event, whichever of the suggested reactions accounts for the alteration of the electrophoretic mobility of haemoglobin as a function of the age of the cell from which it is derived may well be similarly responsible for the altered mobilities of the G-6-PD and GOT, since all these proteins are contained in the same changing cellular environment.

That the reaction leading to the increased electrophoretic mobilities of erythrocytic enzymes is not one that occurs with all these enzymes is shown by the fact that LDH isozymes display neither a storage effect nor do they have altered mobilities as a function of the age of the red cell from which they are obtained¹¹.

Finally, investigation of the behaviour of G-6-PD from horse erythrocytes indicates that this enzyme (which in the horse consists of two isozymes) shows neither a storage effect nor an altered electrophoretic mobility as a

function of the age of the red blood cell from which it is derived.

It may well be that those enzymes which have certain reactive groups on their surface (for example, sulphhydryl) react with some reagent (for example, glutathione) as the cell ages and display the altered mobility observed on electrophoresis. Conversely, those enzymes not possessing such groups in readily accessible positions do not. If this is so, it might also serve as an indicator of this type of different molecular structure of some enzymes having similar substrate specificities but derived from different species (for example, G-6-PD from human and from horse). We are at present investigating some of these possibilities.

In conclusion: the examination of the electrophoretic mobilities of G-6-PD, GOT and LDH from young or old human erythrocytes reveals that G-6-PD and GOT derived from the older cells have increased electrophoretic mobilities while the LDH isozymes do not.

This work was supported in part by a grant (HE-08304) from the National Heart Institute, U.S. Public Health Service.

Note added in proof. Experiments conducted since this communication was submitted for publication in which human and horse haemolysates were reacted with oxidized glutathione and the resulting electrophoretic mobilities of the aforementioned enzymes compared to those from unreacted lysates indicate that human G-6-PD and GOT have increased electrophoretic mobilities after reacting with glutathione while human LDH and horse G-6-PD have not.

HARRY WALTER
FRED W. SELBY
JUANITA R. FRANCISCO

Laboratory of Chemical Biology,
Veterans Administration Hospital,
Long Beach, California, and
Department of Biological Chemistry,
University of California,
Los Angeles, California.

¹ Tildes, R. A., and Parr, C. W., *Biochem. J.*, **87**, 45P (1963).

² Hopkinson, D. A., Spencer, N., and Harris, H., *Nature*, **199**, 969 (1963).

³ Huisman, T. H. J., and Dcozy, A. M., *J. Lab. Clin. Med.*, **60**, 302 (1962).

⁴ Prankerd, T. A. J., *J. Physiol.*, **143**, 325 (1958).

⁵ Walter, H., Selby, F. W., and Brake, J. M., *Biochem. Biophys. Res. Commun.*, **15**, 497 (1964).

⁶ Simon, E. R., and Topper, Y. J., *Nature*, **180**, 1211 (1957).

⁷ Kornberg, A., *J. Biol. Chem.*, **182**, 805 (1950).

⁸ Karmen, A., *J. Clin. Invest.*, **34**, 131 (1955).

⁹ Sass, M. D., and Spear, P. W., *J. Lab. Clin. Med.*, **51**, 926 (1958).

¹⁰ Schwartz, M. K., Nisselbaum, J. S., and Bodansky, O., *Amer. J. Clin. Path.*, **40**, 103 (1963).

¹¹ Walter, H., and Selby, F. W. (in preparation).

¹² Kunkel, H. G., and Bearn, A. G., *Fed. Proc.*, **16**, 780 (1957).

¹³ Muller, C. J., thesis, Groningen (Van Gorcum and Co., N V Assen, The Netherlands, 1961).

¹⁴ Walter, H., *Nature*, **198**, 189 (1963).

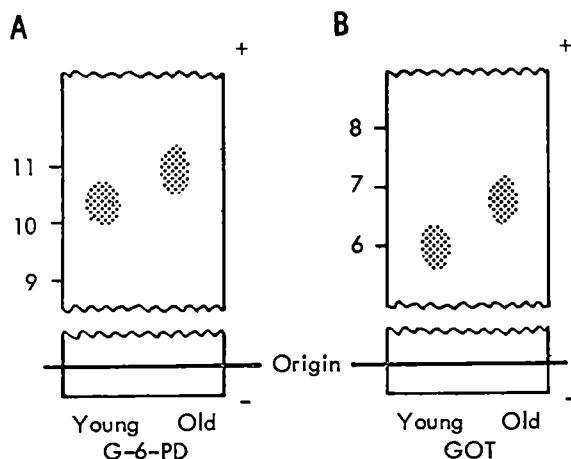


Fig. 1. Electrophoretic mobilities, on horizontal starch-gel, of enzymes derived from young or old human erythrocytes. A, Glucose-6-phosphate dehydrogenase, 0.01 M phosphate buffer, 5 V/cm, 16 h. B, Glutamic-oxaloacetic transaminase, 0.01 M phosphate buffer, 6 V/cm, 16 h. Distance of migration in cm.

Deterioration of Stored Inulin Solutions

WHILE using a batch of inulin for determination of glomerular filtration rate in man, we found that some ampoules were giving much lower serum inulin-levels than were expected from the dose given. The inulin had been stored in the dark at 20°–25° C, in sealed ampoules, and from the batch number it was established that it had been manufactured thirteen years ago by Thomas Kerfoot and Co., Barnsley. We investigated the deterioration of inulin with time by determining the inulin content by the acid hydrolysis and resorcinol technique¹ in fifteen ampoules of the thirteen-year-old batch of inulin, in seven ampoules of inulin one-year old and in inulin recrystallized from the one-year-old batch, after washing with distilled water at 20° C. The ages of the inulin batches were kindly provided by the manufacturers.

Table 1 shows the results of total inulin content; taking recrystallized inulin as 100 per cent, the thirteen-year-old

INULIN ¹ CONTENT OF STORED INULIN AMPOULES		
Inulin sample	Per cent inulin determined by acid hydrolysis-resorcinol technique ¹	
Recrystallized inulin	100	
13-year-old inulin:		
Ampoule 1	54.8	
2	75.8	
3	60.6	
4	69.5	
5	74.1	
6	64.0	
7	83.0	
8	64.0	
9	78.2	
10	92.0	
11	81.0	
12	75.6	
13	74.5	
14	75.3	
15	72.6	
	Mean 73.0	S.D. 9.07
1-year-old inulin:		
Ampoule 1	83.9	
2	87.1	
3	89.2	
4	89.7	
5	90.3	
6	92.8	
7	89.2	
	Mean 88.9	S.D. 2.36

Table 2. AMOUNT OF REDUCING SUBSTANCES IN 10 PER CENT INULIN SOLUTIONS EXPRESSED AS FRUCTOSE (ref. 2)

Type of inulin	Fructose g/100 ml. in 10^{-4} per cent solution of inulin
(1) 13-year-old batch. First ampoule	5.05
(2) 13-year-old batch. Second ampoule	4.83
(3) 1-year-old batch. First ampoule	0.97
(4) 1-year-old batch. Second ampoule	1.42
(5) Recrystallized inulin	0.32

batch had a mean inulin content of 73.0 per cent *S.D.* 9.07, and the one-year-old batch had a mean inulin content of 88.9 per cent *S.D.* 2.36. Because the acid hydrolysis-resorcinol technique measures fructose and non-inulin fructose polymers, there must be considerable degradation of inulin to products other than fructose during prolonged storage. The true picture of how much inulin remains unaltered can be seen indirectly from Table 2, in which reducing substances were estimated by Benedict's quantitative method² in samples of thirteen-year-old inulin, one-year-old inulin and recrystallized inulin. It will be seen that about 5 g per cent of reducing substances (expressed as fructose) was present in 10 per cent inulin solutions thirteen years old, whereas 10 per cent inulin solutions one year old have between 1 and 1.5 g per cent reducing substances (as fructose) and 10 per cent solutions of recrystallized material contain 0.3 g per cent of reducing substances.

This work suggests that even one-year-old inulin contains appreciable amounts of non-inulin reducing substances, presumably fructose or fructose polymers; these may give falsely high values for plasma inulin re-absorption by the tubules³; a considerable error may be introduced into the measurement of glomerular filtration rate. It is concluded that for inulin-clearance measurements, solutions of inulin freshly prepared from recrystallized inulin should be used, and that inulin samples more than one year old should be discarded.

We thank Thomas Kerfoot and Co. for their help, and H. Varley for advice.

S. NILWARANGKUR
G. M. BERLYNE

University Department of Medicine,
Royal Infirmary,
Manchester, 13.

¹ Varley, H., *Practical Clinical Biochemistry*, 128 (Heinemann, 1954).

² Varley, H., *Practical Clinical Biochemistry*, 69 (Heinemann, 1954).

³ Gametoft, A., and Kjerulf-Jensen, K., *Acta Physiol. Scand.*, 6, 368 (1943).

Interaction between Chlorophyll and Hydroquinone

CHLOROPHYLL has a unique characteristic in being photo-oxidized or photo-reduced by reactants which can serve as electron acceptors or donors to light-activated chlorophylls¹. The photo-reduction of chlorophyll occurs

when ascorbic acid is the reductant². Quinones act as electron acceptors for the photo-oxidation of chlorophyll³, but hydroquinones have not been shown to function as electron donors to the excited chlorophyll. It is known that hydroquinones are photo-oxidized by oxygen in chlorophyll *a* and *b* solutions⁴. The work recorded here consists of an experimental investigation of the direct interaction between excited chlorophyll and hydroquinone. The effect of oxygen on this system is also shown in this communication.

Chlorophyll *a* was prepared and purified by Zscheile's method⁵. Hydroquinone (Merck) was recrystallized four times from methanol solution. Photovoltaic changes of chlorophyll *a* with and without hydroquinone in methanol were measured by means of a Kintel digital voltmeter, model 456 B, with read-out, model 473 A. The photovoltaic cell consisted of a platinum electrode with a saturated calomel electrode as reference. The cell was irradiated through a collimating lens at a distance of 25 cm by a 1 kW-tungsten projection lamp. A Corning red filter OS-2-61 was used together with a 2 per cent copper sulphate solution. The solutions were flushed before and during measurements with nitrogen gas pre-treated with a chromous chloride solution. Electron spin resonance absorptions were determined by a Varian V-4502 equipped with 100-ke/s field modulation. The cavity of the EPR spectrometer was irradiated in the same way as in the measurement of photovoltaic changes. The solutions prior to irradiation were degassed on a vacuum line as described in the studies of the triplet state of chlorophyll⁶.

Fig. 1 shows the small photovoltaic change with chlorophyll *a* (7.4×10^{-4} M) in de-aerated methanol. Addition of hydroquinone to the same solution at a concentration of 10^{-4} M greatly enhances the photovoltaic change. The redox-potential of this system decreases in light and reverts to approximately the initial value when the light is turned off. This reversible change indicates an electron

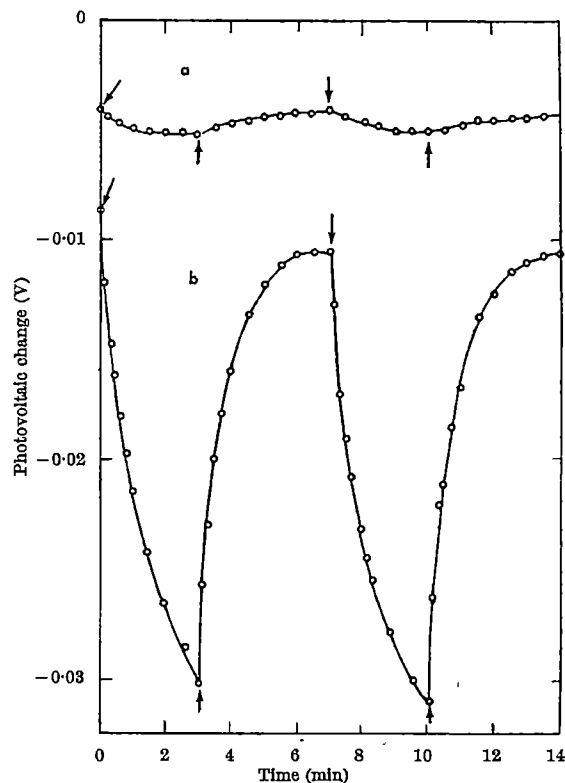


Fig. 1. Photovoltaic change of chlorophyll *a* (a) and chlorophyll *a*-hydroquinone (b) in methanol: 7.4×10^{-4} M chlorophyll *a* and 10^{-4} M hydroquinone. Downward-pointing arrows show light on and upward-pointing arrows light off. Photovoltaic change is represented by $E_h - E_{cal}$, where E_h and E_{cal} are the redox-potential of the system and the electrode-potential of saturated calomel electrode, respectively

exchange between excited chlorophyll and hydroquinone. The negative change of the redox-potential on irradiation suggests that chlorophyll undergoes photo-reduction and its couple is responsible for the photovoltaic effect. The electron spin resonance measurements exhibited a greater rate of electron transfer than that found in the photovoltaic study. This difference is attributed to the slow electrochemical process at the electrode responding to the redox-reaction involving excited molecules in solution.

Electron spin resonance investigations showed that this photo-reversible change is due to one-electron transfer from hydroquinone to chlorophyll. Degassed solutions of chlorophyll *a* ($4.5\text{--}4.9 \times 10^{-4}$ M) with hydroquinone (10^{-2} M) in methanol exhibit two types of electron spin resonance signal when irradiated with red light as shown in Fig. 2*a*. One shows a hyperfine structure, which is characteristic of *p*-benzosemiquinone free radical, and the other is a broader signal probably from reduced chlorophyll. Fig. 3*a* illustrates a characteristic rapid rise and a quick decay of the signals.

In the presence of air, two similar types of signal were obtained and both were greatly enhanced (Fig. 2*b*). One, a single absorption, is a little broader than that in the air-free system. The other is the same as the afore-

mentioned signal having the hyperfine structure. The time to attain saturation of the signals was slower in the aerated solution than that found for the de-aerated solution as shown in Fig. 3*c*, especially the signal with the hyperfine structure which grew slowly during irradiation even after the broad signal had reached its saturation. The hyperfine signal decayed rapidly, while the decay of the broader signal was slower. With aerated chlorophyll *a*-methanol solution without hydroquinone, a smaller broad electron spin resonance signal is observed which is similar to the broad signal of the aerated chlorophyll *a*-hydroquinone solution. This indicates that these broad signals originate from an interaction between excited chlorophyll and oxygen. Chlorophyll *a* in methanol solution in air undergoes a permanent oxidation as evidenced by the decrease in electron spin resonance signal with continuous irradiation. The electron spin resonance decay of this solution was intermediate between that of chlorophyll *a* with hydroquinone and that with hydroquinone and oxygen (Fig. 3*b*). Calvin also showed this kind of electron spin resonance absorption in aerated methanol extract from *Chlorella*⁷.

Small photovoltaic changes obtained in anaerobic solutions of chlorophyll *a* in methanol are attributed to a reversible photo-bleaching of chlorophyll. This is consistent with a mechanism proposed by Livingston and Stockman⁸, where a very small amount of chlorophyll is reversibly photo-reduced by complexed methanol molecules.

The interaction between excited chlorophyll and hydroquinone shown in anaerobic solutions is significant for specific aspects of chlorophyll photochemistry. Very recently chlorophyll-photosensitized reduction of cytochrome *c* has been shown by chloroplasts or isolated chlorophyll *a* in the presence of hydroquinones. These reactions have been assumed to proceed via an initial photochemical reduction of quinone present as an impurity in hydroquinone⁹. Flash-illumination of chlorophyll solution containing reduced ubiquinone induced a rapid absorption change at 430 mμ, followed by a rapid decay which is slower than a decay of triplet state¹⁰. The work recorded here shows that the direct reaction of excited chlorophyll with hydroquinone is possible. Our investigation on the role of hydroquinone in the photo-reactions occurring in chloroplasts is now in progress.

The excited chlorophyll molecule undergoes reactions with oxygen to form an unstable, reactive complex^{11,12}. This occurs with the formation of free radicals and the subsequent permanent oxidation of chlorophyll¹³. In the presence of hydroquinone, the free radicals that are formed in the quick reaction of excited chlorophyll with oxygen probably undergo further reactions with hydroquinone to produce *p*-benzosemiquinone-free radicals in the oxidation of hydroquinone.

EIJI FUJIMORI
MARIA TAVLA

Photobiology Section,
Energetics Branch,
Space Physics Laboratory,
Air Force Cambridge Research Laboratories,
Bedford, Massachusetts.

¹ Livingston, R., *Quart. Rev.*, **14**, 174 (1960).

² Krasnovsky, A. A., *Ann. R.-v. Plant. Physiol.*, **11**, 363 (1960).

³ Tollin, G., and Green, G., *Biochim. Biophys. Acta*, **60**, 524 (1962); **66**, 308 (1963).

⁴ Okayama, S., and Chiba, Y., *Nature*, **205**, 172 (1965).

⁵ Zschelle, F. P., and Comar, J. L., *Bot. Gaz.*, **102**, 463 (1941).

⁶ Fujimori, E., and Livingston, R., *Nature*, **180**, 1036 (1957).

⁷ Calvin, M., in *Biophysical Science—A Study Program*, edit. by Oncley, J. L., 157 (J. Wiley and Sons, Inc., New York, 1959).

⁸ Livingston, R., and Stockman, D., *J. Phys. Chem.*, **66**, 2531 (1962).

⁹ Vernon, L. P., and Shaw, E. R., *Biochemistry*, **4**, 133 (1965).

¹⁰ Ke, B., Vernon, L. P., and Shaw, E. R., *Biochemistry*, **4**, 137 (1965).

¹¹ Schenck, G. O., *Naturwiss.*, **40**, 205, 229 (1953).

¹² Livingston, R., and Owens, K. E., *J. Amer. Chem. Soc.*, **78**, 3301 (1956).

¹³ Claes, H., *Biochem. Biophys. Res. Comm.*, **3**, 585 (1960); *Z. Naturforsch.*, **16**, 446 (1961).

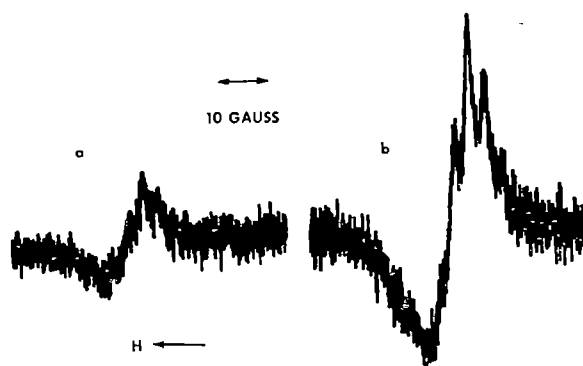


Fig. 2. Light-induced electron spin resonance spectra of chlorophyll *a*-hydroquinone system in methanol in the absence of oxygen, taken immediately after irradiation (modulation amplitude: 2.4 gauss) (*a*) and in the presence of oxygen, taken after irradiation for 7 min (modulation amplitude: 0.6 gauss) (*b*): 4.6×10^{-4} M chlorophyll and 10^{-2} M hydroquinone

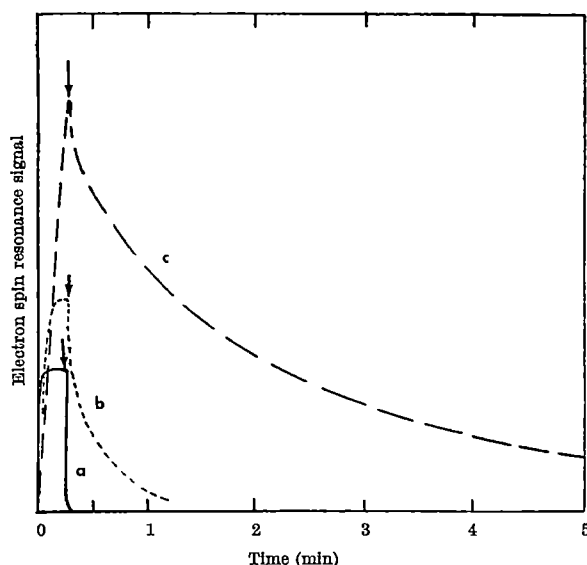


Fig. 3. Rise and decay curves of electron spin resonance signals of chlorophyll *a* (4.9×10^{-4} M)-hydroquinone in the absence of oxygen (*a*), chlorophyll *a* (4.5×10^{-4} M) in air (*b*), and chlorophyll *a* (4.5×10^{-4} M)-hydroquinone in air (*c*), in methanol: 10^{-2} M hydroquinone (modulation amplitude: 4.8 gauss). Downward-pointing arrows show light off after irradiation for 15 sec

Biosynthesis of Triiodothyronine Sulphate by Beef Thyroid *in vitro*

It is well recognized that the thyroid gland contains the L-amino acids, triiodothyronine (T_3), thyroxine (T_4), monoiodotyrosine (MIT), diiodotyrosine (DIT) and iodinated thyronines which are free (unconjugated) but are bound to thyroglobulin. The release of these iodinated amino-acids occurs after digestion by protein-hydrolysing enzymes or chemicals, but the possibility of observing any T_3 and/or T_4 sulphate conjugates *per se* in thyroid tissues seems unlikely since hydrolytic procedures would have precluded their isolation.

The conversion of triiodothyronine (T_3) to triiodothyronine sulphate (T_3S) presumably occurs in the liver¹ since Roche, Michel, Closon, and Michel demonstrated the presence of T_3S in the bile and plasma of thyroidectomized rats. However, the well-established evidence for extra-hepatic mammalian steroid sulphokinase activity in endocrine glands such as adrenal^{2,3} and ovary⁴ stimulated an investigation of T_3 sulphokinase activity in mammalian thyroid tissues.

Fresh beef thyroid glands were homogenized at 4° C in 0.25 M sucrose, containing 0.05 M *tris* (tris hydroxymethyl aminomethane) and 0.002 M EDTA (ethylenediaminetetraacetate) at pH 7.5. The crude homogenate was filtered through cheese cloth and fractionated by ultracentrifugation according to the method of Fischer, Schultz and Oliner⁵. The fractions I-IV (see Table 1) were stored frozen until assayed.

The assay mixture included 100 μ mol imidazole HCl buffer, pH 7.0, 12.5 μ mol $MgCl_2$, 1.0 μ mol triiodothyronine (Sigma), 10 μ mol cysteine (neutralized), 10 μ mol K_4ATP , 10 μ mol K_2SO_4 , 0.2 ml. yeast sulphate-activating enzyme⁶ and 0.2 ml. thyroid sulphokinase fraction in a final volume of 1.0 ml. Incubation was carried out for 35 min in air at 37° C in the Dubnoff metabolic shaker.

Control flasks included zero time, and mixtures incubated without various components including enzymes. The T_3S was determined by the methylene blue method of Nose and Lipmann⁷. A standard curve was prepared by the use of the ammonium T_3S which was synthesized by the method of Fieser⁸. Protein was measured spectrophotometrically by the method of Gornall and colleagues⁹.

Table 1. T_3 SULPHOKINASE ACTIVITY OF BEEF THYROID
Fractional ultracentrifugation $m\mu$ mol T_3S formed per mg
of protein

Fraction I	Filtered crude homogenate	92
Fraction II	700g for 10 min supernatant	161
Fraction III	12,000g for 10 min supernatant	182
Fraction IV	105,000g for 60 min supernatant	216

The results of a typical incubation are listed in Table 1. The identification of ammonium T_3S sulphate in the incubation mixture followed the addition of concentrated ammonium hydroxide and extraction with *n*-butyl alcohol. Identical mobilities of the unknown and standard were observed after descending paper chromatography in the *n*-butyl alcohol, 2 N, NH_4OH system and paper electrophoresis in the $(NH_4)_2CO_3$, 0.05 M, pH 9 according to the methods of Roche *et al.*¹. The T_3S was located by positive ninhydrin stain and Schneider, Lewbart modification of the rhodizonic acid test for sulphate ions¹⁰.

The data indicate that T_3 sulphokinase activity is present in beef thyroid tissue and that the highest enzymatic activity is in the microsome-free supernatant. It is of interest that sulphokinase activity is also highest in the hepatic and adrenal microsome-free fraction, that is 105,000g supernatant. The thyroid T_3 sulphokinase activity remained constant for at least 3 months despite frequent freezing and thawing.

Preliminary studies suggest that beef thyroid microsome-free supernatant synthesizes T_4 sulphate (T_4S) in the same order of magnitude as T_3S (210 $m\mu$ mol per mg of protein per 35 min). Additional studies are in progress to purify T_3 and T_4 sulphokinase(s) and to study the enzyme kinetics involved.

I thank Drs. Arthur Schultz and Leo Oliner for supplying the initial frozen beef thyroid preparations. This work was supported by grant A-4783, National Institutes of Health, Division of Arthritis and Metabolism, Bethesda, Maryland.

GEORGE L. COHN

Department of Internal Medicine,
Yale University School of Medicine,
New Haven 11, Connecticut.

¹ Roche, J., Michel, R., Closon, J., and Michel, O., *Biochim. Biophys. Acta*, **33**, 461 (1959).

² Wallace, E., and Lieberman, S., *J. Clin. Endocrinol. Metab.*, **23**, 90 (1963).

³ Cohn, G. L., Mulrow, P. J., and Dunne, V. C., *J. Clin. Endocrinol. Metab.*, **23**, 671 (1963).

⁴ Wallace, E., and Silberman, N., *J. Biol. Chem.*, **239**, 2809 (1964).

⁵ Fischer, A. G., Schultz, A. R., and Oliner, L., *Biochem. Biophys. Res. Comm.*, **14**, 39 (1964).

⁶ Robbins, P. W., and Lipmann, F., *J. Amer. Chem. Soc.*, **78**, 6409 (1956).

⁷ Nose, Y., and Lipmann, F., *J. Biol. Chem.*, **233**, 1348 (1958).

⁸ Fieser, L. F., *J. Amer. Chem. Soc.*, **70**, 3232 (1948).

⁹ Gornall, A. G., Bardawill, C. J., and David, M. M., *J. Biol. Chem.*, **177**, 751 (1949).

¹⁰ Schneider, J. J., and Lenbart, M. L., *J. Biol. Chem.*, **222**, 787 (1956).

PHYSIOLOGY

Effect of 'Furosemid' on Chloride and Water Excretion in Single Nephrons of the Kidney of the Rat

It has been shown by Muschaweck and Hajdú¹ that in the dog, at doses of 10 mg/kg body-wt. and more, 'Furosemid' (4-chloro-*N*-(2-furylmethyl)-5-sulphamoyl-anthranic acid) markedly increases the excretion of water, sodium and chloride. These authors also showed that the increase in chloride excretion was proportionally greater than that of sodium and water. On the other hand, Karger and Nagel² found a reduced permeability to anions, especially chloride, in the frog skin after addition of 'Furosemid' at concentrations of 25-500 mg/l. to the bathing solution, as evidenced by increased transmembrane potential differences without change in the short circuit current. Deetjen³ obtained proximal and distal tubular fluid samples in rats with an intravenous infusion of 2 mg/kg/min and observed significantly decreased proximal fluid/plasma (F/P) inulin ratios, as well as ratios below 2 in the distal tubule, concluding that 'Furosemid' depresses water and salt re-absorption in the proximal tubule and ascending limb of Henle's loop. Clearance studies by Suki, Rector and Seldin⁴ also indicated that 'Furosemid' might have an effect on the activity of the re-absorptive mechanisms of the proximal tubule and the ascending limb of Henle's loop.

Having this evidence in mind, we decided to investigate the fate of chloride along the nephron of the rat kidney, using micropuncture methods as described by Windhager and Giebisch⁵. Extreme care was taken in the collection of distal tubular samples, using rather long oil blocks (at least four times the tubular diameter), and checking the direction of flow by subsequent microdissection. Chloride and inulin were measured simultaneously, the first by the second coulometric method of Ramsay *et al.*⁶, the second by a modification of the microanthrone method of Hilger *et al.*⁷. 'Furosemid' was infused in saline (0.05 ml./min) at a rate of 0.8 to 1 mg/kg/min, sufficient to produce a marked diuresis. In parallel experiments the same measurements were done in control rats receiving saline infusion (0.05 ml./min) and in animals receiving a 4 per cent sodium chloride infusion at a rate of 0.1 ml./min.

Mean glomerular filtration rate (GFR) was 3.21 ml./kg/min in the 'Furosemid' group, 4.22 in the control group, and 5.77 in the 4 per cent sodium chloride group. Mean urinary pH was 6.32 (range 5.95-6.71) in the control group, 6.50 (5.99-6.83) in the 4 per cent sodium chloride infused, and 5.26 (4.76-5.82) in the 'Furosemid' group.

In Table 1a mean chloride F/P ratios in proximal and distal tubules, as well as chloride U/P ratios, are shown

Table 1

	(a) Chloride F/P ratios*						Urine		
	Proximal			Distal					
	Mean	Range	No.	Mean	Range	No.	Mean	Range	No.
Control	1.29	1.07-1.43	(12)	0.30	0.12-0.56	(15)	0.29	0.05-1.16	(11)
NaCl 4%	1.24	1.15-1.41	(8)	0.46	0.25-0.78	(10)	2.34	2.01-2.80	(7)
'Furosemid'	1.29	1.20-1.41	(10)	1.27	1.13-1.47	(15)	1.45	1.36-1.65	(15)

	(b) Inulin F/P ratios						Urine		
	Proximal			First 1/2 distal					
	Mean	Range	No.	Mean	Range	No.	Mean	Range	No.
Control	2.08	1.15-3.78	(11)	6.01	3.73-7.80	(10)	201.2	56-356	(12)
NaCl 4%	1.39	1.04-1.85	(8)	2.28	1.87-3.28	(9)	17.5	8.8-32.4	(7)
'Furosemid'	1.75	1.21-2.51	(7)	4.51	2.97-5.92	(9)	7.6	5.2-10.7	(15)

* Not corrected for an experimental ultrafiltrate/plasma ratio of 1.06.

n the 'Furosemid' and in the control groups. It can be seen that all three groups show similar proximal ratios, above unity. In the distal tubule, however, there is a marked difference between the control groups, having ratios significantly below unity, and the 'Furosemid' group, showing ratios consistently above one, similar to those observed in proximal tubules. There is a considerable rise in chloride ratios between distal tubules and collecting duct urine in the group receiving 4 per cent sodium chloride, but only very little further increase in the 'Furosemid' group. In Table 1b the corresponding inulin concentration ratios are seen. The proximal tubular inulin ratios are somewhat reduced, though not so much as those in the experiments of Deetjen³, probably due to our lower rate of infusion of 'Furosemid'. A larger reduction is observed in the early distal samples. On the other hand, it is noteworthy that there is little difference between the distal and urine inulin ratios in the 'Furosemid' group, as compared with the 4 per cent sodium chloride infused rats. The inulin ratios obtained in the latter group are similar to those obtained by Giebisch *et al.*⁸. Electrical potential difference measurements with glass microelectrodes in the 'Furosemid' infused rats showed proximal and distal transtubular potential differences within the range of controls (means -19.0 and -50.6 mV, respectively).

The results obtained in these experiments show that the mechanism of diuresis in rats undergoing 4 per cent sodium chloride infusion and during 'Furosemid' infusion is a quite different one, in spite of roughly similar overall chloride excretion rates (mean urinary Cl/inulin excretion rates of 0.13 in sodium chloride infused, and 0.19 in 'Furosemid' infused rats).

It appears that at our infusion rates of 'Furosemid' there is a relatively small inhibition of proximal and distal tubular fluid re-absorption. The major effect observed appears to be an impairment of the re-absorption of sodium chloride in excess of water in the ascending limb of Henle's loop, a process which constitutes the 'single effect' for the counter-current multiplier system of the renal medulla⁹. As shown by the high early distal chloride ratios in the 'Furosemid' group, the normally occurring hypotonicity of the tubular fluid at this site is absent, a further indication of impairment of the transport mechanisms in the ascending limb of Henle's loop. From our results, it appears that, due to inhibition of chloride and fluid re-absorption at this site, the build-up of a medullary salt concentration gradient might be impaired, as evidenced by the reduced further increase in the chloride and inulin F/P ratios between distal tubule and final urine. These experiments suggest, therefore, that 'Furosemid' in doses of 0.8-1 mg/kg body-wt./min acts preferentially on the sodium chloride transport system of the ascending limb of Henle's loop and the early distal convolution, preventing the establishment of a considerable concentration gradient for sodium chloride along these tubular segments.

This work was supported by a grant from Fund. de Amparo à Pesquisa do E. S. P. We thank Dr. Phyllis

Bott and Dr. Gerhard Giebisch for the loan of equipment and for their advice. We also thank Dr. Wilhelm Fill, from Hoechst do Brasil, for the supply of 'Furosemid'.

GERHARD MALNIO
FRANCISCO LACAZ VIEIRA
HIROKO ENOKIBARA

Department of Physiology,
University of São Paulo,
Brazil.

- ¹ Muschaweck, R., and Hajdu, P., *Arzneimittel-Forschung*, **14**, 44 (1964).
- ² Karger, W., and Nagel, W., lecture, Twenty-ninth Meeting of German Physiol. Soc., Bad Nauheim (1964).
- ³ Deetjen, P., Symp. on Laax, Bad Homburg (1963).
- ⁴ Suki, W., Rector, F. C., and Seldin, D. W., *Clin. Res.*, **12**, 260 (1964).
- ⁵ Windhager, E. E., and Giebisch, G., *Amer. J. Physiol.*, **200**, 581 (1961).
- ⁶ Ramsay, J. A., Brown, R. H. J., and Croghan, P. C., *J. Exp. Biol.*, **32**, 822 (1955).
- ⁷ Hilger, H. H., Kluemper, J. L., and Ullrich, K. J., *Arch. Ges. Physiol.*, **267**, 218 (1958).
- ⁸ Giebisch, G., Klose, R. M., and Windhager, E. E., *Amer. J. Physiol.*, **206**, 687 (1964).
- ⁹ Ullrich, K. J., *Prog. Cardiovasc. Dis.*, **3**, 395 (1961).

Feeding and Reproduction of Rats fed Calcium Cyclamate

WITH the increased production and consumption of calcium cyclamate as a sweetening agent in foods for dietary uses, a series of feeding trials on rats was initiated at the request of, and under contract with, the Sugar Research Foundation, Inc.

Earlier work in this area was reported in 1951. Fitzhugh *et al.*¹ using rats as test subjects fed sodium cyclamate at levels up to 5 per cent in the diet for a two-year period. At levels below 1 per cent sodium cyclamate displayed little if any effect in the rat. At a level of 5 per cent in the diet, weight loss accompanied by moderate diarrhoea was reported. Histopathological examination of the animals was normal.

As this early work was performed with sodium cyclamate, and as the human consumption of calcium cyclamate has greatly increased during the intervening years, additional feeding trials appeared in order. The use of calcium cyclamate in human dietaries where reduced calorie intake is important led to the inclusion of feeding trials where calorie intake was restricted.

Weanling rats were placed on dietaries containing 5 and 10 per cent calcium cyclamate. Growth, food and water intake, and biochemical parameters were observed. After eight weeks on test, half the animals from each group were placed on restricted intake of food. These latter animals were limited to 60 per cent of the calorie intake of the animals receiving *ad lib.* feeding.

At eight weeks the females from each group were mated with males from their respective groups. Females were allowed to deliver their young and raise them to weanling stage (21 days). Following a rest period the females were mated a second time and allowed to raise their young to weanling age.

Haemoglobin and urine examinations were conducted at 6 and 8 months with selected animals. Selected

animals from the control group and the group receiving 10 per cent calcium cyclamate were killed at 6 months and examined histopathologically.

So far (9 months), body-weight and food consumption data have illustrated an effect of feeding calcium cyclamate in the diet, whether on full feed or on restricted feed intake. Animals receiving 10 per cent calcium cyclamate consumed approximately 10 per cent more diet than control animals. This increase in food intake compensated for the non-caloric cyclamate in the diet. Thus the 10 per cent calcium cyclamate rats' calorie intake was equal to the control animals. Despite equal calorie intake animals receiving calcium cyclamate at the 10 per cent level grew at a rate 20–30 per cent less than control animals. This growth depression results from the inclusion of the calcium cyclamate in the ration, but whether this is due to toxicity *per se* or an interference with nutrient absorption in the gastro-intestinal tract is being determined. Possible effect on metabolic rate is being examined. The same growth depression was noted to a lesser degree in animals receiving 5 per cent calcium cyclamate in their diet.

Animals in all groups were in good health. The faeces of animals receiving calcium cyclamate in the diet were soft and moist. Initial diarrhoea during the early weeks of the experiment disappeared after 8 weeks on test. No remarkable variation was noted among the rats during haematological or urine examination. Animals receiving calcium cyclamate consumed 25–40 per cent more water daily.

Animals receiving *ad lib.* feed conceived and were able to raise their young to weaning (21 days) during both breeding trials. In the first litters the control young averaged 50 g in weight at 21 days of age, the 5 per cent calcium cyclamate young averaged 42 g, and the 10 per cent calcium cyclamate young averaged 32 g.

In the second litter, control young averaged 52 g, 5 per cent calcium cyclamate young 45 g, and 10 per cent calcium cyclamate young 32 g.

Reproduction investigations using animals receiving restricted or limited food intake were carried out. These animals conceived, bore their young, but were unable to maintain them beyond 5 days of age.

In an effort to understand the reduced growth rates in animals receiving calcium cyclamate in the diet despite equal calorie intake a balance study was set up. This investigation indicated complete utilization of the ration based on nitrogen retention and faecal and urinary nutrient excretion.

Other parameters to understand these effects, including possible increased metabolic rates and ultimate fate of cyclamate, are being investigated.

P. O. NEES
P. H. DERSE

Wisconsin Alumni Research Foundation,
Madison, Wisconsin.

¹Fitzhugh, O. G., Nelson, A. A., and Frawley, J. P., *J. Amer. Pharm. Assoc.*, 40, 583 (1951).

Carbon Dioxide Production of Whole Blood *in vitro*

A RECENT publication¹ has shown the mean oxygen consumption of whole blood *in vitro* at 37° C to be 8.5×10^{-5} ml./ml./min, and the mean rate of increase of P_{CO_2} to be 0.11 mm mercury/min. The addition of sodium fluoride was found to reduce the oxygen consumption by 26 per cent and to prevent completely the rise of P_{CO_2} . The authors commented that their findings were paradoxical "because oxygen could not be consumed without production of carbon dioxide".

We believe this statement to be incorrect. Working in separate centres, we have reached the conclusion that carbon dioxide production of whole blood *in vitro* at normal P_{O_2} is negligible compared with its oxygen con-

Table 1

	Temp. (° C)	Method	Rate of fall of O_2 content ml./ml./min	Temp. (° C)	Method	Rate of fall of CO_2 con- tent ml./min
Capel and Fletcher	37	Modified Haldane	10.4×10^{-5}	30	Modified Haldane	2.1×10^{-5}
Nunn	38	Polarography at 100% saturation of haemoglobin	10.5×10^{-5}	38	Van Slyke	5.6×10^{-5}

sumption. We have, in fact, found the carbon dioxide content of blood to fall during storage, although our values for oxygen consumption are of the same order as those of Lenfant and Aucutt¹.

Our results suggest a negative respiratory quotient for whole blood at normal P_{O_2} although we agree closely with the rate of rise of P_{CO_2} reported by Lenfant and Aucutt¹. We believe that this rise cannot be due to carbon dioxide production and suggest that it is due to the production of non-gaseous acids. We have found that blood stored at 37° C develops a metabolic acidosis of the order of 0.03 m.equiv./l./min, a figure which is in close agreement with the rate of increase of lactic acid². It can easily be shown by the addition of lactic acid to blood (*in vitro*) that the production of lactic acid is sufficient to account for the rise in P_{CO_2} without invoking the production of carbon dioxide.

L. H. CAPEL
E. C. FLETCHER

London Chest Hospital.

University of Leeds.

J. F. NUNN

¹Lenfant, C., and Aucutt, C., *J. App. Physiol.*, 20, 503 (1965).

²Pranker, T. A. J., *The Red Cell* (Blackwell, London, 1961).

Transplantation Experiments on Placental Ageing

KROHN¹ has recently re-emphasized the role that transplantation studies can play in the elucidation of the process of ageing in organs and tissues. He has shown that skin and ovary have intrinsic life cycles, to a large extent independent of central mechanisms. These techniques have not been successfully used in the study of rapidly ageing transient tissues like the placenta. Earlier transplantation studies of placenta have always used allogeneic tissues, since the importance of genetic differences in such experiments was not appreciated². The present experiments, to be described in detail elsewhere, were designed to demonstrate the evolution of syngeneic transplants of mouse trophoblast with age.

Adult mice of the highly inbred strains C3H and C57 were mated (C3H female \times C3H male, C3H female \times C57 male, C57 female \times C3H male). The gestational age of each pregnancy was calculated from the day of the appearance of a copulation plug. The pregnancies were interrupted on gestational days 2, 7, 10, 15, 18 and 20. Recipient animals were adult male mice syngeneic to the donor tissue in all cases. At two and seven days, the fertilized ova and the ectoplacental cone were transplanted into the spleen or under the kidney capsule as previously described³. The placental rims of the older pregnancies were divided into 1-mm fragments and implanted into the spleen and under the kidney capsule. The recipients were killed 5, 10, 15 and 20 days after transplantation, and the tissue fixed in formalin. Haematoxylin and eosin staining was routinely carried out.

Trophoblast underwent active proliferation from the implanted ova and ectoplacental cones of the 2- and 7-day-old pregnancies and it was actively invasive of host tissue. A large amount of haemorrhage was present from eroded host vessels by five days after transplantation. Such transplanted trophoblast cells were of large size with long cytoplasmic processes, giant nuclei and prominent nucleoli. Living cells from such transplants could occasionally be found as long as 25 days after transplanta-

ion, but most frequently cells survived for only 15–20 days. Host cellular reaction was minimal. No definite cytolysis or phagocytosis of host tissue as reported by Kirby⁴ was seen nor was there any ingrowth of host capillaries into the graft. Trophoblast-free haemorrhagic cysts interspersed with shreds of eosinophilic hyaline material remained after cell death.

In contrast, no proliferation or invasion of host tissue was present in any of the transplants performed after 10 days gestation. Haemorrhage was elicited by the 10-day gestational placental transplant, but its appearance was delayed until 10 days after transplantation. No haemorrhage was present in any transplant older than 15 days gestation. Vascularization of the transplant did not take place.

A cell pattern similar to that of the original transplanted placental fragments could be seen in the older implants removed 5 days after transplantation. Such patterns disappeared from specimens taken 10 days after implantation. Generally the smaller trophoblastic cells peculiar to the labyrinth of the placenta disappeared with time and the proportion of giant cells increased. In the specimens taken 10–15 days after transplantation, the vacuolized eosinophilic cytoplasm of the remaining cells tended to coalesce with the loss of cell boundaries. Soon afterwards, the giant cells became swollen or disintegrated. The cytoplasm then lost its vacuoles and became hyalinized. In the 15–20 day specimens, viable cells were rare or absent and eosinophilic hyaline bands frequently occupied the entire implant. Extensive hyalinization was present as early as 5 and 10 days after transplantation in the 18-day and 20-day gestational transplants.

In conclusion, it would appear that the transplanted mouse trophoblast has an intrinsic life cycle which extends about 25–30 days after conception. Its ability to proliferate and actively invade tissue is lost by mid-gestation and its ability to elicit haemorrhage by eroding host blood vessels is lost soon thereafter. There is a suggestion that the trophoblast nuclei may increase in size with age and that cellular degeneration may be accompanied by formation of a symplasma, which is not normally found in the rodent placenta⁵. These results are consistent with the early cessation of trophoblastic invasion and its pattern of ageing and hyalinization *in utero*⁶. Since proliferation and invasion are such prominent features of young transplants into the adult male, it is unlikely that the loss of trophoblastic vigour, and death, are due primarily to a changing or unfavourable endocrine environment.

This work was supported by grant CA 07466–01 from the National Institutes of Health, U.S. Public Health Service. One of us (R. L. S.) is a recipient of the Mead-Johnson award from the American College of Surgeons.

RICHARD L. SIMMONS
JOEL WEINTRAUB

Department of Surgery,
Columbia University College of Physicians and Surgeons,
New York.

¹ Krohn, P. L., *Proc. Roy. Soc.*, B, 157, 128 (1962).

² Simmons, R. L., and Russell, P. S., *Transplantation*, 2, 551 (1964).

³ Simmons, R. L., and Russell, P. S., *Ann. N.Y. Acad. Sci.*, 99, 717 (1962).

⁴ Kirby, D. R. S., *J. Reprod. Fertil.*, 5, 1 (1963).

⁵ Kirby, D. R. S., and Malhotra, S. K., *Nature*, 201, 520 (1964).

⁶ Wislocki, G. B., in *Osba Foundation Colloquia on Ageing: Ageing in Transient Tissues*, 105 (Churchill, London, 1956).

PHARMACOLOGY

Serotonin Depletion by Dietary Leucine

MAPLE syrup disease (branched-chain ketonuria) is an apparently familial disease associated with mental retardation and biochemically characterized by enhanced urinary excretion of branched-chain amino-acids and their products.

Few cases of this disease have been reported^{1–4} since its characterization by Menkes, Hurst and Craig in 1954 (ref. 5), and little is known about its biochemical basis beyond suggestive evidence implicating impaired oxidative decarboxylation of branch chain α -keto-acids⁶. Still less is known about the relationship between biochemical features of the disease and the attendant mental retardation.

Mental retardation is a prominent clinical feature both of maple syrup disease and the more familiar phenylketonuria, and attempts have been made, with some success, to establish corresponding biochemical similarities between these diseases. Thus, branched-chain and aromatic amino-acids, and/or their metabolites, inhibit rat brain decarboxylase⁷, serotonin and 5-hydroxytryptophan uptake by rat brain slices⁸, and, when added as a dietary supplement, reduce liver tryptophan hydroxylase activity⁹.

These similarities prompted us to determine whether the brain serotonin-level would be diminished by dietary leucine as it is by dietary phenylalanine¹⁰.

At 17 days of age litter-mate pairs of Sprague-Dawley rats¹¹, matched for age, weight and sex, were divided into control and experimental groups, housed in individual cages and fed *ad lib.* either a control diet¹¹ or the control diet supplemented with 5 or 8 per cent by weight of L-leucine¹². After 30–40 days on the diet, animals were killed by decapitation, the brain removed immediately, sectioned between medulla and spinal cord, rinsed in saline, blotted dry, and analysed for serotonin¹³. Statistical significance was determined using a *t* test for paired differences.

Brain serotonin-levels were diminished by dietary leucine in a manner analogous to that observed using dietary phenylalanine (Table 1). Experimental animals significantly differed from controls in both instances ($P < 0.05$).

Table 1. DEPLETION OF BRAIN SEROTONIN BY DIETARY LEUCINE

	Body-wt. (g) Mean \pm S.D.	Brain-wt. (g) Mean \pm S.D.	Serotonin (μ g/g brain) Mean \pm S.D.
Controls	187 \pm 8	1.726 \pm 0.048	0.609 \pm 0.033
5 per cent leucine	190 \pm 13	1.741 \pm 0.054	0.518 \pm 0.036
8 per cent leucine	170 \pm 8	1.626 \pm 0.024	0.473 \pm 0.010

Growth was impaired somewhat by 8 per cent but not 5 per cent leucine supplementation, although control animals did not significantly differ from those fed the 5 or 8 per cent diets because of the large variance in body-weights. Brain-weights reflected these differences in body-weight—animals on the 5 per cent diet having brains not significantly heavier than controls and animals on the 8 per cent diet having ones significantly lighter. The ratio of brain- to body-weight was essentially the same for all three groups.

Total brain serotonin was 1.084 μ g for controls, 0.969 μ g for the 5 per cent group and 0.863 μ g for the 8 per cent group, again indicating diminished serotonin with increased dietary leucine. Because of the differences in brain weight, however, the controls differed significantly only from the 8 per cent group ($P < 0.001$); the difference from the 5 per cent group reached the 0.1 level of statistical significance. Correcting for body-weight differences by calculating μ g serotonin/g brain/kg body-weight, the control value of 3.21 was significantly greater than 2.69 for the 5 per cent group ($P < 0.05$) but not significantly greater than the 2.78 for the 8 per cent group.

Despite these complications resulting from altered growth rates, it seems clear that dietary leucine supplementation lowers brain serotonin. It has been suggested¹⁴ that the decreased brain serotonin in the phenylalanine-fed animals is related to impairment in problem solving ability. The present finding suggests a possible common basis for the mental deficiency accompanying phenylketonuria and maple syrup disease.

This work was supported in part by U.S. Public Health Service grants HD 01058 and AM 01775.

ARTHUR YUWILER
EDWARD GELLER

Neurobiochemistry Laboratory,
Veterans Administration Center
and Department of Psychiatry,
University of California,
Los Angeles.

- ¹ Westall, R. G., Dancid, J., and Miller, S., *Amer. Med. Assoc. J. Dis. Child.*, **94**, 571 (1957).
- ² Mackenzie, D. Y., and Woolf, L. I., *Brit. Med. J.*, **i**, 90 (1959).
- ³ Tada, K., et al., *Tohoku J. Exp. Med.*, **79**, 142 (1963).
- ⁴ Lane, M. R., *J. Pediatrics*, **58**, 80 (1961).
- ⁵ Menkes, J. H., Hurst, P. L., and Craig, J. M., *Pediatrics*, **14**, 462 (1964).
- ⁶ Daniels, J., Hutzler, J., and Levitz, M., *Pediatrics*, **32**, 234 (1963).
- ⁷ Tashuan, R. E., *Metab. Clin. and Exp.*, **10**, 393 (1961).
- ⁸ Schanberg, S. M. J., *J. Pharmacol.*, **139**, 191 (1963).
- ⁹ Yuwiler, A., Geller, E., and Slater, G. G., *Life Sci.*, **3**, 567 (1964).
- ¹⁰ Yuwiler, A., and Louttit, R. T., *Science*, **134**, 831 (1961).
- ¹¹ Strain obtained for Berkeley Pacific and bred by Dr. DeVols of the Veterans Administration Animal Facility.
- ¹² L-Leucine (methionine-free) obtained from Mann Biochemical Corp.
- ¹³ Bogdanski, D. F., Pletscha, A., Brodie, B. B., and Udenfriend, S., *J. Pharmacol. Exp. Therap.*, **117**, 82 (1956).
- ¹⁴ Wooley, D. W., and Van der Hoeven, Th., *Science*, **144**, 883 (1964).

IMMUNOLOGY

Mathematical Model for the Process of Aggregation in Immune Agglutination

IMMUNE haemagglutination is the combination of bivalent antibody molecules with specific antigenic determinants on the surface of red cells and the coincident collision of red cells resulting in the formation of antibody cross-linkages between cells and visible aggregation^{1,2}. As a means of characterizing immune haemagglutination, rates of red cell aggregation were examined with the aid of a Coulter electronic cell counter³. In the course of this examination it was empirically found that the initial decrease in free cell concentration followed an exponential decay curve. There was no basis, however, for considering aggregation to be a first-order reaction. Subsequent consideration suggested that aggregation involved an orderly sequence which might be expressed mathematically.

The mathematical representation of the rate of red cell aggregation presented in this report was arrived at through several series of experiments in which theoretical and experimental curves were compared. The eventual close approximation achieved between these curves tends to substantiate the validity of the equations from which the theoretical rate curves were derived.

The experimental curves were obtained by a procedure previously described in detail³. The rate of reduction in free cell concentration and total number of particles (total of free cells plus aggregates) was followed by means of the 'Model B' Coulter electronic cell counter (Coulter Electronics, Hialeah, Florida). Rates were determined under controlled conditions of antiserum concentration, cell concentration, temperature and speed of mechanical mixing. The reaction vessel of 150 ml. capacity was secured in a temperature control jacket, which fits on the stand of the Coulter counter. Agitation was accomplished with a spiral stirrer attached to a constant speed motor, 300 r.p.m. The 100- μ aperture tube had a sampling time of 15 sec. The temperature was maintained at 25° C.

With the double thresholds arrangement of the Coulter counter it was possible to follow experimentally the concentration of several different size aggregates, but because of practical limitations concerned with inhomogeneity in the size of red cells, and complications concerned with coincidence, only the total particle count was followed.

An initial lag period in the rate curve, due to the time required for the primary union of antibody and cells, was eliminated by incubating antiserum and cells without mechanical mixing³. At the antiserum and cell concentra-

tions employed in these experiments, a sensitization period between 30 and 80 min was sufficient to eliminate the lag period.

Several immune systems of different specificity were examined in these studies. Human A and B isoantibodies and human red cells of appropriate blood group were used in early experiments. Variation in the degree of fit between theoretical and experimental rate curves obtained with different cell populations suggested a heterogeneity of the red cell population with respect to their agglutinability⁴. It was found that this heterogeneity could be minimized with any of several antigens coupled to tanned red cells⁵. The agglutination obtained with human anti-A sera and purified hog A substance coupled to tanned group C human red cells was most intensively examined.

Immune aggregation can be defined as the change in state from free cells to aggregates due to the random collision of partially antibody-coated (sensitized) red cells. The random collision of sensitized cells leads to a progressive decrease in the number of free cells with a concomitant increase in the number of aggregates of larger size with time. Although the concentration of free cells would decrease throughout the process of aggregation, the concentration of progressively larger size aggregates would consecutively increase and then decrease as aggregation proceeds. The combinations which lead to the formation and subsequent decrease of different size aggregates are shown in Table 1. Based on these considerations a mathematical model for red cell aggregation was progressively developed. The present form of the model will be explained.

Table 1. COMBINATIONS WHICH INCREASE AND DECREASE THE CONCENTRATION OF DIFFERENT SIZE AGGREGATES

Aggregate size	Combinations which:	
	increase number of aggregates	decrease number of aggregates
1	0	(1,1) (1,2) ... (1,N)
2	(1,1)	(2,1) (2,2) ... (2,N)
3	(1,2)	(3,1) (3,2) ... (3,N)
4	(1,3) (2,2)	(4,1) (4,2) ... (4,N)
5	(1,4) (2,3)	(5,1) (5,2) ... (5,N)
6	(1,5) (2,4) (3,3)	(6,1) (6,2) ... (6,N)
7	(1,6) (2,5) (3,4)	(7,1) (7,2) ... (7,N)
8	(1,7) (2,6) (3,5) (4,4)	(8,1) (8,2) ... (8,N)
9	(1,8) (2,7) (3,6) (4,5)	(9,1) (9,2) ... (9,N)
10	(1,9) (2,8) (3,7) (4,6) (5,5)	(10,1) ... (10,N)
...		
N	(1,N-1).....	(N,1).....

It is assumed that there may be aggregates of 1, 2, 3 . . . N cells. The number of aggregates consisting of i cells at time t can be denoted by $X_i(t)$.

To analyse the change in $X_i(t)$ with time, let $A_{ij}(t)$ be the rate at which the number of aggregates of size i combine with aggregates of size j at time t . Notice that if $i=j$, then only $A_{ii}(t)/2$ aggregates of size $2i$ are produced. To make this clear, consider the following two examples. If 5 aggregates of size 3 combine with 5 of size 4, then $A_{34}(t)=5$ and 5 aggregates of size 7 are formed. But if 5 aggregates of size 3 combine with another 5 of size 3, then there are 10 aggregates of size 3 which each combine with an aggregate of size 3, so $A_{33}(t)=10$ whereas only 5 aggregates of size 6 are produced.

The next consideration was those combinations of aggregates which increase the number of aggregates of size i . The combination of an aggregate of size j with one of size k will do so if $j+k=i$. Thus at each time t , $X_i(t)$ is increasing at the rate of the sum of $A_{jk}(t)$ with $j+k=i$. If $j=k$, then the appropriate term is $A_{jj}(t)/2$. In order that each combination be considered only once, the following restriction is added: $j \leq k$; thus $A_{12}(t)$ and $A_{21}(t)$ are not both added to $X_3(t)$.

The combinations of aggregates which decrease $X_i(t)$ are all those of the form $A_{ij}(t)$ subject only to the condition that $i+j \leq N$.

Putting this information together, the following system of differential equations may be written, where $X'_i(t)$ is the rate change of X_i at time t , that is, $dX_i(t)/dt$.

f i is odd, then:

$$X'_i(t) = \sum_{\substack{j < k \\ j+k=i}} A_{jk}(t) - \sum_{i+j \leq N} A_{ij}(t) \quad (1)$$

f i is even, then:

$$X'_i(t) = \sum_{\substack{j < k \\ j+k=i}} A_{jk}(t) + A_{i/2, i/2}(t)/2 - \sum_{i+j \leq N} A_{ij}(t) \quad (2)$$

To complete the model, $A_{ij}(t)$ was derived. The rate of combination of aggregates of size i with aggregates of size j should be proportional, according to the law of mass action, to the product of the concentration of the interacting aggregates. Thus, $A_{ij}(t) = C_{ij} X_i(t) X_j(t)$, where C_{ij} represents the readiness of aggregates of size i to combine with aggregates of size j . It is assumed that C_{ij} is proportional to the product of the average surface area of aggregates of size i and of aggregates of size j , that is $C_{ij} = C s_i s_j$, where s_i and s_j are the average surface of aggregate of size i and j . We thus have:

$$A_{ij}(t) = C s_i s_j X_i(t) X_j(t) \quad (3)$$

Because the shape of the red cell is a non-rigid biconcave disk, a mathematical estimate of the effective surface area of aggregates would be a major undertaking. It was therefore decided to use a physical model consisting of rigid spheres to obtain an approximation of aggregate surface area for small aggregates. Models of different aggregate size, with maximum contact among the spheres, were constructed. The effective surface area of each aggregate was estimated by attempting to fit a free sphere into the aggregate and then tracing around it. The exposed traced areas were cut out and weighed for each model aggregate. Based on the weight of the fragments, the relative effective surface of single spheres and aggregates of sizes between one and nine were calculated (Table 2). For aggregates beyond nine, it was assumed that the aggregates were spherical and the increase in surface was calculated per unit increase in volume. Thus, for sizes greater than nine, surface area was obtained recursively. $s_i = (i/i - 1)^{2/3} s_{i-1}$.

Table 2. THE RELATIVE EFFECTIVE SURFACE AREA OF DIFFERENT SIZE AGGREGATES (I-IX) BASED ON A RIGID SPHERE MODEL

I	II	III	IV	V	VI	VII	VIII	IX
1.00	1.48	1.81	2.07	2.32	2.59	2.88	3.14	3.41

Having obtained values for the surface area of different size aggregates, C of equation (3) remains as a parameter which varies with the aggregating potential of the sensitized cells. This parameter would include any factor which

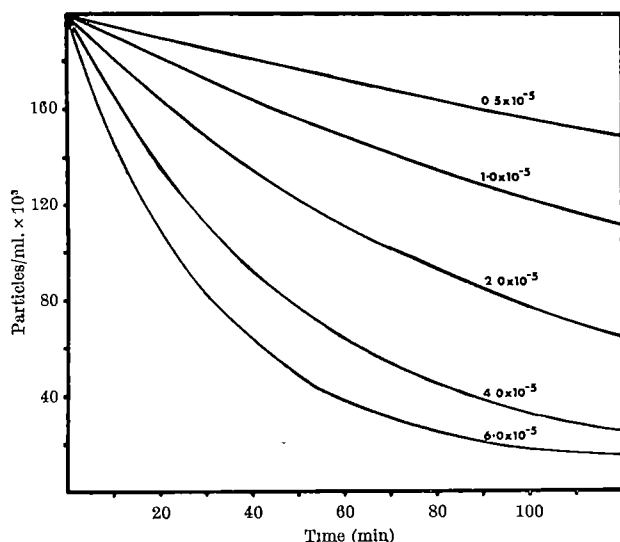


Fig. 1. A plot of curves representing the concentration of particles with time, substituting different values for C (aggregating potential of cells)

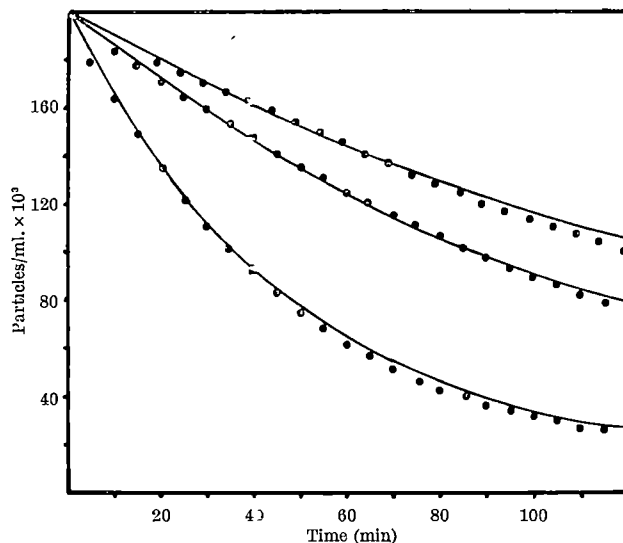


Fig. 2. A comparison of three different theoretical and experimental curves obtained with human anti-A sera and hog A substance coupled to tanned human O cells. The theoretical curves are represented by solid lines and the experimental curves indicated by points. Values of C : 1.1×10^{-5} , 1.6×10^{-5} and 4.0×10^{-5}

affects either the quantitative or qualitative characteristics of the antigen-antibody reactions responsible for agglutination⁶. For example, at different antiserum concentrations the red cells are sensitized to different degrees, altering the rate at which antibody cross-linkages are formed between cells and, therefore, the rate of aggregation. A fit of such different rate curves could be achieved by substituting an appropriate value for C .

The computations required to solve the equations for a theoretical rate curve were performed on an IBM '7094' computer and were obtained by the recursive formula: $X_i(t+1) = X_i(t) + X'_i(t)$, t being in minutes with the boundary conditions that all $X_i(0) = 0$ except for X_1 which equals the initial number of free cells. In these computations, aggregates up to 100 cells in size were allowed. Several theoretical curves of total particle count with different values of C are shown in Fig. 1. At low values of C a nearly linear rate is obtained. With a progressive increase in the value of C there is a decreasing effect on the rate curve. Theoretical curves and experimental curves obtained with different concentrations of antiserum are compared in Fig. 2. It can be seen that the mathematical model does give a reasonably close approximation to the rates of aggregation obtained experimentally.

According to the foregoing considerations, the process of immune aggregation is pictured as a sequence of second-order reactions which can be mathematically expressed as a system of second-degree first-order differential equations with constant coefficients.

This investigation was supported by U.S. Public Health Service research grant AI-03667 and grant FR-00013 from the Division of Research Facilities and Resources.

TAO KUANG MING*

HAROLD S. GOODMAN

Division of Medicine, Blood Center,
Michael Reese Hospital and Medical Center,
Chicago, Illinois.

BARRY BROWN
Biological Computation Center,
University of Chicago,
Chicago, Illinois.

* Present address: Illinois Institute of Technology, Chicago, Illinois.

¹ Gynes, L., and Schon, A. A., *J. Immunol.*, **89**, 483 (1962).

² Fudenberg, H. H., Mandy, W. C., and Nisonoff, A., *J. Clin. Invest.*, **41**, 2123 (1962).

³ Ming, T. K., Severson, C., and Goodman, H. S., *J. Immunol.*, **93**, 567 (1964).

⁴ Atwood, K. C., *Proc. U.S. Nat. Acad. Sci.*, **44**, 1954 (1958).

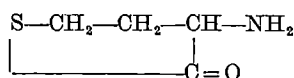
⁵ Rolitt, I. M., and Doniach, D., *Lancet*, **ii**, 1027 (1958).

⁶ Goodman, H. S., *Transfusion*, **22**, 327 (1962).

RADIOBIOLOGY

Electron Spin Resonance Investigations of Ultra-violet and X-irradiated Homocysteine-thiolactone

SOME time ago we discussed the formation of radicals in irradiated samples of methionine¹ and bovine serum albumin². In the case of methionine, in which the bivalent sulphur is present in the form of a thioether, X-irradiation forms radicals of the alkyl-type, but ultra-violet irradiation (254 mμ) localizes them at the sulphur atom. Moreover, ultra-violet light is able to quench the alkyl-type radicals. In the case of bovine serum albumin, X-irradiation as well as ultra-violet light forms a complex electron spin resonance spectrum consisting of a glycylglycine- and a sulphur-radical³. The portion of the sulphur radical part becomes bigger after ultra-violet irradiation. By ultra-violet irradiation of longer wave-length ($\lambda > 300$ mμ) the X-ray conditioned radical state at the carbon atom is transferred to the SS- or SH-groups of the albumin molecule. In the following investigations we controlled the behaviour of the bivalent sulphur in the form of a thiolactone ring compound, that is, a thioester, homocysteine-thiolactone (HCT):



Polycrystalline, *d,l*-homocysteine-thiolactone (HCT)-HCl (Degussa, Konstanz/Germany) with a bulk volume of about 220 cm³/100 g was sealed under vacuum ($< 10^{-3}$ torr) and irradiated at room temperature. The electron spin resonance measurements were carried out under the same conditions as previously described¹. The X-irradiation was carried out with a 200 kV source (half value layer: 0.64 mm copper, dose-rate 920 r./min, dose 100 kr.). The irradiations with ultra-violet were performed with a mercury low-pressure lamp (NN 15/44, Quarzlampengesellschaft, Hanau, Germany) which emits mainly in the 254 mμ line. The intensity of the ultra-violet irradiation at the sample tube amounted to 9×10^3 ergs cm⁻² sec⁻¹. The other technical details have been reported in a former publication¹.

After X-irradiation a radical yield of 10.8/100 eV is obtained. The electron spin resonance signal of the X-irradiated HCT is demonstrated in Fig. 1 (solid line). Though it is not possible to differentiate the obtained spectrum of the polycrystalline material, it is possible to ascertain that there exists no sulphur radical state. The different behaviour of saturation effects of the micro-wave energy on the different parts of the spectrum includes at

least two different radicals. We suppose that the unpaired electrons are localized at a carbon and/or nitrogen atom of the molecule. The radicals are very stable not only in vacuum but also in air. In vacuum and at room temperature the decay of the radical concentration is only about 1 per cent/h.

In contrast to methionine and bovine serum albumin ultra-violet light of 254 mμ has no influence on the X-ray signal of homocysteine-thiolactone. The dotted line in Fig. 1 shows the change of the X-ray spectrum after an ultra-violet irradiation of 7 h. This dotted line spectrum demonstrates clearly that only one part of the former spectrum is influenced by quenching. The same change is obtained by warming up the X-irradiated sample of HCT. In comparison with the solid line spectrum (X-ray alone) the radical concentration was found to be diminished after ultra-violet irradiation to nearly 88 per cent. It is very interesting that no sulphur radical is detectable although the lactone ring is opened by the ultra-violet irradiation. The latter process is certain because hydrogen sulphide gas is liberated. Perhaps the yield is not big enough to detect the sulphur radicals by the sensitivity of the electron spin resonance spectrometer.

If HCT is irradiated only with ultra-violet light of the wave-length of 254 mμ for 8 h, a radical concentration of only 5 per cent is obtained corresponding to that of an X-irradiation with 100 kr. This ultra-violet signal is not decomposed, it might be a quintet and centred at $g = 2.0046$. In contrast to sulphydryl-, disulphid- and thioether-compounds, there is no sulphur radical to be found for the thiolactone.

The results we obtained from bivalent sulphur containing compounds demonstrate quite clearly that not only the primary formation of sulphur radicals but also the transfer of the radical state to the sulphur atom depends on the chemical binding of the bivalent sulphur. In our next publication we hope to describe the influence the whole molecule has on the character of the sulphur radical.

R. KOCH
H. MÖNIG

Radiologisches Institut der Universität,
Freiburg im Breisgau,
Germany.

¹ Monig, H., and Koch, R., *Nature*, **202**, 289 (1964).

² Koch, R., and Monig, H., *Nature*, **203**, 859 (1964).

³ Gordy, W., and Shields, H., *Rad. Res.*, **9**, 611 (1958).

Use of Technetium-99m in Hepatic Scintillography

HARPER *et al.*¹ have recently pointed out the excellent possibilities of technetium-99m in medical scintillography.

Tc-99m is a nucleid with a period of semidisintegration time of 6 h, emitting γ -radiation of 140 keV, obtained by acid elution of molybdenum-99 adsorbed on an aluminium column. It is obtained as a pertechnetate (TcO_4^-), which has a behaviour similar to the I⁻ ion in the body. We have used it with very good results in thyroid scintillography^{2,3}. Harper has also used this compound combined with a colloid which is retained by the reticulo-endothelium in liver scintillography¹. The Production Department of the Comisión Nacional de Energía Atómica, Argentina⁴, has obtained a colloid of antimony sulphide which has a molecular size of 100 to 400 mμ, an adequate take-up of ^{99m}Tc, and appears to be very stable. We have been able to obtain hepatic pictures of excellent resolution by intravenous injection of this substance (Fig. 1). Its concentration in the liver allows us to perform tracing only 10 min after injection and its activity is still at adequate levels after 180 min.

After 24 h the activity eliminated by urine was 10–15 per cent. We have used activities of 0.4–1.0 mc.

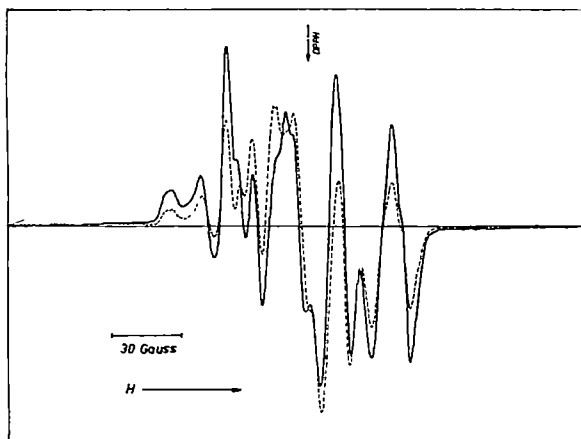


Fig. 1. Electron spin resonance spectra of irradiated homocysteine-thiolactone. The spectra represent the first derivatives of the actual absorption curves. —, 100 kr; ---, 100 kr + 7 h ultra-violet ($\lambda = 254$ mμ)

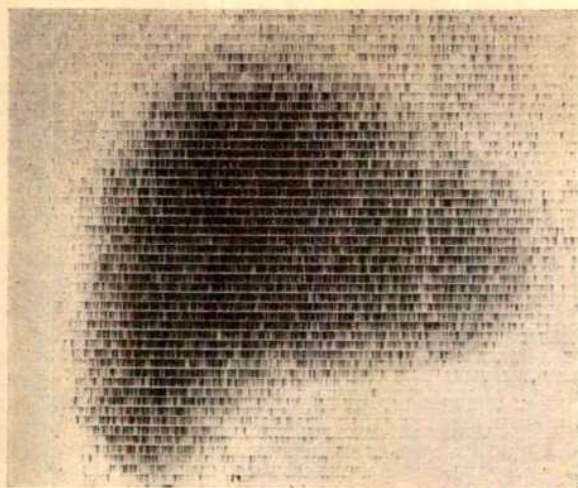


Fig. 1. Normal liver scanning (approximate dose 0.8 mc.) ^{99m}Tc -labelled colloid

which are much smaller than those used by Harper *et al.* The equipment used was a 'PHO-DOT' (Nuclear Chicago). The pictures obtained could be compared satisfactorily with those obtained with rose bengal iodine-131 colloid albumin, the body radiation being several thousand times smaller with ^{99m}Tc .

O. DEGROSSI
J. MARTINEZ SEEGER
H. GOTTA
A. OLIVARI
A. CHWOJNICK

Centro de Medicina Nuclear,
Hospital-Escuela Jose de San Martin,
Cordoba 2351, Buenos Aires.

¹ Harper, P. V., Beck, R., Charleston, D., and Lathrop, K. A., *Nucleonics*, **22**, 50 (1964).

² Degrossi, O., Gotta, H., Olivari, A., Chwojnik, A., and Pecorini, V., *Rev. Arg. Endocrinol. Metab.*, **10**, 134 (1964).

³ Baró, G., Chwojnik, A., Degrossi, O., Olivari, A., and Pecorini, V., *I Simposio Argentino de Medicina Nuclear, Mendoza*, 19-21, November, 1964.

⁴ Garzón, L., Palcos, M., and Radicella, R., *Int. J. Appl. Radioisotopes* (in the press).

BIOLOGY

Multiple Thymus Grafts in Aged Mice

ONE characteristic of the thymus which has not yet been explained completely is the weight loss (primarily lymphoid depletion) of the organ with increasing age. This process, known as thymus involution, is most marked immediately after puberty, but thymus involution continues, albeit at a slower rate, throughout subsequent life.

The recent data of Harris *et al.*¹ from parabiotic mice and Miller² and Metcalf and Wakonig-Vaartaja³ from thymus grafts have made it evident that the lymphoid population of the thymus is replaced continuously during life by precursor cells seeding in the organ from the blood. These precursor cells then proliferate to build up a large new population of primitive and mature thymic lymphocytes. The most probable source of these thymus-seeding precursor cells is the bone marrow.

Analysis of the growth patterns of thymus grafts has suggested that the growth of the lymphoid elements of the thymus is regulated by the non-lymphoid elements of the organ—the specialized thymic epithelial and reticulum cells⁴. The strength of the proliferative stimulus exerted by these elements appears to decrease with advancing age and, if so, this could be the basis for the decrease in size of the organ with advancing age. However, in view of the continuous replacement of the lymphoid

population of the organ throughout life by precursor cells of extrathymic origin, another possible basis for the decline in size of the organ is that, with advancing age, the supply of thymus-seeding precursor cells becomes restricted, leading to a limitation in the total amount of thymic lymphoid tissue which can be built up, and sustained, by the proliferative activity of these cells.

To investigate the possibility that the supply of thymus-seeding lymphoid precursor cells may be limited in old animals, use was made of an earlier observation that when more than one thymus is grafted to a young recipient, each graft behaves autonomously, achieving the same size it would if grafted alone to a normal or even a thymectomized recipient⁵. This allows the possibility of growing unlimited amounts of thymus tissue in the same animal and thereby of testing whether the supply of thymus-seeding cells ever becomes limiting for the growth of such grafts—particularly in very old animals. In the present investigation, 20-month-old C57 BL mice were each grafted subcutaneously with 12 or 24 whole 1-day-old C57 BL thymus glands. One month after grafting the weights of thymus grafts and host lymphoid organs were determined. As controls, C57 BL thymus glands were grafted to young adult C57 BL mice (either 1, 12, 24 or 48 per recipient) and these grafts were also examined one month after grafting. The results obtained are summarized in Table 1. In agreement with previous investigations⁵, in the young hosts, a linear relationship was observed between the number of thymus glands grafted and the total weight of thymus graft tissue recovered. In neither type of host did the multiple thymus grafts significantly affect the weights or the histological appearance of the host thymus or other lymphoid organs. It is evident that the total mass of thymus graft tissue achieved in 4 weeks in the old recipients was somewhat less than for the corresponding number of grafts in young adult recipients. However, the mass of thymus graft tissue achieved far exceeded that of the maximum size of the normal C57 BL thymus in young mice (60 mg). Histologically no difference between grafts in young and old hosts could be detected in the morphology, extensiveness and mitotic activity of the cortical lymphoid regions of the thymus graft tissue.

Table 1. THYMUS GRAFT WEIGHTS ATTAINED BY MULTIPLE WHOLE C57 BL THYMUS GRAFTS IN YOUNG AND OLD C57 BL MICE

Age of recipient at grafting	No. of thymus glands grafted per recipient*	No. of recipients	Mean host thymus weight (mg)	Mean weight of individual thymus grafts (mg)	Mean total thymus graft weight per recipient (mg)
2 months	1	28	47	24	24
	12	41	42	22	262
	24	2	48	25	609
	48	2	36	24	1,160
20 months	12	18	16	16	185
	24	2	15	19	467

* Mean weight of each donor thymus gland, 3 mg.

Since the lymphoid population of thymus grafts is derived from host cells, it is obvious from the present experiments that, although there may be some restriction in the supply of thymus-seeding precursor cells in old age, the available supply is quite capable of supplying thymus-seeding precursor cells for a mass of thymus tissue far exceeding that of even the pre-involutional thymus. Therefore it may be concluded that no significant limitation in the supply of thymus-seeding precursor cells exists in older mice and such a factor cannot be responsible for the decrease in size of the thymus with advancing age. Recently, Makinodan and Peterson⁶ have demonstrated that in old mice the number of cells in the spleen capable of being stimulated by sheep red cells does decline in old age, and we have obtained similar results in C57 BL mice. The cell population in the spleen is replaced continuously by cells entering from the blood in essentially the same manner as is that in the adult thymus. It appears therefore that although the supply of some spleen-seeding cells may become restricted in old age, there is no significant

limitation in the supply of thymus-seeding cells in mice of a similar age.

DONALD METCALF

Cancer Research Laboratory,
Walter and Eliza Hall Institute,
Royal Melbourne Hospital,
Victoria, Australia.

¹ Harris, J. E., Barnes, D. W. H., Ford, C. E., and Evans, E. P., *Nature*, **201**, 884 (1964).

² Miller, J. F. A. P., *CIBA Symp. Tumour Viruses of Murine Origin*, 262 (J. and A. Churchill, London, 1962).

³ Metcalf, D., and Wakonig-Vaartaja, R., *Proc. Soc. Exp. Biol. and Med.*, **115**, 731 (1964).

⁴ Metcalf, D., in *The Thymus*, edit. by Defendi, V., and Metcalf, D., 53 (Wistar Institute Press, Philad., 1964).

⁵ Metcalf, D., *Austral. J. Exp. Biol. and Med. Sci.*, **41**, 437 (1963).

⁶ Makinodan, T., and Peterson, W. J., *J. Immunol.*, **93**, 886 (1964).

Role of Sialic Acid in Potassium Transport of L1210 Leukaemia Cells

THE cell membranes of various tumour strains possess large quantities of sialic acid and a strong, negative surface charge¹⁻³. This charge, which is partly due to the ionized carboxyl group of sialic acid^{2,4,5}, may be important in influencing physiological properties such as transport of ions⁶. Since tumour cells seem to concentrate K⁺ ions better than normal cells⁷⁻⁹, we have begun to investigate the regulation of K⁺ fluxes by sialic acid.

L1210 mouse leukaemia cells, which had been maintained in the ascitic form, were washed at 3° C with isotonic saline until free of red blood cells. The L1210 cells were then incubated for 90 min at 37° C in the incubation media shown in Table 1. Sialic acid was removed from the leukaemia cells by the addition of neuraminidase (*Vibrio cholerae*), which was present at a final concentration of 50 units/ml. Under these conditions the sialic acid released is derived essentially from the cell membrane⁴. Free sialic acid was determined by the method of Warren¹⁰. Na⁺ and K⁺ ions were assayed by means of a flame spectrophotometer. The optimum wave-lengths were 589 mμ for Na⁺ and 766.5 mμ for K⁺. ¹⁴C-D-Glucose (5.2 × 10³ c.p.m./μmole) and ¹⁴C-L-lysine (1.6 × 10⁵ c.p.m./μmole) were utilized in measuring glucose and lysine uptakes.

Table 1. CONSTITUENTS OF THE INCUBATION MEDIA

Substance	Conc. (mM)	K-free	Incubation medium K-low _g	K-free _{g-1}	K-low _{g-1}
L-Lysine	0.2	—	—	+	+
D-Glucose	4.9	—	+	+	+
KCl	10.0	—	+	—	+
NaCl	130.2	—	+	—	+
NaCl	140.2	+	+	—	—
Na ₂ HPO ₄	4.8	+	+	+	+
NaH ₂ PO ₄	5.2	+	+	+	+
CaCl ₂	0.9	+	+	+	+
CH ₃ COONa	5.0	+	+	+	+

The amounts of sialic acid removed by neuraminidase in the K-free medium were 0.21 μmole/100 mg dry wt of cells after 45 min of incubation and 0.23 μmole/100 mg dry wt at 90 min. The quantities of sialic acid released were nearly the same in any of the incubation media listed in Table 1, and the values obtained approximated those determined for the Ehrlich ascites tumour cell⁴.

Different incubation media were utilized in the transport investigations, so that the direction of ion flow could be altered. In the K-free medium, K⁺ release from the L1210 cells accompanied Na⁺ uptake; with the K-low_g medium, K⁺ uptake accompanied Na⁺ release. Table 2 includes the results of typical experiments, in which the removal of sialic acid inhibited K⁺ transport, regardless of direction of flow. When the results from a number of experiments were pooled, the differences between the control and the experimental groups were found to be statistically significant (*P* < 0.05). Contrary to the relatively sharp response of K⁺ ions to the removal of sialic acid, Na⁺ transport was only slightly inhibited when neuraminidase was present, and in this case the results did not yield significant differences.

Table 2. TRANSPORT IN L1210 CELLS STRIPPED OF SIALIC ACID

Medium	Substance measured	Time (min)	μmoles/100 mg dry wt. of cell	Control	+ Neuraminidase
K-free	K ⁺	45	—	-2.56	-1.97 (23)
		90	—	-4.60	-3.40 (26)
	Na ⁺	45	—	+15.1	+13.4 (11)
		90	—	+13.8	+12.0 (13)
K-low _g	K ⁺	45	—	+25.0	+16.0 (36)
		90	—	+26.0	+17.0 (35)
	Na ⁺	45	—	-50.0	-45.0 (10)
		90	—	+3.13	+3.09 (1)
K-free _{g-1}	D-glucose	45	—	+6.31	+6.25 (1)
		90	—	+0.208	+0.204 (2)
	L-lysine	45	—	+0.206	+0.207 (0)
		90	—	—	—

(+) Indicates flow into the cells; (−) indicates flow out of the cells. Percentage decreases from the control values are given in parentheses.

Glucose uptake was determined for both the control cells and the enzyme-treated cells and did not appear to differ between the two groups. This finding suggests that transport in general was not affected by sialic acid and that K⁺ transport in particular was not regulated by the action of membrane-bound sialic acid on glucose utilization. Furthermore, although glucose was absorbed from the K-free medium, the removal of sialic acid inhibited K⁺ outflow.

Finally, sialic acid was found not to affect lysine uptake in the absence or presence of K⁺ ions, thereby indicating that sialic acid does not influence transport of positively charged substances in general.

Pardee¹¹ has postulated that altered surface properties of tumour cells may cause a loss of growth control mechanisms. The accumulation of K⁺ ions in malignant cells⁷⁻⁹ may well be such a mechanism, which is partly responsible for the adaptation of these cells to a state of uncontrolled growth. The presence of K⁺ ions is necessary for optimal growth of certain bacteria strains^{12,13} and plant tumours^{14,15}. Lubin¹⁶ has demonstrated a control of protein synthesis by K⁺ accumulation. The work recorded here in turn indicates that sialic acid apparently mediates both the inward and outward diffusion of K⁺ ions in a leukaemia cell.

We thank Prof. Arthur B. Pardee for his advice. This work was supported by grants 1-F2-CA-24245-01 and AI-04409 from the U.S. Public Health Service.

J. LESLIE GLICK

SHERWOOD GITHENS III

Department of Biology,
Princeton University,
Princeton, New Jersey.

¹ Defendi, V., and Gasic, G., *J. Cell. Comp. Physiol.*, **62**, 23 (1963).

² Forrester, J. A., Ambrose, E. J., and Macpherson, J. A., *Nature*, **196**, 106 (1962).

³ Miller, A., Sullivan, J. F., and Katz, J. H., *Cancer Res.*, **23**, 485 (1963).

⁴ Wallach, D. F. H., and Eylar, E. H., *Biochim. Biophys. Acta*, **52**, 594 (1961).

⁵ Wallach, D. F. H., and de Perez Esandi, M. V., *Biochim. Biophys. Acta*, **83**, 363 (1964).

⁶ Hempling, H. G., *J. Cell. Comp. Physiol.*, **60**, 181 (1962).

⁷ Brunschwig, A., Dunham, L. J., and Nichols, S., *Cancer Res.*, **6**, 230 (1946).

⁸ Dunham, L. J., Nichols, S., and Brunschwig, A., *Cancer Res.*, **6**, 233 (1946).

⁹ DeLong, R. P., Coman, D. R., and Zeidman, I., *Cancer*, **3**, 718 (1950).

¹⁰ Warren, L., *J. Biol. Chem.*, **234**, 1971 (1959).

¹¹ Pardee, A. B., *Nat. Cancer Inst. Monograph*, **14**, 7 (1964).

¹² Lubin, M., and Kessel, D., *Biochem. Biophys. Res. Comm.*, **2**, 249 (1960).

¹³ Damadian, R., and Solomon, A. K., *Science*, **145**, 1327 (1964).

¹⁴ Wood, H. N., and Braun, A. C., *Proc. U.S. Nat. Acad. Sci.*, **47**, 1907 (1961).

¹⁵ Braun, A. C., and Wood, H. N., *Proc. U.S. Nat. Acad. Sci.*, **48**, 1776 (1962).

¹⁶ Lubin, M., *Fed. Proc.*, **23**, 994 (1964).

Occurrence of Fructose-1-phosphate in Tissues of Higher Plants

THERE is considerable evidence for a role of fructose-1-phosphate in the metabolism of some animal tissues¹. Relatively little is known about the occurrence of this compound in higher plants. Cardini² has shown that preparations of jack bean seeds can cleave fructose-1-phosphate to dihydroxyacetone phosphate and a compound which is probably glyceraldehyde. On the basis of electrophoretic separation, Schwimmer, Bevenue and Weston^{3,4} tentatively identified fructose-1-phosphate in extracts of potatoes. We report here evidence for the

presence of fructose-1-phosphate in storage tissue of carrot (*Daucus carota* L.) and white turnip (*Brassica rapa* ssp. *rapa*).

Disks (1 mm × 10 mm) of the tissues were aerated at 25° C for 17 h in distilled water and then in 0.1 mM KH_2PO_4 for a further 24 h. The tissue was rinsed and extracted successively with boiling 80 per cent ethanol, 20 per cent ethanol and water. The phosphate esters in the combined extracts were fractionated by ion-exchange resin column chromatography using a technique⁵ similar to that of Bartlett⁶. The hexose monophosphates, free from hexose diphosphates, were located by their radioactivity, by reaction with anthrone reagent (for hexoses)⁷ and by a slightly modified cysteine-carbazole reaction (for fructose)⁸. Fractions containing mainly fructose phosphates were subjected to one-dimensional paper chromatography in each of two solvents. Solvent 1 was ethyl acetate-glacial acetic acid-water, 3 : 3 : 1 by volume⁸, and was used with Whatman No. 1 paper. Solvent 2 was *tert.*-butyl alcohol-0.1 N hydrochloric acid, 80:20, v/v (ref. 9), and was used with Whatman No. 4 paper. These solvents separated glucose-1- and glucose-6-phosphates from fructose-1- and fructose-6-phosphates and also gave a partial separation of the two fructose phosphates. Although the separation of fructose phosphates on paper chromatograms was not complete, it was sufficient to enable the two compounds to be distinguished by their characteristic ultra-violet fluorescent reactions as described below.

These procedures revealed the presence of a compound in carrot and turnip extracts which behaved similarly to authentic fructose-1-phosphate. The results in Table 1 compare the P constants:

$$\left\{ \frac{\text{distance travelled by compound}}{\text{distance travelled by orthophosphate}} \times 100 \right\}$$

of the extracted compounds with the P constants of authentic fructose-1- and fructose-6-phosphates and those of other sugar phosphates which could have been components of the eluates obtained from ion-exchange resin column chromatography.

Table 1. P CONSTANTS AND ULTRA-VIOLET FLUORESCENT REACTION WITH GLYCEROL/HCl/ACETONE REAGENT OF SOME CARBOHYDRATE PHOSPHATES COMPARED WITH COMPOUNDS ISOLATED FROM CARROT AND WHITE TURNIP

	P constants Solvent 1	Solvent 2	Ultra-violet fluorescent reactions in each solvent
F-1-P	63	73	yellow
F-6-P	66	81	red-brown
G-1-P	52	67	none
G-6-P	47	67	none
R-5-P	75	92	none
P _i	100	100	none
Carrot	62	75	yellow
	67	82	red-brown
Turnip	62	74	yellow
	67	82	red-brown

Evidence that the compound obtained from carrot and turnip was fructose-1-phosphate was obtained by a modification of the technique of Steinitz⁹. Under our conditions in which the reagent was sprayed onto the chromatogram, authentic fructose-1-phosphate gave an intense yellow fluorescence and authentic fructose-6-phosphate a dull, red-brown fluorescence which changed to yellow on heating for a further 3-4 min. The ultra-violet fluorescent reactions of the isolated compounds obtained under a 'Chromatolite' (Hanovia Lamps, Slough, England) were compared with those of authentic samples of fructose phosphates and other sugar phosphates likely to be present in the column eluate fractions (Table 1). Subsequent development of the chromatograms with the reagent of Hanes and Isherwood¹⁰, as modified by Bandurski and Axelrod¹¹, confirmed that the isolated compounds contained phosphate.

Additional evidence that the plant extracts contained fructose-1-phosphate was provided by the demonstration

that column eluate fractions, suspected of containing fructose-1-phosphate, contained a substrate for an aldolase preparation isolated from rat liver by the method of Leuthardt and Wolf¹². This preparation, which is known to cleave both fructose-1,6-diphosphate and fructose-1-phosphate, was used to assay the column eluate fractions for fructose-1-phosphate by linking the production of dihydroxyacetone phosphate to the oxidation of NADH, on the addition of purified α -glycerophosphate dehydrogenase. Values of 1.95 and 2.11 μ moles of fructose-1-phosphate/100 g fresh weight were obtained for carrot and turnip tissue respectively.

D. GRAHAM
T. AP REES*

C.S.I.R.O. Plant Physiology Unit,
Division of Food Preservation and
School of Biological Sciences,
University of Sydney.

* Present address: Botany School, University of Cambridge.

¹ Hers, H. G., *Le metabolisme du fructose* (Editions Arscia, Bruxelles, 1957).

² Cardini, C. E., *Enzymologia*, **15**, 303 (1952).

³ Schwimmer, S., Bevenue, A., and Weston, W. J., *Arch. Biochem. Biophys.*, **60**, 279 (1956).

⁴ Schwimmer, S., Bevenue, A., and Weston, W. J., *J. Agric. Food Chem.*, **3**, 257 (1955).

⁵ Graham, D., and ap Rees, T. (in preparation).

⁶ Bartlett, G. R., *J. Biol. Chem.*, **234**, 459 (1959).

⁷ Graham, D., and Smydzuk, J., *Anal. Biochem.*, **11**, 246 (1965).

⁸ Mortimer, D. C., *Canad. J. Chem.*, **30**, 653 (1952).

⁹ Steinitz, K., *Anal. Biochem.*, **2**, 497 (1961).

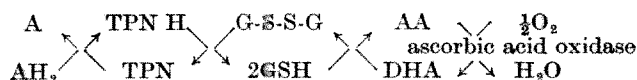
¹⁰ Hanes, C. S., and Isherwood, F. A., *Nature*, **164**, 1107 (1949).

¹¹ Bandurski, R. S., and Axelrod, B., *J. Biol. Chem.*, **193**, 405 (1951).

¹² Leuthardt, F., and Wolf, H. P., *Methods in Enzymology*, **1**, 320 (Academic Press, New York, 1955).

Some Postulated Relationships among Autumn Phenomena in Woody Plants

Most research on winter hardiness (cold acclimation) of plants has been concerned with changes in amounts of organic compounds such as sugars, amino-acids and proteins. These autumnal alterations in organic constituents of the cell can only be brought about by changes in enzymes. The question as to what initial factor causes this change in the metabolism is still not solved. It is tempting to think of a hormone action which could influence a great many enzyme systems¹, especially since cold acclimation and de-acclimation are closely associated with the growth-regulator controlled phenomenon of growth cessation in autumn, and growth resumption in spring. Recently, Levitt² has suggested that protein and glutathione sulphydryls bear a direct relationship to the state of winter hardiness of plants. Sulphydryl groups in cells are essential for the three-dimensional structure of cell proteins³ and for cell growth⁴. Glutathione is also an essential part of the ascorbic acid oxidase system which is involved in growth processes, that is, ascorbic acid oxidizing capacity is low in growing plants and high in dormant plants. Marré and Arrigoni⁵ presented evidence that auxins inhibited the ascorbic acid oxidase and thereby increased the ratio of reduced to oxidized glutathione. A postulated relationship between ascorbic acid oxidase and glutathione can be represented as follows:



AH₂, metabolic substrate; G-S-S-G, oxidized glutathione; GSH, reduced glutathione; AA, ascorbic acid; DHA, dehydroascorbic acid.

It has also been found⁶ that there is a shift in wheat caryopses from the cytochrome oxidase system to the ascorbic acid oxidase system after cold treatment. An increase in glutathione oxidizing activity, along with an apparent rise in ascorbic acid oxidizing activity, was observed in cold acclimating plants⁷. This high rate of

ascorbic acid oxidizing activity is contrary to the low capacity in vigorously growing tissue and thus must be due to an absence or inhibition of growth-promoting substances in cold treated tissue.

It has been reported by several investigators^{8,9} that the biosynthesis of growth promoters in woody plants levels off during the growing season, and that the concentration of growth inhibitors increases toward autumn. It has also been reported that there was a significant increase of tryptophan in dormant tissue after low-temperature treatment¹⁰. As tryptophan is considered to be the precursor of indolyl-3-acetic acid¹¹ its accumulation would suggest an arrest of auxin synthesis. An actual decrease of auxin has been reported to occur in cold-acclimated plants¹².

Changes in growth regulators may influence many processes. It has been reported that they are able to affect the bioelectric potential of the plant¹³ and thereby may change the cell membrane permeability¹⁴ which is a prerequisite for the cold acclimation process¹⁵. They may also affect the properties of proteins¹⁶ as well as the enzyme action already mentioned.

Growth inhibitors may affect the hardiness process as the synthetic growth inhibitor maleic hydrazide does¹⁷, via the sulphydryl groups of the mitotic cell¹⁸. Several natural growth inhibitors have been reported to occur¹⁹; many are flavonoid-like structures^{20,21}; others, such as β -inhibitor²², have only been tentatively identified²³. Flavonoids have been known to influence plant metabolism in several ways²⁴.

The phenomena in red-osier dogwood during autumn occur in the following order²⁵: irreversible rest inducement, cold acclimation, and loss of rest. The sequence of this process could be in these steps. An inducement of rest is brought about by some kind of phytochrome system²⁶. At this time, a hormone is produced which causes an increase in RNA content²⁷ and a subsequent increase of water-soluble proteins. Although the nature of these water-soluble proteins is obscure, some of them may represent an increase of enzymes which are responsible for the increase of carbohydrates and other organic products in cold acclimating plants.

R. B. VAN HUUSTEE*
C. J. WEISER
P. H. LI

Department of Horticultural Science,
University of Minnesota.

* Present address: Department of Horticulture, Purdue University, Lafayette, Indiana.

- ¹ Pilet, P.-E., in *Les Phytohormones de Croissance*, 485-514 (Masson et Cie, Paris, 1961).
- ² Levitt, J., *J. Theoret. Biol.*, **3**, 335 (1962).
- ³ Boyer, P. D., *Brookhaven Symp. Biol.*, **13**, 1 (1960).
- ⁴ Stern, H., *Science*, **124**, 1292 (1956).
- ⁵ Marré, E., and Arrigoni, O., *Physiol. Plant.*, **10**, 289 (1957).
- ⁶ Gunther, G., *Deutsche. Bot. Ges.*, **72**, 25 (1959).
- ⁷ Waisel, Y., Kohn, H., and Levitt, J., *Plant Physiol.*, **37**, 272 (1962).
- ⁸ Kawase, M., *Proc. Amer. Soc. Hort. Sci.*, **78**, 532 (1961).
- ⁹ Doerffling, K., *Planta*, **60**, 390 (1963).
- ¹⁰ Asen, S., and Stuart, N. W., *Proc. Amer. Soc. Hort. Sci.*, **71**, 563 (1958).
- ¹¹ Wightman, F., *Canad. J. Botany*, **40**, 689 (1962).
- ¹² Sulakadze, T. S., *Izvest. Akad. Nauk, S.S.S.R., Ser. Biol.*, **4**, 551 (1961).
- ¹³ Key, J. L., and Wold, W., *J. Biol. Chem.*, **236**, 549 (1961).
- ¹⁴ Fensom, D. S., *Canad. J. Botany*, **37**, 1003 (1959).
- ¹⁵ Levitt, J., and Scarth, G. W., *Canad. J. Res.*, **C14**, 285 (1933).
- ¹⁶ Galston, A. W., Kaur, R., Maheshwari, N., and Maheshwari, S. C., *Amer. J. Bot.*, **50**, 487 (1963).
- ¹⁷ Stewart, I., and Leonhardt, C. D., *Proc. Amer. Soc. Hort. Sci.*, **75**, 253 (1960).
- ¹⁸ Hughes, C., and Spragg, S. P., *Biochem. J.*, **70**, 205 (1959).
- ¹⁹ Giertych, M. M., *Bot. Rev.*, **30**, 292 (1964).
- ²⁰ Hendershott, C. H., and Walker, D. R., *Science*, **130**, 798 (1959).
- ²¹ Furuya, M. A., Galston, W., and Stowe, B. E., *Nature*, **193**, 456 (1962).
- ²² Doerffling, K., *Planta*, **60**, 412 (1964).
- ²³ Lane, F. E., and Bailey, L. F., *Physiol. Plant.*, **17**, 91 (1964).
- ²⁴ Stenlid, G., *Physiol. Plant.*, **60**, 110 (1963).
- ²⁵ Van Huystee, R., Ph.D. thesis, University of Minnesota, St. Paul, Minnesota (1964).
- ²⁶ Wilson, J. R., and Schwabe, W. W., *J. Exp. Bot.*, **15**, 368 (1964).
- ²⁷ Siminovitch, D., *Canad. J. Bot.*, **41**, 1301 (1963).

Freeze-fixation, a New Method for Electron Microscopy

FREEZE-FIXATION implies rapid freezing of small specimens followed by vapour fixation at a low temperature. This method differs from freeze-drying by not removing the water of the specimen before fixation, and from the freeze-substitution by not replacing the water during fixation. All these three methods work with frozen tissue, but since the mode of fixation varies the ultrastructure of the cellular constituents also varies. Therefore, further information of the cellular constituents might be obtained.

For freezing, small specimens are rapidly frozen in liquid nitrogen according to the usual technique for freeze-drying. The frozen specimens are collected in a small weighing bottle with grounded stopper. The bottle contains a substance suitable for vapour fixation and serves as a fixation chamber after freezing.

For fixation, the bottle containing specimens and fixation substance, and filled with liquid nitrogen, is placed in dry ice. At this temperature, the liquid nitrogen evaporates. The bottle is then firmly closed with its stopper and left in a freezer for 2-3 weeks for vapour fixation. The only fixation substance as yet tried in the experiment is crystalline osmium tetroxide. After the period in dry ice, the specimens are either left in the bottle for further fixation at room temperature or brought to the appropriate solution of the embedding chemicals used. In the present experiments, plastic embedding has been started in 70 per cent ethanol for the 'Epon' procedure or 25 per cent component 'A' of the 'Durcupan' procedure.

The main drawbacks of freeze-fixation are the low vapour pressure of the fixation substance at -30°C and the slow penetration of the fixative into the frozen tissue. The specimens therefore must be small, preferably single cells. The morphological quality of the tissue is inferior to that obtained after freeze-drying. Thus, the several artefacts impede investigations of the whole cell, but they do permit analysis of, for example, the cell membrane.

This work was supported by "Riksföreningen mot Cancer", Sweden.

O. NILSSON

Department of Human Anatomy,
University of Uppsala,
Sweden.

MICROBIOLOGY

Endogenous Bud Formation in the Hypotrichida

As a general rule, ciliated protozoans reproduce asexually by transverse binary fission. Suctorians, however, characteristically produce unequal endogenous buds which develop into migratory ciliated larval forms. Endogenous budding has not been reported in the remaining subclasses of the Ciliata. Observations made by Kent suggest that one member of the Hypotrichida has a larval stage in its life-cycle¹. He observed the development of an embryonic hypotrich, which closely resembled *Glaucoma margaritaceum* Ehr., into *Aspidisca costata* Ehr. In the same treatise Kent considers the holotrich genera *Glaucoma* and *Microthorax* to be embryonic or transitional phases in the life-cycles of higher hypotrichous forms. No other reference to such life-cycles in the Hypotrichida have been found.

This communication presents preliminary observations made on monoxenic clonal cultures of the hypotrichous ciliate *Histriculus vorax* Corliss. *Bacillus cereus* was used as the sole food organism, and the ciliate culture was originally separated from activated sludge.

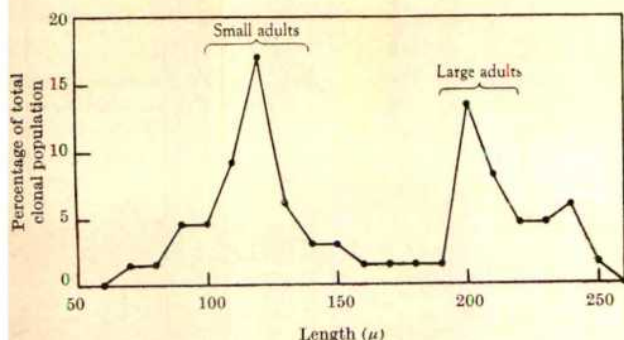


Fig. 1. Size frequency curve showing the presence of two distinct adult cell sizes within a well-fed clonal population of *Histriculus vorax*

Great variations in size have been noted in clonal populations, but 70 per cent of the cells in such populations fall within two definite ranges of size (Fig. 1). Large adult cells (190–220 μ long, 80–100 μ wide) are bluntly shaped, whereas small adult cells (100–140 μ long, 50–60 μ wide) are streamlined in their appearance. Both adult forms are capable of normal transverse division resulting in the production of two daughter cells the dimensions of which, after a short period of growth, resemble those of the mother-cell. The large adult cells form 20–30 per cent of a well-fed clonal population, but under conditions of starvation they are absent.

Observation has shown that the large adult cells (Fig. 2) develop endogenous bi-macronucleate buds (20–40 μ diameter) which, when mature, are extruded through the cell wall. After extrusion, the mother-cell may either then produce more buds endogenously or divide transversely. Newly deposited buds may either develop directly into a ciliated larval form or, if conditions are unfavourable, the bud will encyst.

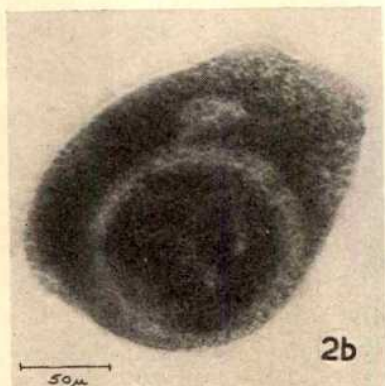
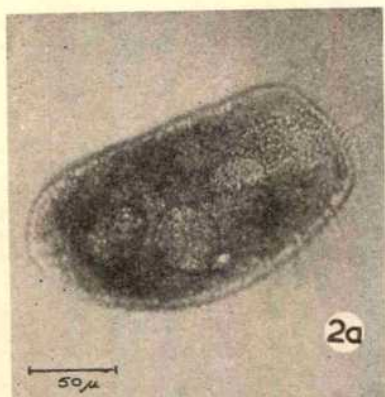


Fig. 2. Formation of endogenous buds in *Histriculus vorax* Corliss. a, Large adult containing two developing endogenous buds; b, large adult mother-cell containing a mature endogenous bud

Isolated encysted buds have been excysted by placing them into fresh culture media. Excystment takes approximately 48 h to complete at room temperatures, and terminates when a non-trophic migratory larval form, which bears only caudal and frontal cirri, emerges (Fig. 3). Under favourable conditions newly deposited buds develop directly into larval forms which cannot be distinguished from the embryos which emerge from encysted buds. The non-trophic embryos rapidly develop feeding membranelles and a full adult complement of cirri. The trophic larval stage grows and quickly develops into a small adult capable of transverse binary fission.

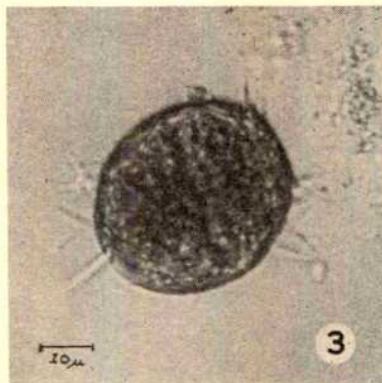


Fig. 3. Non-trophic embryonic stage of *Histriculus vorax* Corliss

This work was supported by the Department of Scientific and Industrial Research while I was holding a research fellowship.

COLIN R. CURDS

Water Pollution Research Laboratory,
Elder Way,
Stevenage, Hertfordshire.

¹ Kent, W. S., *A Manual of the Infusoria*, 794 (David Bogue, London, 1880–1882).

Variation of the X-ray Sensitivity of *Serratia marcescens* with Relative Humidity

In a recent article¹ describing the effects of humidity on the inactivation of micro-organisms, the results of some preliminary experiments with X-rays were presented. The present communication reports an extension of this work in which the effect of atmospheric oxygen in modifying the radiation sensitivity at various humidities was investigated in addition to the protective effect of inositol. Inositol was of particular interest because of its apparent ability to replace water in biologically important macromolecules² and in this way to protect cells against inactivation by drying, ultra-violet and X-rays^{1,3}.

The effect of inositol appears to be similar to that of glycerol, which has been the subject of a number of recent investigations^{4–10}. The role of water in the radiation inactivation of bacterial spores and vegetative bacteria has been extensively studied by Powers and his colleagues^{6,7,11,12}.

For the experiments reported here, a sub-culture of *Serratia marcescens* was prepared by incubation in heart infusion broth for 48 h. Cells from the culture were centrifuged, washed, and re-suspended in either distilled water or a 5 per cent (0.3 M) solution of meso-inositol. One ml. samples from the foregoing suspensions containing about 5×10^9 cells were deposited on 'Millipore' filters. The filters were placed in an irradiation chamber through which air or nitrogen of the desired relative humidity could be passed. The relative humidity of the gas which had passed over the filters was measured with a membrane-type meter ('Serdex'). The meter reading was dependent not

only on the relative humidity of the gas entering the irradiation chamber, but also on the degree to which the cells had come into equilibrium with the relative humidity of this gas. For experiments with a nitrogen atmosphere, commercial nitrogen (oxygen concentration less than 70 parts per million) was passed through the irradiation chamber for at least 1 h before beginning the irradiations.

After equilibration, cells were irradiated with unfiltered 200 kVp. X-rays (half-value thickness, 8 mm aluminium) at an exposure rate of 790 r./min. The irradiations were carried out at room temperature (22°C–25°C). In some experiments, cells were irradiated in suspension after aeration of the suspension by bubbling air through it. After irradiation, the cells were plated on heart infusion agar. Visible colonies were scored after 24 h incubation at 35°C.

The survival curves obtained under all experimental conditions appeared to be exponential and were fitted by an expression of the form $N/N_0 = \exp(-kD)$, where N and N_0 are the number of colony-producing cells in the irradiated samples and the unirradiated control, respectively, D is the absorbed dose and k is an inactivation constant, which was used as an index of radiation sensitivity under the various conditions. In the control samples it was found that 30–50 per cent of the cells deposited on the filter would later produce colonies even after storage at a relative humidity as low as 25 per cent for several hours.

Values obtained for the inactivation constant for *Serratia marcescens* irradiated in air are shown in Fig. 1. The relative humidity values on the abscissa scale refer to the atmosphere above the cells. The circles and upper curve give values for k obtained for cells deposited from a water suspension, the triangles and the lower curve for cells deposited from a 5 per cent inositol solution. It may be seen that inositol has a protective effect at all relative humidities and that this protection is nearly constant at humidities below 70 per cent. This is in contrast to the results for ultra-violet irradiation of cells in aerosols in which a marked increase in the protective effect of inositol with decreasing relative humidity was found¹. The abrupt decrease in sensitivity at a relative humidity of about 80 per cent in both the curves shown in Fig. 1 is believed to be due to the removal of 'free' water from the cell and therefore to a decrease in the 'indirect' effect produced by the radiolysis of water.

This decrease is in contrast to the results of Tallentire and Powers¹² for the bacterial spores and those of Webb⁸ for vegetative cells of *Staphylococcus aureus*. These workers observe an increase in sensitivity with decreasing water vapour pressure if oxygen is present during and after the irradiation. The difference may be due to differences in the procedure by which the cells are dried.

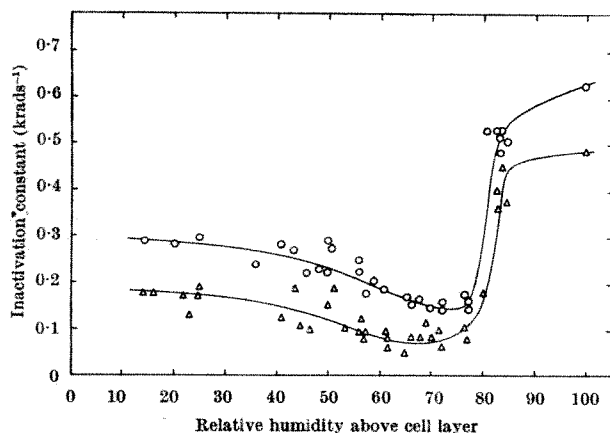


Fig. 1. X-ray sensitivity of *Serratia marcescens* cells irradiated under an atmosphere of air, having previously been suspended in water (○) or in 5 per cent inositol (Δ).

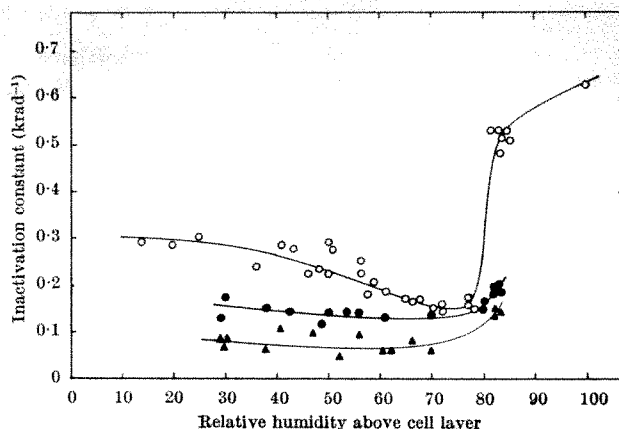


Fig. 2. X-ray sensitivity of *Serratia marcescens* under various conditions indicated. ○, Water-air; ●, water-nitrogen; ▲, inositol-nitrogen.

In Fig. 1 the shape of the curves below 70 per cent is consistent with a model in which the inactivation constant is assumed to have two components one of which, k_0 , is independent of water in the cell and the other, k_1 , is proportional to the amount of water which has been removed by drying, taking the amount of water at various relative humidities from data measured by Webb⁸. The increase in k_1 with decreasing humidity does not appear to correspond directly to the much greater increase observed by Powers and his colleagues for bacteria and spores irradiated and stored in the presence of oxygen^{8,12}.

The measured inactivation constants for cells irradiated under a nitrogen atmosphere are shown in Fig. 2. The water-air curve from Fig. 1 has been included in Fig. 2 to facilitate comparison. For cells deposited from a water suspension and irradiated at high humidities (above 80 per cent) there is a marked decrease in sensitivity when air is replaced by nitrogen. This 'oxygen effect' nearly disappears at a relative humidity of about 70 per cent, suggesting that the damage due to the 'indirect' effect, postulated here, requires the presence of oxygen. Below 70 per cent the inactivation constant for cells irradiated in air increases with decreasing relative humidity whereas that for cells irradiated in nitrogen appears to remain nearly constant. It therefore appears that the inactivation described by k_1 , which we have associated with the amount of water in the cell, requires the presence of oxygen. The humidity-independent component, k_0 , appears also to be independent of oxygen and therefore to correspond to the Class I damage described by Powers and his colleagues^{8,11,12}.

The triangles and the lowest curve in Fig. 2 give the inactivation constants for cells deposited from an inositol suspension and irradiated in nitrogen. These points fall considerably below those for cells deposited from a water suspension and irradiated in nitrogen. The fact that there is a considerable protective effect in the absence of oxygen indicates that the action of inositol is not simply in interfering with oxygen concentration in the cell. Below 70 per cent relative humidity the inactivation constant for inositol-treated cells irradiated in nitrogen is nearly independent of relative humidity and has a value approximately equal to the minimum value for inositol-treated cells irradiated in air. It therefore appears that at the lower humidities there are two kinds of radiation damage both in inositol-treated cells and in unprotected cells, with the humidity-dependent component in each case requiring the presence of oxygen.

These experiments indicate that, at the lower humidities, inositol exerts most of its protective effect by reducing the oxygen-independent component of the radiation damage. This is in contrast to the observed protection by glycerol of cells in suspension which is considerably greater in the presence of oxygen than in its absence^{7,9,10}.

We thank Dr. S. J. Webb for his assistance in this work, which was supported by a grant from the National Cancer Institute of Canada.

H. G. MORRISON
D. V. CORMACK

Departments of Physics and Cancer Research,
University of Saskatchewan,
Saskatoon, Canada.

- Webb, S. J., Cormack, D. V., and Morrison, H. G., *Nature*, **201**, 1103 (1964).
Warner, D. T., *Nature*, **196**, 1055 (1962).
Webb, S. J., *Canad. J. Microbiol.*, **6**, 8a (1960).
Webb, S. J., and Dumasla, M. D., *Canad. J. Microbiol.*, **10**, 877 (1964).
Webb, R. B., *Rad. Res.*, **18**, 607 (1963).
Webb, R. B., *Physical Processes in Radiation Biology* (Academic Press, New York, 1964).
Webb, R. B., and Powers, E. L., *Intern. J. Rad. Biol.*, **7**, 481 (1963).
Manney, T. R., Brustad, T., and Tobias, C. A., *Rad. Res.*, **18**, 374 (1963).
Dewey, D. L., *Rad. Res.*, **19**, 64 (1963).
Dewey, D. L., *Inter. J. Rad. Biol.*, **7**, 151 (1963).
Powers, E. L., Webb, R. B., and Ehret, C. F., *Rad. Res. Supp.*, **2**, 94 (1960).
Tallentire, A., and Powers, E. L., *Rad. Res.*, **20**, 270 (1963).

elucidate this point experiments involving a small RNA virus (Mengo) and a DNA virus (adenovirus) are in progress.

This work was supported, in part, by the U.S. Army Medical Research and Development Command (contract DA-49-193-MD-2589 and National Institutes of Health grant AI-01799 from the National Institute of Allergy and Infectious Diseases and research career award 5-K6-HE-7342 from the National Heart Institute.

RICHARD I. CARP
DAVID KRITCHEVSKY

Wistar Institute of Anatomy and Biology,
Philadelphia, Pennsylvania.

- ¹ Manson, L. A., Carp, R. I., Defendi, V., Rothstein, E. L., Hartzell, jun., R. W., and Kritchevsky, D., *Ann. N.Y. Acad. Sci.*, **84**, 685 (1960).
² Kritchevsky, D., Carp, R. I., and Koprowski, H., *Nature*, **191**, 250 (1961).
³ Lwoff, A., and Lwoff, M., *C.R. Acad. Sci., Paris*, **251**, 3131 (1960).
⁴ Carp, R. I., Chudnow, I., Koprowski, H., and Kritchevsky, D., *Proc. Soc. Exp. Biol. and Med.*, **113**, 569 (1963).
⁵ Wecker, E., and Schonne, E., *J. Cell. Comp. Physiol.*, **63**, 101 (1964).

VIROLOGY

Influence of Heavy Water (Deuterium Oxide) on Plaque Size of Western Equine Encephalitis Virus

THE influence of deuterium oxide (D_2O) on virus replication in tissue culture cells is variable. When poliomyelitis virus (a small, RNA virus) is grown in 20–30 per cent D_2O its proliferation is enhanced^{1–3}, whereas simian virus 40 (a DNA virus) does not replicate as well in deuterated medium as it does in protonated medium⁴. In an attempt to ascertain whether the size of a virus or its nucleic acid composition may be correlated with the D_2O effect, we have examined the effects on the replication of western equine encephalitis (WEE), a relatively large, RNA-containing virus.

The influence of 25 per cent D_2O on the replication of two mutants of WEE was studied. The two mutants are distinguished by the size of the plaques which they yield when grown on chick embryo fibroblasts. The method of growing the virus was essentially that of Wecker and Schonne⁵. Fibroblasts of ten-day-old chick embryos were cultivated in 100-mm Petri dishes in a growth medium consisting of Eagle's medium in Earle's balanced salt solution and 10 per cent calf serum. The WEE virus was diluted in Hanks's salt solution and 0.2 ml. of the diluted WEE was added to each monolayer. Virus absorption was permitted to proceed for 1 h at 37° C, then the cells were overlaid with nutrient agar (12 ml. of 1 per cent agar in Eagle-Earle's medium containing 10 per cent calf serum). Half the fibroblast monolayers were overlaid with nutrient agar made up in medium containing 25 per cent D_2O . After 3 days of incubation in an atmosphere of 4 per cent carbon dioxide in air, the plaques caused by the WEE virus were counted and measured. The results of two experiments are presented in Table 1. In every case plaque size was smaller in the D_2O medium. The observed differences are highly significant ($P < 0.001$).

The data presented here indicate that the nucleic acid moiety of a virus does not necessarily determine the nature of the viral growth response in D_2O . To further

CYTOLOGY

Barr Bodies in the Mouse

UP to the present, sex chromatin has not been identified in the nuclei of mouse cells. This is probably due to the fact that such nuclei contain multiple chromocentres, of varying size and number, the presence of which tends to mask the appearance of a sex-specific chromatin mass in females. Moore and Barr¹, in a systematic survey of the nerve cell nuclei of many different mammals, were unable to demonstrate sexual dimorphism in those of the mouse. Later, Hay², examining twenty non-nervous tissues of the mouse, could not identify sex chromatin in any of them. Himrichsen and Gothe³ analysed the multiple chromocentres of murine Purkinje cells, organizing them into eleven arbitrary patterns in accord with their sizes and distribution. Among these patterns there were two configurations with respect to which male and female mice seemed to differ, but neither of these was that of the single nucleolar satellite common to the neurones of female mammals.

A search for peripheral sex chromatin masses—Barr bodies—was made in nuclei of fibroblasts which had grown out from small fragments of mouse peritoneum explanted on to cover slips. This method provided a population of young cells with flattened nuclei which were in a monolayer. Six-week-old male and female CBA/C57BL mice were killed by stunning and neck fracture. Small (2 mm) pieces of peritoneum were excised and placed on cover slips mounted on agar drops in a Petri dish. Each explant was allowed to become adherent in a thin clot which formed spontaneously. The explants were covered with a medium composed of NCTC-109 and foetal calf serum (4:1), and were incubated at 37° C in an atmosphere of 5 per cent carbon dioxide in air. Outgrowth was generally visible on the fourth day, and on the sixth day the tissue fragments were carefully picked off the cover slips, leaving behind a corona of fibroblasts. These were fixed in 95 per cent ethanol, hydrolysed in 1 N hydrochloric acid for 10 min at 60° C, stained in a standardized Feulgen stain for 1 h, dehydrated and mounted in 'XAM' for examination at times 900 under oil immersion using a Wratten B green filter.

In order to avoid the problem presented by the multiple chromocentres (Fig. 1) it was decided to search for Barr bodies only in those nuclei which contained either no chromocentres at all, or the chromocentres of which were sufficiently small so as not to be confused with a Barr body. Such nuclei were designated 'clear' (Fig. 2). To be identified as a Barr body a chromatin mass had to be large (about 1 μ), compact, well defined, and tangent to the nuclear membrane all along its base. The aspect of it which faced inward on the cell was allowed to vary from

Table 1. INFLUENCE OF 25 PER CENT D_2O ON GROWTH OF WESTERN EQUINE ENCEPHALITIS (WEE) ON CHICK EMBRYO FIBROBLASTS

Exp. No.	Plaque mutant	Medium	No. plaques measured	Plaque size (mm) (avg.)
1	Large	H_2O	19	4.4 ± 0.14*
		D_2O	24	3.2 ± 0.16
	Small	H_2O	24	2.6 ± 0.05
		D_2O	20	2.0 ± 0.04
2	Large	H_2O	21	4.7 ± 0.09
		D_2O	24	3.7 ± 0.14
	Small	H_2O	†	2.2–5.†
		D_2O	†	1.1–5.†

* Standard error.
† Not quantitated.

Fig. 1

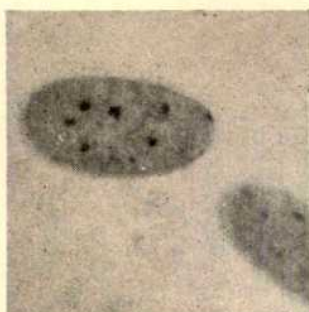


Fig. 2

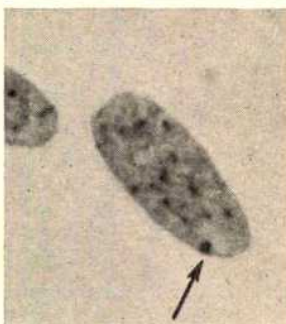
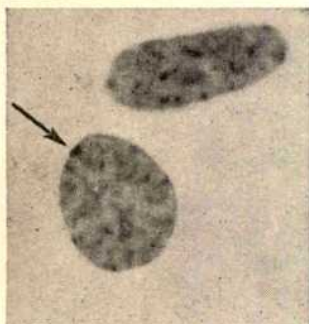
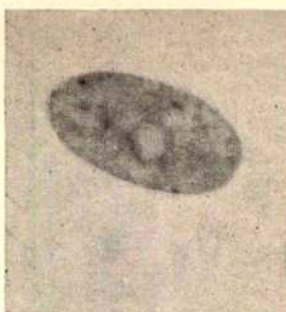


Fig. 3

Fig. 4

Fig. 1. A fibroblast nucleus showing multiple chromocentres
Fig. 2. A 'clear' nucleus with a few very small chromocentres
Figs. 3 and 4. Nuclei containing Barr bodies

a short to a tall pyramid, or to be convex (Figs. 3 and 4). Rectangular masses and masses only slightly tangent to the nuclear membrane were not accepted. All nuclei were examined throughout their volumes.

A preliminary examination of many cover-slip specimens from both male and female animals in accord with these criteria revealed that there was a readily recognizable proportion of female cell nuclei which contained Barr bodies. The corresponding clear nuclei of male cells, on the other hand, did not contain any. These findings were tested further by a review of a series of 'unknown' specimens. Nine mounts were examined independently by two observers who did not know the sex of a given specimen of cells or how many specimens of each sex had been selected for review. Both observers were able readily to identify the sexes of the cell populations. Because subjective factors were numerous it was not felt possible to state exactly the percentage of female nuclei which contained Barr bodies; but after lengthy counts it seemed that about 10 per cent of the clear nuclei contained them. The proportion of nuclei which had to be rejected because of their chromocentre content varied from one preparation to another. In general, somewhat more than half of them were inappropriate for the identification of sex chromatin.

These findings complement the description by Ohno and Hauschka⁴ of a heteropycnotic X chromosome in late prophase in normal and tumorous mouse tissues, and the recent identifications of a late labelling X chromosome in tissue culture of mouse embryo by Galton and Holt⁵ and in the living adult mouse by Evans, Ford, Lyon and Gray⁶. It is recognized that the occurrence of Barr bodies in only 10 per cent of fibroblast nuclei is low as compared with the incidence in tissue cultures from other mammals. This seems due in part to the unavoidable loss of some Barr bodies by disregarding all nuclei which bore chromocentres. By the same token it may be related to the low incidence of late-labelling X chromosomes reported by Evans and his colleagues, for the recognition of a late-labelling X must have been difficult in the presence of the proximal portions of autosomes which labelled equally late. Ohno and Hauschka⁴ have suggested that the

chromocentres of mouse cell nuclei represent allocyely portions of many or all the chromosomes of the complement. The variable size of the chromocentres could be attributed to fusion of some of these heterochromatin regions. The 10 per cent of clear nuclei which we have observed to contain Barr bodies may be those of a proportion of cells in which allocyely of one X is out of phase with allocyely of the remainder of the chromosomes.

We thank Prof. H. Grüneberg for providing the micrograph used in the study. One of us (J. D. K.) was supported by the U.S. Public Health Service as an indirect trainee of the National Institutes of Health.

J. D. KENNEY
URSULA MITTWOCH

Galton Laboratory,
University College, London.

¹ Moore, K. L., and Barr, M. L., *J. Comp. Neurol.*, **98**, 213 (1953).

² Hay, J. C., *Anat. Rec.*, **136**, 315 (1960).

³ Hinrichsen, K., and Gothe, H.-D., *Z. Zellforsch.*, **48**, 429 (1958).

⁴ Ohno, S., and Hauschka, T. S., *Cancer Res.*, **20**, 541 (1960).

⁵ Galton, M., and Holt, S. F., *Exp. Cell Res.*, **37**, 111 (1965).

⁶ Evans, H. J., Ford, C. E., Lyon, M. F., and Gray, J., *Nature*, **206**, 90 (1965).

Electron Microscope Observations of Neurosecretory Elements in the Neurointermediate Lobe in Skates

In this report, observations of ultrastructural organization of neurosecretory elements of the neurointermediate lobe in two types of the Black Sea skates, *Raja clavata* and *Trygon pastinaca*, are considered. As was shown in our earlier work with the light microscope¹, the axoadena neurosecretory contacts in these types of skates are well developed and very distinct. In the literature there appears to be only one report² in which the axoadena contacts with the adenohypophysis elements in *Scyliorhinus caniculus* (L) were examined with the electron microscope, and some other work done on teleosts³⁻⁵.

With the light microscope, it was found that bundles of the neurosecretory fibres were between the strands of the epithelial glandular cells of the intermediate lobe and elements of connective tissue, and vessels were not found in these spaces. The terminal branches of the neurosecretory fibres are found among the epithelial cells, but never reach the sinusoid capillaries located in the epithelial strands. Numerous swellings of the neurosecretory fibres make intimate contact with the glandular epithelial cells.

During investigations with the electron microscope two kinds of unmyelinated nerve fibres—fibres with elementary neurosecretory granules and those without them—were found in the tissue of the neurointermediate lobe.

The terminal swellings of the neurosecretory fibres—neurosecretory endings—have diameters of considerable size (1.5–2.5 μ , more seldom 4–5 μ) as compared with the fibres. Neurosecretory granules with various ultra-thin structure and tiny vesicles (diam., 350–500 Å), resembling 'synaptic' vesicles in typical nerve endings, and also mitochondria are found in the terminal swellings in skates as in all other vertebrates, the mitochondria having various but often irregularly curved shapes.

Three main types of neurosecretory granules are observed in the neurosecretory endings (Figs. 1 and 2): (1) Typical elementary granules ranging from 1100 to 2800 Å. (2) Granules of the same size with their central substance having smaller electron density (the degree varying from one granule to another). The distinctness of the light space between the membrane and the central substance is reduced with the osmophilicity of the central substance of granules.

(3) 'Residual' neurosecretory granules, as we call them⁶, are usually of the same size, but they have membranes which are practically only lipoprotein and are free of the electron-dense central substance. In their structure they resemble vesicles. In some cases, however, we observed the 'residual' granules which are considerably smaller than

mentary granules. Tiny 'residual' granules are almost of same size as the 'synaptic' vesicles of the neurosecretory endings.

Between all three types of the neurosecretory granules there are connecting transitional forms which, as is considered in the literature, demonstrate the process of solving or diluting the substance of a granule during the process of secreting the biologically active substance of the neurosecretory material.

Single neuroglial cells of an elongated form—pituicytes are found among the neurosecretory fibres (Fig. 2). They are poor in cytoplasm, which surrounds the nucleus only as a thin border (100–300 m μ) and turns into relatively thin branches at its poles. It is in these parts of the cytoplasm that the substantial mass of small mitochondria, in canals of endoplasmic reticulum and ribosomes, is concentrated.

The nuclei of pituicytes always have an elongated form—some invaginations often are found on their surface. The nuclei are characterized by a considerable electron density—they contain a large number of chromatin grains. The nucleolus is, as a rule, of a rounded or an oval form and is characterized by high electron density.

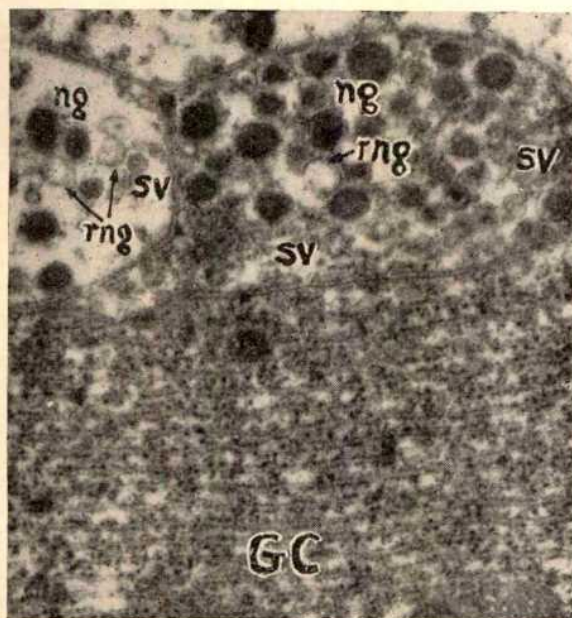


Fig. 1. The contact of two neurosecretory endings with a glandular epithelial cell (GC) from the neurointermediate lobe of the hypophysis of *Trygon pastinaca*. Plasma membranes of the terminals and of the glandular cell are divided by a narrow intercellular space about 150 Å. ($\times 37,500$)

ng, neurosecretory granules; rng, 'residual' neurosecretory granules; sv, synaptic vesicles

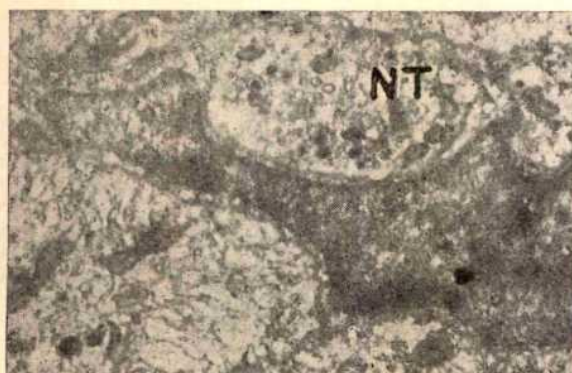


Fig. 2. The contact of the neurosecretory terminal (NT) with a pituicyte from a neurointermediate lobe of the hypophysis of *Raja clavata*. ($\times 18,000$)

Pituicytes come into close contact with the neurosecretory fibres and sometimes with their terminals. Fig. 2 shows the closest form of such contact where the neurosecretory terminal seems to be invaginated into the body of the pituicytes—the nucleus at the same time being deformed and resembling the invagination form. In their morphology, the pituicytes of skates resemble the oligopituicytes of white mice⁷.

The neurosecretory endings are surrounded by glandular cells on all sides. Their plasma membranes are divided only by a thin slit about 150 Å. Some invaginations are found on the surface of glandular cells at the points of contact. No peculiarities have been observed in ultrastructural organization of bounding membrane and organization of glandular cells in the region of contact with the neurosecretory endings. Compared with typical nerve endings, in the neurosecretory endings there is no concentration of 'synaptic' vesicles near plasmatic membranes which come into direct contact with the surface of a glandular cell. Moreover, no swellings of these sections of membranes similar to those of the presynaptic membrane of typical nerve endings are found.

The foregoing findings concerning the ultrastructure of the neurosecretory endings and the places of contact with the glandular cells testify, we believe, the opinion that the biologically active substances of the neurosecretory granules have a direct influence on the functional activity of the glandular cells of the intermediate lobe. The absence of morphological evidence showing that the neurosecretory granules directly penetrate beyond the endings supports the generally accepted opinion that the biologically active substances of the neurosecretory material—neurohormones—are secreted not as entire granules but as small molecular components.

A. L. POLENOV
M. A. BELENKY

Institute of Cytology,
Academy of Sciences of the U.S.S.R.,
Leningrad.

¹ Polenov, A. L., *Second Sci. Conf. Inst. Cytology, Acad. Sci., U.S.S.R. Edition, Acad. Sci. U.S.S.R., Leningrad*, 59 (1962).

² Meilinger, J., thesis, Strasburg (1963).

³ Legait, E., and Legait, H., *C.R. Soc. Biol., Paris*, **151**, 1943 (1957).

⁴ Bargmann, W., and Knoop, A., *Z. Zellforsch.*, **52**, 256 (1960).

⁵ Follenius, E., and Porte, A., *Mém. Soc. Endocrinol.*, **12**, 51 (1962).

⁶ Polenov, A. L., and Belenky, M. A., *Doklady A.N.S.S.S.R.*, **154**, 940 (1964).

⁷ Belenky, M. A., and Polenov, A. L., *Tsitologiya*, **5**, 651 (1963).

Interspecific Karyotypic Variation as a Systematic Character in the Genus *Anolis* (Sauria: Iguanidae)

THE examination of animal chromosomes has recently gained impetus from the development of techniques for the short-term culture of blood¹. Modifications of this technique have been used successfully on several species of reptiles².

Most of the classical literature on reptile chromosomes indicates a conservativeness of karyotype at the familial or super-familial level³. Subsequent work by Matthey⁴ on the chamaeleontids indicates that in that family the differences in karyotype can be used to help establish species groups.

Using a technique of whole blood tissue culture utilizing only 2–3 drops of blood⁵ I have made a preliminary examination of the chromosomes of nine species of *Anolis* lizards in order to assess the value of the karyotype as an additional systematic character.

The genus *Anolis* is taxonomically complex, consisting of approximately 200 species, distributed from South America to Central America and Mexico; and throughout the West Indies and the south-eastern United States. On the basis of differences in the structure of the caudal vertebrae, the genus has recently been divided by Etheridge⁶ into two major groups (termed α and β) and

both these groups have been sub-divided into major series of species.

The chromosomes of many reptile species fall into two sharply defined size classes termed macro- and micro-chromosomes³. Here only the macrochromosomes will be discussed, for my chromosome spreads of *Anolis* so far give equivocal results for the smaller elements, some of which may be masked by the arms of the larger elements.

The chromosome spreads obtained from blood cultures indicate that *A. carolinensis* (from Louisiana), *A. equestris* (Cuba), *A. luciae* (St. Lucia) and *A. bonaiensis* (Bonaire) all have twelve macrochromosomes (Fig. 1). This is in agreement with the published karyotype of *A. carolinensis*², the only member of the genus previously examined.

A second group, *Anolis grahami* and *A. lineatopus* (from Jamaica), *A. lineatus* (Curacao), and *A. chrysolepis* (Trinidad), have fourteen macrochromosomes (Fig. 2).

Finally, *A. bimaculatus* (St. Kitts) has still another kind of karyotype in which eighteen to twenty chromosomes appear larger than dots. However, there is no sharp distinction between macro- and micro-chromosomes but instead a graded series from large to small (Fig. 3).

Table 1		
Major group	Species	Macrochromosomes
Alpha	Carolinensis series	
	<i>carolinensis</i>	12
Alpha	Latifrons series	
	<i>luciae</i>	12
Alpha	Bimaculatus series	
	<i>bimaculatus</i>	18-20 (see text)
Beta	Grahami series	
	<i>grahami</i>	14
Beta	Chrysolepis series	
	<i>lineatus</i>	14
	<i>chrysolepis</i>	14

Macrochromosomal number in 9 species of *Anolis*. Alpha and beta are the major groups of *Anolis*, and the various series are series of species within the groups, all as defined by osteological characters⁴.

Table 1 shows that in the four cases in which pairs of species belonging to the same series as defined by Etheridge⁶ were examined, there is no variation in macrochromosomal number. The major groups of Etheridge can be distinguished only by the fact that betas have 14



Fig. 1. Chromosomes of *Anolis carolinensis*, showing twelve macrochromosomes

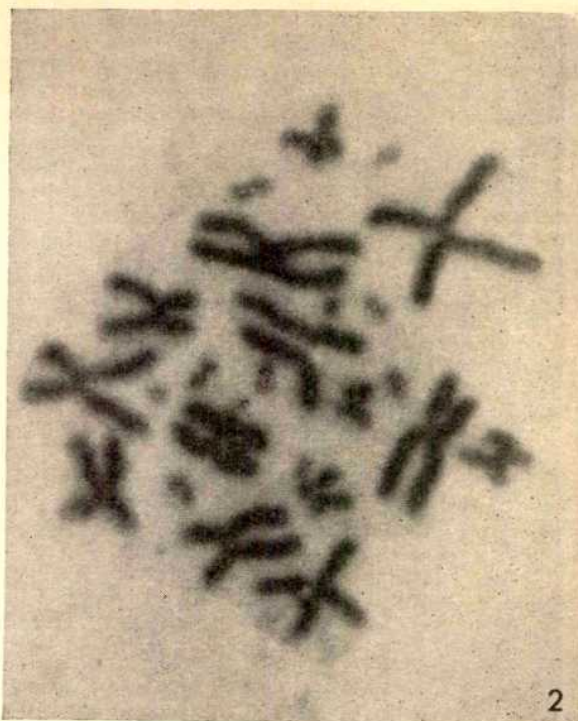


Fig. 2. Chromosomes of *Anolis lineatus*, showing fourteen macrochromosomes

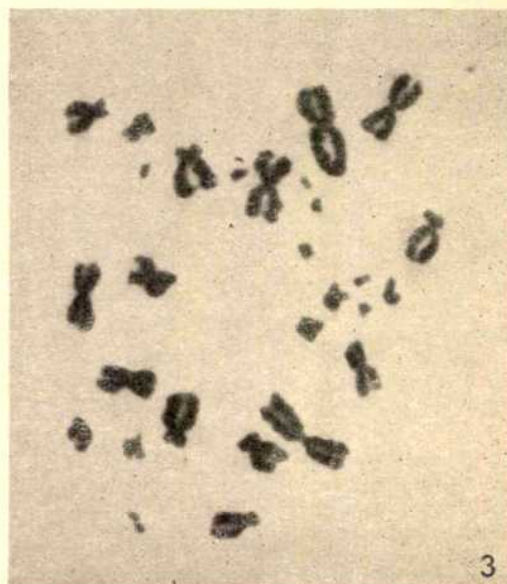


Fig. 3. Chromosomes of *Anolis bimaculatus*, showing a series of chromosomes grading from large to small with no sharp distinction between macro- and micro-chromosomes

macrochromosomes and alphas do not. Obviously further generalizations must await the examination of additional species within the genus.

These preliminary results suggest that karyotype will be a valuable taxonomic character for the elucidation of relationships and evolution of species groups within the genus *Anolis* as well as in other groups of the Reptilia. The karyotype can be obtained by the relatively simple techniques of blood culture.

I thank Dr. E. E. Williams, Dr. N. Feder and Dr. E. MacLeod of Harvard University for their advice and assistance; and Dr. P. Gerald and Miss S. Warner of the Boston Children's Hospital for introducing me to the techniques of blood culture and for use of their facilities. The photomicrographs were prepared by Dr. Feder. The

rk was carried out while I held a pre-doctoral fellowship in the U.S. National Science Foundation.

GEORGE C. GORMAN

seum of Comparative Zoology,
Cambridge, Massachusetts.

orhead, P. S., Nowell, P. C., Mellman, W. J., Battips, D. M., and Hungerford, D. A., *Exp. Cell Res.*, **20**, 613 (1960).

acak, W. J., Becak, M. L., Nazareth, H. R. S., and Pecchini, D., *Resumo XVI Reuniao-SBPC-Ciencia E Cultura*, **16**, 122 (1964); Becak, W., Becak, M. L., and Nazareth, H. R. S., *Amer. Naturalist*, **97**, 249 (1963).

atthey, R., *Rev. Suisse de Zoologie*, **38**, 118 (1931)

atthey, R., *Rev. Suisse de Zoologie*, **64**, 709 (1957).

akaki, D. T., and Sparkes, R. S., *Cytogenetics*, **2**, 57 (1963).

theridge, R. E., *The Relationships of the Anoles (Reptilia: Sauria: Iguaniidae): An Interpretation Based on Skeletal Morphology* (Univ. Microfilms, Inc., Ann Arbor, Michigan, 1960)

GENETICS

Measurements of Bovine Chromosomes

WE recently had an opportunity to study the karyogram a cell from a bovine male. Since we are interested in sperm nuclear areas in connexion with estimates of DNA individual sperm nuclei, it was of interest to measure projected areas of the chromosome images. The comments that follow all derive from the planimetric measurements (three times as complete sets by one observer, without reference to previously recorded measurements) from one karyogram.

The mean total area of the 60 chromosomes (corrected for magnification) was $281.25\mu^2$, making the mean area per chromosome $4.69\mu^2$. The smallest chromosome area is 0.203 times that of the largest, 0.400 times that of the mean. The largest area was 1.970 times that of the mean. Bovine chromosomes, except for the presumed sex chromosomes, are so morphologically similar that they cannot always be paired with certainty, so the cumulative distribution of chromosome sizes was plotted on probability semi-log graph) paper in ascending order of their calculated areas. Except for the seven largest chromosomes, the plot on log-probability paper approximates to a straight line. This is indicative of a skewed distribution sizes of bovine chromosomes.

The area of the presumed X chromosome was $7.85\mu^2$; the presumed Y it was $3.47\mu^2$. From these figures, and the total given above, one may compute the expected area of haploid (gametic) sets. A set containing the presumed X would have an area of $143.31\mu^2$ compared with $138.93\mu^2$ for a set containing the presumed Y. Thus there is an expected difference of 3.1 per cent in the area (and probably the mass) of haploid sets of chromosomes bearing either the X or Y. Could this difference (in X- and Y-bearing bovine spermatozoa) be detected by measurement of Feulgen-stained chromatin in bovine sperm nuclei? We think not. Using a Barr and Stroud integrating microdensitometer, we obtained a mean standard deviation of 0.033 units for 145 samples of 20 sperm nuclei each. From this one may calculate the expected least significant differences (*L.S.D.*) for different sample sizes. For samples of 1,000 the *L.S.D.* 0.01 turns out to be 0.234 units. For a sample mean of 15 units this is a difference of 1.6 per cent; for a sample mean of 20 units, the difference is 1.2 per cent. Thus it might be possible to detect a difference of 3.1 per cent between two populations of cells provided they were separated. The pooling of two normally distributed populations of equal size, with only a small separation in their means, results in a single population that approximates normality, but may be leptokurtic. To our knowledge there is no means of separating or identifying the X and Y gametes in bovine sperm populations, despite the attempts of Lindahl¹ by differential centrifugation and Gordon² by electrophoresis. The results of Nevo *et al.*³ would argue against the acceptance of electrophoresis as a general solution to the problem.

One other aspect was considered. Is it within the realm of possibility to see chromosomes in the sperm cell? We think not, despite the claims of Shettles⁴ for human

material. The measured area in plane of focus of Feulgen-stained bovine sperm nuclei varies from 20 to $35\mu^2$ (depending on individual variation, treatment of the semen sample, and method of fixation), which is about one-seventh to one-fourth of the area here estimated for haploid sets. Assuming equivalence of mass in the chromosomes of leucocyte cultures and the chromosomes of sperm nuclei, the chromosomes in the sperm nuclei must be layered 4-7 deep, making it unlikely that any optical system could resolve the individual chromosomes. Of course, the calculation above is an over-simplification which does not take into account the range of variability to be expected in the total areas of chromosomes from leucocyte cultures, but we have no reason to believe that the karyogram used was in any way unusual. The work reported was supported in part by a grant, GM-08753-03, from the U.S. Public Health Service, National Institutes of Health.

F. N. BAKER

G. W. SALISBURY

University of Illinois, Urbana.

N. S. FECHHEIMER

The Ohio State University, Columbus.

¹ Lindahl, P. E., *Nature*, **181**, 78 (1958).

² Gordon, M. J., *Proc. U.S. Nat. Acad. Sci.*, **43**, 913 (1957).

³ Nevo, A. C., Michaeli, I., and Echindler, H., *Exp. Cell Res.*, **23**, 69 (1961).

⁴ Shettles, L. B., *Nature*, **186**, 64 (1960); **188**, 918 (1960).

SOIL SCIENCE

Fixation of Metabolic Products in the Soil during Decomposition of Carbohydrates

SOME of the protein or amino-acids synthesized in the soil exist for a long time because they become resistant towards decomposition. About 40 per cent of the total amount of nitrogen in soils are thus found in amino-acids¹. This communication describes experiments dealing with the transformation of carbohydrate carbon into amino-acids and other compounds in soils.

Carbon-14-labelled cellulose and glucose were prepared from material originating from barley plants grown to maturity in a growth chamber in an atmosphere enriched with $^{14}\text{CO}_2$ (ref. 2). The specific activity of the preparations was about 80 $\mu\text{C/g}$ carbon; the nitrogen content was nil or insignificant. Cellulose (ground to pass a 40 mesh screen) and glucose respectively were added to soil portions in amounts corresponding to 500 mg carbon/100 g of dry soil, besides 10 mg nitrogen in NH_4NO_3 and water to 40 per cent of the water-holding capacity. Samples of 50 g were placed in 100-ml. Erlenmeyer flasks which were stored at 20° C in the dark connected to a respiration apparatus enabling absorption and measurement of the carbon dioxide evolved from the soil. Water evaporated was replenished every second week.

Soil samples were removed with intervals during the decomposition period and analysed as follows. Portions of 50 g without previous drying were shaken for 30 min with 200 ml. of 0.1 N $\text{Ba}(\text{OH})_2$ at room temperature. The filtrate of the suspension plus the washings of the residue was neutralized with sulphuric acid. The filtrate from the BaSO_4 precipitate was concentrated *in vacuo*, after which the carbon content and the radioactivity of the residue were determined². The soil extracted with $\text{Ba}(\text{OH})_2$ was boiled with 6 N HCl for 16 h under reflux, 10 g of air-dry soil plus 100 ml. of acid. The acid extract of the soil combined with the washings of the residue was repeatedly evaporated to dryness *in vacuo*. The part of the residue which was soluble in water was run through a H^+ saturated column of a cation exchange resin ('Amberlite IR-120'). The amino-acids retained by the column were desorbed by 2 N ammonia. After removal of the ammonia the concentration of α -amino-acid-N (ref. 3), carbon and radioactivity of the amino-acid solution were determined². Individual amino-acids were

detected on paper chromatograms. Carbon content and radioactivity of the untreated soil and the soil extracted with 6 N HCl were also determined.

Fig. 1 shows the results from an experiment in which cellulose was added to a medium loam soil (pH 6.1, carbon 1.4 per cent). It is seen that about 10 per cent of the original cellulose carbon was recovered in amino-acids after 12 and 30 days of incubation; after 90 days of incubation this value had decreased to 7 per cent, and after 300 days to 5 per cent. The amount of original cellulose-carbon incorporated in amino-acids constituted a considerable part of the total amount of original cellulose carbon remaining in the soil, about 40 per cent after 90 days of incubation and 30 per cent after 300 days. Maximum amount of original cellulose carbon in $\text{Ba}(\text{OH})_2$ extractable material (2.5 per cent) was observed after 12 days of incubation.

Addition of glucose to the soil mentioned above resulted in a larger incorporation of original glucose carbon in $\text{Ba}(\text{OH})_2$ extractable compounds, and in amino-acids during the first 5 days of incubation than observed for cellulose, but after 30 days of incubation the amounts incorporated were of the same size as mentioned for cellulose.

The $\text{Ba}(\text{OH})_2$ extracted material included a number of free amino-acids and polysaccharides which on hydrolysis yielded glucose and smaller amounts of other sugars. The amino-acids liberated during the treatment with 6 N HCl included 13 normal protein amino-acids besides glucosamine and galactosamine.

The clay content of the soil might be of importance for the fixation of metabolic products⁴. This relation was investigated in a few experiments. A sandy soil (pH 5.8, carbon 2.2 per cent) was mixed with washed quartz sand; to the sand was added a clay mineral, Wyoming montmorillonite, American Petroleum Institute Clay Mineral Standard No. 25, obtained from Ward's Natural Science Establishment, Rochester 3, New York. The clay was added in such amounts that the soil-sand mixtures con-

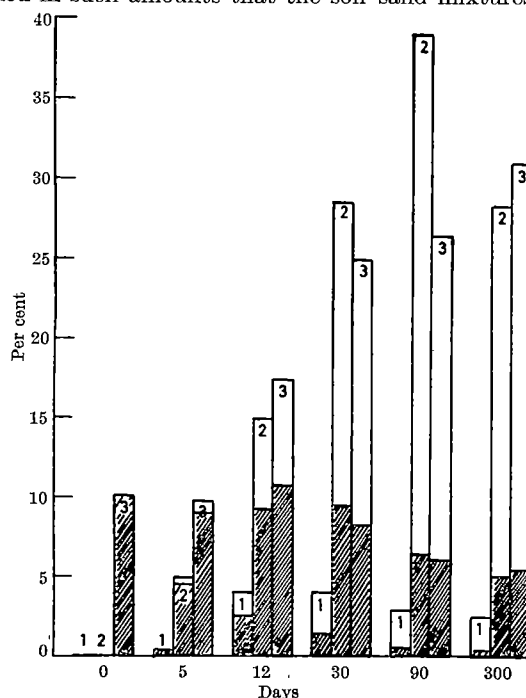


Fig. 1 Transformation of carbon added in cellulose into other compounds during decomposition in soil. The shaded areas of the columns indicate the amounts in per cent of the cellulose carbon added, the total areas of the columns indicate the amounts in per cent of original cellulose carbon remaining in the soil. 'Days' means days of incubation at 20° C. Columns marked 1 indicate original cellulose carbon in material extracted with 0.1 N $\text{Ba}(\text{OH})_2$, columns marked 2 indicate original cellulose carbon in amino-acids dissolved during treatment with 6 N HCl, columns marked 3 indicate original cellulose carbon remaining in the soil treated with 6 N HCl.

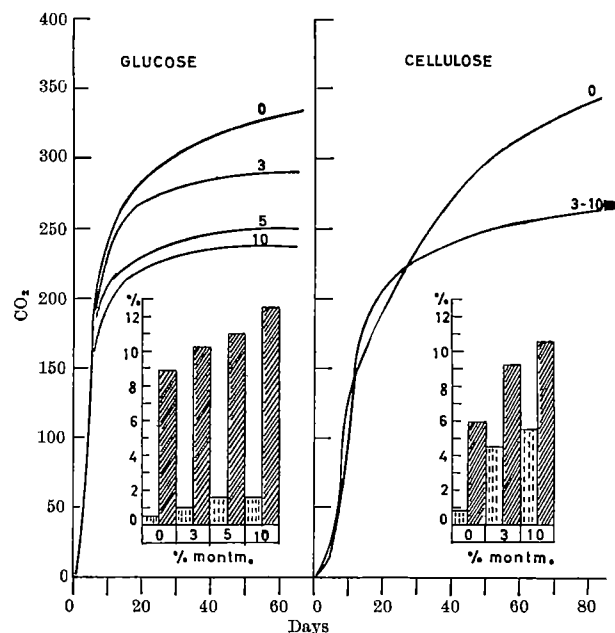


Fig. 2 Influence of added montmorillonite on the decomposition of glucose and cellulose in a soil-sand mixture. CO_2 means mg of carbon dioxide evolved per 100 g of dry soil-sand, 'days' means days of incubation at 20° C. The curves are summation curves and the numbers indicate per cent of montmorillonite added to the soil-sand from which the carbon dioxide production originates. The inserted histograms show the results of the chemical analyses of the soil-sand performed after 60 (glucose) and 90 (cellulose) days of incubation. The areas of the columns indicate the amounts of original glucose or cellulose carbon recovered in material extracted with 0.1 N $\text{Ba}(\text{OH})_2$ (dotted columns), and in amino acids dissolved during treatment with 6 N HCl (shaded columns). The values are stated in per cent of glucose or cellulose carbon added.

tained 0, 3, 5 and 10 per cent respectively of the clay besides 50 per cent of the soil, and sand to 100 per cent. Cellulose and glucose were added and the experiment performed and analysed as described above. Before addition the clay was prepared as follows. A 5 per cent suspension of the raw mineral was centrifuged for 10 min at 2,500 r.p.m. The suspension of particles not sedimented was run through columns of Na^+ and H^+ saturation exchange resin. The effluent suspension which had a pH of 2.2 was titrated to pH 5.8 (the pH of the soil with 0.5 N NaOH and centrifuged at 20,000 r.p.m. until all material had come down. The sediment was dried at room temperature *in vacuo* and ground thoroughly.

Fig. 2 shows that the presence of montmorillonite reduced the rate of the production of carbon dioxide from the soil-sand during the later stage of the decomposition, whereas it seemed to be of no importance during the first stage of decomposition during which about 40 per cent of the added carbon was evolved as carbon dioxide. The rate of carbon dioxide calculated to originate from soil humus was not, or only very slightly, influenced by the addition of the clay mineral.

The chemical analyses of the soil-sand mixtures after the decomposition period (Fig. 2, insets) showed that a larger percentage of the added carbon was recovered in amino-acids where the montmorillonite was added than in the controls without added montmorillonite. This seems to indicate that the reduced rate of production of carbon dioxide was a result of a protection of metabolic products towards microbial decomposition by adsorption to the montmorillonite⁵.

HENNING SØRENSEN

Department of Agricultural Research,
Atomic Energy Commission
Research Establishment,
Riso, Roskilde, Denmark.

¹ Stevenson, F. J., *Soil Sci. Soc. Amer. Proc.*, **21**, 283 (1957).

² Sørensen, H., *Soil Sci.*, **95**, 45 (1963).

³ Van Slyke, D. D., *et al.*, *J. Biol. Chem.*, **141**, 627 (1941).

⁴ Allison, F. E., *et al.*, *Soil Sci.*, **68**, 463 (1949).

⁵ Esminger, L. E., and Gieseking, J. E., *Soil Sci.*, **53**, 205 (1942).

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, October 4

BRITISH BIOPHYSICAL SOCIETY, PHYSICAL BIOCHEMISTRY GROUP (at the School of Pharmacy, Brunswick Square, London, W.C.1), from 11 a.m. to 6 p.m.—Meeting on "Light Scattering"

INSTITUTION OF MECHANICAL ENGINEERS, EDUCATION AND TRAINING GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion Meeting on "The Teaching of Mechanics of Machines".

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (in the Shell Centre, Shell Centre, London, S.E.1), at 6.30 p.m.—Scientific Film Evening.

Monday, October 4—Wednesday, October 6

IRON AND STEEL INSTITUTE and the INSTITUTE OF METALS (at the Royal Commonwealth Society, Northumberland Avenue, London, W.C.2)—Joint Conference on "Machinability".

Tuesday, October 5

INSTITUTION OF MECHANICAL ENGINEERS, APPLIED MECHANICS GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion Meeting on "Acoustic Excitation and Mechanical Resonance".

Wednesday, October 6

COLOUR GROUP (Great Britain) (in the Physics Department, Imperial College, London, S.W.7), at 3.15 p.m.—Twenty-ninth Science Meeting part on the International Colour Conference in Lucerne (June 1965) and on the C.I.E. Committee Meeting in Basle. Speakers: Dr. B. H. Crawford, D. A. Palmer, Dr. R. W. G. Nunt and Mr. F. J. B. Wall.

ROYAL SOCIETY OF MEDICINE, HISTORY OF MEDICINE SECTION (at 1 Wimpole Street, London, W.1), at 5.15 p.m.—Mr. N. Capener: "An English seventeenth Century Emergency Hospital"; Mr. J. Shepherd: "The Civil Hospitals in the Crimea, 1855–1856"; Mr. D. L. Griffiths: "Medicine and Surgery in the American Civil War".

INSTITUTE OF PETROLEUM (at 61 New Cavendish Street, London, W.1) at 3.0 p.m.—Mr. R. Hollinghurst: "Radiation-Resistant Fluids and Lubricants".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at 100 Fenchurch Lane, London, E.C.3), at 5.30 p.m.—Discussion on "Filling Gaps in H.F./U.H.F. Service Areas" opened by Mr. L. F. Tagholm.

ROYAL MICROSCOPICAL SOCIETY (at the Royal Society, Burlington House, Piccadilly, London, W.1), at 5.30 p.m.—"Photomicrography—a Brain's Trust".

ENVIRONMENTAL GROUP (in the Department of Electrical Engineering, Imperial College of Science and Technology, Exhibition Road, London, W.7), at 5.45 p.m.—Extraordinary General Meeting. 6.30 p.m.—Discussion Meeting on "Existing Methods of Appraisal".

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, RADAR GROUP (at Bedford Square, London, W.C.1), at 6 p.m.—Mr. H. W. Cole: "SECAR"—Modern SSR Ground Interrogator and Decoding Equipment".

PHARMACEUTICAL SOCIETY OF GREAT BRITAIN (at 17 Bloomsbury Square, London, W.C.1), at 6 p.m.—Mr. L. G. Goodwin: "Comparative Pharmacology".

SOCIETY OF ENVIRONMENTAL ENGINEERS, PACKAGING GROUP (in the Mechanical Engineering Department, Imperial College, Exhibition Road, London, S.W.7), at 6 p.m.—Dr. J. Pendered: "Shock Response Analysis under Shock Spectrum".

SOCIETY FOR ANALYTICAL CHEMISTRY (at the Chemical Society, Burlington House, Piccadilly, London, W.1), at 7 p.m.—Prof. T. S. West: "Some Special, Sensitive and Selective Reactions in Inorganic Trace Analysis".

TELEVISION SOCIETY (at the I.T.A. Conference Suite, 70 Brompton Road, London, S.W.8), at 7 p.m.—Discussion Meeting on "The Technical Future of British Television".

Thursday, October 7

ROYAL SOCIETY OF MEDICINE, UNITED SERVICES SECTION (at 1 Wimpole Street, London, W.1), at 5 p.m.—Surgeon Commander J. D. Walters and Dr. J. J. Brand: "Motion Sickness".

SOCIETY OF COSMETIC CHEMISTS OF GREAT BRITAIN (at the Royal Society of Arts, John Adam Street, Adelphi, London, W.C.2), at 7.30 p.m.—Dr. F. W. Scott Blair: "The Subjective Assessment of the Consistency of Materials in Relation to Physical Measurements".

Friday, October 8

BIOCHEMICAL SOCIETY (at the Middlesex Hospital Medical School, London, W.1)—453rd Meeting. Programme includes a Colloquium on "The Effects of Hormones on Nucleic Acid Synthesis".

Saturday, October 9

INNER LONDON EDUCATION AUTHORITY (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Dr. J. Eric S. Thompson: "The Ancient Maya Civilization of Central America".

Monday, October 11

INSTITUTION OF MECHANICAL ENGINEERS, EDUCATION AND TRAINING GROUP (Joint Conference with the Schools Council, at 1 Birdcage Walk, Westminster, London, S.W.1), at 2.30 p.m.—Conference on "Applied Science and Engineering Activities in Schools".

UNIVERSITY OF LONDON (in the Botany Lecture Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. G. Drews (University of Freiburg): "The Cell of the Photosynthetic Bacteria in Light- and Dark-Metabolism" (further lecture on October 13).

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 6 p.m.—"Germany: a Regional Geography" (colour film).

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

PRINCIPAL BIOCHEMIST (with a good honours degree in chemistry or biochemistry and/or a Ph.D. or other higher qualification) in the Department of Chemical Pathology, Ashton Street, Pembroke Place, Liverpool, 3—The Secretary, The United Liverpool Hospitals, 80 Rodney Street, Liverpool, 1 (October 12).

PHYSICIST, BASIC GRADE, IN THE PHYSICS DEPARTMENT, to assist with the routine, development and research work of the department in the application of physics to medical problems (if necessary training will be given in all aspects of this work)—The House Governor, The London Hospital, Whitechapel, London, E.1 (October 14).

ASSISTANT LIBRARIAN (qualified librarian preferably with some experience in a college library)—The Clerk to the Governors, Woolwich Polytechnic, Wellington Street, Woolwich, London, S.E.18 (October 15).

PROGRAMMING ASSISTANT IN THE COMPUTING DEPARTMENT, to write programmes for the University EDF 9 computer and to assist in the general supervisory service of the Department—The Secretary of the University Court, The University of Glasgow, Glasgow (October 15).

RESEARCH ASSISTANT IN THE DEPARTMENT OF GEOGRAPHY to undertake the field work and photogrammetric plotting of aerial photographs necessary to compile a series of maps of an area in the Inner Hebrides—The Secretary of the University Court, The University of Glasgow, Glasgow, W.2 (October 15).

LECTURER (initiated, designed, constructed and used electronic devices, not necessarily medical) IN BIOLOGICAL ENGINEERING—The Secretary, The University, Aberdeen (October 16).

LECTURER (with special qualifications in social geography or social anthropology) IN GEOGRAPHY—The Registrar (Room 39, O.R.B.), The University, Reading (October 16).

SENIOR LECTURER IN MATHEMATICS; a LECTURER or ASSISTANT LECTURER IN MATHEMATICS, a LECTURER or ASSISTANT LECTURER IN STATISTICS; a LECTURER IN COMPUTATION, and a LECTURER or ASSISTANT LECTURER IN OPERATIONAL RESEARCH—The Registrar, Ref. 124Y/E, Bradford Institute of Technology, Bradford, 7, Yorkshire (October 18).

ASSISTANT STATISTICIAN (with an honours degree in statistics, or a first- or second-class honours degree (or equivalent qualification) in which statistics is a principal subject) in the Department's Headquarters in London—B. Johnston, Department of Education and Science, Curzon Street, London, W.1 (October 19).

LECTURER or ASSISTANT LECTURER (preferably with research experience, interest or qualifications in platyhelminth or nematode parasitology or the ecology and systematics of small animals) IN THE DEPARTMENT OF ZOOLOGY, Ahmadu Bello University, Northern Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (October 20).

RESEARCH ASSISTANT (with an honours degree in botany or an equivalent qualification, with an interest in taxonomy and preferably with some knowledge of bryophytes) IN THE DEPARTMENT OF BOTANY for participation in a project involving floristic and phytogeographical studies in antarctic bryophytes—The Assistant Registrar (Science), The University, Birmingham 15 (October 22).

LECTURER (with special qualifications in economic geography and quantitative methods) IN THE DEPARTMENT OF GEOGRAPHY, University of Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (October 25).

SENIOR LECTURERS and LECTURERS IN THE DEPARTMENT OF PHYSICS, University of Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (October 25).

LECTURER IN GEOLOGY AT FOURAH BAY COLLEGE, The University College of Sierra Leone—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (October 28).

READER IN COMPUTER SCIENCE AT THE INSTITUTE OF COMPUTER SCIENCE, to lead a small research group concerned with the general design and use of future computing systems including problems of man-machine communication—The Academic Registrar, University of London, Senate House, London, W.C.1 (October 29).

SCIENTIFIC INFORMATION OFFICER (with a botanical or agricultural degree, the ability to write correct English, and preferably a knowledge of foreign languages) AT THE COMMONWEALTH BUREAU OF PLANT BREEDING AND GENETICS, Cambridge, for work which includes abstracting, editing, classification of biological literature and dealing with scientific enquiries—The Director, Commonwealth Agricultural Bureaux, School of Agriculture, Cambridge (October 30).

JUNIOR LECTURER or LECTURER (with at least a B.Sc. (Hons.) degree, and preferably some experience in radioisotope work) IN SOIL SCIENCE, Massey University of Manawatu, Palmerston North, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, October 31).

LECTURER or SENIOR LECTURER (with a degree in agricultural science or science, and experience in soil fertility work, either in the field or the laboratory) IN SOIL SCIENCE at Massey University of Manawatu, Palmerston North, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, October 31).

SENIOR LECTURER, LECTUREL or JUNIOR LECTURER (one or two appointments) (preferably with research experience or special interests in (a) plant physiology, particularly of the cell and tissue levels, (b) morphology and physiology of lower plants; or (c) cytology and histochemistry) IN THE DEPARTMENT OF BOTANY, Massey University of Manawatu, Palmerston North, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, October 31).

CHAIR OF HAEMATOLOGY at St. Bartholomew's Hospital Medical College—The Academic Registrar, University of London, Senate House, London, W.C.1 (November 1).

CHAIR OF PLANT PHYSIOLOGY within the Waite Agricultural Research Institute, University of Adelaide, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, November 1).

LECTURER (graduate in microbiology or biochemistry) IN BACTERIOLOGY—The Secretary, Trinity College, Dublin, 2, Republic of Ireland (November 1).

LECTURER (with a higher degree in anthropology/sociology, experience in conducting independent research, and preferably some teaching experience) IN ANTHROPOLOGY at the University of Western Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 13).

SENIOR LECTURER (competent circuit engineer with modern experience and a knowledge of digital computers, and preferably analytical ability and lecturing experience) IN ELECTRICAL ENGINEERING at the University of

Adelaide, South Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W. 1 (Australia, November 15).

ASSISTANT LECTURERS or LECTURERS (3) IN THE DEPARTMENT OF PHYSICS—The Registrar, The University, Liverpool, quoting Ref. CV/290 (November 27).

LECTURERS (with a good honours degree and a keen research interest in any branch of electronics and control engineering) IN THE SCHOOL OF ENGINEERING SCIENCE, ELECTRONICS AND CONTROL DIVISION—The Registrar, University College of North Wales, Bangor, North Wales (November 30).

ASSISTANT LECTURER, GRADE B, to teach human anatomy, physiology and hygiene and possibly some general science, together with biology to G.C.E. Advanced Level—The Registrar, Derby and District College of Technology, Kedleston Road, Derby.

ASSISTANT LECTURER (honours graduate with suitable postgraduate experience) IN PHYSICS—The Academic Registrar, Brunel College, Woodlands Avenue, Acton, London, W.3.

ASSISTANT LECTURER (with appropriate qualifications in biology, mycology or botany, and preferably special qualifications in plant mycology or general mycology) IN BIOLOGY IN THE DEPARTMENT OF APPLIED MICROBIOLOGY AND BIOLOGY—The Registrar, University of Strathclyde, Glasgow, C.1.

CHIEF TECHNICIAN (with appropriate technical qualifications and wide practical experience) IN THE SCHOOL OF BIOLOGICAL SCIENCES AT BATH, to be responsible for the overall control of technical services and the ordering of materials and equipment in the School—The Secretary and Registrar, Bristol College of Science and Technology, Ashley Down, Bristol, 7, quoting Ref. CST. 65/90.

ELECTRONICS TECHNICIAN (preferably with a National Certificate in Electrical Engineering or equivalent) IN THE BIOLOGICAL LABORATORY, to be responsible for the maintenance and construction of electronic instruments used in research and teaching—The Assistant Registrar (Establishment), University of Sussex, Stanmer House, Stanmer, Brighton, Sussex.

FIELD ASSISTANT (Female) (with a degree in zoology, preferably with an interest in geography or geology) at Preston Montford Field Centre, near Shrewsbury—The Secretary, Field Studies Council, 9 Devereux Court, Strand, London, W.C.2.

GRADUATE RESEARCH OFFICER (preferably with experience in medical statistics, biometry or genetics) IN STATISTICS in the Oxford Record Linkage Study and Unit of Clinical Epidemiology—The Medical Director, Oxford Record Linkage Study, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Oxford.

LECTURER (honours graduate in zoology with subsidiary qualifications or interest in physiology) IN ZOOLOGY IN THE BIOLOGY AND ZOOLOGY DEPARTMENT—The Principal, Norwood Technical College, Knight's Hill, London, S.E.27.

LECTURER or ASSISTANT LECTURER (preferably with a special interest in social statistics, social administration or industrial sociology) IN SOCIOLOGY, to assist with teaching for London honours degrees in Sociology—The Registrar, Portsmouth College of Technology, Hampshire Terrace, Portsmouth, Hampshire.

LIBRARIAN (qualified person or graduate)—The Administrator, Department of Biochemistry, University of Oxford, South Parks Road, Oxford.

MASTER (preferably graduate in mechanical sciences or engineering) TO TEACH MATHEMATICS AND SOME PHYSICS—The Headmaster, Oundle School, Peterborough.

MASTER TO TEACH MATHEMATICS to scholarship level—The Headmaster, Monkton Combe School, near Bath, Somerset.

ORNITHOLOGIST (university graduate in zoology) with the Canadian Wildlife Service, Quebec City, to undertake ecological studies of migratory bird populations with particular emphasis on the distribution, numbers, locations and migrant patterns of waterfowl and the evaluation of their habitat—Technical and Scientific Requirements Group, Civil Service Commission of Canada, Ottawa 4, Ontario, Canada, quoting Ref. 65-1350K.

PHYSICIST (well qualified and experienced, able to teach to university scholarship level)—The High Master, Manchester Grammar School, Manchester, 13.

PHYSICIST (with an honours degree) IN THE PHYSICS DEPARTMENT for work involving the clinical and biological uses of ionizing radiation—The Physics Department, Westminster Medical School, Horseferry Road, London, S.W.1.

PROFESSOR IN PHYSICAL CHEMISTRY—Prof. Harry E. Gunning, Head, Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada.

RESEARCH ASSISTANT (civil or chemical engineering graduate) IN THE DEPARTMENT OF CIVIL ENGINEERING, to investigate fluidization-flocculation processes in water purification—The Secretary, University College, Gower Street, London, W.C.1.

RESEARCH ASSISTANT IN THE DEPARTMENT OF PHYSICS for research on plasma generation by high power lasers—The Registrar, University of Essex, Wivenhoe Park, Colchester, Essex.

RESEARCH ASSISTANT (with a good honours degree in sociology) IN SOCIOLOGY—The Registrar, Portsmouth College of Technology, Hampshire Terrace, Portsmouth, Hampshire.

SOIL CHEMIST (national of the United Kingdom or the Republic of Ireland, with either an honours degree in agricultural chemistry (soils and plants), or a degree in chemistry with postgraduate experience in agricultural analysis (soils and plants), and preferably experience in soil and plant analysis) with the East African Common Services Organization, to carry out and supervise soil and plant analysis and to help develop and investigate new methods—The Appointments Officer, Ministry of Overseas Development, Room 301, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 213/214/04.

TECHNICIAN or SENIOR TECHNICIAN (with a good knowledge of general laboratory practice, and preferably special experience in experimental and microscopical cell studies or electron microscopy) in the Analytical Cytology Section, to assist in work on cell ultrastructure and function—A. D. Greenwood, Department of Botany, Imperial College, London, S.W.7.

TECHNICIAN (preferably with experience in the food or pharmaceutical industries) TO TAKE CHARGE OF BIOCHEMICAL ENGINEERING PILOT PLANT—The Establishment Officer, University College, Gower Street, London, W.C.1, quoting Ref. Chem. Eng./3.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Agricultural Research Council. A Bibliography of Farm Buildings Research. Part 1: Buildings for Pigs, 2nd Supplement, 1962-64. Pp. 80. (London: Agricultural Research Council, 1965. Obtainable from H.M. Stationery Office.) 5s. 6d. net.

Political and Economic Planning. Annual Report 1964-65. Pp. 12. (London: Political and Economic Planning, 1965.)

Automatic Computing: Its Problems and Prizes. By Prof. Stanley (Inaugural Lecture, 26 January, 1965). Pp. 69-93+6 plates. (Long Imperial College of Science and Technology, 1965.) 4s. 6d.

Building Research Station Digest (Second Series). No. 61: Strength Brickwork, Blockwork and Concrete Walls. Pp. 6. (London: Building Research Station, 1965.) 4d.

Agricultural Research Council: Soil Survey Research Board. Soil Survey of Great Britain—Report No. 17, 1964. Pp. v+46. (London: Agricultural Research Council, 1965. Obtainable from H.M. Stationery Office.) 6s. net.

Wye College (University of London). Annual Report of the Department of Hop Research 1964 (from 1st April, 1964, to 31st March, 1965). Pp. v+ (Wye, Ashford, Kent: Wye College, 1965.) 6s.

Ministry of Technology. Laboratory of the Government Chemist. Report of the Government Chemist 1964. Pp. viii+108+4 plates. (London: H.M. Stationery Office, 1965.) 8s. net.

Geological Survey and Museum. Memoirs of the Geological Survey Great Britain—England and Wales. Geology of the Country around Huntingdon and Biggleswade (Explanation of One-inch Geological Sheet 187 and 204, New Series). By E. A. Edmonds and the late C. H. Dinoh with contributions by J. B. W. Day and Dr. R. Casey. Pp. viii+90+3 plates. (London: H.M. Stationery Office, 1965.) 25s. net.

The British Drug Houses, Ltd. 2: 4-Dinitro-phenyl-hydrazine. Pp. (Poole, Dorset: The British Drug Houses, Ltd., 1965.)

Other Countries

United States Department of the Interior: Geological Survey Bulletin 1183: The Crystalline Rocks of South Carolina. By William C. Overst and Henry Bell, III. Pp. v+126+4 plates. Bulletin 1188: Geology Northwestern North Park, Colorado. By William J. Hall, Jr. Pp. v+133 plates 1-5. Bulletin 1199-B: Bauxite and Kaolin Deposits of Mississippi Exclusive of the Tippah-Benton District. By Louis C. Conant. Pp. v+70+plates 1-5. (Washington, D.C.: Government Printing Office, 1965.)

Proceedings of the United States National Museum, Smithsonian Institution. Vol. 117, No. 3508: Microlepidoptera of Juan Fernandez Islands. J. F. Gates Clarke. Pp. 1-106. Vol. 117, No. 3509: Neotropical Hemepterididae in the United States National Museum. By W. Nakahara. Pp. 107-122. Vol. 117, No. 3510: Hemipterobates, a New Generic Record for the Atlantic Ocean, with Descriptions of New Species (Hemiptera: Gerridae). By Jon L. Herring. Pp. 123-130. (Washington, D.C.: Government Printing Office, 1965.)

Western Australia. Report of the Government Chemical Laboratories for the year 1963. (Extract from the Report of the Department of Mines Pp. 39. (Perth: Government Printer, 1965.)

Contributi dell'Istituto Geofisico e Geodetico dell'Università di Genova. Anno Geofisico Internazionale 1967-68 e 1959-1963. Memoria di Ricerche sulla Radiazione Solare. Da M. Boscellasco, G. Cicconi, I. Dagnin A. Elena e G. Flocchini. Pp. 56. (Genova: Istituto Geofisico e Geodetico dell'Università di Genova, 1965.)

Progress in Protozoology: Abstracts of Papers read at the Second International Conference on Protozoology, London, 29th July-5th August, 1964 (International Congress Series, No. 91.) Pp. 278. (Amsterdam, New York, London, Milan, Tokyo and Buenos Aires: Excerpta Medica Foundation, 1965.) 65s.

Proceedings of the United States-Japan Conference on Research Related to Earthquake Prediction Problems. (Sponsored by the National Science Foundation and Japan Society for Promotion of Science.) Pp. 105. (Tokyo: Dr T. Hagiwara, Earthquake Research Institute; New York: Dr J. Oliver Lamont Geological Observatory, Palisades, 1964.)

Year Book of the National Institute of Sciences of India, 1965. Pp. vi+239. (New Delhi: National Institute of Sciences of India, 1965.) Rs. 15.

United States Department of the Interior: Geological Survey. Professional Paper 490: Geology of the Flaming Gorge Area, Utah-Colorado-Wyoming. By Wallace R. Hansen. Pp. viii+196+plates 1-3. (Washington, D.C.: Government Printing Office, 1965.)

Metropolitan Life Insurance Company. Statistical Bulletin, Vol. 4 (June 1965): Education and the Occupation of Men. Geographic Patterns of Fatal Accidents: Further Reduction in Maternal Mortality. Hospital Care of Company Personnel in 1964. Pp. 12. (New York: Metropolitan Life Insurance Company, 1965.)

Cenco Instruments Corporation. 1965 Annual Report. Pp. 34. (Chicago: Cenco Instruments Corporation, 1965.)

Atlantide Report No. 8: Scientific Results of the Danish Expedition to the Coasts of Tropical West Africa, 1945-1946. By Jørgen Knudsen and Torben Wolff. Pp. 255. (Copenhagen: Danish Science Press; London: British Museum (Natural History), 1965.) 65 D.kr.; 65s.

University of Lagos. Change in Vice-Chancellorship. Pp. 28. (Lagos: The University, 1965.)

Records of the Queen Victoria Museum, Launceston. New Series, No. 18: Thomas Bock's Portraits of the Tasmanian Aborigines. Pp. 24 (10 plates). New Series, No. 19: Two Skink Lizards Newly Recorded from Tasmania. By R. H. Green. Pp. 4. (Launceston: The Queen Victoria Museum, 1965.)

Records of the Australian Museum. Vol. 26, No. 6 (5th June, 1964): The Archaeology of the Capertee Valley, New South Wales. By Frederick I. McCarthy. Pp. 197-246+plates 11-24. 16s. 6d. Vol. 26, No. 14 (6th January, 1965): Some Nephthyidae (Polychaeta) from Australian Waters. By Kristian Fauchald. Pp. 333-340. 2s. (Sydney: The Australian Museum, 1964 and 1965.)

Editorial and Publishing Offices of "NATURE"

MACMILLAN (JOURNALS) LIMITED

4 LITTLE ESSEX STREET, LONDON, W.C.2

Telephone Number: Temple Bar 6633. Telegrams: Phusis London W.C.2

Annual subscription: Inland £14, Overseas £13 15s., payable in advance, postage paid to any part of the world.

Advertisements only should be addressed to

T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2.

Telephone Number: Holborn 4743

Registered as a newspaper at the General Post Office

Copyright © Macmillan (Journals) Limited, October 2, 1965

NATURE

No. 5006

SATURDAY, OCTOBER 9, 1965

Vol. 208

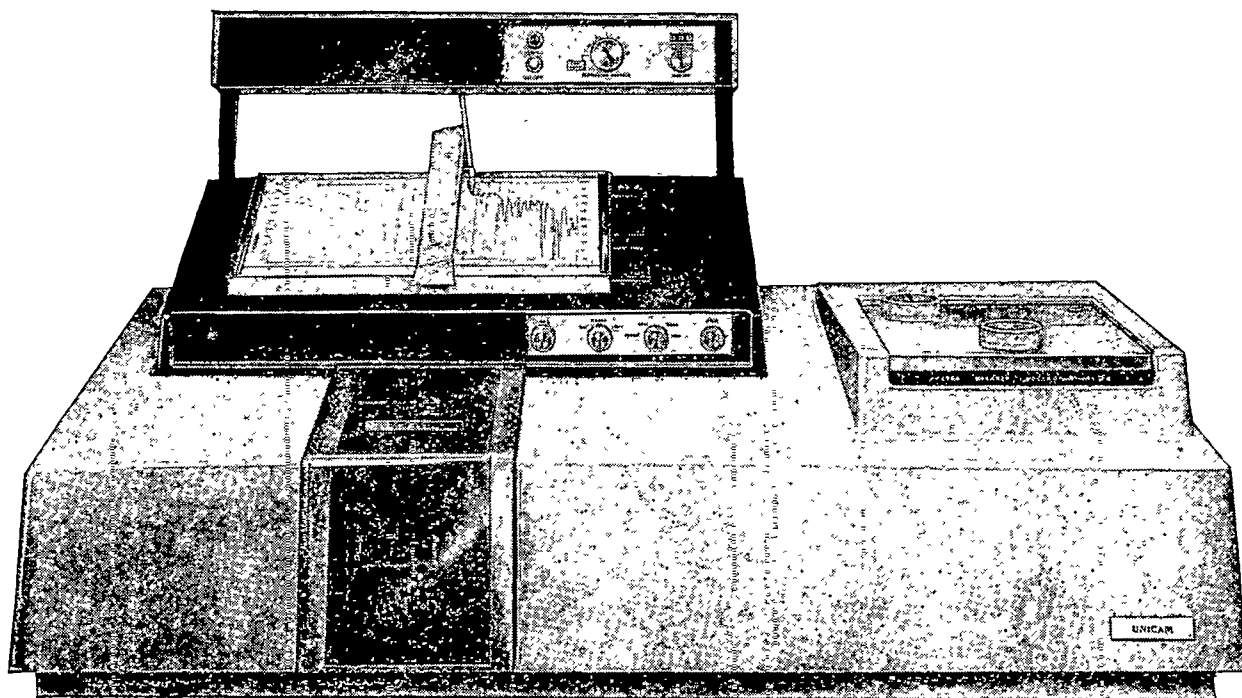
Published by
MACMILLAN (JOURNALS) LTD.

CONTENTS

	<i>Page</i>
RESEARCH AND DEVELOPMENT IN BRITISH INDUSTRY	101
NOBEL LECTURES AND SCIENTIFIC METHOD. By Dr. G. Burniston Brown	103
ELECTROCHEMISTRY. By Dr. R. F. Phillips and N. A. Hampson	104
NUCLEAR AND RADIOCHEMISTRY. By Dr. H. A. C. McKay	105
FOURIER THEORY. By Prof. T. A. A. Broadbent	106
ERGONOMICS. By C. E. Fleming	106
REGULATION OF GROWTH. By Dr. R. A. Weiss	107
MICROLEPIDOPTERA UNDER THE MICROSCOPE. By Prof. G. C. Varley	108
RECENT DEVELOPMENTS IN COSMOLOGY. By Prof. F. Hoyle, F.R.S.	111
A VISIT TO THE LOWER AMAZON. By I. R. Bishop and Prof. G. E. H. Foxon	114
NUTRITION AND ITS INTERNATIONAL RECOGNITION. By Sir Rudolph A. Peters, F.R.S.	117
NEWS AND VIEWS	117
SPECTROSCOPY IN AUSTRALIA. By Dr. G. C. Barraclough, Dr. P. J. Black, D. J. David, Dr. A. D. E. Pullin and J. S. Shannon	125
BRITISH AID TO DEVELOPING COUNTRIES	128
SOLAR MOTION AND SUNSPOT COMPARISON. By Dr. Robert M. Wood and Prof. Karl D. Wood	129
STRATOSPHERIC TRANSPORT OF VOLCANIC DUST INFERRED FROM SOLAR RADIATION MEASURE- MENTS. By Dr. A. J. Dyer and B. B. Hicks	131
EXPERIMENTAL OBSERVATIONS ON THE STRUCTURE OF COLLISIONLESS SHOCK WAVES IN A MAGNETIZED PLASMA. By Dr. J. W. M. Paul, L. S. Holmes, Dr. M. J. Parkinson and J. Sheffield	133
PLEISTOCENE GLACIAL-MARINE ZONES IN NORTH ATLANTIC DEEP-SEA SEDIMENTS. By Dr. John R. Conolly and Dr. Maurice Ewing	135
AN INFRA-RED INVESTIGATION OF THE EFFECT OF PRESSURE ON SILICA POWDERS, AS REVEALED BY DEUTERIUM OXIDE EXCHANGE. By F. H. Hambleton, Dr. J. A. Hockey and J. A. G. Taylor	138
HEXAGONAL-MESH STRUCTURES AND STABLE POLYPEPTIDE CHAIN CONFIGURATIONS. By Prof. R. E. Dickerson	139
A NEW INHERITED COAGULATION DISORDER CAUSED BY AN ABNORMAL FIBRINOGEN ('FIB- RINOGEN BALTIMORE'). By Dr. Eugene A. Beck, Patricia Charache and Prof. Dudley P. Jackson	143
MECHANISM OF INTESTINAL ABSORPTION: TRANSPORT OF METHIONINE AND SODIUM BUTYRATE BY INTRACELLULAR PLASMA. By S. L. Hart and Dr. J. A. Nissim	145
STOICHIOMETRY OF PROTON TRANSLOCATION THROUGH THE RESPIRATORY CHAIN AND ADENO- SINE TRIPHOSPHATASE SYSTEMS OF RAT LIVER MITOCHONDRIA. By Dr. Peter Mitchell and Dr. Jennifer Moyle	147
STRUCTURE OF CONDENSED TANNINS. By L. L. Creasy and Dr. T. Swain	151
INTERSTIMULUS INTERVAL AND NUMBER JUDGMENT. By D. Jones and Ir. A. Elithorn	153
CONTROL OF THE COMPETENT STATE IN <i>Pneumococcus</i> BY A HORMONE-LIKE CELL PRODUCT: AN EXAMPLE FOR A NEW TYPE OF REGULATORY MECHANISM IN BACTERIA. By Dr. Alexander Tomasz	155
RATE OF CHROMOSOME DUPLICATION AT THE END OF THE DEOXYRIBONUCLEIC ACID SYN- THETIC PERIOD IN HUMAN BLOOD CELLS. By Dr. C. W. Gilbert, S. Muldal and Dr. L. G. Lajtha	159
SHADE TOLERANCE IN FLOWERING PLANTS. By Dr. J. P. Grime	161
THE CONCEPT OF THE 'CENTRON' By Dr. D. W. Menzies	163
IMMUNOLOGY OF THE MOUSE MAMMARY TUMOUR VIRUS (MTV): A QUALITATIVE <i>in vitro</i> ASSAY FOR MTV. By Phyllis B. Blair	165
INCREASED SENSITIVITY OF <i>in vitro</i> MURINE LEUKAEMIA CELLS TO FRACTIONATED X-RAYS AND FAST NEUTRONS. By Dr. William L. Caldwell, Prof. Leonard F. Lamerton and Dr. David K. Bewley	168
EFFECT OF GASTRIN ON ISOLATED SMOOTH MUSCLE PREPARATIONS. By Dr. A. Bennett	170

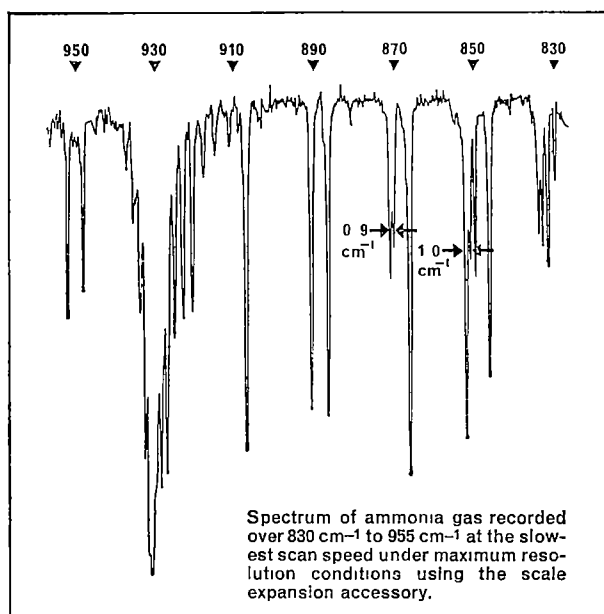
(Continued overleaf)

NEW grating SP200G infrared spectrophotometer



high resolution

Unicam now offers all the advantages of a grating filter monochromator combined with the proved features of the SP200 prism spectrophotometer. The first order of the NPL grating is isolated by four interference filters, brought into play by an automatic mechanism exclusive to Unicam. The resolution is as high as 3 cm^{-1} at 3000 cm^{-1} and stray light is less than 1% throughout most of the range and never exceeds 2%. The wavenumber accuracy is better than 2 cm^{-1} at 1000 cm^{-1} and 4 cm^{-1} at 3000 cm^{-1} . To this performance are added the advantages of the flat bed recorder, the accessible sample compartment and the convenient operation of the SP200. The new SP200G is constructed and finished to the high degree of precision for which Unicam instruments are famous. Descriptive literature is available on request.



Precision Spectrophotometers

UNICAM

LETTERS TO THE EDITOR

	Page		Page
PHYSICS		PHYSIOLOGY	
Structural Basis of Neutron and Proton Magic Numbers in Atomic Nuclei.—Prof. Linus Pauling, For.Mem. R.S.	174	Monocular versus Binocular Visual Acuity.—Dr. F. W. Campbell and Dr. L. G. Green	191
Size Analysis in the Sub-sieve Range by Electronic Counter.—Prof. C. C. Harris and Dr. A. Jowett	175	A Central Action of Hemicholinium.—Dr. D. M. J. Quastel and Prof. D. R. Curtis	192
Ionization Times in Shock-heated Potassium Vapour.—R. M. Hill and B. Capp	176	HAEMATOLOGY	
METALLURGY		Kinetic Differences between Human Red Cell and Leucocyte Pyruvate Kinase.—José O. Campos, Robert D. Koler and Robert H. Bigley	194
Interconnexion of Silicon in Modified Aluminium-Silicon Eutectic.—J. A. E. Bell and Prof. W. C. Winegard	177	Inhibition of Haemolysis by 'Cremophor' in Conserved Blood.—Dr. K. Reber	195
GEOLOGY		RADIOBIOLOGY	
Re-classification of the Ethiopian Cainozoic Volcanic Succession.—P. A. Mohr	177	Uptake by Plants of Radiostrontium from Contaminated Soils.—A. J. Andersen	195
Palaeomagnetism of the Isachsen Diabasic Rocks.—A. Laroche, R. F. Black and R. K. Wanless	179	Mechanism of Uptake of Trace Elements by Plant Roots.—G. Findenegg and Prof. E. Broda	196
MINERALOGY		BIOLOGY	
Interstratified Mineral of Illite and Montmorillonite.—Prof. Mitsuo Sato, Kaoru Oinuma and Kazuo Kobayashi	179	Prolactin and the Incidence of Brood Patch Formation and Incubation Behaviour of the Two Sexes in Certain Birds with Special Reference to Phalaropes.—Prof. E. O. Hohn and S. C. Cheng	197
CRYSTALLOGRAPHY		Relation of Population Density to Sodium Availability and Sodium Selection by Microtine Rodents.—Glenn D. Aumann and Prof. John T. Emlen	198
Pairs of Spiral Etch Pits in Ice Crystals.—J. Muguruma	180	Simple Technique for embedding and supporting Delicate Biological Specimens.—R. H. Harris	199
CHEMISTRY		Selective Effect of Particulate Insecticides on <i>Simulium</i> among Stream Fauna.—Prof. W. E. Kershaw, T. R. Williams, S. Frost and Prof. H. B. N. Hynes	199
Simultaneous Oxidation and Reduction of Diphenyl Picrylhydrazyl on Surfaces: Evidence for Catalytic Activity of <i>F</i> -centres.—Prof. John G. Aston, Dr. Dwarka N. Misra and Katherine M. Cresswell	181	MICROBIOLOGY	
Correlation of Sodium Atom Reaction Rates with Electron Capture Cross-sections.—K. R. Wilson and Prof. D. R. Herschbach	182	Bacterial Count and Cell Differentiation in Milk.—C. L. Duetschaeffer	200
A Preparation of Cementite.—J. Freel and Dr. A. K. Galwey	183	Formation of Protocatechuic from Quinic Acid by Fluorescent Pseudomonads.—Dr. D. J. Stewart	200
Oxidation of Some 2,4-Dinitrophenylamines with Ceric Salts.—Miss Susan Haworth and Dr. J. R. Holker	184	VIROLOGY	
BIOCHEMISTRY		Rubella Virus Complement-fixation Test.—Dr. H. Stern	200
Enzyme Regulatory Mechanisms at the Level of Lactate-oxidoreductase in Erythrocytes and Ascites Tumour Cells.—S. Rapoport, L. Ababei, C. Wagenknecht and S. R. Sarkar	185	GENETICS	
Effect of <i>Trans</i> -Solutes on the Fluxes of Chloride Ions across Artificial Membranes.—S. Kitahara, Dr. E. Heinz and C. Stahlmann	187	Phenocopies of the <i>ma-l</i> and <i>ry</i> Mutants of <i>Drosophila melanogaster</i> : Inhibition <i>in vivo</i> of Xanthine Dehydrogenase by 4-Hydroxypyrazolo(3,4-d)pyrimidine.—E. C. Keller, jun., and Dr. Edward Glassman	202
Identification of Dinitro-octylphenols in Certain Commercial Fungicides.—Dr. A. H. M. Kirby and L. D. Hunter	189	Induction of Crossing-over in <i>Drosophila</i> Males by Means of Ovarian Extract.—O. S. Reddi, G. M. Reddy and M. S. Rao	203
<i>n</i> -Alkan-1-ols in Butter after Oxidative Deterioration.—W. Stark and D. A. Forss	190	Regression of a Phenotypic Value on the Values for the Parents and Grandparents.—Dr. I. J. Good	203
Dependence of Photophosphorylation by Isolated Chloroplasts on the Oxidation-Reduction State of <i>N</i> -Methylphenazinium Methyl Sulphate (Phenazine Methosulphate).—Gunter Zweig and Dr. Mordhay Avron	190	AGRICULTURE	
		Objective Evaluation of Quality in Poultry Meat.—Dr. A. W. Khan	204
		ANTHROPOLOGY	
		Lower Palaeolithic Hominid and Pebble-industry in Hungary.—Dr. M. Kretzoi and Dr. L. Vértés	205
		Cranial Capacity of the Hominine from Olduvai Bed I.—Prof. Ralph L. Holloway, jun.; Prof. P. V. Tobias	205

AGAROSE

A neutral galactose polymer obtained from Agar by a unique method using reagents having no toxicity.

- * Negligible electroendosmotic properties.
- * High gel strength.
- * Non-toxic.
- * Minimum adsorption properties.

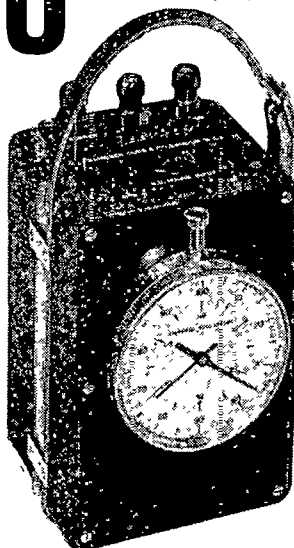
This product and many ENZYMES and RELATED BIOCHEMICALS of the highest possible integrity are produced entirely in our own laboratories.

Send for descriptive brochure

SERAVAC LABORATORIES (PTY.) LTD.

Moneyrow Green, Holport,
Maidenhead, Berks., England.

SYNCHRONOUS CHRONOSCOPE



for scientific and industrial use supplied to industrial organizations and research establishments. Available for panel mounting. Enquiries are welcomed.

Camerer Cuss supply all types of timing units, and sectional catalogues and leaflets are available for all types of timers. Special equipment can also be manufactured to suit specific requirements.

A superbly accurate instrument, this fully portable chronoscope is suitable for AC mains operation 110/220v. It measures:

- Duration of an Impulse
- Interval between two impulses
- Duration of an Interruption
- Duration between two interruptions



CAMERER CUSS

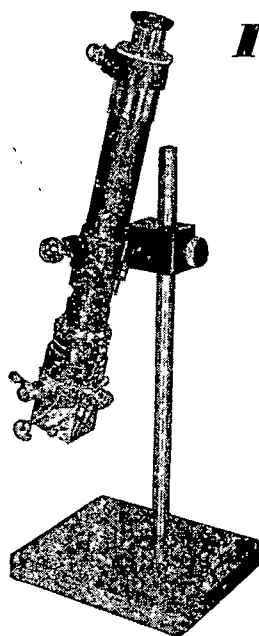
TIMING AND INSTRUMENT DIVISION

54/56 NEW OXFORD STREET,
LONDON W.C.1 Tel: MUSEUM 4861

also at 5 New Cavendish Street W.1



PRECISION INSTRUMENTS



Immersion Refractometer

The Immersion or 'dipping' refractometer, as it is sometimes known, is a versatile instrument yielding a high accuracy—0.00004 on the majority of liquids in common use in science and industry. The principal function is of a comparative nature, measuring small differences in index between similar samples. The normal range of 1.325 to 1.597 is made up using 7 interchangeable prisms, each covering a span of approximately 0.04. The first range prism (1.325 to 1.367) is also available in a water jacketed prism box as illustrated. A recent addition is a flow cell holding about 4 ml. of sample, intended for repetitive measurements where the fresh sample flushes out and replaces the previous sample. The unit is fully water jacketed for close temperature control and may also be used for continuous flow measurements.

SOLE DISTRIBUTORS IN U.K. OF SCHOTT INTERFERENCE FILTERS

Bellingham & Stanley Limited

71 HORNSEY RISE, LONDON, N.19. PHONE: ARCHWAY 2270

RESEARCH AND DEVELOPMENT IN BRITISH INDUSTRY

PROF. J. W. MITCHELL'S stimulating Jubilee Memorial Lecture to the Society of Chemical Industry (*Nature*, 207, 113; 1965) on the organization of basic research for the British chemical industry was followed in May by Sir Ronald Holroyd's Brotherton Memorial Lecture to the same Society at Leeds, in which he discussed some aspects of research and development in that industry. More recently, at the annual general meeting of this Society in Cardiff in July, Mr. K. Piercy considered the broader picture of the chemical industry in a changing world, touching on such topics as the problems presented by technological change, research policy, logistics, long-range planning and the demands made on management and staff. Similar ground was covered by Dr. J. Taylor in a lively and provocative address entitled "Restrictive Practices", when he was awarded the Society's Medal at that meeting. Taken with Mr. J. C. Duckworth's eleventh Graham Clark Lecture to the Institution of Mechanical Engineers, "Incentives to Innovation and Invention", these lectures provide a highly stimulating and suggestive commentary on British industry in general and on the chemical industry in particular, which is worth examining in the context of the organization and activities of the Ministry of Technology and of Britain's economic situation generally.

Early in his address, Sir Ronald Holroyd, for example, points out that research in industry aimed primarily at commercial objectives has contributed greatly to the general body of scientific knowledge, and that such fields as colloid science, crystal-habit control and the chemistry of photography have developed very largely through industrial contributions. Such research work inside industry has called for the same degree of ingenuity and skill as any other form of research, and has involved more fundamental work than is commonly imagined. Indeed, the possibilities of conducting fundamental research in industry are considerably greater than is often appreciated, especially perhaps by teachers and others who may influence the choice of a career by a young graduate.

Sir Ronald is not, however, concerned with this point more than to emphasize the advantage which the greater resources of the industrial firm may confer in an integrated attack and the dependence of the future growth, diversification and productivity of chemical industry on the volume and efficiency of its own internal research effort. He is not unmindful, however, of the benefit derived from academic research or that in some fields Government institutions could provide valuable, if strictly limited, assistance. He is concerned, though, more especially with the way in which new discoveries and inventions in industry emerge: here his address runs parallel with that of Mr. Duckworth's.

Sir Ronald points out that the really successful company is one which, in addition to seeking new applications for existing knowledge, has the imagination to pick out potentially profitable objectives not obtainable with existing knowledge and the ability to work out an approach to these objectives through its own facilities for research and development. Success in identifying such objectives depends very largely on the early recognition of existing or future needs, whether in the company itself, in the industries it serves or in the community at large, and

Sir Ronald adds that this responsibility must be spread over all branches of the company. The ordinary research scientist is not the most likely person to recognize or to assess the importance of such needs: his skill and inspiration are the dominant factors in seeking a scientific breakthrough towards a selected objective.

Accordingly, Sir Ronald reminds us that research, including technical development, cannot be treated in an industrial setting as an isolated function, but must be closely integrated with the other activities of the firm. Scientists in industry must recognize that they are a part of the overall management team and look to the practical and economic achievements of the company for their main personal satisfaction in their work. This he regards as the essential difference between research within and without industry, and it obviously has a close bearing on the ability of an industry to attract competent scientists. However, in his lecture, Sir Ronald stresses the responsibility it places on management for making effective use of its scientists, including the deliberate diversification of their expert experience. Neglect of this could be a potent factor in the shortcomings of the chemical industry which have led it to lose ground to foreign competitors to an extent for which the Economic Development Council could find no obvious explanation.

The Council did indeed suggest that uncertainty about the future growth of the economy was leading to hesitant investment, and that the high cost of British coal and electricity, as well as the comparatively low British tariff on the newer plastics, might be hidden obstacles. None of these, however, bears closely on the point discussed by Sir Ronald and by Mr. Duckworth, and it is worth noting that Mr. Duckworth, so far as the individual is concerned, discounts the primary importance of material incentives. Both here and in his comment that significant technological advances now tend to be made by teams rather than by individuals, his views support Sir Ronald's, at least by implication. He challenges the academic attitude towards patenting, as perhaps might be expected from the chairman of the National Research Development Corporation, and stoutly champions the policy of energetic filing and exploitation of patents.

Mr. Duckworth's views on industrial and national incentives are likewise largely derived from his experience with that Corporation. However, he points out that inventions and innovation are not necessarily meritorious in themselves but only so far as they contribute to higher efficiency and enable us to compete more effectively in world markets. While all significant technological information available in the United States is also available in Britain, the output per worker in the United States is in general 2.5-3 times that in British industry. Britain's immediate problems, he believes, are concerned mostly with management and with the attitude of workers to their jobs. He suggests that a thorough study of the reasons underlying this difference would be a most potentially rewarding research programme.

Mr. Duckworth again agrees with Sir Ronald when he remarks that, in the long run, achievement of a steady increase in productivity can be achieved only by the introduction of new processes and products. It is in this context that he suggests that a main purpose of taxation

should be to ensure, so far as possible, that individual firms, while looking after their own interests, act in the national interest also. Nevertheless, a recent survey has shown that when deciding whether or not to install a new process only 16 per cent of firms make prior allowance for all available tax incentives in their calculations. This may be an indictment of management but, as Mr. Duckworth observes, it makes it difficult to see what the next stage should be.

Mr. Duckworth sees four areas where direct Government action is, or would be, advisable in the national interest. First are those few projects of great significance and public interest where the research and development expenditure or capital cost involved are too great to be shouldered by an individual firm or groups of firms—as in the development of civil aircraft or nuclear power. It might be added that we have yet to elaborate really satisfactory procedures to provide the basis for even Government decisions in such major technological developments. Mr. Duckworth suggests that scientifically trained executives would make a great contribution in Government by ensuring that scientific method was applied in Government procedures so that the maximum possible information was available before decisions were taken.

Secondly, there is the analogous situation of a medium-sized project for which, as with the hovercraft, there is no particular industry in existence with the potential or expertise to develop it fully. The third area which he instances is concerned with the fact that a relatively long-term view is required as compared with the relatively short-term view which directors are usually compelled to take in the interests of their shareholders. The fourth is that where the estimated benefit to the innovating industry may provide insufficient incentive to proceed, although the benefit to the national economy as a whole may be considerable. Here a start has already been made with the use of cost/benefit analysis, and Mr. Duckworth concludes his lecture by stressing the vital part which the engineer or scientist who turns to management could play. It is to the application of scientific method and results to management and to new products and processes that we must look for increased productivity, though the creation of a society which welcomes innovation also depends largely on a re-orientation of our sense of values.

Much of what Mr. Duckworth says in this lecture is reflected in the closing part of Sir Ronald Holroyd's lecture, particularly in reference to management and the methods of assessments available or in use. Both are also conscious of the critical importance of recruitment, of ensuring that a due proportion of talent is attracted to engineering and technology and that the idea that industrial scientific activities are intellectually second-rate should be firmly dispelled. Mr. Piercy does not refer to this recruitment problem, but in referring to management and staff he stresses the exacting demands made on them in the chemical industry, with the result that their jobs are correspondingly more intellectually satisfying and interesting than in the past. He believes that the industry could do more to ensure that its able young men receive first-rate training in management at appropriate stages in their career and that, after giving them the opportunity to broaden their experience, the best use is made of their abilities.

Mr. Piercy's confidence in the ability and professional competence of such management in chemical industry is the more significant in that his address treats more

specifically the policy and export performance of the industry which the Economic Development Council for the industry has since criticized. In referring to long range planning, he does not mention the uncertainty stressed by the Council, but he admits that its export performance does not compare well with similar bodies in other countries. He is also concerned at the rise in chemical imports, for which he suggests no specific reason. Commenting on the trend towards larger plants he believes that the risk of excess capacity has been over exaggerated; while this trend makes the choice of a site more difficult, he agrees with Sir Paul Chambers as to the importance of really large markets.

On all this Dr. Taylor's lively address makes a most stimulating gloss. He puts the question of restrictive practices very fairly and pertinently in its proper context and in a way that indicates where the particular responsibility lies. Restrictive practices are, he points out among the restrictions and inhibitions on our adaptability and rate of progress imposed by the illogical forces derived from man's past which dominate our behaviour. To recognize this is a first step toward eliminating the restrictive practices which, Dr. Taylor agrees, must be cut out if we really mean to improve our material prosperity. They are, in fact, widely present throughout our society and in so far as they do, as some argue, arise out of social and economic cleavages. Dr. Taylor considers that our troubles are due in greater part to a lack of leadership.

Dr. Taylor makes one point that is often overlooked in discussing our economic situation to-day: as a people, we made two all-out efforts in war in little more than thirty years, and whereas the defeated nations had help from outside in their subsequent struggle to survive, the British people have never had the opportunity to relax. It is equally important to note that Dr. Taylor does not commit himself that a materialistic objective is necessarily wise and this should be remembered when he emphasizes that British industry is more highly manned than its American counterpart and that only if the profit per person in productive industry is high can the standard of living in Britain be improved and capital resources built up. That the chemical industry is so highly manned should induce a careful study with the view of reducing manpower. To cut out restrictive practices is one way of achieving this, provided our diagnosis of those practices is accurate and complete, and takes account of those which arise from outside—more particularly from Government regulation or interference and tax systems.

Dr. Taylor faces the difficulties of this problem fairly, putting his finger on some of the real sources of friction, such as the differentiation between the salaried staff and the pay-roll, particularly in respect of redundancy. Without defending obsolete ideas and practices in the Trade Unions, he points out that their critics also sometimes fail to take account of changed conditions and that on the staff side too restrictive practices are to be found quite inconsistent with responsibilities which are the counterpart of their privileges. Moreover, if it is difficult enough for people of advanced education to adapt themselves to change and even the *tempo* of change, it is much more difficult for those who left school at the age of fourteen to make the transition from a well-established routine to fundamentally different methods of working. Adaptation and innovation in an industrial society present essentially an educational problem. A much clearer understanding of the problems of adaptation in an industrial society is needed and, as Dr. Taylor adds, a more

sitive approach to the educational problems involved overdue.

There is sense and insight in Dr. Taylor's comments on management, on the inherent weakness of controls and of organization, necessary as they are. If management must bear the major share of responsibility for stricture practices, the fundamental need is leadership at all levels, and, in this, individuality and personality must to an extent that educational authorities—and others—are liable to forget. Moreover, the type of leadership we need to-day will not concentrate on economic efficiency to the detriment of the rights and dignities of the individual. However, here Dr. Taylor recognizes that national policy also has a part to play, particularly if we are to take work to the workers rather than transfer workers to the work.

While this group of lectures and addresses is of interest in illustrating the outlook and approach of responsible leaders within the chemical industry as much as for their diagnosis of some defects on which the National Economic Development Council has commented, the importance attached to management is impressive and supports much that was said at the recent Cambridge meeting of the British Association for the Advancement of Science. The idea that the chemical industry generally is backward is scarcely enable in the light of these addresses, but their challenge to education and to Government is unmistakable. Neither in industry nor in Government is there room for complacency, and in the chemical industry as elsewhere the challenge of Britain's economic position will only be met by the response of efficient management and of workers and staff is matched by appropriate Government policy and structure for the assessments on which policy should be based.

NOBEL LECTURES AND SCIENTIFIC METHOD

Physics

Nobel Lectures including presentation speeches and laureates' Biographies 1922–1941. Pp. xii + 456. Amsterdam, London and New York: Elsevier Publishing Company, 1965.) 160s.

THIS handsome volume is the second of three volumes which present the Nobel Prize Lectures in Physics in the English language accompanied by the presentation addresses and short biographical notes. The Nobel Foundation hopes that they will serve to supplement its annual publication *Les Prix Nobel*. The publishers state that the translations of Lectures which were not given in English were prepared by the Babylon Translation Service of London: it is to be regretted that the English is often irritatingly bad, for example, "Allow me to refresh your memories what is the Laue phenomenon" (p. 254). It is curious, also, to have the short sentence which justifies the bestowal of the Prize listed, in each case, as "Motivation" in the contents. The Lectures are preceded by presentation addresses which are a valuable aid to the reader, although these sometimes exceed in length the lectures by the laureates.

The period 1922–41 is a fascinating one, since it contains the development of revolutionary ideas associated with the names of Bohr, de Broglie, Heisenberg, Schrödinger and Dirac, as well as vital experiments concerned with the ultimate structure of matter made by laureates from Millikan (1923) to Fermi (1938) and Lawrence (1939). The Lectures are therefore particularly interesting from the point of view of scientific method and the philosophy of science, since they exemplify the modern method of

starting with mathematical equations and then attempting to interpret their solutions later. This, of course, does not supply causal explanations but permits predictions which, it is nowadays claimed, are all that can be hoped for.

Prince Louis Victor de Broglie (laureate 1929) was the pioneer of the hypothesis of matter-waves. He says, "experiment which is the final judge of theories has shown that the phenomenon of electron diffraction by crystals actually exists and that it obeys exactly and quantitatively the laws of wave mechanics" (p. 255), and concludes "this wave is no myth". He nowhere actually states what the waves are waves of, and he accepts the prevailing word-magic which, by using the Latin word *quanta*, avoids saying what the quantities are quantities of. He appears to avoid actually calling these quantities 'energy'—rightly, because this is a purely metrical term which, therefore, could never be absorbed by any atom and cannot constitute a causal explanation. In recent years de Broglie has resumed the attempt he made in 1927 to give a causal interpretation to wave-mechanics; he has now, he says, "The growing impression that the majority of physicists who have yielded to these exaggerated abstract tendencies have too easily desisted from forming an intelligible representation of the phenomena of quanta physics"¹.

In 1932 the laureate was Werner Heisenberg—"for the creation of quantum mechanics . . .". He put forward the well-known thesis that we must forgo a visual description of the atom and maintained that the laws of Nature are basically statistical.

Erwin Schrödinger (1933) summed up his epistemological views somewhat obscurely as follows: "I would define the present state of our knowledge as follows. The ray or particle path corresponds to a *longitudinal* relationship of the propagation process (i.e., in the *direction* of propagation), the wave surface on the other hand to a *transversal* relationship i.e., *normal* to it). Both relationships are without doubt real: one is proved by photographed particle paths, the other by interference experiments. To combine both in a uniform system has proved impossible so far. Only in extreme cases does either the transversal, shell-shaped or the radial, longitudinal relationship predominate to such an extent that we *think* we can make do with the wave theory alone or with the particle theory alone".

Paul A. M. Dirac, who shared the 1933 Prize with Schrödinger, explains how he derived a relativistic wave-equation involving the invention of new mathematical variables, and states that these "give rise to the spin of the electron" (p. 322), but he also admits frankly that these variables also "give rise" to some rather unexpected phenomena. (Mathematical terms and operators cannot, of course, "give rise" to anything physical. They can only give rise to other mathematical expressions. This illustrates the confusing misuse of language in which this subject is involved.) One unexpected phenomenon is that "an electron . . . must actually have a very high-frequency oscillatory motion of small amplitude superimposed on the regular motion which appears to us". As a result, "the velocity of the electron at any time equals the velocity of light" (p. 322): he does not comment on the fact that this contradicts the theory of relativity from which the conclusion has been derived, "but one must believe in this consequence of the theory, since consequences of the theory which are inseparably bound up with this one, such as the law of scattering of light by an electron, are confirmed by experiment". Scientists have not always agreed with this attitude to a theory: it raises the difficult problem of the status of an equation some of the interpretations of which are verified, and some are not, or are meaningless, or even contradictory. With equal frankness, Dirac discusses another difficulty—that the equations which led to the prediction of the positron also predicted negative energy-states, which are, of course, physically meaningless; but once again "we must

find some meaning for these states". In this case he is driven to the *ad hoc* hypothesis that they are nearly all "occupied" by electrons and to the further *ad hoc* hypothesis that if they were fully occupied they would be completely unobservable. Unoccupied negative energy-states are first called "holes" ("for brevity") and then a hole is said to be "just like an ordinary particle", and finally, the hole is called a positron. But despite the *ad hoc* hypotheses and the word-magic, energy-states are not particles, whether occupied or not, and are not observed but calculated.

In 1937, the laureates were Clinton J. Davisson and G. P. Thomson. In Thomson we see the spirit of British empiricism and a cautious and critical approach: "I am predisposed by nature in favour of the most mechanical explanation possible . . . it might be best, and it is certainly safer, to keep strictly to the facts and regard the wave-equation as merely a way of predicting the results of experiments".

After several unsuccessful attempts to give some physical meaning to matter-waves, mathematical physicists have realized that Eddington was right in calling wave mechanics "a dodge—and a very good dodge too"². But dodges are not physical theories, and diffraction does not necessarily imply waves³. We still await a Nobel Lecture which will give us a physical theory of the atom.

G. BURNISTON BROWN

¹ *The Current Interpretation of Wave Mechanics—A Critical Study*, preface (Elsevier, 1964).

² *The Nature of the Physical World*, 219 (Cambridge University Press, 1928).

³ *Contemporary Physics*, 5, 1, 15 (1963).

ELECTROCHEMISTRY

The Encyclopedia of Electrochemistry

Edited by Clifford A. Hampel. Pp. xviii + 1206. (New York: Reinhold Publishing Corporation; London: Chapman and Hall, Ltd., 1964.) 35 dollars.

Proceedings of the First Australian Conference on Electrochemistry

Held in Sydney, 13th–15th February, and Hobart, 18th–20th February, 1963. Edited by J. A. Friend and F. Gutmann, assisted by J. W. Hayes. Pp. xvi + 954. (London and New York: Pergamon Press, 1965.) 200s. net.

MANY of us will have our own, we suspect, limited ideas as to the scope of electrochemistry, and an 'encyclopaedia' in this field might accordingly appear to be a publishers' 'gimmick' rather than a worth-while proposition to the potential user. An evening's perusal of the present volume, however, will convince even the most complacent reader that here is a book which not only fully justifies the title "*Encyclopedia*" but is also an authoritative up-to-date reference book of high scientific standard. The individual items, numbering 400–500 and written by nearly 300 special contributors, are substantial articles; they average about 2,000 words or more, are well supported by block diagrams, photographs and data tables and in addition in most cases conclude with relevant and important literature references. The items covered are theoretical and practical, laboratory and industrial, as indicated briefly by the following range quoted in the introduction: batteries, corrosion, insulation, electrodeposition, luminescence, electro-organic, electrothermics, industrial processes, theoretical electrochemistry and electrochemistry in biology and medicine. But such a classification does not convey anything of the wide range of substantial articles in each of these fields, nor prepare the reader for those no less important articles that cannot be so classified. Anyone whose work or interests impinge on matters in any way connected with electrochemistry in its widest sense is sure to find articles of direct interest in this book. The editor is by no means exaggerating when he states that this book will be especially useful to physical, inorganic and

analytical chemists, to chemical corrosion, electronic or electroplating engineers, to metallurgists concerned with non-ferrous metals and with basic studies of high temperature and surface phenomena, to physicists specializing in electronics and to workers in biophysics and neurology.

To proceed from the general to the particular and discuss specific articles may not be helpful because of the wide range of readers catered for. The university student, for example, will be attracted by such articles as those by Dr. Parsons on the double-layer and on electrode kinetics, by Dr. Eisenberg on concentration polarization and by Prof. Read on hydrogen embrittlement. Nor will he be confused by electrode sign conventions; the European and American conventions are both clearly discussed and unambiguous, used throughout with the American convention reserved for oxidation potentials. Thus those to whom the use of the American sign convention in text-books of electrochemistry is anathema may safely refer their student charges to this book. But to mention in this way items of particular interest to one class of reader is to single out just a few to the detriment of the hundreds of other items equally demanding recognition. One high-light which must be mentioned, however, is the inclusion of a most comprehensive "Table of Electrode Potentials and Temperature Coefficients".

This, then, is a book which, though it may have arrived unasked for and unexpectedly, nevertheless has arrived and is here to stay. The contributors are all acknowledged specialists in their fields and each has now confirmed and enhanced his own reputation. Not least in this respect the editor, C. A. Hampel, who may feel justly proud of his success in harnessing so much electrochemical knowledge in one substantial volume. Typographical errors are insignificant; one might, however, wonder what is meant by "incomprehensible" liquid (p. 159), and one could be misled by the use of "free enthalpy" (p. 429) when referring to Gibbs free energy.

THE justification for the First Australian Conference on Electrochemistry embracing virtually all the modern facets of electrochemistry, pure and applied, lies in two facts. The first is the present considerable interest in electrochemistry, reflected in a four-fold post-war increase in the percentage of physical chemistry research papers appearing in the field of electrochemistry; the second is the significant proportion of this work which is being carried out in Australia. The volume under review represents a substantial contribution to present-day electrochemical literature and offers unequivocal evidence of the success of this Conference. It is certainly a publication which should be read in part or in its entirety by all those interested in electrochemistry; our only reservation springs from the fact that *Proceedings* of conferences, even international ones such as this, often do not find their way into published abstracts. One wonders if the many original contributions would eventually reach wider public had they been published in the usual journal and the present *Proceedings* limited to extended abstracts. This, however, is a fault of the system, not of the Conference.

The *Proceedings* contain, with the exception of two papers, a full and detailed record of all the contributions amounting to some 60 papers. About one-half of these were contributed by visiting scientists mainly from the United States and Canada, although several were from European, Japanese, Indian and Egyptian workers. Contributions by Russian workers were unfortunately absent.

The Conference was held partly in Sydney and partly in Hobart. Each of the twelve sections of the Conference was introduced by a different chairman; this provided the key to the success of the Conference and contributed in no small measure to the justification for this book. The choice of chairmen, all distinguished in their field, was excellent and each in his address provided a lucid and detailed review

f the main theme and so prepared the way for the succeeding contributed papers. The range of topics covered is well illustrated by the following list of the twelve sections: solid-state chemistry, thermodynamics of electrolytes, corrosion, theory of double-layers, electro-analytical methods, applications (electroplating, anodizing), non-aqueous electrolytes, molten salts, fuel cells, electrode processes, electrochemical processes, electrowinning and electrorefining. The major sections are those concerned with fundamental research, and these give substance to Prof. J. O'M. Bockris's comments in the foreword that electrochemical research is now heavily concerned with electrode kinetics ('electrodics') and with kinetics and theories of electrode processes to the virtual exclusion of thermodynamic equilibrium studies. The sections on applied electrochemistry will prove no less interesting to those concerned with applications, but we consider that these sections might with advantage have been brought together, so permitting a greater continuity in the theoretical sections.

It is invidious perhaps to single out individual contributions. The review papers by chairmen Ross Macdonald (and C. A. Barlow) and by J. O'M. Bockris on the theory of the double-layer and electrode processes respectively are of particular interest because of the difficulties attending work in these fields. Perhaps not unexpectedly the number of contributions to these sections were not as numerous as in some of the other sections. In the section on electrode processes, a paper by Horuti, Matsuda, Enyo and Kita on the mechanism of the hydrogen evolution reaction is of considerable interest, as also is that by Christov on the application of quantum mechanics to electrode kinetics. In the section on electrochemical processes mention should be made of the chairman's address and a stimulating paper by Bockris, Devanathan and Müller on the structure of charged interfaces. But this is to mention a few among the many excellent papers the degree of importance of which will vary according to the particular interests of the reader.

The production of the book is excellent, and Pergamon Press have fully maintained the high standard set by F. Gutmann and M. Bloom, who organized the Conference and, with J. A. Friend, edited the *Proceedings*. The price at £10 is not excessive for such a book.

R. F. PHILLIPS
N. A. HAMPSON

NUCLEAR AND RADIOCHEMISTRY

Nuclear and Radiochemistry

By Dr. Gerhart Friedlander, Joseph W. Kennedy and Dr. Julian Malcolm Miller. Second edition. Pp. xi + 585. (New York and London: John Wiley and Sons, Inc., 1964.) 68s.

"FRIEDLANDER, Kennedy and Miller" is still recognizably the same excellent text as "Friedlander and Kennedy", though the format is new, the order of the chapters has been changed, and there are substantial additions to the subject-matter, as well as some deletions. On balance, the new edition is longer by roughly one-third compared with its predecessor. Prof. J. M. Miller of Columbia University has replaced the late Prof. J. W. Kennedy as author, though Kennedy's name has deservedly been retained on the title-page. Not unnaturally, the book still has an American slant, but this in no way invalidates it for other countries.

In their preface the authors state that "the book is still intended as a text for an advanced undergraduate or first-year graduate course; but a significant aspect of its modernization is found in the greater depth of treatment accorded to many topics, particularly theoretical ones". The new material includes greatly expanded treatments of nuclear reactions and radioactive decay processes, accounts of new types of accelerators, counters and other instru-

ments, and two completely new chapters, the first on models of the nucleus, and the second on the Mössbauer effect and other nuclear processes that can be made to yield chemical information.

It is characteristic of the book that, whatever topic one turns to, one finds the essential points well and clearly stated. It has been my experience more than once, after wrestling with a tricky point, to find the conclusion that I have extracted with difficulty from other sources set down in simple terms in *Nuclear and Radiochemistry*.

The title and contents of the book prompt the questions: What is nuclear chemistry? and What is radiochemistry? The claim that the book is "written by chemists for chemistry students" is a valid one, yet of the fifteen chapters, only three deal with specifically chemical topics, and only two others contain sections of any length on chemical subjects. Moreover, the chapter on radioactive tracers has actually been shortened in the new edition.

I first remember the term 'nuclear chemistry' in the annual reports of the Chemical Society for 1935, when it was used to refer to the science of nuclear transmutations, on the analogy between chemical reactions, in which atoms are conserved, and nuclear reactions, in which nucleons are conserved. It is now often used to mean something like "nuclear studies carried out by chemists". Some of the properties investigated in this context undoubtedly belong to the realm of physics rather than chemistry, but chemists do a large share of the work because success depends on preparing pure specimens and on carrying out efficient chemical separations at the end of the experiment.

However, the term is also used in a much wider sense. Coryell, in his foreword to the English edition of Haissinsky's *Nuclear Chemistry and its Applications*, speaks of it including "nuclear reactions, radioelements, radiochemistry, isotope chemistry, radiation chemistry, and tracer applications", and all those fields and more are indeed covered in Haissinsky's book, even though Haissinsky's own definition of nuclear chemistry is merely "the study of the transmutations and transformations of nuclei".

'Radiochemistry' is likewise used in both broad and restricted senses. Originally it referred to the chemical effects of ionizing radiations, but this is now a matter of history only. Later it was applied to the chemistry associated with the natural radioelements, though excluding the ordinary chemistry of thorium and uranium, in which radioactivity played no part. When artificial radioactivity was discovered, the term was of course extended to the new radioactive species. A further extension was to the radiotracer field, in which both natural and artificial radioelements find numerous applications. Many authors still stop at this point, and leave the lively subjects of hot atom chemistry and nuclear chemistry (in the limited sense) largely outside the scope of 'radiochemistry'. Others again treat the subject as if it were primarily a collection of manipulatory and measuring techniques. *Radiochimica Acta*, on the other hand, adopts a more eclectic view—one which I also favour. There seems indeed no good reason why 'radiochemistry' should not include all aspects of the interaction of chemistry with radioactivity, with the sole exception of most of the radiation chemistry aspects, which involve by and large a distinct set of concepts and techniques.

This discussion is relevant to the book under review, because it needs to be appreciated that *Nuclear and Radiochemistry* is primarily a text-book of nuclear chemistry in the restricted sense, and only secondarily a general text-book of radiochemistry. The nucleus itself, nuclear reactions and radioactivity are discussed very fully, since they form the principal subject of investigation in nuclear chemistry, and so are the techniques needed. Other radiochemical topics are treated much more briefly, and very largely from the point of view of the nuclear chemist. In saying this, no criticism is intended, but rather a definition of the scope of this important work. H. A. C. MCKAY

FOURIER THEORY

A Treatise on Trigonometric Series

By N. K. Bary. Vol. 1: Pp. xxiii + 553. 84s. net. Vol. 2: Pp. xix + 508. 105s. net. (London and New York: Pergamon Press, 1964.)

PROF. Nina Bary, distinguished for her contributions to the theory of functions of a real variable, died in 1961, the year of publication of her book on trigonometric series of which this is a translation. Zygmund's much-extended two-volume revised edition of his book on the same topic appeared in 1959, when Prof. Bary's was already in proof, so that we now have two independent and up-to-date treatises, each with much the same aim: to lay firm foundations of the theory and to carry the work, in suitably selected regions, to the frontiers of present knowledge. Both books are intended for the pure mathematician who can claim some acquaintance with set theory and the Lebesgue integral; there is nothing for the applied mathematician or physicist. The total amount of material is about the same in the two books; but Zygmund deals with several topics which Bary does not touch, while she has only two chapters not covered in Zygmund. The difference here is due to the slower pace of the Russian book; Zygmund demands throughout a fairly high level of mathematical sophistication, whereas Bary takes things at a more leisurely pace, particularly in her first volume. The phrase "it is easily seen" has been used, often in a Pickwickian sense, by many authors since Laplace set a bad example; in her preface, Bary protests against its frequent misuse, and has endeavoured to make her text, at least in the first volume, easily readable by the good undergraduate. To some of the longer and more delicate proofs, she appends informal comment to sketch the main lines and to emphasize the critical points of the argument.

After some preparatory analytical matters, a long first chapter of some 170 pages gives a good general survey of the domain, including the Dini and Jordan tests for convergence, the Gibbs phenomenon, Cesàro and Abel summability, Poisson's integral. Chapter 2 deals with the order of magnitude of Fourier coefficients of certain types of function, and Parseval's theorem. The next three chapters take the natural line of examining the convergence of a Fourier series at a point, the uniform convergence of a Fourier series of continuous functions, and convergence in a point-set. Most of this is to be found in the first half of Zygmund's first volume, but the last chapter deals with work by Men'shov on a problem which Zygmund does not touch: roughly, given a function $f(x)$, can it be altered on a set of arbitrarily small measure to give an 'adjusted' function the Fourier series of which will converge everywhere or almost everywhere, or converge uniformly?

Vol. 2 begins with summability processes, the simpler part of the theory of the conjugate series, absolute convergence, sine and cosine series with coefficients which tend to zero, and the remarkable differences which arise when the series is lacunary, having increasingly large gaps. Then there are chapters on the convergence, absolute convergence, and uniqueness of the general trigonometric series: in the main, these topics centre around what can be said about a point set on which the series has a certain specified behaviour. The final chapter deals with another problem of Men'shov's, again not discussed by Zygmund: this concerns the representation of a function by a summable trigonometric series, arising from work by Lusin and Privalov which showed that if a function is finite almost everywhere (in the basic 2π interval) then a trigonometric series can be found which is summable to the function by the Riemann, Poisson or Cesàro methods.

The techniques used are on the whole not so advanced as those which Zygmund draws on, so that a good deal of his second volume lies outside the range of Bary's book. The student thinking about research in this field might well begin and go a long way with Bary, turning to Zygmund

for the sharper weapons likely to produce a deeper penetration.

There are some infelicities of expression, possibly due to the need to keep the translation close to the original. In some places, it is said that an assertion "cannot" be true when what is meant is that it "need not" be true: this distinction is important, and blurring it may confuse the novice. But in general Mrs. Mullins has coped well with a gigantic task.

T. A. A. BROADBENT

ERGONOMICS

Ergonomics

Man in his Working Environment. By K. F. H. Murrell. Pp. xiv + 396. (London: Chapman and Hall, Ltd 1965.) 63s.

ERGONOMICS: Man in his Working Environment provides an important link between scientific research and industry. It is a text-book both for investigators in the basic sciences concerned with the practical application of their research, and for industrialists and designers who appreciate the importance of the human factor in maximizing industrial efficiency.

Ergonomics has been defined as "the scientific study of the relationship between man and his working environment", but Mr. Murrell has extended the term 'environment'. The key *motif* of ergonomics is that of fitting the work situations as a whole to the man on the basis of scientific knowledge, including knowledge of human physiological and psychological capacities. This is called 'human engineering' in the United States. It is an interdisciplinary applied science relating principles derived from the basic sciences to work design, the control of physical environment factors and the mode of work organization.

With more and more semi-automatic and automatic processes being introduced into industry, the contention is that when equipment is intended for human use, it should, so far as possible, be designed to give effect in a man-machine unit, with the man complementary to the machine and the machine complementary to the abilities of the man. While physical stress has tended to be reduced in modern industry, the shift of emphasis from man as 'doers' to man as 'controllers' has tended to increase psychological stress. Stress can lead to inefficiency and reduced output, through failure to match the requirements of the task with the capacity of the operator. Again, the contribution of ergonomics to work design is invaluable when technical change makes it necessary for operators to move on to jobs which may be variants of their old jobs or entirely new.

As a dual text-book for scientists and industrialists the book is well designed. Part 1 outlines salient data from physiology, anatomy, psychology, etc., while Part 2 deals with practical ergonomics and gives examples of the application of ergonomic principles in work design and manpower utilization. The book is easy reading; technical and scientific jargon having been largely avoided. Mr. Murrell points out that while there is a large body of data in scientific publications which is relevant to industry, there is need for some translation to put this to work. He contributes materially to such a translation, and this publication contains an extensive reference section to his material.

The chapter on the design of seating provides an example of the universality of ergonomic contribution to human well-being. The importance of good seating design is becoming more widely recognized. While considerable research has been done in seating design, surprisingly little of that research has been put into practical application. This chapter sets out the requirements of good seating as ascertained from research, summarizes the relevant anthropometric data and gives practical advice for seating-manufacturers.

Significantly, Mr. Murrell's final chapter deals with the necessity of taking account of ageing in the interests of assuring the maximum efficiency of available manpower. The importance of this is underlined by the fact that more than 50 per cent of the male workpower in British manufacturing industry is already over the age of forty. In certain circumstances efficiency can be affected by the concomitants of ageing from as early as thirty-five. Yet industry has shown little interest in research on ageing in relation to occupation. In our present demographic circumstances there is need to retain or engage older workers. But industrial gerontological research emphasizes the basic necessity of appropriate work-changes, even from the middle-age, if older manpower is to be used to the best effect. The author relates age-indicated work-change to that resulting from technical developments. In both, the use of ergonomic principles would result in a better matching of job demands and physiological and psychological capacities. This may be either through a shift to an alternative job, that is change 'of' the job, or some modification of the work demands, that is change 'in' the job. The latter alternative gives greater opportunity for the continued use of the previously acquired skills and experience.

It is evident that ergonomics can make an important contribution to the more efficient and more considerate use of manpower. This book will also be of interest to non-specialists who wish to be conversant with the relationship between science and its practical application in the modern world

C. E. FLEMING

REGULATION OF GROWTH

Adaptive Growth

By Prof. Richard J. Goss. Pp. 360. (London: Logos Press, Ltd., in association with Elek Books, Ltd. Distributed by Academic Press, London and New York, 1964.) 70s.

THIS book will be welcomed by all biologists and pathologists interested in vertebrate development, for it fills a gap in the bibliography of this subject. Another attraction is that it is written by a single author, who has something definite to say and who says it well.

Prof. Goss has set out to explain diverse kinds of post-embryonic growth in terms of a unifying hypothesis, namely that the regulation of growth is governed by the functional demands of the organism. He is fully aware of the teleological overtones of the word 'demand' and carefully avoids them in his attempt to elucidate the mechanisms which control the homeostasis of growth. The characteristics of different types of developmental phenomena, such as the local control of wound healing, or the systemic mediation of compensatory growth, are interpreted as adaptive responses determined by the function of the organ concerned and the nature of the stimulus. Normal growth is discussed in terms of cell turnover, different modes of proliferation of cell populations are classified, and ageing—"the body's homeostatic depreciation"—in some tissues is briefly reviewed.

Nearly half the chapters of this book are devoted to an analysis of compensatory growth which is exhaustively considered on gross, histological, cellular and molecular levels. Evidence of the action of humoral activators and inhibitors is critically examined. The liver and haematopoietic system predominate in this analysis, as the majority of investigations have been concerned with these tissues, but all other organs which show compensatory growth are dealt with. The adaptive role of the pituitary-thyroid system could have been more fully described, particularly the functional significance of the relay system.

No attempt has been made to review growth in all vertebrate groups. No data on fishes or reptiles are included, and little on birds. Perhaps this is because little space has been devoted to morphogenetic regeneration

(apart from an interesting chapter on amphibian lens regeneration) although the author himself has made outstanding contributions to this subject. He does, however, briefly mention Singer's and Thornton's investigations, commenting on the significance of the role of nerves in limb morphogenesis.

The chief task of this monograph is to re-define all growth phenomena according to functional ideas. As the author points out in his preface: "It has become fashionable to disregard functional considerations in favour of mechanisms by which the regulation of tissue mass might be an end in itself. Consequently, much of to-day's research is predicated on the assumption that humoral agents are responsible for adjusting the sizes of organs to certain predetermined dimensions".

While the author does not deny the participation of humoral agents in the control of mitosis of many tissues, he postulates that they are primarily dependent on the physiological demands of the particular tissue. Thus the stimulation of erythropoiesis is mediated through the same regulatory mechanisms whether the initial functional insufficiency was due to loss of blood or reduced oxygen in the environment. Likewise the mechanisms controlling epidermal hyperplasia are the same in wound-healing and the growth of calluses. This interpretation is controversial. It is not the view, for example, of Prof. Bullough, whose ingenious experiments on mouse epidermis and whose theory of mitotic inhibition by chalones are reviewed by Goss.

Moreover, there seems to me to be a deeper contradiction between a 'chalones' theory of mitotic regulation and the functional demand theory. Prof. Swann, a proponent of the functional demand theory himself, has pointed out that mitosis is a form of differentiation in requiring specialized (but not tissue-specific) protein synthesis which must therefore be antagonistic to tissue-specific synthesis, and Glinos has suggested that functional overload of tissue-specific synthesis (in the liver) switches the synthetic machinery of the cell to elaborate mitotic proteins. The chalone theory recognizes the antagonism, but reverses the action of the products of differentiation (chalones) so that they inhibit mitoses, or, as recently stated in more contemporary terminology, the concentration of a chalone has an effector-like action "which determines whether the 'mitosis-operon' or the 'tissue-operon' is active"¹. In renewing cell systems, such as epidermis or blood cells, a single stimulus could derepress the synthesis of both mitotic proteins and tissue proteins, since proliferation and differentiation represent separated compartments of the cells' life-histories. In expanding cell systems, however, where there is no distinction between stem-cells and functional tissue (for example, liver), partial ablation will cause a substantial rise in the mitotic index of the remaining tissue just at the time when the functional demands for tissue-specific products are greatest (though it is not yet certain whether an increase in the tissue-specific protein synthesis per cell occurs simultaneously). While Prof. Goss reviews in some detail the various theories of mitotic control and the data supporting them, he does not clearly define this conflict between immediate and long-term homeostasis of functional activity, which must occur if mitosis and differentiation are competitive or mutually exclusive. If products of differentiated tissues normally inhibit mitosis and further tissue-specific synthesis, then a fall in their concentration following partial ablation will promote both activities, though it remains to be explained how different cells in an expanding system are allocated to the alternative pathways.

Since this monograph represents a virtuoso performance over a wide repertory, it is not surprising to find occasional factual inaccuracies and interpretative inconsistencies. Thus on p. 88, Prof. Goss is of the opinion that "cell migration is antagonistic to cell division", stating that "Abercrombie and Ambrose suggest that until the motile cells in a healing wound again become station-

ary as a result of contact inhibition, their mitotic propensities are temporarily suspended". In fact they suggest the opposite, which Prof. Goss himself corroborates five pages later when discussing Harding and Srinivasan's observations on small wounds in lens epidermis.

Prof. Goss includes a chapter on maternal-foetal relationships in which he reviews the poorly understood interactions between homologous organs in this "special case of parabiosis". There is also an interesting chapter on allometry in which the author discusses the functional, histological adaptations in some organs relating to the size of different species, and the adjustments in size which may occur following transplantation of organs. In a delightful account of the allometric relationships of urodeles varying in ploidy, we see that although cell size varies according to the number of chromosome sets, the size of gross and histological structures is constant, indicating that it does not matter how the chromosomes are packaged, their total number being the controlling factor. This can lead to certain developmental dilemmas, such as when the size of an 'organ' approximates to the size of the diploid cell. Triploid erythrocytes can pass through capillaries of normal (diploid) diameter (by change in shape, which gives a clue as to how their shape is controlled), but the single pair of Mauthner's cells cannot remain proportional both to ploidy and brain size.

Prof. Goss mentions Fankhauser's discovery that triploid salamanders are less intelligent than diploids, and he states that "the quality of the central nervous system cannot be improved by increasing the size of neurones at the expense of their numbers". It is disappointing that Goss does not actually pose questions about the lack of compensatory growth of the brain, though he does incidentally provide most of the answers! There are several unsolved problems concerning the different sizes of neurones. Whereas hypertrophy of particular nerve cells occurs, usually as a consequence of functional activity, neuronal hyperplasia is not known. Goss prefers to regard mitotic incapability as an adaptation serving to prevent disruption of organized cell communities rather than an unfortunate consequence of specialized differentiation. It is the function of the cell, not its "degree of specialization", that governs its ability to divide. Neither is it functional in the case of nerves and muscles to maintain 'mitotic competence' by recruitment from undifferentiated stem-cells.

Prof. Goss brings a fresh and firmly biological approach to his analysis of regulatory phenomena in growth. His controversial ideas should lead to lively discussions and penetrating research during the next few years.

R. A. WEISS

¹ Bullough, W. S., and Rytomaa, T., *Nature*, 205, 573 (1965).

MICROLEPIDOPTERA UNDER THE MICROSCOPE

Catalogue of the Type Specimens of Microlepidoptera in the British Museum (Natural History) described by Edward Meyrick

By J. F. Gates Clarke. Vol. 5. Pp. 581 (283 plates). (London: British Museum (Natural History), 1965.) 300s.

BIOLOGISTS do not often realize the immensity of the task still to be undertaken and the vast amount of published work needed before even half the species of the world can be named even by specialists. Much of the work was (and a considerable amount still is) done by expert amateur entomologists. Meyrick's position as an amateur entomologist was perhaps unique because of the extent of his work. Although well up to the standards of his time, his failure to illustrate his new species created a world problem. He first published descriptions of new species of microlepidoptera in 1886 when he was a schoolmaster in New Zealand, and he gradually came to dominate their

study after his return to England. He retired in 1914 after many years teaching classics at Marlborough College. Specimens of microlepidoptera were sent to him by collectors and museums from all parts of the world, but he retained in his collection, which is now in London, only about a third of the types. By the time of his death in 1938 he had published descriptions of about 15,000 new species (catalogued in Volume 1).

Dr. Gates Clarke became an authority on the microlepidoptera through his work for the United States Department of Agriculture and the Smithsonian Institution, and found some years ago that further taxonomic progress was impossible until Meyrick's species had been re-examined. He then worked for some years at the British Museum (Natural History) and the Trustees gave him permission to dissect Meyrick's types. The abdomens of the 5,000 moths were carefully removed, softened, dissected and mounted on microscope slides. This has for the most part been carried out with commendable skill, but it would be interesting to know whether the large numbers of Cosmopterygidae male genitalia were mounted sideways from choice, or whether their symmetrical arrangements presented peculiar difficulties.

Volumes 1 and 2 of this work were published as long ago as 1955 and it is very much hoped that the final one or two volumes will soon be completed. This, the fifth volume, dealing with moths of the families Timyridae, Hyponomeutidae, Ethmidae, Metachandidae, Cosmopterygidae, Walshiidae, Blastodacnidae and Scythridae, contains 283 half-tone plates some of which include as many as 15 illustrations. For each species there is a photograph of the wings of one side of the type specimen and sometimes a picture (which gives rather little detailed information) of the head and its associated sense organs. If the species is the type of a genus there is also a clear drawing of the wing venation. Photomicrographs of dissections of the genitalia of the types provide a much-needed supplement to Meyrick's original description. The quality of the photographic illustrations is almost uniformly good, so that in spite of difficulties with depth of focus they are almost as useful as good drawings would be.

Apart from a very few instances where the author synonymizes two species, the text consists of little more than a legend for the illustrations and the reference to the original description of the species concerned. Lectotypes are designated when needed and Meyrick's occasional errors in determining the sex of his specimens are corrected.

When this series of volumes is complete, the stage will be set for the next advance. This will be the revision of genera, including species described by many authors. That there is much to do is clear even from a superficial study of the diversity of form in such genera as *Lecithocera* and *Ethmia*. Dr. Gates Clarke has wisely not tried to do two things at once, for the present work is already large and expensive. The speed with which these much-needed revisions come will depend very largely on the funds available for this basic kind of work in museums and universities. The Trustees of the British Museum (Natural History) already publish a very substantial amount, but publications like this are, unfortunately, still far too rare. Of the hundreds of thousands of type specimens in the museums of the world a surprisingly high proportion must still await critical re-examination. Experience suggests that the original descriptions have been misunderstood in at least one-tenth of the cases. Many of the changes in the names of animals which so baffle the non-specialist are caused by the correction of these old mistakes of identification.

Dr. Gates Clarke is to be congratulated on performing this basic and useful task, and the staff at the British Museum (Natural History), especially Mr. J. D. Bradley, who has assisted in the work at every stage, will earn the thanks of the many specialists in various parts of the world who will find this work indispensable.

G. C. VARLEY

Man's Place in the Island Ecosystem

A Symposium. Edited by F. R. Fosberg. Pp. vii + 264. (Honolulu, Hawaii: Bishop Museum Press, 1963.) 8 dollars.

WHEN the late Sir Arthur Tansley in 1935 introduced the term ecosystem and later defined it as "a unit of vegetation considered as a system . . . includes not only the plants but also the animals habitually associated with them", it may be doubted whether he envisaged man as the most important animal concerned. Yet this is the logical conclusion, and where better to study the problems involved than among the numerous and varied islands of the vast Pacific Ocean? An island is a clearly defined areal unit; in the Pacific there is every stage from the uninhabited coral islet to the crowded sophisticated human atmosphere of the United States' fiftieth State. Under the Humid Tropics programme of Unesco, a symposium was arranged to take place at the tenth Pacific Science Congress held in the University of Hawaii at Honolulu in August 1961. It was very efficiently organized by Dr. F. R. Fosberg (Washington), aided by his able lieutenant, Dr. Marie-Hélène Sachet, and he contributes the short introductory paper on the ecosystem concept.

The major criticism must be that too much was attempted in the time allotted. Each of the thirteen papers is of the nature of an *ex cathedra* statement and each was followed by a comment by a 'discussant'—limited to 3 or 5 min, despite the fact that he might have been flown half-way around the world to take part. It was soon clear that no time would be available for discussion; in a few cases a question or two after the discussant had spoken. Many of the big names in the ecological field from all over the world are there—Marston Bates (Michigan), Pierre Gourou (Brussels), G. Mangenot (Paris), Irene Taeuber (Princeton), William L. Thomas (California), Kenneth Cumberland (New Zealand)—among the contributors; J. A. Steers, H. E. Maude among discussants; while a distinguished audience is revealed by simple questions from Paul Sears and C. F. A. Pantin. The task of a general summing up was left to Oskar Spate (Canberra).

The symposium is a landmark in the present development of thought. The ecosystem was the concept of a botanist mindful of the interaction of plants and animals and of the significance of the complex environment with its various physical factors. Here the influence of man brings in the physical and human geographer with the concept of human ecology fitting into the whole. The time to absorb the several contributions was lacking in Honolulu; this comprehensive report allows time for the proper and careful study of a series of papers of the highest value and significance. The sequence is logical—from physical environment and marine influence to the nature and evolution of the biota, the reciprocal man-nature relationship, the pattern and varied nature of human influence and culture systems to the practical issues of the present problems of human pressure and demographic instabilities. This is an essential volume for every serious library.

L. DUDLEY STAMP (Discussant)

A History of Parasitology

By W. D. Foster. Pp. vi + 202. (London and Edinburgh: E. and S. Livingstone Ltd., 1965.) 35s.

THIS is a well-written and interesting book and I gained much pleasure and information from it. It is the first account of the history of parasitology written in English, and a book that has long been needed.

The author provides, in his first chapter, a succinct account of the growth of parasitology from ancient times. In succeeding chapters, in order to illustrate the basic discoveries of parasitology, he outlines the history of our knowledge of a series of animals parasitic in man.

He begins with the trematodes, and discusses the liver fluke, *Fasciola hepatica*, and the schistosomes, which cause

bilharziasis, which is still one of the major diseases of man and at present the subject of a campaign organized by the World Health Organization. Four nematode types are then discussed, namely the hookworms, *Trichinella spiralis*, the Medina worm (*Dracunculus medinensis*), and *Wuchereria bancrofti* (a cause of filariasis). He next considers, in a separate chapter, the parasitic Protozoa. In the chapters that follow he treats the trypanosomes, *Entamoeba histolytica*, the cause of amoebic dysentery, and those other Protozoa, the babesias, which cause serious diseases of cattle and other farm stock. A final chapter brings the history up to about the year 1920, when parasitology became well established as a branch of biology.

This plan has certain advantages, but it does tend to direct attention to the animals parasitic in man only, and some readers will wish that more attention had been given to the achievements of veterinary parasitologists. It is true that their work on the babesias is discussed, but more space might have been given to the part played in the development of parasitology by co-operation between the veterinarians and workers concerned with diseases of man. Moreover, a chapter on the social and economic effects of parasitic animals and the devastating effects of some of them on the food supplies of the world would not have been out of place, for there is much evidence that animals parasitic in man have, through their social and economic effects, influenced appreciably the course of human history. Diseases caused by them have, for example, greatly influenced, if they have not actually determined, the outcome of wars. But themes such as these were no doubt outside the author's intention, which was, he explains in his preface, "to give an accurate account of the basic discoveries of parasitology . . . at a level comprehensible to the non-professional parasitologist". In this general aim he has certainly succeeded. The book will be read with pleasure and profit by all who are interested in the subject.

G. LAPAGE

Histologie und Mikroskopische Anatomie des Menschen

Von Dr. W. Bargmann. 5. überarbeitete Auflage. Pp. xvi + 856. (Stuttgart: Georg Thieme Verlag, 1964.) 72 D.M.

THE fact that Dr. Bargmann's book is, after fifteen years, already in its fifth German edition, and that the second Spanish edition is in preparation, speaks for the author's thoroughness in revision and his desire to keep up with recent developments. The text is based on a correlation of histology with physiology, biochemistry and submicroscopic morphology on one hand, and where applicable to pathology on the other. The text is divided into two parts. The first deals with general cytology and a descriptive exposé of the tissues: epithelial, supporting, muscular and nervous.

The second part considers the organs and organic systems: locomotor, circulatory and defensive, internal, secretory, digestive, respiratory, urinary, sexual, cutaneous, sensory, and central nervous systems. The text is lucid and integration of the different disciplines is excellent although some might find a more extensive discussion of tissue culture morphology of advantage. Literature references are ample and not confined to German publications. The illustrations are of excellent quality, but, as they are not indicated in the index, are sometimes hard to find if a particular one is sought. Another criticism, which applies to most publications, is that magnifications mentioned do not indicate whether they apply to the finished product or are applicable to some previous stage in their production. Both reference and subject indexes are adequate with the aforementioned reservation.

In conclusion this book is of general interest and, because of the illustrations, might even prove useful to workers who have only a limited knowledge of German.

G. SANDER

Theory of Crystal Dislocations

By Prof. A. H. Cottrell. (Documents on Modern Physics.) Pp. ix + 94. (London and Glasgow: Blackie and Son, Ltd., 1964.) 32s. 6d.

THIS little book (also available in paper-back form) is a kind of *vade-mecum* for dislocationists. It deals with the basic theory of dislocations, and consists mainly of the notes prepared for Prof. Cottrell's lectures to the 1956 summer school of the University of Grenoble at Les Houches. Some measure of the extent to which this theory has now been incorporated into the accepted physical scheme is provided by the fact that only minor changes were necessary when the manuscript was prepared for publication six years later.

It is perhaps unnecessary to add that this rather satisfactory situation does not extend to the complex many-dislocation problems which are encountered in plastic deformation. Developments in the theory of deformation, and in the experimental observation of dislocations by high-resolution techniques, are mentioned briefly in a series of very short notes added to the original manuscript. Although these additions were completed as recently as 1962, they have inevitably dated much more rapidly than the basic theory; the 'jog theory' of work-hardening, for example, is no longer current.

The book is written with Prof. Cottrell's usual clarity, but it should be emphasized that it is not a text-book from which to approach the basic theory for the first time. Its main use will undoubtedly be as a desk-book for quick ready reference, or as a companion of such slender dimensions as not to deter the aeroplane traveller. It is a great convenience to have so many useful results gathered together in compact form, and it may seem ungrateful to ask for more. However, there have been important developments in basic theory in the past few years, dealing, for example, with the stress fields of various types of dislocation aggregates, or with the theory of continuous distributions of dislocations. If Prof. Cottrell could find time to add these to a future edition, the value of the book would be even greater.

J. W. CHRISTIAN

Molecular Orbital Theory

By C. J. Ballhausen and Dr. Harry B. Gray. (Frontiers in Chemistry.) Pp. ix + 273. (New York and Amsterdam: W. A. Benjamin, Inc., 1964.) 9.90 dollars.

THIS book is divided into two approximately equal parts, the first being a text of atomic orbitals using the LCAO approach, while the second part is a collection of previously published papers on the subject. A very simple and short exposition is given of the basic concepts of the LCAO theory, which leads into a group theoretical section on the uses to which these simple concepts may be put. These include symmetry consideration electronic states and hybridization. The types of possible bonding systems are discussed and the results applied to selected diatomic, triatomic and a few of the more simple polyatomic molecules. Finally, the theories developed are applied to more complicated polyatomic molecules making use of ligand field theory. The book is written in a simple manner with the result that the reader is sometimes left with the impression that the stage of knowledge of molecular theory is more advanced than is the case in reality. The illustrations are not only well done but are also so positioned in the text that they are extremely useful to those readers who are not very conversant with the more mathematical form of presentation. The choice of papers in the second part is in some cases a little surprising, but some very useful information has been collected together, making the book valuable as a reference for the reader interested in making molecular calculations.

E. W. T. RICHARDS

The Evolution of the Bristol Channel

By F. J. North. Third edition, revised and enlarged. Pp. vii + 110 + 14 plates. (Cardiff: National Museum of Wales, 1964.) 15s.

OVER a period of many years Dr. North has done much to interest the layman in geology, particularly in South Wales. In the third edition of *The Evolution of the Bristol Channel* one of his books is once more brought up to date, incorporating much recent work in this area. A historical introduction precedes a simple account of the geological processes of erosion, transport and sedimentation, and earth movements; and this is followed by a concise geological history of the region, with particular reference to the Welsh coast. Useful palaeogeographical maps and a geological time scale (the latter unfortunately not brought up to date) accompany accounts of the structure of the Welsh coalfield and of the geological reasons for the distribution of the mines. Similarly a description is given of how the formations exposed on the coast of South Wales control the form of the coast-line. The origin of the South Wales drainage pattern, the evolution of the Bristol Channel since its appearance in Pliocene times, the Recent changes in sea-level, the appearance of man, the accretion of sand dunes, and the great flood of the Wentloog Level in 1607 are among the many topics dealt with in an interesting manner. The plates and diagrams usefully supplement the text, though the standard of reproduction is not high. Visitors to the South Wales coast and the local inhabitants are fortunate in having available this very readable account of the local geological history.

A. R. MACGREGOR

Tizard

By Ronald W. Clark. Pp. xvii + 458 + 16 plates. (London: Methuen and Co., Ltd., 1965.) 50s. net.

SIR HENRY TIZARD is still freshly remembered by many who worked with him or were associated with him in one or more of his varied activities. In the relatively short time since his death numerous tributes have been paid to his work, notably by Prof. P. M. S. Blackett and by Prof. R. V. Jones in Tizard Memorial Lectures, in Sir Harold Hartley's memoir for the Chemical Society, and by Sir W. S. Farren and Prof. R. V. Jones for the Royal Society. Nevertheless, there is a place for Mr. R. W. Clark's book, in which he has made full use of his access to unusually large collections of papers.

With one reservation, Mr. Clark has given us a well-balanced account of Tizard's life and work which does full justice to Tizard's real achievements. The reservation relates to the chapter on Tizard's post-war years as chairman of the Advisory Council on Scientific Policy and of the Defence Research Policy Committee, which scarcely conveys the significance of his positive achievements or the magnitude of his own contribution to the complex problems of the relations between science and Government. Mr. Clark, moreover, has avoided being drawn into personalities of the argument that has stemmed from Sir C. P. (now Lord) Snow's lecture on that theme, and his book points to most of Sir Henry's activities in the field of education. Its value is enhanced by a careful bibliography and references to most of Sir Henry's published papers. There is an error on p. 369, when a long letter to Sir Alan Barlow is attributed to Sir Henry himself instead of Sir Thomas Morton—an error which has since been rectified by Mr. Clark, who notes that the views expressed in it are substantially those of Sir Henry himself.

If the book is one to be studied by all who are concerned with the intricate problems of science and Government, it can scarcely be put down by a British reader without some sense of shame for the way in which Sir Henry was treated by the British Government. The exploitation of the scientific adviser moving in and out of the Civil Service is a reproach which we should be at pains to avoid in any structure established for civil science.

RECENT DEVELOPMENTS IN COSMOLOGY*

By PROF. F. HOYLE, F.R.S.

Professor of Astronomy and Experimental Philosophy, University of Cambridge

I SHALL start from the observed shift of the spectrum lines of galaxies interpreted in terms of the expansion of the universe. The red shift implies that distances between galaxies, measured with an imaginary ruler for example, increase with time. An immediate question is whether the universe was denser in the past than it is to-day. If so, how much denser was the universe?

A definitive answer to this critical problem could be obtained in principle by observing the state of the universe in the past. This is possible because of the finite speed of light. We do not observe a galaxy as it is now but as it was at the moment the light started on its journey. In the case of very distant galaxies the light started several billion years ago, so we have direct evidence of the state of affairs several billion years ago. All that need be done, again in principle, is to observe the density of galaxies as it was a few billion years ago and to compare it with the density in our immediate neighbourhood. This would be a direct way of settling the density problem. This indeed was the way in which Hubble tried to settle it more than thirty years ago. He failed to do so because of the extreme difficulty of making a fair count of distant galaxies. The tendency is to count the brighter galaxies but to miss the fainter ones. Since Hubble's attempt nobody has had the hardihood to make a direct assault on the problem by attempting to count galaxies.

Ryle and his associates have counted radio sources instead of galaxies, and here the result has turned out to be much more clear-cut, at any rate so far as the counting process itself is concerned. The difficulty with radio sources is that we are still far from sure exactly what it is that is being counted. Developments in the past two years, the discovery of the quasi-stellar sources in particular, have shown the situation to be more complex than it was at first thought to be. The indication of the radio counts is that the universe was more dense in the past than it is to-day. However, further knowledge is needed concerning the nature of the radio sources before this conclusion can be regarded as definitive.

If the quasi-stellar objects are truly cosmological a great deal becomes pretty well settled. Imagine an object of fixed intrinsic brightness to be moved to increasingly great distances. Two things will happen. The apparent luminosity of the object will decrease and the red-shift of the spectrum lines will increase. A theoretical relation between these quantities can be determined for any specified cosmological theory. The relation is different in different theories, so that in principle it would be possible to distinguish between one cosmological theory and another if we could experiment in this way with a standard object of fixed brightness. Unfortunately it is impossible to move a single object to increasing distances; so the astronomer must rely on similar objects just happening to lie at different distances. The question then arises of how sure we can be that the objects are really similar to each other. Massive galaxies do seem to be quite remarkably similar, but the theoretical differences we are looking for are rather slight in the case of galaxies. This is because galaxies, even the brightest galaxies, cannot be observed far enough away for the theoretical differences to be more than slight. At greater distances the theoretical differences become much more appreciable, however. The technical problem is that of photographing spectrum lines when the light intensity is very small. What is needed is more light. It is here that

the quasi-stellar sources are of critical importance. Accepting for the moment that the red-shift of the spectrum lines in these sources is cosmological in character, the quasi-stellar sources are brighter than the most massive galaxies by about four magnitudes, a factor of about forty.

At present, red-shift measurements are available for about fifteen quasi-stellar objects. The shifts are dimensionless numbers given by dividing the wave-length shift of any spectrum line by the laboratory wave-length of the same line. The result is the same for all lines. The measured values range from quite small values, for example 0.16 for 3C 273, up to the enormous value of 2 for the source 3C 9. The theoretical differences become quite large for red-shifts as great as this, so that a distinction between different cosmological theories should be straightforward once a sample of the order of a hundred quasi-stellar objects has been obtained. The present indication based on the small sample of fifteen is that the universe has expanded from a state of higher density, although the statistical scatter in the sample is large enough to be comparable to the effects that are being looked for.

For spectral shifts as large as two, the Lyman- α line is displaced from the unobservable ultra-violet into the blue, at about 3700 Å. It is possible to look for a continuum on the blueward side of Lyman- α . This continuum is subject to absorption by neutral hydrogen atoms in intergalactic space. A very small density of neutral atoms would be sufficient to absorb out the light completely. Schmidt's observation of 3C 9 shows the continuum to be present but to be weakened, that is, to be lower than the continuum on the redward side of Lyman- α . The implication is that intergalactic gas, if it exists, must be hot, perhaps above 10^6 °K. The weakening of the continuum is rather strange, for in a sensitive situation like this one would expect either the continuum to be essentially unweakened or to be absent. The intermediate case seems *a priori* unlikely, since it depends on a closely defined value of the hydrogen density. It is rather in the nature of a coincidence that the density has this critical value.

These remarks are all subject to a cosmological interpretation of the red-shift of the spectrum lines of the quasi-stellar objects. If much smaller, fainter objects were fired out of our own galaxy, or out of some neighbouring galaxy, with speeds close to light the same red-shifts would be observed. Can we be sure that such a 'local' interpretation is wrong?

Even though on a 'local' hypothesis the quasi-stellars are much less spectacular objects, the total mass involved in all such objects must be as high as $10^6 M_{\odot}$, or perhaps even more. For such a quantity of matter ejected at speeds close to light, the kinetic energy must be comparable with the rest mass energy, 10^{60} ergs. This is of a similar order of magnitude to the energy involved in the outburst of a major radio galaxy. The energy in the latter case is also in the form of particle motions. The difference is that the particles in the radio galaxies have been thought of in the past as occupying large volumes, not as being condensed into compact objects. However, we now have to ask whether a radio galaxy may not eject compact pieces as well as diffuse clouds of high-speed particles.

Radio galaxies do not eject their material with an initial isotropy. The typical pattern is of two centres of radio emission on opposite sides of a galaxy, with the two centres and the nucleus of the galaxy more or less collinear. To me personally, this has always suggested that an object in the nucleus of the galaxy separates violently into two

* Substance of an Evening Discourse delivered on September 6 at the Annual Meeting in Cambridge of the British Association for the Advancement of Science.

pieces with a large relative motion. The collinear property then follows from conservation of momentum. Exactly the same phenomenon is observed in the quasi-stellar source *MH* 14-121, two regions of radio emission on opposite sides of, and collinear with, a centre of optical emission. The possibility must be considered that a cascade process is involved. An initial object in the centre of a galaxy breaks violently into two pieces. Later, each of these pieces breaks into two further pieces; and so on. As the cascade develops, and as the objects spread out from the parent galaxy, an approximation to an isotropic situation would then gradually develop.

From independent evidence it has been suggested that an explosion occurred in the nucleus of our own galaxy about ten million years ago. If the quasi-stellar objects emerged in this explosion, as Terrell has suggested, the brightest of the objects, *3C* 273, would now be distant about 0.5 million parsecs, about one-thousandth of the cosmological distance. The optical emission, instead of the enormous cosmological value of 10^{46} ergs sec⁻¹, would be 10^{40} ergs sec⁻¹. The burning of some 300 solar masses of hydrogen gives sufficient energy to supply such an output for as long as ten million years. Since the mass of *3C* 273 could be set as $10^3 M_{\odot}$ there would seem to be no difficulty in explaining the optical output. The kinetic energy of *3C* 273 would then be $\sim 10^{56}$ ergs, about one order of magnitude less than the total energy of the galactic explosion, as estimated by Burbidge and Hoyle. The masses of other quasi-stellar objects can be set lower than *3C* 273, because *3C* 273 is intrinsically considerably brighter than the others, at any rate for the sources so far observed. Setting $10^3 M_{\odot}$ as the mass per object, and taking the mean speed as half the velocity of light, the kinetic energy per object is comparable with that of *3C* 273. The total energy estimated by Burbidge and Hoyle, 10^{57} ergs, would provide only for ~ 10 objects. It would seem therefore that either Burbidge and Hoyle underestimated the violence of the galactic explosion, or an energy difficulty arises.

The same difficulty does not arise in the case of the galaxy *NGC* 5128. This is a nearby massive elliptical, distant about four million parsecs. At least one major outburst is known to have occurred in the nucleus of this galaxy within the past ten million years. The probability must also be considered that both *NGC* 5128 and our galaxy are involved, with our galaxy contributing the comparatively low-speed objects and *NGC* 5128 contributing the high-speed objects.

These questions can undoubtedly be resolved by observation. Observations leading to size estimates for the quasi-stellar objects are coming along with rapidly mounting impetus. The light from *3C* 273 has been known to be variable in a characteristic time of about ten years. This sets the maximum radius of the optical object at ten light years. In addition, rapid flashes in the light over only a few weeks have been suggested. Largely because the maximum radius would have to be reduced to a tenth of a light-year, or even less, there has been a disposition not to believe this evidence in the case of *3C* 273. On the cosmological hypothesis, how can one have an optical emission a hundred times brighter than the most luminous galaxies pouring out of an object only a tenth of a light-year in diameter? The issue appears to have been resolved by a recent observation of a doubling of the light of a quasi-stellar source in less than a month.

On the radio side, fluctuations from *3C* 273 have been found, first by Dent and more recently by Moffet and Maltby. The radio data set stronger constraints than the optical data, particularly for the cosmological theory. The present state of the argument is that the cosmological theory just survives the existing data. Whether it will continue to do so remains to be seen. My judgment of the situation is that survival for the cosmological theory depends on there being a sharp saturation in the accumulation of fluctuation data, that not much more in the way of fluctuations can be tolerated. If we are already near the

end of the road, the theory will survive and will then probably turn out the correct theory. But if we are still near the beginning of the road, the prospects for the theory will be slight. I would say we have to do with a fifty-fifty situation.

I would like to turn now to quite different issues; but still bearing on the question I asked at the beginning: Has the universe emerged from a more dense state? The kinds of observation I have discussed so far all relate to great distances. Observations can be made in our own neighbourhood which also bear on the problem. I am now going to describe three such observations, together with the related arguments. The three are utterly different in character, illustrating how wide are the issues in cosmology, and how very many phenomena have to be made to fit into a consistent picture.

Recently, Penzias and Wilson have observed a radio background at a wave-length of about 7 cm, which they do not believe to be due to their equipment or to the nearby terrestrial environment. The intensity is between 10 and 100 times greater than can be attributed to radio sources. The suggestion is that the universe has a thermodynamic radiation background corresponding to about 3.5° K. Observations at two other wave-lengths at least are needed to confirm this suggestion. One such observation is now being planned by Dicke at Princeton.

There seems no way in which such a background can be explained in terms of current astrophysical processes. Hence, if we accept the suggestion of Penzias and Wilson, the immediate implication is that the universe must have been different in the past from what it is to-day. Particularly, a higher density is needed to generate the background.

A similar result follows from the entirely different consideration of the helium-to-hydrogen ratio in stars and gaseous nebulae within our galaxy. Determinations of this ratio range from 0.08 to 0.18; and the ratio seems to be just as high in old stars as in most young stars. The ratio to be expected from current stellar activity is only 0.01. So either activity in the galaxy was much greater in the past, or the helium cannot be explained in terms of production from hydrogen through thermonuclear processes within the galaxy. Failure to observe any stars or any object with a low helium content points to the second of these possibilities.

It is possible to show by detailed calculation that, if matter in the universe has emerged from a state in which the temperature was above 10^{10} °K, the helium-to-hydrogen ratio must be about 0.14, a value which falls in the centre of the observed range. However, no values less than a truly universal value should be found. Two independent determinations for the Sun, one from structure calculations, one from observations of solar cosmic rays, give concordant results close to 0.09. This seems significantly below the expected universal value; but further work is needed to establish whether the discrepancy is real or not.

All the lines of investigation which I have mentioned so far point to an affirmative answer to the initial question: they point to the universal density in the past being higher than it is at present. Yet in every case the argument has been fraught with uncertainty. The probability seems against a negative answer, yet the possibility cannot be excluded. Speaking personally, I believe the case for a negative answer would still be arguable if it were not for the third of my three lines of attack. This again is entirely different in character from the helium/hydrogen ratio and from the microwave observation of Penzias and Wilson. I refer to the problem of the origin of elliptical galaxies. In my view, a consideration of this problem points decisively toward the universe having been very much denser in the past than it is at present.

Galaxies have been broadly classified into two types—ellipticals and spirals. There is an incontrovertible argument to show that spirals must have condensed from a more diffuse form. The spirals are rotating. Their angular

oments prevent them from being compressed into more compact forms. The flattened ellipticals have always been supposed to be similarly in rotation, but this has never been properly checked by observation. Because the ellipticals were thought to be in rotation it was similarly supposed that they were formed by a condensation process. This, I am now convinced, is wrong. I believe ellipticals have formed through expansion from a higher density state.

Elliptical galaxies are remarkably amorphous. The star distribution is everywhere smooth. If one measures the surface brightness it is found to behave very nearly as an inverse square law, rising with great steepness towards the centre. The centres possess extremely bright central spots. How sharp these centres really are, how star-like, is impossible to say at the moment, for atmospheric seeing effects smear the central pip into an apparent disk.

Suppose the universe expanded from a much denser state, say $10^{-12} \text{ g cm}^{-3}$, and suppose the gas at the beginning of the expansion was not entirely smooth, suppose there were condensation knots already within it. It appears that such condensation knots can restrain the expanding gas to a degree which can be subject to precise calculation. A knot of mass $10^9 M_\odot$ can restrain a total mass of $10^{12} M_\odot$ within a region of galactic dimensions. A knot of $10^7 M_\odot$ can restrain a mass of about $5 \cdot 10^{10} M_\odot$. The critical point now emerges, that the surface brightness of the resulting aggregation can be calculated (assuming the material forms into stars) and the calculations yield quite unambiguously a law close to the inverse square, in fact just what is observed.

The point I wish to make is that whereas the steep rise towards the centre is expected, and is predicted, by the expansion picture, this characteristic feature of elliptical galaxies cannot, I believe, be understood at all within the condensation picture.

The clinching factor, it seems to me, is that a condensation knot—a memory of the initial dense state—is to be expected at the centre of every major elliptical galaxy. It is these condensation knots that give rise to the phenomenon of the radio galaxy. These are the massive objects which Fowler and I postulated some three years ago. Questions were asked of us at the time as to how our objects ever managed to form. Difficulties of angular momentum were raised. The answer which can now be given is that the objects never formed, in the sense in which the questions were asked. They are relics of a much higher density phase of the universe. They have been there since the galaxies themselves were formed, and in the sense of the radio astronomer they have been smouldering throughout the lifetimes of the galaxies. They are systems which remain at the very edge of stability. Whenever instabilities occur, violent outbursts serve to restabilize them.

Why is my initial question so important? Why make such a fuss about whether the universe has been in a more dense state? Because the present physical theory suggests that there is no limit to how great the density must have been in the past. I use the word 'suggests' because the physical divergence of the density was first demonstrated for a homogeneous and isotropic universe. Divergence also occurs when the isotropic restriction is removed. Does it also occur when homogeneity is removed? I have always believed the answer to this question was also affirmative, but my belief was based more on the failure of those who maintained the opposite to demonstrate their case than on any positive demonstration on the affirmative side. However, progress on the affirmative side has been made very recently, and opinion has generally moved toward the view that the equations of physics contain a universal singularity.

I have always had a rooted objection to this conclusion. It seems as objectionable to me as if phenomena should be discovered in the laboratory which not only defied present physical laws but which also defied all possible physical

laws. On the other hand, I see no objection to supposing that present laws are incomplete, for they are almost surely incomplete. The issue therefore presents itself as to how the physical laws must be modified in order to prevent a universal singularity, in other words how to prevent a collapse of physics.

It was with this background to the problem that several of us suggested, some twenty years ago, that matter might be created continuously. The idea was to keep the universe in a steady-state with creation of matter compensating the effects of expansion. In such a theory the density in the universe would not be higher in the past than it is at present. From the data I have presented here it seems likely that the idea will now have to be discarded, at any rate in the form it has become widely known—the steady-state universe. But let me proceed with the theoretical ideas which have grown out of the notions of twenty years ago, for they may turn out to have a value going beyond the first suggestions.

During the past ten years the struggle has been to invent a form of mathematics operating in the manner customary in physics, namely, starting from an action principle. It was found possible to represent the creation of matter through the introduction of a new field. The manner in which the field was treated was quite normal. What was different from ordinary physics was the motive underlying the investigation, the avoidance of a universal singularity, rather than an experiment in the laboratory. Physicists will introduce a new field at the drop of a hat, if experiments in the laboratory should direct them so; but the physicist is unhappy to do so for any other reason.

Having obtained the mathematical structure of the new field it was found that singularities never occur, quite regardless of whether matter is being created or not. So long as the new field exists there will be no singularity either of the universe or of a local imploding body. In other words, the models available for investigation, the models without singularities, were very much wider than the old steady-state theory. During the past few years it is these other models which have been under investigation. What has turned out?

The simplest case is that in which the new field exists but in which there is no creation of matter. It is then possible to obtain a finite, oscillating universe of the kind that has been sought for so long in the usual theory. The universe alternately expands and contracts. Gravitation causes the reversal from expansion to contraction, while the new field causes the rebound from contraction to expansion.

So far as I am aware, such an oscillating model is in satisfactory agreement with all available data. The model is less dull than it seems at first sight, for it contains the possibility of some carry-through from one cycle to the next. Suppose the universe as we observe it eventually stops expanding. Suppose it falls back to a state of comparatively high density, a state in which stars are evaporated, a state in which even the nuclei of heavy elements are disrupted, a state from which matter emerges with the helium-to-hydrogen ratio I described before, about 0.14. In the state of high density things will not be quite uniform. Because of the existence of galaxies, and of clusters of galaxies, there will inevitably be some departures from uniformity. These departures will form the condensation knots round which a new generation of galaxies will form. Thus the condensation knots of which I spoke at an earlier stage are not merely random perturbations. One generation of galaxies acts as the seeds for the next generation. Magnetic fields will also persist from cycle to cycle.

There are two objections to this model. The new field is without sources. It is introduced *ad hoc*, along with the matter. There is never any coupling between the matter and the field. Then there is the subtle, but I believe the correct, objection that a series of oscillations must eventually damp out. Unless dissipative processes are precisely zero,

which seems unlikely, the amplitude of the oscillation will gradually die away and the universe will come to rest in an intermediate static state.

For these reasons it is of interest to examine the models with creation of matter, noticing there is no specification from theory as to what the density must be in the steady-state situation. In the past the density was set empirically, by requiring it to be equal to the present-day density. Perhaps this step was wrong. Perhaps the true steady-state density should be very much higher.

During the past year, Dr. Narlikar and I managed to investigate a possibility which had previously proved too difficult to handle, the case in which there are departures from homogeneity, the case in which there are fluctuations from one region of space to another. To our surprise we found that under certain conditions the creation of matter could fall away in a localized region, and that if it did so the region would break into a series of oscillations of a kind that were closely analogous to the oscillations I have just been describing. In the former case we had oscillations of the whole universe, but of a universe of finite volume and finite mass. In this new case we have oscillations of a finite region of an infinite universe. From the point of view of an observer living in such a region it would be difficult to tell the difference. The oscillations would eventually damp away, but in this second case there would simply be a return to the steady-state condition. In this second case there will be many localized oscillating regions, not merely one. The regions will not in general be

in juxtaposition with each other; they will usually separated like islands in an ocean: and, like islands, they will be of different sizes and the amplitudes of their oscillations will be different.

I have already mentioned the philosophy of the physicist that the whole of physics is discoverable in the laboratory. What has been discovered is a remarkable mixture of elegance—invariance properties for example—and ugliness, the fine structure constant being 137 . . . for example. The properties of matter depend critically on the dimensionless numbers of physics, as well as on the structure of the laws. One can take three views on the dimensionless numbers:

(1) They just happen to have the values we find for them and no explanation of these values will ever be found.

(2) The observed values are necessary to the logic consistency of physics.

(3) The observed values are of non-local origin.

I imagine few will be satisfied with the first of the possibilities. I also imagine most physicists prefer (2). But what if (3) should be correct? Could the curious values we observe for the dimensionless numbers be connected with the particular oscillating and finite region in which we happen to live? If this were so, the universe would be far richer in its possibilities and content than we normally imagine. In other regions the numbers would be different and the gross properties of matter, the science of chemistry for example, would be entirely changed.

A VISIT TO THE LOWER AMAZON

IN the summer of 1964 a visit was made to the lower reaches of the Amazon by a party of four naturalists, namely, I. R. Bishop of the Department of Biology, Guy's Hospital Medical School, who acted as leader of the expedition, E. C. Tatchell of the same department, D. Walliker of the London School of Hygiene and Tropical Medicine and Dr. J. C. Garnham who acted as medical officer.

The party had a number of objectives the most definite being to make collections of parasites of the blood and tissues of various vertebrates, to collect a variety of vertebrate animals and also to attempt to arrange for the return to Great Britain of some living specimens of the South American lungfish, *Lepidosiren*. Other objectives were to introduce the members of the party to various scientists, laboratories and working conditions in Brazil and also to see whether air travel now made it possible for a biologist to go from Britain to Brazil for a comparatively short time, such as a long vacation, and accomplish a worth-while amount of work.

The heavy baggage of the party, including collecting gear and some hydrographic apparatus, was shipped from Britain in May, but the first member of the party to leave was I. R. Bishop, who flew out on June 16; he arrived in Manaus, approximately 1,000 miles up the River Amazon, on June 19, having made a short stop at Belém, where he visited the Goeldi Museum (director, Dr. D. Albuquerque) and the Instituto Evandro Chagas (director, Dr. Orlando Costa) and confirmed arrangements which had been agreed by correspondence that the last three weeks of the trip should be spent at these institutes in Belém (Fig. 1).

On arriving at Manaus, Bishop received a very cordial welcome from Dr. W. Rodrigues, the acting director of the Instituto Nacional de Pesquisas da Amazonia (I.N.P.A.) the director (Dr. Djalma Batista) being on leave. The remaining three members of the party left London on June 30 and arrived in Manaus on July 2.

While the party was in Manaus it was based on the Instituto, Dr. Batista and his staff giving them every possible facility, including living accommodation, laboratory space, and most important the use of a launch, technical assistant and crew to make collecting excursions up and down the river.

The tentative plans laid before leaving London now suffered a minor reverse as the boat carrying the collecting gear, etc., was much delayed and, instead of arriving at Manaus almost at the same time as the main party, was more than a fortnight late and collecting was entirely dependent on facilities provided by others. Fortunately Bishop had soon after his arrival been introduced to Dr. Rudolph Friburgo, who, besides being a keen amateur

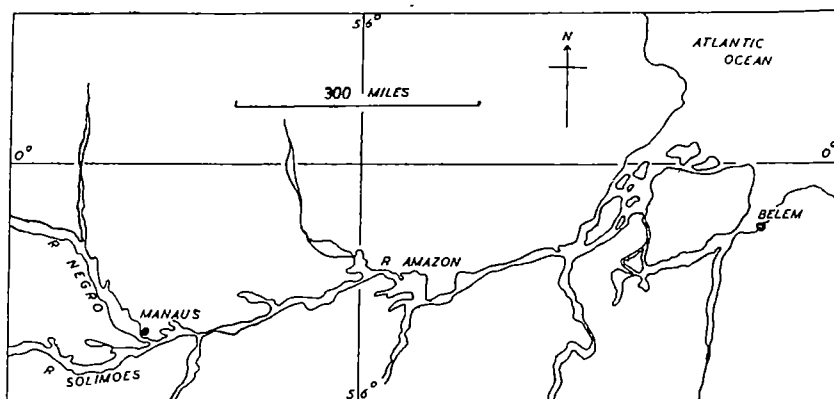


Fig. 1. Sketch map of the Lower Amazon showing localities visited

naturalist, is a civil engineer and at that time was in charge of the construction of a road being built through the forest and which had reached a point 130 km north-east of Manaus. At 105 km from Manaus Dr. Friburgo had a temporary home and camp to which he invited the party so that while awaiting their stores they could become acquainted with the forest. A technician was lent by I.N.P.A., and in his company the party spent eleven days collecting birds, mammals and amphibians and acquiring a first-hand knowledge of the forest. The darkness under the leaf canopy was what had been expected, but what came as a surprise were both the undulating nature of the forest floor and the ease with which it was possible to travel through the forest as the undergrowth, except for a few yards on the edge of any clearing where the sun could penetrate, was very thin under the dense leaf canopy and there was also a paucity of animal life. The forest was also extremely silent, the stillness being broken only by the cries of toucans and parrots and the outbursts of howling monkeys the calls of which could be heard over large distances. One member of the party (J. C. G.), who has had experience of the African tropical forest, has remarked that he was particularly impressed by the contrast between the forest in the two continents.

This phase of the work came to an end when the heavy baggage arrived at Manaus and the party returned to see it through the customs, to unpack and to select the equipment required for the next stage which was an excursion up the Rio Negro. As has been mentioned, a launch with crew and sleeping accommodation for all was lent by I.N.P.A. and on July 25 the journey began (Fig. 2).

The rainy season had ended only shortly before the party arrived in Brazil and the water-level was still high; the Rio Negro has low banks and thus the high river floods into the adjacent forest, in some places for distances of several hundred yards. This has the effect of driving from the area all the truly terrestrial animals and there is little in the way of animal life. This applies to man also, and there are few habitations on this river and only in those few places where the land is above flood-level can any settlement be attempted. Attention was mainly confined to the river itself, in which the notorious flesh-eating fish, the *Piranha* (used as food by the crew), is abundant, as are also dolphins, and to the birds—numerous parrots and toucans were seen and occasionally a hawk.

This journey was continued with frequent halts until on the fifth day the confluence of the Rio Negro with the Rio Branco (about 290 km from Manaus) was reached. Here the bank is higher and a suitable place was found to make fast for several days. Collections made here consisted

of fish, lizards and frogs, and hydrological observations were made on a nearby lake. A film record of the swarming behaviour of 'mud puddle clubs' of butterflies was also made. After a stop of five days the return commenced and Manaus was reached on August 3.

Before leaving for Brazil several lines of enquiry had been followed with the view of ascertaining whether it would be possible for the expedition to charter a suitable boat for work in the Manaus region which could then be used for the journey down stream to Belém so that further collections could be made on this journey if opportunity offered. The advice that was received before departure was very conflicting, and, in fact, this course of action proved impossible. Small boats of suitable construction and robustness are all engaged in working for their owners. It was therefore reluctantly decided that the journey to Belém would have to be made by passenger ship. This, however, meant that departure for Belém need not be immediate and the extended use of the I.N.P.A. launch was gratefully accepted and a journey made up the main stream of the Amazon, which here changes its name to the Rio Solimões. This journey took the party to the village of Codajaz (about 250 km from Manaus) (Fig. 2).

The banks of the Rio Solimões were visibly higher than those of the Rio Negro. This had the effect of preventing the seasonal flooding and also allowed for considerable agriculture on the banks themselves. Large areas of the forest along the riverside had been cleared to provide grazing for cattle. Houses, farming communities and townships were frequently passed and it was rare to be out of sight of some habitation whereas on the Rio Negro this had been the rule. From the banks large quantities of floating vegetation extended into the mainstream of the river. At first sight this appeared solid, but as the boat passed, the wash caused the floating vegetation to rise and fall and as the waves passed beneath it the surface of the floating vegetation appeared to ripple as if blown by a strong wind. From time to time portions of these floating mats broke away and became islands drifting downstream, and they appeared to remain intact for many miles. This may be of practical significance in animal transport. Collections made from these floating islands show that mosquito larvae are not infrequent and by this means are transported over long distances, a fact that must be remembered if local mosquito eradication schemes are attempted.

Owing to the fact that Codajaz is frequently visited by research workers from I.N.P.A. the technician, Mozarth, who had been attached to the party, knew several families in the village and enlisted their help in getting together a number of *caçadores* (hunters), and a team of four went off on hunting trips each morning and returned with their catch in the afternoons; it consisted mainly of mammals which were examined for parasites, their skins and skulls being preserved for further examination.

The small boys of the district added various animals to the collection from butterflies to sloths, bats and alligators. These collections were supervised by Walliker and Bishop while Tatchell and Garnham made several trips to various lakes and rivers to study the freshwater biology and hydrology of the region. Dr. Garnham was also able, with help from the officials of the public service (S.E.S.P.), to undertake some clinical helminthological investigations.

The stay at Codajaz was most productive, both of specimens and of scientific information, and it was with some reluctance that on August 18 the return journey to Manaus was begun. Five live specimens of *Iguana*, two more than 3 ft. long, and two Tegu were flown back to London. One *Iguana* died shortly after arrival but the other four were presented to the Zoological Society of London and placed on display in the menagerie. A photograph of one of these specimens appeared in the Society's annual report.

After arriving at Manaus, Walliker and Garnham paid another visit to Dr. Friburgo (at Camp 105 km) while Bishop and Tatchell repacked the equipment for the journey to Belém. This voyage started on August 29, and

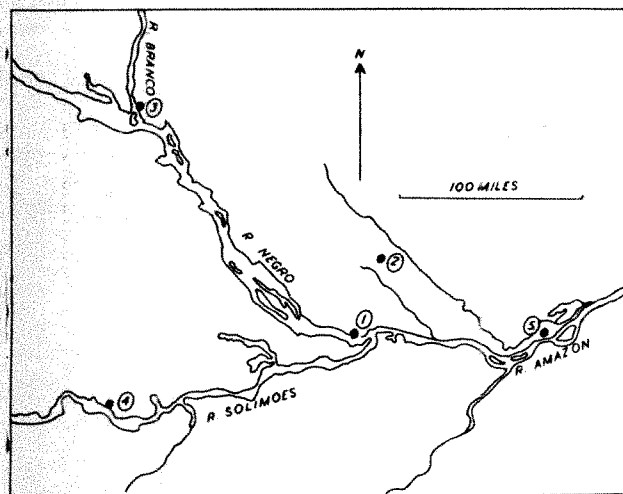


Fig. 2. Sketch map of Manaus region showing localities visited. (1) Manaus; (2) camp 105 km from Manaus; (3) mouth of Rio Branco; (4) Codajaz; (5) Itacoatiara.

after a stop of three days at Itacoatiara (about 100 km downstream) to load brazil-nuts, Belém was reached on September 5. Now the party found itself in considerable difficulty as, having come down an international waterway on a British steamer, the chief customs official at Belém held that the party were seeking to re-enter Brazil and questioned the validity of the documents presented. The equipment was impounded and only after a fortnight was the position clarified and the equipment released on September 19, and after a few days it had to be repacked for the journey home. During the stay at Belém the party was almost entirely dependent on borrowed equipment, and it is a pleasure to record that the party was made extremely welcome by Dr. Orlando Costa of the Evandro Chagas Institute, who provided laboratory facilities, and by Dr. Daley Albuquerque of the Goeldi Museum, who provided more laboratory facilities and excellent living accommodation in the zoological garden attached to the Museum. If it had not been for the kindness of these two directors no scientific work would have been possible during the time spent separated from the equipment.

Here Walliker had the assistance of the Belém Virus Laboratory in taking blood smears from animals in the Utinga Forest. Garnham turned to the problem of chloroquine-resistant malaria, which had been reported to be present in the area and, with the aid of Dr. Lins of the Evandro Chagas Institute, visited the hospitals and made valuable contacts with physicians dealing with malaria infection. Whether drug-resistant malaria is present or not is a matter of debate among the experts in Brazil, and the matter remains unresolved.

Throughout the visit to Amazonia constant enquiry had been made concerning the possibility of obtaining specimens of the South American lungfish, and in the Manaus region the answers to such enquiries were always that it could be found but not in this precise locality or

season. At Belém hopes were higher, for it is well known that it occurs in the island of Marajó which is close by. Eventually a local fisherman was persuaded to try to attempt to find some in one of the many waterways which run through the outlying parts of Belém. These are shallow ditches which are kept full of water by the influence of the tides in the delta; these ditches contain much vegetation (see Fig. 3) and are used by the local inhabitants as places in which to dispose of household refuse and rubbish of all kinds and the water is turbid, of unpleasant odour and, as shown by the electronic recording gear, of negligible oxygen content. At night the fisherman stretched a net across such a ditch and then by working it along the stream and beating the rooted vegetation drove fish into the net. In one night's fishing fifteen specimens of *Lepidosiren* were taken of which twelve were flown back to London in suitable containers. Six were sent independently and six accompanied the members of the expedition on the return journey and all arrived in good condition.

Thus the objective of obtaining living lungfish was attained. What of the other objectives? It is now, at the time of writing, some ten months since the party returned. During this time work on the material has proceeded and a preliminary scientific report was made at a meeting of the Linnean Society of London on February 18. A series of reports on the parasitological materials, some of which on protozoa are in the press, is being prepared by Walliker, and in a joint report on some aspects of filariasis he is being joined by Garnham. Bishop and Tatchell will make their own reports in due course; Bishop has also been responsible for sending material to experts on various aspects of the work, largely systematic, for their detailed investigation. In addition, a colour 16-mm film recording the expedition and its work is nearing completion. Work on the lungfish is proceeding in the Biology Department at



Fig. 3. The habitat of *Lepidosiren* in Belém, Pará, Brazil

Luoy's Hospital Medical School under the guidance of Prof. G. E. H. Foxon.

Of the more indefinite objectives, that of getting to know and working in conjunction with Brazilian scientists has most certainly been achieved. All the members of the party are grateful for the splendid assistance and co-operation received at every turn, not only from those who have been named here, but also from all those whom they met both professionally and socially who helped to make their visit so pleasant; in particular, mention should be made of the British vice-consuls at Manaus and Belém.

In conclusion, the members of the party and all concerned with the expedition thank the many individuals, commercial companies and public bodies who by their financial support and other aid enabled this journey to be undertaken. Among the public bodies the following should be especially mentioned: the Zoological Society of London, the Medical Research Council and particularly the Wellcome Trust, which by a generous guarantee made the project possible.

I. R. BISHOP
G. E. H. FOXON

NUTRITION AND ITS INTERNATIONAL RECOGNITION

By SIR RUDOLPH A. PETERS, F.R.S.

THIS subject has a very long history, but in spite of this the International Union of Nutritional Sciences is not yet accepted as one of the 'international unions' of the International Council of Scientific Unions—even at a time when we all know that a proper knowledge of nutrition is more than ever necessary to save the increasing population of the world from famine. A haphazard use of food does not matter so much when there is plenty, because the body can adapt itself to a badly balanced diet to some extent. It is, however, now vital to know how to use our food with the greatest economy. Many years ago, for example, Chittenden and his colleagues (Chittenden, R. H., *Physiological Economy in Nutrition*, New York, 1904) showed that protein requirements of adults could be much reduced without damage to health.

Why, then, is there delay in accepting nutritional science as a separate science? Probably part of the reason is the complexity of the subject. It involves physics, chemistry, physiology, biochemistry, pharmacology, pathology and medicine, and extends to sociology especially in regard to food habits and the preparation of foodstuffs. Each of these sciences sees only a small part of a complexity. Fifty years ago, nutrition seemed almost synonymous with biochemistry and Gowland Hopkins shared a Nobel Prize with Eijkmann for his part in delineating vitamins, and for showing so clearly that nutrition was a qualitative subject and not just a matter of energetics, the so-called isocaloric value of foodstuffs. This was followed by an outburst of activity in isolating and synthesizing various vitamin factors, in the furtherance of which the U.K. Medical Research Council played a considerable part. For the past eighteen years, however, those concerned with granting finance for research have turned to 'pastures new', witness: The isolation of enzymes, the unravelling of protein structure and the excitement of the nucleic acids and genetics. The result is that at the present time few research workers have been trained in the rigorous and lengthy discipline of nutritional research—which is emphatically not just 'the feeding of a

few rats'. From many points of view, even from that of space travel, we want to know much more about the interaction of the various compounds making up a diet.

The problems extend, of course, beyond man into the animal kingdom. In agriculture, for example, there is the problem of digestion in the ruminant. The micro-organisms in the rumen, bacteria and protozoa, by a controlled fermentation set up a digestion of substances such as cellulose, which the secreted enzymes of the digestive tract cannot digest; and so they turn inedible substances into food for man. Anatomy, physiology, biochemistry and microbiology are all involved in this work; but none of these sciences, as such, is interested in the implications of ruminant digestion for the utilization of the world's grassland to feed its populations.

Again, it is the business of the soil scientist, who may be geographer, physicist and chemist, to show that certain strata of the world's surface lack particular elements, such as copper or cobalt; and the biochemist will relate this to plant growth and to enzymes present. It is the business of the nutritionist to relate these discoveries to the health of the grazing animal, and so ultimately to the food supply of populations. Actually, it has often been investigated in reverse, starting with the ill-health of the animal on the natural vegetation.

It is to be noted that the International Union of Nutritional Sciences has been in existence since 1948; and it has at present the support of seventeen national members of the International Council of Scientific Unions. At intervals of three years, it has held six successful international congresses; the last one was in Edinburgh under the presidency of Sir David Cuthbertson. There will always be a need for close relations with organizations such as the Food and Agriculture Organization, the World Health Organization and Unesco; but this very necessity makes it all the more important for nutrition to have a firm scientific basis within the organization of the International Council of Scientific Unions.

NEWS and VIEWS

Science Research Council Support for University Science and Technology

THE Science Research Council has set up a new University Science and Technology Board under the chairmanship of Sir Ewart Jones, Waynflete professor of organic chemistry in the University of Oxford, to advise on the support which the Council should give to science and technology in the universities. The new Board will advise the Council on the award of grants for the support of university research, as well as studentships and fellow-

ships for the training of young postgraduate scientists: support for university scientific research and advanced training will thus be closely integrated. The scope of the Board will include science and technology; it will therefore work in close touch with the other Research Councils and the Ministry of Technology. The Council has already set up a Nuclear Physics Board under the chairmanship of Prof. C. F. Powell, and an Astronomy, Space and Radio Board under the chairmanship of Sir Bernard Lovell. Part of the support previously given to university research and training by the Department of Scientific and Industrial

Research, namely that concerned with earth sciences and ecology, has been taken over by the Natural Environment Research Council; and most of the support given in the past by the Department of Scientific and Industrial Research to university research and training in some parts of the human sciences (particularly sociology and psychology applied in industry) will be taken over by the new Social Science Research Council which is being set up by the Government following the recommendation of the Heyworth Committee.

The new Board replaces the Research Grants Committee and the Postgraduate Training Awards Committee of the Department of Scientific and Industrial Research. The membership of the Board is as follows: *Chairman*, Sir Ewart Jones. *Members*, Prof. B. Bleaney, Dr. Lee's professor of physics, University of Oxford; Prof. R. Brown, Regius professor of botany, University of Edinburgh; Dr. A. Caress, Imperial Chemical Industries, Ltd.; Prof. G. C. Drew, professor of psychology, University College, London; Dr. H. M. Finnis, International Research and Development Co., Ltd.; Prof. H. Ford, professor of applied mechanics, Imperial College of Science and Technology; Prof. M. R. Gavin, professor of electronic engineering, University College of North Wales, Bangor; Lord Halsbury; Dr. J. W. Menter, Tube Investments Ltd.; Prof. R. S. Nyholm, professor of chemistry, University College, London; Prof. G. Porter, Firth professor of chemistry, University of Sheffield; Prof. E. J. Richards, professor of applied acoustics, director of Institute of Sound and Vibration Research, University of Southampton; Prof. A. L. Roberts, Livesey professor of fuel technology, University of Leeds; Dr. J. E. Smith, secretary of the Marine Biological Association of the United Kingdom and director of the Laboratory; Prof. I. N. Sneddon, Simson professor of mathematics, University of Glasgow; Sir Peter Venables, vice-chancellor, University of Aston in Birmingham. *Assessors*, University Grants Committee and the Ministry of Technology.

Directorate of Overseas Surveys :

Mr. G. J. Humphries, C.M.G., O.B.E.

MR. G. J. HUMPHRIES retires this month as director of Overseas Surveys of the Ministry of Overseas Development, and Surveys adviser to the Minister. Mr. Humphries was born in 1900, and after three years at the University of Reading joined the Colonial Survey service in Nigeria in 1928. He was with the Royal Engineer Survey from 1939 until 1946, attaining the rank of lieutenant-colonel. From 1944 he was responsible (under Brigadier Hotine, director of Military Surveys) for the planning and organization of what was originally known as the Directorate of Colonial Surveys. Mr. Humphries was deputy director of this from its start at Bushy Park in 1946 with responsibility for the field side of the work. He was appointed director in 1963 on the retirement of Brigadier Hotine. When the tellurometer was being perfected in South Africa in 1957 he played a major part in its acceptance by geodesists and land surveyors by his advocacy and by organizing the completion by tellurometer in one month of a 400-mile precise traverse in Kenya which would have taken two years or more by conventional methods—the first major field task for which this revolutionary survey instrument was used. In addition to his control of the field side of the Directorate with parties working all over the Commonwealth, Mr. Humphries was responsible for the technical side of the selection and training of British surveyors for the Colonial Survey departments and of the training of African and other overseas surveyors in Britain. His experience and judgment of character have played an important part in the growth of these departments and in their effective evolution after the independence of the countries concerned. Mr. Humphries was awarded an O.B.E. in 1954 and a C.M.G. in 1965.

Mr. W. D. C. Wiggins, O.B.E.

MR. W. D. C. WIGGINS has been appointed to succeed Mr. Humphries as director of Overseas Surveys and Surveys adviser. Born in 1905, Mr. Wiggins was a scholar of King's School, Canterbury, and went to University College, London. He joined the Nigerian Survey Department in 1928 and served in the Royal Engineers from 1939 until 1946, attaining the rank of lieutenant-colonel. He was appointed assistant director to the Directorate on its formation in 1946 and deputy director in 1948 with responsibility mainly for the mapping side at Tolworth. He was awarded an O.B.E. in 1957.

Standards Division, National Physical Laboratory, Teddington :
Dr. H. Barrell, C.B.E.

DR. H. BARRELL, who will be retiring from his post of superintendent of the Standards Division of the National Physical Laboratory at the end of March 1966, joined the Metrology Division, as it then was, in 1923. He took his degree in physics at the Royal College of Science, where as a demonstrator he later worked with A. Fowler on ultra-violet spectroscopy in the early years of quantum theory. Dr. Barrell's own experimental work has been largely devoted to precise measurements of wave-lengths in the visible spectrum and their applications to the measurements of length. An outstanding contribution, made jointly with Mr. J. E. Sears during 1932–38, was the determination of the metre and the yard in wave-lengths of light and of the refractive index and dispersion of air. From 1939 until 1945 he was responsible for mechanical gauging of ammunition to which he applied pneumatic methods, so initiating an important development in the work of the Division. Dr. Barrell was appointed superintendent of the Metrology Division in 1953 in succession to Dr. F. H. Rolt. In 1958, in a re-organization of the National Physical Laboratory, the Metrology Division was combined with other groups to form the Standards Division. Dr. Barrell has been particularly concerned with the decision, taken in 1960, to define the metre in terms of the wave-length of an orange line in the spectrum of krypton-86: he has directed the Division's contribution to the international co-operative programme of research organized by the International Committee for Weights and Measures and he is a member of that Committee's Advisory Committee for the Definition of the Metre. In 1954, following the death of Mr. J. E. Sears, he was elected to the International Committee. He was a member of the British delegations to the General Conferences of Weights and Measures of 1948, 1954, 1960 and 1964. As chairman of the international Advisory Committee for the Definition of the Second, Dr. Barrell has been influential in securing agreement on the definition of time in terms of an atomic frequency, a decision which, like that on the metre, depends heavily on the experimental work of his own Division. Dr. Barrell's superintendency has been a time of rapid change. From a small group concerned mainly with measurements in classical physics and engineering, the Division has grown to be the largest in the Laboratory with responsibility for the fundamental units, for the most precise measurements of length, mass, time and frequency, electrical quantities and temperature, and for much of the routine test work of the Laboratory as well. This growth Dr. Barrell has taken in his stride while maintaining and encouraging the many research activities and manifold international responsibilities of the Division. He retires at a time when the international repute of the Division is high in the field, a true reflexion of the imaginative guidance he has given. He was awarded a C.B.E. in 1962.

Dr. A. H. Cook

DR. BARRELL is being succeeded next April by Dr. A. H. Cook, who was educated at Westcliff High School and Corpus Christi College, Cambridge. After graduating

in physics in 1943, Dr. Cook worked at the Admiralty Signal Establishment on electrical measurements at micro-wave frequencies and returned to Cambridge in 1946 to specialize in the Department of Geodesy and Geophysics on pendulum and gravity meter surveys of the gravitational field in Britain and continental Europe. He joined the staff of the National Physical Laboratory in 1952 and has gained international renown for his outstandingly accurate determinations of fundamental quantities, in particular the density of mercury, wave-lengths of light and gravitational acceleration at Teddington, which are of basic importance in the establishment of more precise standards of measurement. During recent years his interests have extended to metrological applications of gas lasers and to the development of advanced methods and equipment for interferometric spectroscopy in the extreme ultra-violet region. He is planning, in association with the University of Trieste, a new determination of the gravitational constant, using for the experiment a torsion pendulum 80 m long suspended in a large cave near Trieste. Dr. Cook is a member of the British National Committees for Geodesy and Geophysics and for Space Research and holds official positions in the International Union of Geodesy and Geophysics and the International Astronomical Union. He has served on the Council of the Royal Astronomical Society and is joint editor of the *Geophysical Journal*. He is now being granted sabbatical leave until he takes up his new appointment and will be a Visiting Fellow at the Joint Institute for Laboratory Astrophysics at Boulder, Colorado, which is directed by the University of Colorado and the American National Bureau of Standards.

U.S. National Bureau of Standards :

Dr. W. J. Youden

DR. WILLIAM J. YOUSEN has retired from the Applied Mathematics Division at the National Bureau of Standards. Dr. Youden, who had been with the Bureau since 1948, was responsible for significant research in mathematical statistics, especially in the field of experiment design, and vigorously promoted sound understanding and increased utilization of modern statistical techniques throughout science and industry. His most outstanding achievements are in the field of combinatorial arrangements for the conduct of scientific experiments. One class of these designs, constructed for use when test conditions vary from one group of tests to another, is known as the Youden Square. He has also contributed methods for interlaboratory tests that are used in biological and medical applications as well as in the physical sciences. Before joining the Bureau staff, Dr. Youden carried out scientific and statistical research at the Boyce Thompson Institute for Plant Research, Inc. He was an instructor at the University of Rochester, and has had temporary professional appointments at Columbia University, the University of North Carolina, and the University of Chicago. Born in Australia, Dr. Youden received a B.S. in chemistry, mathematics, and engineering from the University of Rochester in 1921 and an M.A. and Ph.D. from Columbia University in 1924 in chemistry. Dr. Youden plans in the future to take up a teaching position in the College of Engineering at the George Washington University.

Dr. P. H. Verdier

DR. PETER H. VERDIER recently joined the staff of the Molecular Properties Section, Polymers Division, of the Institute for Materials Research of the National Bureau of Standards as a physical chemist. He will undertake research of the dynamics of polymer chains by means of the Monte Carlo system, and will specialize in the effects of excluded volume interactions on the motion of polymer chains. Dr. Verdier joined the Bureau from the Union Carbide Research Institute where he was engaged in

research on molecular structure and dynamics. Born in 1931 in Pasadena, California, Dr. Verdier received his B.S., with honours, in chemistry from the California Institute of Technology in 1952. In 1957 he received his Ph.D. in physical chemistry from Harvard University. He was a Research Associate in chemistry at the Massachusetts Institute of Technology during 1957-58, and a Research Fellow at Harvard during 1958-59.

Chemistry at Battersea College of Technology :

Prof. J. A. Elvidge

DR. J. A. ELVIDGE has been appointed to the second chair in the Department of Chemistry at Battersea College of Technology, the proposed University of Surrey. Educated at Habercashers' Aske's Hampstead School (now at Elstree) and the Imperial College of Science and Technology, he graduated with honours in 1943 and gained the Hofmann Prize. As a William Gilles and then Beit Fellow, he carried out research on penicillin in a team under Sir Ian Heilbron and Dr. A. H. Cook, which gained him a London Ph.D. in 1946. Appointed lecturer at the (then) Royal Technical College, Glasgow, in 1947, he worked with Prof. F. S. Spring on gliotoxin and aspergillus acid. On returning to the Imperial College of Science and Technology in 1949, he collaborated with (then) Prof. R. P. Linstead in research on unsaturated acids and lactones, and macrocyclic compounds and intermediates, and took charge of the newly instituted third-year course for specialists in organic chemistry, which remained his major and expanding teaching interest. He was promoted to senior lecturer in 1956 and to a University readership in organic chemistry in 1962, and at this time received a London D.Sc. Dr. Elvidge's research interests include heterocyclic syntheses from malonyl chloride, reactions of nitrogen heterocycles, tetrazaporphins, metal derivatives, and applications of proton magnetic resonance to structural problems and in the determination of aromaticity. He has collaborated with chemists at the Brewing Industry Research Foundation on the structure and enolization of acylcyclopentanone products from hop resin. He is a consultant to Fisons Pest Control, Ltd., and prizes close relations with Imperial Chemical Industries Dyestuffs Division. He has been a lecturer and tutor at the recent summer schools on nuclear magnetic resonance spectroscopy organized by the Royal Institute of Chemistry. In his new post, Dr. Elvidge looks forward to introducing nuclear magnetic resonance techniques to an established research school, to further contacts with industry, and participation in the arrangements for the proposed new University at Guildford.

1965 Guggenheim International Astronautics Award :

Prof. M. V. Keldysh

THE Daniel and Florence Guggenheim International Astronautics Award for 1965 has been conferred on Prof. Mstislav V. Keldysh, of the U.S.S.R. Academy of Sciences in Moscow. Prof. Keldysh has made valuable contributions to the progress of astronautics and he is well known for his extensive research in problems of aerodynamics, fluid mechanics, rocket propulsion, and vibration theory. For several years he has been directing work on rocket propulsion and the mechanics of space flight. Prof. Keldysh has been president of the U.S.S.R. Academy of Sciences since 1961 and is also a member of the Praesidium of that Academy's Committee on Theoretical and Applied Mechanics and of the editorial boards of several scientific journals. He has received numerous awards in recognition of his achievements. He is a Corresponding Member of the Engineering Sciences Section of the International Academy of Astronautics. The Guggenheim International Astronautics Award, which is offered by the International Academy of Astronautics, carries with it a prize of 1,000 dollars.

U.K. Atomic Energy Authority and Central Electricity Generating Board Co-operation Scheme

To facilitate the close co-operation which already exists between the United Kingdom Atomic Energy Authority and the Central Electricity Generating Board, it has been decided that there should be cross-membership between the two bodies. Mr. F. Cousins, Minister of Technology, has accordingly appointed Mr. E. S. Booth, the engineering member of the Central Electricity Generating Board, to be a part-time member of the Atomic Energy Authority, and Mr. F. Lee, Minister of Power, has appointed Mr. J. C. C. Stewart, the Member for Reactors of the Atomic Energy Authority, to be a part-time member of the Central Electricity Generating Board.

Professional and Technical Staff in the Civil Service

IN a recent issue of *State Service*, the journal of the Institution of Professional Civil Servants (August 1965), the general secretary, M. W. McCall, commenting on the 1964 report of the Civil Service Commission, stresses the continuous failure to recruit sufficient professional and technical staff. Candidates could only be recommended for about a third of basic grade vacancies for engineers, although two-thirds of the vacancies at main grade and higher levels were filled. Similarly, while two-thirds of the vacancies for architects in these grades were filled, only one-sixth of those at the basic grade were filled. Only 85 out of 220 vacancies for mechanical and electrical engineers were filled and only 327 out of 546 vacancies in the technical classes. The Board of Inland Revenue is stated to be short of a fifth of its complement of professional valuers, the work of the Patent Office is delayed for a like reason, and the shortage of Factory Inspectors has been reflected for years in the low frequency of inspections. To remedy this situation, Mr. McCall urges that pay and careers must be offered to scientists, technologists and technicians which indicate that they are no longer regarded as inferior or second- and third-rate. Their training and talents should be used to the field in terms of the work they are qualified to do and they should have full opportunity of promotion to the highest posts.

Aid to Education Overseas

THE Overseas Development Institute has issued the report of a Ditchley Foundation Conference held at Ditchley Park during March 26-29, 1965 (*Aid to Education: An Anglo-American Appraisal*. Report of a Ditchley Foundation Conference held at Ditchley Park, March 26-29, 1965. Rapporteur: Peter Williams. Pp. 52. London: The Overseas Development Institute, in association with The Ditchley Foundation, 1965. 3s. 6d.). Dealing with educational aid, especially in Africa, Asia, and the Caribbean, it considers first the donor programmes and policies of educational aid, and second the requirements of the recipient countries. Next it sets forth some conclusions about priorities and the principles of educational aid before making specific suggestions for future Anglo-American co-operation in this field. Recognizing the need for a balance between education and the economy, the Conference gave much thought to the determination of priorities between types and levels of education. It also emphasized that educational aid is a two-way process and that the developing countries should not copy too rigidly the institutions and techniques of developed countries. It is emphasized that the developing countries will need educational assistance for a very long time ahead. The Conference strongly urged that Britain and the United States should, as the major donor countries, prepare themselves to meet the challenge by establishing permanent resource centres on which to draw in their aid programmes.

It suggests that it would be wise to establish small cadres of experts on a permanent footing and to create

supernumerary posts in Government departments, universities and other institutions. Moreover, appropriate arrangements should be made for training the personnel involved, so that specialists could be seconded for overseas assignments as required. In this way a permanent supply of experts would be ensured. The advantages of involving non-government institutions are also emphasized, as well as the need for a strategy of educational aid. It is suggested that existing forms of co-operation, like the Teachers for East Africa Scheme and programmes of joint support for overseas universities, should be extended and supplemented by new schemes. Specific areas for future co-operation should include the development of curricula and text-books, the teaching of English (application of modern techniques (like educational television and programme learning), educational research, the training of teachers, experiments in comprehensive secondary school education and work on methods of reducing the costs of education. Pilot projects and experiments should be financed so far as possible by aid donors. Direct linking of American, British and overseas teacher training institutes for various purposes is recommended as well as co-ordination of British and American programmes for teacher refresher courses in developing countries.

Water Affairs in Zambia

THE independence of Zambia came about in 1964 and inevitably brought with it great changes in the administration and conduct of the work of official organizations concerned with development of natural resources, including water. The Department of Water Affairs had to adjust itself to the new conditions, and how it set about this task is revealed in the recently published annual report of the Department of Water Affairs for the year 1964 (Pp. 9. Lusaka: Government Printer, 1965. 2s.). One of the major difficulties which had to be faced was the loss to the Water Department of many well-trained and experienced members of the professional and technical staff who decided to leave the country and seek a new life elsewhere. The report devotes considerable attention, and rightly so, to the serious staff position thus created. Notwithstanding this handicap, and the rather alarming number of vacancies existing in the various grades of water engineers and technical staff, actually 39 at the close of 1964, the amount of development work accomplished in many areas now under regional planning, including investigation and construction of new water works, appears quite remarkable. Special mention is made of the work of the Hydrological Branch in respect of the Kafue Basin Survey, where an investigation of the river system was considerably extended by installation of many new stations chiefly in the area between Mumbwa and the Copperbelt. Good progress was also made in rehabilitation of old drainage canals and also in construction of new canals in Barotseland. It is recorded that there has since been a notable improvement in drainage of areas affected by these works; crops can now be grown where formerly the land was far too wet. Further sections of the report summarize the present position of the Copperbelt water supply; other work of the Hydrological Branch; water projects in view; water communications and drainage in the Bangweulu Swamps; drainage and communications in Barotseland; the functions of the Water Board and of the Geological and Drilling Sections.

Scientific Research in the Development of Polish Industry

IN the *Review of the Polish Academy of Sciences* for January-March 1965, I. Malecki describes the role of scientific research in the development of Polish industry since the Second World War. The period 1945-52 was

largely one of reconstruction. During 1953-57 the technical base was expanded, and this period was marked by an extension of the industrial institutes, which in 1957 numbered 79, employing 22,600 scientific and technical workers, compared with 1,000 in the research institutes of the Polish Academy of Sciences. The next period, 1958-63, was notable for modernization and a general raising of the level of production, and has since been followed by one of systematization and development of the work of the research centres. Throughout these phases, Dr. Malecki emphasizes the attention given to the exploitation of natural resources and to the creation of the technical infra-structure; to improving the properties and production of novel technical materials; to the design of buildings and of major technological processes; and to the development of electronics and machine production. The situation of the medical sciences in Poland is described by K. Rowinski in a further article in the same issue. The physiological sciences are still inadequately developed in Poland, but the pharmacological and pharmaceutical sciences have contributed considerably to the supply of drugs. In biochemistry developments have been more in fundamental research than in the clinical field. Valuable work has been done in histology and in medical microbiology, and the clinical sciences are stated to be progressing favourably.

The Pharmaceutical Industry

In recent years the pharmaceutical industry in Britain, and, for that matter, overseas also, has been the target for much adverse criticism on several counts, notably high prices of some new drugs, inadequate clinical testing of newly marketed products, the problem of 'side effects', and a belief that profits in the industry overall are excessive and nationally unwarranted. That much of this criticism is ill-informed and unjustified is made clear in two recent publications which are worthy of a much wider circulation than they will probably achieve. The first is *Astra Annual Report 1964* (Pp. 40. Södertälje: Astra, 1965). This is in English and, in the course of his annual review, the President of the Astra Group says: "During the year there was a continued trend in most countries toward more rigorous legislation for pharmaceutical products. Behind this is the urgent desire to improve safety conditions for the consumer. However, the divergent regulations in various countries and the growing bureaucratic interference create great difficulties for the industry and delay the introduction of new drugs, yet have failed to ensure a corresponding degree of safety. Efforts are being made to produce more unified rules governing new drug registration". A much more detailed exposition of the problems of the industry to-day appears in a recent issue of *CIBA Journal* which, in a series of impressive articles lavishly illustrated, is devoted to the subject (33. Pp. 62. CIBA Ltd., Basle, Switzerland; Spring 1965). This is again in English and the titles of the articles are self-explanatory. The editorial foreword discusses at some length "The Intricate Mechanism of Drug Discovery, Manufacturing, and Control". R. Larose (CIBA Dorval) details something of "The Canadian Pharmaceutical Industry at Work". "The Viability of the British Pharmaceutical Industry" is ably revealed by A. W. Morrison (CIBA Horsham). An anonymous article entitled "Working in the Light: The New Drug Research Unit at Horsham" describes the modern building there now at the disposal of those engaged in the search for new and better pharmaceuticals (*Nature*, 207, 236; 1965). Prof. H. Bloch (CIBA Basle) writes on "The High and Rising Importance of Biology in Pharma Research". Dr. Matthys Staehelin (CIBA Basle) contributes an account of "Biochemistry—Cornerstone of Biological Research". It is impossible to read these articles without coming to the conclusion that, in spite of sins of omission and commission such as there may have been in the past, the international outlook of the industry

to-day is to work for the betterment of mankind and to spare neither energy nor money in giving the medical profession the tools it needs to alleviate human suffering. One gets the impression that Government interference in what is after all pure scientific research, if carried to excess, must inevitably defeat its own object. Freedom to explore, to formulate, to think ahead, untrammelled by obstructive regulations, is the privilege of every qualified worker in fundamental scientific research; in no case is this freedom more vital to the community than in the practice of pharmacology.

Load-bearing Brickwork

At a meeting of the Building Materials Section of the British Ceramic Society held at the Building Centre, Store Street, London, during November 18-19, 1964, nine papers were presented dealing with some fundamental properties of structural brickwork. These have now been published under the collective title *Load-Bearing Brickwork* (*Proc. Brit. Ceramic Soc.*, No. 4. (July 1965): *Load-Bearing Brickwork*. Pp. 121. Stoke-on-Trent: British Ceramic Society. 25s.). The first paper, by D. Lenzner, is on "Design of Creep Machines for Brickwork" and deals with the creep characteristics of brickwork and particularly the various creep machines designed for testing brick piers, walls and brick mortar. Next, M. E. C. Stedham contributes results of his further work to establish crushing tests of 9-in. brickwork cubes as an indication of the strength of brickwork built of the same materials, under the title of "Quality Control for Load-Bearing Brickwork". The third paper entitled "Measurement of Strains in Load-Bearing Brickwork", by R. N. Picken and A. W. Hendry, describes the construction, mode of use, and application of the vibrating-wire strain-gauge to the measurement of strains in the brickwork at the base of a brick chimney during dismantling at Hem Heath Colliery, Stoke-on-Trent. J. M. Plowman in "The Modulus of Elasticity of Brickwork" compares the elastic moduli obtained from brickwork made from bricks and mortar of wide ranges of strength. "Comparative Tests on $\frac{1}{4}$ - and $\frac{1}{2}$ -scale Model Brickwork Piers and Walls", by A. W. Hendry and C. K. Murthy, describes their investigations carried out to ascertain the suitability of model bricks; tests that had been carried out on full-size brick piers at the Building Research Station and on full-size walls at the Department of Building Science, University of Liverpool, were repeated on $\frac{1}{4}$ - and $\frac{1}{2}$ -scale models; their results show that, for given strengths of brick and mortar, subject to certain provisos, the strength of full-size brickwork can be reproduced with reasonable accuracy by means of model tests. The sixth paper by S. Prasan, A. W. Hendry and F. E. Bradshaw, entitled "Crushing Tests on Storey-height Walls 4.5 in. Thick", discusses the results of tests on seventeen walls 4.5 in. thick loaded between reinforced concrete slabs and their significance in terms of the various mechanical factors involved. A paper by L. G. Simms on "The Strength of Walls built in the Laboratory with some Types of Clay Bricks and Blocks" briefly describes loading-tests on walls of storey-height built in the laboratory (Building Research Station) with clay bricks and blocks. F. H. Clews discusses "Experiments to Assess the Durability of Bricks and Brickwork": an account of natural exposure tests, with examples of techniques applied by other workers, and of the methods used at the Mellor-Grøn Laboratories on fifty-nine batches of bricks and thirty-two batches of roofing tiles. Finally, a paper entitled "The Design and Economics of High Load-Bearing Brick Structures", by R. A. Sefton-Jenkins, dealing with the design of highly stressed brickwork, includes comparison of various codes of practice with the British, discussion of economics of load-bearing brickwork compared with other structural forms, and a plea for more research in this subject as a basis of a new British Code of Practice.

Thermal Comfort in Industry

In everyday circumstances of industrial and, for that matter, domestic life, the range of temperature and humidity that can be tolerated by the human body is really quite small. Human reaction to different thermal conditions, especially in factories and offices, is a subject of research falling within the ever-widening scope of ergonomics. In this context, there has recently been published a booklet by Dr. R. H. Fox, of the Division of Human Physiology, Medical Research Council (Ministry of Technology). *Ergonomics for Industry*, No. 8: *Thermal Comfort in Industry*. Pp. 52. London: H.M.S.O. Obtainable from Warren Spring Laboratory, Stevenage, Herts. 1965. *Gratis*. The essential theme of this guide-book (for that is the underlying purpose of each issue in the *Ergonomics for Industry* series so far available) is not only a discussion of human reactions to environmental conditions of temperature and humidity, but also the ways and means of ensuring that optimum conditions may be established. Quoting one specific instance (well illustrated), the author says: "Few people would choose to live in a greenhouse all the year round, yet the indoor climate in this modern office building is not very different". Thus attention is directed to the different conditions that different types of work require. It is recommended that buildings should be planned with the comfort and working efficiency of the occupants in mind. It stresses the problems encountered in some modern office buildings where large areas of glass prevail, with relatively little, if any, provision for air conditioning; such conditions result in overheating and consequent fatigue in summer, and coldness and discomfort in winter. Either of these extremes is inimical in the long run to welfare and efficiency. It is admitted that in some cases extremes of temperature occur and thermal control is impracticable; in the event, protective clothing and training are necessary; the principles involved in insulating individuals in such environments are outlined. The illustrations in this booklet are impressive, so is the advice and practical guidance throughout the text. Suggestions are made for further reading on this important subject of human and environmental relationship, while sources of specialist advice, and information on general ergonomic questions and on heating, ventilation and insulation of buildings, conclude a most practical exposition of the problems involved.

Science Information and Documentation Services

A *World Guide to Science Information and Documentation Services*, issued by Unesco, is intended primarily as a guide to the location of such services dealing with the natural sciences (Pp. 211. Paris: Unesco, 1965. 9 francs; 13s.; 2.50 dollars). Technology is to be dealt with in a separate volume, but services for agriculture and medicine are expressly included because of their paramount importance for the developing countries. The *Guide*, which is in both French and English, is based on 403 completed forms returned in a detailed questionnaire, and covers 144 institutions representing 85 countries. The institutions are listed under countries in alphabetical order of their titles and the entries indicate the subjects covered, approximate holdings and services offered. There is also an alphabetical list of institutions and a subject index. It might be noted that there are only three entries for the United Kingdom: Aslib; the National Lending Library for Science and Technology; and the Science Museum Library.

British Art, 1740-70

THE American Philosophical Society has rendered a notable service to art-history by publishing Prof. E. K. Waterhouse's Jayne Lectures for 1964 under the title *Three Decades of British Art, 1740-70 (Memoirs of the*

American Philosophical Society, 63. Pp. xii+77. Philadelphia: American Philosophical Society, 1965. 2 dollars). But these discourses can be interpreted more widely than that, namely, as a conspectus of what a certain cross-section of society was like at that period. In general, we note the advent of Italian influence, culminating in the Grand Style, and with it the works of such masters as Hogarth, Reynolds and Gainsborough. To men of science, there are several points of interest, but two stand out clearly. One is the statue of Handel by Roubiliac (illustrated), so complex and rather voluptuous, when one recalls that it is by the same hand as the ascetic and sublime "Newton" in Trinity College Chapel. How versatile and sympathetic the sculptor must have been to respond like that in his portrayal of two utterly different characters. The second matter is the 'character' (by Reynolds) of Raphael's *School of Athens*. This is indeed an odd fantasy—the great Greek philosophers and mathematicians replaced by eighteenth-century milords and their fellow-travellers, seen against a background of Gothic niches (in place of Raphael's classical statues). Sir Joshua was far too great a man to perpetrate this merely for fun. He was showing up his contemporaries, and incidentally instructing himself quite effectively. Altogether a most intriguing episode, described with exceptional insight.

University of Leicester, Department of Museum Studies

WITH the assistance of a £15,000 grant from the Gulbankian Foundation, the University of Leicester has established a postgraduate Department of Museum Studies. The University has appointed H. R. Singleton, at present director of the City Museum, Sheffield, to be the first director of this new Department. His duties will commence on January 1, 1966, and it is expected that the first students will be admitted in the following October. Initially, the intake will be confined to graduates with qualifications in archaeology or geology, but in subsequent years it is hoped to accept graduates from other disciplines. The one-year, full-time course for a Certificate in Museum Studies will be designed to offer intending entrants to the museum profession a preliminary training in museum administration and organization, in the museum application of their particular subject, and in the practical side of museum work.

National Museums of Southern Rhodesia

THE report of the Trustees and Directors of the National Museums of Southern Rhodesia for the year ended December 31, 1964, records the probably unique event of the opening of three new museum buildings within one year, namely, the National Museum, Bulawayo, the Queen Victoria Museum, Salisbury, and the Umtali Museum (Pp. 25. Salisbury: National Museums of Southern Rhodesia, 1965). With the future in mind, each of the buildings is designed to allow for expansion, and the sites have sufficient ground reserved for extension. Although the fittings in the exhibition galleries were far advanced for the opening ceremonies, much still remains to be done. This great development has come at a time when political considerations in Africa have disturbed the progress which took place in the immediate post-war years. As a result, funds are not so freely available as formerly, and so, with Government sanction, it was decided to levy an entrance charge to help to offset the greatly increased maintenance costs on buildings some two-and-a-half times the size of the old. In view of the educational nature of the organization, school parties are admitted free.

Meat from Wild Animals in Africa

In the past decade increased attention has been directed towards the management and potential utilization of wild

imals in Africa as a natural resource in order to assist filling the world's larder. The more scientific investigation work which has been carried out in this field has been brought together by the Commonwealth Agricultural Bureaux in a recent publication, issued as *Technical Communication* No. 16, entitled *The Meat Production Potential of Wild Animals in Africa—A Review of Biological Knowledge* (By Lee M. Talbot, W. J. A. Payne, H. P. Ledger, Norma D. Verdcourt and Martha H. Talbot. Pp. vii+42. Barnham Royal, Bucks.: Commonwealth Agricultural Bureaux, 1965. 13s. 6d.). This directs attention to the possibilities of exploiting some of the game animals in under-developed countries. The review points out that the African continent contains the greatest diversity of indigenous large mammals to be found anywhere in the world. The wild ungulates considered range from the 10-lb. dik-dik to the 5-ton African elephant. The determination of the numbers of animals present in an area has represented a major problem. In general, the most accurate estimations were made from light aircraft, and the results agreed closely with those obtained by Royal Air Force photo-reconnaissance planes. Some animals were marked so that they could be recognized later and their movements and growth-rates followed. Agriculturists and meat technologists will be interested in the figures given under the heading "Carcase Composition". Killing-out percentages quoted include the following: East African zebu steers, 40 to more than 60; wildebeest, 50; topi and xongoni, 53; and impala and gazelle, both about 60. In general, the amount of fat is consistently low, so that the carcase yield is relatively constant within a given species.

Soil Catena

SINCE Milne used the word 'catena' as a mapping unit in East Africa, some thirty years ago, to describe a sequence of soils, extending from hill top to valley bottom, which is repeated across the contours of the landscape, it has been interpreted in various ways in different countries. Such matters as parent material, erosion, intensity of leaching and mobility of sesquioxides have tended to complicate the original concept, which was intended to apply to one climatic zone, a medium-rainfall area of the tropics, and to differences in level. It is most useful, therefore, to have a documented statement on the subject by J. P. Watson in *Soils and Fertilizers* (28, No. 4, 307; 1965). The same number contains a review by Dan H. Yaalon of a Russian book on *Soil Geographical Zoning* of the U.S.S.R., and a description by W. E. Calton of a soil-map of Africa (scale 1 : 5,000,000) with an explanatory monograph by J. L. d'Hooze. The former is a systematic inventory of the natural soil and climatic resources of the U.S.S.R.; the latter is a large inventory of the soils of Africa with seven sheets of soil maps.

Soil Survey of Great Britain

THE work completed and in progress during 1964, under the auspices of the Soil Survey Research Board of the Agricultural Research Council, is described in its seventeenth report (Pp. v+46. London: Agricultural Research Council. Obtainable from H.M.S.O. 1965. 6s. net). Publications include a memoir on "The Soils and Land Use of the District around Aylesbury and Hemel Hempstead", two bulletins on the soils of the Middle Teign Valley and of six counties in the West Midlands, and a soil map of 432 square miles (sheet 57) around Forfar. Two more memoirs on the Mendip District and on the country around Haddington and Eyemouth, and eleven sheets of soil maps, are in the press. Members of staff of the Soil Survey also contributed 21 papers to scientific journals and conferences. This report contains short accounts of the nature of the soils found in various parts of Britain, with emphasis on unusual features, and some classifications of soil series. There is also a summary of

the laboratory investigations that have been carried out. The Soil Survey is frequently called on to make special surveys, and the assistance rendered to such authorities as the Forestry Commission, the Nature Conservancy, the Agricultural Advisory Services, and planning and archaeological departments is briefly recorded. There are also two maps showing the areas in which field work has been completed or is in progress.

Pituitary Transplants in the Teleost

J. N. BALL and his colleagues have taken very effective advantage of the development of a technique for hypophysectomizing the viviparous teleost *Poecilia (Mollinnesia) formosa*, and for establishing homografts of the pituitary (*Phil. Trans. Roy. Soc., B: Functional Capacity of Ectopic Pituitary Transplants in the Teleost Poecilia formosa, Comparative Discussion on the Transplanted Pituitary*. By J. N. Ball, Madeleine Oliverreau, Anna M. Slicher, and K. D. Kallman. Pp. 69-99+plates 18-21. London: The Royal Society, 1964. 18s.; 2.70 dollars). The ectopic gland survives, but without the direct link with the hypothalamus on which its relationships with the central nervous system normally depend. It has thus become possible to analyse with some precision the degree to which the various ad-nohypophysial functions are influenced by signals from the central nervous system. In some respects the results are similar to those that have been reported from studies of the ectopic pituitary in mammals. Abundant prolactin is secreted by the ectopic graft, but there is a marked reduction or elimination of the secretion of growth hormone and gonadotrophins. But the teleosts have pursued their own independent line of evolution, and it is not surprising, therefore, to find some interesting points of difference in their hypothalamo-adenohypophysial relationships. Corticotrophin secretion seems to be less dependent on hypothalamic stimulation than it is in mammals and in this respect there is some resemblance between *Poecilia* and amphibians. More unexpected is the finding that the ectopic adenohypophysis of the fish secretes thyrotrophic hormone at a higher rate than normal. This means that in the intact animal the hypothalamus must be exerting an inhibitory influence on this function, whereas in mammals, as is well known, the influence is excitatory. The situation in amphibians in this respect is somewhat obscure, although there is certainly some evidence for an excitatory influence. The authors conclude that at present *Poecilia* seems to be peculiar in the way in which its thyrotrophin secretion is regulated.

New Zealand Geochemical Group

At the University of Otago on August 18, 1965, during the annual conference of the New Zealand Institute of Chemistry, a meeting attended by twenty-one interested persons unanimously approved a motion by Prof. D. S. Coombs, seconded by Mr. A. H. Horn, to form a New Zealand Geochemical Group. Officers elected were Dr. J. Rogers, chairman, Mr. S. H. Wilson, secretary, and Dr. A. Ewart, with power to co-opt. This motion crystallized a recommendation from a meeting chaired by Dr. A. J. Ellis at Lower Hutt during the centenary of the New Zealand Geological Survey. The New Zealand Geochemical Group plans to promote discussion and co-operation in geochemical problems between scientists of different disciplines and backgrounds by a newsletter and symposia at conferences. Liaison is also proposed with geochemical societies overseas. It is hoped to arrange a meeting of the Group during the international symposium on "Volcanology" in New Zealand between November 21 and December 3, which a considerable number of geochemists from overseas will be attending. Anyone interested in the Group is invited to register with Mr. S. H. Wilson, Institute of Nuclear Sciences, Private Bag, Lower Hutt.

Bryozoan Research

SIXTEEN zoologists and palaeontologists from nine countries were participants in the first international conference on "Post-Palaeozoic Bryozoa" held at the University of Stockholm, Sweden, during May 24-31, 1965, with Ivar Hesseland and the Geological Institute as hosts. To assure continuation of frequent and informal exchange of information, ideas, and techniques, the conference established the International Bryozoology Association, membership in which is open to all workers engaged in research on Bryozoa, Palaeozoic as well as post-Palaeozoic. Plans are being made for a second conference in 1968. The Stockholm conference included four days of informal discussion (led by A. H. Cheetham) of morphology, systematics, biometrics, ecology-palaeoecology, and stratigraphy of post-Palaeozoic Bryozoa and four days of collecting (led by Sten Schager and Ole Berthelsen) of Bryozoa from Senonian-Danian deposits in southern Sweden (Scania) and eastern Denmark (Zealand). Further information about the International Bryozoology Association or the *Proceedings* of the Stockholm conference can be obtained from A. H. Cheetham, Geological Institute, Kungstensgatan 45, Stockholm Va.; or from P. L. Cook, Department of Zoology, British Museum (Natural History), London, S.W.7.

The Official University of the Congo at Elisabethville

THE July 1965 issue of Publications of the Official University of the Congo at Elisabethville contains an "Introduction to the Study of Economic Development: General Theories" by A. Cecchella, who describes representative theories of the evolution of economic thought on problems of development (*Publications de l'Université Officielle du Congo à Elisabethville*, 8. *Introduction à l'Étude du Développement Économique les Théories Générales*, by Aldo Cecchella. Pp. 158. Elisabethville: Université Officielle du Congo à Elisabethville, 1965). There are four chapters. The first deals with economic development in the classic thought of J. B. Say, D. Ricardo and R. Malthus, and the second with the Marxist analysis of economic development. The third is directed to the work of J. Schumpeter and the fourth to the Keynesian analysis of modern theories of economic development.

Announcements

DR. F. M. LEVER has been appointed manager of the Research Laboratories of Johnson, Matthey and Co., Ltd. He succeeds Dr. J. C. Chaston, who has retired from the Company. For the past eighteen years Dr. Lever has been head of Chemical and Extraction Metallurgy Research in the Laboratories.

MR. P. M. J. WOODHEAD, senior scientific officer at the Fisheries Laboratories of the Ministry of Agriculture, Fisheries and Food, Lowestoft, has been appointed to the position of scientific and administrative officer at the Heron Island Research Station of the Great Barrier Reef Committee in Queensland, by the University of Queensland.

THE second edition of the "Biographical Index of Members, Correspondents and Associates of the Royal Academy of Belgium" covers the years 1769-1963, correcting and completing the first edition published in 1948 (*Index Biographique des Membres, Correspondants et Associés de l'Académie Royale de Belgique de 1769 à 1963*. Pp. 299. Bruxelles: Académie Royale des Sciences, des Lettres et des Beaux-Arts de Belgique, 1964). The entries indicate the field of work of the member; for deceased members, reference is given to obituary notices or other tributes.

THE *Annals of the Library Association, 1877 to 1960*, edited on behalf of the Library History Group by W. A.

Munford, is a chronological record of the main even affecting the work of the Association since its foundation in 1877 (Pp. 128. London: The Library Association, 1965. 22s.; L.A. members, 16s. 6d.). Mainly of interest to librarians, it includes dates of publication of certain reports, books or pamphlets which may be of wider utility.

A SYMPOSIUM on "Cellular Plastics", the next in the series of Borough Polytechnic symposia on "Plastics and Polymer Technology", will be held at the Borough Polytechnic during November 4-5. Further information can be obtained from the Secretary, Borough Polytechnic Borough Road, London, S.E.1.

A CELEBRATION conference, to mark the 200th anniversary of the founding of the Freiberg Mining Academy in East Germany, will be held at the Academy during November 9-14. Further information can be obtained from Mardie Henry, Lex Hornsby and Partners, Ltd. Wellington House, 125 Strand, London, W.C.2.

A ONE-DAY conference on "Access to Information", to discuss the implications of a national bibliographical centre and any other ideas which might improve access to information, will be held in the Beveridge Hall, Senate House, University of London, on October 26. Further information can be obtained from the Research Officer Library Association, Malet Place, London, W.C.1.

A MEETING of the Photobiology Group will be held at the Chester Beatty Research Institute, London, on October 27. The programme will include a paper and conversation on monochromators for photobiology. Further information can be obtained from Dr. J. D. Moreland, Institute of Ophthalmology, Judd Street, London, W.C.1.

THE eighteenth annual conference and exhibit on "Engineering in Medicine and Biology", sponsored by the Institute of Electronic and Electrical Engineers and the Instrument Society of America, with participation of the American Society of Mechanical Engineers, will be held in Philadelphia during November 10-12. Further information can be obtained from Dr. H. P. Schwan, University of Pennsylvania, Philadelphia, Pennsylvania.

THE third international conference on "Hyperbaric Medicine", sponsored jointly by the Duke University Medical Center and the Committee on Hyperbaric Oxygenation of the National Academy of Sciences—National Research Council, will be held in Durham, North Carolina, during November 17-20. Further information can be obtained from the Secretariat, Box 2928, Duke University Medical Center, Durham, North Carolina.

THE annual Pittsburgh Diffraction Conference will be held at the Mellon Institute, Pittsburgh, during November 3-5. The programme will include (1) special sessions on metals, ceramics, structures, crystal physics (lattice dynamics and crystalline imperfections) and instrumentation and techniques, and (2) a symposium on "Texture and Preferred Orientation in Polymers". Further information can be obtained from Paul A. Flinn, Carnegie Institute of Technology, Pittsburgh, Pennsylvania 15213.

ERRATUM. In the communication entitled "Response of Time-dependent Materials to Oscillatory Motion" by Dr. J. Harris, which was published on p. 744 of the August 14, 1965, issue of *Nature*, equation (5) should read as follows:

$$M(t - t') = \int_0^{\infty} \frac{R(\Pi)}{\Pi} \exp - \left(\frac{t - t'}{\Pi} \right) d\Pi \quad (5)$$

SPECTROSCOPY IN AUSTRALIA

THE fifth Australian Spectroscopy Conference, sponsored by the Spectroscopy Committee of the Australian Academy of Science and organized by a Committee under the chairmanship of Dr. A. R. H. Cole, was held at Perth, at the University of Western Australia, during May 31–June 2.

The Conference was opened by Dr. A. L. G. Rees, who welcomed those attending of whom fourteen were from overseas. The programme consisted of morning and afternoon sessions on each of the three days, usually with concurrent lectures on topics in different sections of spectroscopy. The sections were infra-red and Raman spectroscopy; visible, ultra-violet and theoretical spectroscopy; nuclear and electron spin resonance spectroscopy; atomic absorption spectroscopy; mass spectroscopy and X-ray fluorescence and electron absorption spectroscopy. More than eighty-five papers were presented, of which nine were review papers by invited speakers.

The section on infra-red and Raman spectroscopy opened with a review paper given by Prof. N. S. Sheppard (University of East Anglia) on infra-red band shapes and rotational motion in condensed phases. It was shown that simple molecules such as methyl chloride undergo at least hindered rotation in solution while molecules adsorbed on to surfaces may rotate about the bond linking the molecule to the surface.

The first of the succeeding papers of this section was a report by A. D. E. Pullin (Monash University) on the operation of a commercially made interferometer designed to measure infra-red spectra down to 20 cm^{-1} . A brief outline of the theory of operation and the factors determining spectral range and resolution was given. Examples of spectra obtained were shown.

Several succeeding papers discussed the use of infra-red and Raman spectra to assign particular molecules to their correct point group or to indicate preferred conformations of flexible molecules. J. F. Horwood (C.S.I.R.O., Division of Dairy Research, Melbourne) and J. K. Wilmschurst (C.S.I.R.O., Division of Chemical Physics, Melbourne) suggested that dimethyl oxalate in the liquid and vapour phases was non-planar with point group symmetry C_2 . E. Spinner (Australian National University) reported Raman polarization data for aqueous solutions of formates and pointed out that these were consistent with C_2 rather than C_{2v} symmetry for the formate ion. N. S. Ham (C.S.I.R.O., Division of Chemical Physics, Melbourne) discussed the spectra of thionyl aniline, C_6H_5NSO , and showed that it was consistent with a *cis* planar CNSO structure though the *trans* structure could not be eliminated. R. A. Cummins (Defence Standards Laboratories, Melbourne) discussed the preferred conformations present in alkyl tin compounds of the type R_nSnCl_{4-n} , and H. Lee and J. K. Wilmschurst (C.S.I.R.O., Division of Chemical Physics, Melbourne) the conformations of trimethyl orthoformate and tetramethyl orthocarbonate. The vibrational data are consistent with an unsymmetrical *trans-gauche-gauche* arrangement of the methyl carbons with respect to the formate hydrogen in trimethyl orthoformate and with a distorted S_4 structure for the tetramethyl carbonate.

In a second paper concerned with the infra-red spectra of several acetates (hydroxyanthraquinone acetate, phenyl acetate, α and β naphthyl acetates) these authors attribute the observed doubling of the carbonyl stretching absorption to Fermi resonance with combination tones. A. R. H. Cole and L. M. Engelhardt (University of Western Australia) analysed the rotational fine structure of several bands of acrolein in terms of an approximate symmetric top and showed that the data are consistent with the existence of only the *trans* form. Analyses of vibration-rotation bands of methylphosphine and isotopic deriva-

tives, which are also approximate symmetric tops, leading to values of $\frac{1}{2}(B + C)$ in agreement with microwave data were reported by A. G. Moritz (Defence Standards Laboratories, Melbourne). In a later paper A. G. Moritz discussed the vibrational assignment of CH_3SCN and related molecules. G. C. Barraclough and M. Sinclair (University of Melbourne) discussed the vibrational assignment of dicarbonyldinitrosyl iron, and D. E. Clegg (University of Queensland) that of $(CH_3)_3Pt(NH_3)_2^+$ ($(CH_3)_3Pt(NH_3)_2Cl$ in water and deuterium oxide). M. S. Farrell and T. L. Whateley (A.A.E.C. Research Establishment, Lucas Heights) reported the infra-red spectra of beryllium nitrate complexes with organic-phosphorus compounds such as tri-*n*-butyl phosphate, tri-*n*-octyl phosphine oxide and di-(2-ethylhexyl)-phosphoric acid. B. H. James and P. E. Rogasch (Weapons Research Establishment, Adelaide) discussed the changes of the in-plane bending frequencies of substituted phenols with respect to electronic effects and hydrogen bond formation, and R. H. Laby (Department of Agriculture, Melbourne) and T. C. Morton (School of Biochemistry, University of Melbourne) discussed inter- and intra-molecular hydrogen bonding in hydroxy- and nitro-hydroxy-coumarins.

Three papers on the use of infra-red spectroscopy in adsorption studies were presented: M. Clark, B. A. Morrow, N. Sheppard and J. W. Ward (University of Cambridge and the University of East Anglia) discussed the infra-red spectra obtained from the examination of the systems olefine-silica supported nickel (or supported platinum)-hydrogen; N. W. Cant and L. H. Little (University of Western Australia) the spectra for ammonia and deuterio ammonia adsorbed on silica; and W. I. Stuart and T. L. Whateley (A.A.E.C. Research Establishment, Lucas Heights, N.S.W.) the spectra of carbon monoxide, of carbon dioxide, and of water on magnesium oxide and on beryllium oxide. Below 200°C carbon dioxide is chemisorbed on beryllium oxide, to form a carbonate and also a singly charged anion, probably CO_2^- . Above 300°C only the carbonate is formed.

Several infra-red investigations of compounds of biological interest were described. A. J. Michell described polarized infra-red studies of some monosaccharides, related to cellulose and xylan, which were chosen because their crystal structures had been investigated and because they were more easily crystallizable than higher members of the sugar series. E. G. Bendit (C.S.I.R.O., Division of Textile Physics, Sydney) discussed the changes of the infra-red spectra of α - and β -keratin of horse-tail hair on hydration and deuteration. Deuteration tests on α -keratin showed that helical peptide NH groups begin to deuterate immediately, while non-helical peptide NH groups continue to exchange throughout the process. R. D. B. Fraser and E. Suzuk (C.S.I.R.O., Division of Protein Chemistry, Melbourne) considered the roles of inter- and intra-molecular hydrogen bonds in modifying the characteristic vibrations of polypeptides. In an interesting paper R. H. Laby (Department of Agriculture, Melbourne) discussed the infra-red spectra of glycine, glycyglycine and β -alanine in aqueous salt solutions and related spectral changes to conformational changes brought out by differing salt solutions.

Two interesting papers in this section were contributed by visiting Indian scientists. Prof. R. S. Krishnan (Indian Institute of Science, Bangalore) gave an account of the theory of Raman spectra of cubic diatomic crystals; and Prof. C. N. R. Rao (Indian Institute of Technology, Kanpur), in a paper on charge transfer and hydrogen-bonded complexes of π -donors, surveyed correlations of spectroscopic and thermodynamic data with substituent effects and other structural parameters. Also concerned with complexes of the charge-transfer type was a paper by

N. F. Cheetham and A. D. E. Pullin (Monash University), who described an infra-red study of molecular complexes between perfluoroalkyl bromides (and iodides) or perfluoroaryl bromides (or iodides) and tertiary amines. The stoichiometry of the complexes had been previously determined by phase studies.

Of wide interest was a paper by G. R. Hunt and J. W. Salisbury (Lunar and Planetary Research Station, Bedford, Massachusetts) on the infra-red techniques used in an attempt to gain information about the silicates present in selected areas of the Moon's surface. These techniques were based on selective reflexion of the radiation by silicates of the various chemical types that might be present on the Moon's surface. A standard spectrometer, suitably modified, was used in conjunction with 42-in. and 69-in. reflecting telescopes.

Many of the papers presented in section B, that on visible, ultra-violet and theoretical spectroscopy, reflected the present-day strong interest in problems peculiar to transitions in condensed phases on one hand, and on the other to the new problems that obtrude when the spectra of larger molecules are examined in the vapour phase by high resolution methods.

Prof. D. P. Craig (University College, London), in an invited paper, reviewed the recent advances in the theory of molecular excitons. Prof. Craig described first the modifications of the theory of crystal spectra consequent on taking into account the finite time of propagation of electrical fields in solids, and then discussed, among other topics, how application of theory to crystal spectra could give information on long-range interactions between molecules. Prof. Craig ended his lecture by showing how information about the degree of order in melts could be obtained from their electronic spectra, taking azulene as his example. In a subsequent paper R. G. Body (University of Sydney) gave the results of a mathematical investigation, based on the principles of coupled oscillators, of the positions and polarization of absorptions in crystals due to impurities. Approximate formulae derived enabled the normalized second moment of the energy-level distribution in the pure crystal to be deduced from the spectra of isotopic impurities. Under certain conditions non isotopic impurities can display striking intensity anomalies. The theme of solid-state spectra was also the subject of papers by G. W. Robinson (California Institute of Technology) on exciton structure in benzene, who gave experimental data on the exciton splitting in the benzene ${}^1B_{2u}$ electronic state and in a number of vibrational levels in the ground electronic state, and by M. Chowdhury, D. McClure and E. Zalewski (University of Chicago), who described an investigation of the Zeeman effect on the singlet triplet spectrum of pyrazine. Single crystals at $\sim 4^\circ \text{K}$ and pulsed magnetic fields up to 200,000 gauss were used. Two bands, believed to be two Davydov components, at 26,254 and 26,261 cm^{-1} were assigned, from their polarization and Zeeman effect characteristics, to an electric dipole transition along the N—N axis and to a magnetic dipole transition respectively. Later in the Conference, in an invited address, Prof. McClure described the way in which spectroscopic states of various ions are split when they are subjected to crystal fields of cubic lower symmetry. He illustrated this for various transition elements and rare earth ions, and showed how some complex crystal spectra yielded to this form of analysis. B. N. Figgis and L. G. B. Wadley presented preliminary polarization data on a charge transfer band at about 24,000 cm^{-1} in dilute solid solutions of potassium ferrioxalate in potassium cobalt cyanide. Several papers concerned with solvent effects on electronic bands were presented. N. S. Bayliss and G. Wills-Johnson (University of Western Australia) examined the effect of solvents on the intensities of electronic bands of molecules in solution by perturbation methods, deriving the perturbation from the solvent reaction field. The shortcomings of, and possible improvements to, this approach were outlined.

The predictions of the theory were compared with experimental results for the $n \rightarrow \pi^*$ transitions in aliphatic ketones and nitroparaffins in solution. D. W. Jamieson (University of Queensland) and C. R. Boston and G. M. Smith (Oak Ridge National Laboratory, U.S.A.) described the effects of change of temperature and change of cation species on the weak band at $\sim 195 \text{ m}\mu$ and the strong band at $\sim 300 \text{ m}\mu$ in molten nitrates in terms of coulombic interactions, variations in environmental symmetry and variation in vibrational energy.

The third invited speaker was Dr. D. A. Ramsay (National Research Council, Ottawa). Dr. Ramsay began his address by pointing out that in general, in electronic spectra, doppler motion broadens lines sufficiently to limit useful resolution to one in half a million, a natural limit attained by the best present-day spectrographs. For larger molecules, the lines of which are closer than this the observed band contour can be compared with contour computed from overlapping line intensities. (Later in the conference the application of this procedure to part of the azulene spectrum was described by A. J. McHugh of the University of Sydney.) Dr. Ramsay then turned his attention to spectra of smaller molecules recently obtained at the Ottawa laboratories. The structures of glyoxal in the ground and excited states were being deduced from its spectra. Preliminary estimates of bond-lengths for the first excited singlet state were in agreement with predictions of molecular orbital theory for conjugated molecules of this type (glyoxal, butadiene, etc.). After reference to recently obtained spectra of the allyl and glyoxal radicals, Dr. Ramsay described techniques being developed at the Ottawa laboratories to aid the analysis of spectra. The 'J sorting' technique was based on the injection of microwave power to disturb the rotational levels of one of the combining states. This technique was limited to molecules having a microwave spectrum. In a later paper with Prof. A. D. Buckingham (University of Bristol), Dr. Ramsay described experiments carried out at the Ottawa laboratories on the use of Stark modulation to help identify the type of transitions. An alternating voltage was applied to the plates of a Stark cell at 400 c/s and the Stark-modulated spectrum recorded at high resolution with the photomultiplier detector connected to a lock-in amplifier tuned to 800 c/s so that only lines with appreciable Stark effect were recorded. Up to 80 kV was applied across the 3-cm width of the 2-m long cell. A relatively high pressure ($\sim 300 \text{ cm}$) of SF_6 was introduced in the cell to prevent electrical breakdown. For a symmetric top the Stark splittings are dependent on $\mu^2 K M / J(J+1)$ so that Stark modulation favours the selection of lines of higher K and lower J values. By use of polarized light the Q lines can be identified as those more prominent in parallel polarization and those more prominent in perpendicular polarization as P and R lines. As well as helping to assign lines as P , Q or R , dipole moments of molecules in excited states could be obtained from Stark splittings.

In the field of vapour phase aromatic spectra G. C. Morris (University of Melbourne) presented results on band positions, oscillator strengths and assigned transitions for the region 20,000–54,000 cm^{-1} of the absorption spectra of naphthalene. J. P. Byrne and I. G. Ross (University of Sydney) considered the cause of spectral diffuseness: not all diffuseness is due to predissociation; plain congestion of the spectrum can be a cause in larger molecules. L. M. Logan and I. G. Ross (University of Sydney), in a paper concerning molecular fluorescence in the vapour state, considered the interaction between the original excited vapour state and the vibronic states of the lower electronic levels. R. H. Kennet (Weapons Research Establishment, Adelaide) described a method for the determination of the intensity distribution in an unresolved molecular band, and described features of the profile that are dependent only on temperature and from which the temperature may be deduced.

In an interesting paper C. E. Kendall (National Biological Standards Laboratory, Canberra) attempted to delineate more closely the possible connexion between the carcinogenic activity of molecules and their electron donor characteristics by using experimental data and energies of the highest occupied molecular orbitals of the donors to prepare a common index of donor activity for comparison with charge transfer data. When this comparison is made—for example, in the form of a plot—complexes of early all carcinogens lie in a fairly narrow range, especially when the common acceptor is chloranil. Possibly some type of electron transfer may be involved in a cell, with an electron acceptor of similar affinity to chloranil. K. Selinger (School of General Studies, Canberra) in a paper on excimer formation described an investigation of the effect of pressure on the kinetics of formation of excited dimers by molecules in the excited state. Where the activation energy of dimer formation is low, the increase in viscosity with pressure does not affect the process and the decrease in volume accompanying excited dimer formation can be measured.

The diversity of uses of spectroscopic techniques was exemplified by a paper by K. J. Taylor and P. D. Jarman (University of Western Australia) on an aspect of sonoluminescent spectra. When cavities in liquids, produced ultrasonically, collapse, thermally excited radiation is emitted. If the liquid contains a dissolved alkali metal salt, the alkali lines appear in emission displaced and much broadened by interactions with the cavity contents. From these data the relative density of the cavity contents at the time of light emission can be calculated.

Instrumental aspects of spectroscopy were represented by several papers. Dr. S. E. Williams (University of Western Australia), in a review of the development of spectroscopy in the spectral range 1000–20 Å, described the many experimental problems encountered and showed examples of spectra in this region. Dr. Williams emphasized the importance of isoelectronic sequences in the interpretation of such spectra. S. C. Baker (University of Newcastle) described an Ebert-type scanning spectrometer with a resolving power of 500,000 in the tenth order green, and K. J. Taylor and P. D. Jarman described an auto-spectrophotometer for a low-intensity source. The prism can be rotated in either direction in steps by a motor driven by electric pulses derived from a logic circuit which also addresses the integrated output pulses from the photomultiplier into a 400-channel analyser. The memory of the analyser can then be read out as desired.

In a paper on the electronic emission spectrum of the diatomic molecule IF, R. A. Durie (C.S.I.R.O., Division of Coal Research, Sydney) summarized the analysis of the $A'E \rightarrow X_{\pi}^2$ transition and the derived molecular constants. C. Candler (Bendigo Technical College, Bendigo) presented a paper on the mechanism of dye sensitization of photographic emulsions.

Two other invited review papers related to electronic spectroscopy were given during the conference. Dr. Wilse Robinson (California Institute of Technology) gave a stimulating account of photosynthesis from a spectroscopist's point of view, stressing the essentials and the two-quantum process involved; and Prof. R. D. Brown (Monash University) outlined the best methods now available for calculating the energies of excited states of molecules. Introduction of configuration interaction led to a large number of integrals. Their number could be reduced by the approximation of zero differential overlap. Improved energies could be obtained by semi-empirical corrections to decrease nearest neighbour repulsion integrals to allow for the effects of electron correlation. Prof. Brown ended his address by showing how the calculated energies of the electronic states of benzene varied according to which of the up-to-date methods of correcting these integrals was adopted.

Two papers in this section formed a close link with those in the mass spectroscopy section. J. D. Morrison

(C.S.I.R.O., Division of Chemical Physics, Melbourne) discussed the application of extended Hückel type calculations on simple molecules to determine if possible the location from which an electron will be ionized and, if the resultant ion dissociated, the most probable fragments and their structures, and A. J. C. Nicholson (C.S.I.R.O., Division of Chemical Physics, Melbourne) reported an investigation of photo-ionization efficiency curves. Ionization potentials of methane, ethane and propane, claimed to be closer to the adiabatic values than those extant, were reported, and it was shown how, from a comparison of photo-ionization efficiency curves, it is possible to identify vibrational and excited states of specific molecular ions.

Section C, dealing with nuclear and electron spin resonance spectroscopy, covered a fairly wide range of interests despite the small number of papers presented.

The analysis of high-resolution nuclear magnetic resonance spectra was considered in a paper by S. N. Stuart (C.S.I.R.O., Division of Chemical Physics, Melbourne), who showed how it was possible to systematize the use of quantum mechanical trace relations in such analyses and illustrated some of the simplifications thus arising.

In the field of proton resonance spectroscopy, the paper by P. J. Black, R. D. Ewens and M. L. Heffernan (Monash University) showed how it was possible, after appropriate corrections had been applied, to correlate proton shifts with molecular orbital electron densities of aromatic heterocycles. R. G. Hillis (Defence Standards Laboratories, Victoria) and N. S. Ham (C.S.I.R.O., Division of Chemical Physics, Melbourne), in studies on a number of methyl-sulphur compounds, used the relation between proton chemical shifts and $^{13}\text{C-H}$ coupling constants to estimate the anisotropy effects present in these substances. The effectiveness of natural abundance ^{13}C -nuclear magnetic resonance spectroscopy as a tool for the analysis of coal derivatives was well illustrated in the paper by R. A. Friedel and H. L. Retcofsky (Bureau of Mines, Pittsburgh Coal Research Centre).

Broad line NMR was well represented by three papers. C. K. Coogan (C.S.I.R.O., Division of Chemical Physics, Melbourne) discussed the investigation of charge distributions in the ionic crystals LiOH and NaSH and showed how a number of other crystal properties could be investigated with NMR. A similar investigation of ionic crystal with a linear triatomic anion was reported by I. D. Campbell (C.S.I.R.O. Division of Chemical Physics, Melbourne). G. J. Jenks (Defence Standards Laboratories, Melbourne) demonstrated the use of tin and nitrogen resonances in the assignment of a structure to tri-*n*-butyltin thiocyanate and reported the presence of an ^{119}Sn - ^{15}N coupling constant.

Electron spin resonance spectroscopy was represented by one paper. K. Kenny (Washington University, St. Louis, U.S.A.) indicated how the use of multi-channel analyses has facilitated the detection of transient free radicals. The application of the technique to living aquarium plants was described.

The section on atomic absorption revealed exciting developments in all aspects of the field. In his invited contribution, A. Walsby (C.S.I.R.O., Division of Chemical Physics, Melbourne) described the successful development by him and his colleagues of several new systems for enhancing and isolating resonance radiation. These include a hollow cathode lamp having a subsidiary discharge that selectively intensified resonance lines 100-fold without increasing line width, and resonance monochromators that can be used both with conventional and high-intensity lamps and that offer the possibility of simultaneous determination of a number of elements. A. Hell (Beckman Instruments, U.S.A.) discussed the optical and mechanical factors involved in obtaining optimum performance from a laminar flow burner and described means of extending analytical range by the use of an infra-red heated spray chamber and solvent conden-

sation system. The addition of 12 elements to those previously determinable in aqueous solution and a general alleviation of chemical interference by the use of high-temperature flames employing O_2-N_2 mixtures and N_2O with acetylene was revealed by J. B. Willis (C.S.I.R.O., Division of Chemical Physics, Melbourne) in a joint paper with M. D. Amos (Techtron, Victoria). In another paper, J. B. Willis stated that examination of atomizer design has resulted in considerable improvement in performance being achieved by precision in construction and by providing means of accurately positioning the capillary. J. V. Sullivan (C.S.I.R.O., Division of Chemical Physics, Melbourne) discussed the construction and performance of high-intensity lamps. The increase in the intensity of the resonance lines has been achieved by exciting the metal atoms, produced by a hollow-cathode discharge with a secondary discharge electrically isolated from the former. He then described a high-intensity lamp which incorporates an additional hollow cathode which selectively modulates the resonance lines and permits the use of low-resolution monochromators for the isolation of resonance lines in complex spectra. W. Slavin (Perkin-Elmer, U.S.A.), in tracing the development of burners, described a design having multiple slots for producing an $N_2O-C_2H_2$ flame of high temperature and stability by the operation of sheathing flames on each side. Examination of the performance of this burner has shown that good agreement with other methods is obtained, that a number of transitions from excited states absorb and that phosphate enhances vanadium absorption. Mrs. J. A. Bowman (C.S.I.R.O., Division of Chemical Physics, Melbourne), in collaboration with J. C. Heerdt (Commonwealth Railways, Port Augusta, South Australia) and J. B. Willis, reported that good agreement could be obtained between atomic absorption measurement on oil dissolved in 2-methyl-4-pentanone and both atomic absorption and colorimetric results on dissolved ash for Cu, Cr, Fe, Pb and Ag, but it was agreed in discussion that the greatest hazard in diagnosis of engine breakdown lies in the sampling of the oil. N. J. Marshall (Bureau of Mineral Resources, Canberra) reported success in the extraction of various substituted thiocarbamate complexes of Cu, Pb, Ni, Co and Te into amyl acetate for the determination of these elements, at parts-per-hundred-million levels, in rocks by atomic absorption; and D. C. Bowditch, jointly with J. A. Powell (both of the Australian Mineral Development Laboratories, Adelaide), that atomic absorption could compete favourably with other means of analysis in the determination of Cu, Zn, Co, Ni, Cd, Mn, Sb, Ag and Bi both at low concentration in geochemical and mill tailing samples and at high concentration in mill feed and product control. After outlining his methods of soil and plant analysis for some 25 major and minor elements by means of flame emission, porous cup spark emission, rotating disk spark emission on a direct reader and chemical concentration followed by cathode layer arc emission (improved by image converter scanning of plates), R. L. Mitchell (Macaulay Institute for Soil Research, Scotland) described, in an invited address, an atomic absorption method for the determination of extractable cobalt at deficiency-levels in soils, stating that considerable gain in sensitivity had recently been achieved by use of a special atomizing system, tuned a.c. amplifier and recorder read-out.

A successful mass spectrometry section was a late addition to the Conference programme. Seven papers were presented; six of these, including an introductory lecture by J. S. Shannon (C.S.I.R.O., Division of Coal Research, Sydney), dealt with structural applications in organic, metal-organic and co-ordination chemistry, and the seventh with an electric quadrupole mass spectrometer (D. Swinger, C.S.I.R.O., Division of Chemical Physics, Melbourne). The structural applications reported included a structural assignment for the complex fungal metabolite sporidesmin C, a tentative structure for the cell growth factor 'Zeatin', a characteristic reaction of 2,6-diaryl oxazole systems in which CO and HCN is eliminated (W. D. Crow, J. Hodgkin, R. Hodges, D. Letham and J. S. Shannon), structures for the Schiff Base bisbenzoylacetone-ethylenediamine and its metal chelates, and the mass spectra of Ph_3M ($M = N, P, As, Sb, Bi$) and related compounds (J. S. Shannon) and of macrorungia alkaloids (R. R. Arndt, K. Biemann, A. Jordaan, V. P. Joynt and J. L. Occolowicz (C.S.I.R.O., Division of Chemical Physics, Melbourne). N. Wasada and I. Tsuchiya (Government Chemical Industrial Research Institute, Tokyo), E. Yoshii (Faculty of Pharmaceutical Sciences, University of Toyama, Japan), T. Fukuzumi (Japan Monopoly Corporation) and E. Watanabe (Japan Electron Optics Laboratory) reported on fragmentation patterns and discussed their mechanisms for santonins and their derivatives and also for solanone, an unsaturated branched aliphatic ketone from tobacco leaf. J. L. Occolowicz (Defence Research Laboratories, Melbourne) and J. M. Swan (C.S.I.R.O., Division of Organic Chemistry, Melbourne) described the mass spectra of phosphonic esters $RPO(OR')$, and dichlorides $RPOCl_2$, and emphasized the value of keeping an accurate electron balance in the formulation of ionic equations. General patterns for the decomposition of odd electron (radical) ions and even electron ions were described. In a stimulating discussion of these papers the approach of the organic chemists to the interpretation of mass spectra was criticized as lacking quantitative energy factors; in reply it was maintained that, provided the approach was utilized correctly, there was at present no better method available.

The remaining section of the Conference consisted of four papers on X-ray spectroscopy. D. McKenzie (Australian Iron and Steel, Wollongong, N.S.W.) described the analysis of coals by X-ray spectroscopy, and in a paper by K. Norrish (C.S.I.R.O., Division of Soils, Adelaide) a technique for silicate analysis was described in which the sample was fused with lithium borate glass, with added lanthanum oxide and cast in a penny-shaped disk. Advantages of this technique include durability of sample and uniform background. K. Norrish and C. G. Gurr (C.S.I.R.O., Division of Soils, Adelaide) described an automatic pulse-height analyser for use in X-ray spectrography, and J. B. Swann (University of Western Australia) the energy of electrons scattered by silver with surface contamination.

The sixth Australian Spectroscopy Conference will be held in Brisbane in 1967.

G. C. BARRACLOUGH
P. J. BLACK
D. J. DAVID
A. D. E. PULLIN
J. S. SHANNON

BRITISH AID TO DEVELOPING COUNTRIES

A WHITE PAPER from the Ministry of Overseas Development*, outlining the work of the new Ministry, describes the policies by which the British Government

proposes to be guided and the ways in which it hopes to help to meet the needs of the developing countries, without, however, specifying the extent of Britain's aid in particular periods. Successive chapters outline motives and objectives, the pace of overseas development, the transfer of resources and Britain's capacity to help—in

* Ministry of Overseas Development. *Overseas Development: The Work of the New Ministry*. (Cmd. 2786.) Pp. 74. (London H.M.S.O., 1965.) 5s. 6d. net.

which the Department of Technical Co-operation had laid firm foundations. However, it does not really meet the criticism which has come from the Overseas Development Institute, or from the Estimates Committee in its tenth report for the session 1963-64. Mr. R. Carr, then head of the Department of Technical Co-operation, had indicated that Britain's contribution, £190 millions in 1964, would rise to about £220 millions, and since the White Paper was published it is understood that this figure will probably be increased to £250 millions.

The White Paper makes scarcely any reference to the application of science and particularly of research, and this weakness, exemplified notably in the disbandment of the Overseas Research Council, therefore persists. The initiatives in technical assistance which are being taken in the economic services seem a little inadequate. Certainly, it is unfortunate that in a statement on the White Paper in the House of Commons on August 3 the Minister should have been so petulant and inclined to make a party issue of a matter which has the support of all parties and for which Mr. Carr had laid such good foundations. Mrs. Castle said that the levels of expenditure on aid would be contained in the national plan. It was proposed to establish a careers service in the Ministry for a corps of specialists directly employed by the Ministry who would normally be on loan overseas. The Ministry was also setting up an Overseas Service Pensions Fund to provide pension rights for persons serving overseas for which such provision was not at present made.

Much in the White Paper supplements an earlier statement from the Department of Technical Co-operation in 1963, but further chapters deal with the lines of future policy, the new terms of aid, the private sector and the Commonwealth Development Corporation, the management of aid, and with what are described as "new initiatives in technical assistance", including recruitment and voluntary service, economic services, education, training and public health. It is emphasized that the Ministry would like to see it widely accepted in Britain that a professional career should normally include a period of work overseas in a developing country. The Government has decided, therefore, that in fields of recruitment where it is important to ensure that British help is available, the home establishments of Government departments and public bodies should be strengthened so as to make it possible to release people more readily. Discussions with other Government departments and the University Grants Committee have shown that there is scope for strengthening certain establishments and that the organizations concerned are willing to co-operate. A total of at least 400 posts will be added for this purpose to home establishments, in Government departments, universities, technical colleges, etc.

On service overseas by volunteers, the White Paper, noting that the number of such volunteers is expected to

rise from 1,400 in the autumn of 1965, to 1,800 in 1966, points out that it is important to widen the range of work which volunteers are doing and to draw them from wider sections of our society. On the economic services, more economists and statisticians are being supplied for periods of service overseas, directly attached to Governments; it is hoped to increase this number by the recruitment measures just described. A survey of British universities is being undertaken to learn more about the number and type of economists interested in, and available for, work overseas; the supply of such people will be increased by a new Institute of Development Studies in accordance with the recommendation of the Bridges Committee in 1962. Up to 1,000 non-university teachers are to be recruited in 1965 and almost £200 millions a year has been spent on the supply of teachers under the Overseas Service Aid Scheme alone. The provisions of the Overseas and Development Service Bill will help to increase new recruitment and retain the services overseas of British staff employed other than by Governments. The Commonwealth Teacher Training Bursary Scheme has been stepped up to 500 and will be increased to 550 next year. The number of students and trainees coming to Britain under the Ministry's programme has risen from just more than 2,000 in 1962-63 to nearly 2,800 in 1964-65.

Under the terms of aid, the White Paper refers to the decision to make development loans free of interest where appropriate. It is claimed that the new structure under the Ministry of Overseas Development has strengthened Britain's work on aid in various ways and, after detailing the extent of British aid, there is a brief appendix on the organization of the Ministry. The objective of Britain's aid programme is to help developing countries in their efforts to raise standards of living. Its basis is accordingly a moral one, but it is recognized that provision of aid is to Britain's own long-term economic advantage and that she gives aid because, in the widest sense, it is believed to be in her interests to do so as a member of the world community. British or any other aid is most effective where it forms an integral part of a coherent and co-operative effort to implement a well-prepared development plan.

The White Paper is well written and cogently argued. It directs attention to such obstacles to the development of the poorer countries as the shortage of professional and technical personnel, which in many countries had been even more serious than shortage of foreign exchange in inhibiting development. It also notes the difficulties caused by the accelerated growth of population and, as a consequence of economic and demographic trends, a general failure to find productive employment for the labour force. Its silence in regard to scientific research is its outstanding defect, and from this it is apparent how vulnerable some of the proposals are to the incidence of the Government's new measures of restricted expenditure.

SOLAR MOTION AND SUNSPOT COMPARISON

By DR. ROBERT M. WOOD*

Assistant Director, Research and Development, Douglas Missile and Space Systems Division, Santa Monica, California

AND

PROF. KARL D. WOOD

Aerospace Engineering Sciences Department, University of Colorado, Boulder

SEVERAL attempts have been made to associate the position of the planets with the 11.1-year cycle of solar activity¹. This article presents some preliminary results of a solar dynamic investigation based on classical dynamics. The inertial frame for bodies in the solar system is the centre of mass of the solar system, not the

centre of the Sun². It is known that the centre of the Sun can be greater than one solar radius distant from the solar system centre of mass if Jupiter and Saturn are in the same general direction in space³. This motion of the Sun with respect to the solar system centre of mass will lead to forces on and in the Sun which vary with time and heliographic position. Although the spatial relationships between the Sun and the solar system centre of mass

* Mailing address: Dr. R. M. Wood, 580 Swarthmore Avenue, Pacific Palisades, California 90272.

have been examined and reported, we are aware of no systematic investigation of the attendant velocity, acceleration, or jerk, an accepted term for rate of change of acceleration.

To accomplish such an investigation, the appropriate three-dimensional vector operations were coded for use with the IBM 7094 computer, using an autogenerating ephemerides sub-routine as an input. For example, the position, velocity, acceleration, and jerk of the Sun are given, respectively, by:

$$\begin{aligned}\vec{cmr}_1 &= - \sum_{i=2}^{10} (m_i) \frac{\vec{r}_{1i}}{|\vec{r}_{1i}|^3} \\ \vec{cmv}_1 &= - \sum_{i=2}^{10} (m_i) \frac{\dot{\vec{r}}_{1i}}{|\vec{r}_{1i}|^3} \\ \vec{cma}_1 &= - G \sum_{i=2}^{10} (m_i) \frac{\vec{r}_{1i}}{|\vec{r}_{1i}|^3} \\ \vec{cmj}_1 &= - G \sum_{i=2}^{10} m_i \left(\frac{\ddot{\vec{r}}_{1i}}{|\vec{r}_{1i}|^3} - \frac{3(\dot{\vec{r}}_{1i})(\dot{\vec{r}}_{1i})}{|\vec{r}_{1i}|^4} \right)\end{aligned}$$

where \vec{r}_i and $\dot{\vec{r}}_i$ are outputs of the autogenerating ephemerides sub-routine, a pre-subscript refers to the co-ordinate system, and a post-subscript refers to the body in question ($i = 1$ for Sun, $i = 2$ for Mercury, etc.). The body mass is given by m_i , position by \vec{r}_i , and acceleration by $\ddot{\vec{r}}_i$, and G is the universal constant of gravitation. Pluto ($i = 10$) was omitted because its influence is negligible for the calculations performed.

The four large outer planets (Jupiter, Saturn, Uranus, Neptune) are the most important in determining the position of the Sun⁴, whereas the three closest inner

Table 1. RANGES OF SOLAR DYNAMIC QUANTITIES

Planets	Position (10^8 m)	Velocity (m/sec)	Acceleration (10^{-7} m/sec ²)	Jerk (10^{-14} m/sec ³)
All	0-15	9-16	1.4-2.9	0-2.8
Outer	0-15	9-16	1.7-2.5	0.8-0.4
Inner	0-0.01	0.01-0.2	0-0.6	0-2.4

These ranges were obtained by the examination of 100 years of output most of it at 8-day intervals. IBM 7094 running time is about 1 min per decade. Note the increasing importance of the inner planets with each successively higher time derivative.

planets (Mercury, Venus, Earth) are the most important in determining the jerk of the Sun. Table 1 illustrates this point by summarizing the ranges of the magnitudes of these dynamic quantities. It is particularly noteworthy that the fluctuation of the acceleration due to all the planets is approximately twice that due to the outer planets only; furthermore, the inner planets predominate in their influence on the jerk. Although it is not known whether the jerk itself is a physically important quantity, it represents a useful function which is related to rate of change of force.

The solar acceleration and jerk vectors can be usefully described by three scalars each: magnitude, component along the Sun's axis, and the longitude. The latter is defined as that angle between the planes defined by the zero longitude vector and the Sun's axis of rotation, and the vector of interest and the Sun's axis. The zero longitude vector is essentially fixed in direction in inertial space and lies at the intersection between the plane of the solar equator and the plane of the ecliptic.

The jerk longitude in inertial heliographic co-ordinates is plotted for the beginning of sunspot cycle 17 (1933) and cycle 18 (1944) in Fig. 1.

The detailed similarity of the patterns for the times beginning with 1933 and 1944 does not occur at any intermediate times, although space prevents a display of this point. The jerk along the Sun's axis and the magnitude of the jerk also repeat in a similar fashion, and all these quantities have patterns which repeat with the same period

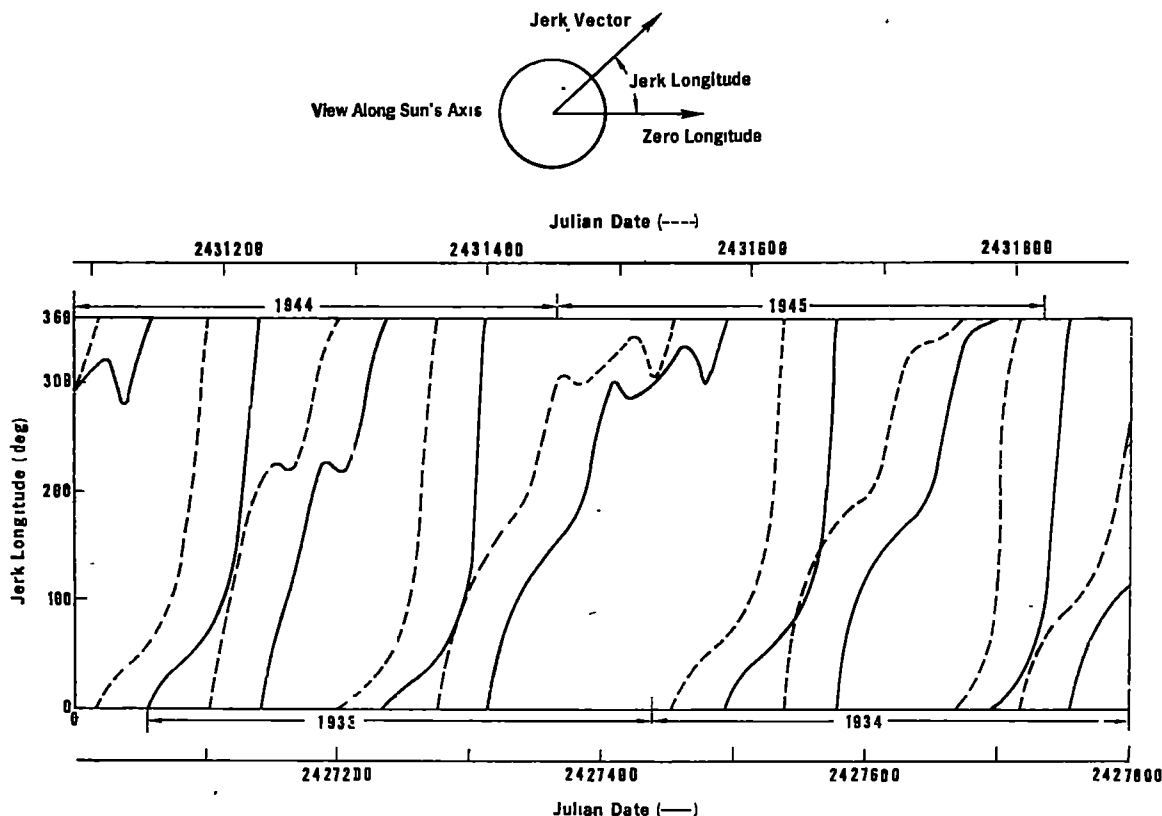


Fig. 1. Jerk longitude during portions of cycle 17 and cycle 18. The longitude is measured in the plane of the solar equator from the ascending node of the intersection between the plane of the solar equator and the ecliptic. The time axes, shown in Julian days, are displaced by 4,085 days. The 1933-34 time axis goes with the solid curves and the 1944-45 time axis with the dashed curves. The pattern separation is determined to be 4,047 days.

for cycles 19 and 20. Moreover, although the acceleration has a maximum approximately every 11.86 years (due to Jupiter) the fine structure of the acceleration due to the inner planets has the same repetition period as the jerk. Careful measurements of accurate curves indicate a separation between the patterns of 4,047 days, corresponding to 11.08 years. The average length of the solar sunspot cycle from Waldmeier⁵, using maxima from 1610 to 1960, is 11.04 ± 0.25 years, and using minima is 11.08 ± 0.20 years. This correspondence, while not conclusive, seems unlikely to be accidental.

Fig. 2 shows the comparison between smoothed sunspot Wolf number and the rate of change of jerk longitude. The Wolf number is the daily count of the number of spots plus ten times the number of sunspot groups, and is roughly proportional to the total sunspot area. The similarity of the curves is interesting. The use of rate of change of jerk longitude followed considerable examination of other dynamic function comparisons, namely, acceleration magnitude, jerk magnitude, axial components of jerk and acceleration, and longitude of acceleration. The Wolf number peaks of 1947 were selected to illustrate a case of favourable comparison. Since for other years the similarity of the curves is weaker, this suggests that the rate of change of jerk longitude is not the main variable responsible for an effect on solar activity, if indeed such a causal effect does exist.

There are several ways in which the Sun's motion and attendant accelerations, forces, or their rate of change may trigger solar activity. Three typical ones are: (a) small photosphere velocities could result in Coriolis terms leading to cyclones and anticyclones; (b) the very unstable convective region several hundred km below the visible surface could be influenced by acceleration, leading to different buoyant forces⁶; (c) dynamic effects could push the material associated with strong magnetic fields through the solar surface, as in Babcock's theory⁷. Such mechanisms all seem plausible.

If the motion of the Sun as determined by the gravitational forces of the planets is a causal element for solar activity⁴, then many observed facts about solar activity could be explained. The alternation of sunspot polarities with each cycle, the decrease in latitude of activity as the cycle progresses, the multiplicity of cycles⁸, the east-west asymmetry of activity, and the observed recurrence of active regions fixed in inertial space all require explanation. By demonstrating a physical link between solar dynamics and solar activity, a more comprehensive understanding of solar physics could be realized. This could ultimately lead to realistic solar activity prediction.

In conclusion, the following points can be clearly stated: (a) the influence of the inner planets on the fluctuation of the acceleration of the Sun in inertial space is as important as that of the outer planets; (b) the short-time

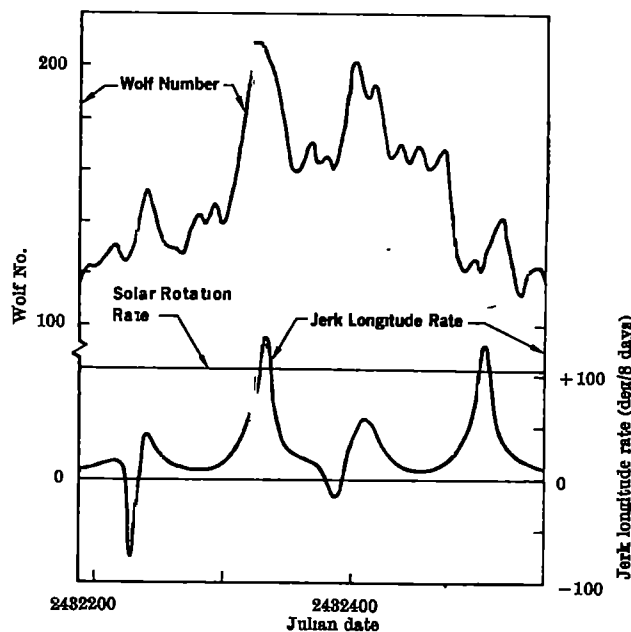


Fig. 2. Comparison between Wolf number and jerk longitude rate for 1947. A 27-day moving average for Wolf number has been used to reduce greatly the effects of solar rotation as seen from the Earth. The rate of change of jerk longitude is the slope of curves like those of Fig. 1. The average solar rotation rate is shown for reference.

acceleration and jerk patterns are repetitive with a clear 11.08-year period, agreeing well with the mean sunspot cycle.

This work was partially supported by the Douglas Aircraft Co., Inc., Independent Research and Development Program. We thank Dr. M. J. Abzug, A. D. Goedeke and Dr. L. Larmore for their advice, and J. P. Harland for analytical and digital simulation assistance.

¹ Johnson, M., *Correlation of Cycles in Weather, Solar Activity, Geomagnetic Values, and Planetary Configurations* (Phillips and Van Orden, San Francisco, California, 1953). Sanford, F., *Smithsonian Misc. Coll.*, 95 (1936). Nelson, J., *Trans. Inst. Rad. Eng.*, CS-2, 19 (1954).

² Kurth, R., *Introduction to the Mechanics of the Solar System* (Pergamon Press, New York, 1959).

³ Flammarion, G., *The Flammarion Book of Astronomy*, 51 (George Allen and Unwin, Ltd., London, 1944).

⁴ Jose, P., *Astron. J.*, 70, 198 (1965). This recent article suggests a connexion between a 178-year cycle of Wolf number maxima and the rate of change of solar angular momentum. However, his results were based on the five outer planets only, and our investigation shows that the inner planets have a major effect on the rate of change of angular momentum.

⁵ Waldmeier, M., *The Sunspot-Activity in the Years 1610-1960* (Zurich Schultheiss and Co., Zurich, Switzerland, 1961).

⁶ Aller, L. H., *Atmospheres of the Sun and Stars*, 449 (The Ronald Press, New York, 1963).

⁷ Bray, R., and Loughhead, L., *Sunspots*, 281 (John Wiley and Sons, New York, 1965).

⁸ Rabashov, B., *Problems of Solar Activity*, 48 (NASA TTF-244, translated by National Aeronautics and Space Administration, Washington, D.C., 1964).

STRATOSPHERIC TRANSPORT OF VOLCANIC DUST INFERRED FROM SOLAR RADIATION MEASUREMENTS

By DR. A. J. DYER and B. B. HICKS

C.S.I.R.O. Division of Meteorological Physics, Aspendale, Victoria, Australia

RECENTLY, a number of authors have reported observations of volcanic dust in the southern hemisphere following the eruption of Mount Agung, Bali (8° S., 115° E.), on March 17, 1963. Hogg¹ discussed several optical effects observed at Mount Stromlo and elsewhere. Moreno and Stock² measured extinction effects at Cerro Tololo, Chile. Mossop³ obtained dust samples from aircraft flights at a height of 20 km in latitude belts 15°-35° S. and 40°-45° S. Flowers and Viebrock⁴ have reported an anomalous decrease in solar

radiation at the South Pole and also at Mauna Loa, Hawaii.

In the northern hemisphere, Meinel and Meinel⁵ have estimated the height of the dust layer over Tucson, Arizona, to be 17.8 km in late 1963.

These various accounts contain some features of interest to the meteorologist concerned with atmospheric transport on a global scale. It appears that the volcanic dust moved toward the South Pole in the lower stratosphere at such a rate that the initial detection in middle

latitudes occurred after 5-7 weeks and the maximum concentration appeared after about 4-6 months. These findings bear a remarkable similarity to observations at 38° S. of the arrival of fission debris following the atomic tests in equatorial regions in 1958-62 (refs. 6 and 7).

A very extensive record of solar radiation data has been continuously maintained since 1959 at this laboratory. This has been examined to see if information could be deduced concerning the movement of the dust over a number of years. Similar investigations following the Krakatoa, Mount Pelée, Katmai and other explosions suggested that absorption of the direct solar beam by the dust layer might be observable for several years⁸⁻¹¹.

The data used exist in the form of continuous chart records of the total radiation T and the diffuse radiation D falling on a horizontal surface. These observations are made with Kipp and Zonen pyranometers periodically calibrated against a Linke Feussner pyrheliometer. From the chart records, 'clear' days were selected, that is, days for which there was no evidence of cloud within 1 h of noon, and the maximum values about noon of total and diffuse radiation noted. On the average seven 'clear' days were available for each month with slightly more in summer than in winter.

The direct solar radiation, I , at normal incidence, can be readily calculated from $I = (T - D)/\cos \zeta$ where ζ is the zenith angle. Subsequent analyses were then carried out in terms of two-month averages.

The data for the years 1959-62 were used to establish the norms \bar{T} , \bar{D} and \bar{I} for each two-month period. Fig. 1 presents a plot of the quantities D/\bar{D} , I/\bar{I} , T/\bar{T} and D/\bar{T} for 1959-65. The time of the Bali eruption is indicated.

It is obvious that some remarkable effects occurred in the radiation levels during 1963 and to a lesser extent in 1964 and 1965. In the light of observations elsewhere it is plausible to associate this with the presence of a layer of volcanic dust from the Bali eruption. The gradual removal of the dust over a period of several years is evident.

The maximum effect occurred during July-August of 1963 when the direct solar radiation was reduced by 24 per cent. A reduction of 16 per cent occurred during July-August of 1964 and there is a suggestion of similar behaviour in 1965.

The most spectacular feature appears in the diffuse radiation record which shows twice normal values during July-August 1963 and continues to show abnormally high values in 1964 and 1965.

The total radiation values show a significant, but less-obvious, reduction in 1963 and 1964. The information contained in Fig. 1, if taken at face value, would indicate that, of the radiation removed from the direct solar beam, more is scattered in a forward than in a backward direction. This would imply only a minor modification of climate as a result of scattering by the dust layer, even when such dramatic effects are evident in the direct and diffuse radiation.

It is clear, however, that the 'signal-to-noise ratio' of the total radiation data in Fig. 1 is not very high. This is caused partly by the presence of pollutants in the urban atmosphere and partly by the use of data from routine observations instead of a specifically oriented experiment. A similar analysis of higher quality data would materially assist the foregoing considerations.

From the point of view of using the volcanic dust as a tracer of atmospheric motions, it is necessary to estimate the amount of dust present at various times.

The change in the direct solar beam dI' along an element at slant path dl is given, in simple scattering theory, by:

$$dI' = -knI'dl - k_0n_0I'dl$$

where k is a scattering coefficient and n is the concentration of dust particles in a thin layer. The term $k_0n_0I'dl$ is the reduction due to scattering by aerosols normally present.

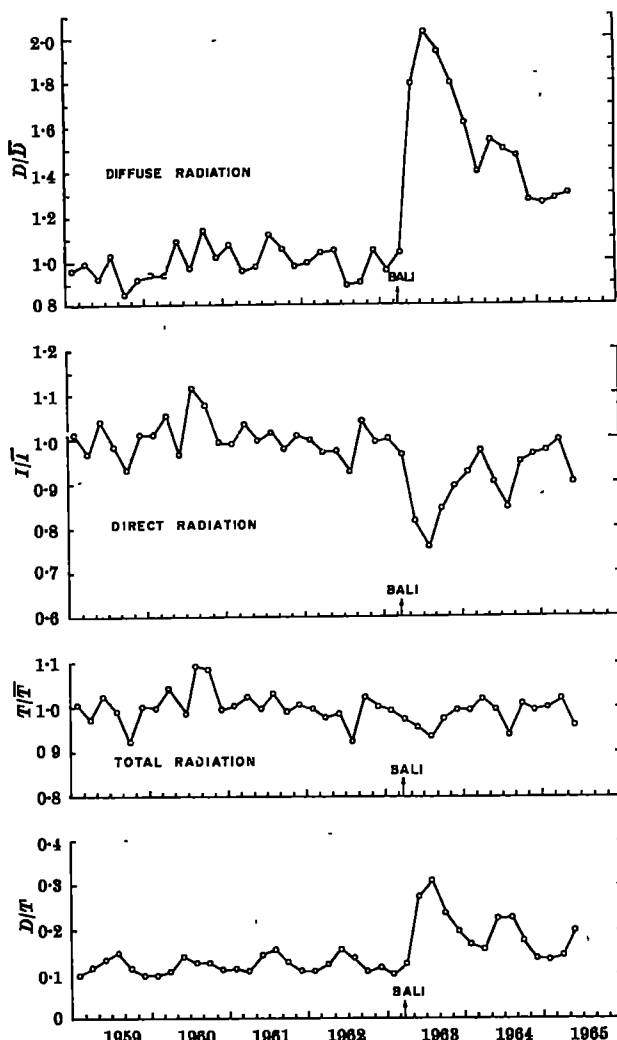


Fig. 1. Deviations from the mean of the diffuse, direct and total solar radiation and the contribution of scattered radiation to the total, 1959-65.

Introducing the Earth radius R , the solar zenith angle ζ , the height of the dust layer h and its depth Δh , we have on integration:

$$\ln(I/I_0) = -kn \left\{ \sqrt{(R+h)^2 - R^2 \sin^2 \zeta} \right\}_h^{h+\Delta h} - C$$

where I_0 and I are the values of I' outside the atmosphere and at the Earth's surface respectively. C refers to the integral of k_0n_0dl taken throughout the atmosphere. Making the simplifying approximation $R \gg h \gg \Delta h$, we have:

$$\ln(I_0/I) = kn\Delta h/(\cos^2 \zeta + 2h/R)^{1/2} + C$$

\bar{I} , as previously defined, is the average value of I in the absence of volcanic dust so that $\ln(I_0/\bar{I}) = C$ and hence:

$$\ln(\bar{I}/I) = kn\Delta h/(\cos^2 \zeta + 2h/R)^{1/2}$$

Since $n\Delta h$ represents the total number of dust particles in a vertical path we can, by assuming k constant, use the quantity $(\cos^2 \zeta + 2h/R)^{1/2} \ln(\bar{I}/I)$ as an index of the amount of dust present at any time. We may, in fact, expect k to change slowly with time owing to the gradual removal of the larger particles. The term $2h/R$ is only important when the Sun is very close to the horizon. At all other zenith angles, $(\cos^2 \zeta + 2h/R)^{1/2}$ reduces to $\cos \zeta$.

In Fig. 2 the quantity $(\cos^2 \zeta + 2h/R)^{1/2} \ln(\bar{I}/I)$ is plotted from the present data and for the South Pole data of

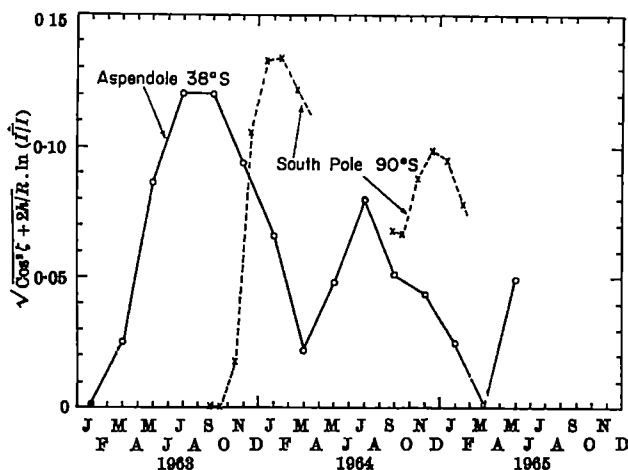


Fig. 2. The dust content of the atmosphere above Aspendale and the South Pole following the eruption of Mt. Agung on March 17, 1963

Flowers and Viebrock^{4,5}. In the latter case, a dust layer altitude of 10 km has been assumed where necessary.

The first major arrival of volcanic dust is seen to occur during July–August of 1963 at 38° S. and during January–February of 1964 at 90° S. At almost the same times in the following year a new influx of dust is apparent at both latitudes, indicating a pronounced seasonal effect in the poleward stratospheric transfer. The existence of an enhanced ‘winter’ transfer revealed in 1964 would no doubt have contributed to the initial arrival of the volcanic dust in 1963. The indications of Fig. 2 are supported by the occurrence of vivid sunsets over Southern Australia during the winters of 1963 and 1964, and their reappearance at reduced intensity during the present 1965 winter. Similar seasonal effects were observed in 1884 and 1885 following the Krakatoa eruption in 1883 (ref. 8).

Time-lags of similar amounts between temperate and polar latitudes have been observed in studies with other

tracers. The maximum in total ozone occurs at 38° S. in September, at 54° S. in October and at 75° S. in December¹². The maximum in radioactive fall-out in rain occurs over southern Australia during November–December¹. All these tracers have in common that they originate in equatorial regions. Ozone is also produced at other latitudes, but in reduced amounts.)

It is clear that stratospheric transfer processes from equator to pole involve times of the order of five months to middle and eleven months to polar latitudes. Whether these take the form of mean motion or eddy diffusion, or some combination of both, cannot be determined from the present studies. However, for the South Pole data Flowers and Viebrock have commented that the sudden arrival of dust in November 1963 coincided with a change in the stratospheric circulation from circumpolar to meridional. The volcanic dust observations are consistent with the presence of an equatorial reservoir with gradual depletion by enhanced poleward transfer during the winter.

The different times of occurrence of the maxima of the various tracers are probably associated with the different heights at which the tracers occur and at which the transport takes place. A complete understanding, however, requires considerably more detailed information than we have at present.

We thank Dr. Herbert J. Viebrock for permission to use his unpublished results, and Mr. B. G. Collins for his assistance in analysing the Aspendale radiation data.

- ¹ Hogg, A. R., *Austral. J. Sci.*, **26**, 119 (1963).
- ² Moreno, H., and Stock, J., *Pub. Astron. Soc. Pacific*, **76**, 55 (1964).
- ³ Mossop, S. O., *Nature*, **203**, 324 (1964).
- ⁴ Flowers, B. O., and Viebrock, H. J., *Science*, **148**, 498 (1965).
- ⁵ Melnel, A. B., and Melnel, R. P., *Nature*, **201**, 657 (1964).
- ⁶ Dyer, A. J., and Yeo, S.-A., *Tellus*, **12**, 195 (1960).
- ⁷ Dyer, A. J., and Hicks, B. E., *J. Geophys. Res.*, **70**, 3376 (1965).
- ⁸ Symons, G. J., *The Eruption of Krakatoa* (1888).
- ⁹ Kimball, H. H., *Mon. Weather Rev.*, **46**, 355 (1918).
- ¹⁰ Blümmner, W. B., *Gerl. Beitr. Geophys.*, **50**, 388 (1937).
- ¹¹ Humphreys, W. J., *Physics of the Air* (1940).
- ¹² Viebrock, H. J. (personal communication).
- ¹³ Kulkarni, R. N., *Quart. J. Roy. Meteor. Soc.*, **88**, 522 (1962).

EXPERIMENTAL OBSERVATIONS ON THE STRUCTURE OF COLLISIONLESS SHOCK WAVES IN A MAGNETIZED PLASMA

By DR. J. W. M. PAUL, L. S. HOLMES, DR. M. J. PARKINSON and J. SHEFFIELD
U.K. Atomic Energy Authority Research Group, Culham Laboratory, Abingdon, Berkshire

THIS article reports measurements of the structure of shock waves propagating through a plasma in a direction perpendicular to an initial magnetic field. The shock wave is produced by a fast linear ‘Z-pinch’ between electrodes with 100 cm separation in a fused silica tube of 50 cm diameter. The applied electric field of 320 V/cm produces an axial current which rises to 300 kA in 0.7 μsec, and flows in a thin annular layer (~1 cm). The resulting pinch forces drive this skin current radially inwards at a velocity exceeding the Alfvén velocity for the undisturbed plasma. This rapid radial compression generates an imploding shock wave which propagates ahead of the driving current layer.

The radial distribution of electron density in the initial plasma has been measured by using a double Langmuir probe and by infra-red interferometry. For the particular case of a hydrogen plasma with central electron density $n_0 = 7 \times 10^{14} \text{ cm}^{-3}$ and initial axial magnetic field $B_{z0} = 1.2 \text{ kG}$, the total density (ions and neutrals) has also been measured. This was obtained from the measured velocity of propagation of a low-frequency, small amplitude, magnetosonic wave. The deduced degree of ionization is more than 85 per cent for the central 20 cm diameter.

The plasma collapse has been examined in detail for the foregoing conditions by using magnetic probes. The shock front, a sharp jump in axial magnetic field strength, is clearly separated from the thin skin current (Figs. 1 and 2). For most of the implosion the Alfvén Mach number (shock velocity (V_s) /initial Alfvén velocity), $M_A = 2.5$. The measured magnetic fields have been compared with numerical computations in which the plasma is treated in the magneto-hydrodynamic (MHD) fluid approximation¹. With this model the shock structure broadens rapidly as viscous effects increase. If viscosity is omitted the structure steepens as the electron temperature rises and resistivity decreases. These structure effects, which disagree with the experimental results, are avoided by introducing into the computation an artificially fixed shock width². Good agreement is then obtained between the experimental and computed dynamics if it is assumed that the neutral gas in the outer 10 cm takes part in the collapse (Figs. 1 and 2).

The structure of the shock front has been examined in detail using magnetic probes of diameter down to 0.9 mm and a coaxial double electric probe with an effective tip of 0.25 mm and variable electrode separation. The electric probe records the radial voltage (V_r) through the shock

front. For the foregoing conditions ($M_A = 2.5$), the radial voltage rises in less than 6 nsec as the shock moves on to the tip of the electric probe. The magnetic probe records a coincident but slightly slower rise of magnetic field. Typical oscillograms are shown in Fig. 3. The measured radial voltage (V_R) and change of magnetic field ($B_z - B_{z0}$) in the shock front are plotted in Fig. 4a against time for 9 cm radius. As the measured shock velocity is sensibly constant at 2.5×10^7 cm/sec, Fig. 4 also represents the spatial variation.

The measured shock width (1.4 mm) is appreciably shorter than the appropriate ion-ion and ion-neutral mean free paths (5 cm and 2.5 cm, respectively). Consequently, collisional viscosity can be neglected as reported previously³. Resistive dissipation through the azimuthal current in the shock front, which is the only other collisional process (electron-ion collisions) capable of sustaining such a shock⁴, cannot be eliminated by such a simple criterion. The measured 6-nsec rise time of the voltage is less than two electron-ion collision times. The latter is calculated for the region behind the shock by assuming that the shock heating goes entirely to the electrons, raising their temperature to 50 eV. It is unlikely that this energy can be lost from the shock front in such a short time. As several collisions are required within a collision-dominated shock front it is probable that a collisionless resistivity, such as is provided by turbulence from electron drift instabilities^{5,6}, is important. The measurements indicate an electron drift velocity of about 10 per cent of the predicted electron thermal velocity. This would correspond to the critical drift velocity for instability given by Stringer⁷ if $T_i \sim 0.1 T_e$. The shock width is about $7(c/\omega_{pe})$ for the initial conditions. This is appreciably less than $25(c/\omega_{pe})$ found by Adam⁸. As further evidence for collisionless interaction, the measured rise time of 13 nsec in deuterium at about the same mass density and M_A corresponds to only half an electron-ion collision time while the shock width is about $10(c/\omega_{pe})$.

The change in directed energy of an ion passing through the shock front (considered at rest) is $\Delta W = \frac{1}{2} M[V_s^2 -$

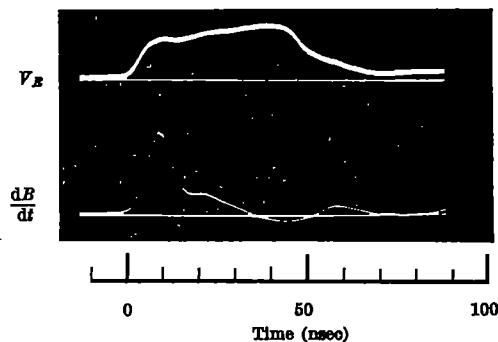


Fig. 3. Typical oscillograms of V_R and dB/dt at 9 cm radius for $M_A = 2.5$. Electric probe tip separation, 1 cm

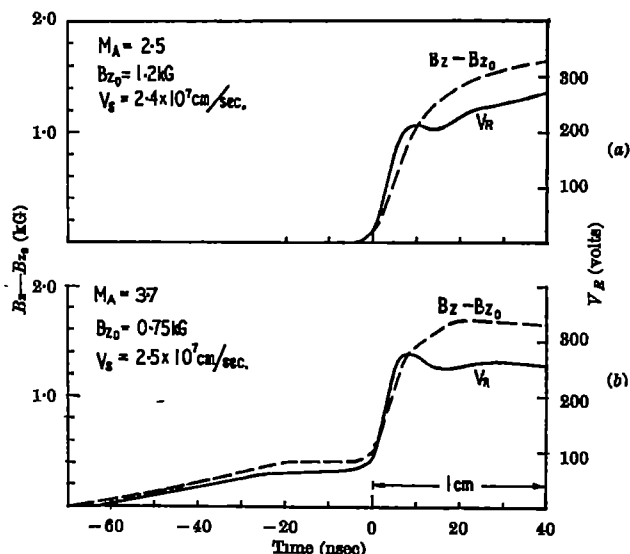


Fig. 4. Structure of magnetic field and electric potential through the shock front for $M_A = 2.5$ (top); $M_A = 3.7$ (bottom)

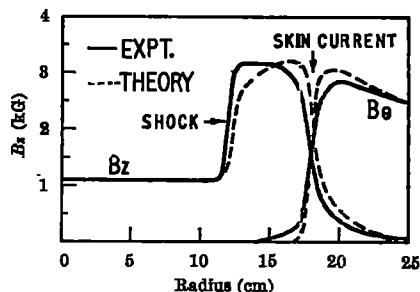


Fig. 1. Radial variation of B_z and B_θ at 0.9 μ sec from a magnetic probe of 7 mm diameter and from computation with a 7 mm shock width

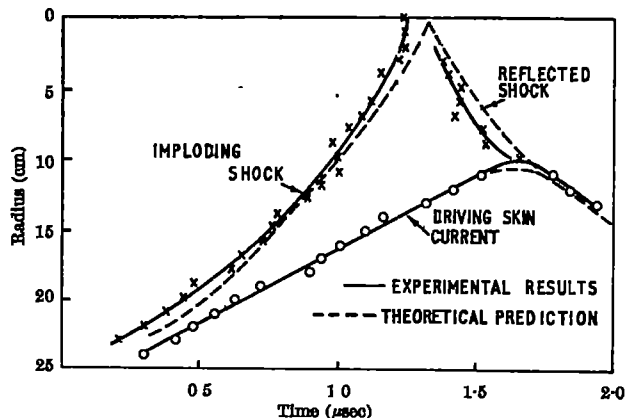


Fig. 2. Space-time 'streak' diagrams of plasma collapse from magnetic probes and computation

$(V_s - V_p)^2]$, where M is the ion mass and V_p is the plasma velocity behind the shock (laboratory co-ordinates). The latter has been derived from the measured magnetic field profiles using the fact that (B_z/n_0) is constant on both sides of the shock (though not within it). The measured azimuthal electric field E_θ behind the shock also provides a measure of $V_p (=E_\theta/B_z)$. The measured voltage across the shock is such that the ratio $R = eV_R/\Delta W = 1$. This means that the ion motion through the shock is entirely accounted for by electric field effects. By implication, the change of ion pressure across the shock is negligible. The measured V_R can be accounted for, in accordance with the generalized Ohm's law⁹, by the known force ($J_\theta \times B_z$) acting on the electron current (J_θ) and the predicted electron pressure gradient.

With resistivity (either collisional or not) as the only dissipative mechanism, continuum theory predicts the occurrence of a discontinuity (iso-magnetic jump) in the fluid velocity within the shock structure for M_A greater than a critical value $M_A^* \sim 3$ (ref. 4). Physically beyond this limit slip of field and plasma (resistive dissipation) alone cannot provide sufficient dissipation of energy. Theories based on particle orbits predict the occurrence of multiple streaming of the ions above a similar limit⁸.

Experimentally M_A is increased by reducing the initial magnetic field. For $M_A = 2.5$ and 3.7 the initial plasma density and the shock velocity are effectively the same. However, at $M_A = 3.7$ the shock has a clear double structure (Fig. 4b) with the sharp transition preceded by a broad one (~ 1.5 cm) corresponding to $2c/\omega_{pi}$. This broad feature of the structure appears for $M_A \geq 3$ over a range of parameters and is interpreted as evidence for the additional mechanism required theoretically. The

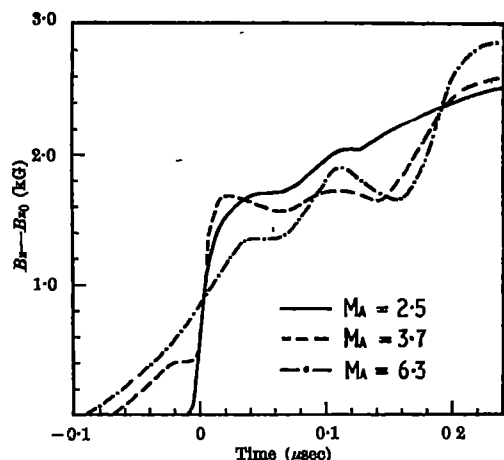


Fig. 5. Structure of magnetic field through the shock front for $M_A = 2.5$, 3.7 and 6.3. (For the latter $B_0 = 0.48$ kG, $n_0 = 4.5 \times 10^{14}$ cm $^{-3}$, $V = 2.8 \times 10^8$ cm/sec)

measured voltage across the shock front again corresponds to $R = 1$, implying that the electrons are heated.

At still higher M_A , the broad structures forward and to the rear of the sharp transition become dominant and at $M_A = 6$ (Fig. 5) the sharp transition is absent. The total structure is then so broad that cylindrical effects are important.

The broad additional feature is shorter than the ion-ion mean free path but is comparable with the ion-neutral mean free path. Even so, this latter mechanism can be neglected because at least five such collisions are required to collect the undisturbed ions (> 80 per cent ionized). Electron thermal conductivity, another relevant collisional mechanism¹⁰, is made negligible by the presence of the axial magnetic field¹¹. Consequently, all collisional mechanisms appear to be eliminated for $M_A > M_A^*$.

Of the many theories of collisionless shocks, only a few are valid for $M_A > M_A^*$. Theories involving ion-ion streaming instabilities^{12,13} can be eliminated because they

predict a sharp transition of width $L \sim \frac{2\pi V_s}{\omega_{pi}}$. The theory of Colgate¹⁴, involving Landau damping of electron oscillation, also predicts a sharp transition with $L \sim \frac{c}{\omega_{pe}}$. Auer

*et al.*¹⁵ treat the problem of ion streaming without instabilities but derive an effective entropy increase from ion gyration phase mixing and assume that the electrons are not heated. This approach leads to a time-dependent

structure with $L \sim \frac{c}{\omega_{pi}} M_A$ (ω_{pi} for compressed plasma) and $R = 0.25$ (for $M_A = 6$). Carmac *et al.*¹⁶ describe a model based on non-linear wave-wave interaction within a front of width $L \sim \frac{2c}{\omega_{pi}}$ for $M_A \sim 4$ but steepening with increasing M_A . The particular analysis, though not the mechanism, is limited to low M_A ($< M_A^*$). Although no explicit prediction is made, it is reasonable to assume that the waves involved ($\omega \gg \omega_{ci}$) heat the electrons and consequently $R = 1$. These values of L and R are comparable with those of the broad feature, but the dependence on M_A appears inconsistent.

Experimental imperfections make it possible that the magnetic field makes a small angle ($\theta > \sqrt{\frac{m_0}{M_A}} \sim 1^\circ$) to the shock front. For the foregoing conditions with $M_A > M_A^*$ this leads to a shock structure^{17,18} more than an order of magnitude shorter than that observed and consequently has been neglected.

In summary, at low Mach numbers ($M_A < 3$) the results indicate that a resistive shock is formed which probably involves collisionless turbulence. Above the predicted critical M_A (~ 3) the observed change of structure is interpreted as due to an additional, but unidentified, collisionless mechanism. None of the present theories of shock structure gives convincing agreement over the range of observations, but some can be eliminated.

We thank Dr. R. J. Bickerton for his advice and Mr. C. A. Steed for his assistance.

¹ Hain, K., Hain, G., Roberts, K. V., Roberts, S. J., and Koppendorfer, W., *Z. Nat.*, **15**, 1089 (1960).

² Richtmeyer, R. D., *Difference Methods for Initial Value Problems*, 208 (Interscience, N.Y., 1957).

³ Paul, J. W. M., Holmes, L. S., Parkinson, M. J., and Sheffield, J. (abst. only), *Bull. Amer. Phys. Soc.*, Ser. 2, **10**, 223 (1965).

⁴ Golden, K. I., Sen, H. K., and Treve, Y. M., *Fifth Conf. Ionization Phenomena in Gases*, **2**, 2109 (1961).

⁵ Zavolski, B. K., *J. Nucl. Energy*, **0**, **5**, 381 (1963).

⁶ Adlam, J. H., and Holmes, L. S., *Nucl. Fusion*, **3**, 62 (1963).

⁷ Stringer, T. R., *J. Nucl. Energy*, **0**, **8**, 267 (1964).

⁸ Spitzer, L., *Physics of Fully Ionized Gases*, **21** (Interscience, N.Y., 1956).

⁹ Morawitz, C. S., *Phys. Fluids*, **4**, 988 (1961).

¹⁰ Marshall, W., *Proc. Roy. Soc., A*, **233**, 367 (1956).

¹¹ Chapman, S., and Cowling, T. G., *Mathematical Theory of Non-uniform Gases* (Camb. Univ. Press, 1939).

¹² Kahn, F. D., *J. Fluid Mech.*, **2**, 601 (1957).

¹³ Parker, E. N., *Astrophys. J.*, **129**, 217 (1959).

¹⁴ Colgate, S. A., *Phys. Fluids*, **2**, 435 (1959).

¹⁵ Auer, P. I., Hurwitz, H., and Kille, B. W., *Phys. Fluids*, **5**, 298 (1962).

¹⁶ Carmac, M., Kantrowitz, A. B., Izyvak, M. M., Patrick, R. M., and Petachek, H. B., *Nuclear Fusion Supp.* Pt. 2, 423 (1962).

¹⁷ Karpman, V. I., *Sov. Phys. Tech. Phys.*, **8**, 715 (1964).

¹⁸ Morton, K. W., *Phys. Fluids*, **7**, 1800 (1964).

PLEISTOCENE GLACIAL-MARINE ZONES IN NORTH ATLANTIC DEEP-SEA SEDIMENTS

By DR. JOHN R. CONOLLY* and DR. MAURICE EWING

Lamont Geological Observatory of Columbia University, Palisades, New York

SINCE Bramlette and Bradley¹ first described glacial marine sediment zones in deep-sea cores across the North Atlantic, there has been no further attempt to use these zones as criteria for interpretation of Pleistocene climates. The occurrence of glacial erratics in the North Atlantic has been described by several authors²⁻⁴. One of the most recent accounts was given by Pratt⁵, who described erratic boulders of granite, schist, diorite and quartzite from cores and dredges on the Great Meteor Seamount. These accounts indicate that icebergs floated much farther south in the North Atlantic during the cold

periods of the Pleistocene, dropping considerable continental material as far south as 30° N.

An investigation was made of the occurrence of ice-rafted material in the Lamont cores from the North Atlantic to find cores in pelagic sediment which contained large variations in the vertical distribution of ice-rafted debris. In general all cores taken in pelagic sediment north of 50° N. contained abundant glacial debris. In many areas near Labrador, Greenland and Iceland there was so much glacial debris that obvious variations in the amount of intensity of ice-rafting could not be measured. Farther south, cores described by Ericson *et al.*⁷ consist of zones of sediment containing abundant ice-rafted grains.

* Present address: Department of Geology, Louisiana State University, Baton Rouge, Louisiana

Cores taken south of 35° N. contain little or no ice-rafted grains. Hence it was decided to examine the variation in ice-rafted material in the cores between 40° and 60° N., particularly since Ericson *et al.* had already shown that they contained zones consisting of different Foraminifera assemblages deposited during cold and warm periods of the Pleistocene. The zones delineated by Ericson *et al.* can also be defined using the relative abundance of ice-rafted detritus present in the cores.

The amount of ice-rafted detritus present in the sand fraction of 13 cores taken from the North Atlantic (Fig. 1) previously described by Ericson *et al.*⁷ was estimated at intervals down the cores (Fig. 2) by examination of the sand fraction under a binocular microscope. The cores lie between latitudes 40° and 60° N and consist almost entirely of pelagic sediment containing varying percentages of pelagic organisms. Cores A157-5 and A180-14 were taken near the edge of the Sohms Abyssal Plain and contain one or two very thin quartz silt beds, presumably deposited by turbidity currents; but the rest of the sediment in these two cores is mainly pelagic. The remainder of the cores is located in positions in the deep-sea floor favourable for pelagic deposition.

In general, the sand fraction consists mainly of tests of pelagic Foraminifera mixed with ice-rafted sand and minor amounts of volcanic ash. The ice-rafted detritus is easily identifiable, as it characteristically consists of very poorly sorted, and mainly angular, sand and pebbles of a variety of mineral and rock types. The size, poor sorting, general angularity and wide lithological range of this detritus that occurs in pelagic sediment, far from

land, indicate that it has dropped from melting ice. Many of the large pebbles are striated or faceted and large quartz grains are commonly frosted and pitted. Quartz is most abundant and commonly makes up 50-70 per cent of the sand fraction. Pebbles of granitic, gneissic, regional metamorphic and sedimentary rocks of all kinds make up the bulk of the fraction coarser than 1 mm.

Black pumice and brown and black glass occur associated with ice-rafted sand in cores from the eastern North Atlantic. Since the amount of black volcanic detritus present bears a constant relation to the amount of poorly sorted ice-rafted sand, it must also be ice-rafted. The black pumice and glass are most abundant in cores taken close to Iceland (Fig. 1), as has been previously noted by Ericson *et al.*⁸, and the amount of black pumice decreases southwards, indicating dilution of this volcanic detritus from Iceland with detritus from eastern Greenland and the British Isles. In the western North Atlantic, quartz is less common and makes up only 30-50 per cent of the ice-rafted sand. Large quantities of hornblende and garnet are particularly characteristic of the sand in cores A157-5 and A157-6.

The amount of ice-rafted sand present increases from a few grains in the southernmost cores to 20-30 per cent of the total volume of sediment in the glacial marine zones in cores SP10-1 and SP9-3. The variation in the percentage of ice-rafted detritus in the sand fraction at intervals down the cores (Fig. 2) can be interpreted as a measure of the effects of climate on deposition. Zones containing a relatively high percentage of ice-rafted detritus correlate very closely with zones containing

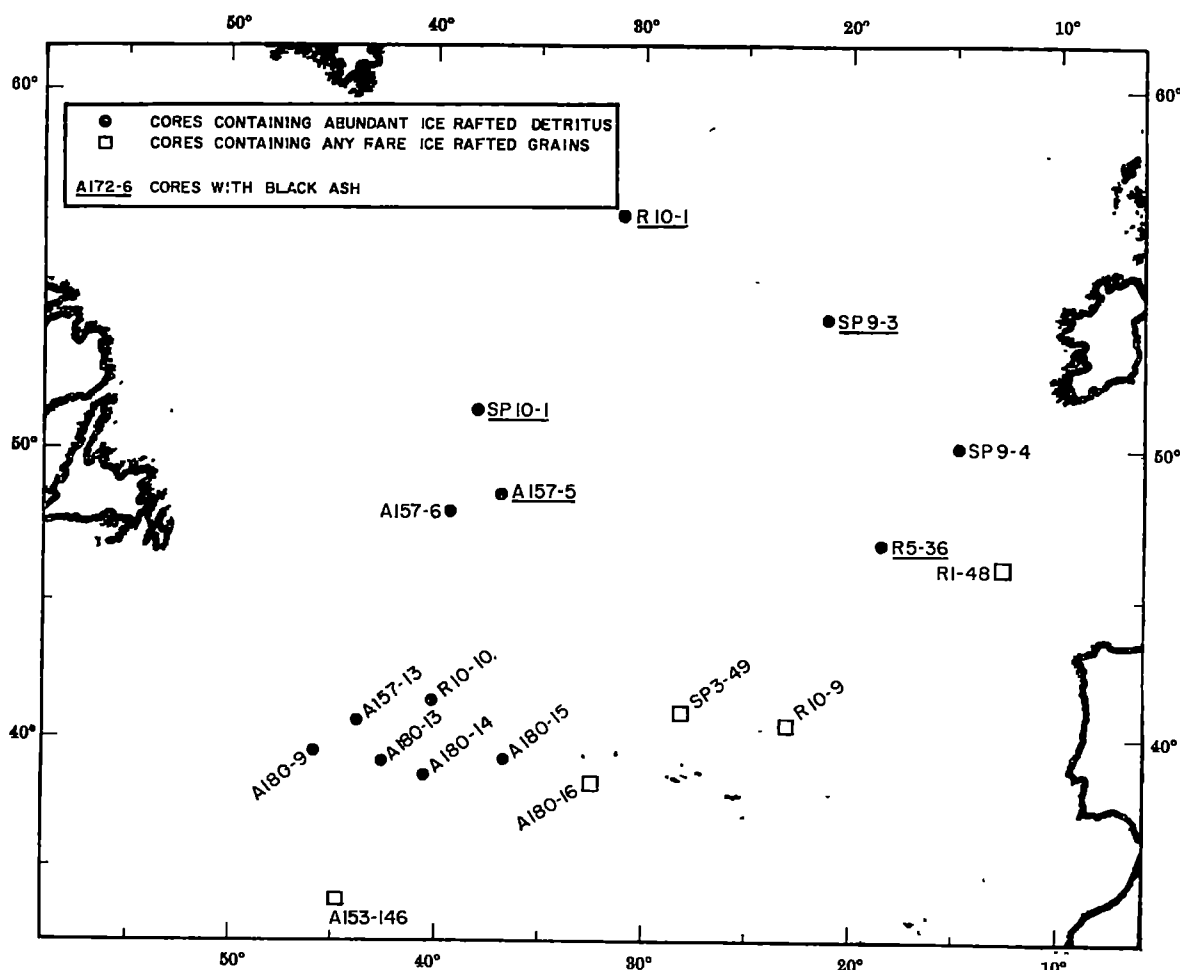


Fig. 1. North Atlantic Ocean showing the location of cores described in this investigation with abundant ice-rafted sand and pebbles and the location of cores containing only rare ice-rafted grains. Cores in the north and north-western North Atlantic contain black pumice and brown and black glass probably derived by ice-rafting from near Iceland.

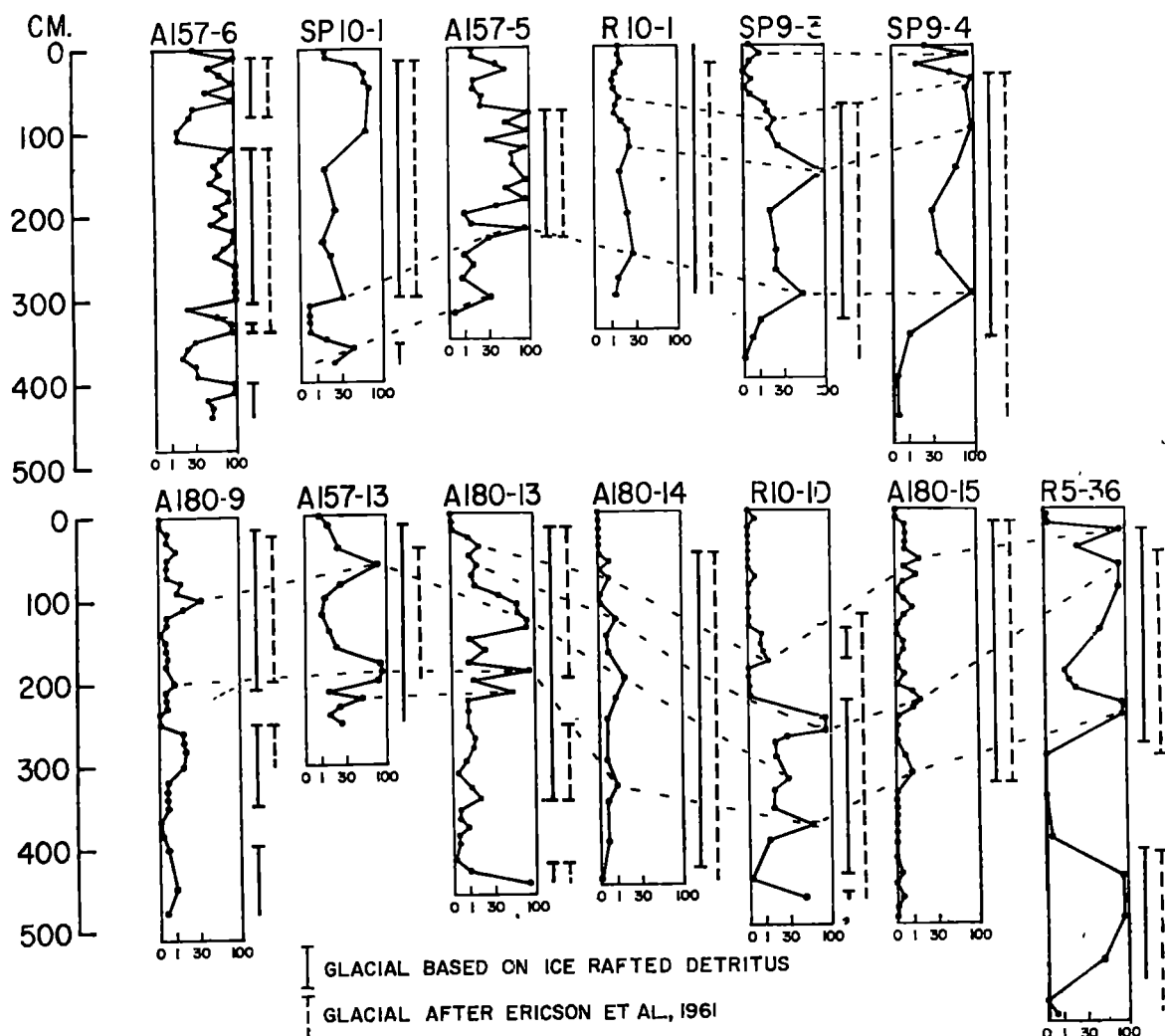


Fig. 2. The amount of ice-rafted detritus in the sand fraction is expressed as a percentage of the total sand fraction at intervals down 13 cores from the North Atlantic. Zones defined as glacial by Ericson *et al.* (ref. 7) in these cores can also be defined on the relative abundance of ice-rafted detritus. Tentative correlations are made between areas of cores containing high amounts of ice-rafted detritus. These areas may represent deposition during the major cold periods of the late Pleistocene.

colder-water foraminiferal assemblages described by Ericson *et al.*⁷

There is considerable fluctuation in the amount of ice-rafted detritus deposited during these cold periods, suggesting that it might be possible to determine the major cold and warm zones within each glacial by correlating areas representing maximum and minimum amounts of ice-rafted detritus in different cores across the North Atlantic.

If it is assumed that the zones which Ericson *et al.*⁷ considered to have been deposited during the last glacial (the period from about 11,000 to 60,000 years ago), and shown in Fig. 2, are correct, then it is possible to sub-divide fur-

ther this cold period on the basis of the relative abundance of ice-rafted detritus. For example, core R10-10 contains a distinct zone containing abundant ice-rafted detritus between 150 and 130 cm from the top of the core. This zone occurs between areas dated as $11,800 \pm 480$ and $15,820 \pm 200$ years old⁷ and probably contains ice-rafted detritus deposited during the last major glaciation of the last glacial period. Beneath this zone there are three more zones in which the sand fraction is made up almost entirely of ice-rafted detritus, suggesting that there were probably three more major cold periods during the last glacial period. Similar zones containing high amounts of ice-rafted detritus occur in many of the other cores and possible correlations between them are shown in Fig. 2.

These results indicate that the abundance and distribution of ice-rafted detritus in deep-sea cores in the North Atlantic could provide a powerful tool for delineating even minor fluctuations in the Pleistocene climate. Cores taken in pelagic sediment in the vicinity of cores R5-36, R10-10 and A180-13 in particular should contain easily determinable ice-rafted zones that might eventually lead to a better understanding of the fluctuations in climate and rates of sedimentation during the Pleistocene in the North Atlantic. Furthermore, it may be possible to delineate the Pliocene-Pleistocene boundary, using the first appearance of ice-rafted detritus in deep-sea pelagic cores as a criterion. The disappearance of ice-rafted

Table 1. LOCATION OF CORES USED FOR ICE-RAFTING DISTRIBUTION ANALYSES (Fig. 2)

Core	Latitude	Longitude	Water depth (metres)
A157-5	48° 35' N	36° 51' W	4,500
A157-6	48° 08' N	39° 20' W	4,500
A157-13	40° 34' N	43° 51' W	4,680
A180-9	39° 27' N	45° 57' W	4,060
A180-13	39° 08' N	42° 39' W	4,880
A180-14	38° 41' N	40° 40' W	5,020
A180-15	39° 16' N	36° 42' W	4,610
R5-36	48° 55' N	18° 35' W	4,500
R10-1	56° 47' N	31° 00' W	2,375
R10-10	41° 24' N	40° 08' W	4,755
SP9-3	53° 53' N	21° 06' W	2,745
SP9-4	50° 02' N	14° 46' W	4,205
SP10-1	51° 28' N	88° 04' W	3,695

detritus with depth occurs in sediment cores raised from the deep ocean floor surrounding Antarctica^{9,10} and occurs just beneath the Pliocene-Pleistocene boundary based on radiolarian assemblages¹¹.

So far, none of the cores taken in the North Atlantic ice-rafting area passes by continuous section through the Pleistocene and none has been identified as containing the Pliocene-Pleistocene boundary; but further attempts to take such cores are now in progress.

We thank D. B. Ericson, who kindly lent us samples of sand from Lamont cores. Important support for this work was provided by a Ford Foundation fellowship and a Fulbright travel grant for Dr. J. R. Conolly. This

work was also supported by the Office of Naval Research and by the U.S. National Science Foundation.

¹ Bramlette, M. N., and Bradley, W. H., *U.S. Geol. Surv., Prof. Paper*, 196, A, Part 1 (1940).

² Murray, J., and Benard, A. F., *Challenger Rep.*, 322 (1891).

³ Andree, K., *Geologie des Meeresboden*, 2 (Gebouder Borntraeger, Leipzig, 1891).

⁴ Ewing, M., *Nat. Geog. Mag.*, 66, 611 (1949).

⁵ Heezen, B. O., *Geol. Soc. Amer. Spec. Paper*, 65, 99 (1959).

⁶ Pratt, B. M., *Deep-Sea Res.*, 8, 152 (1961).

⁷ Ericson, D. B., Ewing, M., Wollin, G., and Heezen, B. O., *Geol. Soc. Amer.*, 72, 193 (1961).

⁸ Ericson, D. B., Ewing, M., and Wollin, G., *Science*, 144, 1185 (1964).

⁹ Krinsley, D. H., and Newman, W. S., *Science*, 149, 442 (1965).

¹⁰ Conolly, J. R., and Ewing, M., *Science* (in the press).

¹¹ Hays, J. D., *Amer. Geophys. Union Antarctic Research Series*, 6, 125 (1965).

AN INFRA-RED INVESTIGATION OF THE EFFECT OF PRESSURE ON SILICA POWDERS, AS REVEALED BY DEUTERIUM OXIDE EXCHANGE

By F. H. HAMBLETON, Dr. J. A. HOCKEY and J. A. G. TAYLOR*

Department of Chemistry, Faculty of Technology, University of Manchester

IN recent years, infra-red spectroscopic techniques have been widely used to examine the surface properties of a variety of solids. The direct examination of finely divided solids is hampered by scattering of the incident radiation. This problem is often overcome by pressing self-supporting disks which have much higher transmission and, additionally, have a higher number of functional groups present in the beam. Infra-red investigations of the exchange properties of the surface hydroxyl groups present on silica, with deuterium oxide (D_2O) vapour, show that the high pressures used in disk formation can significantly alter the behaviour of the sample.

The pretreatment and surface properties of the re-hydrated 'Aerosil' used in the work recorded here have been described elsewhere¹. Disks were prepared from about 50-mg samples which were placed in a die and evacuated (on a conventional vacuum-line fitted with liquid nitrogen traps) to a pressure of 2×10^{-4} mm for 30 min. Two disks, RA2/5 and RA2/10, were formed by pressing at 5 tons/cm² and 10 tons/cm² respectively for 30 min. Two further disks, DA2/5 and DA2/10, were pressed after six-fold exchange in the die with saturated D_2O vapour (Norsk Hydro, purity 99.7 per cent); each contact lasted 120 sec and the sample was evacuated to 2×10^{-4}

* Present address: Unilever Research Laboratory, Port Sunlight, Cheshire.

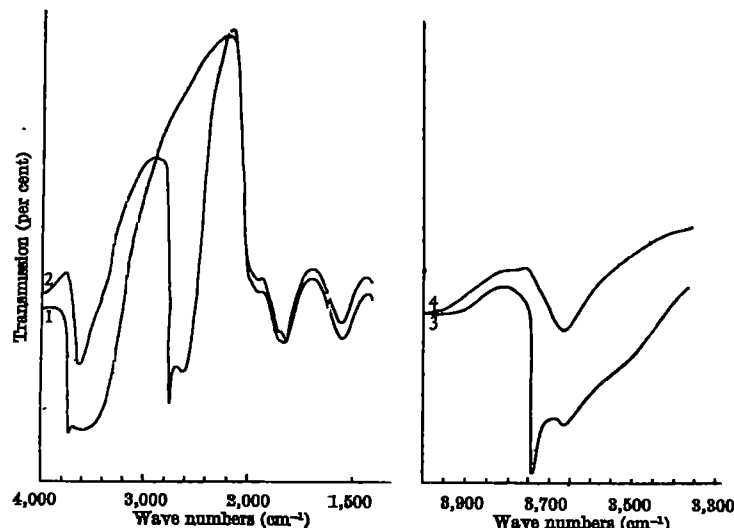


Fig. 1. (1) Infra-red spectrum of original disk RA2/5; (2) spectrum after D_2O exchange; (3) infra-red spectrum of original powder RA—ordinate expanded 50 times; (4) spectrum after D_2O exchange

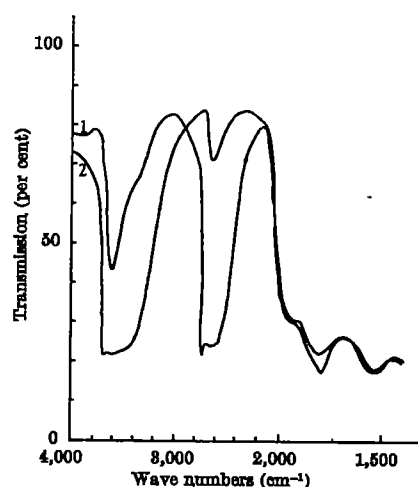


Fig. 2 (1) Infra-red spectrum of disk made from deuterated powder—DA2/5; (2) spectrum after H_2O exchange

mm mercury between each exchange, before final evacuation for 30 min at the same pressure.

The disks were mounted in a stainless-steel cell, similar to that described by Harrison and Lawrence², and spectra were recorded between 4,000 cm^{-1} and 1,500 cm^{-1} , using a Perkin-Elmer 125 double-beam grating spectrometer. The cell was connected to a vacuum line fitted with facilities for treating the sample, *in situ*, with water and heavy water vapour. Disks DA2/5 and DA2/10 were further treated with D_2O vapour to replace the surface OD groups lost during the transfer of the sample from the die to the cells.

The spectra of RA2/5 before and after D_2O exchange are given in Fig. 1; these show that an appreciable proportion of the 3,650 cm^{-1} hydroxyl band is unchanged; this proportion is even greater in the case of an RA2/10 disk. The absence of a peak at 3,737 cm^{-1} indicates that all the single hydroxyl groups are exchanged. Similar spectra for the unpressed RA2 powder are also shown; these indicate that a proportion of the unexchangeable hydroxyl groups are present in the powder.

That additional unexchangeable surface groups result from compression is illustrated by the spectra given in Fig. 2 for

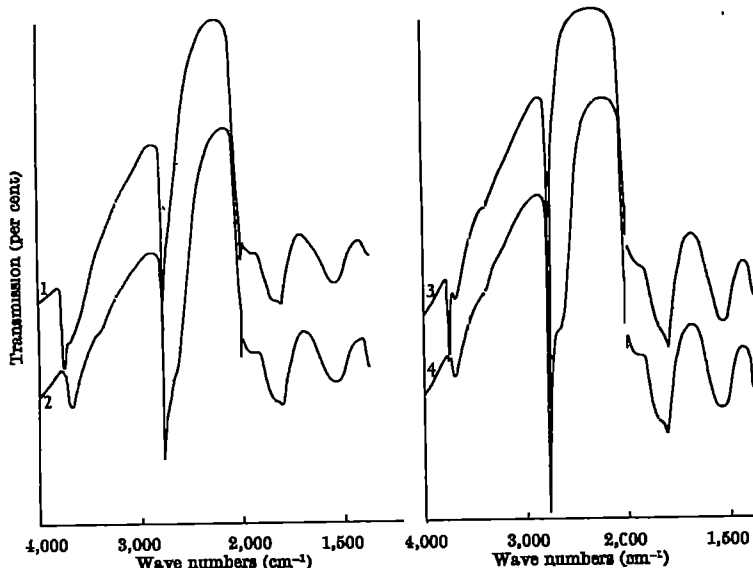


Fig. 3. (1) Disk RA2/5 heated to 300°C; (2) after D₂O exchange; (3) disk RA2/5 heated to 450°C; (4) after D₂O exchange

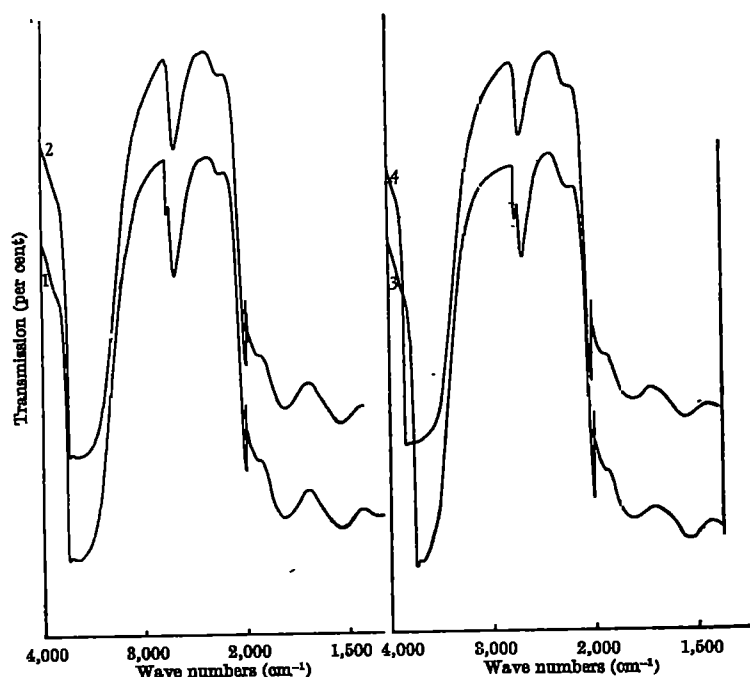


Fig. 4. (1) Disk DA2/5 heated to 150°C; (2) after H₂O exchange; (3) disk DA2/5 heated to 300°C; (4) after H₂O exchange

DA2/5. As with the powder sample, there are unexchangeable hydroxyl groups, but in addition there are also OD groups resulting from exchange of the powder, characterized by a band at 2,710 cm⁻¹, which cannot be exchanged with water vapour. The intensity of this band

is greater for DA2/10 than for DA2/5. Control experiments show that all exchanges carried out on the disks are reversible. No change in the 1,640 cm⁻¹ band as a result of the exchange was noted.

Figs. 3 and 4 show respectively the effects of heating the exchanged RA2/5 and DA2/5. Surface dehydration is accompanied by a decrease in intensity of the low-frequency component of the vibrational absorption bands for both the exchanged and unexchangeable groups. However, there is an increase in the absorption intensity of the 3,737 cm⁻¹ band, assigned to single hydroxyls, for RA2/5, and in that of the 2,755 cm⁻¹ band, assigned to single deuteriohydroxyls, for DA2/5. These new 'single' absorption bands can now be readily changed, together with some of the low-frequency tail. When samples are heated to higher temperatures, exchange is accompanied by a large degree of chemisorption even with contact times of < 60 sec at room temperature. The spectrum of the D₂O-exchanged RA2/5, after heating to 450°C, was stable after further evacuation for 60 h, showing that the growth of the 3,737 cm⁻¹ band did not result from leakage of the cell.

The spectral results presented show that formation of a silica disk results in a product having certain important differences from the original powder. It is clear that it may be erroneous to make direct comparison between the infra-red results obtained from such pressed disks, and gravimetric and adsorption data determined on the corresponding powders. The evidence suggests that either regions inaccessible to H₂O or D₂O vapour are formed, or that bringing the particles closer together causes enhanced hydrogen bonding which prevents exchange occurring. However, as it is thought that it is the most closely adjacent OH groups which are first lost on dehydration, a fact confirmed by the more rapid decrease in the low-frequency tail of the ν_{OH} band, then such strongly bonded groups would be expected to be more rapidly lost than the exchangeable hydroxyl groups. This is shown not to be the case. Additionally, the increase in the single ν_{OH} (or ν_{OD}), observed on heating, would not be expected by this mechanism. Disks made at lower pressures exhibit the same effects, but to a lesser extent. It is realized that pressures described elsewhere for the preparation of 'Aerosil' disks are sometimes lower; however, 'Aerosil' probably represents an optimum case and other oxides require higher pressures.

¹ Taylor, J. A. G., Hockley, J. A., and Pethica, B. A., paper presented at Brit. Ceramic Soc. Conf. Physics and Chemistry of Ceramic Surfaces (in the press).

² Harrison, F. R., and Lawrence, J. J., *J. Sci. Instrum.*, **41**, 693 (1964).

HEXAGONAL-MESH STRUCTURES AND STABLE POLYPEPTIDE CHAIN CONFIGURATIONS

By PROF. R. E. DICKERSON

Gates and Crellin Laboratories of Chemistry, California Institute of Technology, Pasadena

HECHTER¹ has recently proposed a hexagonal-sub-unit model for the protein component of cell membranes, based on polypeptide chain structure principles set forth by Warner^{2,3}. However, available experimental and theoretical evidence shows such structures to

be exceedingly unlikely, and the basis for the membrane model therefore should be reconsidered.

Warner has proposed that simple peptide antibiotics such as gramicidin S and actinomycin are built up from a basic hexapeptide unit in which the carbonyl oxygens

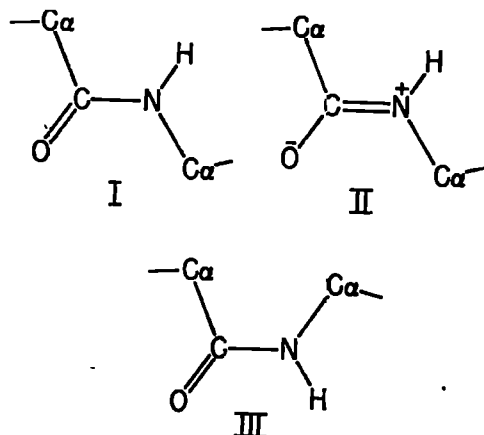


Fig. 1. Two resonance forms of the *trans* configuration of the planar peptide bond (I and II) and the *cis* configuration (III)

lie 4.8 Å apart at the corners of a hexagon. The hydrophobic side-chains project from one face of the flat molecule in this model, and the hydrophilic carbonyl and NH groups extend from the other face. Decapeptide antibiotics are then related to the basic hexamer as naphthalene is to benzene, and larger units are built up on a flat hexagonal mesh. The two essential features of the model are the separated hydrophilic and hydrophobic sides, and the regular 4.8 Å hexagonal spacing of carbonyl oxygens on the hydrophilic face, related to the second-neighbour oxygen spacings in the structure of ice. This flat hexagonal mesh model has been extended to the

158-residue protein sub-unit of TMV as well⁴, although the stacking model shown does not agree well with electron micrographs of the TMV molecule.

Hechter's membrane model uses flat spiral hexagonal sub-units of a similar size packed in an overlapping double layer with hydrophobic faces paired between layers, and with channels or pores through the membrane. Several properties of the membrane are then explained with considerable ingenuity from this model. There may well be elements of truth in the cell membrane model, but the peptide hexamer principles on which it is based are energetically untenable.

The fundamental flaw in the peptide model is that it cannot be built with the desired properties without deforming the amide or peptide linkages severely out of their stable planar configurations (Fig. 1). In contrast, there is considerable evidence for the true planarity of peptide bonds in peptides and proteins.

Theoretical Evidence

Early structural work on small compounds by Corey and Pauling^{5,6} and others showed the amide bonds to be uniformly planar. Pauling explained this in terms of the joint contribution of two resonance structures, I and II in Fig. 1. The central C—N bond would be shortened from its normal 1.475 Å as it assumed a partial double-bond character and the C=O distance would increase beyond 1.205 Å as it lost double-bond character. Assuming a 60:40 contribution of I and II, the expected C—N distance was calculated to be 1.32 Å and the expected C=O distance to be 1.24 Å.

The resonance stabilization energy of the amide bond has been calculated by Pauling and Sherman^{7a,b} from

Table 1. RESULTS OF THE X-RAY ANALYSES OF SIMPLE COMPOUNDS CONTAINING PEPTIDE BONDS
The order is roughly chronological, with references only to the most recent or most complete analyses

Compound	C—N distance in Å	O—N per cent double-bond character (a)	C=O distance in Å	Max. deviation from amide planarity (b)	S.D. in bond-lengths in Å (c)	Ref.
Acetamide	1.88		1.28	—	—	13
α -glycylglycine	1.82		1.24	Planar	—	14
β -glycylglycine	1.29		1.23	< 6°	—	15
N-acetylglutamine	1.82		1.24	< 1.3°	—	16
L-glutamine	1.28		1.27	—	0.024	17
Urea	1.84		1.26	—	0.012	18
Glycyl-L-tyrosine hydrochloride	1.85		1.16	0.20 Å	~0.08	19
Glycyl-L-asparagine	1.82	~25	1.23	< 0.01 Å	~0.014	20, 26
N,N'-diglycyl-L-cystine dihydrate	1.85	25	1.21	6°	~0.08	21
Formamide	1.80	45	1.26	—	0.018	22
Oxamide	1.815	39	1.243	—	0.005	23
N,N'-diacetylhexamethylene diamine	1.85	25	1.22	0.01 Å	~0.008	24
Glycyl-L-tryptophan dihydrate	1.83	32	1.23	0.02 Å	~0.014	25
Succinamide	1.833	33	1.238	—	0.002	26
L-leucyl-L-prolyl-glycine	1.84	42	1.27	0.04 Å	0.015	27
Glutathione (γ -L-glutamyl-L-cysteinyl-glycine)	1.81	38	1.24	0.03 Å	—	28
Diketopiperazine	1.82	~42	1.23	0.12 Å	0.028	29
N-methyl acetamide	1.825	~38	1.24	0.05 Å	—	30
Glycyl-phenylalanyl-glycine	1.89	(d)	1.21	0.034 Å	0.02	31
Cu(II) monoglycylglycine trihydrate	1.84	~30	1.23	0.011 Å	—	47
Tosyl-L-proline-L-hydroxyproline monohydrate	1.276	~55	1.264	Planar	0.016	48
Cyclohexaglycyl hemihydrate	1.82	~55	1.277	Planar	0.014	49
Glycylglycylglycine Cu(II) chloride sesquihydrate	1.84	~29	1.24	6.5°	—	32
Sodium glycylglycylglycine cuprate(II) monohydrate	1.804	~42	1.228	~0.06 Å	—	48
Disodium glycylglycylglycylglycine cuprate decahydrate	1.807	~41	1.190	0.057 Å	0.012	49
	1.29	~50	1.23	0.024 Å	—	50
	1.86	(e)	1.24	0.05 Å	0.012	50
	1.290	~50	1.276	0.16 Å	—	33
	1.816	~39	1.267	0.015 Å	0.008	33
Potassium benzylpenicillin	1.801	~45	1.272	0.004 Å	—	34
	1.83	~34	1.25	0.013 Å	(f)	34
				Planar	—	

(a) Per cent double-bond character is taken from Hahn (ref. 8), based on both O—N and C=O bond-lengths. Later approximate values are interpolations based on C—N distances only. The limits of error in these figures are wide. The difference in bond-length between complete single-bond character and complete double-bond character is only about 0.24 Å, and an uncertainty (three standard deviations) of 0.08 Å in bond-length would correspond to an uncertainty in per cent double-bond character of ± 12 per cent.

(b) Maximum deviation in Å of any atom from the best least-squares plane fitted to the amide group, or alternatively, angle of twist about the C—N bond in degrees. For small angles of twist, β , it is approximately true that, $\sin \beta = 0.8 d$, where d is the deviation in Å of one of the corner atoms of the amide plane from co-planarity. The greatest observed non-planarity is 0.20 Å or 9°, corresponding to a strain energy of only 0.5 kcal/mole.

(c) Standard deviations in most cases have been obtained from least squares refinement. There is a 95 per cent chance that a variation of as much as three times the standard deviation is not statistically significant. This should be kept in mind when assessing limits of error in the per cent double-bond figure.

(d) The value of only 16 per cent double-bond character obtained from this 1.39 Å bond-length is misleading. The authors themselves state that, because of experimental errors in the data, the difference of 0.07 Å between this value and the expected one of 1.32 Å is not significant (ref. 31).

(e) The second C—N bond is badly distorted in the crystal because of the chelation of all three nitrogens to the same copper atom. Note that the C=O bonds, farther removed from the copper atom, are as expected for about 40 per cent double-bond character. In spite of the considerable lengthening of the second C—N bond this amide is only twisted 7.4° out of planarity.

(f) No standard deviations are given, but single bonds are observed to have a mean variation of 0.08 Å from their expected values.

(g) Cysteinyl glycine sodium iodide has been reported (ref. 11) as having a non-planar amide. The original structure analysis is definitely in error and the data are being re-analysed by Dr. J. Donohue (ref. 12).

(h) For further discussions of amide planarity, see refs. 8, 9 and 10.

thermodynamic heats of formation of formamide, acetamide and oxamide. Values of 21.4, 20.5 and 23.7 kilocalories per mole of amide bonds, respectively, were obtained. In view of the similarity of C=O and C—N bond-lengths in these compounds and in peptides, the resonance energy per amide in a polypeptide chain is unlikely to differ from 21 kcal by more than a few kcal. A reasonable measure of the strain energy involved in twisting an amide linkage out of planarity about its C—N bond by an angle β is then: $E = 21 \sin^2 \beta$ kcal/mole. Hence even a twist of 30° will require a strain energy of more than 5 kcal.

Experimental Evidence

The experimental evidence for the planarity of the peptide bonds falls into three categories: di- and tripeptides and related compounds, fibrous proteins, and globular proteins. The results of careful X-ray analysis of compounds in the first category are given in Table 1. In all cases the amide bond has been found to be planar, and in all the peptides it is in the *trans* configuration (I rather than III in Fig. 1). In all cases the expected shortening of the C—N and lengthening of the C=O bond are found, and the contribution of resonance form II usually lies in the 30–40 per cent range.

These observations are given added significance by the X-ray examination of potassium benzylpenicillin, a larger molecule containing one amide bond³⁴. The bond is *trans* and planar, and showed the expected changes in bond-lengths (Table 1).

In X-ray analyses of fibrous proteins, accurate bond parameters are not sought, and analysis consists essentially in fitting sterically acceptable models to the observed diffraction pattern³⁵. Nevertheless, the field only began to produce results after the work of Corey *et al.* had produced standard parameters for polypeptide chain geometry^{3,6}. In all work since then, whether with α -helices, β -sheets, collagen triple helices or more complex structures, it has never been necessary to invoke non-planar amides to satisfy the diffraction pattern, and in only one case (polyproline I (ref. 36)) have *cis* amides been found.

Complete structure analyses are only beginning to appear for globular proteins, and the only ones at near-atomic resolution (2 Å or better) so far are myoglobin^{37,38} and lysozyme³⁹. Two-angstrom resolution does not permit measurement of accurate atomic parameters, but geometry of the peptide links is clear. In all cases, for both myoglobin⁵¹ and a preliminary examination of lysozyme⁵², only *trans* amides were found, planar within the limits of accuracy of the structure (of the order of $\pm 5^\circ$ for myoglobin). The fact that such restrictions are observed in both a high-helix and a moderately low-helix protein, and in both hydrophobic and hydrophilic regions of the molecules, suggests that violations of planarity in other structures will be difficult to justify.

In summary, there is no evidence from any structure analysis, from simple dipeptides to proteins, that the amide bond ever deviates more than a few degrees from planarity, and in all but two or three small cyclic compounds such as diketopiperazine and cyanuric acid and one fibrous polypeptide only the *trans* configuration has been found.

Hexagonal Peptide Models

In contrast to the foregoing evidence, the hexagonal-unit peptide models proposed by Warner²⁻⁴ require the amide bonds to be deformed out of planarity, in some instances by nearly 90° . Of the eighteen amide bonds in a nonadeca-peptide fragment of ACTH¹, only one appears to be *trans* and planar. Four are planar but *cis*, four are skew but nearer *cis* than *trans*, seven are skew but nearer *trans* than *cis*, and two appear to be at right angles. A rough calculation of strain energy based on Pauling's formula, and assigning

twist angles no more precisely than 0° , 45° , 90° , 135° , and 180° to the categories mentioned here as determined from the photograph, gives a value of about 160 kcal/mole.

As part of an X-ray analysis of actinomycin⁴¹ we have tried building Warner's actinomycin molecule shown in Fig. 6b of ref. 2. The total strain energy in this molecule, based on amide deformations measured from the model and accurate to $\pm 10^\circ$, is 120 kcal/mole. An unstrained configuration with eight planar *trans* and two planar *cis* linkages is possible in a model of the same size and shape as Warner's (Fig. 11, ref. 41). However, the structural advantages of Warner's model are largely lost. Only six of the ten carbonyl oxygens extend out from the 'hydrophilic' face, two extend out from the edges and two more now project from the 'hydrophobic' side. Nothing at all resembling a regular hexagonal array of carbonyl oxygens can be seen. Extensive adjustments of the space-filling models have shown no way to segregate hydrophobic and hydrophilic groups or to re-create the oxygen arrangement without deforming the amides from planarity.

If the planarity of the amide bond is accepted as a fundamental principle, then the larger the structure the more difficult it becomes to retain the specific features of Warner's structures. The basic hexapeptide with *cis* amides (Fig. 2b) has α -carbons so close that the structure is only usable for hexaglycine. The *trans* hexamer (Fig. 2a) does place the carbonyl oxygens 4.8 Å apart in a hexagon and extend the side-chains out the opposite side. However, the NH groups fall on the 'hydrophobic' rather than the 'hydrophilic' side.

The decapeptide built up from two fused hexagons is less tractable. The planar *trans* model is shown in Fig. 3 with a bond sketch in the corner. The two re-entrant angles at positions 1 and 6 pose a problem. The amide linkage considered as a unit does not alter the direction of the polypeptide chain (Fig. 4a), and any bending of

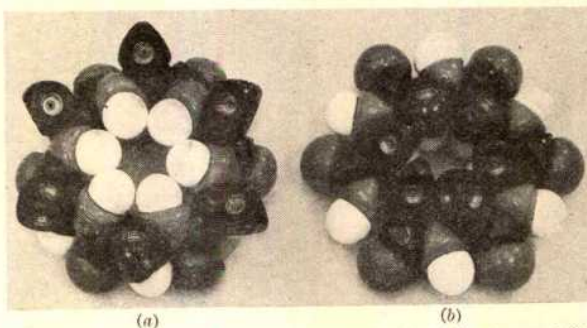


Fig. 2. Hexapeptide models with planar amides, in (a) the *trans* and (b) the *cis* configuration. Side-chains are represented only by single carbon atoms attached to the main chain or α -carbon. Note the steric hindrance between these side-chain carbons in the *cis* form.

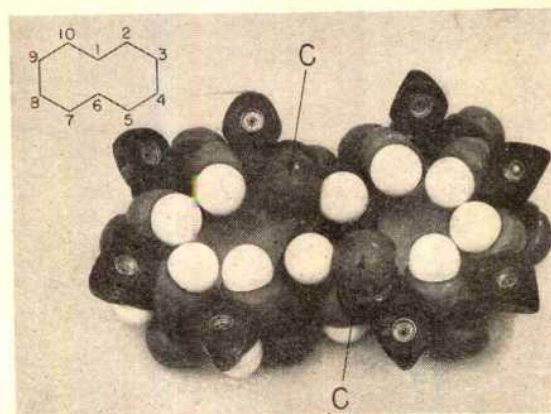


Fig. 3. Decapeptide with planar *trans* amide bonds, showing the 'hydrophobic' side. Side-chains are represented only by single (β) carbons. Note the presence of the two carbonyl groups (c) on the 'wrong' side of the molecule.

the chain must be produced at the α -carbons (Fig. 4b, c). At re-entrant angles the C_α must be rotated 180° from the position of Fig. 4b to 4c, bringing the side-chain over to the hydrophilic side, where it collides with the carbonyl oxygen.

At any re-entrant angle, therefore, one must either move the side-chain of one residue to the hydrophilic side or replace the L-amino-acid by a D-form. If the L-form is retained, then the adjacent amide group must be rotated so as to move the carbonyl oxygen to the hydrophobic side to get it out of the way unless the residue involved is glycine. Fig. 5 shows the decapeptide with the carbonyl rotated in this manner to the 'wrong' side, and Fig. 6 shows the steric hindrance between carbonyl and side-chain if this is not done. A brief examination of sequences of decapeptide antibiotics⁴² shows that except for gramicidin S (and actinomycin if it is one decapeptide ring), one does not generally find D-amino-acids or glycines paired across the ring.

Larger structures become increasingly difficult. Three rings of Warner's flat spiral TMV structure (Fig. 1 of ref. 4) were built, and the preceding comments about re-entrant angles were checked. A further extension of model building was not felt to be worth while. No less than 64 of the 158 residues in the TMV structure are involved in re-entrant angles in this model, meaning that almost as many carbonyls and side-chains will project from each side unless many of these re-entrant sites are glycine or D-amino-acids. The known amino-acid sequence of TMV (ref. 43) lends no support to this latter idea.

Energy Requirements for Non-planar Amide Structures

It has recently been suggested^{44,45} that, even though the non-planar amide structures (hereafter referred to as 'strained' structures) were not the lowest energy states for small peptides and antibiotics in solution, these structures might be stabilized by hydrogen bonding and hydrophobic interactions when in contact with enzyme

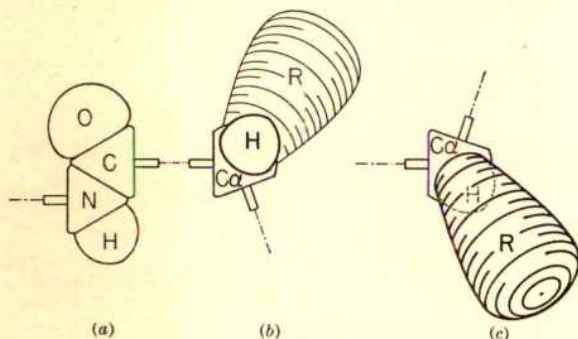


Fig. 4. Space-filling models of: (a) planar *trans* amide group, (b) and (c) α -carbon with hydrogen (H) and side-chain (R). Note that any change in chain direction must occur at an α -carbon site, as the planar *trans* amide group taken as a unit leaves the chain direction unaltered.

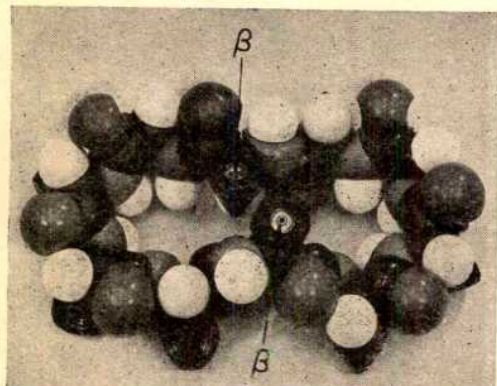


Fig. 5. Decapeptide of Fig. 3, showing the 'hydrophilic' side. Note the presence of two side-chains (β , represented by β -carbon atoms only) on the 'wrong' side, and the fact that there is barely room for them.

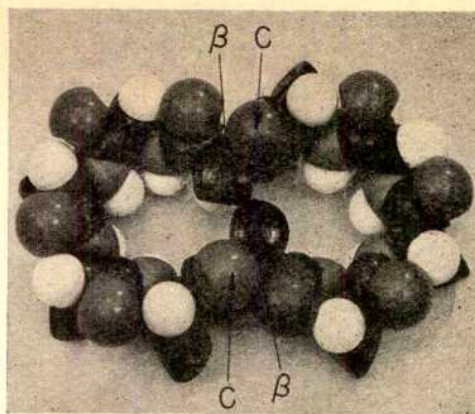


Fig. 6. Decapeptide of Figs. 3 and 5, 'hydrophilic' side, with the two displaced carbonyls (c) rotated back to the proper side. Note the severe steric hindrance between these two carbonyls and the neighbouring side-chains (β).

surfaces, cell membranes or even like molecules in the form of dimers. By this argument, two peptide configurations, each inherently unstable when the units are isolated, would become a stable system when the units were locked together through the collective effect of a number of weak forces. This is a perfectly valid concept if it is made quantitative, but dangerous if it is not. Undoubtedly the exact conformation of a polypeptide chain can be strongly influenced by binding to a supporting surface or to a smaller substrate molecule. But it is not difficult to show that neither hydrogen bonding nor hydrophobic interactions are sufficiently strong to compensate for the strain energies encountered in these non-planar amides.

In such a quantitative comparison, it is not enough to compare the amide strain energy with the total energy obtained by hydrogen bonds and hydrophobic interactions, for an unstrained polypeptide configuration can always be found with much the same binding properties if not the same precise configuration (as with the two actinomycin models). One must therefore compare the strained molecule in a given set of circumstances with an unstrained molecule in an equivalent environment.

(1) *Average strain energy per amide bond.* With a fragment of ACTH containing 18 amide bonds, a crude strain energy estimate of 160 kcal was obtained. The actinomycin figure of 120 kcal for ten amides is more accurate. Thus, although there is a maximum possible strain energy of 21 kcal per bond for bonds wrenched to right angles, an estimate of 10 kcal per bond as an average in hexagonal-type structures is certainly safe. If such strained structures are to be stable, some factors must be found which will contribute an average energy of at least 10 kcal per amino-acid residue.

(2) *Stabilization by favourable hydrogen bonding with solvent.* If the same number of hydrogen bonds can be formed with the strained and unstrained structures, then there is no stabilization. But now suppose that a fraction y of the possible bonds in the strained form are impossible in the unstrained form. A simple inversion of a carbonyl or NH group from one face of the molecule to the other is not sufficient, and to be inaccessible the group must be buried. Examination of models suggests that certainly not more than 30 per cent of the possible groups will be so buried.

The enthalpy of formation of a hydrogen bond with water is about -5 kcal. The entropy change is less precise, but it will certainly be negative, making the free energy change greater than -5 kcal (less in absolute magnitude). The exact value does not matter, for the conclusions hold even if one takes the least favourable case and neglects the entropy entirely. In this case, the stabilizing contribution of the extra hydrogen bonds in the strained form will be $\Delta F = -5y$ kcal per mole of

amide bonds, or -1.5 kcal at the most. This is quite insufficient to counteract the free energy change of -10 kcal per bond in going to the strained form.

(3) *Stabilization by pairing of hydrophobic faces.* Pairing of hydrophobic faces is energetically favourable not because of van der Waals interactions between hydrophobic side-chains, which amount to only a few tenths of a kilocalorie, but because of the removal of such groups from the aqueous environment. Kauzmann⁴⁶ has shown that the most important term involved is the entropy one, and has calculated that the removal of one hydrophobic side-chain from water to a non-polar environment is accompanied by a free-energy decrease of no more than about 4 kcal.

Let us assume that in the strained model all hydrogen-bonding and hydrophobic groups are properly segregated to their respective sides of the flat molecule, while in the unstrained version a fraction x of each type is on the wrong side. The free energy of stabilization of the strained molecules by pairing of hydrophobic faces will be $\Delta F_{h,s} = -4$ kcal per amino-acid residue. In the unstrained molecule, only a fraction $(1-x)$ of the hydrophobic groups will be buried, for a stabilization energy of $-4(1-x)$ kcal. But a fraction x of the previously formed hydrogen bonds will be broken because they occurred to groups on the 'hydrophobic' face, producing a destabilization of $+5x$ kcal in the most unfavourable circumstances. The net free energy of stabilization is then $\Delta F_{h,u} = -4 + 9x$ kcal per mole. The lowest reasonable value of x is 0.5, that for a random distribution of types of groups between faces.

The net free energy change in going from unstrained to strained molecules, both with the maximum possible use of hydrophobic interactions, is then:

$$\Delta F = \Delta F_{\text{strain}} + \Delta F_{h,s} - \Delta F_{h,u} = (+10 - 4 + 4 - 9x) \text{ kcal per bond.}$$

If the worst possible case is assumed, comparing a completely ordered strained molecule with an unstrained molecule having random occurrence of side-chains on either side, then $x = 0.5$ and $\Delta F = +5.5$ kcal.

The conclusion is therefore that even if the most favourable hydrogen bonding and hydrophobic interactions are used, the flat, hexagonal-mesh, non-planar-amide structures are unstable relative to a planar-amide form by 5–10 kcal per amide bond.

Summary

The hexagonal-mesh models proposed by Warner, if made with planar amides, do not create distinct hydrophobic and hydrophilic sides and do not place the carbonyl oxygens at the corners of a hexagonal mesh 4.8 \AA on a side as proposed. Moreover, the strain involved in twisting the amides out of planarity is so great that even the best hydrogen bonding and hydrophobic bonding cannot make these strained structures stable. If models of this general type are proposed, they should be based on planar (and probably *trans*) amide groups, and tested with space-filling models rather than hexagonal-mesh diagrams, for that which is true for single or double rings is not necessarily extendable to a semi-infinite mesh.

It is probably reasonable to say that any peptide model with non-planar amides can be regarded from the outset as extremely improbable. But if it proves useful for certain purposes to hypothesize models of this type, then their instability must be kept clearly in mind, and at some point a quantitative numerical justification for such a distorted structure must be made. At present there is no direct evidence for the hexagonal-mesh structures considered here and overwhelming evidence against them.

- ¹ Hechter, O., *Neurosciences Research Program Bulletin*, **2**, No. 5, 36 (1964).
- ² Warner, D. T., *Nature*, **190**, 120 (1961).
- ³ Warner, D. T., *J. Theor. Biol.*, **1**, 514 (1961).
- ⁴ Warner, D. T., *J. Theor. Biol.*, **6**, 118 (1964).
- ⁵ Corey, R. B., and Pauling, L., *Proc. Roy. Soc., B*, **141**, 10 (1953).
- ⁶ Pauling, L., and Corey, R. E., *Fortschr. Chem. Org. Nat.*, **11**, 180 (1954).
- ⁷ (a) Pauling, L., *The Nature of the Chemical Bond*, third ed. (Cornell Univ. Press, 1960). (b) Pauling, L., and Sherman, J., *J. Chem. Phys.*, **1**, 606 (1933).
- ⁸ Hahn, T., *Z. Krist.*, **109**, 438 (1957).
- ⁹ Kendrew, J. C., and Perutz, M. F., *Ann. Rev. Biochem.*, **26**, 327 (1957).
- ¹⁰ Rich, A., and Green, D. W., *Ann. Rev. Biochem.*, **30**, 93 (1961).
- ¹¹ Dyer, H. B., *Acta Cryst.*, **4**, 42 (1951).
- ¹² Donohue, J. (personal communication).
- ¹³ Senti, F., and Harker, D., *J. Amer. Chem. Soc.*, **62**, 2008 (1940).
- ¹⁴ Hughes, E. W. (unpublished results).
- ¹⁵ Hughes, E. W., and Moore, W. J., *J. Amer. Chem. Soc.*, **71**, 2618 (1949).
- ¹⁶ Carpenter, G. B., and Donohue, J., *J. Amer. Chem. Soc.*, **72**, 2315 (1950).
- ¹⁷ Cochran, W., and Penfold, E. R., *Acta Cryst.*, **5**, 644 (1952).
- ¹⁸ Vaughan, P., and Donohue, J., *Acta Cryst.*, **5**, 530 (1952).
- ¹⁹ Smits, D. W., and Wiebenga, E. H., *Acta Cryst.*, **6**, 531 (1953).
- ²⁰ Pasternak, R. A., Katz, L., and Corey, R. B., *Acta Cryst.*, **7**, 225 (1954).
- ²¹ Yakel, H. L., and Hughes, E. W., *Acta Cryst.*, **7**, 291 (1954).
- ²² Ladell, J., and Post, B., *Acta Cryst.*, **7**, 559 (1954).
- ²³ Ayerst, E. M., and Duke, J. E. C., *Acta Cryst.*, **7**, 588 (1954).
- ²⁴ Bailey, M., *Acta Cryst.*, **8**, 575 (1955).
- ²⁵ Pasternak, R. A., *Acta Cryst.*, **9**, 341 (1956).
- ²⁶ Davies, D. R., and Pasternak, R. A., *Acta Cryst.*, **9**, 334 (1956).
- ²⁷ Leung, Y. C., and Marsh, R. E., *Acta Cryst.*, **11**, 17 (1958).
- ²⁸ Wright, W. B., *Acta Cryst.*, **11**, 632 (1958).
- ²⁹ Degellh, R., and Marsh, R. E., *Acta Cryst.*, **12**, 1007 (1959).
- ³⁰ Katz, J. L., and Post, B., *Acta Cryst.*, **13**, 624 (1960).
- ³¹ Marsh, R., and Glusker, J. E., *Acta Cryst.*, **14**, 1110 (1961).
- ³² Fridrichsons, J., and Mathieson, A. McL., *Acta Cryst.*, **15**, 569 (1962).
- ³³ Freeman, H. C., and Taylor, M. R., *Acta Cryst.*, **13**, 939 (1965).
- ³⁴ Pitt, G. J., *Acta Cryst.*, **5**, 776 (1952).
- ³⁵ Dickerson, R. E., in *The Proteins*, edit. by Neurath, H., **2**, 603 (Academic Press, 1964).
- ³⁶ Traub, W., and Shmueli, W., in *Aspects of Protein Structure*, edit. by Ramachandran, G. N., **81** (Academic Press, 1963).
- ³⁷ Kendrew, J. C., Dickerson, R. E., Strandberg, B. E., Hart, R. G., Davies, D. R., Phillips, D. C., and Shore, V. C., *Nature*, **185**, 422 (1960).
- ³⁸ Kendrew, J. C., *Science*, **139**, 1259 (1963).
- ³⁹ Blake, C. C. F., Koenig, D. E., Mair, G. A., North, A. C. T., Phillips, D. C., and Sarma, V. R., *Nature*, **206**, 757 (1965).
- ⁴⁰ Kendrew, J. C., in *Enzyme Models and Enzyme Structure*, *Brook. Symp. Biol.*, **15**, 216 (1962).
- ⁴¹ Palmer, H. T., Palmer, R. A., and Dickerson, R. E., *Nature*, **202**, 1052 (1964).
- ⁴² Miller, M. W., *Pfizer Handbook of Microbial Metabolites* (McGraw-Hill, New York, 1961).
- ⁴³ Tsugita, A., Gish, D. T., Young, J., Fraenkel-Conrat, H., Knight, C. A., and Stanley, W. M., *Proc. U.S. Nat. Acad. Sci.*, **46**, 1463 (1960).
- ⁴⁴ Hechter, O., in *Mechanisms of Hormone Action*, edit. by Karlson, P., **61** (Academic Press, 1965).
- ⁴⁵ Hechter, O., *Fed. Proc.*, **24**, No. 2, Part 3, S-91 (1965).
- ⁴⁶ Kauzmann, W., *Adv. Protein Chem.*, **14**, 1 (1959).
- ⁴⁷ Strandberg, Bror, Lindquist, L., and Rosenstein, R., *Z. Krist.*, **116**, 286 (1961).
- ⁴⁸ Karle, I., and Karle, J., *Acta Cryst.*, **18**, 969 (1963).
- ⁴⁹ Freeman, H., Robinson, G., and Schoone, J. C., *Acta Cryst.*, **17**, 719 (1964).
- ⁵⁰ Freeman, H., Schoone, J. C., and Seine, J. G., *Acta Cryst.*, **18**, 331 (1965).
- ⁵¹ Kendrew, J. C. (personal communication).
- ⁵² Blake, Colin (personal communication).

A NEW INHERITED COAGULATION DISORDER CAUSED BY AN ABNORMAL FIBRINOGEN ('FIBRINOGEN BALTIMORE')

By DR. EUGENE A. BECK, PATRICIA CHARACHE and PROF. DUDLEY P. JACKSON

Department of Medicine, The Johns Hopkins University School of Medicine and Hospital, Baltimore, Maryland

An inherited abnormality of fibrinogen resulting in an asymptomatic clotting defect was described by Ménaché¹. Another congenital qualitative defect of fibrinogen associated with a symptomatic coagulation

disorder has been discovered in a 29-year-old woman of Anglo-Saxon origin². The patient was admitted to the Johns Hopkins Hospital because of femoral vein thrombosis following minor trauma. She had a history of a

Table 1. COAGULATION STUDIES

	Patient %	Normal control %
Fibrinogen assays:		
(a) Thrombin clotting time (ref. 3).	35 mg	175 mg
(b) Tyrosine determination (ref. 4)	104 mg	180 mg
(c) Immunodiffusion (ref. 5)	180 mg	180 mg
Prothrombin time (one-stage)	45	60-100
Prothrombin time after addition of normal fibrinogen	80	
Specific assays for factors II, V, VIII, IX, XIII	Normal	
Euglobulin lysis time	Normal	
Platelet count	224,000-284,000	
Platelet function (aggregation and clot retraction)	Normal	

mild haemorrhagic diathesis characterized by frequent bruising, epistaxis and menorrhagia, and a history of a previous severe thrombotic episode of several months' duration.

On preliminary investigation a friable whole blood clot was noted. Assays of fibrinogen (Table 1) indicated the presence of a poorly clottable fibrinogen in the patient's plasma: subnormal values were obtained by a method that depends on the rate of clotting in the presence of standard amounts of thrombin³ whereas the amount of immunologically detectable fibrinogen was within normal limits. Further studies of coagulation demonstrated that fibrinogen alone was responsible for the clotting disorder. An abnormally prolonged prothrombin time was corrected by the addition of normal fibrinogen to the patient's plasma. Fibrinolytic activity as measured by euglobulin lysis was not increased. Recalcified plasma clots were insoluble in urea, indicating that factor XIII (fibrin-stabilizing factor) activity was present.

The rate of clotting of the patient's plasma by thrombin¹ (Thrombin Topical, Parke, Davis and Company, Detroit, Michigan) was retarded, especially at low concentrations of thrombin (Fig. 1). Addition of calcium chloride (0.025 M) partially corrected the prolonged thrombin clotting time of the patient's plasma but had little effect on the thrombin clotting time of normal plasma.

The rate of clotting of normal plasma or fibrinogen by thrombin was not inhibited by the patient's plasma or by her fibrinogen. In contrast to acquired defibrination syndromes due to intravascular coagulation, the clotting defect was not corrected by therapy with heparin and infused normal fibrinogen had a normal survival time in the patient's circulation.

The venom of *Bothrops jararaca* ('Reptilase', Pentafarm, Basle, Switzerland), which is known to clot fibrinogen by splitting off predominantly peptide A (ref. 6), did not clot the patient's prothrombin-free plasma. On addition of 'Reptilase' and calcium ions a clot formed, but at a comparatively slow rate (Table 2).

The thromboelastogram (prepared by Dr. S. Attar, University of Maryland, Baltimore, Maryland) showed an abnormally small maximal amplitude (Fig. 2). The rate of clot lysis was not increased. In the absence of platelet abnormalities an abnormal structure of the final clot was therefore suggested.

The patient's fibrinogen could not be distinguished from normal fibrinogen on paper electrophoresis and so far consistent differences of migration have not been observed on starch-gel electrophoresis. On immunodiffusion using rabbit antibody prepared against normal human fibrinogen, the precipitation lines that formed in the presence of normal and patient plasma fused without observable spurring (Fig. 3). However, on immunoelectrophoresis minor but reproducible migration differences were found. The abnormal fibrinogen, therefore, appeared to be

immunochemically similar to, but not identical with normal fibrinogen.

These experiments indicated that the defect represented a dysfibrinogenaemia rather than hypofibrinogenaemia and suggested that the disorder might be inherited. Investigation revealed the presence of a similar abnormal fibrinogen in all three daughters of the probanda, whereas the two sons had normally clottable fibrinogen. Two of the daughters had a history of a significant bleeding tendency. The disorder appears to be transmitted by dominant gene (Fig. 4). No further abnormal family members were found in three generations of the patient's mother's family. The father's family was not available for investigation.

Qualitative abnormalities of fibrinogen may be overlooked, particularly if the defect does not produce symptoms. The defect described by Ménaché¹ was discovered in a patient with no haemorrhagic symptoms. In our probanda and in two of her children an abnormal bleeding tendency is clearly present. It remains to be shown

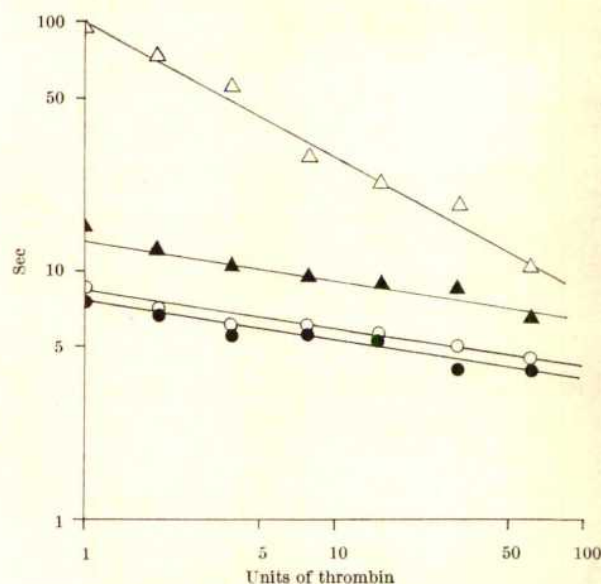


Fig. 1. Thrombin clotting times using serial dilutions of bovine thrombin were performed on normal and patient citrated plasma. Thrombin was diluted with barbital buffer (pH 7.35) alone or with 50 vol. per cent 0.050 M CaCl_2 and barbital buffer. \circ , Normal + thrombin + buffer; \bullet , normal + thrombin + calcium; \triangle , patient + thrombin + buffer; \blacktriangle , patient + thrombin + calcium.



Fig. 2. Thromboelastogram on re-calcified platelet-rich citrated plasma. Maximal amplitude: normal, 34.0 mm; patient, 4.5 mm.

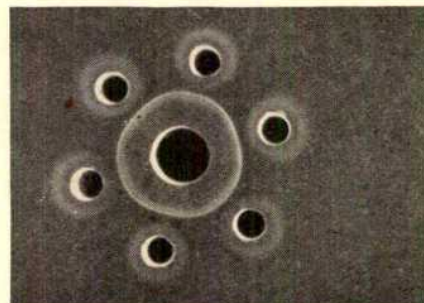


Fig. 3. Immunodiffusion studies. Centre well: antibody against normal fibrinogen. Peripheral wells: citrated plasma, undiluted. Normal plasma and patient plasma are in alternate wells. Complete fusion, shown by a smooth ring, is present indicating antigenic identity in this system.

Table 2. CLOTTING TIME OF PROTHROMBIN-FREE CITRATE PLASMA BY 'REPTILASE'

	Plasma (ml.)	'Reptilase' (ml.)	Calcium 0.025 M (ml.)	Buffer (ml.)	Clotting time (sec.)
Normal:	0.1	0.05	—	0.05	53
	0.1	0.05	0.05	—	27
	0.1	—	0.1	—	No clot*
	0.1	0.05	—	0.05	No clot*
Patient:	0.1	0.05	0.05	—	84
	0.1	—	0.1	—	No clot*

* Observed for 24 h.

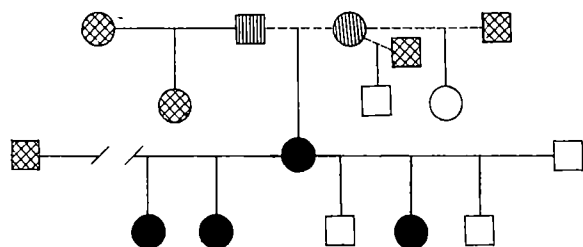


Fig. 4. Family distribution of abnormal fibrinogen. White, tested, normal; black, abnormal; cross-hatched, not tested; hatched, deceased.

whether the thrombotic episodes are related to the defective fibrinogen.

The exact sites of the structural defects in the abnormal fibrinogens described by Ménaché and by us are not known. In contrast to the fibrinogen described here, the abnormal fibrinogen described by Ménaché interfered with the conversion of normal fibrinogen by thrombin, suggesting that the two abnormal proteins may be dissimilar. It has been shown that the amino-acid sequence of the peptides which are split off from fibrinogen by thrombin affects the interaction between thrombin and fibrinogen⁷. The absence of clot formation of our patient's fibrinogen in the presence of 'Reptilase' suggests a possible abnormality of peptide A. The effect of calcium ions in partially correcting the clotting defect cannot be explained on the basis of our present knowledge.

The abnormal fibrinogen described here is, so far, characterized as follows: (1) it clots at an abnormally low rate in the presence of thrombin; (2) in the absence

of calcium ions it does not clot on addition of 'Reptilase'; (3) calcium partially corrects the defect in the presence of both thrombin and 'Reptilase'; (4) the structure of the final clot is abnormal, but the transamidation mechanism induced by factor XIII appears not to be involved; (5) the abnormal fibrinogen does not interfere with clotting of normal fibrinogen; (6) it can be distinguished from normal fibrinogen by differences in migration on immunoelectrophoresis; (7) its presence is controlled by an abnormal dominant gene and leads to a mild haemorrhagic disorder.

As a consequence of the rapidly increasing knowledge concerning the structure of fibrinogen and the fibrinopeptides it can be assumed that further structural abnormalities may be found in the future. Some of the cases which have been thought to have a dominantly inherited hypofibrinogenaemia⁸ may in fact be found to have a dysfibrinogenaemia. A suggested nomenclature to distinguish molecular changes of a protein is that in use for the characterization of abnormal haemoglobins. Accordingly, we would like to designate the fibrinogen described here as 'fibrinogen Baltimore'.

This investigation was supported in part by a contract with the U.S. Atomic Energy Commission and grants from the National Institutes of Health of the U.S. Public Health Service.

¹ Ménaché, D., *Thromb. Diath. Haemorrh. (Stuttg.)*, **10**, Supp. 13, 173 (1964).

² Beck, E. A., *Blood*, **24**, 853 (1964).

³ Clauss, A., *Acta Haemat. (Basel)*, **17**, 237 (1957).

⁴ Ratnoff, O. D., and Menzie, C. J., *Lab. Clin. Med.*, **37**, 316 (1951).

⁵ Feinberg, J. G., *Nature*, **177**, 530 (1956).

⁶ Blomback, B., *Ark. Kem.*, **12**, 321 (1958).

⁷ Laki, K., and Gladner, J. A., *Physiol. Rev.*, **44**, 127 (1964).

⁸ Hasselback, R., Marion, R. G., and Thomas, J. W., *Canad. Med. Assoc. J. (Toronto)*, **88**, 19 (1963).

MECHANISM OF INTESTINAL ABSORPTION: TRANSPORT OF METHIONINE AND SODIUM BUTYRATE BY INTRACELLULAR PLASMA

By S. L. HART and DR J. A. NISSIM

Pharmacology Department, Guy's Hospital Medical School, London, S.E.1

A THEORY of the mechanism of intestinal absorption involving active transport by a spectrum of mobile intracellular proteins was put forward in a previous article¹. This resulted from the analysis of the effects of cetrime and other protein-binding substances, such as chloroquine and thevetin, on the intestinal absorption of glucose in the mouse during intraluminal perfusion of the small intestine. The main points of the theory are: (a) phloridzin acts on a first receptor, blocking a portal of entry for sugars and short-chain fatty acids in the cell membrane; (b) phloridzin is attached to a protein moiety of the first receptor, since phloridzin itself can be displaced, and thus inactivated, by high concentrations of cetrime²; (c) cetrime acts on a second, mobile, receptor, which is the one concerned in active transport. Its inhibition results in leakage of blood glucose to the intestinal lumen; (d) stimulation and inhibition of absorption can be produced by small and large concentrations, respectively, of substances competing with the nutrient for this second receptor; (e) the second receptor is located on intracellular mobile proteins which are characterized by a spectrum of affinities for nutrients and drugs; (f) the association-dissociation constants for proteins, drugs and nutrients are controlled by a pH or electrochemical gradient, which is maintained by the metabolic activities of the cell and requiring the expenditure of energy.

The localization of the second receptor within the cell membrane was considered, but was rejected as unlikely for two reasons. Effective potentiation of phloridzin by cetrime, which removes the former from silent receptors, took half an hour to become established. The phloridzin receptor could not, therefore, be on the surface of the cell

membrane, for potentiation would have been immediate. Furthermore, the second receptor must lie more deeply still from the surface of the cell, and it seemed inconceivable to accommodate a whole spectrum of mobile proteins and their gradient-establishing enzymes, in addition to the distinct protein of the first receptor, within the 75-Å wide cell membrane, of which some 40 Å consists of a lipid outer and inner layer.

The general applicability of the theory was next investigated. It was decided to seek parallel evidence for other species and nutrients. The effect of the cationic cetrime and the anionic sodium lauryl sulphate (B.P.) on the absorption of the amino-acid methionine, the short-chain fatty acid compound sodium butyrate, and glucose, was therefore studied in albino rats. Appreciable inhibition of the absorption of the three nutrients had been demonstrated previously in this species¹, as well as the potentiation of the phloridzin effect on glucose absorption by cetrime. The important problem of stimulation of absorption by low concentrations of different protein-binding inhibitor drugs had not been investigated in the rat. Stimulation was important, because it indicated mobility of the second receptor^{3,4}, while its poorly specific interaction with protein-binding drugs indicated its intracellular localization on the mobile proteins.

The drugs were dissolved in 0.9 per cent sodium chloride containing 0.2 per cent (w/v) D-glucose, DL-methionine and sodium butyrate. 50 ml. of solution was perfused through the lumen of the proximal 60 cm of the small intestine for 0.5 h. The percentage absorption of each nutrient was calculated from estimations of their final concentrations. The methods used in the estimations

Table 1. EFFECT OF CETRIMIDE ON THE PERCENTAGE INTESTINAL ABSORPTION (MEAN \pm S.E.) OF GLUCOSE, BUTYRATE AND METHIONINE IN THE RAT
No. of experiments in parentheses

	Controls	Concentration of cetrimide (w/v)					
		10 ⁻³	10 ⁻⁴	2.5 \times 10 ⁻⁵	10 ⁻⁵	5.0 \times 10 ⁻⁶	10 ⁻⁶
Glucose <i>P</i>	41.2 \pm 0.7 (45)	17.4 \pm 2.0 (7) \leq 0.001	38.1 \pm 3.5 (4)	45.1 \pm 0.8 (21) \leq 0.001	44.8 \pm 1.3 (12) \leq 0.05	41.9 \pm 1.2 (20)	40.7 \pm 1.8 (4)
Butyrate <i>P</i>	43.0 \pm 0.9 (40)	33.0 \pm 1.1 (7) \leq 0.001	44.0 \pm 3.1 (4)	45.4 \pm 1.2 (8) \leq 0.3 > 0.2	45.6 \pm 1.5 (10) \leq 0.2 > 0.1	46.5 \pm 1.3 (20) \leq 0.03	43.5 \pm 2.2 (4)
Methionine <i>P</i>	29.4 \pm 0.7 (37)	20.3 \pm 1.7 (7) \leq 0.001	25.3 \pm 3.1 (4)	29.5 \pm 1.2 (9)	35.2 \pm 1.9 (11) \leq 0.001	25.4 \pm 1.7 (10)	27.4 \pm 3.6 (4)

Table 2. EFFECT OF SODIUM LAURYL SULPHATE ON PERCENTAGE INTESTINAL ABSORPTION (MEAN \pm S.E.) OF GLUCOSE, BUTYRATE AND METHIONINE IN THE RAT
No. of experiments in parentheses

	Controls	Concentration of sodium lauryl sulphate (w/v)									
		2.5 \times 10 ⁻³	10 ⁻³	10 ⁻⁴	7.5 \times 10 ⁻⁵	5.0 \times 10 ⁻⁵	2.5 \times 10 ⁻⁵	10 ⁻⁵	7.5 \times 10 ⁻⁶	5.0 \times 10 ⁻⁶	10 ⁻⁶
Glucose <i>P</i>	41.2 \pm 0.7 (45)	30.0 \pm 2.6 (6) \leq 0.001	36.0 \pm 2.6 (6)	37.2 \pm 2.1 (4)	39.9 \pm 2.4 (4)	44.0 \pm 0.9 (12) \leq 0.1 > 0.05	43.6 \pm 1.7 (10) \leq 0.2 > 0.1	42.8 \pm 1.5 (10)	41.9 \pm 0.9 (12)	47.1 \pm 1.7 (12) \leq 0.001	38.4 \pm 2.4 (4)
Butyrate <i>P</i>	43.0 \pm 0.9 (40)		33.9 \pm 5.7 (6) \leq 0.01	51.0 \pm 3.7 (4) \leq 0.02	50.6 \pm 3.6 (4) \leq 0.02	49.1 \pm 1.8 (11) \leq 0.004	49.8 \pm 2.9 (10) \leq 0.005	47.2 \pm 1.7 (10) \leq 0.05	47.6 \pm 2.0 (12)	47.7 \pm 1.3 (12)	43.8 \pm 3.3 (4)
Methionine <i>P</i>	29.4 \pm 0.7 (37)		17.3 \pm 2.9 (6) \leq 0.001	24.2 \pm 2.3 (4)	26.6 \pm 1.2 (4)	36.4 \pm 1.7 (12) \leq 0.001	34.9 \pm 1.8 (10) \leq 0.005	30.2 \pm 1.5 (10)	35.3 \pm 1.6 (12) \leq 0.001	33.6 \pm 1.9 (12) \leq 0.02 > 0.01	32.4 \pm 2.2 (4)

were essentially as before¹, but a slight modification of the methionine estimation requires the reduction of previously published values by a factor of 1.4, before they can be compared with the present values.

The results obtained at six concentrations of cetrimide are shown in Table 1. At a concentration of 10⁻³, significant inhibition of absorption of all three nutrients occurred, although the inhibition was smaller in each case than that obtained with previous samples of cetrimide^{1,2}. At lower concentrations, there was definite stimulation of absorption, which was maximal at 2.5 \times 10⁻⁵ for glucose, 5.0 \times 10⁻⁶ for butyrate, and 10⁻⁵ for methionine. At lower concentrations still, absorption returned to its level in control animals.

Sodium lauryl sulphate had only a moderate inhibitory effect on the absorption of glucose compared with that of cetrimide. The results obtained with ten concentrations of sodium lauryl sulphate are given in Table 2. Maximal stimulation of glucose absorption occurred at 5.0 \times 10⁻⁶, although there was also some stimulation at 5.0 \times 10⁻⁵ and 2.5 \times 10⁻⁵. Marked stimulation of butyrate absorption was observed with sodium lauryl sulphate at 10⁻⁴, 2.5 \times 10⁻⁵, and combination of the four *P* values from the individual *t*-tests in this region gave *P* < 0.0005. Stimulation of methionine absorption occurred between 5.0 \times 10⁻⁵ and 5.0 \times 10⁻⁶, although not at the intermediate concentration of 10⁻⁵, which gave a value no different from that of the control.

Although the stimulation of absorption obtained with these two drugs was not as marked as the stimulation of glucose absorption in the mouse, the low *P* values fully establish the stimulating effect at low concentrations for all three nutrients. These results assume greater significance when considered alongside the parallel results in the mouse, since they constitute further confirmatory evidence for the proposed theory of the mechanism of intestinal absorption. The observation that both a cationic and an anionic compound, known to bind protein in an unspecific reaction, stimulate the absorption of the three nutrients at different concentrations, lends strong support to the concept that active transport involves the intracellular proteins which have different affinities for individual nutrients and drugs. It is possible that different nutrients bind to different proteins, but they may also bind to different sites on the same protein.

Inspection of the concentrations at which stimulation occurred with cetrimide shows that for all three nutrients there was a single peak, though the range at which butyrate absorption was stimulated was rather wide. By contrast, stimulation with sodium lauryl sulphate occurred

at more than one peak for glucose and methionine, and the range of concentrations for butyrate stimulation was much wider. Both peak multiplicity and range width suggest that more than one protein carrier might be involved, the carriers exhibiting particularly different affinities for sodium lauryl sulphate, but the presence of impurities cannot be excluded.

The first receptor in intestinal sugar transport was previously studied in some detail in the mouse and rat^{1,2}. It was concluded that stimulation of sugar absorption in the mouse was due to the action of small doses of a competitive substrate (or inhibitor) on the second, not the first, receptor. This must also be true in the rat, for the results of experiments in the two species are similar. As for sodium butyrate and methionine little is known about their entry mechanisms. The partial block to butyrate absorption by phloridzin^{1,2} may be exerted on one of the entry mechanisms, for butyrate might enter partly through a pore and partly through the lipid of the cell membrane. For amino-acids, entry through the lipid part of the cell membrane has been suggested⁶, and a two-stage system of absorption has been proposed⁷, involving entry and subsequent active transport. The evidence presented here strongly suggests that for all three nutrients stimulation and inhibition of absorption, by low and high concentrations of cetrimide and sodium lauryl sulphate, occur by competition at the second receptor, concerned with active transport. We shall therefore confine our discussion to the various possible modes of stimulation that may be produced by an inhibitor of the second receptor. Since the simple model of carrier transport capable of achieving this was proposed by Wilbrandt and Rosenberg^{3,4}, other possible mechanisms have been suggested or demonstrated. These different stimulation-inhibition models will therefore be considered to see which of them would be applicable to our findings.

The first is the mobile carrier transport model of Wilbrandt and Rosenberg. According to this, stimulation or inhibition depends on whether the factor ($I' + 1 - S_1/S_2$) in their formula is negative or positive. Here S_1 and S_2 represent substrate or nutrient concentration at the two ends of the carrier path, and I' is the equilibrium value of the inhibitor substance, all three being divided by the respective equilibrium association-dissociation constant for substance and carrier. This is the model adopted previously in the new theory for the mechanism of intestinal absorption.

The second hypothesis is that of an exchange reaction at a membrane surface in a model of carrier transport

ving a 1:1 nutrient-carrier stoichiometry (Jacquez^{6,8}, ong⁹, Guroff⁸, Christensen *et al.*⁹). If *C* indicates carrier, ternary combination *CSI* with subsequent breakdown *CS* + *I* leads to more rapid formation of *CS* than by ect combination. Employing a more comprehensive rome for his models, Jacquez obtained equations for ich simple analytic solutions could not be found. nsideration of idealized stationary states, however, d him to the conclusion that the simple 1:1 nutrient- rrier stoichiometry of Wilbrandt and Rosenberg could t produce stimulation at any concentration of the ibitor, when the value of *S*₂, the concentration of the bstrate at the inner end of the transport system, remains ro. The same result is obtained by putting zero for in the Wilbrandt-Rosenberg formula. This state of airs may conceivably apply for certain cells, such as e Ehrlich ascites cells, if the rate of utilization of *S* by e cell is more rapid than its absorption, but it scarcely plies to the intestinal absorption of nutrients. Further- ore, it seems unlikely that there is ternary complex rmation allowing exchange reaction in the case of all ee nutrients of the present investigation.

Thirdly, a model of carrier-transport having a 2:1 rrient-carrier stoichiometry was proposed by Jacquez⁶. his allows the formation of *CSS*, *CSI* and *CII* at the same ne. The derived equations again permit early stimula- on and subsequent inhibition. While this may be the se for some nutrients, it would seem unlikely to represent e standard form for the general carrier-transport action.

Fourthly, in a model of polymer enzyme splitting into idividual units by low concentrations of the inhibitor bstance, Gerhart and Pardee¹⁰ have explained the tivation and inhibition of aspartic transcarbamylase by aleate on the hypothesis that the enzyme was composed f four units, forming a tetramer, and that low concentra- ons of maleate broke the tetramer, enabling the aspartate o become bound to the enzyme units. At higher concentra- ns, the maleate not only broke the tetramer, but so competed with the aspartate for the enzyme sites, thus ctng as an inhibitor. Such a system shows a sigmoidal ose-response curve, while the single enzyme or carrier- ransport units, which follow Michaelis-Menten kinetics, ow a hyperbolic type of curve. Michaelis-Menten

kinetics have been shown to hold for the intestinal absorption of most nutrients of the type studied here¹¹⁻¹³. That the polymer type of system is general, and would thus apply to all three nutrients, again seems unlikely.

The fifth model involves the facilitation of transfer by increase of oppositely charged groups along pore or path of entry. Rummel and Stupp¹⁴ found that mersalyl stimulated intestinal calcium transport at 5.0×10^{-4} M and inhibited it at 5.0×10^{-3} M. They postulated that mersalyl molecules were bound in charged pores, and the increase in anionic groups, thus produced, stimulated calcium transfer. This may evidently be important for ionized particles, but unlikely to constitute a general mechanism of the type observed in this investigation.

In conclusion, analysis of the results reveals the stimula- tion-inhibition transport pattern to be general in its applicability to species and nutrients. Among the five different possible models, that described by Wilbrandt and Rosenberg is the simplest, and seems the only one that could fit all the results and qualify for a general carrier-transport system with interactions that are not highly specific. The present investigation, therefore, gives a more general significance to the mechanism of intestinal absorption proposed previously, which attributes active transport to a spectrum of intracellular carrier proteins in the cell plasma.

We thank the Medical Research Council for a personal grant to S. L. H. and a grant for expenses.

¹ Nissim, J. A., *Nature*, **204**, 148 (1964); **187**, 308 (1960); *Brit. J. Pharmacol.* **24**, 205 (1965); also observations to be published elsewhere.

² Hart, S. L., and Nissim, J. A., *Nature*, **204**, 51 (1964); *J. Physiol.*, **169**, 119P (1963).

³ Wilbrandt, W., and Rosenberg, T., *Pharmacol. Rev.*, **13**, 109 (1961).

⁴ Wilbrandt, W., *Enzymes and Drug Action* (Ciba Found. Symp.), **47** (Churchill, London, 1962).

⁵ Smyth, D. H., and Taylor, C. B., *J. Physiol.*, **141**, 73 (1958).

⁶ Jacquez, J. A., *Biochim. Biophys. Acta*, **71**, 15 (1963).

⁷ Newey, H., and Smyth, D. H., *J. Physiol.*, **170**, 328 (1964).

⁸ Jacquez, J. A., *Biochim. Biophys. Acta*, **79**, 318 (1964).

⁹ Christensen, H. N., Akado, H., Oxender, D. L., and Winter, C. G., *Amino-Acid Pools*, 527 (Elsevier, Amsterdam, 1962).

¹⁰ Gerhart, J. C., and Pardee, A. B., *Cold Spr. Harb. Symp. Quant. Biol.*, **28**, 491 (1963).

¹¹ Fisher, R. B., and Parsons, D. S., *J. Physiol.*, **119**, 210 (1953).

¹² Barry, R. J. C., and Smyth, D. H., *J. Physiol.*, **152**, 48 (1960).

¹³ Jervis, E. L., and Smyth, D. H., *J. Physiol.*, **149**, 433 (1959).

¹⁴ Rummel, W., and Stupp, H. E., *Experientia*, **18**, 303 (1962).

STOICHIOMETRY OF PROTON TRANSLOCATION THROUGH THE RESPIRATORY CHAIN AND ADENOSINE TRIPHOSPHATASE SYSTEMS OF RAT LIVER MITOCHONDRIA

By DR PETER MITCHELL and DR. JENNIFER MCYLE

Glynn Research Laboratories, Bodmin, Cornwall

ACCORDING to the chemiosmotic hypothesis of oxidative and photosynthetic phosphorylation proposed by Mitchell¹⁻⁴, the linkage between electron transport and phosphorylation occurs not because of hypothetical energy-rich chemical intermediaries as in the orthodox view, but because oxido-reduction and adenosine triphosphate (ATP) hydrolysis are each separately associated with the net translocation of a certain number of electrons in one direction and the net translocation of the same number of hydrogen atoms in the opposite direction across a relatively ion-, acid- and base-impermeable coupling membrane (see also ref. 5). Thus, the chemiosmotic hypothesis, unlike the orthodox chemical hypothesis, predicts that oxido-reduction through the respiratory chain of the mitochondrial membrane system, and ATP hydrolysis through the membrane-located ATPase, should each be accompanied by the stoichiometric translocation of protons (written $\rightarrow H^+$) across the coupling membrane; and that the P/O

quotients observed during respiratory chain phosphorylation should be the product of the $P/\rightarrow H^+$ quotient of the ATPase system and the $\rightarrow H^+/O$ quotient for oxidation of the substrate through the respiratory chain system. It was thought that the direct measurement of $P/\rightarrow H^+$ and $\rightarrow H^+/O$ quotients in rat liver mitochondria would afford a means of testing the chemiosmotic hypothesis, and the present article gives a preliminary report of the results of this quantitative test.

Two main conditions must be satisfied for the accurate measurement of $P/\rightarrow H^+$ and $\rightarrow H^+/O$ quotients: (i) the $\rightarrow H^+$ measured must correspond to a known amount of ATP hydrolysed or of oxygen consumed; (ii) the measurements of $\rightarrow H^+$ must be done in such a way as to minimize the unknown extent of back flow or exchange of H^+ ions and OH^- ions across the membrane system during ATP hydrolysis or substrate oxidation. Estimates of the stoichiometry of H^+ 'secretion' accompanying divalent cation uptake^{6,7} or during monovalent cation uptake in presence

of valinomycin or gramicidin^{8,9} in steadily respiring or ATP-hydrolysing mitochondria are qualitatively interesting, but cannot be relied on quantitatively because they do not satisfy the foregoing conditions. We have met condition (i) by adding a known small quantity of ATP or oxygen to a lightly buffered anaerobic mitochondrial suspension and recording the consequent change of pH (ΔpH) in the suspension medium with a glass electrode during virtually complete hydrolysis of ATP to ADP and P_i , or virtually complete reduction of the added oxygen by substrate present in the suspension medium. Condition (ii) was fulfilled by using quantities of ATP or oxygen such that the electrochemical potential gradient of H^+ ion developed across the mitochondrial membrane system during the transient ATPase or electron transport reaction was sufficiently small and brief to permit ΔpH recordings to be made before much back flow of H^+ or OH^- ions had occurred.

The buffering capacity of the system that we have used was almost independent of pH within a given range of 0.1 pH unit, and readings of virtually instantaneous pH change within the given range were proportional to the amount of H^+ ion (ΔH^+) effectively added to or removed from the phase in acid-base equilibrium with the mitochondrial suspension medium (henceforth called the 'outer phase'). It was thus possible, by suitable calibrations of ΔpH versus ΔH^+ with standard acid or base, to compute ΔH^+ values from the observed respiration-driven and ATPase-driven ΔpH values. As we shall show later, there are sound reasons for concluding that the ΔH^+ values obtained in this way represent the protons translocated ($\rightarrow H^+$) across the mitochondrial coupling membrane that separates an inner mitochondrial phase (probably the interior of the cristae) from the outer phase.

For the measurements of $\Delta H^+/O$, the mitochondria were liberated from male Wistar rat livers by homogenization in ice-cold 0.25 M sucrose, and the mitochondria were largely freed of cell debris, microsomes, and 'fluffy layer' by conventional differential centrifugation in 0.25 M sucrose at 4°C. For the measurements of $P/\Delta H^+$, the mitochondria were prepared by a similar procedure, except that a solution containing 0.21 M sucrose, 1 mM potassium chloride, and 10 mM sodium ethylenediamine tetraacetate (EDTA) was substituted for 0.25 M sucrose in order to reduce the concentration of free Mg^{++} . The stock mitochondrial suspensions (60 mg protein/ml.) were stored briefly in 0.25 M sucrose under semi-anaerobic conditions at 4°C before use.

The ΔH^+ measurements were done in a thermostatically controlled cylindrical glass cell of 6 ml. capacity containing an H^+ -sensitive glass electrode, a saturated potassium chloride junction leading to a calomel half-cell, a Clark oxygen electrode, and a magnetically driven glass impeller giving vigorous stirring. The cell could be closed by a glass piston, but a shallow groove in the piston, parallel to the axis of the cell, permitted the insertion of a fine glass needle so that nitrogen could be bubbled in to flush oxygen from the cell, and reagents could be added from calibrated micro syringes. Oxygen from the air was excluded by a jet of nitrogen continuously directed at the top of the piston. The normal procedure was to flush the oxygen from the suspension medium with oxygen-free nitrogen while the glass piston was at the top of the cell. The cold mitochondrial suspension (0.6 ml.) was then introduced to give a total volume of 6 ml., the piston was lowered on to the surface of the suspension, and 20 min was allowed for temperature and ionic equilibration before commencement of ΔpH measurements. The H^+ -sensitive glass electrode and the potassium chloride junction/calomel half-cell reference system were connected through conventional valve-potentiometer circuitry, the output of which drove a strip-chart recorder giving a full-scale deflexion corresponding to 0.10 pH unit. The measurements were routinely made between pH 7.0 and 7.2 at 25°C in a medium containing 25 mM sucrose, 150 mM potassium

chloride, and 3.3 mM glycyl-glycine-potassium hydroxide buffer, and any variations of these conditions or additions to or changes in this 'normal medium' are referred to the text. The system generally exhibited a small constant rate of pH drift after equilibration, and the time course of ΔpH was corrected for the drift by plotting from a baseline having the slope of the pH drift. Oxygen was added as air-saturated or oxygen-saturated carbon-dioxide-free 0.15 M potassium chloride from a micro-syringe (injection time < 1 sec), and the amounts of oxygen were computed from the relevant temperature-solubility tables for pure water¹⁰, corrected by a factor of 0.9 for the effect of ionic strength¹¹. Mitochondrial protein was estimated by the method of Itzhaki and Gill¹².

Electron transport system. Fig. 1a shows a typical time course of ΔpH and ΔH^+ resulting from the oxidation of β -hydroxybutyrate at 25°C. There was a rapid acidification of the suspension medium, beginning immediately after the addition of oxygen, and a slower, approximate exponential decay of ΔpH to zero. The value of ΔH^+ , corresponding to the peak of the curve of Fig. 1a is 5.4. The controlled and uncontrolled rates of oxygen reduction by the mitochondria under these conditions were found to be respectively 10 and 25 μg atom O/g protein min, and thus it can be estimated that in the experiment of Fig. 1 the oxygen would be consumed within 5 sec. The ΔH^+ quotient for the experiment of Fig. 1a was accordingly corrected for ΔpH decay by a small (linear) extrapolation to time 5 sec, giving a value of 5.96. Sixteen measurements of the corrected $\Delta H^+/O$ quotient on four different batches of mitochondria have yielded a mean value 5.90 ± 0.33 . When the amount of oxygen injected in the 6-ml. mitochondrial suspension is varied between 1 and 5 μg atom O there is little effect on the appropriately corrected $\Delta H^+/O$ quotient, but for larger amounts of oxygen the $\Delta H^+/O$ quotient falls to relatively low values, becoming zero in the steady state. Similar measurements on the $\Delta H^+/O$ quotient for oxidation of 2 mM succinate at 25°C in presence of 0.4 μM rotenone (to stop NAD-linked oxidations) have given a mean corrected value of 3.25 ± 0.08 . However, as shown in the typical pair of experiments described by Fig. 1, succinate causes an enhanced ΔpH decay rate (probably due to specific succinate/ OH^- antiport), which can be largely suppressed at 5°C. Our measurements of the corrected $\Delta H^+/O$ quotient for succinate oxidation at 5°C give a mean value of 4.07 ± 0.11 . Unlike the value for succinate oxidation there is no increase in the $\Delta H^+/O$ quotient for β -hydroxybutyrate oxidation on lowering the temperature from 25°C to 5°C, and this is consistent with the fact that the rate of ΔpH decay is not appreciably temperature dependent in the presence of β -hydroxybutyrate.

The $\Delta H^+/O$ quotient for succinate oxidation at 5°C depressed towards zero as the amount of oxygen increased, just as in the case of β -hydroxybutyrate oxidation.

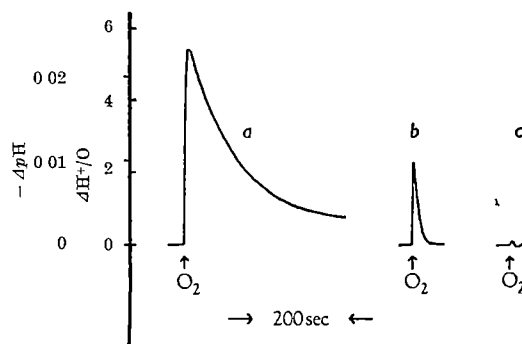


Fig. 1 Time course of ΔpH and ΔH^+ during oxidation of β -hydroxybutyrate at 25°C. Oxygen (23.5 μg atom in 50 μl . 0.15 M potassium chloride) injected into 6 ml anaerobic mitochondrial suspension in 'normal medium' containing 2 mM β -hydroxybutyrate at vertical arrows. Curve a, no addition; curve b, plus 5×10^{-6} M 2, 4-dinitrophenol, curve c, plus 'Triton X-100' (1 mg/ml.)

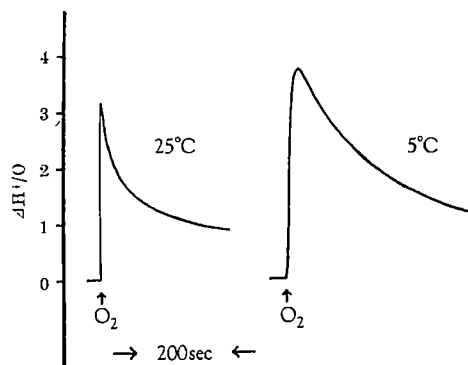


Fig. 2. Time course of $\Delta H^+/O$ during oxidation of succinate at 25°C and at 5°C. Oxygen (23.5 μg atom in 50 μl . 0.15 M potassium chloride) injected into 6 ml anaerobic mitochondrial suspension in 'normal medium' containing 2 mM succinate plus 0.4 μM rotenone at vertical arrows.

n. The presence of 6 μg oligomycin per ml. mitochondrial suspension enhanced the $\Delta H^+/O$ values for both hydroxybutyrate and succinate by 0.8 per cent in ferent experiments. Our results suggest that, ideally, $\Delta H^+/O$ quotients for succinate and β -hydroxybutyrate oxidation are respectively 4 and 6.

We have interpreted the observed acidification of the suspension medium in the experiments described here as a consequence of a stoichiometric net translocation of protons through the mitochondrial coupling membrane. In order, however, to justify this interpretation, we must include the possibility that the observed $\Delta H^+/O$ values might be due to the stoichiometric accumulation of an anhydride¹³ (for example, $X \sim I$) the synthesis of which would involve the net dissociation of $2H^+$ ions per \sim bond. Fig. 1b shows that when 5×10^{-5} M 2:4-dinitrophenol is present, in an experiment otherwise identical to that of Fig. 1a, there is a much faster ΔpH decay, and the height of the ΔH^+ peak is reduced. Similarly, as shown in Fig. 1c, Triton X-100 (1 mg/ml.) in place of dinitrophenol, virtually abolishes ΔpH and ΔH^+ . Other uncoupling and membrane-lytic agents have been found to behave similarly to DNP and Triton X-100 respectively in this type of experiment. These observations suggest that the respiration-driven pH displacement and its subsequent decay are due to the effective translocation of H^+ or OH^- across the coupling membrane and their subsequent equilibration by diffusion or exchange through the membrane, for dinitrophenol and other uncouplers are known to catalyse the equilibration of H^+ ions across the mitochondrial membrane system^{3,14} and the membrane-lytic agents permit general ionic equilibration between the phases originally separated by the membrane. However, these observations do not completely eliminate the aforementioned possibility that the respiration-driven pH displacement and its subsequent decay might be due to the formation of an $X \sim I$ anhydride in a lipid phase, the subsequent hydrolysis of $X \sim I$ being catalysed by uncouplers and by the detergent membrane-lytic reagents. A different experimental approach is required to shed more light on this question.

Fig. 3 shows a group of four observations under conditions identical to those of Fig. 1a, except that in all but the control (A) stoichiometric amounts of potassium hydroxide were present in the air-saturated saline injected into the mitochondrial suspension. We have observed in a number of such experiments that, after the respiration-driven pH displacement: (i) the rapid pH shift caused by the potassium hydroxide present in the saline is proportional to the amount of potassium hydroxide added and is not evidently enhanced or decreased by any chemical reaction consequent on changing the pH with the potassium hydroxide solution; (ii) the subsequent change of the pH of the outer medium with time can be either positive or negative, depending on the outer pH , within a total range of only 0.15 pH unit; (iii) there is one particular

pH at which the outer medium will remain constant after the respiration-driven pH displacement.

If the initial respiration-driven acidification of the outer medium were due to the formation of an $X \sim I$ anhydride, and the subsequent hydrolysis of $X \sim I$ were responsible for the ΔpH decay, it is difficult to account for the fact that the upward pH movement corresponding to the ΔpH decay is eliminated by raising the outer pH by only 0.1 unit relative to the initial pH , and that it is actually reversed by raising the outer pH by 0.2 unit—especially as the normal $\Delta H^+/O$ values have been found in other experiments to be almost independent of pH between pH 6 and 8. On the other hand, if there is no net production or consumption of H^+ ions during the respiration-driven pH displacement, but simply an effective displacement of H^+ ions outwards (or OH^- inwards) across the coupling membrane, the fall in the external pH ($-\Delta pH_o$) should be accompanied by a rise in the internal pH (ΔpH_i), and the magnitudes of ΔpH_i and $-\Delta pH_o$ should be in the inverse ratio of the buffering powers ($\Delta H^+/\Delta pH$) of the respective phases. Further, the ΔpH_o decay, being due to the inward diffusion or exchange of H^+ ions (or outward diffusion or exchange of OH^- ions), should depend on the pH differential across the membrane, and the ΔpH_o decay should therefore be arrested or reversed by raising the external pH (pH_o) appropriately. Assuming that the pH differential across the membrane ($pH_i - pH_o$, or $pH_i + \Delta pH_i - \{pH_o + \Delta pH_o\}$) tends to decay to zero or to some constant value, the internal respiration-driven pH displacement will be given by the total shift of the outer pH required to result in a stable outer pH value after the respiration-driven H^+ displacement. It will be seen from Fig. 3 that the respiration-driven ΔpH_o is 0.0120 unit, giving a corrected value of 0.0135 unit, and that ΔpH_i , equated with the total ΔpH_o required to give a stable pH after the respiration-driven H^+ displacement, is approximately 0.11 pH unit. The ratio $\Delta pH_i/\Delta pH_o$ due to the respiration-driven H^+ displacement is thus about 8. It has been possible to estimate the buffering powers of the inner and outer phases over the appropriate pH range by titrating mitochondrial suspensions with standard hydrochloric acid or potassium hydroxide, (a) adding aliquots of acid or alkali quickly and extrapolating the titre to zero time, giving the buffering power of the outer phase ($\Delta H^+/\Delta pH_o$); (b) titrating in presence of reagents catalysing equilibration of H^+ across the membrane (for example, 5×10^{-5} M DNP + 200 μg

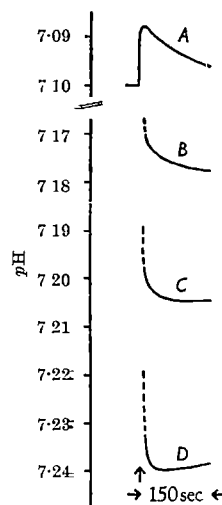


Fig. 3. Effect of injection (at vertical arrows) of 25 μl . 0.15 M potassium chloride containing oxygen (1.8 μg atom) and different amounts of potassium hydroxide on the pH of an anaerobic mitochondrial suspension in the presence of β -hydroxybutyrate at 25°C. A, No potassium hydroxide; B, plus 475 μM equiv. potassium hydroxide; C, plus 665 μM equiv. potassium hydroxide; D, plus 855 μM equiv. potassium hydroxide. External buffering power of the mitochondrial suspension across pH 7.15 in this experiment is 5.7 $\mu\text{equiv.}/pH$ unit.

'Triton X-100' per ml.) to obtain the sum of inner and outer buffering powers ($\Delta H^+/\Delta p\text{H}_o + \Delta H^+/\Delta p\text{H}_i$). The buffering power of the inner phase ($\Delta H^+/\Delta p\text{H}_i$) is given by difference. For the mitochondria used in these experiments in the 'normal medium' the approximate values of $\Delta H^+/\Delta p\text{H}_i$ and $\Delta H^+/\Delta p\text{H}_o$ are 130 and 950 $\mu\text{equiv. H}^+/\text{pH unit ml. mitochondrial suspension (containing 6 mg protein/ml.)}$ respectively across $\text{pH } 7.15$, giving a buffering power ratio of 7.3. This agrees satisfactorily with the $\Delta p\text{H}_i/\Delta p\text{H}_o$ of 8 estimated above.

There appear from the foregoing experiments to be rather convincing grounds, both qualitative and quantitative, for concluding that the respiration-driven pH changes described in this article are caused by the effective stoichiometric displacement of H^+ ions (or OH^- ions) across the coupling membrane of the mitochondria, and that the $\Delta p\text{H}/\text{O}$ and $\Delta \text{H}^+/\text{O}$ values can be properly used to derive the $\rightarrow \text{H}^+/\text{O}$ quotients.

ATPase system. Fig. 4a shows a typical time course of $\Delta p\text{H}$ and $\Delta \text{H}^+/\text{P}$ on injecting $0.1 \mu\text{mole ATP}$ in $10 \mu\text{l. } 0.15 \text{ M oxygen-free potassium chloride}$ into $6 \text{ ml. mitochondrial suspension (6 mg protein/ml.)}$ in 'normal medium' containing 1.0 mM EDTA , but no substrate, at 25°C . Under these conditions the activity of the adenylate kinase is very low¹¹. There is a fairly rapid acidification of the suspension medium, followed by a slower, approximately exponential decay of $\Delta p\text{H}$ towards a value corresponding to $\Delta \text{H}^+/\text{P} = 0.8$. Experiments such as that of Fig. 4b, in which $6 \mu\text{g oligomycin per ml. plus } 5 \times 10^{-5} \text{ M DNP}$ were present, confirm that the final value of $\Delta \text{H}^+/\text{P}$ represents the acidification due to the chemical reaction $\text{ATP} + \text{H}_2\text{O} \rightarrow \text{ADP} + \text{P}_i + (0.76-0.80)\text{H}^+$ at $\text{pH } 7.0-7.1$. The uncorrected height of the peak in the experiments of Fig. 4a represents a total $\Delta \text{H}^+/\text{P}$ quotient of 2.08, or a quotient of 1.30 on subtracting the base-line value of 0.78. Extrapolation to correct for $\Delta p\text{H}$ decay is more difficult in this type of experiment than in the oxidation-reduction experiments because the rate of acidification is considerably slower initially and decreases as the ATP concentration falls during hydrolysis. We adopted the empirical method of extrapolating to the point of intersection of the straight lines representing the initial rate of pH fall and the initial straight portion of the subsequent $\Delta p\text{H}$ decay. The value is thus corrected to 1.49 in the experiment of Fig. 4. The extent of the side reactions for ATP hydrolysis was determined by the fraction of the overall rate that is oligomycin ($6 \mu\text{g/ml.}$) insensitive. In the typical mitochondrial preparation used for the experiment of Fig. 4 the oligomycin-insensitive ATPase represented 22 per cent of the total ATPase, and, accordingly, the corrected value of the $\Delta \text{H}^+/\text{P}$ quotient is 1.91. Several groups of similar experiments on four different batches of mitochondria have given a mean corrected $\Delta \text{H}^+/\text{P}$ quotient of 1.88 ± 0.09 . Conversely to the $\Delta \text{H}^+/\text{O}$ quotients, the $\Delta \text{H}^+/\text{P}$ quotients have been found to be suppressed by oligomycin ($6 \mu\text{g/ml.}$), but

unaffected or slightly enhanced by antimycin A (0.12 ml.) or by rotenone ($0.4 \mu\text{M}$). As described for the case of the electron transport system, by shifting the peak of the electron transport system, by shifting the pH upward as the peak of $\Delta p\text{H}$ is reached during ATP hydrolysis, the pH changes in the suspension medium caused by the ATPase system can be shown to be due to an effective displacement of H^+ ions through the coupling membrane and not to the net production or absorption of H^+ ions during the synthesis or breakdown of a hypothetical $\text{X} \sim \text{I}$ compound.

Ion secretion and electron translocation. There is a difference of opinion as to whether the 'secretion of H^+ ions' by continuously respiring mitochondria in response to the addition of divalent cations or of valinomycin, gramicidin is a primary process⁸, or whether it is a consequence of the 'active' uptake of the cations⁸; and we have therefore made a preliminary examination of this question for the case of the stoichiometric respiration-driven H^+ ion translocation observed in our experiments. Substituting a miniature flowing liquid junction (to be described in detail elsewhere) for the normal saturated potassium chloride junction of the reference electrochemical system, we have observed that, under appropriate conditions of pH and temperature, the limiting $\Delta \text{H}^+/\text{O}$ quotient for β -hydroxybutyrate oxidation is not significantly affected by replacing the $0.15 \text{ M potassium chloride}$ of the suspension medium by an equal concentration of sodium chloride or choline chloride, or by substituting 0.25 M sucrose for the salt. The presence or absence of $3 \text{ mM magnesium chloride}$ in the foregoing media was found to be without effect on the $\Delta \text{H}^+/\text{O}$ quotient. The K^+ content of the mitochondria in these experiments gave an equilibrium external K^+ concentration of $5 \times 10^{-4} \text{ M}$; it was observed, by means of a K^+ -sensitive glass electrode, that in the sucrose medium containing $3 \text{ mM magnesium chloride}$, and in the choline chloride medium, the respiration-driven outward H^+ displacement was accompanied by an uptake of K^+ ions. However, the K^+ ion uptake commenced slightly later than the H^+ displacement, was much less in magnitude than the H^+ displacement, and the subsequent K^+ extrusion was complete or showed a significant reversal about half-way through the time course of the $\Delta p\text{H}$ decay. We conclude that the respiration-driven H^+ translocation is probably an integral consequence of the passage of electrons and hydrogen atoms in opposite directions through the vectorially organized respiratory chain system of the mitochondrial membrane; and in this sense the respiration-driven displacement of H^+ or acid equivalents across the coupling membrane can probably be classed as "primary secretion of H^+ ions".

In the case of the ATPase-driven H^+ translocation, also seems probable, as will be described elsewhere, the cyclic transitions of mobile chemical groups through different states of ionization and hydration give rise to the passage of electrons and hydrogen atoms across the ATPase system in opposite directions.

Summarizing the experimental findings: (1) Oxidation-reduction through the electron-transport chain and ATP hydrolysis through the ATPase system each results in the translocation of protons outwards through the mitochondrial membrane system. (2) The stoichiometry of the ATPase system closely approximates to a $\text{P}/\rightarrow \text{H}^+$ quotient of $1/2$, while the $\rightarrow \text{H}^+/\text{O}$ quotients for succinate and β -hydroxybutyrate oxidation approximate closely to 2 and 6 respectively; thus making the product of the $\text{P}/\rightarrow \text{H}^+$ and $\rightarrow \text{H}^+/\text{O}$ quotients for succinate and β -hydroxybutyrate close to the known P/O quotients of 2 and 3.

These observations are in close accord with the basic principles of the chemiosmotic hypothesis of oxidative and photosynthetic phosphorylation, but show that the following changes of detail are required in Mitchell's original formulation¹: (i) As already surmised², the polarity of the system corresponds to a lower electrochemical potential of H^+ inside than outside the coupling membrane (higher pH

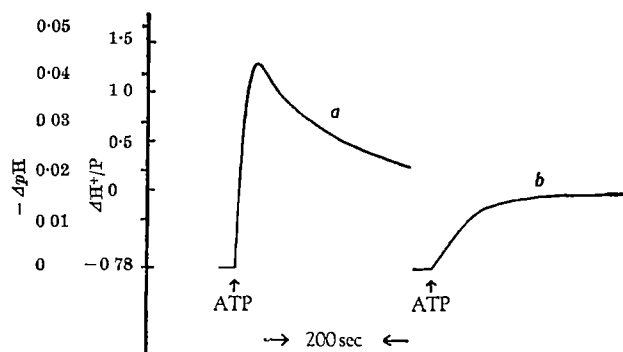


Fig. 4. Time course of $\Delta p\text{H}$ and $\Delta \text{H}^+/\text{P}$ during hydrolysis of ATP at 25°C . ATP ($0.1 \mu\text{mole}$ in $10 \mu\text{l. } 0.15 \text{ M oxygen-free potassium chloride}$) injected into $6 \text{ ml. anaerobic mitochondrial suspension in 'normal medium' containing } 1.0 \text{ mM EDTA}$ at vertical arrows. Curve a, no addition; curve b, plus $5 \times 10^{-5} \text{ M DNP}$ and oligomycin ($6 \mu\text{g/ml.}$)

re negative electric potential), the reverse of that originally assumed. (ii) The $\rightarrow H^+/O$ and $\rightarrow H^+/P$ stoichiometries are double those originally assumed. (iii) Owing to the electrochemical potential differential of H^+ ions across the coupling membrane required to cause a given ising effect on the oxido-reduction and ATPase equilibria only half that previously estimated; that is, a differential of 3.5 pH units with no membrane potential or membrane potential of 210 mV with no pH differential could be sufficient to poise the ATP/ADP quotient central a P_i concentration of 10 mM.

The latter point is particularly helpful to the chemi- nical interpretation of the coupling mechanism because brings the pH and electric potential differentials required the hypothesis within the physiological limits usually accepted for these variables (see Mitchell⁴). As shown elsewhere, the chemiosmotic hypothesis is now able to provide as realistic a model of oxidative phosphorylation the more fashionable chemical hypothesis, and in particular it affords a very simple explanation of the tion of classical uncoupling agents such as dinitrophenol, mitochondrial swelling phenomena, of the uptake and coupling effects of polyvalent metal ions, of the special mechanism of uncoupling by valinomycin and gramicidin, and of the pseudo site-specific inhibition of respiration by organic cations such as guanidine and its derivatives⁴. Other accounts of the work presented in this article and a

separate account of the present status of the theory of chemiosmotic coupling will be published in due course.

We thank Dr. Brian Chappell, Dr. G. D. Greville and Dr. Andre Jagendorf for helpful discussion, Prof. E. C. Slater for encouragement, and Mr. R. Mitchell for assistance.

¹ Mitchell, P., *Nature*, **191**, 144 (1961).

² Mitchell, P., *Biochem. Soc. Symp.*, **22**, 142 (1962).

³ Mitchell, P., in *Cell Interface Reactions*, edit. by Brown, H. D., 33 (Scholar's Library, 1963).

⁴ Mitchell, P., *Symp. Regulation of Metabolic Processes in Mitochondria*, Bari, 1965 (in the press).

⁵ Ogston, A. G., and Smithies, O., *Physiol. Rev.*, **28**, 283 (1948). Davies, R. E., and Ogston, A. G., *Biochem. J.*, **48**, 324 (1950). Davies, R. E., and Krebs, H. A., *Biochem. Soc. Symp.*, **8**, 77 (1952). Robertson, R. N., *Biol. Rev.*, **35**, 231 (1960).

⁶ Saris, N., *Societas Scientiarum Fennica, Commentationes Physico-Mathematicae*, **28**, II, 1 (1963).

⁷ Chappell, J. B., Greville, G. D., and Bicknell, K. E., *Biochem. J.*, **84**, 61P (1962). Chappell, J. B., Coen, M., and Greville, G. D., in *Energy-Linked Functions of Mitochondria*, edit. by Chance, B., 219 (Academic Press, 1963). Brierley, G. P., Murci, E., and O'Brien, K. L., *Biochim. Biophys. Acta*, **88**, 646 (1964).

⁸ Pressman, B. C., *Proc. U.S. Nat. Acad. Sci.*, **53**, 1076 (1965). Moore, C., and Pressman, B. C., *Biochem. Biophys. Res. Commun.*, **15**, 562 (1964).

⁹ Chappell, J. B., and Crofts, A. E., *Biochem. J.*, **95**, 393 (1965).

¹⁰ *International Critical Tables*, **3**, 271 (McGraw-Hill, 1928). *Handbook of Chemistry and Physics*, forty-fourth ed., 1706 (Chemical Rubber Publishing Co., 1962-63).

¹¹ Chappell, J. B., *Biochem. J.*, **9**, 225 (1964).

¹² Itzhaki, R. F., and Gill, D. M., *Analyt. Biochem.*, **9**, 401 (1964).

¹³ Ernster, L., and Lee, C. P., *Ann. Rev. Biochem.*, **33**, 729 (1964).

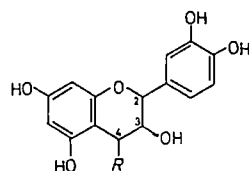
¹⁴ Mitchell, P., *Biochem. J.*, **81**, 24P (1961).

STRUCTURE OF CONDENSED TANNINS

By L. L. CREASY* and Dr. T. SWAIN

Low Temperature Research Station, Cambridge

MOST of the work that has so far been carried out on the structure of condensed tannins (flavolans) has been concentrated on the possible modes of polymerization of the hydroxyflavan-3-ols (for example, (+) catechin, 2)¹⁻⁵. The polymerization of hydroxyflavan-3,4-diols (for example, IIa,b), on the other hand, has been less completely investigated, although it is believed that they are intimately concerned in the formation of flavolans⁶⁻⁸, and model experiments have shown that the hydroxyl group at C-4 readily forms carbonium ions in acid solution (for example, III) which react with nucleophilic centres similar to those at C-6 and C-8 in the commonly occurring flavan-3-ols and -3,4-diols (for example, Ia, Ib, IIa and Ib)⁹⁻¹⁰. Indeed, several workers^{2,11,12} have suggested that the flavolans are formed from flavan-3,4-diols by linkages between C-4 of one unit and C-8 (ref. 13) (or C-6, ref. 9) of another (for example, IV). Freudenberg² has further postulated that such structures would be readily cleaved by acid treatment to yield the parent diols (for example, II) or its reaction products (for example, the corresponding anthocyanidin, VI).

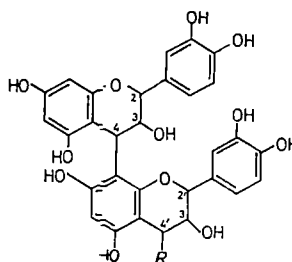


- I: R = H
a, 2,3-trans (+)-catechin
b, 2,3-cis (-)-epicatechin
II: R = OH
a, 3,4-trans
b, 3,4-cis
III: R = \oplus
(Carbonium ion)

In spite of this hypothesis, it has been suggested that the naturally occurring flavan dimers (biflavans) which have so far been investigated¹⁴⁻¹⁶, and which yield on treatment with dilute acid under relatively mild conditions (for example, 0.1 N HCl for 5 min at 100°)¹⁴ a flavan-3-ol (either Ia¹⁶ or Ib^{14,15}) and a second flavan moiety (which in all cases¹⁴⁻¹⁶ gives cyanidin (VI) on heating in stronger acid), have structures involving either a hemiacetal¹⁴,

ether^{15,17} or hemiketal¹¹ linkage between the hydroxyl group at C-3 of the flavan-3-ol and one of the hydroxyl groups of the pro-anthocyanidin moiety. Geissman and Dittmar^{18,19}, on the other hand, have adduced evidence that the biflavan from avocado seed (*Persea gratissima*), which yields catechin (Ia) on mild acid hydrolysis, has the structure Va. They have further suggested that the most commonly occurring polymeric leucoanthocyanins (that is, flavolans which yield cyanidin on acid treatment) are formed by extensive self-condensation of (III) via (IV), but that when a flavan-3-ol (for example, catechin (Ia)) is present a biflavan can be produced (for example, Va-e) which is relatively stable and is incapable of further condensation since it lacks a hydroxyl group at C-4' (ref. 1).

We have been studying the physiology of flavanol formation in the leaves of the wild strawberry (*Fragaria vesca*) and have shown that this tissue produces, besides (+)-catechin (Ia) and a number of flavolans of apparently increasing complexity, a biflavan which yields (+)-catechin (Ia) and cyanidin (VI) by suitable acid treatment, and is thus similar to the biflavans from avocado^{18,19} and *Gleditsia triacanthos*¹⁸. The λ max and molecular



- IV: R = OH
V: R = H
a, Stereochemistry at C-2, -3 and -4 undetermined 2',3'-trans
b, 2,3-trans-3,4-trans-2',3'-trans
c, 2,3-trans-3,4-cis-2',3'-trans
d, 2,3-trans-3,4-trans-2',3'-cis
e, 2,3-trans-3,4-cis-2',3'-cis

* Present address: Department of Pomology, Cornell University, Ithaca, New York.

absorptivity of the solutions of the biflavans from strawberry and avocado, both in methanol and 70 per cent sulphuric acid²⁰, and of their adduct with vanillin in 70 per cent sulphuric acid²¹ are closely similar, as is the yield of cyanidin formed by heating either of them in *n*-butanol-concentrated hydrochloric acid (9:1)²¹ for 1 h (Table 1). However, the R_F values of the two compounds are different in most solvent systems and it must be assumed that the two compounds are stereoisomers. Support for this assumption comes from the fact that, after epimerization in water at 120° for 2 h²², the strawberry biflavan gives six components separable on cellulose thin-layer plates in 5 per cent methanol, three of which have identical R_F values with the products of epimerization of the avocado compound.

In order to obtain more evidence as to the nature of the strawberry biflavan, we have examined the products obtained by treating an aqueous solution of a mixture of (+)-catechin (Ia) and the flavan-3,4-diol racemate (IIa,b) produced by the reduction of naturally occurring 2,3-dihydroquercetin (2,3-*trans*) with sodium borohydride²³, with cold 0.4 *N* hydrochloric acid for 5 min. The acid was removed with di-*N*-octyl methylamine in chloroform²⁴, and the resultant mixture separated by preparative paper-chromatography in butanol/acetic acid/water (BAW, Table 1), giving two compounds *CA* and *CB* (see Table 1) in yields of 50 per cent and 37 per cent respectively (based on the (+)-catechin used). The R_F value of the faster-running major component *CA* on paper chromatograms and on three different thin-layer supports in various suitable solvent systems is identical with that of the strawberry biflavan (Table 1). Furthermore, both *CA* and *CB* have the same spectral properties as the strawberry compound (Table 1) and produce the same six components on epimerization²². It should be noted that both *CA* and *CB* give (+)-catechin (Ia) on treatment with dilute acid and are presumably stereoisomers at C-4 (Vb and Vc). From the chromatographic behaviour of the known isomeric flavan-3,4-diols²², and the fact that sodium borohydride reduction of the dihydroflavonols gives mainly 3,4-*trans* compounds²⁵, it appears possible that the major synthetic dimer, and therefore the strawberry biflavan, is the 2,3-*trans*-3,4-*trans*-2',3'-*trans* isomer (Vb); *CB* therefore being Vc. Naturally, more exact criteria^{22,25,26} are required to confirm this possibility.

When (-)-epicatechin (Ib) was mixed with the flavan-3,4-diol racemate (IIa,b) and treated as above, two different compounds were produced, *EA* and *EB* (50 per cent and 22 per cent yield of each based on the (-)-epicatechin). With suitable acid treatments, these compounds gave (-)-epicatechin (Ib) and cyanidin (V) and both had almost identical spectral properties as those of the biflavan isolated from the seeds of *Theobroma cacao*²⁷. However, the R_F values of both synthetic compounds are different from that of the natural product. Epimerization

of both *EA* and *EB* gives six separable products (two which are identical in R_F value with two of those produced from *CA* or *CB*, but the cocoa biflavan only yields the separable components, one of which corresponds with while another corresponds with *CA*. Since *EA* and must be IVd and IVe, it appears probable that the co-biflavan may differ in the stereochemistry at C-2 and (V), and is perhaps the 2,3-*cis* isomer. If this were the case both flavan moieties would have the same configuration at C-2 and C-3. It is, of course, possible that some other structure is involved, but since the octamethyl ether of the cocoa compound yields 5,7,3',4'-tetra-*O*-methyl-epicatechin and 5,7,3,4'-tetra-*O*-methylecyanidin on treatment with hot acid¹⁴ it must be concluded that the phenolic hydroxyl groups are not involved in the linkage between the flavan nuclei, and structures such as those suggested by Roux¹¹ and Hergert¹² for the formation of polymers involving a link between C-4 and the oxygen at C-7 of the second flavan are unlikely.

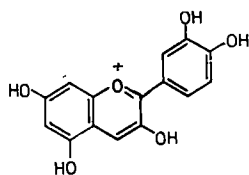
Finally we have examined the properties of the water insoluble, methanol-soluble polymer *LP* which is produced when the flavan-3,4-diol (IIa,b) is treated with cold acid¹² in the absence of any other nucleophile. This compound is immobile in all solvent systems and shows the expected lower reactivity with vanillin in 70 per cent sulphuric acid^{21,28}, but gives a somewhat higher yield of cyanidin than found with natural polymers of this type^{7,13} (Table 1). It also yields no (+)-catechin or (-)-epicatechin on mild hydrolysis: when the hydrolysis is carried out in the presence of (+)-catechin, however, it does give small yields of both *CA* and *CB*, thus indicating that the C-links are broken by such treatment. Since Quesnel²⁹ has shown that the natural cocoa flavanol yields (-)-epicatechin on mild acid hydrolysis, it must be concluded that Geissman and Dittmar's hypothesis with regard to the structure of the flavanols is not wholly correct.

It appears probable, instead, that condensed tannins of the flavanol type are complex, branched, three-dimensional structures in which the majority of the repeating units are nuclei derived from hydroxyflavan-3-ols and 3,4-diols. It is also possible that nuclei derived from flavan-4-ols and flavan-4-ols³⁰ (flavylium pseudobase) may be involved. It is considered that the flavan nuclei will be linked, initially, mainly by bonds between C-4 of one of the flavan-3,4-diol units, and either of the nucleophilic sites, C-8 (ref. 13) or C-6 (especially the latter for those hydroxy flavans having a resorcinol-like A-ring, for example, VII) of another hydroxyflavan. In the case of flavans having a pyrogallol-type of substitution in the B-ring (for example, VIII) there will also be the possibility of links to the nucleophilic C-2' and C-6' positions (cf. refs. 3 and 4). In such polymers flavan-3-ol nuclei linked via C-4 of a flavan-3,4-diol, would form relatively stable end units to branches, and the parent flavan-3-ol would thus be released on mild acid treatment²⁹; similarly,

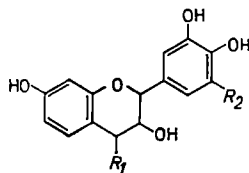
Table 1. PROPERTIES OF NATURAL AND SYNTHETIC BIFLAVANS

Compound	R_F on thin-layer plates*				$\epsilon \times 10^{-3}$			Yield of cyanidin (molar %)	Products of mild hydrolysis
	Cellulose			Silica gel-H Bz : Ac : W** 10 : 10 : 1	MeOH†	70% H ₂ SO ₄ ‡	1% Vanillin in 70% H ₂ SO ₄ §		
	BAW 4 : 1 : 2 : 2	5% aq MeOH	80% phenol						
Avocado biflavan	0.47	0.23	0.10	0.15	6.2	9.0	27.6	38	Epicatechin and catechin
Strawberry biflavan	0.45	0.30	0.13	0.31	6.6	8.4	24.5	35	Catechin
Cocoa biflavan	0.55	0.50	0.19	0.31	6.0	8.8	29.5	30	Epicatechin
CA	0.45	0.30	0.13	0.31	6.3	8.7	20.8	28	Catechin
CB	0.35	0.28	0.06	0.12	††	—	—	—	Catechin
EA	0.52	0.39	0.21	0.31	5.7	10.0	33.0	28	Epicatechin
EB	0.40	0.25	0.09	0.13	††	—	—	—	Epicatechin
(+) Catechin	0.68	0.33	0.37	0.79	3.6	8.7	30.1	—	—
(-) Epicatechin	0.54	0.31	0.38	0.72	3.6	10.8	35.6	—	—
Leucocyanidin	0.57	0.41	—	0.64	3.2	3.9	15.8	40	—
LP	0	0	0	0	4.8¶	6.1¶	13.9¶	17¶	—

* Thin-layer chromatography on polyamide in 70 per cent aq. MeOH; all biflavans had R_F 0.63, monomers 0.50, and *LP* 0. Solvents: BAW, *n*-butanol/acetic acid-water. Bz:Ac:W, benzene-acetic acid-water. ** Developed twice. † At 280 m μ (max. in each case). ‡ At 355 m μ (max. in each case). § At 500 m μ . ¶ The ϵ values for both *CB* and *EB* in MeOH were ~20 per cent lower than *CA* and *EA* respectively, perhaps due to the presence of interfering materials which were not resolved chromatographically: the relative absorptivities in MeOH, 70 per cent H₂SO₄, and with vanillin in 70 per cent H₂SO₄ and the molar yield of cyanidin, however, were more or less the same for *CA*, *CB* and *EB*. ¶ Based on monomer.



VI. Cyanidin

VII. $R_1 = \text{H or OH}$ VIII. $R_1 = \text{H}$ IX. $R_1 = \text{H or OH}$ X. $R_1 = \text{OH}$

van-3,4-diol nuclei present in the structure linked as di units, or only through other diols, would also be released on acid treatment to give in part anthocyanidins. Branch points would occur wherever two or more of the potential reactive centres in any one flavan were involved in linkage. Subsequent changes in the initial polymer may be induced by oxidation^{3,4} (which might be better regarded as being due to one-electron coupling reactions; compare refs. 31, 32), by the formation of bonds between the electrophilic centre at C-2^{1,2} and the nucleophilic sites mentioned earlier, and by the formation of internal ether-type links^{30,33,34}. Of course, other suitable nucleophiles or electrophiles (such as lignin precursors, amino-acids, etc.) may also be incorporated into the whole flavanol molecule, and links may be formed to other macromolecules in the cell²⁸.

In spite of our doubts about Giessman and Dittmar's proposals^{18,19} for the flavanols, we believe that the results presented here support their hypothesis regarding the structure and stability of the biflavans. Furthermore, this theory may be extended to explain why the only natural flavan-3,4-diols so far isolated which have had their structure and stereochemistry unequivocally proven lack a hydroxyl group at C-5 (for example, VII, $R_1 = \text{OH}$). The lack of this hydroxyl group not only

enhances the stability of the flavan-3,4-diol itself by reducing the nucleophilic activity of C-6 and C-8, but also reduces the probability of its reacting with a corresponding flavan-3-ol. It is noteworthy that in spite of extensive investigations⁶ no biflavans corresponding to IV having a resorcinol-type substitution in either or both A rings have so far been isolated.

One of us (L. L. C.) thanks the National Science Foundation for support (grant 43129). We thank Prof. T. A. Giessman and Dr. H. K. F. Dittmar for allowing us to see their results before publication, and for a gift of avocado biflavan from Dr. Dittmar.

¹ Freudenberg, K., and Weinges, K., *Ann. Chem.*, **668**, 92 (1963).

² Freudenberg, K., *Experientia*, **13**, 104 (1960).

³ Hathway, D. E., *J. Soc. Leather-Trade Chemists*, **42**, 108 (1958).

⁴ Hathway, D. E., *Biochem. J.*, **70**, 34 (1958).

⁵ Haworth, R. D., and Haslam, E., *Prog. Org. Chem.*, **6**, 1 (1964).

⁶ Bate-Smith, E. C., and Metcalfe, C. R., *J. Linn. Soc. (Bot.)*, **55**, 669 (1957).

⁷ Roux, D. G., and Paulus, E., *Biochem. J.*, **82**, 320 (1962).

⁸ Roux, D. G., *Chem. and Indust.*, 278 (1962).

⁹ Brown, B. R., Cummings, W., and Newbould, J., *J. Chem. Soc.*, 3677 (1961).

¹⁰ Roux, D. G., and Paulus, E., *Biochem. J.*, **80**, 476 (1962).

¹¹ Roux, D. G., *J. Amer. Leath. Chem. Assoc.*, **54**, 614 (1959).

¹² Hergert, H. L., in *The Chemistry of Flavonoid Compounds*, 553, edit. by Giessman, T. A. (Pergamon, Oxford, 1962).

¹³ Hillis, W. E., and Urbach, G., *J. App. Chem.*, **9**, 665 (1959).

¹⁴ Forsyth, W. G. C., and Roberts, J. B., *Biochem. J.*, **74**, 374 (1960).

¹⁵ Freudenberg, K., and Weinges, K., *Tetrahedron Lett.*, 267 (1961).

¹⁶ Freudenberg, K., and Weinges, K., *Angew. Chem.*, **74**, 182 (1962).

¹⁷ Weinges, K., *Chem. Ber.*, **94**, 3032 (1961).

¹⁸ Giessman, T. A., Meeting of the Plant Phenolics Group of North America (1963).

¹⁹ Giessman, T. A., and Dittmar, H. K. F., *Phytochemistry*, **4** (1965).

²⁰ Creasy, L. L., and Swain, T. (in preparation).

²¹ Swain, T., and Hillis, W. E., *J. Sci. Food Agric.*, **10**, 63 (1959).

²² Drewes, S. E., and Roux, D. G., *Biochem. J.*, **94**, 482 (1965).

²³ Swain, T., *Chem. and Indust.*, 1144 (1964).

²⁴ Harborne, J. B., and Sherratt, H. S. A., *Biochem. J.*, **65**, 238 (1957).

²⁵ Clark-Lewis, J. W., Jackman, L. M., and Williams, L. R., *J. Chem. Soc.*, 3858 (1962).

²⁶ Clark-Lewis, J. W., *Rev. Pure and Appl. Chem.*, **12**, 96 (1962).

²⁷ Forsyth, W. G. C., *Biochem. J.*, **60**, 109 (1955).

²⁸ Goldstein, J. L., and Swain, T., *Phytochemistry*, **2**, 371 (1963).

²⁹ Quesnel, V. C., *Tetrahedron Lett.*, 3699 (1964).

³⁰ Jurd, L., and Wais, jun., *Chem. and Indust.*, 1708 (1964).

³¹ Hassall, C. H., and Scott, A. I., in *Chemistry of Natural Phenolic Compounds*, 119, edit. by Ollis, W. D. (Pergamon, Oxford, 1961).

³² Lewis, J. R., *Chem. and Indust.*, 1672 (1964).

³³ Mayer, W., Merger, F., and Frank, G., *Ann. Chem.*, **675**, 126 (1964).

³⁴ Weinges, K., and Toribio, F., *Ann. Chem.*, **681**, 161 (1964).

INTERSTIMULUS INTERVAL AND NUMBER JUDGMENT

By D. JONES and DR. A. ELITHORN

External Scientific Staff, Medical Research Council, Institute of Neurology and Royal Free Hospital, London

ON the basis of previous experiments it was suggested by one of us that the interference which occurs when subjects respond to two single stimuli, separated by very short time-intervals, was determined by both perceptual and motor components¹. In a more recent paper it has been shown that some subjects can, under certain conditions, process simple stimuli simultaneously and independently². In the experiments reported here we examined some aspects of the perceptual field which may determine the effective size of the so-called span of apprehension. The size of the span of visual apprehension is a subject which has attracted a fair amount of psychological investigation. Less work, however, has been done on the problem of the necessary length of the time-interval needed between two stimuli to allow two independent judgments, or on the interaction between this interval and the content of the two stimuli. The many investigations into the span of visual apprehension all agree that the accuracy of judging the number of briefly displayed objects decreases with increasing stimulus number, and

that, under most conditions of presentation, the threshold criterion of 50 per cent correct responses occurs with 6-7 stimulus objects. Three experiments on the effect of interstimulus interval on the perception of numerosity are described.

In the first experiment 6 subjects were asked to report the number of neon bulbs illuminated in two presentations, separated by time-intervals of 50, 100, 200, 350, 600 and 900 msec. Because earlier experimental findings suggested that some subjects could process simple stimuli independently if they were presented in the opposite half-fields, the display was designed so that pairs of stimuli could be presented in the same half-field or in different half-fields.

Sixty-four small neon bulbs were mounted behind small apertures in a black display board in the form of four small 4 in. x 4 in. square arrays which formed the corners of a square of side 1 ft. At the centre of the display board there was a small warning light which the subject was instructed to use as a fixation point. The warning light

came on 1,200 msec before the start of each stimulus situation and remained on for 800 msec.

The pairs of stimuli were arranged in sets of 36, each set consisting of all combinations of 1-6 lights in the first stimulus, with 1-6 lights in the second stimulus. The number sequences, the spatial relationships and the time separation of the stimuli were determined by a balanced, randomized design. Each subject was tested twice, each session consisting of 6 sets of stimuli, separated by a rest interval of 2 min. The subjects were 6 female medical students at the Royal Free Hospital, their mean age being 24 years. The subject's instructions were to give a verbal report of the presented number for both stimuli in each situation.

The first of each pair of stimuli could be presented in any of the 4 positions and the second followed it at the selected interval in any of the other 3 positions. The duration of the neon flash was 40 msec and the time intervals were measured from the start of the first stimulus to the start of the second. The subject was seated 8 ft. from the display; the visual angle sub-tended by the distance between the warning light and the neon positioned at each outermost corner was 5 deg.

The results of this first experiment are given in Table 1. The findings are disappointing. An analysis of variance showed no significant position effect and that the effect of the value of the time-interval on the number of correct responses was significant only at the 5 per cent level. It was, however, clear from the overall performance of our subjects, and from their introspective reports, that the effects of the variables were obscured by the fact that the task was too difficult under all conditions. It seemed likely that at least three factors were contributing to this. In the first place, the stimulus arrays were outside central vision, making the resolution of each stimulus difficult. This was confirmed in a subsidiary experiment in which the subjects reported the number of neons presented in single groups, in one condition with central fixation and random presentation of the array, and in a second condition in which they were told in which array the stimulus would appear and were allowed to fixate on that position. A second difficulty was that the use of a peripheral stimulus and the unbalance of expectancy, created by the fact that the second stimulus occurred with equal frequency in two contralateral and one unilateral quadrants, made it very difficult for subjects to inhibit eye movements occurring between the two stimuli. These eye movements were both reflex towards the first stimulus and, if this was inhibited, 'expectant' towards the contralateral half-field. Thirdly, the experimental design did not allow enough practice.

In order to overcome these difficulties, we decided to leave for the time being the examination of the half-field effect and to design an experiment which would provide reliable information on the effect of the time-interval. An alternative display was designed and built.

In the second experiment 60 small neon bulbs were arrayed in 3 concentric hexagonal rings. The outermost hexagon had a radius of 2 in. and was made up of 30 neons which formed 1 functional group. The middle hexagon had a radius of 1.2 in. and contained 18 neons and the inner one, with a radius of 0.8 in., was made up of 12 neons. The 30 neons in the 2 inner hexagons formed the second functional group. As in the first experiment, there was a warning light at the centre of the display.

Table 1

	50	100	Interval (msec)		600	900	Average for all intervals
			200	350			
Stimuli in opposite half-fields	28.1	34.7	28.8	32.2	36.8	36.1	32.8
Stimuli in same half-fields	30.6	26.4	31.9	31.9	35.4	41.0	32.9
Average for all conditions	28.9	31.9	29.9	32.2	36.3	37.7	32.8

Percentage of correct pairs of responses for all 6 subjects in Experiment 1 at each of the time-intervals is given separately for the presentations made in the same and the opposite visual half-fields.

The visual angle sub-tended by the display at the view distance of 8 ft. was less than 2.5°.

The stimuli were arranged in basic sets of 36 pairs consisting of all combinations of 1-6 neons in the first stimulus, with 1-6 neons in the second stimulus. The sets stimuli were presented in the 2 sequences inside followed by outside and outside followed by inside. Each subject was required to judge the presented number for 2 practice and 4 test sets of stimuli, at each of the following intervals: 0, 50, 100, 150, 200, 250, 300, 350, 400, 500, 700 and 900 msec.

Each time-condition was examined separately, half the subjects taking the series in ascending order and half the descending order. The subjects were free to report the total number of lights for both stimuli or to make separate estimates. The subjects were students or members of staff at the Royal Free Hospital. Eight were female and 4 were male, the mean age for the group being 24 years.

The results of this experiment are summarized in Fig. 1. At intervals of 200 msec and longer, all 12 subjects made separate reports for each member of the stimulus pairs. At the shorter time-intervals most of the subjects made single reports in response to each pair of stimuli. If the analysis presented here the report is regarded as error when the total number reported was different from the total number presented. In Fig. 1 the results are presented for both the descending and ascending groups. At all intervals, except 300, 700 and 900 msec, the ascending group made more errors than the group taking the time-intervals in descending order.

The differences in the error curves for the 2 groups of subjects strongly support the view that different mechanisms or strategies are employed at the very brief and longer time-intervals. Both groups show evidence of practice effect at the task of judging stimuli separately by the longer time-intervals, that is, in the range from about 100 to 900 msec. The ascending group performs relatively badly at the first intervals they encounter in this range; but at the longest intervals which are the first encountered by the descending group, the performance levels are reversed. At the simultaneous condition and the 50 msec time-interval the prior experience of the groups of the shorter intervals is about the same and the performance levels are almost identical. Both curves show fewer errors when the stimuli are presented simultaneously.

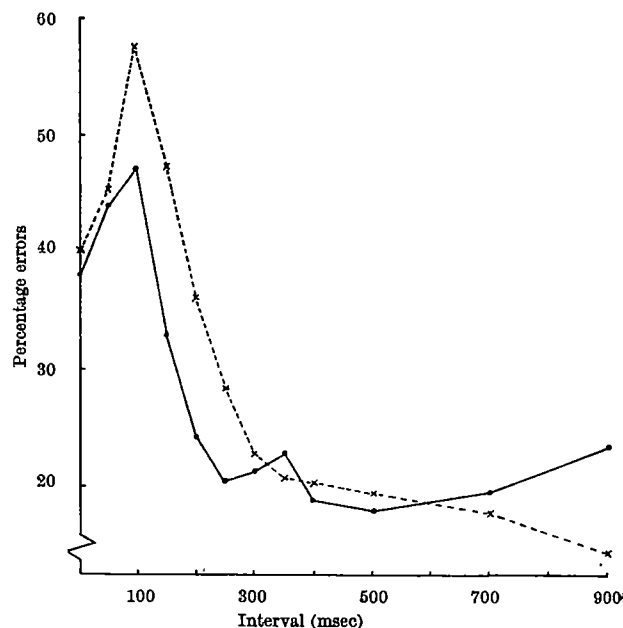


Fig. 1. The error curves obtained in the second experiment for the two groups of 8 subjects—ascending and descending orders of time-intervals. ---, Group 1 ascending; —, group 2 descending

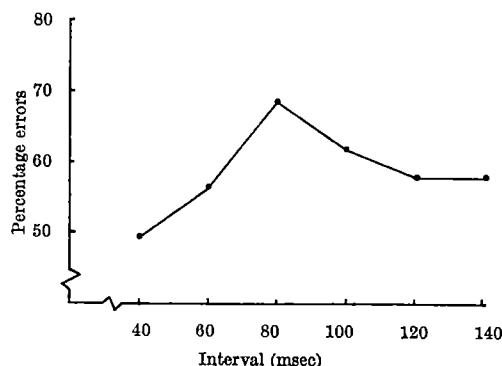


Fig. 2. Relationship between percentage errors and time-interval for all 6 subjects in the third experiment

taneously or with an interval of 50 msec than they do when the interval is 100 msec, both curves showing maximum errors at this time. Studying the error curves for each subject showed that 10 out of the 12 had a peak in their error scores at the 100 msec interval. The other 2 subjects were both in the descending group and both had a peak in their error curves at the 50 msec interval.

In an attempt to narrow the limits within which the peak difficulty occurred, a third experiment was undertaken. Using a Latin square design to balance practice and fatigue effects at the different time-intervals, 6 new subjects undertook 6 sessions, each of 2 practice sets and 4 test sets, each set consisting of 36 pairs of stimuli, arranged in a balanced design, as in the previous experiment. Each session was at one of the following 6 intervals: 40, 60, 80, 100, 120 and 140 msec.

The error curve for the group is given in Fig. 2. The results clearly confirm the findings of the previous experiment and indicate that the peak difficulties lie between 60 and 100 msec.

In this last experiment each subject undertook one session at each time-interval, the order of time-intervals being different for each subject. Practice effects between sessions were marked and the experiment provides no evidence on individual differences.

The analysis presented here takes no account of the numerosity of the stimuli or of order effects in relation to the relative numbers of neons in the first and second stimuli. Errors, as might be expected, were more frequent with the larger total numbers and were greater when the larger number was presented first, as contrasted with the

situation when the second stimulus was the larger. These findings will be presented and discussed in detail later, but clearly the time-course of the error curve calls for brief comment.

In an earlier investigation on reaction time responses to successive stimuli separated by short intervals, it was observed that delays in the second response were not maximal at the shortest intervals³. This observation was critical to Welford's theory of a psychological refractory period, although Hick and Welford⁴ suggested that the apparent exceptions to the theory might be due to subjects' grouping, or responding to both stimuli as a single unit, at the very short intervals. In the work recorded here it is obvious that, with almost no clues for a spatial separation, subjects must be grouping the stimuli when presented simultaneously. It is also clear that at the longest intervals the stimuli are treated as independent. The poor performance at the 50 and 100 msec intervals cannot be due to a grouping strategy. For the ascending group it represents a failure to maintain such a strategy as the interval increases. For the descending group it may be seen as a failure, as the intervals decrease to attain a grouping strategy which is achieved at the shortest intervals.

As will be reported elsewhere, each stimulus impairs the perception of the other and this can be related to the phenomena of metacontrast and of apparent movement. Subjects frequently reported apparent movement effects at the short time-intervals. The perception of two discrete stimuli as a single stimulus in motion would clearly impair the perception of numerosity. Unfortunately, the literature on the time relationships of both metacontrast and apparent movement is, in both instances, complex. However Alpern⁵, who among others has reviewed the work on metacontrast, himself demonstrated a maximum masking effect when the interval between the stimuli was in the region of 100 msec.

While it is tempting to interpret the failures in the perception of numerosity, in terms of the apparently more basic processes of metacontrast and apparent movement, it is perhaps more useful to relate all these to the characteristics of central interpretative mechanisms. It is hoped to develop this approach in relation to a more detailed description of the present findings.

¹ Ellthorn, A., *Quart. J. Exp. Psychol.*, **13**, 240 (1961).

² Kerr, M., Mingay, R., and Ellthorn, A., *Brit. J. Psychol.*, **56**, 53 (1965).

³ Ellthorn, A., and Lawrence, C., *Quart. J. Exp. Psychol.*, **7**, 116 (1955).

⁴ Hick, W. E., and Welford, L. T., *Quart. J. Exp. Psychol.*, **8**, 39 (1956).

⁵ Alpern, M., *J. Opt. Soc. Am.*, **43**, 648 (1953).

CONTROL OF THE COMPETENT STATE IN *Pneumococcus* BY A HORMONE-LIKE CELL PRODUCT: AN EXAMPLE FOR A NEW TYPE OF REGULATORY MECHANISM IN BACTERIA

By DR. ALEXANDER TOMASZ
The Rockefeller Institute, New York

COMPETENCE or 'transformability'—the ability of some bacterial cells to take up biologically active DNA molecules and undergo genetic transformation—is a genetically determined property of some *Pneumococcus* strains. It has been known for some time that in pneumococcal cultures passing through a typical growth cycle this genetic potency gains phenotypic expression in most of the cells only during a relatively short 'competent' phase of growth¹. During this short phase, the cell population becomes highly uniform with respect to competence: the majority of the cells can be transformed by multiply-marked DNA, and there is evidence that all the individual cells present express the competence property.

The time course of this expression during growth shows some unique features: once an induction period is passed in which competence is low or undetectable, there is an abrupt and fairly 'synchronous' development of transformability at a rate very much faster than the growth rate of the culture. Under these conditions, the maximal competence is usually followed by a rapid decay of this property. A typical time course of such an expression process is illustrated in Fig. 1.

The cause of the delayed and abrupt appearance of competent cells in pneumococcal cultures has been a puzzle ever since the discovery of the existence of a 'competent phase' during which these bacteria are capable

of undergoing genetic transformation². Since in some complex growth media the competent phase of pneumococci seemed to develop close to the onset of the stationary phase of growth, it was suggested that transformable cells are physiologically abnormal organisms (for example, cells deficient in ability to grow) which accumulate in the culture as the growth medium becomes exhausted in some nutrients^{3,4}.

However, more recent investigations revealed no support to this interpretation. Instead, it was proposed that the rapid accumulation of competent cells should be regarded as a shift to a distinct physiological state⁵. This point of view has already been expressed by a number of authors^{2,6}, but our experiments yielded new evidence in support. (1) It was found that pneumococcal cultures grown in chemically defined media develop the competent phase in a critical cell concentration range, but independent of the 'closeness' of the stationary phase or the growth rate of the culture^{7,8}. (2) Competent pneumococci seem to be actively growing cells as indicated by their sensitivity to penicillin⁹ and by the fact that the competence of a culture can be stabilized in a chemostat under conditions where slow-growing cells would be rapidly diluted out⁷. (3) Finally, the detection of small quantities of biologically active DNA in sterile filtrates of growing pneumococcal cultures near their peak of competence suggests that this property has a biological 'value' for *Pneumococcus* in that it may equip these bacteria with a mechanism for gene exchange¹⁰. In this unique recombination mechanism, gene transfer depends on the random collision of DNA molecules and cells. Since the rate of this process is a function of the cell concentration and the DNA concentration as well, the synchronous appearance of competence in the majority of the cell population makes the chances for genetic transformation optimum.

If one accepts this interpretation, then the development of competence may be regarded as the phenotypic expression of an important pneumococcal character which at low cell concentrations is 'repressed' in most members of the cell population.

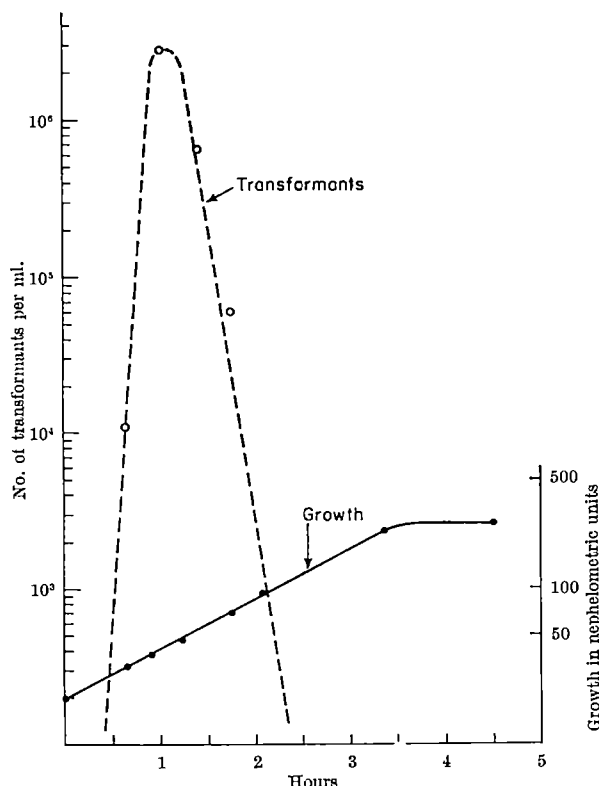


Fig. 1. The time course of development of competence during growth of a *Pneumococcus* culture

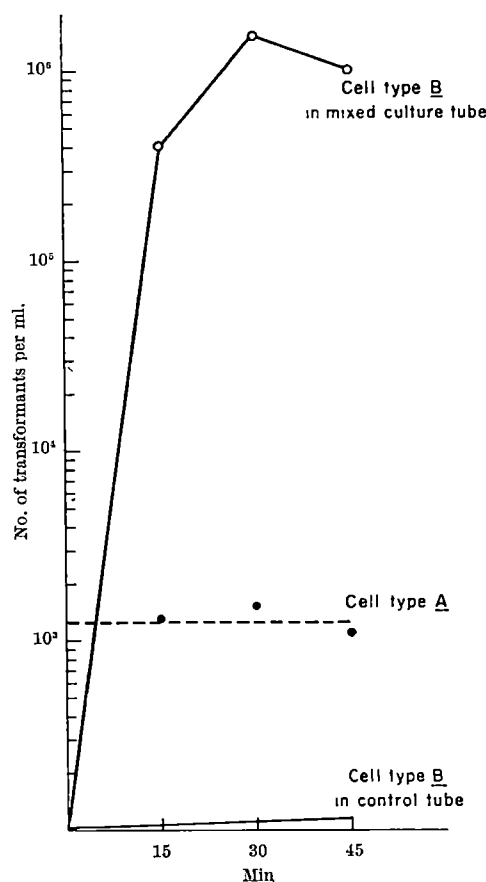


Fig. 2. Cell-to-cell transfer of the competence property

In the past three years, the mechanism of this delayed and synchronous expression was the subject of systematic investigations undertaken in our laboratory. It became apparent that the abrupt expression of competence does not depend on a general phasing of the life-cycles of the individual cells, since there is no detectable sign of massive division-synchrony in cultures during the development of competence.

It seemed likely, therefore, that the uniform and synchronous expression of this pneumococcal character is ultimately controlled by some environmental or endogenous factor able to affect randomly dividing cells.

Investigations of the effect of culture conditions on the time course of development of competence have clearly shown that alteration of the medium composition by the bacterial growth (for example, 'detoxification' or selective removal of some medium components) was not responsible for the synchronous 'expression' of this property⁸. Furthermore, as has already been mentioned, cultures of pneumococci growing in synthetic media in which growth was regulated by varying levels of enrichment were found to develop competence at about the same cell concentrations regardless of the phase of growth or rate of growth of culture^{7,8}.

Recent attempts to identify the mechanism controlling the expression of competence led to the discovery of a unique regulatory mechanism. It was found that the expression occurs through a co-operative process in which cells in the competent state can transfer this property to incompetent bacteria in a rapid process mediated by a specific macromolecular product of the competent cells⁵. The essential features of this observation are reproduced in the experiment illustrated in Fig. 2. Washed suspensions of two pneumococcal cell types which were distinguishable by their different antibiotic resistance (resistance against sulphamylamide in cell-type A and resistance against micrococcin in cell-type B) were mixed in a test-

tube in fresh medium. Cells of type *A* were highly competent and were introduced into the test-tube at a concentration of 1×10^6 cells per ml.; cells of type *B* were incompetent and were present at a concentration of 1×10^7 cells per ml. The same concentration of the incompetent cells was also introduced separately into a (control) test-tube without cell-type *A*. Both tubes were incubated at 30° C, a temperature at which the spontaneous development of competence is inhibited⁵, and the transformability of the two cell types was determined by exposing aliquots to a DNA carrying the streptomycin resistance marker. The transformants were selectively scored in two different antibiotic-containing media: in medium *A* (containing sulphanilamide and streptomycin) to count transformed *A* cells and in medium *B* (containing micrococin and streptomycin) to count transformed *B* cells.

Fig. 2 shows that whereas cell-type *B* incubated alone in the control tube remained incompetent, the same bacteria in the mixed culture tube rapidly developed a high level of competence as a result of some interaction with the competent cells of type *A*. There was no significant change in the numbers of cells present during the short span of the experiment. It was calculated that about 80–90 per cent of all the type *B* cells have become competent by the 'activating' effect of cell-type *A*, in spite of the fact that these latter made up only about 10 per cent of the total population. The competence of the type *A* culture did not change during this process.

Next the mixed culture experiment was repeated in a modified form: the cultures of both cell types were introduced into the separate compartments of a U-shaped glass tube which was divided in two compartments by a 'Millipore' membrane. During incubation at 30° C, the filtrates of the two cultures were mixed by continuous pumping of the medium through the 'Millipore' membrane. Under these conditions, physical contact between the competent and incompetent bacteria was excluded. Table 1 shows that the cell-to-cell transfer of competence did not necessarily require physical contact of the organism but could occur through the mediation of some filterable cell product. The relatively low efficiency of competence induction was attributed to the low concentration of cell-free 'activator' substance and its extreme lability⁵.

Table 1

Cell type	No. of transformants per ml after incubation for	
	30 min	60 min
<i>B</i> (incompetent) alone	0	0
<i>B</i> in mixed culture with <i>A</i> (competent)	5.7×10^4	5.7×10^4
<i>B</i> in U-tube with <i>A</i>	300	1.3×10^4

Cell-to-cell transfer of the competence property by way of a soluble cell product.

The activator substance, responsible for the cell-to-cell transfer of competence, was afterwards isolated from competent pneumococci. Purified preparations of this heat-labile material could convert incompetent bacteria to competence in a fast 'reaction', the rate depending on the concentrations of both the activator and the cells⁵.

The pH optimum of this process was about 7.7, and the biological activity of activator preparations could be greatly enhanced in the presence of 0.05–0.07 M mercaptoethanol. Low concentrations of various proteolytic enzymes (0.1 µg/ml.), such as trypsin, chymotrypsin and subtilisin, inhibited the activation of incompetent cells by activator preparations, whereas these enzymes had no effect on the transformation of already competent cells by DNA. This observation suggested that the activator is a protein. Fractional elution of the activator substance from the cell debris yielded preparations with high specific activity: extracts with less than 0.2 µg protein/ml. could convert 10^6 cells to the competent state at 37° C in 10 min. The activator substance appears to be of fairly high molecular weight, since it is not retained during chromatography on 'Sephadex G25' columns. A small retention can be achieved on 'Sephadex G75' (Fig. 3).

The activator seems to be specific for *Pneumococcus*, since *Bacillus subtilis* and *Haemophilus influenzae* cultures in their incompetent phase of growth could not be converted to competence by pneumococcal activator. During the activation process the surface of the pneumococcal cells seems to undergo significant changes: a new antigenic determinant appears¹ and the bacteria develop a capacity to bind DNA and become transformed by it¹². Subsequent investigations have shown that the rapid disappearance of competence which usually follows closely the brief maximal competence of the culture can be correlated with another cell-produced factor, called 'inhibitor'. After pneumococcal cultures reach a certain density their filtrates rapidly become enriched in this inhibitor which can block the action of the activator and prevent further spread of competence⁵. Thus, it appears that the transient and synchronous expression of the competence character during growth is controlled at the level of the pneumococcal population by extracellular factors manufactured by the cells themselves.

The attainment of a transient physiological state in a population by such an 'induction' process seems to be a novel phenomenon among bacteria, and it raises a number of interesting questions concerning the nature of the cell interactions involved in such a process. (It is important to distinguish between the cellular 'communication' involved in the propagation of competence and the cell interactions which occur during the cell-to-cell transfer of episomal or chromosomal material. These latter interactions serve the 'dispersal' of genetic material in a population of bacteria while in the case of competence a physiological state is propagated.)

In the traditional view bacterial populations are characteristically lacking mechanisms for specific inter-cellular communication. This is contrasted with cell populations of higher organisms in which hormonal mechanisms are known to induce and reinforce the expression of many physiological properties and control differentiation.

The control of competent state in pneumococci forces one to revise this view. Since the activator—a cell-produced chemical—seems to impose a high degree of physiological homogeneity in a pneumococcal population with respect to competence, one is forced to conclude that in this case bacterial population can behave as a biological unit with considerable, although only temporary, co-ordination among its members. In a sense, this situation then resembles hormonal control—an example for a primitive, temporary 'differentiation' in bacterial populations.

One wonders whether this kind of control may not be operative in some other microbial phenomena also. It is

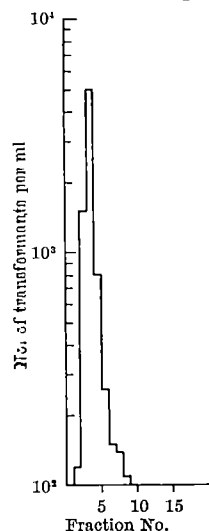


Fig. 3. Chromatography of pneumococcal activator on 'Sephadex G75'

well known that bacterial populations are able to undergo drastic changes in the physiological properties of the majority of the cells. Numerous examples for such massive non-genetic variability or 'modulations'¹³ are known: the list includes the variation in cellular components such as amounts of capsular material, intracellular lipid, carbohydrate and polyphosphate inclusions, qualitative and quantitative changes in the surface antigens and fimbriae (for review see ref. 14), cyst formation¹⁵, cyclic morphogenetic changes in cell shape¹⁶, sporulation¹⁷, changes in virulence¹⁸, changes in the biosynthetic capacity as it occurs in the production of tyrocidine¹⁹, bacitracin²⁰, edein²¹, and other bacterial polypeptide antibiotics.

In the absence of known endogenous causes, such as genetic variation or biological clock mechanisms, such gross physiological changes in a whole bacterial population are usually considered as the results of interactions between cells and their environment (using the term 'environment' in its broadest sense), even though in the majority of the cases the environmental signal invoking them is not known and the physiological significance of the change is usually little understood.

For the interaction between environment and cell population, two general and contrasting types of mechanisms can be considered. In the first type, each individual in the population would receive and recognize the environmental stimulus and respond to it in a continuous interaction with the environment and without any interaction with other individuals in the population. The homogeneity of the population with respect to the particular physiological state would then be the result of the basic uniformity of individuals as well as the uniformity of the environmental change. The derepression of metabolic pathways by the depletion of an end-product is at present considered as having such a mechanism.

In the second type of mechanism, a similar physiological response would originate in a radically different way. The environmental change would be first recognized by a small number of cells only; these cells would respond by 'converting' this environmental signal to a second and specific extracellular (chemical) signal, for example, the activator substance in the case of competence, which in turn would interact with the rest of the individuals of the population and convert them to the appropriate physiological state. In this 'hormone-like' mechanism, the physiological change in the majority of the cells does not occur by virtue of a continuous interaction with the environment but rather is called forth by a specific cell product. The homogeneity of the population with respect to the physiological character is determined here primarily by the specificity and effectiveness of the activator. (The terms 'hormone' and 'hormone-like' are used here in their broadest sense to indicate specific cellular products which have the biological function of co-ordinating individual cells of a cell population with respect to a physiological property. While this formulation includes the essence of the hormonal function, it clearly differs from the traditional definition of 'hormone' which restricts the use of this term to internal secretions of multicellular organisms. The need for a less stringently defined term for hormone-like functions was discussed in detail by J. Huxley, who proposed that such substances could, perhaps, be generally called 'activators'. 'Activator' should include all "chemical substances produced by organisms which exert specific functions in regard to correlation or differentiation"²².)

The 'hormone-like' role of activator in the cell-to-cell propagation of competence seems well established. Practically nothing is known, however, about the nature of the stimulus invoking the production of this substance. It was found that in a number of experimental systems, the prolongation of the induction period preceding the appearance of competent cells is caused by the apparent failure in the production of endogenous activator⁸.

Control by an extracellular activator is not restricted to the transformation system of *Pneumococcus*. A similar

induction system has been discovered independently by Pakula and Walczak²³ among some transformable strains of *Streptococcus*, and we found evidence for the existence of an activator of the pneumococcal type in a streptococcus strain¹² which is closely related to *Pneumococcus* (*Streptococcus viridans* D used in the work of Bracco *et al.*²⁴). More recently it was reported that the competence of a transformable *Bacillus cereus* strain also is regulated by an activator substance²⁵. Possibly, other transformation systems will be found to have similar cellular regulatory mechanisms.

At present, however, there seem to be only a few other well-established examples of such activator-mediated 'cell communications' among micro-organisms, and the control of competence in *Pneumococcus* and *Streptococcus* seem to be the first examples among bacteria in which such a mechanism can be clearly outlined.

Perhaps the most clearly understood other case is the chemotactic movement of some myxomycetes during their morphogenetic aggregation^{26,27}. A somewhat similar effect has been described in the induction of sporulation of vegetatively growing *Bacillus subtilis* by extracts prepared from sporulating cells²⁸. It may be worth while, however, to suggest the possibility that activators may also participate in the regulation of some bacterial 'phenotypic variations', a number of which have already been listed. Judging from the homogeneity of the competence property which the activator produces as well as from the extremely fast rate of the activation process, such activator mechanisms could greatly improve the uniformity and efficiency of cellular responses to some environmental change and in this way improve the adaptive potential of populations.

Another phenomenon which may perhaps be interpreted within the framework of such an activator mechanism is the loss of virulence of group-A streptococci during growth under reducing conditions. The value of this physiological change for the organism is not clear, but the mechanism of this effect may be taken as an example of how a rapid change in a property of a bacterial population may be coupled to an environmental change. Elliott has shown²⁹ that, in this case, the loss of virulence is associated with the loss of the antigenic M-protein from the cell surface as a result of the action of an extracellular protease. This enzyme is excreted by the cells in an inactive zymogen form and becomes activated by the reducing conditions produced in the anaerobic environment.

Clearly, an enzyme could be an ideal activator for the efficient regulation of a property in a cell population, and it may be interesting to consider the role of some bacterial exoenzymes as instruments in the regulation of some as yet unrecognized physiological states. If this were valid, then it is likely that the targets of such exoenzymes or other specific extracellular cell products would be primarily surface components of the bacteria. Interactions with bacterial surface structures can have extremely specific and far-reaching intracellular consequences, as is known from investigations of the mechanism of action of colicins³⁰ and adsorbed bacteriophage ghosts³¹.

Interactions between groups of cells through specific cell-products is, of course, quite common among higher organisms on all levels of cell associations and even in ecological relationships³². There are numerous examples of hormonal control in morphogenesis and differentiation, as well as in the 'integration of individuals of a population' through specific extracellular chemicals³³. The results discussed in this article suggest that the capacity for such a population-level control may already exist among micro-organisms. In addition to the highly efficient enzymatic control mechanisms (such as end-product control or repression), which operate in 'individual' cells of bacteria, 'populations' of these organisms may exhibit supra-cellular regulatory mechanisms also, which can efficiently control a physiological property in a large number of cells. At present, it is not known whether the pneumococcal

activator participates in the regulation of gene activity or operates entirely on the physiological level.

The fact that the transformable state in *Pneumococcus* is regulated by a specific cell product may be interpreted as emphasizing the evolutionary importance of transformation for *Pneumococcus*. This investigation was aided by a grant from the National Science Foundation (GB-2083) to Dr. R. D. Hotchkiss.

- ¹ Schaeffer, P., in *The Bacteria*, edit. by Gunsalus, I. C., and Stanier, R. Y., 5, 87 (Academic Press, New York, 1964).
- ² McCarty, M., Taylor, H. E., and Avery, O. T., *Cold Spring Harbor Symp. Quant. Biol.*, 11, 177 (1946).
- ³ Alexander, H. E., in discussion under Zinder, N., *Cold Spring Harbor Symp. Quant. Biol.*, 18, 269 (1953).
- ⁴ Neester, E. W., *J. Bact.*, 87, 887 (1964).
- ⁵ Tomasz, A., and Hotchkiss, R. D., *Proc. U.S. Nat. Acad. Sci.*, 51, 480 (1964).
- ⁶ Hotchkiss, R. D., *Proc. U.S. Nat. Acad. Sci.*, 40, 49 (1954).
- ⁷ Tomasz, A., abstr. *Meeting of the Amer. Soc. Bacteriol.*, Washington (1964); *Bact. Proc.*, 29 (1964).
- ⁸ Tomasz, A. (in preparation).
- ⁹ Tomasz, A., abstr. *Fourth Meeting of the Amer. Soc. Cell Biol.*, Cleveland (September, 1964); *J. Cell Biol.*, 23, 97a (1964).
- ¹⁰ Ottolenghi, E., and Hotchkiss, R. D., *J. Exp. Med.*, 116, 491 (1962).
- ¹¹ Tomasz, A., and Belser, S. (submitted for publication in *J. Bact.*)
- ¹² Tomasz, A. (in preparation).

- ¹³ Lacey, B. W., *J. Hyg., Camb.*, 58, 57 (1960).
- ¹⁴ Duguld, J. P., and Wilkinson, J. F., in *Microbial Reaction to Environment, Eleventh Symp. Soc. Gen. Microbiol.*, 69 (1961).
- ¹⁵ Socolofsky, M. D., and Weiss, D., *J. Bact.*, 81, 946 (1961).
- ¹⁶ Ensign, J. C., and Wolfe, R. S., *J. Bact.*, 87, 924 (1964).
- ¹⁷ Murrell, W. G., in *Microbial Reaction to Environment, Eleventh Symp. Soc. Gen. Microbiol.*, 100 (1961).
- ¹⁸ Elliott, S. D., *J. Exp. Med.*, 81, 573 (1945).
- ¹⁹ Mach, B., and Tatum, E. L., *Proc. U.S. Nat. Acad. Sci.*, 52, 876 (1964).
- ²⁰ Bernlohr, R. W., and Nowell, G. D., *Arch. Biochem. Biophys.*, 103, 94 (1964).
- ²¹ Borowska, Z. K., and Tatum, E. L., abstr. *Sixth Intern. Cong. Biochem.*, New York, 441 (1964).
- ²² Huxley, J., *Biol. Revs.*, 10, 427 (1935).
- ²³ Pakula, R., and Walczak, W., *J. Gen. Microbiol.*, 31, 125 (1963).
- ²⁴ Bracco, R. M., Krauss, M. E., Roe, A. S., and McLeod, C. M., *J. Exp. Med.*, 106, 247 (1957).
- ²⁵ Felkner, I. C., and Wyss, C., *Biochem. Biophys. Res. Comm.*, 16, 94 (1964).
- ²⁶ Sussman, M., *Ann. Rev. Microbiol.*, 10, 21 (1956).
- ²⁷ Bonner, J. T., in *Developing Cell Systems and their Control, Twenty-eighth Symp. Soc. Study Development and Growth*, 3 (1960).
- ²⁸ Kerauala, Z. J., Srinivasan, V. R., and Halvorson, N. O., *J. Bact.*, 88, 374 (1964).
- ²⁹ Liu, T. Y., and Elliott, S. D., *Nature*, 206, 33 (1965).
- ³⁰ Nomura, M., *Proc. U.S. Nat. Acad. Sci.*, 52, 1514 (1964).
- ³¹ Sher, I. H., and Mallette, I. F., *Arch. Biochem. Biophys.*, 52, 331 (1964).
- ³² Parks, A. S., and Bruce, H. M., *Science*, 134, 1094 (1961).
- ³³ Lucas, C. E., *Biol. Revs.*, 32, 270 (1947).

RATE OF CHROMOSOME DUPLICATION AT THE END OF THE DEOXYRIBONUCLEIC ACID SYNTHETIC PERIOD IN HUMAN BLOOD CELLS

By DR. C. W. GILBERT, S. MULDAL and DR. L. G. LAJTHA

Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester

IN an earlier publication we considered the pattern of chromosome duplication at the end of the DNA synthesis period (*S* period) in human blood cells¹. One of the notable features was a difference in the rate of DNA synthesis between certain chromosomes, particularly so in one of the X-chromosomes of females, the 'hot' X-chromosome. Because of the intensive labelling of this chromosome at a time when most other chromosomes were unlabelled or only weakly labelled, the 'hot' X has been considered as being delayed in its duplication¹⁻⁴. This article analyses in more detail the labelling behaviour of the autosomes as compared with the 'hot' X-chromosome at the end of the *S* period.

Cultures of female peripheral leucocytes were prepared by the usual method⁵ with the difference that red cell agglutination was carried out by letting the sample stand (1 h) at room temperature and using phytohaemagglutinin *M* (Difco) only as a mitotic stimulant.

Labelling was carried out at minus 3 h with 0.2 μ Ci/ml. medium of tritiated thymidine (Radiochemical Centre, Amersham, spec. act. 12.3 mCi/ μ M). 'Colcemid' (Ciba, 0.04 ml./ml. medium) was added 1.5 h before termination of the cultures. Slides were prepared for autoradiography with Kodak 'AR 10' stripping film and exposed for 8 days.

A random sample of 224 cells in metaphase was selected for analysis. To ensure a proper random sample, cells in metaphase were rejected only for one or more of the following reasons: (a) an incomplete set of chromosomes; (b) an excessive overlap of any chromosomes that prevented them from being individually identified; (c) the cell crossed by a prominent 'background streak' of grains. Except for the criterion (c) the autoradiographic grains were ignored during selection.

After the selection of the cells had been made the autoradiographic grains over the whole sample were counted by two observers. For each cell the grains were put into three mutually exclusive classes: (a) *background grains*, grains which were not over any chromosome but within the boundaries of the metaphase plate; (b) *'hot' X grains*,

grains over the most heavily labelled chromosome in Group III (Denver classification); (c) *autosome grains*, all the other grains, that is, grains over all chromosomes except the 'hottest' in Group III.

A grain was taken to be over a chromosome if it was within a distance of one chromatid width. For the sake of brevity 'autosomes' will include the 'cold' X chromosome.

The results are shown in Table 1 and Figs. 1-3. For the purpose of analysis two distributions are shown: (a) cumulative distribution of the total grains over the cell, excluding the 'hot' X, that is, the sum of classes (a) and (c) (Table 1 and Figs. 1 and 2); (b) cumulative distribution of the grains over the 'hottest' chromosomes in Group III, that is, class (b) (Figs. 1 and 2).

(1) *Unlabelled cells*. From Table 1 we can infer that between 160 and 165 cells were unlabelled, and that the grains observed over these cells arise from a random distribution of background. Table 1 shows a distinct change of pattern for cells with 12 or more grains. These cells show labelling over the 'hot' X, which is markedly higher than for cells with less than 12 grains, indicating positive labelling. The background is about 2 grains per cell,

Table 1. GRAIN COUNT DATA OF THE 224 CELLS

Grains over autosomes + background	Cumulative No. of cells	Autosomes + background	Background	'Hot' X
0	3	0	0	0.3
1	14	1	0.6	0.7
2	41	2	1.1	0.9
3	65	3	1.5	0.9
4	94	4	2.2	0.9
5	113	5	2.2	1.3
6	129	6	2.9	1.2
7	145	7	3.2	1.1
8	155	8	4.2	0.9
9	161	9	5.5	1.8
10	164	10	5.3	2.6
11	167	11	5.0	1.3
12-13	171	12-7	1.75	9.3
14	176	14	1.0	14.5
15-20	184	17-1	2.0	8.8
21-100	205	50.9	3.2	22.6
> 100	224	242.1	5.1	40.3
0-9	161	4.28	2.12	1.01

agreeing with a random background of 2.12, the mean for cells with 9 grains or less. That the majority of the 167 cells with 11 grains or less are unlabelled is indicated by the fact that the 'hottest' in Group III has only on average about one grain, and also that about half the grains over the cells are background. However, a few of these 167 cells will nevertheless have some real label as four of them had a Group III chromosome with 5 or more grains.

Taking therefore the 161 cells with 9 grains or less as representative of the background population, a detailed analysis was made to check that these grains were randomly distributed over the cell and over the chromosomes. The mean grains per cell for this population is 4.28 (Table 1) with a mean background of 2.12, giving therefore 2.16 over the autosomes. This latter, together with 1.01 grains per cell over the 'hottest' in Group III, makes a total of 3.17 grains per cell over all the chromosomes. Since Group III contains 37.8 per cent of the chromosome material, a random distribution of grains would lead to an expected mean over Group III of 1.20. Since even

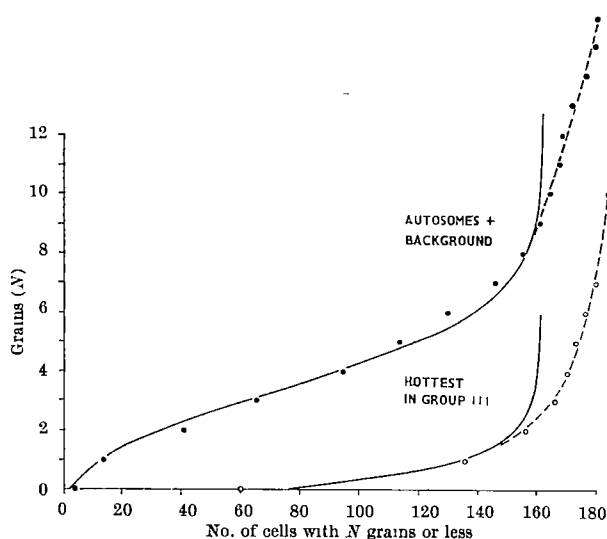


Fig. 1. Cumulative distributions of grains over the unlabelled cells. Solid lines show the Poisson distributions of 161 cells with mean grains of 4.28 and 0.72.

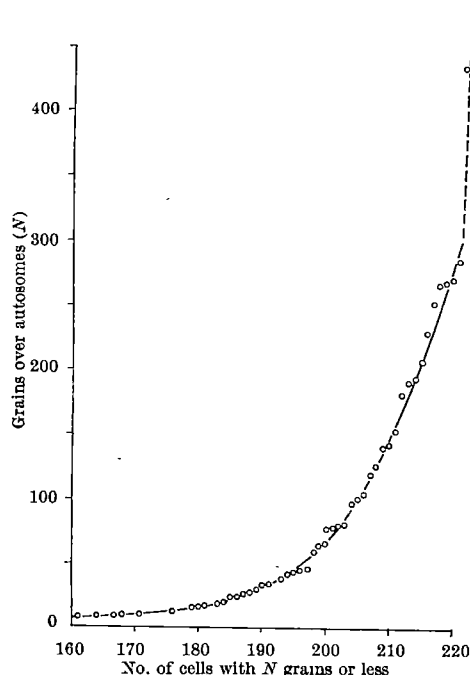


Fig. 2. Continuation of the cumulative distribution of the grains over the autosomes (and background) to include the labelled cells.

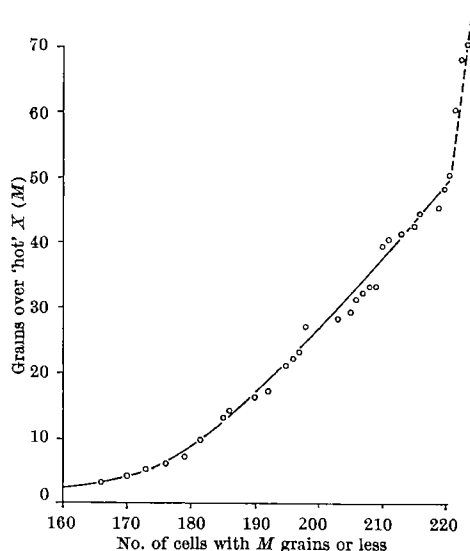


Fig. 3. Continuation of the cumulative distribution of grains over the 'hot' X to include the labelled cells.

with this low density, occasionally more than one chromosome in Group III will be labelled, the observed value of 1.01 over the 'hottest' is consistent with an expected value of 1.20 over the whole group. Furthermore, the distribution of grains among these cells agrees with a random distribution as is shown in Fig. 1, where the solid lines are the calculated Poisson distributions for a population of 161 unlabelled cells with a total mean count of 4.28 grains, and for a population of 161 Group III chromosomes with a mean grain count of 0.72. This latter is the mean of the smallest 161 counts over the 'hottest' chromosome in Group III; this distribution of cells being not necessarily identical with the tail of the autosome and background distribution.

(2) *Labelled cells.* From these arguments we conclude that the cumulative distributions shown in Figs. 2 and 3 are those for the labelled cells. These distributions can be interpreted by considering the consequences of the 'Colcemid' block. Without 'Colcemid', only cells which were 180 min from metaphase when the label was added will give metaphase plates. However, with 'Colcemid' added 90 min before fixation, all cells which were between 90 and 180 min before metaphase when the label was added will give metaphase plates. The proportion of these plates which show label will depend on where in the interval, 90–180 min, the DNA synthesis ends.

Since we have observed a cohort of 224 cells by the 90 min 'Colcemid' block, and since we can also assume these to be distributed uniformly in time, then these cells were coming into metaphase at $224/90 = 2.49$ cells per min. Thus, a time-scale can be put on the cumulative distributions of Figs. 2 and 3, the abscissa now becoming a linear time-scale going backwards into the *S* period. The ordinate will be proportional to the total DNA synthesis still to be completed, since the tritium label is continuously available during this incubation period, and when once incorporated in the chromosome is permanent, at least until the next cell division.

The tangents to the cumulative distribution curves, therefore, give a measure of the rate of DNA synthesis at the corresponding times. In this way the rate of DNA synthesis at the later part of the *S* period can be calculated for the 'hot' X and for the autosomes, and the results are shown in Fig. 4. Since the cumulative distributions give the total grains over the 'hot' X and over all the autosomes, the rate of increase of these grains has been divided by the length of the X chromosome, or by the total length of the autosomes, to get a specific rate of DNA synthesis, that is, a mean rate of synthesis per unit length of chromosome.

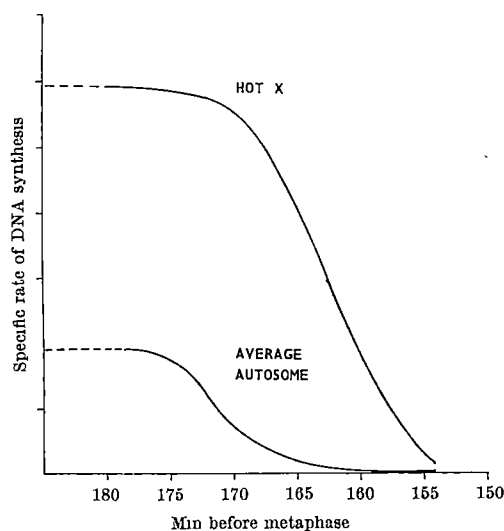


Fig. 4. The specific rate of DNA synthesis at the end of the S period

Individual cells will show a variation about the mean values of Fig. 4. One effect of this variation is to give the sharp upward tilt to the ends of curves of Figs. 2 and 3. This is due to a few cells being farther back in time in the S period than the average cell at 180 min before metaphase. From these few cells we can make a rough estimate of the average value of this time, by taking the time between the extrapolated curve and the actual curve at a grain count equal to the mean for these cells. This time corresponds to about 5–6 min for both the 'hot' X and for the autosomes.

Since the two cumulative distributions for the 'hot' X and the autosomes are independent of each other, we can examine where each individual cell occurs in the two distributions. There is a good degree of correlation and this can be expressed as the mean ranking difference of a cell in the two distributions and was calculated to be 10.8 cells. Since the cohort is progressing at 2.49 cells/min, this corresponds to $10.8/2.49 = 4.4$ min internal timing variation between the state of the 'hot' X and of the autosome DNA synthesis within a single cell.

The results obtained in these experiments with continuous labelling by tritiated thymidine indicate that the rate of DNA synthesis is constant towards the end of the

S period and then falls off sharply during the last 20 min. Our observations were limited to the last 25–30 min, therefore we cannot say how far this constant rate extends back into the S period.

Several publications have commented on the 'lateness' in labelling of the 'hot' X chromosome^{1–4,6}. This was shown by an increasing proportion of the grains located over the 'hot' X as the total grain count over the cell decreases^{1,2,6}.

The results recorded here offer a quantitative explanation of this phenomenon. The specific rate of DNA synthesis is about three times higher in the 'hot' X than in the average autosome (during the last constant part of the rates). This constant rate is maintained somewhat longer in the 'hot' X than in the average autosomes (by about 5 min). The rate in the 'hot' X then falls sharply so that both the 'hot' X and the average autosomes finally finish at about the same time, in this culture about 155 min before metaphase.

Although the two rates of synthesis end together, the ratio of the areas under the two curves in Fig. 4 is about 6, corresponding to the specific grain density of 6 for the 'hot' X reported earlier^{1,6}.

In order to single out the 'hot' X, all other chromosomes have been grouped together under the term 'average autosome'. The known differences in grain density between the various autosomes and parts of autosomes indicate that any one segment of any chromosome may have its own 'end of S' pattern. Some of these may not differ greatly from the 'hot' X, others may be greatly displaced early in time. For these reasons the curve for the average autosome might well be expected to have a more gradual ending than that of the more homogeneous 'hot' X.

It would require a very considerable amount of grain counting to provide data for any one autosome, let alone for parts of autosomes; but with suitably longer exposure of the autoradiographs it is possible to determine similar patterns at least for some of the autosomes.

¹ Gilbert, C. W., Muldal, S., Lajtha, L. G., and Rowley, J., *Nature*, **195**, 869 (1962).

² Rowley, J., Muldal, S., Gilbert, C. W., Lajtha, L. G., Lindsten, J., Fraccaro, M., and Kausser, K., *Nature*, **197**, 251 (1963).

³ German, J. L., *Trans. N.Y. Acad. Sci.*, **24**, 395 (1962).

⁴ Morishima, A., Grumbach, H. M., and Taylor, J. H., *Proc. U.S. Nat. Acad. Sci.*, **48**, 758 (1962).

⁵ Moorhead, P. S., Nowell, P. C., Mellman, W. J., Batipps, D. M., and Hungerford, D. A., *Exp. Cell Res.*, **20**, 613 (1960).

⁶ Muldal, S., Gilbert, C. W., Lajtha, L. G., Lindsten, J., Rowley, J., and Fraccaro, M., *Lancet*, **i**, 361 (1963).

SHADE TOLERANCE IN FLOWERING PLANTS

By DR. J. P. GRIME

Department of Soils and Climatology, Connecticut Agricultural Experimental Station, New Haven, Connecticut

SHADE tolerance and shade avoidance. A distinction has been drawn^{1,2} between species with seedlings which are able to penetrate rapidly through grassland shade (shade-avoiding plants) and species which are capable of surviving long periods in the dim light of the forest floor (shade-tolerant plants). Certain characteristics which facilitate rapid emergence from grassland shade have been recognized³. In this article, experimental results suggesting a physiological basis for shade tolerance are summarized.

Fungal attack and shade fatalities. Failure of seedlings in shade is almost invariably associated with fungal attack^{2–7} and often the effect of shading is to increase infection^{2,8–11}. Experimental evidence with tree seedlings² suggests that in comparison with tolerant species, shade-intolerant plants are more susceptible to infection both above and below the compensation point.

Predisposition to fungal attack. Correlations have been established between decreasing sugar content and increased susceptibility in shaded foliage^{11–13} and a causal role in resistance to infection has been ascribed to sugar content¹⁴. Consistent with this theory is a recent observation that the longevity of tree seedlings in deep shade is closely related to the quantity of material stored in the seed (Table 1).

The carbohydrate economy of the shade-tolerant seedling is therefore of interest for two reasons. If high levels of sugars are maintained in shade, continued growth may be possible; in addition we may suspect that fungal attack will be resisted.

Morphogenetic adaptation to shade. Morphogenetic changes induced by shading include increased leaf area, reduced leaf thickness and laminae inclined at right-angles to the light source^{2,15–17}. These changes appear

Table 1. NUMBER OF FATALITIES RECORDED IN SEEDLINGS OF EIGHT NORTH AMERICAN TREE SPECIES GROWN IN DEEP SHADE

	Seed reserve (mg dry wt.)	No. of fatalities
<i>Betula populifolia</i>	0.1	4.6
<i>Betula lenta</i>	0.7	5.4
<i>Rhus glabra</i>	1.5	4.8
<i>Acer rubrum</i>	7.3	3.2
<i>Larodendron tulipifera</i>	8.5	3.4
<i>Pinus strobus</i>	14.8	3.6
<i>Gleditsia triacanthos</i>	40.3	1.8
<i>Quercus rubra</i>	1,969.2	1.4

Each value tabulated is the mean number of fatalities per container over a period of twelve weeks. Initially, a single seedling was sown into each of five containers. Casualties were replaced by freshly germinated seedlings throughout the rest of the experimental period.

to be advantageous in that they increase the amount of energy intercepted by the shoot. They are not confined to shade-tolerant species¹⁸, however, although in some investigations more effective responses to shading have been observed in shade-tolerant plants¹⁷.

Metabolic adaptation to shade. Many shade-tolerant genera, for example, *Pachysandra* and *Hedera*, have thick leaves and leaf arrangements which permit considerable self-shading. Clearly this indicates that the carbohydrate solvency of shade plants may depend on conservation of energy rather than the efficiency of its capture.

Initial study of the metabolism of shade-tolerant plants has been carried out in Connecticut by measuring: (a) relative growth-rate of small plants of tolerant and intolerant species growing under full sunlight; (b) respiration in the dark in leaves of species differing widely in shade tolerance.

(a) Under the conditions of the experiment (Table 2) all the shade-tolerant species examined grew extremely slowly in comparison with the species intolerant of shade, crop plants and various weeds.

(b) In shade-tolerant species respiration rates of leaf disks in darkness were consistently lower than those measured in species from open habitats (Table 3).

From these data it would appear that shade plants have slow metabolic rates. It is not clear whether these are imposed biochemically or arise through reduced permeability of the leaf. As suggested by D. N. Moss, dark respiration may be inhibited by accumulated carbon

Table 2. RELATIVE GROWTH-RATE (MG/G/H) OF SHADE-TOLERANT SPECIES. CROP PLANTS AND SPECIES FROM UNSHADED HABITATS

Shade-tolerant species		
<i>Tsuga canadensis</i>	1.79	<i>Acer saccharum</i> 0.99
<i>Castanea mollissima</i>	1.41	<i>Pachysandra</i> spp. 0.92
<i>Quercus rubra</i>	1.66	<i>Deschampsia flexuosa</i> 0.57
Shade-intolerant trees		
<i>Ailanthus altissima</i>	3.32	<i>Rhus glabra</i> 2.97
<i>Betula populifolia</i>	9.24	<i>Fraxinus americana</i> 3.44
<i>Betula alleghaniensis</i>	5.02	<i>Paulownia tomentosa</i> 8.02
<i>Betula lenta</i>	4.75	
Crop plants		
Sunflower	6.53	Tomato 12.55
Cucumber	7.45	Sugar beet 11.64
Tobacco	31.39	Kentucky bluegrass 13.20
Dallis grass	16.71	
Arable weeds		
<i>Ambrosia artemisiifolia</i>	15.46	<i>Portulaca oleracea</i> 12.43
<i>Polygonum persicaria</i>	10.19	<i>Rumex acetosella</i> 10.19

Each value tabulated is the mean of measurements taken with five replicates, over three consecutive five-day periods in June, 1964, New Haven, Conn. Plants grown on Arnon solution in sand culture in sunny weather.

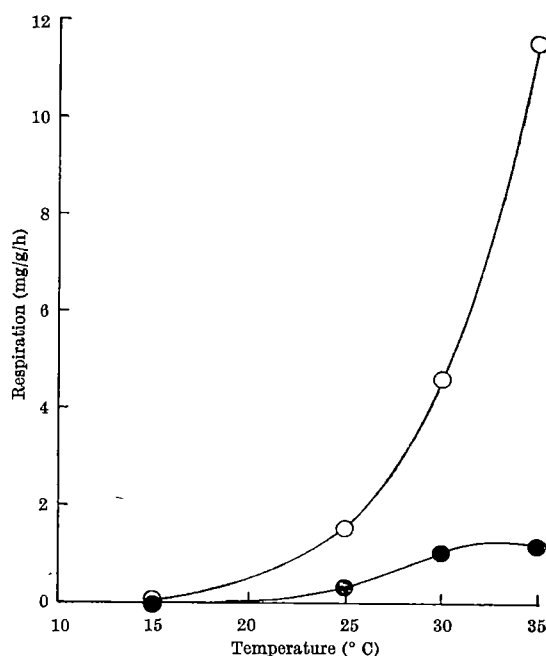
Table 3. DARK RESPIRATION (MG/G DRY WT. LAMINA/H) AT 25° C IN LEAF DISKS OF SHADE-TOLERANT SPECIES AND A VARIETY OF PLANTS FROM UNSHADED HABITATS

Shade-tolerant species		
<i>Tsuga canadensis</i>	2.19	<i>Acer saccharum</i> 1.80
<i>Castanea mollissima</i>	1.20	<i>Acer rubrum</i> 2.12
<i>Quercus rubra</i>	1.76	<i>Fagus grandifolia</i> 2.58
<i>Taxus brevifolia</i>	1.17	<i>Pachysandra</i> spp. 2.14
<i>Hedera helix</i>	2.36	<i>Viola</i> spp. 1.41
Shade-intolerant trees		
<i>Larodendron tulipifera</i>	3.89	<i>Populus tremuloides</i> 4.17
<i>Juglans nigra</i>	2.70	<i>Betula lenta</i> 3.62
<i>Paulownia tomentosa</i>	5.13	<i>Rhus copallina</i> 6.62
<i>Prunus serotina</i>	4.80	
Crop plants		
Tobacco	9.00	Arable weeds
Sunflower	3.51	<i>Rumex acetosella</i> 7.26
Bushbean	3.01	<i>Atrplex</i> spp. 3.33
		<i>Amaranthus retroflexus</i> 5.37

Respiration was measured as the loss in dry weight of disks from young fully expanded leaves floated on water for 12 h. Leaf samples were freshly collected from trees growing in unshaded habitats or from seedlings grown in sand culture in a greenhouse.

dioxide within the leaf and photosynthesis by slow inward diffusion of carbon dioxide. As demonstrated by Heath¹⁹, respiration may be inhibited by depletion of oxygen in leaves with tightly closed stomata. However, the alternative of control of photosynthetic and respiratory rates through common or linked slow biochemical processes is suggested by data, such as those of Thomas and Hill²⁰, which demonstrate a close correlation between rates of photosynthesis and respiration in species with high gas-exchange capacity.

Recently high-temperature optima have been observed for photosynthesis in certain sun plants^{21,22}. A high-temperature optimum for some fraction of respiration may be concomitant with this adaptation. Accordingly, in seedlings of *Paulownia tomentosa*, a shade-intolerant tree with an exceptionally high relative growth-rate in full sunlight (Table 2), leaf respiration was accelerated to a high rate over the temperature range 25°–35° C (Fig. 1). Values for a tolerant species of *Pachysandra* were comparatively low.

Fig. 1. The effect of temperature on respiration of leaf disks in darkness in *Pachysandra* (shade tolerant, ●) and *Paulownia* (intolerant, ○)

This investigation was carried out using plants grown in full sunlight. Provided the differences between tolerant and intolerant species, which have been observed in respiration rate, obtain in shade-grown plants, we may assume that depletion of carbohydrate below the compensation point will be more rapid in intolerant species. Furthermore, where intolerant species have a high Q_{10} for respiration, their compensation point will be most sensitive to changes in temperature. This was apparent in an experiment with *Paulownia*, in which a rise in temperature from 25° to 35° C caused a large depression in net photosynthesis at low light intensities (Fig. 2) and an elevation of light intensity compensation point from 300 to 1,200 ft. candles.

The contrast between tolerant and intolerant species in relative growth-rate and leaf respiration rate supports the suggestion of Went²³ that an important adaptation of shade plants has arisen through selection for slow respiration in shade with concomitant selection for slow photosynthetic rates and hence slow growth-rates in all environments. In accordance with this, photosynthetic rates of intolerant species exceed those measured in tolerant plants even when the comparison is made at low light intensities^{17,24–26}. Apparently, these high rates of photosynthesis do not confer shade tolerance. This is

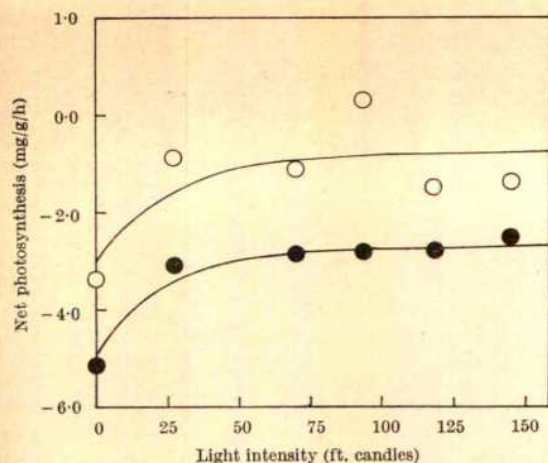


Fig. 2. Net photosynthetic rate in leaf disks of *Paulownia tomentosa* incubated in a range of low light intensities at 25°C (○) and 35°C (●).

probably because, in intolerant species, they are offset by high rates of respiration and by a rapid and continuous conversion of photosynthate into plant structure.

In a wider context, it has been proposed²⁷ that the plant characteristics which lead to the failure of a genotype or species in one environment may be an indirect consequence of adaptations necessary for survival in another. Hence high respiration rate, a liability in shaded habitats, is a feature of the high metabolic rate necessary in colonization of unshaded productive environments such as aban-

doned arable fields. The distinction between tolerant and intolerant plants may, therefore, arise through a physiological dilemma; adaptation for high productivity under full sunlight involves the assumption of characteristics limiting in shade and vice versa.

- ¹ Boysen, Jensen P., *Dansk. Skovfor. Tidsskr.*, **14**, 5 (1929).
- ² Grime, J. P., and Jeffrey, L. W., *J. Ecol.*, **53**, 621 (1965).
- ³ Vaartaja, O., *Phytopath.*, **42**, 501 (1952).
- ⁴ Vaartaja, O., *Canad. Dept. Agric. Forest. Biol. Div. Bi-monthly Prog. Rep.*, **9**, 3 (1953).
- ⁵ Vaartaja, O., and Cran, H. W., *Phytopath.*, **46**, 391 (1956).
- ⁶ Vaartaja, O., *Ecology*, **43**, 47 (1962).
- ⁷ Wardle, P., *J. Ecol.*, **47**, 483 (1959).
- ⁸ Brooks, F. T., *Ann. Bot.*, **22**, 479 (1908).
- ⁹ Bewley, W. F., *Diseases of Glasshouse Plants* (Benn, London, 1923).
- ¹⁰ Foster, R. E., and Walker, J. C., *J. Agric. Res.*, **74**, 165 (1947).
- ¹¹ Read, D. J., Ph.D. thesis, Univ. Hull (1963).
- ¹² Baker, K. F., *Plant Dis. Report*, **30**, 145 (1946).
- ¹³ Sucharukov, K. T., *Proc. Second Intern. Conf. Plant Protect.*, Fernhurst Research Sta., England, **42** (1957).
- ¹⁴ Horsfall, J. G., and Dimond, A. E., *Z. Pflanzenkrankh. und Pflanzenschutz* (1957).
- ¹⁵ Blackman, G. E., and Rutter, A. J., *Ann. Bot.*, N.S., **12**, 1 (1948).
- ¹⁶ Blackman, G. E., and Wilson, G. L., *Ann. Bot.*, N.S., **15**, 63 (1951).
- ¹⁷ Bourdeau, R. F., and Laverick, M. L., *Forest Sci.*, **4**, 196 (1958).
- ¹⁸ Blackman, G. E., and Black, J. N., *Ann. Bot.*, N.S., **23**, 51 (1959).
- ¹⁹ Heath, O. V. S., *J. Exp. Bot.*, **1**, 29 (1950).
- ²⁰ Thomas, N. D., and Hill, G. R., in *Photosynthesis in Plants*, edit. by Franck, J., and Loomis, W. E. (Iowa State Univ. Press, Ames, Iowa, 1949).
- ²¹ Murata, Y., and Iyama, J., *Proc. Crop. Sci. Soc., Japan*, **31**, 315 (1963).
- ²² Moss, D. N., *Crop Sci.* (in the press).
- ²³ Went, F. W., *The Experimental Control of Plant Growth* (Chron. Bot. **17**, 343, 1957).
- ²⁴ Bohning, R. H., and Burnside, C. A., *Ann. Bot.*, **43**, 557 (1956).
- ²⁵ Hesketh, J. D., and Moss, D. N., *Crop Sci.*, **3**, 107 (1963).
- ²⁶ Hesketh, J. D., *Crop Sci.*, **3**, 493 (1963).
- ²⁷ Grime, J. P., *Ecology*, **46**, 513 (1965).

THE CONCEPT OF THE 'CENTRON'

By DR. D. W. MENZIES

Department of Pathology, University of Melbourne, Australia

THIS communication proposes a structural concept which has been found useful in accounting for the behaviour of cortical follicles during antigen stimulation of rat lymph node. Such changes can be explained by supposing that the follicular lymphoid cells are grouped in relation to a pre-existent spherical stromal structure. This stromal 'centron' is considered to be constant, though its contained lymphoid population is very variable.

The case for the existence of the centron depends on consideration of the following: (a) visible changes in follicular lymphoid cells under antigen stimulation; (b) visible changes in cortical macrophage processes under antigen stimulation; (c) flow patterns of afferent lymph-borne carbon and flagellar antigen in the lymphoid cortex.

Visible changes in lymphoid cortical follicles under antigen stimulation. Within the cortex of the rat lymph node can be found a series of follicles of variable cell population, depending on the age of the animal. These age changes in follicular cytology appear to be due to the accumulation of natural antigenic stimuli, as they can be reproduced by appropriate experimental lymph-borne antigen.

In young rats (70–100 g) the follicular system is represented by a series of surface mounds. Beneath each mound can be seen in the cortex a condensation of small lymphocytes, showing in many cases the beginning of a circular edge (Fig. 1). These small lymphocyte condensations are difficult to observe in ordinary paraffin sections, as they differ from the rest of the cortical lymphocytes only in the tightness of their packing. They are best seen in unfixed frozen sections cut from a node placed in a gutter in a block of liver or other supporting material.

In older rats, these follicles are more easily defined because they differentiate into a peripheral rim of closely adherent small lymphocytes, and a central area which contains numerous primitive blast cells with prominent

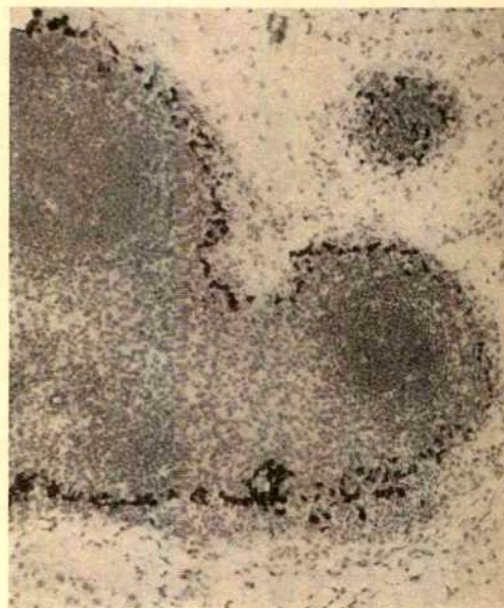


Fig. 1. Lymph node cortex from 100 g rat, with subcapsular sinus macrophages marked by afferent lymph-borne carbon. Note (a) the surface mounds, (b) the spherical masses of packed lymphocytes lying below. H. and E. 5μ frozen section. (× c. 112)

nucleoli. However, this blast cell concentration can be more controllably reproduced by experimental antigen stimulation of the 70–100-g rat (0.1 c.c. TAB vaccine, Commonwealth Serum Laboratories, into the right hind footpad twice a day for 5 days).

By the fifth day of this 'mass antigen' course, examination of the right para-aortic lymph node will show in the outer cortex a series of blast cell masses, forming incomplete circles, with the outer part of their circumference obscured by packed small lymphocytes extending to the subcapsular sinus (Fig. 2). If placed more deeply, or perhaps cut obliquely, these follicles are no longer obscured by lymphocytes, and appear as a full circle (Fig. 2). In this case there is a sharp contrast between the packed blast cells of the follicle and the loose lymphocytes of the cortex outside. The smooth and well-defined edge of the blast cell mass is very obvious (Fig. 2).

Observations of serial sections of many lymph nodes under mass antigen stimulation suggest that these blast cell circles correspond to sections of a sphere 200–250 μ in diameter. Their constancy of shape and size, and the sharpness of their edge, are contrary to the idea that they are random aggregations. Instead, the appearances suggest that the blast cells fill pre-formed cortical stromal spheres, thus making them visible.

Visible changes in cortical macrophage processes under antigen stimulation. Pioneer researches of Bunting¹ and

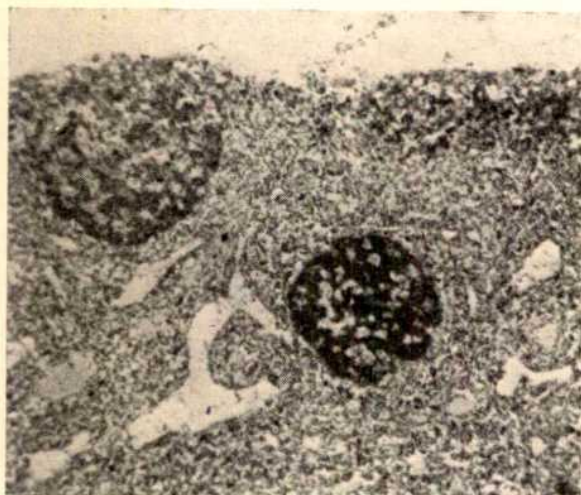


Fig. 2. Lymph node cortex after 5 days 'mass antigen' (see text), stained by a nucleic acid method (ref. 4) showing blast cells as dark masses. Two blast cell follicles are shown. On the left, one follicle is partly obscured on its outer aspect by packed lymphocytes. On the right, another follicle is seen with a fully circular shape. Note the sharpness and smoothness of the edge of this blast cell mass. Basic fuchsin-azure B, 3 μ paraffin section. ($\times 75$)

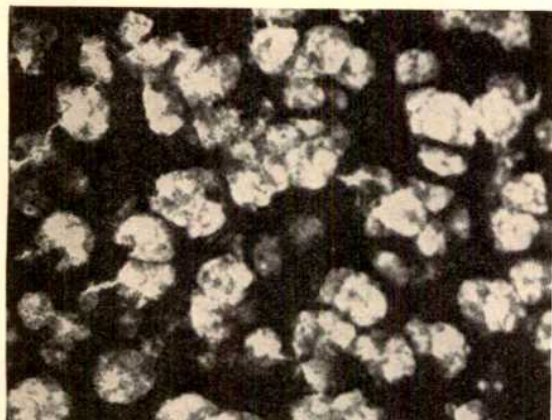


Fig. 3. Macrophages in the lymph node cortex, the processes of which have been outlined by adherent carbon, as described in the text. The ribbon-like processes broaden into macrophage bodies containing indistinctly visible nuclei. 10 μ frozen section. ($\times 900$)



Fig. 4. Follicular mound (see left) from 100 g rat. Adherent carbon shows the general uniformity of the dendritic macrophage pattern, with no sign of follicular organization. 10 μ frozen section. ($\times c. 170$)

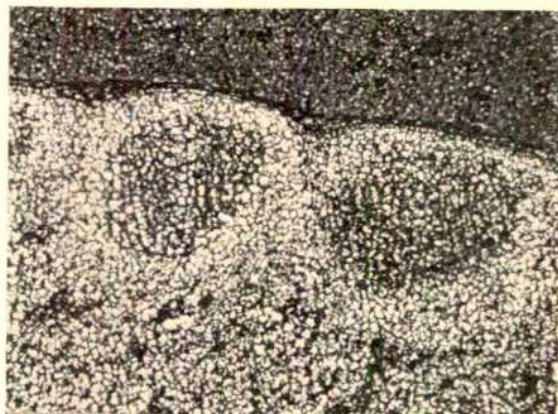


Fig. 5. Adherent carbon preparation of rat lymph node cortex one week after cessation of 'mass antigen'. Follicular areas of thickened dendritic macrophage processes are now easily seen. 10 μ frozen section. ($\times 75$)

others at the turn of the century have recently been reinforced by modern electron microscopy^{2,6} and the skeleton of the lymph node shown to be a protoplasmic pseudo-synctium, made up of the interlacing membranous processes of dendritic macrophages, with fibres running in extra-cytoplasmic tunnels and providing occasional reinforcement.

These stromal macrophage extensions can be outlined by the application of colloidal carbon. 10 μ frozen unfixed sections of lymph node, dried on the slide at room temperature for 6–8 h, are flooded with 'Pelikan' indian ink (Günther Wagner C11/1431a), for 10 sec and washed with a 4 per cent aqueous dilution of the same ink. This dilution is finally used as a mounting medium under a coverslip.

The carbon adheres selectively to the bodies and processes of macrophages, leaving lymphoid cells almost completely unmarked (Fig. 3). Observation has to be speedy, as the carbon particles dislocate from the macrophages after 15–20 min. Washing the preparation with water, as in any attempt to counterstain, results in the same dislocation. Drying may have the same effect. Before or after carbon adhesion, fixation by such agents as alcohol, formalin, or mercuric chloride abolishes the specific localization.

The method shows lymph node stroma as a network of narrow ribbons which broaden out at intervals into macrophage bodies with recognizable, but indistinctly visible, negatively staining nuclei (Fig. 3).

In the 80–100-g rat, the follicular mounds exhibit no distinctive stromal morphology in the sites where primary follicular lymphocyte condensations could normally be

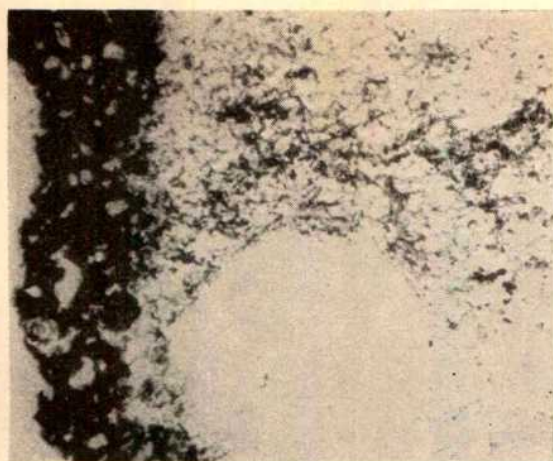


Fig. 6. Rat lymph node cortex, 2.5 h after carbon injection of corresponding footpad. Note how the carbon has penetrated the inner wall of the heavily marked subcapsular sinus (see left) and is spreading through the cortex along the arms of dendritic macrophages. Carbon does not penetrate into the circularly outlined primary follicle. 10μ frozen section. ($\times c. 170$)

expected (Fig. 4). Even under mass antigen stimulation, there is no stromal counterpart of the blast cell circles which appear so early and consistently under these conditions. About the fifth and final day of the antigen course, and especially during the week following, follicular organization does appear, in the form of circular clusters of thickened macrophage processes (Fig. 5). It must be emphasized that this is a late event, only produced by intense and prolonged antigen stimulation.

Flow patterns of afferent lymph-borne carbon and flagellar antigen in the lymphoid cortex. Stromal follicles can thus be produced by antigen, but the evidence so far does not prove whether such follicles constitute a pre-formed structure in the unstimulated cortex. Studies of the early localization of lymph-borne carbon and flagellar antigen do, however, provide pointers in this direction.

0.2 c.c. 'Pelikan' indian ink is injected into the right hind footpad of the 80–100 g rat which has received no previous antigen. Two and a half hours later, the right para-aortic node is cut into frozen sections. These show the whole sinus system of the node outlined in black, and some direct penetration of the cortex by the carbon through the inner wall of the subcapsular sinus. Both these observations agree with Drinker *et al.*³

As the carbon penetrates the outer cortex, it outlines the processes of dendritic macrophages. It also negatively outlines circular follicular areas beneath the subcapsular

sinus (Fig. 6). Nuclear counterstaining is possible, as carbon, adherent to dendritic macrophages *in vivo*, resists subsequent water dislocation in frozen sections. Such counterstaining shows that the area of minimum penetration corresponds with primary lymphocytic follicles.

Further information has been supplied by Nossal *et al.*⁶, who have recently investigated the cortical distribution of afferent lymph-borne flagellar antigen marked by radio-iodine. Within 24 h of footpad injection of primary antigen, the corresponding popliteal lymph node shows circular or ovoid areas of tagged protein, which in terms of their contained lymphoid population may be indistinguishable from the rest of the cortex. At a later stage, blast cells are seen to appear and become numerous in each protein area, a classical lymphoid germinal centre being the end product.

Radio-iodine does not give sufficiently sharp localization of the actual cells involved in this protein localization. However, fluorescent antigen marking⁵ has since shown that these cortical areas are composed of circular clusters of protein-marked dendritic macrophages identical in morphology to the antigen-induced follicles demonstrated by carbon adhesion, and presumably representing the same structure.

Conclusion. The combined weight of this evidence supports the idea of a pre-existent stromal macrophage centre, with a contained lymphoid population varying from small lymphocytes to blast cells according to the conditions of antigen stimulation. The problem is how to define this structure anatomically.

The difficulty is that the macrophages inside this structure do not appear to differ morphologically from the cortical macrophages outside, nor are they arranged in any constant or obvious way as a boundary structure.

Yet such a boundary seems very likely, whether in terms of the processes of macrophages, or in terms of some type of basement membrane. If such a barrier does exist, particles carried by lymph through the cortex³ would be deflected from the outside of the stromal sphere (short-term carbon) or concentrated in the inside (antigenic protein), depending on complex physical factors such as barrier porosity, speed of lymph flow, and size of the particle concerned.

¹ Bunting, T. L., *J. Anat., Lond.*, **39**, 55 (1905).

² Clark, S. L., *Amer. J. Anat.*, **110**, 217 (1962).

³ Drinker, C. K., Wislocki, G. B., and Field, M. E., *Anat. Rec.*, **56**, 261 (1933).

⁴ Menzies, D. W., *Stain Technol.*, **38**, 157 (1963).

⁵ Miller, J. J., III, and Nossal, G. J. V. (personal communication).

⁶ Moe, R. E., *Amer. J. Anat.*, **112**, 311 (1963).

⁷ Nossal, G. J. V., Ada, G. J., and Austin, C. M., *Nature*, **199**, 1257 (1963).

IMMUNOLOGY OF THE MOUSE MAMMARY TUMOUR VIRUS (MTV): A QUALITATIVE *in vitro* ASSAY FOR MTV

By PHYLLIS B. BLAIR

Department of Bacteriology and Immunology and Cancer Research Genetics Laboratory,
University of California, Berkeley

EXPERIMENTAL investigations of MTV are considerably hampered by the absence of a short-term *in vitro* assay. The conventional assay for the presence of infectious MTV has been the measurement of tumour development in susceptible test mice injected while young with extracts of infected mammary tissues or of milk¹. This test requires 1–2 years for its completion. This time-interval has been shortened in a recently developed technique in which the end-point is the development of hyperplastic alveolar nodules in the mammary glands of test mice², but even this assay requires 4–5 months.

It is feasible to shorten the assay time still further by the development of techniques which measure the antigenicity rather than the biological activity of MTV. A number of attempts to demonstrate MTV by standard immunological techniques have been reported, using antisera obtained from rabbits or guinea-pigs immunized with MTV-containing tissue extracts or milk. It is well established that such antisera are capable of neutralizing the biological activity of MTV³. *In vitro* techniques have included passive haemagglutination, precipitin, and complement-fixation tests⁴. Precipitation reactions specific

for MTV in immunodiffusion agar plates have also been reported⁵.

This article reports the development of the immunodiffusion technique as a qualitative assay for the presence of MTV. In its present form, the assay requires six weeks for its completion, including the time necessary for the preparation of the rabbit antiserum.

The methods used in the preparation of the tissue extracts and of the rabbit antisera were similar to those previously reported for work on the neutralization of MTV infectivity¹. The tissues were homogenized in saline and clarified by a 30-min centrifugation at 1,500*g*. The supernatant was then centrifuged at 30,000*g* for 90 min. The resulting pellet was resuspended in saline, so that 1 ml. saline contained the material obtained from either 1 or 2 g. wet weight, of tissue. This suspension was clarified by centrifugation at 1,500*g* for 15 min, and the supernatant was frozen at 4° C until needed. Material prepared by this method was used as the antigen preparation for injection into rabbits, for absorption of the rabbit antisera, and in the immunodiffusion plates.

Young adult female New Zealand rabbits were used for the production of antiserum. Each rabbit received a total of tissue extract equivalent to 2.5–4.0 g of original tissue, in 4 weekly intramuscular injections, and was bled one week after the final injection. The resulting sera were frozen at 4° C until used. The antiserum from each rabbit was stored and used separately. Each antiserum was used undiluted in the plates except in the absorption experiments. Antisera were obtained from rabbits which had been immunized with one of the following tissues:

(A) Tissues obtained from isogenic *BALB/cCrgl* and *BALB/cfC3H/Crgl* mice.

(1) Lactating mammary glands from *BALB/c* females. These mice are not infected with MTV, and antisera from rabbits immunized with preparations of this material do not neutralize the biological activity of MTV¹.

(2) Transplant generations 3 through 7 of a *BALB/c* mammary tumour which arose spontaneously in a 21-month-old *BALB/c* female. No virus particles were observed when samples of the tumour were examined in the electron microscope⁶.

(3) Lactating mammary glands from *BALB/cfC3H* females. This strain, in its 3rd to 5th generations, was started by fostering new-born *BALB/c* mice on *C3H/Crgl* lactating females, and using the babies when adult as generation 1 of the strain. Thus, these mice are isogenic with *BALB/c* mice, but are infected with MTV.

(4) Transplanted outgrowths of hyperplastic alveolar nodules and transplanted mammary tumours obtained from *BALB/cfC3H* females. These tissues contain virus particles as observed in the electron microscope and also biologically active MTV.

(B) Tissue obtained from a strain of mice which is not infected with MTV.

(1) Lactating mammary glands from *C57BL/Crgl* females.

(C) Tissues obtained from strains of mice which are infected with MTV.

(1) Lactating mammary glands from *C3H* females.

(2) Spontaneous and transplanted mammary tumours from *C3H* females.

(3) Spontaneous mammary tumours from *RIII/Crgl* females.

(4) Spontaneous mammary tumours from *A/Crgl* females.

(5) Spontaneous mammary tumours from *DBA/2Crgl* females.

The tissue preparations routinely used as antigen in the immunodiffusion plates were obtained from *BALB/cfC3H* spontaneous and transplanted mammary tumours. In some experiments, tissue preparations of *DBA/2* mammary tumours were also used as the antigen. Preparations of mammary tumours from the other strains of mice (*C3H*,

RIII and *A*) have so far not been used successfully as antigens in the immunodiffusion plates.

In absorption experiments, the various antisera were absorbed with MTV-free or MTV-containing tissue preparations. The antisera were combined with the tissue extracts and then maintained at room temperature for 2 with occasional manual agitation. The mixtures were clarified by centrifugation at 1,500*g* for 15 min, and the serum-containing supernatants were then added to the immunodiffusion plates.

The agar plates were prepared with 0.6 per cent Noble agar, 0.85 per cent sodium chloride, and 1:10,000 merthiolate in distilled water, at pH 7. Wells were cut with a 6-mm diameter cork borer in the pattern illustrated in Fig. 1. The distance from the centre of the antigen well to the centre of the antiserum well was 11 mm. Six antisera could be tested on each plate, and the experiments were designed so that the plates contained both positive and a negative control. The standard antigen preparation was placed in the two centre wells one day before the antisera were added to the other wells. The centre wells were refilled with saline once daily for two days after the introduction of the antigen preparation. The plates were maintained at room temperature. Precipitate lines were visible within a few days, but the plates were routinely examined for a period of two weeks.

Each of the antisera already described here was tested against the standard MTV-containing antigen preparation in several separate experiments. In most experiments no absorption of antiserum was carried out, and each antiserum produced several precipitate lines with the antigen. This is an expected result, since the standard antigen preparation contains not only MTV or MTV-induced antigen but also normal tissue antigens. The precipitate line characteristic of MTV was initially found by a comparison of the precipitate lines made by antisera obtained from rabbits immunized with *BALB/cfC3H* tissues (which contain MTV) with those made by antisera obtained from rabbits immunized with the isogenic but virus-free *BALB/c* tissues. Precipitate lines specific for

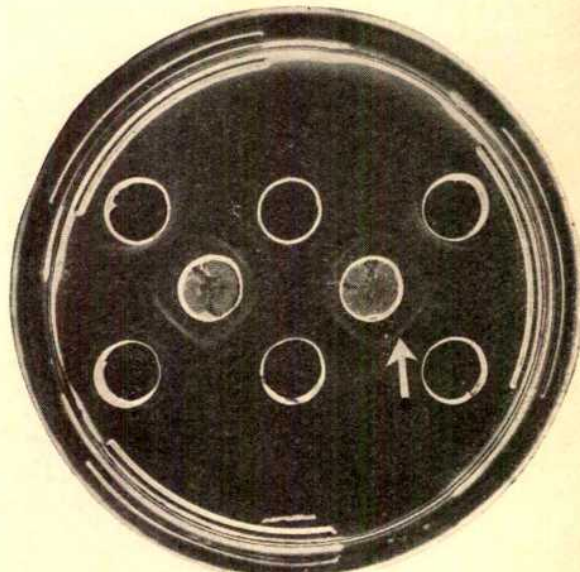


Fig. 1. Immunodiffusion of rabbit antisera and extracts of *BALB/cfC3H* mammary tumours. The three top wells were filled with, left to right, (1) rabbit antiserum against *BALB/c* lactating gland, (2) rabbit antiserum against *BALB/cfC3H* mammary tumour, and (3) rabbit antiserum against *BALB/c* lactating gland. The two centre antigen wells were filled with the standard antigen preparation of *BALB/cfC3H* mammary tumour. The three bottom wells were filled with, left to right, (1) rabbit antiserum against *C3H* mammary tumour, (2) rabbit antiserum against *DBA/2* mammary tumour, and (3) rabbit antiserum against *C3H* mammary tumour. The characteristic precipitate lines (indicated by the arrow) are found near the antigen wells wherever the advancing antiserum contained antibodies against MTV, but not in the two areas where the advancing antisera were from rabbits immunized with virus-free *BALB/c* tissues ($\times 1.3$).

sue antigens were observed in the vicinity of the antigen wells; the precipitate line characteristic of MTV was found near the antigen wells (Fig. 1). The MTV specificity of the line was tested by using antisera from rabbits immunized with tissues from other strains of mice known to contain MTV. The specificity was further tested by using antisera which had been absorbed with virus-free tissue preparations; the absorbed antisera produced only the line characteristic of MTV in the immunodiffusion plates.

As recorded in Table 1, all the antisera from rabbits immunized with normal or neoplastic tissues containing MTV (obtained from 5 different strains of mice) produced a characteristic precipitate line. On the other hand, none of the antisera from rabbits immunized with BALB/c C57BL tissues (which do not contain MTV) produced this line.

Further, in the absorption experiments completed so far (Table 2), extracts of tissues which do not contain MTV are not capable of absorbing the antibodies responsible for the formation of the characteristic precipitate line. In contrast, the antibodies were removed by absorption with preparations of either normal or neoplastic tissues which do contain MTV.

The fact that antisera from rabbits immunized with tissues obtained from a variety of mouse strains infected with MTV all produced the characteristic precipitate line in this test system indicates that the MTVs carried by different strains of mice contain at least one antigen in common. This is in agreement with the results of previous neutralization studies, in which antisera from rabbits immunized with the MTV from one strain of mice were capable of neutralizing the biological activity of the MTV from other strains of mice^{1,7}. Based on this common antigenicity, the immunodiffusion technique has a potential usefulness as an assay system for the MTV from any strain.

Not only similarities but also differences in biological activity⁸, morphology⁹, and antigenicity¹⁰ of the MTVs from various strains of mice have been reported. The scope of such investigations has been limited by the cumbersome *in vivo* assay for the presence of infectious MTV. Moreover, there frequently is little correlation between the presence of the type B virus particles considered to be MTV and the presence of biologically active MTV, and various explanations for this lack of correlation have been proposed¹¹. An assay method which is based on the presence of a common MTV antigenicity, rather

than on biological activity, can be expected to resolve some of these differences in interpretation.

The antigenic component responsible for the production of the characteristic precipitate in the immunodiffusion assay appears to be the intact type B virus particle¹². This particulate antigenic component can be sedimented by centrifugation at 30,000g for 1.5 h or less. The MTV-associated antigen found by Lezhneva³ in the lactating mammary glands of FIII mice was a much smaller component. It remained in the supernatant even after centrifugation at 100,000g for 1.5 h. These two MTV-associated components may represent the two sizes of infective MTV reported by Moore¹³. It is also quite possible that the smaller component may represent fragments of the type B particle, or a virus-induced antigen.

As reported here, all MTV-containing antigen preparations tested were successfully used for the production of antisera in rabbits which then produced the characteristic precipitate line when tested against the standard MTV-containing antigen preparation in the immunodiffusion plates. However, some of the same preparations did not produce the characteristic precipitate line when they were used as antigens in the plates, and tested against standard antisera. This is probably related to the concentration of MTV antigen in these preparations. The concentration of the type B virus particle varies considerably in MTV-containing tissues from different sources⁹. As expected, MTV-free BALB/c tissue preparations cannot be used as antigen in immunodiffusion.

The qualitative *in vitro* test described herein is, nevertheless, ideally suited to indicate the presence or absence of MTV in tissues, by using the suspect tissues as antigen for immunization of rabbits and then testing these antisera against standard MTV-containing preparations in the immunodiffusion plates. The assay can also be developed to provide data on the relative amounts of MTV-related antigen in various tissues, by means of absorption of standard antisera with the different tissue preparations. Antigenic differences between the MTVs carried by different strains can also be examined, again utilizing absorption experiments. That such experiments may be subject to quantitation is indicated by work with other viruses¹⁴. Quantitative absorption experiments designed to investigate these possibilities are currently in progress.

I thank Prof. David W. Weiss for his advice and Mrs. Rose Bonhag for her assistance. This work was supported by research grants E-344 from the American Cancer Society and AI-2309 and CA-05388 from the U.S. National Institutes of Health, and by Cancer Research Funds from the University of California.

¹ Blair, P. B., *Proc. Soc. Exp. Biol. and Med.*, **103**, 188 (1960); *Cancer Res.*, **23**, 281 (1963).

² Nandi, S., *J. Nat. Cancer Inst.*, **31**, 75 (1963).

³ Andervont, H. B., and Bryan, W. R., *J. Nat. Cancer Inst.*, **5**, 143 (1944).

⁴ Dmochowski, L., and Passey, E. D., *Ann. N.Y. Acad. Sci.*, **54**, 1035 (1952). Heidelberg, M., Graff, S., and Haagenensen, C. D., *J. Exp. Med.*, **95**, 333 (1952). Heidelberg, M., Leon, M. A., Graff, S., and Haagenensen, C. D., *J. Mt. Sinai Hosp.*, **15**, 210 (1952). Imagawa, D. T., Green, R. G., and Halvorson, H. O., *Proc. Soc. Exp. Biol. and Med.*, **68**, 162 (1948). Plescia, O. J., Doat, P., Pontieri, G., and Bianco, A., *Fed. Proc.*, **18**, 592 (1959). Shabad, L. M., *Amer. Rev. Soc. Med.*, **4**, 166 (1946). Stepien, V. N., *Prob. Oncol.*, **7**, 41 (1961).

⁵ Lezhneva, O. M., *Prob. Oncol.*, **7**, 62 (1961).

⁶ Pitelka, D. R. (unpublished results).

⁷ Blair, P. B., *Proc. Amer. Assoc. Cancer Res.*, **6**, 6 (1965).

⁸ Blair, P. B., *Science*, **127**, 518 (1958). Hummel, K. P., and Little, C. C., *J. Nat. Cancer Inst.*, **23**, 813 (1959). Pitelka, D. R., Bern, H. A., Nandi, S., and DeOme, K. B., *Proc. Amer. Assoc. Cancer Res.*, **5**, 202 (1964). Squarini, F., Rossi, G., and Paoletti, L., *Nature*, **197**, 505 (1963).

⁹ Hairstone, M. A., Sheffield, J. B., and Moore, D. H., *J. Nat. Cancer Inst.*, **33**, 825 (1964).

¹⁰ Bittner, J. J., and Imagawa, D. T., *Cancer Res.*, **15**, 464 (1955).

¹¹ Sykes, J. A., Grey, C. E., Scanlon, M., Young, L., and Dmochowski, L., *Tex. Rep. Biol. Med.*, **22**, 639 (1964). Moore, D. H., *Nature*, **198**, 429 (1963). Pitelka, D. R., Bern, H. A., Nandi, S., and DeOme, K. B., *J. Nat. Cancer Inst.*, **33**, 867 (1964).

¹² Blair, P. B., Weiss, D. W., and Pitelka, D. R. (to be published).

¹³ Moore, D. H., in *Tumors Induced by Viruses*, edit. by Dalton, A. J., and Haguenuau, F., 113 (Academic Press, New York, 1962).

¹⁴ Grogan, R. G., Taylor, R. H., and Kimble, K. A., *Phytopath.*, **54**, 163 (1964).

Table 1. PRODUCTION OF CHARACTERISTIC MTV PRECIPITATE LINE BY VARIOUS RABBIT ANTISERA TESTED IN IMMUNODIFFUSION PLATES AGAINST TISSUE EXTRACTS OF BALB/cC3H MAMMARY TUMOURS

Tissues used for immunization of rabbits	No. of rabbits	No. of sera producing characteristic precipitate line
1) Tissues containing no MTV:		
BALB/c lactating glands	5	0
BALB/c mammary tumours	1	0
C57BL lactating glands	2	0
2) Tissues containing MTV:		
BALB/cC3H lactating glands	4	4
BALB/cC3H nodule outgrowths and mammary tumours	5	5
C3H lactating glands	2	2
C3H mammary tumours	3	3
FIII mammary tumours	1	1
A mammary tumours	2	2
DBA/2 mammary tumours	2	2

Table 2. PRODUCTION OF CHARACTERISTIC MTV PRECIPITATE LINE BY ANTISERA FROM RABBIT IMMUNIZED WITH MTV-CONTAINING TISSUE EXTRACTS, AFTER ABSORPTION OF THE SERA WITH VARIOUS TISSUE PREPARATIONS

The antisera were tested in immunodiffusion plates against tissue extracts of BALB/cC3H mammary tumours

Tissues used for absorption	Antisera from rabbits immunized with	
	BALB/cC3H tumour	C3H tumour
1) Tissues containing no MTV:		
BALB/c lactating glands	+	+
BALB/c mammary tumours	+	+
2) Tissues containing MTV:		
BALB/cC3H lactating glands	0	0
BALB/cC3H mammary tumours	0	0

+, Characteristic precipitate line formed; 0, no characteristic precipitate line.

INCREASED SENSITIVITY OF *IN VITRO* MURINE LEUKAEMIA CELLS TO FRACTIONATED X-RAYS AND FAST NEUTRONS

By DR. WILLIAM L. CALDWELL and PROF. LEONARD F. LAMERTON
Department of Biophysics, Institute of Cancer Research (Surrey Branch), Sutton, Surrey

AND

DR. DAVID K. BEWLEY

Medical Research Council Cyclotron Unit, Hammersmith Hospital, London, W.12

FOLLOWING the original report of Elkind and Sutton¹, the variation with time in radiosensitivity after a single dose of radiation has been studied for a number of different cell systems. They have all shown a similar pattern of change, but with differences in the degree of variation. In no case, however, has the net effect of a given dose delivered in two fractions been greater than that of a similar undivided exposure.

In this article, investigations are reported using a mammalian cell suspension in which it has been found that the radiosensitivity increases above normal following an initial dose of radiation. The data are described and discussed in relation to possible mechanisms operating.

Murine leukaemia cells growing *in vitro* in suspension were used for the investigations. These were made available by Dr. Peter Alexander of the Chester Beatty Research Institute². The cells were grown at 37° C in a specially prepared, balanced medium³, and under control conditions have a doubling time of 9–10 h. The cells were irradiated in polypropylene tubes at 37° C with 230 kVp. X-rays (half value layer, 1.5 mm copper) at a dose rate of 41 rads/min, or with fast neutrons having a mean energy of 6 MeV at about 50 rads/min. The dose-survival curves were obtained by backward extrapolation of growth curves, following varying doses of radiation. Since the growth curves were parallel for all doses approximately 48–60 h after radiation, extrapolation gave the surviving fraction after a correction was made for mitotic delay⁴. This correction is not large, and resulted in an uncorrected D_{37} of 55 rads becoming 62 rads on correction.

In order to evaluate the survival kinetics of the cells in the divided dose experiments, cell survival 100 h after the initial exposure was measured. Survival at this time can be considered a measure of reproductive integrity since, even accounting for mitotic delay, 100 h is equivalent to approximately 10 cell cycles. Radiation doses totalling 200 rads of X-rays or 80 rads of neutrons were used for all experiments. Counts of viable cells were made in standard haemocytometers using phase-contrast microscopy.

Fig. 1 shows dose survival curves for X-rays and fast neutrons respectively. The X-ray curve clearly has no very substantial shoulder, but the scatter of the experimental points does not allow the degree of shoulder to be determined with any precision. Alexander and Mikulski², using the same cells with the same medium, observed a slight shoulder, but a correction for mitotic delay would very largely eliminate it. With the neutrons there would appear to be very little, if any, shoulder. With neutrons the D_{37} is 27 rads; this implies a relative biological effectiveness (RBE) of 2.3, when compared with the D_{37} of 62 rads for 250 kVp. X-rays.

Fig. 2 shows the mitotic delay induced by a range of doses of X-rays. Preliminary measurements have also been made with neutrons, for which the response shows a similar pattern of change with time as for X-rays, with an RBE of 2–2.5.

Fig. 3 shows the relative survival after a pair of equal exposures for varying intervals of time between them. It can be seen that with both X-rays and neutrons there is a brief period of decreased sensitivity to a second exposure, followed by an interval (from about the fourth to tenth hour) in which sensitivity is actually increased

above normal. The form of the curve is similar for the types of radiation with smaller variation in the case of neutrons. With X-rays, and an interval of about 6 h between exposures, survival is approximately 50 per cent of that for cells exposed to the same total amount of radiation at one time, and with neutrons about 70 per cent.

Using X-rays, an investigation was also made of survival when the time-interval between radiation fractions was kept constant at 5 h and the exposure fractions varied. The relative survival curve is given in Fig. 4, and it shows an increased sensitivity for all tested fractionations with the maximum effect when the initial dose was 180 rads and the second dose 180 rads.

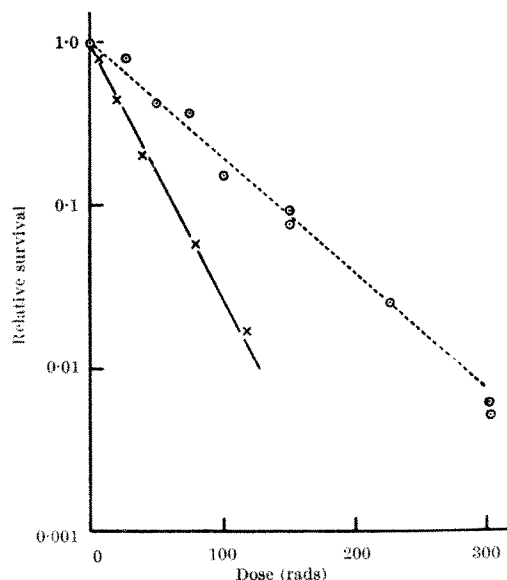


Fig. 1. Dose-survival curves for X-rays and neutrons. ○, X-rays; ×, neutrons.

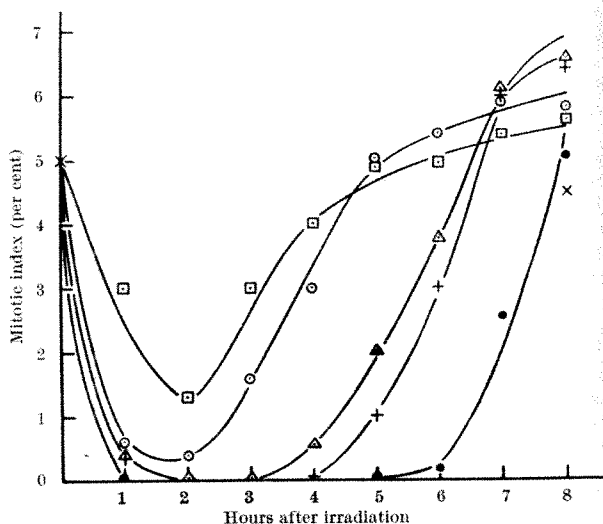


Fig. 2. Mitotic index curves following different doses of X-radiation (rads). □, 20; ○, 25; △, 50; +, 100; ●, 200.

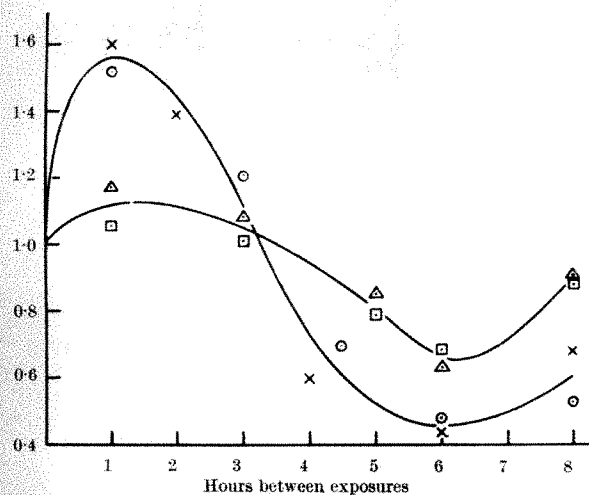


Fig. 3. Cell survival following a pair of equal exposures given at various intervals. Two doses each of 100 rads given for X-rays, and two doses each of 40 rads for neutrons. Points represent results from two separate experiments. Δ , \square , Neutrons (2×40 rads); \times , \circ , X-rays (2×100 rads)

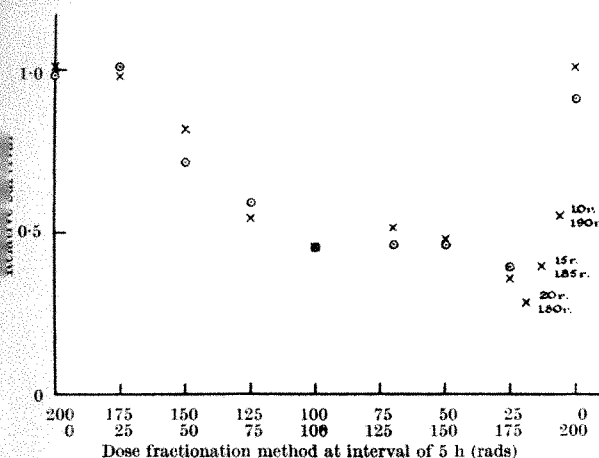


Fig. 4. Cell survival under X-rays following different fractionation schemes at a constant time interval of 5 h, compared with survival following a single exposure of 200 rads. Points represent results from two separate experiments

The two features of particular interest in these results are: (1) that the sensitivity after an initial conditioning dose is actually increased above normal; (2) that with an interval of 5 h between exposures the effect of a total dose of 200 rads is greater when the smaller fraction is given first (the maximum effect on survival being achieved with an initial dose of 20 rads).

In other cell systems in which the response to divided doses has been reported, the increase in sensitivity following the initial decrease has been relative only, sensitivity to the second exposure approaching, but not exceeding, control-levels. However, the dose-survival curves for these systems all have a substantial initial shoulder and therefore it is possible that at least part of the explanation for the difference lies in the lack of a significant initial shoulder in the survival curve of the cells used in the present study.

Elkind⁵ originally concluded that recovery after an initial exposure was associated with reconstruction of the shoulder of the dose-survival curve; that is, with return of the extrapolation number, changed by the conditioning exposure, to its original value. Afterwards, the work of Terasima and Tolmach⁶ gave support to Kallman's hypothesis⁷ that the change in sensitivity was related to a shift in the proportion of cells in different compartments of the mitotic cycle characterized by different sensitivities. Recently, synchronization investigations^{8,9} provide data supporting the model of recovery kinetics for dose fractionation experiments which had been suggested by Elkind,

Sutton and Moses¹⁰; that is, that the initial decrease in sensitivity following a conditioning exposure results from recovery of sub-lethal damage (reconstitution of the shoulder of the dose-survival curve) while the subsequent relative increase in sensitivity is the result of phasing of cells within sensitive phases of the mitotic cycle.

At the same time one must recognize that other processes may also be operative. It is of interest that Beer *et al.*¹¹ and Lett *et al.*¹² have shown that various factors connected with culture conditions can alter the radio-sensitivity of these leukaemia cells, neither D_{37} nor the extrapolation number remaining constant. It is possible that when these cells are given two radiation exposures, the first exposure may modify the growth conditions so as to alter the response to the second exposure.

If the difference between the present results and those of Till and McCulloch¹³, for example, were a consequence of the initial shoulder, then subtraction of the two curves should show the effect of the shoulder. Fig. 5 shows the results of this operation. Making allowance for difference in time intervals (as indicated), subtraction of the leukaemia curve from that obtained by Till and McCulloch for mouse bone marrow cells yields the curve (c), which could possibly represent the 'shoulder' effect. On this basis, the variation in sensitivity found for the leukaemia cells would have to be due to variation in radiation response with cell phase, associated with partial synchronization of the cells following irradiation, or to an entirely new process not so far postulated.

One conclusion that can be drawn from the mitotic index data of Fig. 2 is that the variation of sensitivity following a conditioning dose is not the result of an altered sensitivity during mitosis. So far as phasing effects are concerned, interpretation of the mitotic data is not straightforward since, particularly with the higher doses, many of the cells being counted, including those in mitosis, are 'doomed' (destined to die in a future division cycle). Phasing of the viable cells might be obscured by the presence of 'doomed' cells, in which case, with passage of time, peaks of mitotic activity of viable cells should be detectable, as the 'doomed' cell population diminished, provided the variation in post-irradiation cell-cycle times was not sufficient to obscure such peaks. Preliminary explorations for such peaks in leukaemia cells have been fruitless.

The significance of cell phasing in divided-dose survival kinetics in any cell system still remains to be clarified. Experimental investigations have given very diverse results concerning the change of sensitivity with phase of the cell cycle. A further difficulty in explaining the results by a simple phasing effect is that Whitmore⁸ found, in

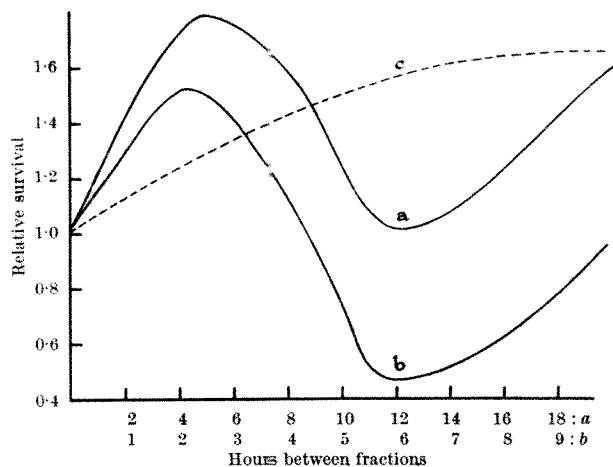


Fig. 5. Comparison of a typical curve (a) of change in sensitivity to a second exposure following a conditioning exposure (ref. 13) with the curve (b) obtained for leukaemia cells. Differences in time between fractions for the two curves are as indicated. Curve (c) results from a subtraction of curves (a) and (b)

his synchronized *L* cells, an initial upswing in the 'recovery curve' following a conditioning exposure during all phases of the mitotic cycle. Apparently unrelated to phasing, this initial upswing was thought to be primarily the result of recovery of that sub-lethal damage which gives rise to the shoulder of the curve. However, since this upswing is also seen in the leukaemia cells with both X-rays and neutrons where the shoulder is either small or absent (Fig. 3), it seems necessary to postulate some other process.

There is, however, another possible interpretation of the results which does not require the assumption of a new process of recovery. If the sensitivity varies over the various phases of the cell cycle, the survival curve for the leukaemia cells cannot be a true exponential, but must represent the combined survival of various fractions. It is possible that one or more of these fractions has an initial shoulder. As a simple model it can be shown, for example, that if the cells consisted of 60 per cent with a D_{37} of 62 rads and an extrapolation number of 1.67 and 40 per cent with a D_{37} of 20 rads and an extrapolation number of 1.00, the combined curve would be practically indistinguishable from an experimental curve with a D_{37} of 62 rads. In this case the component with a shoulder could be responsible for the increased survival at 1 h and the subsequent swing could be due to phasing. The finding that the effect of the split dose was less with the larger fraction given first would be explained if the selective killing by the conditioning exposure were such that, at 5 h, a greater proportion of surviving cells were concentrated in their sensitive phase after a small dose than after a large dose. The maximal effect found with about 20 rads as the initial exposure presumably represents the optimal combination of 'sensitization' and size of the second exposure.

To decide whether the foregoing hypothesis is correct, or whether some other mechanism, such as modification of actual repair processes, is operating requires investigation of the effect of different dose fractions at different

time-intervals and of the response of synchronized populations of the cells.

Studies with high LET radiation could presumably help a great deal in disentangling the various mechanisms operating. Radiation of sufficiently high LET might give a true exponential survival curve instead of synthetic one, and in these circumstances it is generally assumed that no recovery would be expected¹⁴. Fast neutrons give rise to a wide spectrum of LET, and the reduced variation in sensitivity found with neutrons, compared with the lower LET X-rays, is consistent with this idea. On the other hand, the possibility must not be ignored that some recovery processes may operate even with high LET radiation.

We thank Mrs. D. Courtenay, Mr. P. Coultas and I. N. M. Blackett for their advice, and Dr. S. Field and Mr. Parnell for help in the neutron experiments, and Prof. D. W. Smithers, director of the Radiotherapy Department, for his interest in this investigation. One of us (W. L. C.) is a Special Fellow of the National Cancer Institute, U.S. Public Health Service, on leave from the Department of Radiology, Stanford University School of Medicine, Palo Alto, California.

¹ Elkind, M. M., and Sutton, H., *Nature*, **184**, 1293 (1959).

² Alexander, P., and Mikulski, Z. B., *Nature*, **192**, 572 (1961).

³ Fischer, G. A., *Ann. N.Y. Acad. Sci.*, **76**, 673 (1958).

⁴ Dewey, W. C., Humphrey, R. M., and Cork, A., *Intern. J. Rad. Biol.*, **6**, 4 (1963).

⁵ Elkind, M. M., *Brookhaven Symp. Biol.*, **14**, 220 (1961).

⁶ Terasima, T., and Tolmash, L. J., *Nature*, **190**, 1210 (1961).

⁷ Kallman, R. F., *Nature*, **197**, 557 (1963).

⁸ Whitmore, G. F., *Eighteenth Ann. Symp. Fundamentals of Cancer Research: Cellular Radiation Biology* (1964).

⁹ Sinclair, W. K., and Morton, R. A., *Nature*, **203**, 247 (1964).

¹⁰ Elkind, M. M., Sutton, H., and Moses, W. B., *J. Cell and Comp. Phys.*, **5**, Suppl. 1, 113 (1961).

¹¹ Beer, J. Z., Lett, J. T., and Alexander, P., *Nature*, **199**, 193 (1963).

¹² Lett, J. T., Parkins, G., Alexander, P., and Ormerod, M. G., *Nature*, **201**, 593 (1964).

¹³ Till, J. E., and McCulloch, E. A., *Rad. Res.*, **18**, 96 (1963).

¹⁴ Barendsen, J. W., Bensker, T. L. J., Vergroessen A. J., and Budke, W., *Rad. Res.*, **13**, 841 (1960).

EFFECT OF GASTRIN ON ISOLATED SMOOTH MUSCLE PREPARATIONS

By DR. A. BENNETT

Department of Surgery, King's College Hospital Medical School, Denmark Hill, London, S.E.5

EDKINS^{1,2} found that intravenous injections of crude aqueous antral extracts into anaesthetized cats stimulated the secretion of acid gastric juice. Since then many workers have studied the secretory activity of antral extracts. Blair *et al.*³ noticed that the intravenous injection of an antral extract into anaesthetized cats not only stimulated gastric secretion, but also increased the tone of the stomach and small intestine. Gregory and Tracy^{4,5} extracted hog antra and obtained two pure, almost identical, peptides which they called gastrins I and II. These stimulated gastric secretion and also contracted the stomach and jejunum in the dog. Very little is known about this stimulation of gastro-intestinal motility and the present experiments were carried out to examine the effect of pure gastrin II on guinea-pig and rat smooth muscle preparations.

Rat colon and rat uterus preparations were suspended in an organ bath containing de Jalon's fluid at 28° C bubbled with oxygen. All the other smooth muscle preparations were suspended in Krebs's solution at 37° C bubbled with 95 per cent oxygen and 5 per cent carbon dioxide. The responses of the tissues were recorded on a smoked drum by a light frontal writing lever (magnification $\times 12$). In some experiments guinea-pig ileum preparations were stimulated transmurally⁶ through coaxial electrodes. Drugs used were acetylcholine per-

chlorate, cocaine hydrochloride, dimethylphenylpiperazinium iodide (DMPP), hexamethonium bromide, histamin acid phosphate, 5-hydroxytryptamine creatinine sulphate (5-HT), (-) hyosine hydrobromide, gastrin II, mepyramine maleate, methysergide, morphine sulphate and physostigmine sulphate. The concentrations of cocaine hydrochloride, DMPP and methysergide are expressed as the salt. Other concentrations are in terms of base.

Gastrin II (1–2 $\mu\text{g}/\text{ml}$.) caused small contractions of the rat fundus strip preparation⁷, the rat duodenum and the rat colon. Guinea-pig sacculus rotundus and a strip of antrum and body cut from the rat stomach contracted feebly, and the rat uterus not at all in the presence of 1–4 $\mu\text{g}/\text{ml}$ of gastrin. The most responsive preparation encountered was guinea-pig ileum which usually contracted well in response to 0.1–4 $\mu\text{g}/\text{ml}$ of gastrin. Misiewicz and Waller (personal communication) found that 0.5–1 $\mu\text{g}/\text{ml}$ of gastrin I contracted 5 of 11 strips of human isolated stomach and that tachyphylaxis occurred. The analysis of the action of gastrin described here was carried out on the guinea-pig isolated ileum preparation.

Gastrin II produced a contraction of guinea-pig ileum which was rapid in onset and which quickly reached a peak. Sometimes the contraction was maintained, but often the tissue partially relaxed while gastrin was still

the bath. A second dose added soon after the first had been washed out produced a smaller effect. In order to prevent this tachyphylaxis, gastrin was administered at intervals of approximately 15 min. Mepyramine (100 ng/ml.) completely blocked the responses to histamine; it had no effect on the responses to gastrin in 3 experiments, but in another experiment 10 ng/ml. of mepyramine slightly reduced the effect of gastrin. The H_1 antagonist methysergide (which only partially antagonizes 5-HT on the guinea-pig ileum⁸) had no effect on the responses to gastrin in 3 experiments (methysergide 100 ng/ml.). In another 4 experiments methysergide (10 ng/ml.) reduced the contraction produced by gastrin, since the responses to acetylcholine were also a little reduced in 3 of these 4 experiments the antagonism was probably non-specific.

In each of 6 experiments hexamethonium (10 μ g/ml.) almost completely abolished the response of the ileum to DMPP but had no effect on the response to gastrin (Fig. 1). Hyoscine (2–200 ng/ml.), however, completely abolished the contraction produced by acetylcholine in 3 experiments and either completely abolished the response to gastrin (7 experiments) or reduced its effect (3 experiments; hyoscine 4 ng/ml.). When the hyoscine was washed out the effects of acetylcholine and gastrin were restored together (Fig. 2). During a hyoscine block, additions to the bath of 20–40 ng/ml. of eserine (3 experiments) restored or even potentiated the responses to acetylcholine and gastrin (Fig. 2). Eserine (20–100 ng/ml.) added to the bath in the absence of hyoscine potentiated responses to acetylcholine and gastrin equally in 4 experiments, and potentiated gastrin more than acetylcholine in 2 experiments.

These results suggest that gastrin acts either on acetylcholine receptors in the muscle or, more likely, on the postganglionic parasympathetic nerves at a site not blocked by hexamethonium to cause the release of acetylcholine. These possibilities were tested by using: (a) atropine to prevent the release of acetylcholine⁹; (b) anoxia to prevent nervous conduction⁸; (c) cocaine in an attempt to anaesthetize the nervous tissue in the ileum.

The responses to gastrin and DMPP were reduced in 2 experiments or almost abolished (2 experiments, see Fig. 3) by morphine (10–100 ng/ml.). The responses to acetylcholine were slightly depressed in 3 of the 5 experiments, and the responses to transmural stimulation were reduced in each of 3 experiments in which coaxial stimulation was used.

The effects of anoxia on responses to gastrin were studied in 4 experiments by replacing the oxygen/carbon dioxide mixture with nitrogen or with a nitrogen/carbon dioxide mixture. Anoxia abolished the effects of transmural stimulation and almost completely prevented the responses to gastrin and DMPP (Fig. 4). The effects of acetylcholine and histamine were reduced to a much less extent and the responses of the ileum to histamine and acetylcholine approximated their pre-anoxia effects when the doses of the agonists were doubled. On the other hand, increasing the doses of gastrin and DMPP 7–10 times produced only moderate or small contractions. These results indicate that gastrin acts on nervous tissue, but since it produced a small effect on the anoxic ileum it is possible that gastrin also has a small direct effect on muscle cells. In each of 6 experiments cocaine hydrochloride (10–25 μ g/ml.) almost completely blocked the effect of DMPP. In 2 of these experiments the responses to gastrin were unaffected, but in 3 experiments the effect of gastrin was slightly reduced (Fig. 5) and in one instance it was almost completely abolished. The effect of cocaine (20 μ g/ml.) on the response of the ileum to transmural stimulation of the nerves by single shocks (6/min, 0.5 msec and 3 V) was examined in 3 of these 6 experiments. Surprisingly, the contractions were only slightly reduced in 2 experiments and were unaffected in the other, thus indicating that cocaine in this concentration has little or no effect on transmission in postganglionic parasympathetic nerve endings. The finding that cocaine always abolished the effect of DMPP, but only sometimes reduced the effect of gastrin, supports the evidence obtained with hexamethonium that DMPP and gastrin act at different sites. The variation in the effect of cocaine on the response to gastrin is apparently explained by the finding that cocaine only sometimes reduced the effect of electrical stimulation, presumably by partial anaesthesia of the nerves.

All these experiments indicate that gastrin acts on the postganglionic parasympathetic nerves, and suggest that in the guinea-pig ileum the contraction produced by gastrin is caused by the release of acetylcholine. Such a view is consistent with the results of Gregory and Tracy⁵ that atropine reduces the motor effect of gastrin on the stomach and jejunum of the dog. It is of great interest that atropine also inhibits the stimulation of gastric secretion by gastrin in the dog^{10–12} and in man¹². It may follow that gastrin stimulates secretion by acting on nerves to release acetylcholine.

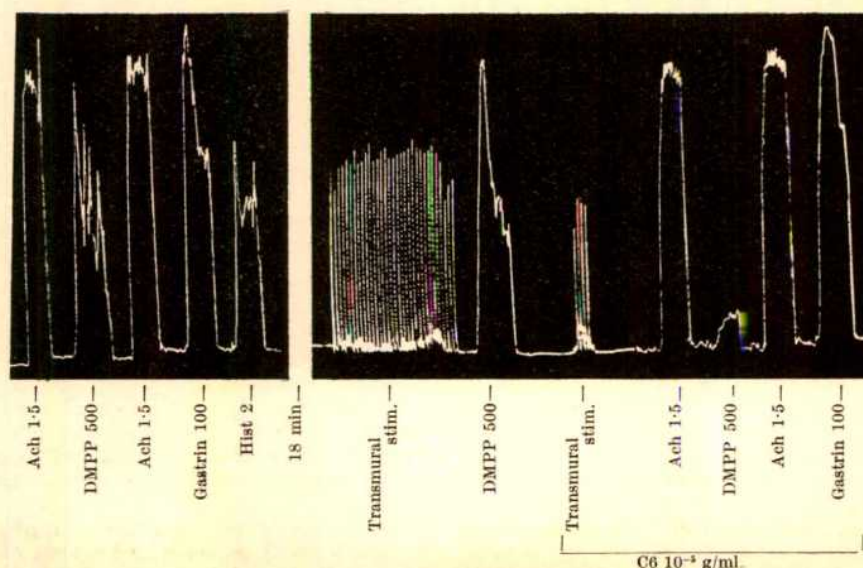


Fig. 1. The lack of effect of hexamethonium on the response of guinea-pig ileum to gastrin. Hexamethonium inhibited the response to DMPP but did not alter the response to gastrin. The numbers by the agonist drugs represent drug concentrations in ng/ml.

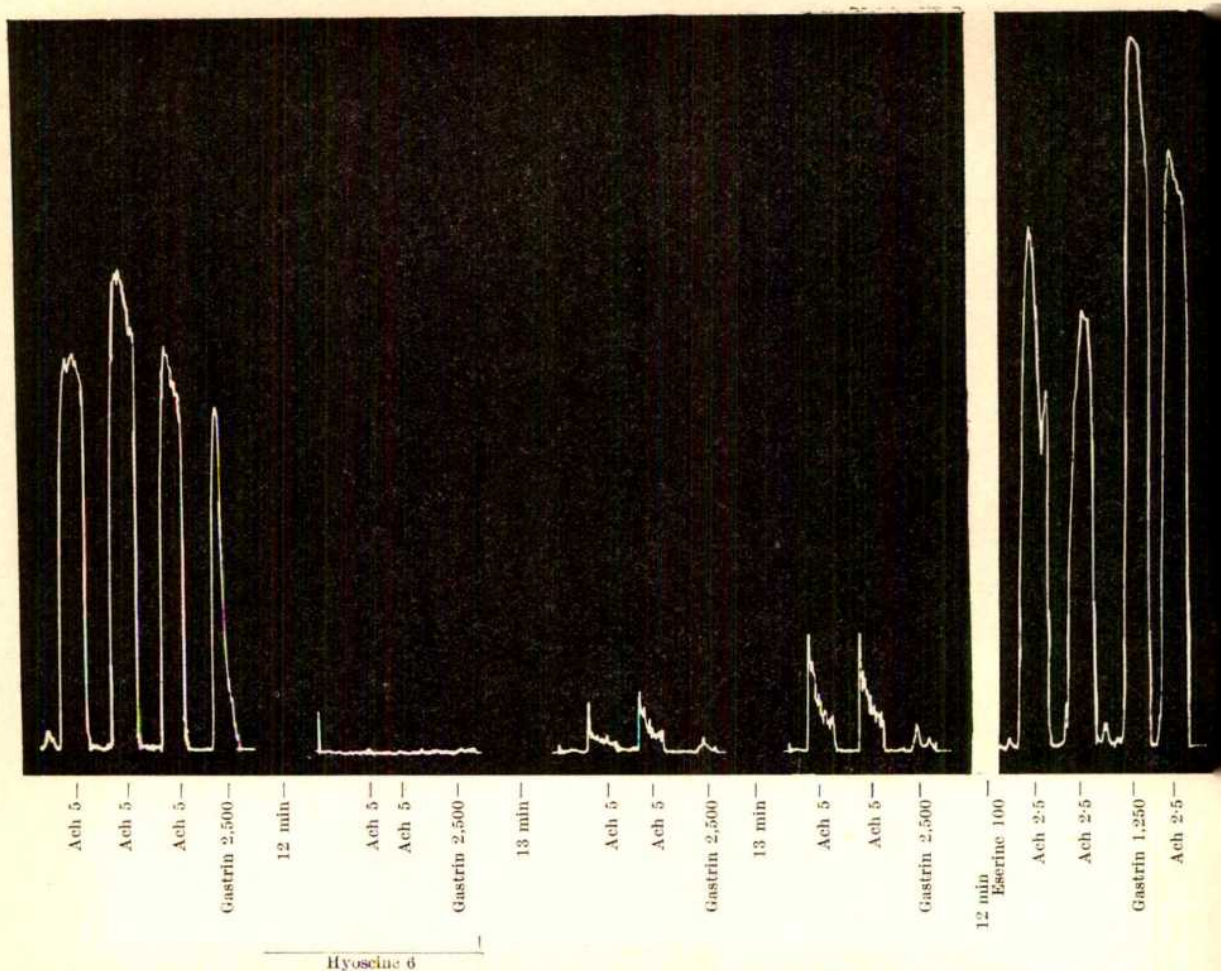


Fig. 2. The effects of hyoscine and of eserine on the response to gastrin. Hyoscine abolished the effects of acetylcholine and gastrin. When the hyoscine was washed out the responses to acetylcholine and gastrin started to return. After the addition of eserine both gastrin and acetylcholine were greatly potentiated. The numbers by the drugs represent concentrations in ng/ml.

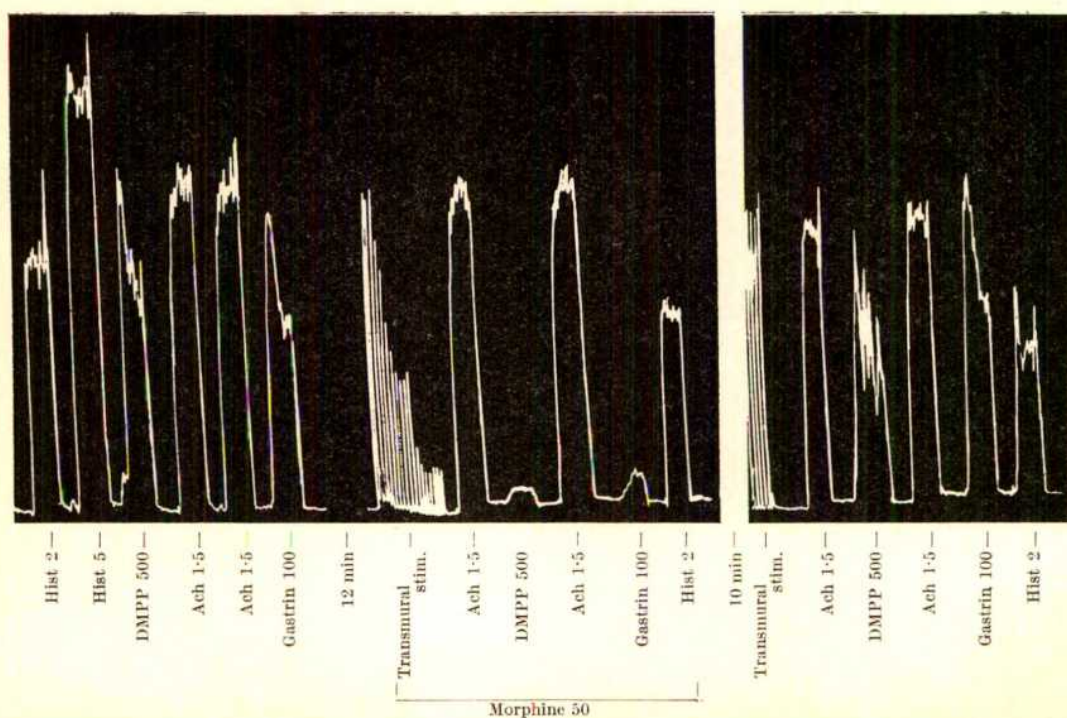


Fig. 3. The effect of morphine on the response to gastrin. Morphine greatly reduced the effects of gastrin, DMPP and transmural stimulation. The numbers represent drug concentrations in ng/ml. During transmural stimulation (3 V, 0.5 msec, 4 shocks/min) the drum speed was reduced to one-eighth.

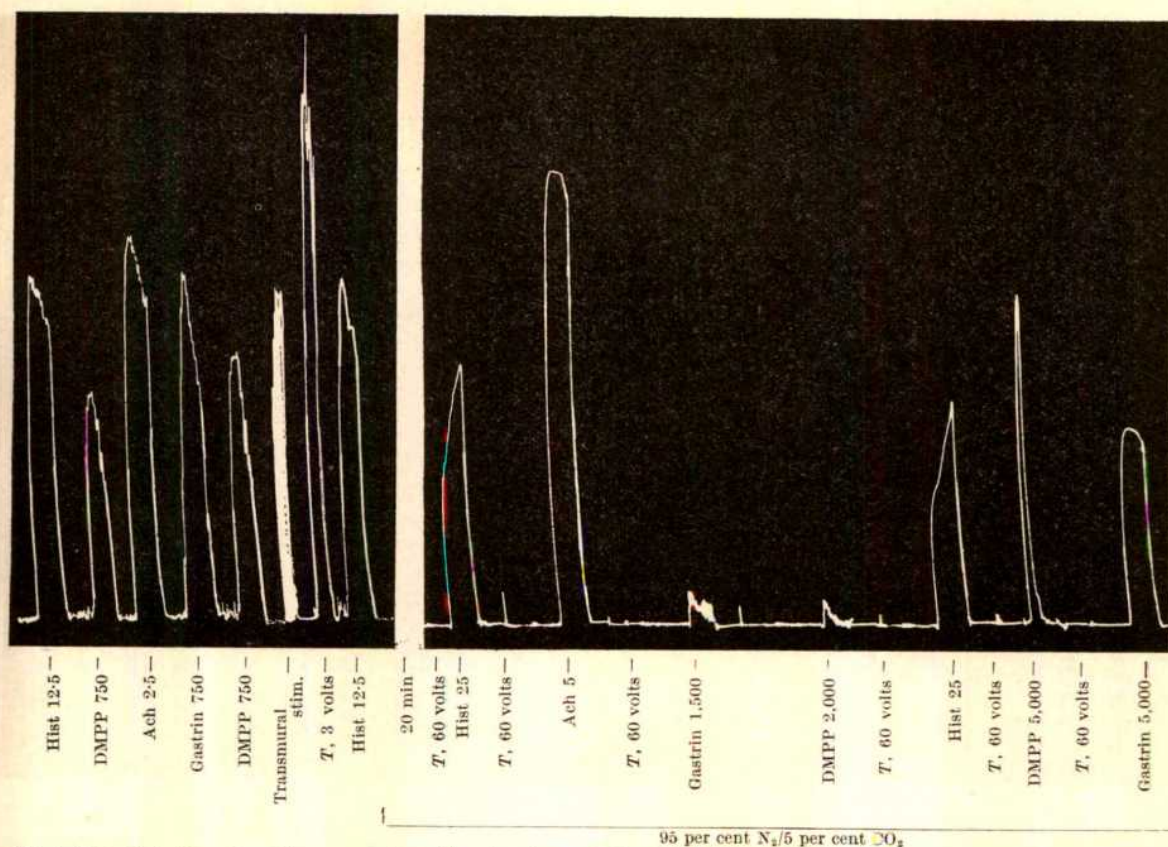


Fig. 4. The effect of anoxia on the response to gastrin. Anoxia completely abolished the effect of transmural stimulation at 3 V, 1 msec, 6 shocks/min; 3 V, 1 msec, 5 shocks/sec (T, 3 volts) and 60 V, 1 msec, 5 shocks/sec (T, 60 volts). Anoxia slightly reduced the responses to acetylcholine and histamine but greatly reduced those to gastrin and DMPP. The responses to gastrin and DMPP approached their pre-anoxia levels when the doses were increased from 750 ng/ml. to 5,000 ng/ml.

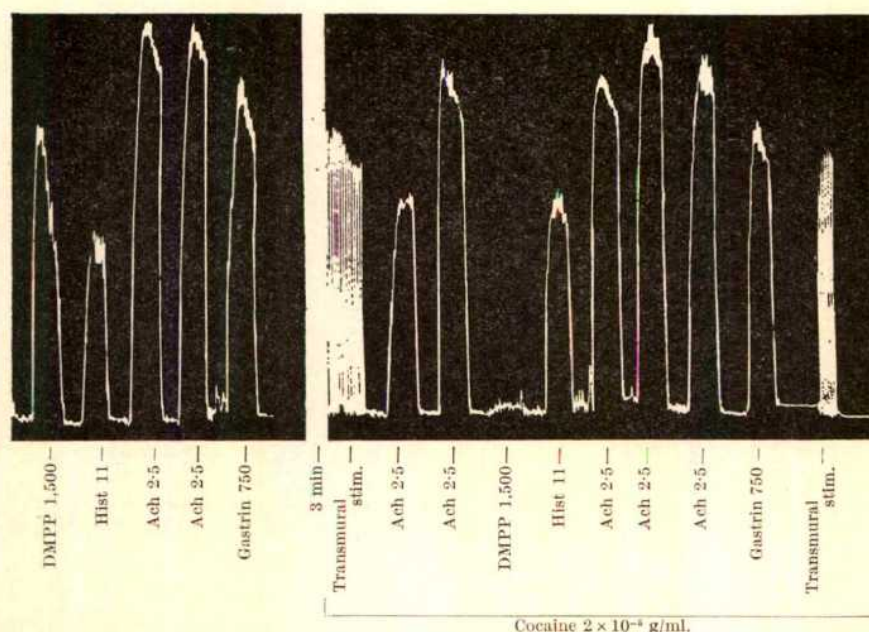


Fig. 5. The effect of cocaine on the response to gastrin. Cocaine almost abolished the response to DMPP but only slightly reduced the effects of transmural stimulation and of gastrin. The numbers by the agonists represent the drug concentrations in ng/ml. During transmural stimulation (3 V, 0.5 msec, 6 shocks/min) the drum speed was reduced to one-eighth.

I thank Prof. R. A. Gregory and Dr. B. A. L. Hurn of the Wellcome Research Laboratories for samples of gastrin II, and Dr. J. R. Vane for his advice.

* Edkins, J. S., *Proc. Roy. Soc., B*, **76**, 376 (1905).

* Edkins, J. S., *J. Physiol.*, **34**, 133 (1906).

* Blair, E. L., Harper, A. A., Lake, H. J., Reed, J. D., and Scratcherd, T., *J. Physiol.*, **156**, 11P (1961).

* Gregory, R. A., and Tracy, H. J., *J. Physiol.*, **169**, 18P (1963).

* Gregory, R. A., and Tracy, H. J., *Gut*, **5**, 103 (1964).

* Paton, W. D. M., *J. Physiol.*, **127**, 40P (1955).

* Vane, J. R., *Brit. J. Pharmacol.*, **12**, 344 (1957).

* Day, M., and Vane, J. R., *Brit. J. Pharmacol.*, **20**, 159 (1963).

* Paton, W. D. M., *Brit. J. Pharmacol.*, **12**, 119 (1957).

* Gregory, R. A., and Tracy, H. J., *J. Physiol.*, **149**, 70P (1959).

* Grossman, M. I., *J. Physiol.*, **157**, 14P (1961).

* Gregory, R. A., and Tracy, H. J., *J. Physiol.*, **156**, 523 (1961).

LETTERS TO THE EDITOR

PHYSICS

Structural Basis of Neutron and Proton Magic Numbers in Atomic Nuclei

In 1933 Elsasser¹ pointed out that some of the properties of atomic nuclei correspond to greater stability for certain numbers of neutrons and protons (given the name magic numbers) than for other numbers; the magic numbers for both N (neutron number) and Z (proton number) are 2, 8, 20, 50, 82 and 126. (Less-pronounced effects are observed also for N or Z equal to 6, 14, 28, 40, and some larger numbers. The set of magic numbers is often assumed to include 28.)

The magic numbers do not have the values $(2n^2)$ for completed shells of fermions (with all states with total quantum number n , azimuthal quantum number $l \leq n-1$, occupied by pairs), which are 2, 8, 18, 32, 50, ..., nor the values for certain shells and sub-shells that lead to maximum stability for electrons in atoms, which are 2, 10, 18, 36, 54 and 86.

It was discovered by Mayer² and by Haxel, Jensen and Suess³ that the magic numbers can be accounted for by use of the sub-sub-shells corresponding to spin-orbit coupling of individual nucleons; that is, to the values of $j = l + 1/2$ and $l - 1/2$ for the two sub-sub-shells of each sub-shell. For example, they⁴ assign to N or $Z = 50$ the configuration $(1s1/2)^2(1p3/2)^4(1p1/2)^2(1d5/2)^6(2s1/2)^2(1d3/2)^4(1f7/2)^8(2p3/2)^4(1f5/2)^6(2p1/2)^2(1g9/2)^{10}$, which may be written more briefly as $1s^21p^61d^{10}2s^22p^61f^{14}(1g9/2)^{10}$.

The evidence for spin-orbit coupling and for the Mayer-Jensen shell model is convincing. It is, however, difficult to understand, on the basis of their arguments, why the six magic numbers should be outstanding among the many numbers corresponding to the completion of spin-orbit sub-sub-shells, which (for the Mayer-Jensen sequence⁴ of energy-levels) are 2, 6, 8, 14, 16, 20, 28, 32, 38, 40, 50, 56, 64, 68, 70, 82, 92, 100, 106, 110, 112, 126, 136, 142,

In the course of developing a theory of nuclear structure based on the assumption of closest packing of clusters of nucleons⁵, I have found that the magic numbers have a very simple structural significance: 2 and 8 correspond to the closed shells $1s^2$ and $1s^21p^6$, and the others to a closed-shell core with an outer layer (the mantle of the nucleus) containing the number of spherons (helions⁶, He⁴, tritons, H³, or dineutrons) required to surround the core in closest packing.

Triangular (icosahedral) closest packing, as found, for example, in the intermetallic compound⁷ $Mg_{32}(Al,Zn)_{49}$, involves the sequence 1, 12, 32, 72 of spheres in successive layers. These numbers are approximated by the equation $n_0 = (n_i^{1/3} + 1.30)^3 - n_i$, in which n_0 is the number of spheres in an outer layer and n_i is the number in the core. (The form of this equation corresponds to assigning equal effective volumes to the spheres, and the value of the constant reflects the nubbly of the surface and the packing of outer spheres into pockets of the core.) This equation can be applied to obtain the number of spherons in the successive layers in a nucleus, and thus to obtain the sequence of nucleonic energy-levels. Sub-shells (with given value of l) occurring once (as $1s$, $1p$, etc.) are assigned to the mantle of spherons, those occurring twice ($1s$ and $2s$, for example) to the mantle and next inner layer, and so on. Thus I interpret the configuration for N or $Z = 50$, given above, as representing 8 neutrons or protons in the

core ($1s^21p^6$) and 42 in the outer layer ($2s^22p^61d^{10}1f^{14}(1g9/2)^{10}$).

The application of the packing equation leads to sequence of levels essentially as given by Mayer and Jensen, but often with sub-sub-shells for different layers being filled over overlapping ranges of values of N or Z . For example, the $3s1/2$, $2d3/2$, and $1h11/2$ sub-sub-shells all begin to be occupied at about N or $Z = 60$ and are completed at about N or $Z = 82$.

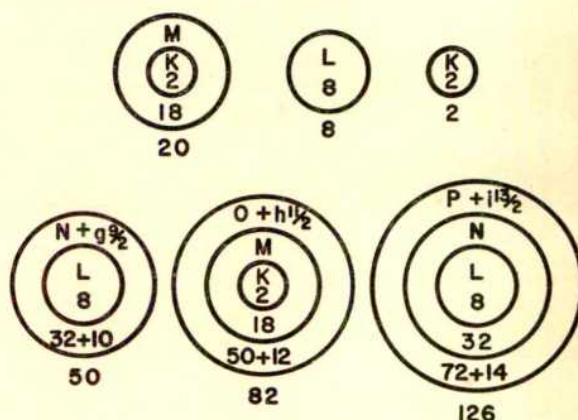


Fig. 1. The magic-number structures of atomic nuclei

The configurations found in this way for the magic numbers are given in Table 1 and Fig. 1. Each of the first two represents a completed shell. The third (20) has a completed shell as core and another as mantle. Each of the others has a core of a completed shell or two completed shells, with a mantle that is required by the packing to include a sub-sub-shell $(1g9/2)^{10}$ for 50, $(1h11/2)^{12}$ for 82, and $(1i13/2)^{14}$ for 126. Until 184 reached, there are no other values of N or Z for which the packing equation leads to a core consisting of layers that are completed shells.

Table 1. NUCLEON CONFIGURATIONS FOR MAGIC NUMBERS

N or Z	Mantle	Core or outer core	Inner core
2	$1s^2$		
8	$1s^21p^6$		
20	$2s^21p^4d^{10}$	$1s^2$	
50	$2s^22p^4d^{10}1f^{14}(1g9/2)^{10}$	$1s^21p^6$	
82	$3s^22p^4d^{10}1f^{14}1g^{12}(1h11/2)^{12}$	$2s^21p^4d^{10}$	$1s^2$
126	$3s^23p^4d^{10}2f^{14}1g^{12}1h^{14}(1i13/2)^{14}$	$2s^22p^4d^{10}1f^{14}$	$1s^21p^6$

I conclude that the stability that characterizes the magic numbers results from the completion of shells of a single layer (2, 8) or two layers (20) of spherons, or, for the larger magic numbers (50, 82, 126), for the core layer the mantle having a completed shell plus a completed sub-sub-shell.

LINUS PAULING

Big Sur, California.

¹ Elsasser, W. M., *J. Phys. et Radium*, **4**, 549 (1933); **5**, 389, 635 (1934).

² Mayer, M. Goeppert, *Phys. Rev.*, **75**, 1969 (1949).

³ Haxel, O., Jensen, J. H. D., and Suess, H. E., *Phys. Rev.*, **75**, 1766 (1949); *Z. Physik*, **128**, 295 (1950).

⁴ Mayer, M. Goeppert, and Jensen, J. H. D., *Elementary Theory of Nuclear Shell Structure*, 58 (John Wiley and Sons, Inc., New York and London, 1955).

⁵ This theory is described in papers to be published in *Phys. Rev. Letters*, *Proc. U.S. Nat. Acad. Sci.*, and *Science*.

⁶ Pauling, L., *Nature*, **201**, 61 (1964): proposal of the name helion for the α -particle.

⁷ Bergman, G., Waugh, J. L. T., and Pauling, L., *Nature*, **169**, 1057 (1952); *Acta Cryst.*, **10**, 254 (1957). Pauling, L., *The Nature of the Chemical Bond*, third ed., 427 (Cornell University Press, Ithaca, New York, 1960).

Size Analysis in the Sub-sieve Range by Electronic Counter

THE electronic apparatus for the rapid measurement of particle content and size distribution, known as the Coulter counter¹, is finding increasing application in many fields. A known volume of an extremely dilute suspension of particles in a conducting fluid flows through a standard interchangeable aperture having an immersed electrode either side. The particles traverse the aperture substantially one at a time, causing an attendant change in electrical resistivity proportional to the volume of the particle passing through the aperture. The amplified signals pass through a gate circuit which ensures that only pulses greater than a pre-selected threshold value are counted; thus, the pulse count records the number of particles greater than a certain size. A typical count requires about 5–25 sec and is performed at a rate up to 10 particles/sec. A size range of about 500–0.5 μ can be covered, therefore overlapping with sieving, microscope and sedimentation methods.

After applying certain corrections (for example, for the effect of coincident particle passage) and calibration constants, the basic data relate the numbers of particles greater than a stated size to the particle volume. This information is readily converted into the relationship between cumulative volume above a stated size (V) and particle size (X).

Size analysis results are usually quoted as cumulative volume (or weight) percentage above (or below) a stated size versus size. For this the total volume, V_∞ , or weight of particles in the measured sample must be known. However, the instrument response to sub-micron particles obscured, if not by insensitivity then by factors such as background noise and insufficient counter frequency response. Thus, the finest material in a distribution may be below the range of measurement, so that the cumulative data cannot be expressed as a fraction or percentage.

It would be possible to obtain the total solid volume gravimetrically, but one of the potential advantages of the Coulter counter is that, in the suspension prepared for analysis, the concentration of solids need not be known; additionally, the gravimetric analysis would need to be highly sensitive. The maker's instruction manual suggests a trial and error method in which the total volume of particles traversing the aperture is estimated by choosing the value providing the best straight line when cumulative percentage is plotted against size on a probability X logarithmic grid. A similar curve-fitting procedure was suggested by Batch². Simple extrapolation of the plot of cumulative volume against size either on simple arithmetic or logarithmic X arithmetic co-ordinates affords another approximate method of estimation. Experience indicates that both methods are unreliable.

The problem is determination of an unknown asymptote³, and a somewhat analogous situation exists with the sedimentation balance where an inordinately long time is necessary for the finest material to sediment, or because it remains permanently in suspension on account of Brownian movement⁴. A direct method for graphically estimating the missing finest material⁵, or what amounts to the same thing, the value of V_∞ , is presented here. While sub-micron material is the main concern here, the method may be applied to any size below the range of the aperture in use.

The equations of size distribution⁶ assumed are the Gates-Gaudin-Schuhmann type:

$$V = V_\infty \left[1 - \left(\frac{X}{X_0} \right)^N \right] \quad (1)$$

and the Rosin-Rammler type:

$$V = V_\infty [1 - \exp(-X/X_0)^n] \quad (2)$$

where X_0 is a parameter of mean size, and n and N parameters of distribution spread. These are closely

similar for the finest 20 per cent of the material and their correspondence at 30 per cent is within 1 per cent absolute. Consequently one or other equation could be used when the missing material is of the order of 20 per cent of the total volume counted. Correspondence with the logistic and probability distributions is less good, being about 0.5 per cent in absolute error over the finest 10–15 per cent of the material.

Equation (1) can be represented by the pair of equations⁴:

$$X_{r-1} = qX_r \quad (3)$$

and:

$$\left(\frac{V_{r-1}}{V_\infty} - 1 \right) = q^N \left(\frac{V_r}{V_\infty} - 1 \right) \quad (4)$$

while equation (2) can be represented by the pair of equations⁴:

$$\frac{V_{r-1}}{V_\infty} = \left(\frac{V_r}{V_\infty} \right)^q \quad (5)$$

together with equation (3), where r is an integer increasing as X decreases and as V increases and $q > 1$ and is constant. A solution of equations (4) and (5) is $V_{r-1} = V_r = V_\infty$; by plotting V_{r-1} against V_r on a squared grid for equation (4) and on a log-log grid for equation (5), V_∞ is obtained from the point of intersection of the resultant line with the line, $V_{r-1} = V_r$.

Table 1. SIZE ANALYSIS OF -300 B.S.-MESH QUARTZ BY COULTER COUNTER

Particle size (X, microns)	Cumulative vol. (V, arbitrary units)	Particle size (X, microns)	Cumulative vol. (V, arbitrary units)
60.40	0	33.12	5,100
38.68	3,528	23.44	9,100
24.19	8,792	16.56	12,700
15.28	13,523	11.72	15,600
10.68	16,365	8.28	17,600
9.33	17,183	5.86	18,669
7.40	18,103		
5.86	18,669		

The procedure for applying this method is to plot the original data V against X and interpolate values of V_{r-1} , V_r , V_{r+1} , etc., corresponding to values of X_{r-1} , X_r , X_{r+1} , etc., diminishing in geometric progression in accordance with equation (3) (see Table 1). The last actual reading of V and X should be included in this sequence. Interpolation at this stage has the advantage of smoothing experimental errors. Having determined V_∞ from a plot of V_{r-1} against V_r (Fig. 1) the size distribution Y against X ($Y = V/V_\infty$) is obtained in the usual manner.

For this particular example, V_∞ has been estimated to lie in the range 20,500–20,600 by independent observers. The ordinary extrapolation method, on the other hand, yielded estimates ranging from 19,500 to 21,200. The estimation of V_∞ from the plot of V_{r-1} versus V_r can be

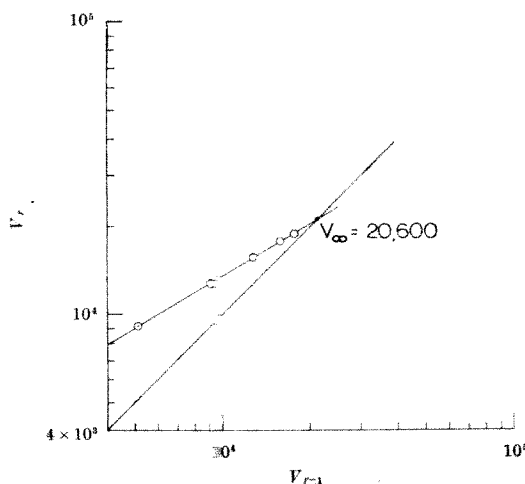


Fig. 1. Graphical determination of V_∞ . Data from Table 1. $q = \sqrt{2}$; last value of $X = 5.86 \mu$; $V = 18,669$

made as accurate as one desires. Larger graphs can be drawn and more points taken, either by diminishing the ratio, q , or by taking overlapping sets of points; for example, for a ratio of $\sqrt{2}$, take the sets 5.86, 8.28, . . . and 6.0, 8.5, . . . and 6.5, 9.2, . . . etc.

If $n = 1$ the values of X may be selected in arithmetic progression; this case has been considered previously⁴. Should the plotted points not lie on a straight line the curve would probably fit another function having an unknown asymptote. However, the last point should be sufficiently close to the line $V_{n-1} = V_r$ for an extrapolated curve to give a reliable estimate of V_∞ .

This graphical procedure has been used in a number of similar applications all involving unknown asymptotes^{4,5,7,9}. Finally, a warning must be issued: this is an extrapolation technique; there is no certainty that the size distribution law outside the measured range is the same as that inside. Thus, results obtained from this method must be used with discretion; it is not a substitute for an effective and rapid particle size analysis technique for the sub-micron region, the search for which must continue.

C. C. HARRIS

Henry Krumb School of Mines,
Columbia University, New York.

A. JOWETT

Department of Applied Mineral Sciences,
University of Leeds.

¹ Coulter, W. H., *Proc. Nat. Elec. Conf.*, **12**, 1034 (1956).

² Batch, B. A., *J. Inst. Fuel*, **37**, 455 (1964).

³ Harris, C. C., *J. App. Phys.*, **31**, 215 (1960).

⁴ Harris, C. C., and Jowett, A., *Nature*, **197**, 1192 (1963).

⁵ Harris, C. C., *Symposium on Coal Preparation*, Leeds University (1957).

⁶ Taggart, A. F., in *Handbook of Mineral Dressing* (John Wiley, New York, 1947).

⁷ Frangiskos, N. Z., Harris, C. C., and Jowett, A., *Third Intern. Congr. Surface Activity* (Cologne, 1960).

⁸ Harris, C. C., *Nature*, **197**, 371 (1963).

⁹ Harris, C. C., *Cement, Lime and Gravel*, **38**, 162 (1963).

Ionization Times in Shock-heated Potassium Vapour

THE sudden heating of a gas in a shock wave has often been used to study relaxation effects¹⁻³. Most experiments on relaxation times for ionization have been carried out with inert gases, which have first ionization potentials in the range 12-24 V. These measurements have suggested^{1,4,5} that the atoms are ionized via excited states; but the results may have been influenced by the unknown effect of impurities.

The monatomic character of inert gases, and the consequent absence of complications due to dissociation, make the inert gases attractive at first sight; but in fact the high ionization potential allows only a small amount of ionization in a pressure-driven shock-tube and almost any impurity may dominate the ionization process. Moreover, the high temperature required to achieve a reasonable degree of ionization results in a short cooling time, which may be less than the ionization time if the density is low; and complications arise because of secondary ionization and ion excitation, since the relevant critical potentials are of the same order.

It has often been pointed out⁶ that these difficulties could be avoided by using alkali metals (these have ionization potentials of 4 or 5 V, whereas the first excitation potential of their ions is several times this figure). But the great technical problems of passing shocks through such vapours have to be overcome. Such a shock tube is now in operation and ionization relaxation times have been determined by measuring the current in probes flush with the sides of the tube.

The shock tube, which was specially developed for alkali metal vapours, is of stainless steel and has ground-in stainless steel valves and a copper diaphragm. It is immersed in an oil vapour furnace at 300°-400° C.

Liquid potassium metal is fed into a boiler. The latter can be isolated from the tube, cooled during evacuation and then opened and allowed to heat up to admit vapour after the shock-tube has been isolated from pumps. The boiler is kept at a temperature some 10° lower than that of the furnace. Initial potassium pressures of 0.3-3 torr can be obtained by varying temperatures of the boiler and furnace. Helium-argon mixtures at a pressure of about 15 atmospheres are used in the compression chamber to produce shocks with Mach numbers of between 3 and 8 in the potassium vapour.

Metal probes of 'Nilo K' (an alloy of iron, cobalt and nickel), about 1 mm diameter and insulated from the shock-tube by a ceramic seal, are fitted flush to the wall of the expansion chamber and connected through a series resistor to the positive terminal of a 4.5-V battery, the other terminal being connected to the metal shock-tube. The series resistor is adjusted by experiment to have about the same value as the resistance of the probe in the equilibrium plasma behind the shock wave. Oscillograms of the probe voltage indicate the electron current to the probe. Probes mounted at two stations 13 cm apart show traces on a double beam oscilloscope from which the shock speed can be measured. The first probe also operates a trigger-amplifier which triggers the oscilloscope immediately and also triggers a fast time-base oscilloscope after a variable delay. This delay is arranged by experiment so that the fast oscilloscope is triggered just before the shock reaches the second station. The fast oscilloscope records on the upper beam the probe voltage at the second station and on the lower beam the voltage on another probe mounted opposite this with a small reflecting obstacle immediately behind it. Fig. 1 shows a typical trace. The lower trace shows a fast rate of ionization, but no change in the onset time. This indicates that there is no dormant period between the shock and the initial fall of the probe voltage.

Relaxation times τ (defined as indicated in Fig. 1) have been measured for shock Mach numbers M in the range 3.2-4.6 and initial particle densities n from 6×10^{16} to 2×10^{18} per c.c. Higher densities and shock Mach numbers resulted in relaxation times less than 1 μ s which could not be resolved by the probe. Lower densities and Mach numbers failed to trigger the amplifier, probably because of a reduction in running time.

Fig. 2 shows a graph of $\log n\tau$ against $1/T$ (where T is the temperature of the shocked gas calculated from M). If the ionization relaxation time obeys an Arrhenius law of the type:

$$\tau \propto (1/n) \exp(eV/kT) \quad (1)$$

where V is some critical potential, the slope of the graph should give V . A least squares fit gives $V = 1.9 \pm 0.3$ V. The first potassium excitation potential is 1.6 V. Since the ionization potential of potassium is 4.3 V, these results indicate a multi-stage ionization process dominated by the rate of excitation of the atom. The measured values of τ are for values of n and T much lower than those used by previous investigators. If the measurements for

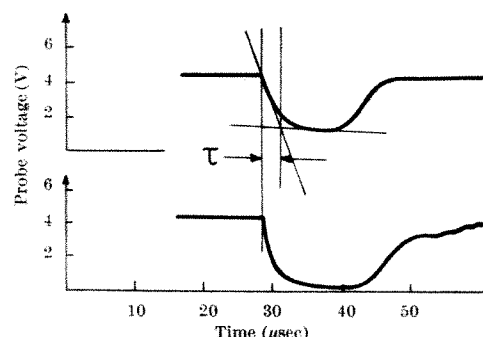


Fig. 1. Typical probe voltage traces. Upper trace, plain probe; lower trace, reflector probe

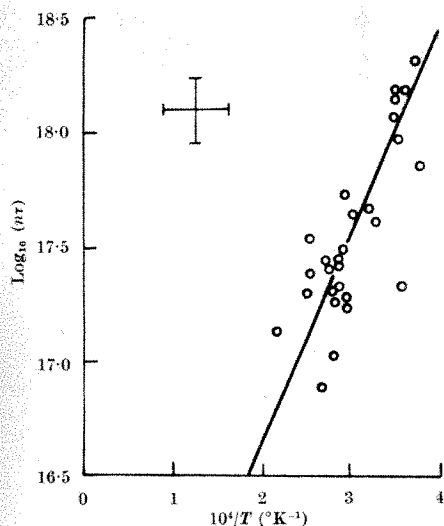


Fig. 2. Ionization times in shock-heated potassium vapour

on^{1,7} are extrapolated according to expression (1) to values of n and T , values of τ some 10^9 times greater than for potassium are obtained. This is reasonable considering the much lower potentials and higher cross-sections of the alkali metals. Though experiments have previously been performed on argon seeded with small amounts of alkali metal⁸ these experiments are, so far as we know, the first of this type to be made in a pure metallic vapour.

We thank the United Kingdom Atomic Energy Authority financing the contract under which this research was formed.

R. M. HILL
B. CAPP

Department of Physics,
University of Newcastle upon Tyne.

- tschek, H. E., and Byron, J., *Ann. Phys.*, **1**, 270 (1957).
blett, B., and Blackman, V., *J. Fluid Mech.*, **4**, 191 (1958).
erson, P., *Phys. of Fluids*, **3**, 857 (1960).
nd, J. W., *Phys. Rev.*, **105**, 1683 (1957).
eymann, H. D., *Univ. of Maryland Techn. Note BN-144* (1958).
ills, R. M., *Proc. High Temp. Gas Physics Symposium*, R. R. E., Malvern (1959).
arwell, K. E., and Jahn, B. G., *Phys. of Fluids*, **7**, 214 (1964).
aught, A. F., *Phys. of Fluids*, **5**, 1337 (1962).

METALLURGY

Interconnexion of Silicon in Modified Aluminium-Silicon Eutectic

WHEN the aluminium-silicon eutectic is rapidly frozen, when sodium is added in small quantities (< 0.017 per cent), the structure apparently is modified from an acicular form to a globular form of the silicon phase¹⁻⁷. The reason for the modification is still a matter of controversy⁸, and a review of present-day theories is given by Chadwick¹.

All the theories presented so far claim that the fine dispersion of silicon particles is produced by an increased nucleation frequency of silicon particles at a temperature lower than the equilibrium eutectic temperature. The lowering of the temperature is attributed to either a reduction in the initial nucleation frequency^{1,3,6,9-12} or restricted growth^{2,4,7,13-15}.

All present theories assume that each of the silicon particles in the modified structure is separate from, and not connected to, a neighbouring particle. The work reported here shows that this assumption is not valid.

A rapidly cooled sample of high-purity aluminium-silicon eutectic was polished metallographically to reveal

the modified structure. The sample was then etched for 24 h in a 10 per cent hydrochloric acid solution, and for 1 h in a solution of 2.5 c.c. hydrochloric acid, 2.0 c.c. nitric acid, 0.75 c.c. 40 per cent hydrofluoric acid, and 40 c.c. water to dissolve the aluminium phase and leave the silicon untouched.

The silicon phase in this modified structure was found to be interconnected in any one grain, even when the grains were 2 mm in diameter. The same result was obtained when the structure of the sodium-modified alloy was examined.

From these observations, it is apparent that in the aluminium-silicon eutectic the silicon phase grows from a common nucleus in each eutectic grain in both the rapidly cooled and sodium-modified structures. In fact, the structure has not been 'modified' in the normal usage of this word. There is no change in the basic growth mechanism, and the structure is not globular. The silicon takes up a very fine interconnected acicular structure.

J. A. E. BELL
W. C. WINEGARD

Department of Metallurgy and Materials Science,
University of Toronto.

- ¹ Chadwick, G. A., *Prog. Mat. Sci.*, **12**, 2 (1963).
² Ghosh, S., and Kondic, U., *T.A. Foundrymen's Soc.*, **71**, 17 (1963).
³ Kim, C. B., and Heine, R. W., *J. Inst. Met.*, **92**, 367 (1964).
⁴ Thall, B. M., and Chalmers, B., *J. Inst. Met.*, **77**, 79 (1950).
⁵ Spengler, H., *Metall.*, **9**, 181 (1955).
⁶ Plumb, R. C., and Lewis, J. E., *J. Inst. Met.*, **86**, 393 (1958).
⁷ Davies, U. de L., and West, J. M., *J. Inst. Met.*, **92**, 175 (1964).
⁸ Discussion meeting, *J. Inst. Met.*, **93**, 233 (1965).
⁹ Tiller, W., *Liquid Metals and Solidification* (Amer. Sci. Met., 1958).
¹⁰ Hardy, H. K., *J. Inst. Met.*, **78**, 729 (1950).
¹¹ Patterson, W., *Giesserei*, **6**, 35E (1952).
¹² Gurtler, G., *Z. Metallk.*, **44**, 50E (1953).
¹³ Archer, R. S., and Kempf, L. V., *Trans. Amer. Inst. Mech. Eng.*, **73**, 581 (1926).
¹⁴ Boom, E. A., *Met. Abst.*, **20**, 81 (1952).
¹⁵ Scheuer, E., *Metal Indust.*, **49**, 556 (1936).

GEOLOGY

Re-classification of the Ethiopian Cainozoic Volcanic Succession

RECENT exploration and field-work in Ethiopia, in particular gravity-geology surveys made by Prof. P. Gouin, Dr. A. S. Rogers and me, have shown the Cainozoic volcanic succession in association with the Ethiopian Rift System to be more complicated than previously suspected.

The classic work of Blanford¹ established the existence of an older, extensive, pre-rift series of lavas, the Trap Series, covered by a younger, post-rift series of lavas, the Aden Series, the latter largely confined to the rift-system itself. Furthermore, Blanford divided the Trap Series into an earlier group of basalts, the Ashanghi Group, unconformably overlain by basaltic and silicic lavas and tuffs, the Magdala Group. This classification was generally accepted until the work of Merla and Minucci², who denied the existence of Blanford's unconformity, considering the tilting of the Ashanghi Group basalts to be due to post-Trappean tectonic movements. Merla and Minucci's revision of the nature of the Trap Series succession has been largely accepted by later workers, including Dainelli³ and myself⁴, though Blanford's terminology has been retained on the basis of lithology.

However, the recent discoveries of unconformities within the Trap Series in various parts of Ethiopia, by Jepsen and Athearn⁵, Rogers⁶ and me⁷, have led Rogers and me to a reconsideration of Merla and Minucci's arguments and a re-examination of the critical Ashanghi-Magdala region. The results of this new work (details to be published elsewhere⁸) have included the re-establishment of Blanford's unconformity, and the implications of this finding together with additional data from recent studies in the Ethiopian Rift System are summarized here.

The Trap Series, as defined by Dainelli², can now be divided into three major units: (3) great shield volcanoes of the Plateaux, composed of basalts, alkaline silicies and undersaturated lavas. Generally conformable on: (2) fissure and central-type basalts, with silicic lavas and pyroclasts becoming more abundant near the top. Unconformable on: (1) fissure basalts, with localized thick silicic lavas in Afar. Unit 1 is the Ashanghi Group of Blanford, and units 2 and 3 the Magdala Group. It would, however, be preferable to exclude unit 3 from the Magdala Group for reasons of age-range, localization and petrology. The term 'Shield Group' might be acceptable, and will be used here.

The re-establishment of Blanford's unconformity has led to the important finding that the Ashanghi Group lavas were down-warped (and flowed down?) into Afar and the Main Ethiopian Rift during and immediately subsequent to their extrusion^{4,8}. The sites of the fissure-feeders for these lavas coincided with the Plateau-Afar structural margin^{4,7}, where down-warping was particularly severe and complicated by strong north-south compressional movements. There is evidence at Amba Alaji (12°58' N., 39°32' E.) that these compressional movements had begun during the deposition of the now isoclinally folded Jurassic marine sediments^{8,10}. Furthermore, the Ashanghi lavas seem to have been largely confined to the region and margins of the down-warped proto-rift system; they have been dated as Eocene by Lipparini¹¹ and Grasty *et al.*¹².

The ensuing initiation of the Arabo-Ethiopian Swell in the Upper Eocene resulted in a moderate uplift of the whole region^{8,13} (cf. Dainelli², who considered the larger part of the uplift of this Swell to have occurred at this time). This uplift was followed, over most of Ethiopia, by a duration of volcanic quiescence sufficient for appreciable peneplanation to occur across the warped Ashanghi lavas along the Plateau-Afar margin, prior to the first Magdala Group eruptions. On the northern Somalian Plateau, however, there was either little or no break between the Ashanghi and Magdala eruptions, or else a very small degree of uplift in the Upper Eocene.

Unlike the Ashanghi lavas the Magdala lavas are thickly developed over much of the Plateaux away from the Rift System, perhaps because of their dominant extrusion from aligned centres on a gently sloping surface. These centres lay just outside the outer structural margin of Afar^{4,7} on a surface suffering contemporaneous slight upwarping towards the Rift System. Important fissure eruptions of basaltic and silicic lavas occurred along the inner structural margins of Afar, lavas which were included by Gortani and Bianchi¹⁴ as the earliest lavas of the Aden Series. The Magdala Group lavas have been dated as Oligocene and early Miocene^{3,12}.

In the Burdigalian-Helvetian period there occurred the major uplift of the Arabo-Ethiopian Swell together with rift faulting on a large scale^{13,15}. The great shield volcanoes, of which the culminating example is that of Semien¹⁶, were initiated at, or shortly after, this tectonic paroxysm. Despite the tectonic intervention, however, the lack of significant unconformity between the Magdala Group and the Shield Group indicates a continuing though modified single volcanic episode. The Shield Group volcanoes all lie outside and in many cases well away from the Rift System. Until more radiometric ages are available it remains uncertain what form, if any, volcanicity took within the Rift System during Shield Group times, but it seems likely that the upper part of the Magdala lavas of the Rift System are temporally (but not petrologically) equivalent to the Shield Group of the Plateaux. The Shield Group is dated as late Miocene-early Pliocene, but isolated volcanoes continued their activity throughout the Pliocene and even into the Pleistocene. A good example of such late activity is provided by the alkaline rhyolitic centre of Mt. Kakka on the Somalian Plateau.

As previously indicated by me⁴, there was no obvious interval between the last Trap Series eruptions and first Aden Series eruptions; indeed, recent work suggests an overlap and a corresponding difficulty drawing a precise line between the two, especially with the Rift System. The earliest lavas ascribed to the Aden Series are the Pliocene hyperalkaline, coarsely crystal lavas of which the famous Adua-Axum suite on northern Ethiopian Plateau is typical. These lavas aligned along approximately east-west lines.

Pleistocene volcanicity occurred throughout at least two phases of renewed uplift of the Arabo-Ethiopian Swell¹³, being characterized by violent silicic eruptions in the Rift System but by basalts on the Ethiopian and Somalian Plateaux. Such basalts dammed back Lake Tana in the down-warped and tilted Tana basin. In the Pleistocene and Holocene times transcurrent faulting along the Main Ethiopian Rift^{7,17} was accompanied by carbonatitic activity, while the latest lavas erupted from the fissures and volcanoes of the Rift System have been either scoriaceous olivine basalts or pantelleritic and comenditic obsidians.

No detailed table of the succession of lavas and tectonic events during the Cainozoic in Ethiopia is presented here as information on the Trap Series succession on the southern part of the Ethiopian Plateau is still being gathered. More important, as hinted in this communication, local variations and complexities both in lava thickness and types and in contemporaneous tectonic movements are proving to be more intricate than can be satisfactorily expressed in any correlation table at present. In this regard the recent work of Merla¹³ on erosive surfaces in south Ethiopia and north Kenya, and particularly his consideration that the Plateau surface is the result of post-Trappean peneplanation during the Oligocene, must be treated cautiously before being extended into central Ethiopia⁸. Indeed, the conclusions of different investigations of the behaviour of erosion surfaces in southern Ethiopia are so contradictory^{13,18,19} as to emphasize the need for a dispassionate resumé to indicate the critical regions needed for further examination.

Finally, while this article has been briefly concerned with the major Cainozoic volcanic episode in Ethiopia it must be noted that there was important volcanicity during Mesozoic times along the present Red Sea coast region. Both north of Massawa and east of the Danakil Alps andesitic lavas occur which are unconformably overlain by the thick Eocene-Miocene Dogali geosynclinal sediments of the proto-Red Sea trough; in the Danakil Alps these same lavas rest unconformably on late Jurassic limestones, thus suggesting an end-Jurassic or early Cretaceous phase of vulcanism.

P. A. MOHR

Geophysical Observatory,
Haile Sellassie I University,
Addis Ababa, Ethiopia.

¹ Blanford, W. T., *Observations on the Geology and Zoology of Abyssinia during the Progress of the British Expedition to that Country in 1867-68* (London, 1870).

² Merla, G., and Minucci, E., *Missione Geologica nell Tigris* (Rome, 1938).

³ Dainelli, G., *Geologia dell'Africa Orientale* (Rome, 1943).

⁴ Mohr, P. A., *Geophys. Obs. Addis Ababa Bull.*, **3**, 103 (1963).

⁵ Jepsen, D. H., and Athearn, M. J., in *Land and Water Resources of the Blue Nile Basin—Ethiopia* (Washington, 1962).

⁶ Rogers, A. S. (unpublished results).

⁷ Gouin, P., and Mohr, P. A., *Geophys. Obs. Addis Ababa Bull.*, **3**, 185 (1964).

⁸ Mohr, P. A., and Rogers, A. S., *Geophys. Obs. Addis Ababa Bull.* (in the press).

⁹ Dainelli, G., *Lexique Stratigraphique Intern.*, **4**, Fasc. 5c (Paris, 1956).

¹⁰ Mohr, P. A., *Geophys. Obs. Addis Ababa Bull.*, **3**, 33 (1962).

¹¹ Lipparini, T., *Serv. Geol. Ital. Bull.*, **76**, 95 (1954).

¹² Grasty, R., Miller, J. A., and Mohr, P. A., *Geophys. Obs. Addis Ababa Bull.*, **3**, 97 (1963).

¹³ Merla, G., *G. Geol. Bologna*, **31**, 1 (1963).

¹⁴ Gortani, M., and Bianchi, A., *Mem. R. Acc. Sci. Ist. Bologna*, **8**, 146 (1941).

¹⁵ Azzaroli, A., *Palaeont. Ital.*, **52**, 1 (1958).

¹⁶ Mohr, P. A., *Geophys. Obs. Addis Ababa Bull.*, **3**, 155 (1963).

¹⁷ Mohr, P. A., *Geophys. Obs. Addis Ababa Bull.*, **2**, 65 (1961).

¹⁸ Dixey, F., *Geol. Surv. Kenya Rep.*, **15** (1948).

¹⁹ Saggerson, E. P., and Baker, B. H., *Quart. J. Geol. Soc. Lond.*, **121**, 51 (1965).

Palaeomagnetism of the Isachsen Diabasic Rocks

In a previous article by two of us¹ it was suggested that a belt of diabasic rocks south of Isachsen in the Canadian Arctic Archipelago appeared to be of Cretaceous on the basis of its palaeomagnetism. On the other hand, whole rock potassium/argon age determinations on two of the samples studied (1-A and 10-B) indicated a Cretaceous age (241 and 249 m.y.), in conflict with the palaeomagnetic data. No reasonable explanation for this incompatibility could be advanced at the time and, since then, one of us (R. K. W.) has re-determined the age of two of the samples (10-B), using a mass spectrometer with higher sensitivity than was used in the original determinations. Another sample (9-B) was sent to the Geophysical Branch laboratories, U.S. Geological Survey, and a third one (9-A) was split and one half of it was sent to N. J. Snelling at the University of Oxford, while the other half was dated in the laboratories of the Geological Survey of Canada with the new high-sensitivity instrument. The ages obtained in these four whole rock determinations are listed in Table 1.

Table 1

Sample	Laboratory	% K	% ⁴⁰ Ar (radio-genic)	⁴⁰ Ar/ ⁴⁰ K	Age* (m.y.)
10-B	Geological Survey of Canada	0.93	39	0.00656	109
1-A	"	0.96	40	0.00660	110
1-A	Oxford (ref. 2)	0.94	39	0.00618	103
10-B	U.S. Geological Survey (ref. 3)	0.75	66	0.00612	102

Constants: $\lambda_{\beta} = 4.72 \times 10^{-10} \text{ y}^{-1}$; $\lambda_{\epsilon} = 0.585 \times 10^{-10} \text{ y}^{-1}$; $^{40}\text{K} = 0.0119 \text{ per cent.}$

It is apparent from the consistency of these determinations that the rocks dealt with in the original paper¹ are of Lower Cretaceous age and that the pole position derived from their mean palaeomagnetic direction should be considered as a fairly reliable one for Lower Cretaceous with respect to the North American Continent.

We thank Dr. N. J. Snelling of the University of Oxford and Dr. S. S. Goldich, formerly of the U.S. Geological Survey, for their assistance.

A. LAROCHELLE
R. F. BLACK
R. K. WANLESS

Geological Survey of Canada,
Ottawa.

Larochelle, A., and Black, R. F., *Nature*, **198**, 1260 (1963).

Snelling, N. J. (personal communication).

Goldich, S. S. (personal communication).

MINERALOGY

Interstratified Mineral of Illite and Montmorillonite

A YELLOWISH-GREY tuff, about 1 m thick, is distributed over a considerable area of the Sorachi coal field, Hokkaido, Japan¹, as a member of the Noborikawa coal-bearing formation. The tuff contains a small amount of quartz and plant fragments, but is generally homogeneous.

Table 1. X-RAY POWDER DATA OF 29 Å INTERSTRATIFIED MINERAL (ILLITE-MONTMORILLONITE)

Air dried	150°C	300°C	450°C	600°C	750°C	E.G.	G.	NH ₄ NO ₃
29.4	29.4	29.4	9.94	10.3	10.5	11.9	12.8	11.1
11.9	11.1	10.3				9.51	9.51	
						7.14		
5.07	5.04	5.01	4.95	5.04	5.10	5.13	5.90	5.13
4.51	4.51		4.48	4.51	4.51	4.46	4.46	4.48
					3.36	3.34	3.34	3.86
					(m)	(m)	(m)	
3.28	3.30	3.35	3.30	3.34				3.28

Values represent the maximum peak positions of reflexions.

(m) means that it may be multiple reflexions of quartz and interstratified mineral.

E.G., treated with ethylene glycol. G., treated with glycerol.

Having dispersed some of the material in water, the minus-two-micron fractions of the tuff were separated by the sedimentation method; Fig. 1 shows its electron micrograph. The X-ray powder patterns and data on the well-oriented specimens are shown in Fig. 2 and Table 1. The specimens were dried in air, heated at 150°C, 300°C, 450°C, 600°C,

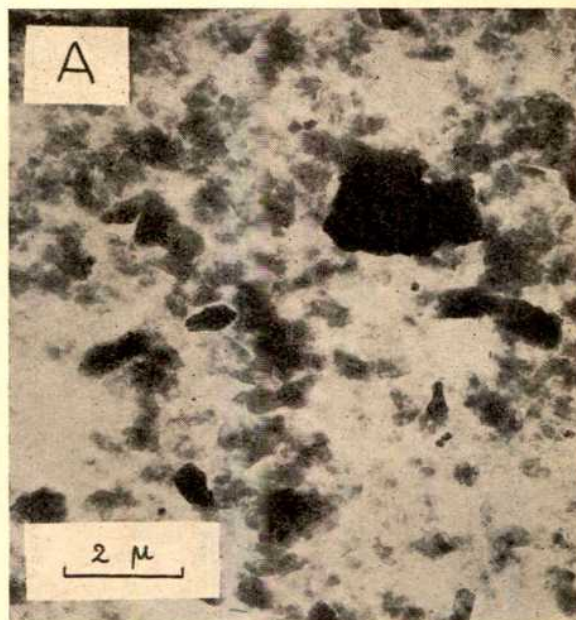


Fig. 1. Electron micrograph of 29 Å interstratified mineral.

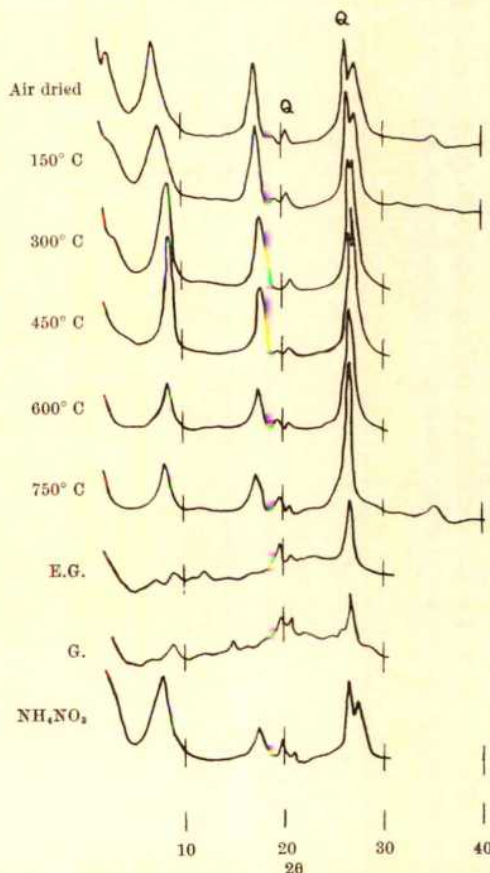


Fig. 2. X-ray powder patterns of the interstratified mineral. Q, reflexions due to quartz; E.G., treated with ethylene glycol; G., treated with glycerol. Operation conditions: Ni filtered copper radiation, 35 kV, 15 m.a.m.p. Scanning speed, 1°/min; scale factor, 16; multiplier, 1; time constant, 4; receiving slit, 0.4 mm.

Table 2. CHEMICAL COMPOSITION OF THE INTERSTRATIFIED MINERAL

	%		%
SiO ₂	52.64	MgO	2.22
TiO ₂	0.38	Na ₂ O	0.24
Al ₂ O ₃	27.77	K ₂ O	3.72
Fe ₂ O ₃	0.89	H ₂ O (110° C)	3.04
FeO	0.98	ig loss	6.50
MnO	0.02	Total	99.58
CaO	1.28		

Table 3. OBSERVED AND CALCULATED SPACINGS

Air dried		450° C	
<i>d</i> _{obs.}	<i>d</i> _{cal.}	<i>d</i> _{obs.}	<i>d</i> _{cal.}
29.4 Å	28.8 Å		
11.95	12.02	9.94 Å	9.94 Å
5.07	5.08	4.95	4.965
3.25	3.247	3.30	3.309

The calculation is based on the model shown in Fig. 4.

750° C, for 1 h, and treated with ethylene glycol, glycerol and ammonium nitrate. The chemical compositions and differential thermal analysis curves of both natural and piperidine-treated specimens are shown in Table 2 and Fig. 3.

All these data correspond to those of the so-called random interstratified minerals of illite and montmorillonite reported by many investigators, but the X-ray reflexion at about 29 Å in the natural specimen (uppermost in Fig. 2) cannot be expected from the random structure. Sato has proposed² that the interstratified mineral would take its layer arrangements to minimize the total interaction energy, and that it should be treated for at least Rechweite

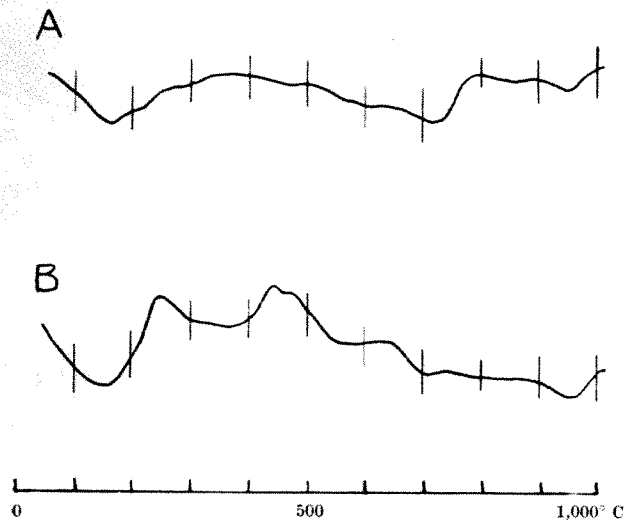


Fig. 3. Differential thermal analysis curves of the interstratified mineral. A, Natural; B, treated with piperidine

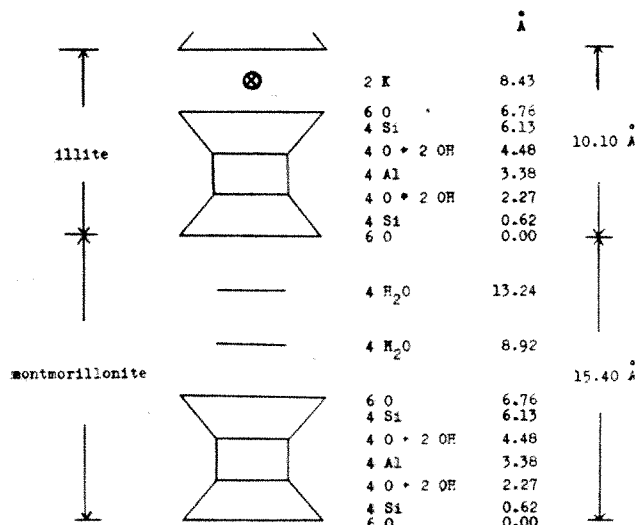


Fig. 4. Illite and montmorillonite models used in calculation of X-ray intensity

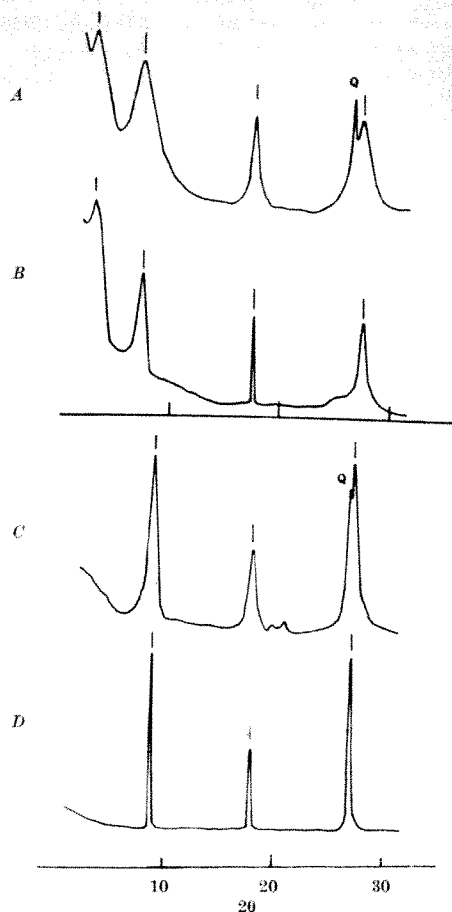


Fig. 5. X-ray reflexion curves along (00ζ). A and B, air dried, observation, calculation, *N* = 60 (total number of layers); C and D, heated at 450° C, observation, calculation, *N* = 150

g = 1, not *g* = 0 (random). With this in mind, X-ray reflexion curves along (00ζ) in the air-dried specimen and the specimen heated at 450° C—the intensity of which was calibrated at low angles in consideration of the slit system used—were carefully compared with mathematical curves. The calculation was based on the models shown in Fig. 4. As a consequence, it was found that the specimens were interstratified mineral of illite and montmorillonite, which the component of illite (*W_I*) is 0.72, that of montmorillonite (*W_M*) is 0.28, the probability of finding illite succeeding illite (*P_{II}*) is 0.611, and the probability of finding montmorillonite succeeding montmorillonite (*P_{MM}*) is 0.0. The result is shown in Fig. 5 and Table 3 (and graph in ref. 2).

We thank Prof. T. Sudo for his advice.

MITSUO SATO
KAORU OINUMA
KAZUO KOBAYASHI

Geological and Mineralogical Institute,
Faculty of Science,
Tokyo University of Education.

¹ Kobayashi, K., and Oinuma, K., *J. Geol. Soc. Japan*, **779**, 506 (1960).
² Sato, M., *Nature*, **208**, 70 (1965).

CRYSTALLOGRAPHY

Pairs of Spiral Etch Pits in Ice Crystals

In an earlier communication¹, I reported observations of spiral etch pits in ice crystals which had quite large step heights (200–2000 Å). At that time no detailed explanation of their origin was offered; but in the course of further investigations² of ice crystal etch pit, these etch pits were postulated to correspond to dislocations. I

ny crystals, however, the step height of the spiral etch is quite large in comparison with the Burgers vector, and it is thus impossible to determine the causal factor³. Recently, an attempt was made to verify the mechanism involved in etching in ice crystals, and it is found that pairs of etch pits may develop on the {001} surface in which the pairs consist of one right-handed and one left-handed spiral. These are often remarkably similar to those one would expect from a Frank-Read source (Figs. 1 and 2). Sometimes, pairs of pits with a unidirectional spiral are observed (Fig. 3). It is of interest to note that each pair of etch pits is closely aligned to crystallographic orientations such as $\langle 10\bar{1}0 \rangle$ or $\langle 11\bar{2}0 \rangle$. In Figs. 1 and 3, the distance between a pair of pits is about 8μ and in Fig. 2 the distance is 50μ . If we assume that these spiral etch pits correspond to screw dislocations lying on a prism plane, the Burgers vector should be $\langle 0001 \rangle$, which vector, although possible, has never been reported in ice crystals or in any other hexagonal crystals. If this assumption is correct, the existence of paired etch pits consisting of one left-handed and one right-handed spiral implies a dislocation half loop on a prism plane (Figs. 1 and 2). With regard to the large angle of the pitch of the spiral, one interpretation, based on the presence of a dislocation or a helical dislocation, has been given by Ellis⁴ and Amelinckx *et al.*⁵, and another by Lang⁶ which is not based on the presence of dislocations. It is possible that the large step heights observed are not formed from screw dislocations with Burgers vectors of this magnitude, but develop during the etching process from aggregations of asymmetrical spirals which develop about the point on the surface at which

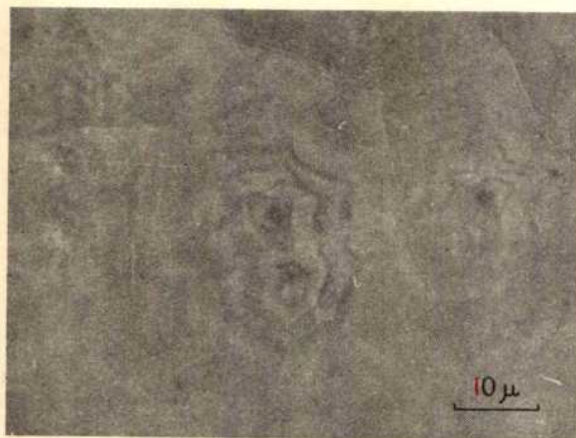


Fig. 1. A pair of etch pits on a {0001} plane. Note that one is right-handed and the other is left-handed

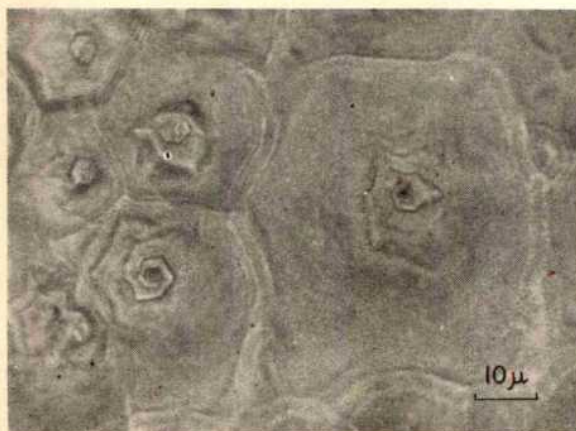


Fig. 2. A pair of fairly widely separated etch pits on a {0001} plane. One spiral is right- and the other left-handed



Fig. 3. A pair of etch pits with unidirectional spirals

the dislocation emerges. This closely resembles the mechanism proposed by Amelinckx *et al.*⁵ which requires a helical dislocation; but there is no reason why a screw dislocation could not develop such a spiral. Although this appears to be a plausible explanation of the large angle of pitch observed, further detailed investigation is required for any definite conclusions.

Recently, the first direct observation of dislocation in ice crystals has been reported by Hayes and Webb⁷, using Lang's X-ray transmission method. They found that dislocation segments in the (0001) plane are quite long and are oriented parallel to $\langle 10\bar{1}0 \rangle$ and $\langle 11\bar{2}0 \rangle$. Judging from the results of direct observations and from successive etching of basal surface, which give a three-dimensional picture of the dislocation arrays, the non-basal segments of these dislocations are probably very short, and one may expect that there would be a long distance between a pair of pits.

If this speculation is correct, dislocations with $\langle 0001 \rangle$ Burgers vectors may exist in ice crystals and may provide a clue to an understanding of some unexplained observations of dislocation movement in ice crystals.

J. MUGURUMA

Department of Applied Physics,
Hokkaido University,
Sapporo, Japan.

¹ Muguruma, J., *Nature*, **190**, 37 (1961).

² Muguruma, J., and Higashi, A., *Nature*, **198**, 573 (1963); *J. Phys. Soc. (Japan)*, **18**, 1261 (1963).

³ Johnston, W. G., *Progress in Ceramic Science*, **2**, 34 (Pergamon Press, 1962).

⁴ Ellis, S. G., *Phil. Mag.*, **2**, 1285 (1957).

⁵ Amelinckx, S., Bontinck, W., and Dekeyser, W., *Phil. Mag.*, **2**, 1264 (1957).

⁶ Lang, A. R., *J. Appl. Phys.*, **28**, 497 (1957).

⁷ Hayes, C. E., and Webb, W. W., *Science*, **147**, 44 (1965).

CHEMISTRY

Simultaneous Oxidation and Reduction of Diphenyl Picrylhydrazyl on Surfaces: Evidence for Catalytic Activity of F-centres

We have recently found that diphenyl picrylhydrazyl undergoes intermolecular oxidation and reduction quite rapidly on carbon black (Cabot: 'Carbolac 2') and on a high area TiO_2 (anatase) which has almost a monolayer of sulphuric acid on its surface.

With 2 g of diphenyl picrylhydrazyl dissolved in 250 c.c. of benzene and 25 g of anatase (263 sq. m/g. containing 7.55 wt. per cent of sulphuric acid) the reaction is more than 90 per cent complete in 90 min.

The non-volatile products obtained after filtering and distilling off the benzene are dissolved in chloroform. The benzene distillate contains considerable quantities of quinone. The non-volatile products can be seen to contain yellow needles of crystalline quinone which can be

picked out (m.p. 114°–115°, mixed m.p. with quinone 114°–115°). Separation was performed by thin-layer chromatography.

The main product is diphenyl picerylhydrazine, m.p. 169°–170°, mixed m.p. with diphenyl picerylhydrazine 169°–170°, and in most runs the amount of it corresponds to a reduction of 75 per cent of the radical. This compound results from self-reduction of the hydrazyl radical while the quinone results from simultaneous oxidation of another molecule of the radical in consecutive steps. 2',4',6'-trinitro-4-aminodiphenylamine (piceryl phenylenediamine) is isolated from one of the bands almost pure; usually about 0.10 g, m.p. 142°–143° (found: C, 45.65, 45.83; H, 2.90, 2.67; N, 21.50, 21.24; mol. wt. (Rast 290); calculated for $C_{12}H_9N_3O_6$: C, 45.15; H, 2.84; N, 21.94; mol. wt., 319).

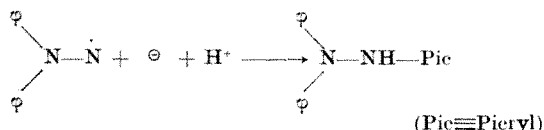
The only obvious way to make this compound is by the action of piceryl chloride on phenylenediamine, but this has been shown to yield a high melting product.

According to Wedekind¹, the product is an addition product (found: C, 40.90; H, 3.14; N, 19.91). He states that this corresponds to the addition product $C_{12}H_{10}N_3O_6Cl$ (calc. C, 40.48; H, 2.81; N, 19.73). Wedekind's work was repeated by Morgan and Micklethwait². The product has a melting point of 185°–187° and forms a sparingly soluble salt with strong hydrochloric acid, but they give no analysis.

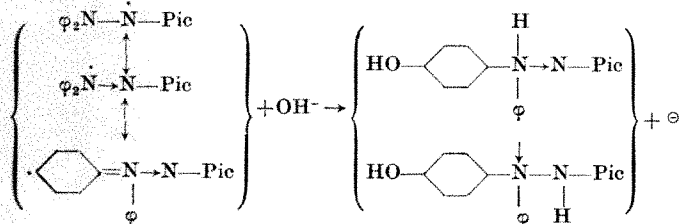
A consideration of the possible resonance forms involving the para groups would lead one to expect that the salt would be unstable. Attempts to hydrolyse the compound, m.p. 142°–143° (which analyses for *N*-piceryl phenylenediamine), to its components yielded a mixture of alkali and acid insoluble products.

The following series of reactions is one explanation of what has obviously happened.

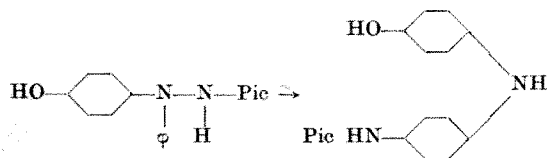
Reduction :



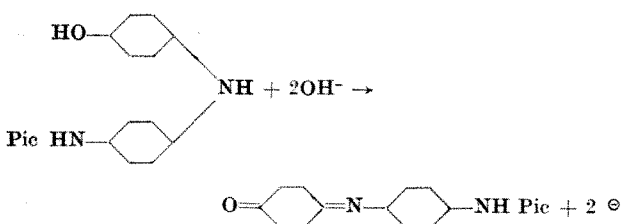
Oxidation :



Rearrangement :



Oxidation :

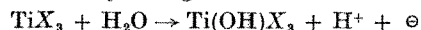


The two oxidation steps require two further moles hydrazyl yielding a product which is the *N*-piceryl substituted indanilin. Hydrolysis yields quinone and piceryl phenylenediamine as found. It could also yield picramid and *p*-aminophenol. If the first oxidation step involves the ortho position the indanilin then has an ortho-quinoid linkage and would yield orthoquinone, a difficult substance to isolate. If the rearrangement (equivalent) of the *p*-hydroxydiphenyl piceryl hydrazine involves the ortho position of the phenyl group, processes involving a cyclic intermediate are possible. Likewise the *p*-hydroxy diphenyl piceryl hydrazine could be oxidized to the substituted hydrazyl. The total amount of *N*-piceryl diphenylamine, if all the reaction went as outlined should be 0.4 g.

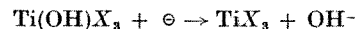
The reaction occurs very slowly on sulphuric-acid-free TiO_2 (anatase) and silica gel. It occurs rapidly in sulphuric acid saturated benzene. On 'Carbolac 2' without any addition of acid or water the reaction occurs at one-fifth the rate of that on the anatase (7.5 per cent sulphuric acid), yielding the same products.

During the reaction on the anatase no sulphuric acid was removed. Results to be reported elsewhere show that on all surfaces the reaction is first order in the radical. This indicates a mechanism involving mobile *F*-centres or the equivalent as the only possibility.

The triggering reaction must be the addition of an electron to the hydrazyl in the reduction part, since the oxidation part involves consecutive reactions. On the anatase the reaction yielding the electron is:



where *X* represents any group covalently linked to titanium. The reaction taking the electron in the oxidation part is:



These two reactions yield a stoichiometric balance with the reduction and oxidation equations written here. The first involves the destruction of an *F*-centre which must appear elsewhere if the surface is mobile. The electron to produce an *F*-centre comes from the oxidation step.

This work was supported by a grant from the U.S. National Science Foundation.

JOHN G. ASTON
DWARIKA N. MISRA
KATHERINE M. CRESSWELL

Department of Chemistry,
Pennsylvania State University,
University Park.

¹ Wedekind, E., *Ber.*, **33**, 435 (1900).

² Morgan, G. T., and Micklethwait, F. M. G., *J. Chem. Soc.*, **93**, 608 (1908).

Correlation of Sodium Atom Reaction Rates with Electron Capture Cross-sections

THE classic Polanyi flame work^{1,2} explored the reactions of sodium atoms with a very wide range of halogen compounds. For some of these the rate constants were found to be extremely large ($\sim 10^{14}$ c.c. mole⁻¹ sec⁻¹), corresponding to "reaction on more than every gas-kinetic collision". This was attributed to an electron-jump mechanism,



Here essentially all collision trajectories that pass within the radius r_c at which formation of the ion-pair becomes possible lead to reaction³. This radius is given by $e^2/r_c = I(Na) - E^v(X-R)$, where *I* denotes the ionization potential of the alkali atom and E^v the vertical electron affinity of the halogen molecule. Recently, several alkali atom reactions of this type (with $X-R = Br_2, ICl, PBr_3, CBr_4, SnCl_4, SF_6$, etc.) have been examined in molecular beam scattering experiments⁴. The form of the angular distribu-

ns of elastic and reactive scattering and other detailed nautical properties observed in these experiments can very plausibly interpreted in terms of the electron-jump mechanism.

However, a much larger class of 'reactions with inertia' is also examined in the Polanyi sodium flame experiments. These cover a broad spectrum of reactivity and many proceed at rates which are $\sim 10^4$ times slower than action at every collision^{1,2}. The simple electron-jump mechanism cannot apply to these reactions, as the cross-sections correspond to such small values of r_c that other chemical interactions must become very important.

is therefore of particular interest to note a simple relation which indicates that even for this class of actions the electron affinity of the reactant molecule plays a dominant part.

Recently, Lovelock has estimated electron absorption coefficients for many compounds from gas chromatography³. Under the conditions of measurement this coefficient is a thermodynamic parameter. It governs the thermal equilibrium between electrons, ions, and neutral molecules and is essentially a measure of the adiabatic electron affinity⁴. (The difference between the vertical and adiabatic electron affinity becomes irrelevant for reactions with small values of r_c as the bond distances in the transition state are expected to be considerably displaced from the equilibrium values in the isolated reactant molecule.) The electron absorption coefficients show systematic variations with chemical structure which Lovelock has mapped out. His results are remarkably similar to those found for Na atom reaction rates in the Polanyi experiments. For example, the following factors markedly enhance both capture of thermal electrons and reaction with Na atoms: (1) change of the halogen atom, in the order $F \rightarrow Cl \rightarrow Br \rightarrow I$; (2) presence of an oxygen atom in addition to the halogen; (3) multiple substitution which increases the number of electrophoric groups or brings them closer together; (4) location of the halogen atom at an allylic or benzylic position (double bond in β -position to X atom). Also, the rates for both processes are depressed when (5) the halogen atom is adjacent to an ethylenic double bond or a benzene ring (double bond in α -position to X atom).

Only order-of-magnitude values are known for both the electron absorption coefficients and the Na reaction rate constants. However, the correlation holds for a wide variety of compounds and in the case of factors (1) and 3) covers a range of $\sim 10^6$ in reactivity. Fig. 1 gives a

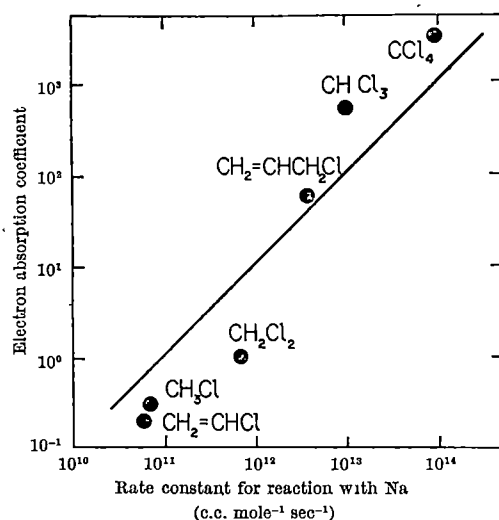


Fig. 1. Correlation between electron absorption coefficients (at 100° C) and rate constants for reaction with sodium atoms (at 250° C). The line is included merely to indicate a slope of unity. The values of the electron absorption coefficients are relative to that for chlorobenzene, which is taken as unity.

comparison for a few chlorinated hydrocarbons and illustrates the operation of factors (3)–(5). The values given for the electron absorption coefficients⁵ are relative to that for chlorobenzene. Approximate absolute values can be assigned since the cross-section for chlorobenzene has been estimated⁷ to be ~ 0.1 to 1 Å^2 . Thus an absorption coefficient of 10^3 corresponds to a cross-section of about $100\text{--}1000 \text{ Å}^2$. For the Na reactions the cross-sections can be roughly estimated from the ratio of the rate constant to the average thermal velocity; a rate constant of 10^{14} corresponds to a cross-section of about 30 Å^2 .

The extended parallelism between electron affinity and reactivity indicated by this correlation is perhaps to be expected from familiar arguments concerning stabilization of the transition state by charge delocalization^{8,9}. For these simple gas phase reactions, it seems likely that the factors (1)–(5) are mainly governed by delocalization. Although these factors sometimes operate in a qualitatively similar way for reactions in solution, they are often overcome by other effects⁹.

We thank Dr. J. E. Lovelock for his advice. This work was supported by the U.S. National Science Foundation.

K. R. WILSON
D. R. HERSCHBACH

Department of Chemistry,
Harvard University,
Cambridge, Massachusetts.

¹ Polanyi, M., *Atomic Reactions* (Williams and Norgate, London, 1932).

² A useful review is given by Trotman-Dickenson, A. F., *Gas Kinetics*, 212 (Butterworths, London, 1956).

³ Magee, J. L., *J. Chem. Phys.*, **5**, 687 (1940).

⁴ A review is given by Herschbach, D. R., in *Advances in Chemical Physics*, **8**, edit. by Ross, J., and Prigogine, I. (Interscience, New York, 1965).

⁵ Lovelock, J. E., and Gregor, N. L., in *Gas Chromatography*, edit by Brenner, N., Callear, J. E., and Weiss, M. D. (Academic Press, New York, 1962).

⁶ Wentworth, W. E., and Becker, R. S., *J. Amer. Chem. Soc.*, **84**, 4263 (1962).

⁷ Lovelock, J. E. (personal communication).

⁸ Evans, M. G., and Polanyi, M., *Nature*, **148**, 436 (1941).

⁹ See, for example, Streitwieser, A., *Solvolytic Displacement Reactions* (McGraw-Hill, New York, 1962).

A Preparation of Cementite

SEVERAL methods for the preparation of the carbides of iron have been reported in the literature. In order to obtain a satisfactory product it may be necessary to use a relatively high temperature¹, or long periods of reaction during which reaction conditions must be controlled within narrow limits for several weeks^{2,3}. Using these methods we found considerable difficulty in preparing a sample of iron carbide which was sufficiently pure to be used in a projected study. During preliminary investigations of reactions of transition metal salts of organic acids, however, it was noticed that the solid residue from the thermal decomposition in vacuum, at about 500° C, of a ferric salt of mellitic acid gave an X-ray diffraction pattern characteristic of cementite. This communication is concerned with the use of this compound for the preparation of cementite. A detailed report of the kinetics of the thermal decomposition reaction will be published elsewhere⁴. Studies of the thermal decomposition of ferric salts of benzoic and of phthalic acids showed that no detectable cementite was present in the non-volatile crystalline products⁵.

For use in the preparation of cementite the ferric salt of mellitic acid was formed by heating two moles of ferric hydroxide with one mole of mellitic acid in a little water at 90° C for 30 min. The ferric hydroxide had been freshly precipitated from ferric chloride solution by the addition of ammonium hydroxide (both 'AnalaR'), boiled and washed by decantation six times. For the present study five preparations of ferric mellitate were investigated, in which the tan-coloured precipitates formed as above were treated as follows:

Sample *A*, filtered immediately after cooling, but not washed.

Samples *B1* and *B2*, washed by decantation; the precipitate remained tan coloured.

Samples *C1* and *C2*, the wet product washed with distilled water on the filter paper. Part of the product darkened, suggesting that some hydrolysis occurred.

Each batch of material was dried and the elements determined by combustion analyses. Iron was calculated from the weight of the residue from ignition in oxygen, which was assumed to be Fe_2O_3 only, and oxygen was found by difference. Results are shown in Table 1. The relatively large iron content of samples *C1* and *C2* is consistent with the foregoing view that some hydrolysis of the product has occurred, possibly yielding some ferric hydroxide.

Table 1. ANALYSES OF IRON SALT OF MELLITIC ACID

Sample	Fe	C	H	O
<i>A</i>	20.78	24.91	2.57	51.74
<i>B1</i>	20.45	24.61	2.53	52.41
<i>B2</i>	20.84	23.91	2.64	52.61
<i>C1</i>	23.51	24.05	2.48	49.93
<i>C2</i>	24.06	24.13	2.52	49.29

The non-volatile products remaining after thermal decomposition of samples of the foregoing salts between 470° and 500° C *in vacuo* or in contact with the gaseous products (excluding water) were pyrophoric, and considerable difficulty in handling was experienced. Products from decompositions between 450° and 470° C did not so readily oxidize in air. Decomposition of samples of salts *C1* and *C2* gave solid products which X-ray examination showed to contain appreciable amounts of Fe_2O_3 and/or Fe_3O_4 together with a diffuse pattern attributable to cementite.

Samples of preparations *A*, *B1* and *B2*, when decomposed for intervals between the limits 6 h at 460° C and 1 h at 500° C, gave cementite as the main crystalline product, but this was only observed for those samples in which atmospheric oxidation was prevented by chilling the solid to -195° C before admission of air to the apparatus, and subsequent storage under benzene. Samples for X-ray investigation were thoroughly mixed with a glue before evaporation of the benzene. Small traces of oxide were detected in the products from those reactions in which the gaseous decomposition products were allowed to remain in contact with the reactant during decomposition.

Samples of cementite, having X-ray diffraction patterns almost identical with a sample of cementite supplied by Dr. L. J. Dry of the S.A. Coal, Oil and Gas Corporation, Ltd., Republic of South Africa, were obtained by decomposition of 15 mg of sample *B1* and of 2 g of sample *B2* at 480° C with continual evacuation of products. Both samples were pyrophoric and an attempt was made to prevent atmospheric oxidation by the methods already described.

Determination of the surface area of the product cementite, from measurements of the adsorption of nitrogen at -195° C and application of the Brunauer-Emmett-Teller equation, showed that both sample *B1*, after decomposition 2 h 480° C with accumulation of gaseous products, and sample *A*, after decomposition 2 h 470° C with continual evacuation, had surface areas $240 \pm 10 \text{ m}^2 \text{ g}^{-1}$. Due to difficulties in handling in air we cannot be certain that no oxidation of these samples occurred, but both results show that a high surface area product is given.

The detailed mechanisms of the decomposition reactions are discussed elsewhere⁴, and only particular points arising from the results presented here will be considered:

(1) The decomposition product contains excess carbon; the composition is believed to be $\text{Fe}:\text{C}::1:2.6$. It is probable that a layer of finely divided carbon over the crystallite surfaces reduces sintering, so stabilizing the high-area product observed.

(2) The product from decomposition in the presence of gaseous oxides of carbon was indistinguishable from that observed from decomposition with continual evacuation. It seems probable, therefore, that the carbide was formed by the interaction of the finely divided iron-carbon mixture (the latter being the non-volatile product from the aromatic ring present in the acid radical) rather than a mechanism in which finely divided iron reacted with gaseous carbon monoxide.

(3) It is suggested⁴ that the decomposition of the ferric salt of mellitic acid 400°-500° C may involve reaction between a carbonaceous residue and an iron oxide. The foregoing results for samples *C1* and *C2*, where it is believed that ferric oxide was present in the reactant, suggest that the presence of this oxide may modify the course of the decomposition reaction to yield a product containing an appreciable amount of oxide. The mechanism described⁴, however, may be applicable to samples containing lower oxides of iron where ferric hydroxide was absent from the reactant.

Cementite may therefore be prepared by the method outlined here. Two disadvantages of the method are that the product contains excess carbon ($\text{Fe}:\text{C}::1:2.6$) and that the pyrophoric material is difficult to handle. Advantages of the method are that cementite may be prepared relatively rapidly at a low temperature (namely < 500° C) in a glass apparatus.

One of us (J. F.) held a postgraduate studentship awarded by the Ministry of Education, Northern Ireland.

J. FREEL

A. K. GALWEY

Department of Chemistry,
The Queen's University of Belfast.

¹ Ephraim, F., *Inorganic Chemistry*, 869 (Gurney and Jackson, 1949)

² Bahr, H. A., and Jessen, V., *Ber.*, **66**, 1238 (1933).

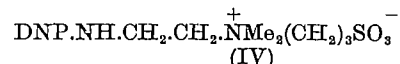
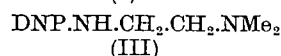
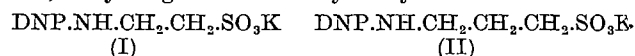
³ Hofer, L. J. E., *The Preparation and Properties of Metal Carbides* (U.S. Bureau of Mines R.I. 3770, 1944).

⁴ Galwey, A. K., (to be published).

⁵ Galwey, A. K., *J. Chem. Soc.*, 4235 (1965).

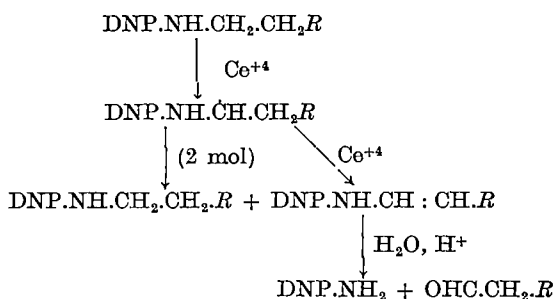
Oxidation of Some 2,4-Dinitrophenylamines with Ceric Salts

DURING an investigation into the mechanism of cerium-initiated graft polymerization of acrylic acid on to nylon, the behaviour of the dinitrophenylated product led us to examine the oxidation with ceric salts of some model 2,4-dinitrophenylamino-compounds. Since we are unable to complete the work, we are reporting the preliminary results, which may arouse interest elsewhere in the possible scope and mechanism of this reaction. The compounds examined were the potassium salts of DNP taurine (I, m.p. 249°-252° C) and 3-DNP-aminopropane sulphonic acid (II, m.p. 231°-233° C), the base (III, m.p. 71°-73° C) and its quaternary derivative (IV, m.p. 276°-277° C), obtained in 70 per cent yield by treatment of III with 1,3-propanesultone in boiling acetonitrile for 7 h; they all gave satisfactory analyses.



Oxidation at room temperature of each DNP-amine (0.01 g-mol) in water (50 ml.) or, for III, N sulphuric acid (50 ml.) with a solution of ceric ammonium sulphate (0.03 g-mol.) in N sulphuric acid (100 ml.) rapidly gave a yellow precipitate, which was isolated after 1 h and shown to be 2,4-dinitroaniline by comparison of its m.p. and infra-red spectrum with those of an authentic specimen. The yields were 95, 88, 75 and 70 per cent, respectively. Limiting the amount of oxidant to 1 mol per mol DNP-amine resulted in only an approximate halving

the yield of 2,4-dinitroaniline and no intermediate product was detected. Taurine did not reduce ceric ammonium sulphate significantly in 7 days at 25° C. Rates of oxidation at 25° C (Fig. 1) were determined by titrating the compounds (0.001 g/mol), dissolved in water with 0.001 N sulphuric acid (50 ml.), with ceric ammonium sulphate (0.005 g/mol) in N sulphuric acid (50 ml.) and titrating aliquots (10 ml.) of the reaction mixture with 0.005 N ferrous ammonium sulphate at suitable intervals. These curves indicate that the rapid primary oxidation of 2,4-dinitroaniline requires two equivalents of cerium, and if an analogy can be drawn with the oxidation of alcohols by ceric salts¹ or of alkylamides by perchlorates² one possible reaction path involves initial formation of a radical by abstraction of hydrogen at the carbon atom; disproportionation of this yields an amine, which is then hydrolysed. Alternatively the amine may result from direct attack of the radical by a second cerium ion:



Since the solutions were not de-aerated, routes involving oxidation of a peroxide derived from the initial radical and oxygen could also be implicated. Further, slow oxidation of the aldehyde would account for consumption of cerium beyond two equivalents.

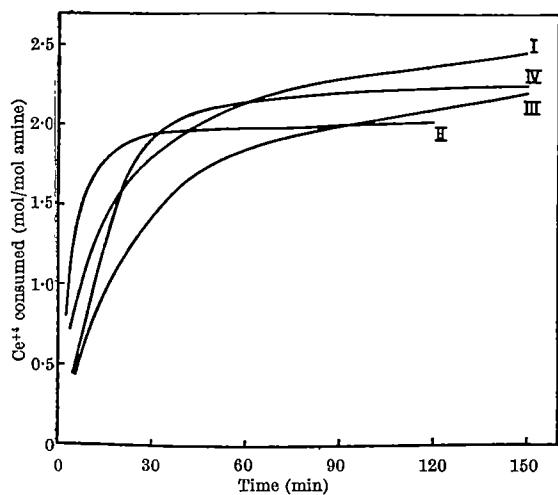


Fig. 1. Oxidation of the 2,4-dinitrophenylamines I-IV with ceric ammonium sulphate at 25° C

The solutions obtained from oxidation of compounds I-III with 3 equivalents of ceric ammonium sulphate, after removal of dinitroaniline and cerium salts, on treatment with 2,4-dinitrophenylhydrazine in 5 N hydrochloric acid at 25° C yielded derivatives which, after recrystallization, melted with decomposition at 239°-244°, 202°-203° and 158°-159° respectively, and gave elemental analyses differing greatly from those of the expected aldehyde 2,4-dinitrophenylhydrazones. However, a concentrated solution from oxidation of compound (III) gave glyoxal bis-2,4-dinitrophenylhydrazone when boiled with the reagent in N hydrochloric acid, as did the authentic diethyl acetal of dimethylaminoacetaldehyde³, thus affording some confirmation that this aldehyde is a product of the oxidation.

We thank Dr. H. R. Cooper and Mr. B. F. Sagar for their advice.

SUSAN HAWORTH
J. R. HOLKER

Cotton Silk and Man-made Fibres
Research Association,
Didsbury, Manchester, 20.

¹ Mino, G., and Kaiserman, S., *J. Polymer Sci.*, **31**, 242 (1958).

² Needles, H. L., and Whitfield, E. E., *J. Org. Chem.*, **29**, 3632 (1964).

³ Jones, L. A., Hancock, C. K., and Seligman, R. B., *J. Org. Chem.*, **26**, 228 (1961).

BIOCHEMISTRY

Enzyme Regulatory Mechanisms at the Level of Lactate-oxidoreductase in Erythrocytes and Ascites Tumour Cells

In recent years several attempts have been made at a complete analysis of metabolic pathways. In comparing complex systems, be they intact cells or homogenates, with isolated enzymes, both similarities and differences¹⁻³ have been found. So far no single pathway has been completely elucidated with respect to its kinetics. For this reason we turned to the erythrocyte, as representing both structurally and metabolically one of the simplest biological systems. One line of research which is to be reported here was concerned with the regulation of glycolysis on the level of lactate oxidoreductase. These studies were extended to ascites tumour cells. In view of the generally high activity of the lactate oxidoreductase (LDH) it may be assumed that the lactate/pyruvate system is at equilibrium within the cell¹. Recently, however, deviations from the expected lactate/pyruvate ratios have been observed in brain homogenates³. We explored this question in a more detailed manner by studying the effect of pH on the formation of pyruvate and lactate.

The pH dependence of the lactate/pyruvate ratio. From the stoichiometry of the LDH reaction one would expect a pH dependence of the lactate/pyruvate ratio such that with increasing pH this ratio is diminished. Table I shows that the experimental results do not correspond to this assumption. As a matter of fact, in erythrocytes just the reverse is observed. For further study of these paradoxical observations the pH dependence of the lactate/pyruvate ratio of stroma-free dialysed haemolysates and of particle-free homogenates of ascites tumour cells was studied. The formation of pyruvate from added NAD and lactate was determined at varying pH values. The amounts of pyruvate observed were compared with calculated values based on the equilibrium constant of LDH. The results were the same as with intact cells. While there was approximate agreement between expected and experimental pyruvate formation at pH 8.4, twenty times the expected value of pyruvate was formed at pH 6.4. To explain these results we postulated the existence of an oxidation factor in erythrocytes and ascites tumour cells which disturbs the lactate/pyruvate equilibrium particularly at lower pH-values.

NADH₂- and NADPH₂-oxidases in erythrocytes and ascites tumour cells. Further experiments have indeed demonstrated, both in erythrocytes and in ascites tumour cells, extramitochondrial enzymes which oxidize NADH₂.

Table 1. LACTATE/PYRUVATE RATIO IN ERYTHROCYTES AND ASCITES TUMOUR CELLS

pH	10 ⁻³ moles/l.				Lactate/ Pyruvate	
	Eryth.	Ascites tumour cells	Eryth.	Ascites tumour cells	Eryth.	Ascites tumour cells
6.4	2.57	28.3	0.148	1.47	17.3	19.2
7.0	—	30.6	—	1.34	—	22.8
7.4	3.03	35.5	0.089	1.30	33.7	27.3
8.0	—	34.5	—	1.08	—	31.9
8.4	4.86	—	0.033	—	148.0	—

Human erythrocytes were incubated with 0.2 M tris-maleate buffer. Ascites tumour cells were incubated in Ringer-phosphate-bicarbonate solution at 37.5° C. The glucose concentration was 250 mg/100 ml.

Table 2. OXIDASE ACTIVITIES WITH NADH₂ AND NADPH₂ IN ERYTHROCYTES AND ASCITES TUMOUR CELLS

	Enzyme activity 10 ⁻³ moles/l.cells/min	
	NADH ₂	NADPH ₂
Human erythrocytes	0.102	0.177
Rabbit erythrocytes	0.113	0.099
Ascites tumour cells	0.017	0.018

The oxidases were measured spectrophotometrically at 340 nm, after 48 h dialysis in 0.1 M phosphate buffer, pH 6.4.

and NADPH₂ (Table 2). In Fig. 1 the pH-dependence of the erythrocyte oxidases is shown in a haemoglobin-free haemolysate of rabbit erythrocytes. It may be seen that both enzyme activities are optimal at pH 6.4. The NADH₂ oxidase activity is inhibited by higher concentrations of its substrate; with 1×10^{-4} M NADH₂ inhibition was practically complete. The inhibitory effect of NADPH₂ on its oxidase was much less pronounced. Under anaerobic conditions the oxidase activities were low whereas cyanide had no effect. From these observations it would appear that the oxidases are flavin enzymes. Formerly the existence of NADH₂- and NADPH₂-dehydrogenases (diaphorases) had been demonstrated in haemolysates¹¹. It might well be that the oxidases here described, and the dehydrogenases, are identical. On the other hand, it could be demonstrated that the oxidation of NADH₂ and NADPH₂ is mediated by at least two different enzymes. From the results in Table 3 it may be seen that a significant separation of the two activities could be obtained, so that a more than twenty-fold shift in the oxidase activity ratios of NADH₂ and NADPH₂ could be obtained. The two enzyme activities also differed in their stability towards lyophilization and dialysis, the NADPH₂ oxidase being the more sensitive. The dehydrogenase activities resembled the oxidases. The activity of these ratios was shifted about fifty-fold by the fractionation. The ratio of the oxidase to diaphorase activity was significantly higher with NADPH₂ as compared with NADH₂. Further purification of the enzymes is in progress.

The lactate-NADP-oxidoreductase in erythrocytes and in ascites tumour cells. The literature contains several reports concerning the reactivity of LDH of different tissues with NADP. With purified enzymes, various ratios of activity with NADP as compared with NAD have been reported, ranging from 0.003 to 0.05. In other communications the importance of this reaction for the generation of NADPH₂ for biosynthesis has been assessed^{4,5}. We have performed similar experiments on erythrocytes and ascites tumour cells. Table 4 summarizes the data from the literature together with our own results. It may be seen that erythrocytes have the highest activity with NADP which has been observed so far. The variation in the activity of the LDH with the two coenzymes led us to search for an NADP-specific LDH. In Table 5 are shown the results of a preliminary separation on DEAE cellulose. Whereas the original ratio of NAD/NADP activities in the dialysate of erythrocytes was 10:1, it increased to 60:1 in the first eluate and was only 1:2 in the second fraction.

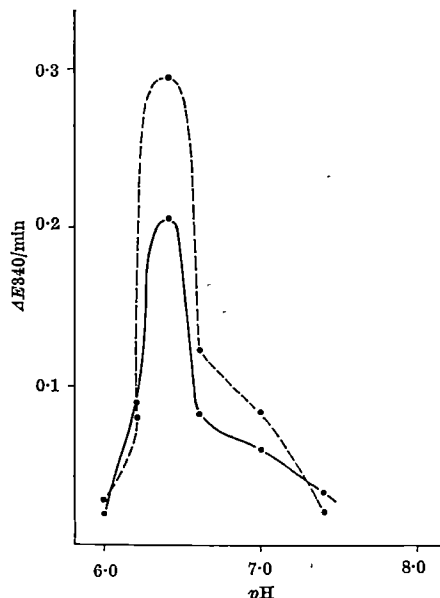


Fig. 1. pH-dependence of NADH₂ (—) and NADPH₂ oxidase (---) in erythrocytes. 0.1 ml. of eluate (DEAE cellulose column) from stroma-free haemolysate from rabbit erythrocytes, 3.0 ml. of a 0.1 M phosphate buffer and 0.02 ml. of NADPH₂ solution, final concentration 5×10^{-3} moles per cuvette

In Fig. 2 are shown the pH optima of the two enzyme activities in erythrocytes. Whereas the main optimum of the NADP-dependent LDH is located at about pH 6, two optima were observed with NAD; the main optimum was at about pH 9.0 and a second one at about pH 5. In a dialysate of ascites tumour cells (Fig. 3), the pH optimum of the NADP-dependent LDH was also at pH 5.8–6.0 and that of the NAD-dependent enzyme at pH 8. It is conceivable that the acid pH-optimum represents an isoenzyme of the NAD-dependent LDH.

From these results it would appear that an NADP-dependent LDH occurs generally and accounts for the

Table 4. LACTATE OXYDOREDUCTASE WITH NADP AND NAD

	NAD	NADP	NADP/NAD
Erythrocytes	8.50	0.72	0.085
Ascites tumour cells	41.70	1.00	0.024
Rabbit muscle	—	—	0.0057
Bovine heart muscle	—	—	0.01–0.003
Cattle retina	—	—	0.05
Bovine cornea	—	—	0.005

1 enzyme unit = 1×10^{-6} mole/l./min.

Table 5. LACTATE OXYDOREDUCTASE IN HAEMOLYSATE OF RABBIT ERYTHROCYTES AFTER DEAE-CELLULOSE FRACTIONATION

Vol. (ml.)	Proteins (mg)	Enzyme units/vol.	NAD	NADP	Specific act. $\times 10^{-3}$	NAD/NADP
70	6,195.0	15.2	1.45	2.5	0.24	10
240	98.6	49.3	0.83	499.0	8.4	60
600	126.0	0.24	0.51	2.0	4.0	0.5

1 unit = 1×10^{-6} mole/ml./min.

Table 3. OXIDASE AND DEHYDROGENASE (DIAPHORASE) ACTIVITY IN ELUATES FROM A DEAE CELLULOSE COLUMN OF A HAEMOLYSATE

Volume (ml.)	Protein (mg)	Coenzyme	Units/vol.*		Specific activity		NADH ₂ /NADPH ₂		
			Oxidase	Diaph.	Oxidase	Diaph.	Oxidase	Diaph.	
70	6,195.0	NADH ₂ NADPH ₂	—† —	63.70 0.67	— —	0.010 0.0001	—	94.0	Dialysate
240	98.6	NADH ₂ NADPH ₂	34.2 21.6	38.40 4.30	0.35 0.22	0.39 0.044	1.6	9.0	Fraction I
600	126.0	NADH ₂ NADPH ₂	7.1 93.8	12.00 7.20	0.057 0.11	0.095 0.057	0.075	1.7	Fraction II
71	28.1	NADH ₂ NADPH ₂	17.3 7.8	14.70 1.26	0.62 0.28	0.53 0.045	2.2	12.0	Fraction Ia

* 1 oxidase unit = 10^{-3} moles/ml./min. 1 diaphorase unit = 10^{-4} moles/ml./min.

† The activity in the dialysate of the stroma-free haemolysate could not be measured because of the presence of haemoglobin. The haemoglobin was eluted from the column with 0.002 M phosphate buffer, pH 7.4, whereas fractions I and II were eluted with 0.2 M buffer at the same pH. Then Fraction I was lyophilized and dialysed again and the activity determined. Diaphorase activities were measured with dichlorophenolindophenol and K₃Fe(CN)₆ as acceptors.

ing activities with NADP reported in the literature. enzyme plays a definite part in the metabolic regulation of the cell. The importance of provision of NADPH₂ various reductive syntheses has been stressed⁴. The DP-dependent LDH is probably the specific mediator of the reduction of NADP by lactate. On the other hand, oxidation of NADPH₂ by pyruvate may also have a regulatory significance. Another aspect of its function is of a cross-link between the Embden-Meyerhof and oxidative pentose pathways.

Relating to the outset of our investigations, an explanation of the deviations of the lactate/pyruvate ratio from the theoretical values has been found in the activities of 2 types of enzymes: (1) the NADH₂ and NADPH₂ oxidases, and (2) the NADP-dependent LDH. In addition we have recently found an LDH which is independent of a pyridine nucleotide in ascites tumour cells⁶.

The effect of the oxidases is probably the more important one of the two. The oxidases represent part of the extramitochondrial oxygen uptake of the cells. For erythrocytes at least, their contribution may be of some importance at lower pH values.

S. RAPOPORT
L. ABABEL
C. WAGENKNECHT
S. R. SARKAR

Physiologisch-chemisches Institut,
Humboldt-Universität, Berlin.

- ¹ Bucher, T., and Klingenberg, M. *Angew. Chem.*, **70**, 552 (1956).
- ² Holzer, H., Schütz, G., and Lyren, F., *Biochem. Z.*, **323**, 251 (1956).
- ³ Lowry, O., Passoneau, J., Hasselberger, F., and Schulz, D., *J. Biol. Chem.*, **239**, 31 (1964).
- ⁴ Navazzo, F., Ernster, B., and Ernster, L., *Biochim. Biophys. Acta*, **26**, 416 (1957).
- ⁵ Rapoport, S., and Ababel, L., *Acta Biol. Med. Germ.* (in the press).
- ⁶ Ababel, L., and Sarkar, S. R., *Acta Biol. Med. Germ.* (in the press).
- ⁷ Mehler, A. H., Kornberg, A., Grisolia, S., and Ochoa, S., *J. Biol. Chem.*, **174**, 961 (1948).
- ⁸ Meister, A., *J. Biol. Chem.*, **184**, 17 (1950).
- ⁹ Futterman, S., and Kinoshita, J. H., *J. Biol. Chem.*, **234**, 3174 (1959).
- ¹⁰ Wortman, B., and Schnelder, A., *Biochem. Biophys. Research Commun.*, **2**, 179 (1960).
- ¹¹ Rapoport, S., and Wagenknecht, C., *Z. Physiol. Chem.*, **309**, 296 (1957).

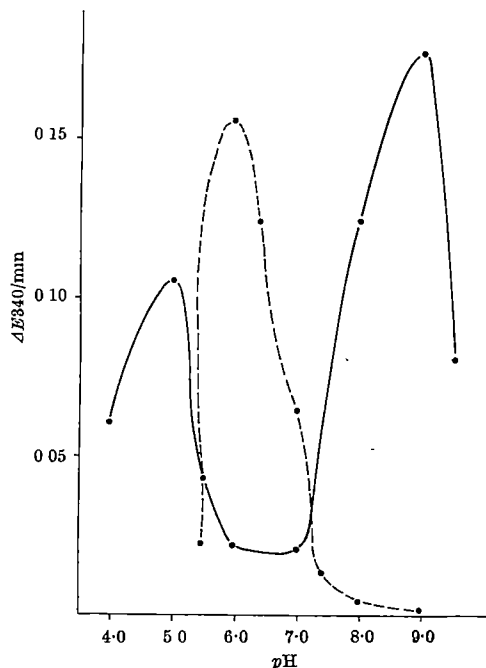


Fig. 2. pH-dependence of LDH in erythrocytes. 0.1 ml. of eluate ADP-LDH/3.2 ml. total volume 0.05 ml. of eluate from NAD-LDH :10/3.2 ml. total volume. For the range of pH 4-5 0.1 M citrate as employed. 0.1 M phosphate buffers of varying pH were used. The final coenzyme concentration was 5×10^{-4} M. —, NADH₂-LDH; ---, NADPH₂-LDH

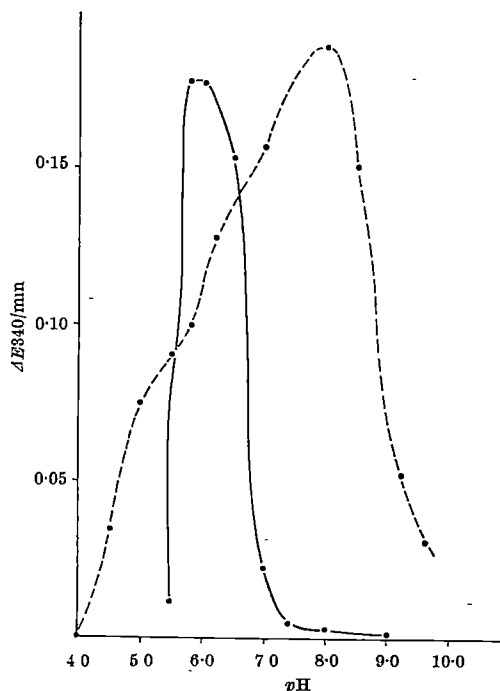


Fig. 3. pH-dependence of LDH in ascites tumour cells. Experimental conditions as described for Fig. 2. —, NADH₂-LDH; ---, NADPH₂-LDH

Effect of Trans-Clutes on the Fluxes of Chloride Ions across Artificial Membranes

IN free diffusion across membranes the unidirectional fluxes of a solute are usually treated as mutually independent movements: each flux being a function only of the electrochemical potential of its solute in the compartment from which it originates (*cis* side). Across biological membranes, however, such as the gastric mucosa of the frog¹, or some cellular membranes²⁻⁴, the fluxes of various solutes strongly depend on the nature of the solutes on the *trans* side. These *trans* effects have been interpreted in terms of a carrier exchange mechanism with different affinity to different ions and molecules^{5,2}. In the present work such *trans* effects have been studied in artificial membranes, in which transport or exchange carriers can be excluded.

Commercial packing 'Celophane' or dialysing membranes were mounted between 'Lucite' chambers, as described earlier¹. In order to keep the experimental conditions comparable to those with the gastric mucosa of the frog, the *cis* side, to which the radioactive material was added, was bathed by (frog) Krebs bicarbonate ringer ('nutrient') solution (84.6 mM NaCl, 3.2 mM KCl, 1.8 mM CaCl₂, 0.8 mM KH₂PO₄, 0.8 mM MgSO₄, 17.8 mM NaHCO₃). For the control experiments the *trans* side was bathed with 'secretory' solution (102.1 mM NaCl, 4.3 mM KCl, 1.8 mM CaCl₂, 0.8 mM MgSO₄). In the test experiments the chloride of the *trans* solution was equivalently replaced by other anions. In the case of di- and tri-valent anions iso-osmolarity was restored by mannitol. In some experiments, all but 10.2 mM of the NaCl was replaced by iso-osmolar concentrations of non-electrolytes. Both solutions were bubbled by 5 per cent CO₂ and 95 per cent O₂. The unidirectional fluxes were measured with radioactive tracers as described elsewhere¹, and corrected for variations of self-absorption.

Contrary to the assumption of free diffusion the flux of chloride ions across artificial membranes decreased if the chloride ions of the *trans* side were replaced by heavier ions or molecules (Figs. 1 and 2). The effect seems to be an almost linear function of the cube root of the *trans* particle weight.

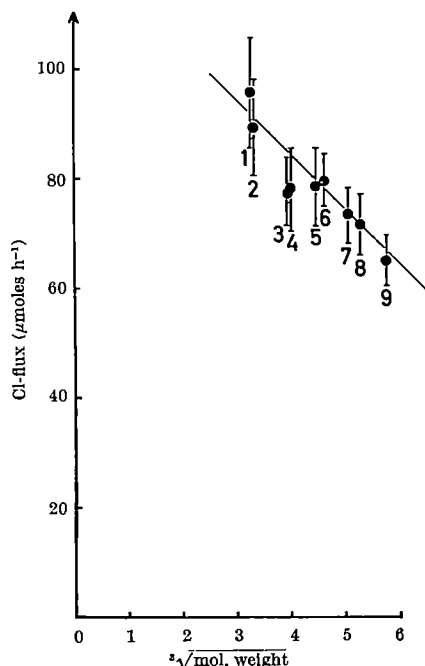


Fig. 1. *Trans* effect of anions on the Cl-flux across the short-circuited 'Cellophane' membrane. Composition of the solutions is given in the text. The *trans* ions are; 1, Cl; 2, formate; 3, HCO_3^- ; 4, NO_3^- ; 5, pyruvate; 6, SO_4^{2-} ; 7, I $^-$; 8, tartrate; 9, citrate. Room temperature. Correlation coefficient $r = 0.76$ ($P < 0.001$)

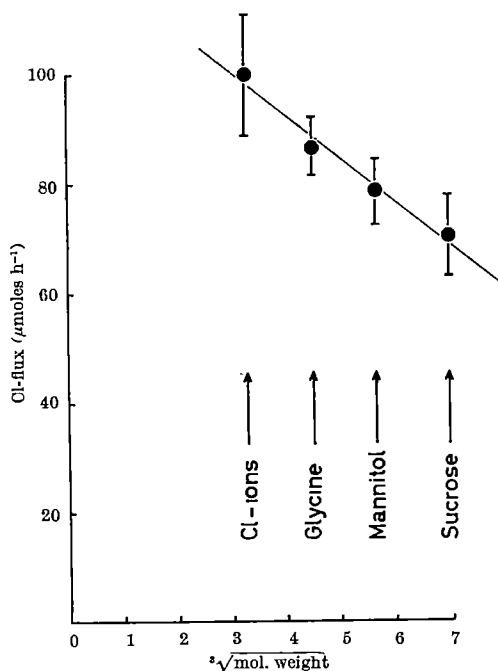


Fig. 2. *Trans* effect of non-electrolytes on the Cl-flux across the short-circuited 'Cellophane' membrane. Composition of the solutions is given in the text. The *trans* solutes used are indicated below. Room temperature. Correlation coefficient $r = -0.88$ ($P < 0.001$)

These *trans* effects are scarcely due to changes of the hydration of the membrane. Although dialysing membranes seem to take up slightly less water if the solute weight of the bathing solutions increases, these differences scarcely exceed 5 per cent of the control and may be still smaller if the *trans* solution, as in the flux experiments, bathes only one side of the membrane (Table 1). Furthermore, no correlation was found for a given *cis-trans* combination of solutes between the *trans* effect and the accidentally varying hydration of different membranes.

The *trans* effects cannot be accounted for by changes of viscosity within the membrane pores either, since the

Table 1. EFFECT OF VARIOUS ELECTROLYTES AND NON-ELECTROLYTES WATER CONTENT OF DIALYSING MEMBRANE

Bathing solution	No. membranes tested	H ₂ O content ml g ⁻¹ (dry weigh)
110 mM NaCl	3	1.26 ± 0.05
55 mM Na ₂ SO ₄ + 55 mM Mannitol	3	1.25 ± 0.04
55 mM Na ₂ Citrate	3	1.22 ± 0.06
220 mM Glycerol	4	1.30 ± 0.06
220 mM Mannitol	4	1.24 ± 0.03
220 mM Sucrose	4	1.21 ± 0.05

Pieces of dialysing membrane (No. 4465-A₂) were bathed for 1 h in solution at room temperature. The H₂O content was determined by weight before and after evaporation (at 105°) to dryness with a correction for so residues.

Table 2. EFFECT OF VARIOUS NON-ELECTROLYTES ON THE CONDUCTANCE OF 'CELLOPHANE' MEMBRANES FOR 10 mM NaCl

Bathing solution	2 sheets	Conductances 4 sheets mmhos per 0.785 cm ²	6 sheets
170 mM Glycerol + 10 mM NaCl	8.0 ± 0.3	4.1 ± 0	2.9 ±
170 mM Mannitol + 10 mM NaCl	8.0 ± 0.3	4.3 ± 0	2.9 ±
170 mM Sucrose + 10 mM NaCl	7.9 ± 0.2	4.1 ± 0	2.7 ±

All measurements were made at room temperature with three different membranes. In order to amplify the effects each membrane was made up of 2, 4 or 6 sheets of packing 'Cellophane'.

conductance of NaCl solutions decreased only slightly with increasing weight of added non-electrolyte molecule (Table 2).

According to Onsager's principles the flux of a given solute across the membrane may be altered by interaction with other fluxes, for example with the solvent flux or bulk flow⁶. Even between iso-osmolar solutions bulk flow might result if *cis* and *trans* solutes differ with respect either to the osmotic or the Stavermann deflection coefficient. The osmotic coefficients of the binary salts, as derived from the molar freezing point depressions, are rather close to those of NaCl⁷. Those of the non-electrolytes, however, very similar to each other, are all greater than that of NaCl, so that their effect on Cl-fluxes, if any, would be in the 'wrong' direction. The Stavermann coefficients, on the other hand, increase with the particle size⁸. Any bulk flow resulting from differences in these coefficients should be directed towards the side with the larger particles, that is, also in the 'wrong' direction. Hence by

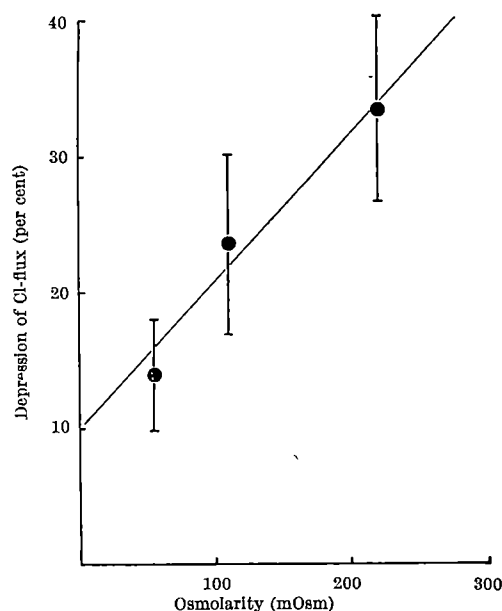


Fig. 3. Effect of total solute concentration on *trans* effect of raffinose on the Cl-flux across the short-circuited 'Cellophane' membrane. On the *cis* side, NaCl in osmolar concentrations as indicated. On the *trans* side 10 mM NaCl and raffinose to complete iso-osmolarity with the *cis* side. Room temperature. Correlation coefficient $r = 0.81$ ($P < 0.01$)

with solute-solvent interaction cannot be the cause of observed *trans* effect.

solute-solute interaction, namely between Cl-ions and *trans* solutes within the membrane pores, seems improbable in view of the large pore radii, which are about 100 Å for dialysing tube and 40 Å for 'Cellophane'. Were it, however, effective, one would expect that the *trans* effect would also occur with non-ionic fluxes, and would increase with the total solute concentration. The *trans* effect on flux of ¹⁴C-urea caused by heavier molecules is almost negligible and may be accounted for by changes in membrane hydration or viscosity. On the other hand, the *trans* effect on Cl-ions does increase if the total solute concentration is raised on both sides of the membrane (Fig. 3). In summary, the observed *trans* effects cannot be attributed as yet to a single cause. Since they are negligible with non-ionic fluxes, the ionic pattern of the membrane may be important. Although the *trans* effects observed with urea mucosa are much greater than those with artificial membranes⁵, they clearly cannot be exclusively due to a simple transport or exchange mechanism, and to assume free diffusion for passive fluxes across any membrane is to be a dangerous over-simplification.

This work was supported by grants from the U.S. National Science Foundation (NSF-G-10812) and the National Institute of Arthritis and Diseases of the National Institutes of Health (AM 06276-01 and AM 06276-02).

S. KITAHARA
E. HEINZ
C. STAHLMANN

Institute for Vegetative Physiology,
University of Frankfurt a. Main.

Heinz, E., and Durbin, R. P., *J. Gen. Physiol.*, **41**, 101 (1957)

Heinz, E., *J. Biol. Chem.*, **211**, 781 (1954).

Heinz, E., and Walsh, P. M., *J. Biol. Chem.*, **233**, 1488 (1958)

Sensenberg, T., and Wilbrandt, W., *J. Gen. Physiol.*, **41**, 289 (1957).

Durbin, R. P., Stahlmann, C., Kitahara, S., and Heinz, E., *Amer. J. Physiol.* (in the press).

Adam, O., in *Membrane Transport and Metabolism*, 87 (Czechoslovak Academy of Sciences, Prague, 1960).

Handbook of Chemistry and Physics (Chemical Rubber Publishing Co., Cleveland, Ohio, 1952).

Durbin, R. P., *J. Gen. Physiol.*, **44**, 315 (1960).

Identification of Dinitro-octylphenols in Certain Commercial Fungicides

A MIXTURE of nitrated alkylphenols is made by various manufacturers in the United States, Great Britain, and The Netherlands, who then esterify it with crotonic or other acids for the preparation of fungicides useful in the control of powdery mildews and also spider mites on various crops. Fractionation of this mixture, hereinafter called DNOP, on alumina (Spence type 'H') in benzene, followed by 2 per cent methanol in benzene, followed by 1 per cent acetic acid in 49 : 1 benzene-methanol, led to the isolation of three eluates¹. From each of these, amine salts were prepared and analyses showed them all to be of dinitro-octylphenols; the melting points, however, showed them to differ from each other and also from those of 2,4-dinitro-6-*n*-octylphenol and 2,6-dinitro-4-*n*-octylphenol². The melting points of salts of eluate II, which formed not more than 5 per cent of any of the DNOP samples, were close to those of the corresponding salts of dinocap phenol (4-dinitro-6-(1-methylheptyl)phenol)^{1,3}. As the biological activities (towards powdery mildews, spider mites, and test plants) of eluates I and II were similar, and those of eluate III were dissimilar, it appeared that eluate I, which formed about 60 per cent of one sample, was likely to be another isomer of dinocap phenol, and eluate III, which formed about 25 per cent of the same sample, an isomer of 2,6-dinitro-4-octylphenol other than the *n*-octyl or methylheptyl^{1,3}. The 1-ethylhexyl members of both series (2,4-dinitro-6-octylphenol and 2,6-dinitro-4-octylphenol) have therefore been synthesized, together with the

1-ethylpentyl and 1-ethylheptyl homologues, and the isomeric 2,6-dinitro-4-(1-propylpentyl)phenol. The ethyl-alkyl compounds were prepared by reaction of the appropriate alkyl magnesium bromide with *o*- or *p*-hydroxypropionophenone to give the *o*- or *p*-hydroxy-alkylphenol, respectively⁴. Dehydration by refluxing with toluene and a few crystals of iodine under a Dean and Stark tube yielded the alkene, which was reduced catalytically (palladium charcoal) at about 50° C under 500 lb./in.² pressure in 4-6 h to the 1-ethylalkylphenol. Nitration was done in glacial acetic acid, and the dinitro-(1-ethylalkyl)phenols isolated as various amine salts. Salts of the 2,4-dinitro-6-(1-ethylalkyl)phenols were easily recrystallized from benzene or methanol; those of the 2,6-dinitro-compounds required cyclohexene to give a tar-free product economically. The 2,4-dinitrophenols formed suitable salts with a greater range of amines than the 2,6-dinitrophenols. Melting points and analyses are given in Table 1.

Table 1

Phenol	M.p. of amine salt [†] °C.	Analysis [†]			
		C		H	
		Calc.	Found	Calc.	Found
2,4-Dinitro-6-(1-ethylpentyl)	{ M 138-9 CHA 151	55.3	55.3	7.3	7.4
	{ CHA 138	60.7	60.2	8.4	8.2
2,4-Dinitro-6-(1-ethylhexyl)	{ Pip. 143-4 M 154-6	59.8	60.4	8.1	8.6
	{ DCHA 153-4	56.4	56.4	7.6	7.8
	{ M 150-1	65.4	65.0	9.0	8.7
2,6-Dinitro-4-(1-ethylpentyl)	{ M 150-1	55.3	55.1	7.3	7.7
	{ CHA 173-4	—	—	—	—
2,6-Dinitro-4-(1-ethylhexyl)†	{ CHA 163-5	60.7	60.7	8.4	8.5
	{ M 125	56.4	56.1	7.6	7.6
	{ Pip. 114	—	—	—	—
2,6-Dinitro-4-(1-ethylheptyl)	{ CHA 167-8	61.7	61.7	8.6	8.5
	{ M 107-8	—	—	—	—
2,6-Dinitro-4-(1-propylpentyl)	{ CHA 181-3	60.7	61.9	8.4	8.6
	{ Pip. 109-1	—	—	—	—

* CHA = cyclohexylamine; Pip = piperidine; M = morpholine; DCHA = dicyclohexylamine

† Analyses by Weller and Strauss, 164 Banbury Road, Oxford.

‡ Forms salts with DOHA (m.p. 60° C) and *N*-ethylpiperidine (m.p. 90° C).

A comparison of the melting-points of the amine salts obtained from DNOP eluates with those of the salts of authentic dinitro-octylphenols (Table 2) led to the conclusion that eluate I was 2,4-dinitro-6-(1-ethylhexyl)phenol. Four of the five salts of eluate III had melting points agreeing well with those of the corresponding salts of 2,6-dinitro-4-(1-ethylhexyl)phenol, but there was a large discrepancy in the case of the morpholine salts.

Table 2

Phenol	CHA	M.p. of amine salt, °C			
		Pip.	M	DCHA	NEP
Eluate I*	39-41	141-2	150-1	152-3	—
2,4-Dinitro-6-(1-ethylhexyl)	38	143-4	154-6	153-4	—
Eluate II	45-7	107-10	97	—	—
2,4-Dinitro-6-(1-methylheptyl)	51-3	108-10	97-8	149-50	—
Eluate III	60-1	117	161-3	< 60	< 90
2,6-Dinitro-4-(1-methylheptyl)	29-30	144	127	—	—
2,6-Dinitro-4-(1-ethylhexyl)	53-5	114	125	< 60	90

* Eluate I also yielded salts with diamylamine, m.p. 39°-70° C, and 2-ethylpiperidine, m.p. 117° C.

To clear up the doubt about the identity of eluate III, nuclear magnetic resonance spectra in carbon tetrachloride of all three eluates, after purification via amine salts, were prepared by Mr. P. J. Ayres, Middlesex Hospital, and the band positions were computed and assigned by Dr. J. A. Elvidge, Imperial College of Science and Technology. Spectra were also prepared of the six dinitro-octylphenols, two *n*-octyl and four *s*-octyl, available at that time. Data obtained are given in τ values (and J c/s) in Tables 3 and 4. The inferences drawn are: (i) in spite of the discrepancy in the melting points of the morpholine salts, eluate III from the DNOP provided by Rohm and Haas Co. is 2,6-dinitro-4-(1-ethylhexyl)phenol; (ii) although the melting points of the piperidine salts of eluate III from May and Baker, Ltd., DNOP and of 2,6-dinitro-4-(1-ethylhexyl)phenol are so similar, the former was a mixture, approximately 1:1, of the latter and the isomer, 2,6-dinitro-4-(1-methylheptyl)phenol (a 1:1 mixture of the two

Table 3

2,4-Dinitro-6-(1-ethylhexyl)phenol	Eluate I, purified via piperidine salt	2,4-Dinitro-6-(1-methylheptyl)phenol	2,4-Dinitro-6-n-octylphenol	Assignments
9-13 (7t)	9-15 (6-5t)	9-12 (ca.5t)	9-11 ca.t	ω -Me
9-09 (7 5d)	9-08 (6d)	8-67 (7d)	8-67s	1-Me [CH ₂] ₆ [CH ₂] ₄
8-72 ca.s 8-25m	8-73 ca.s 8-26m	8-72 ca.s	8-35m	2-CH ₃ +CH ₃ of 1-Et
6-72 (7g)	6-75 (6-8g)	6-59 (6-8se)	7-14 (7 5t)	1-CH ₃ OH
2-68s 1-65 (2-5d) 1-03 (2-5d)	2-69s 1-68 (2-5d) 1-09 (2-5d)	2-71s 1-65 (2-5d) 1-10 (2-5d)	2-69s 1-66 (2-5d) 1-10 (2-5d)	3-H

Table 4

2,6-Dinitro-4-(1-ethylhexyl)phenol	Eluate III*	Eluate III†	2,6-Dinitro-4-(1-methylheptyl)phenol	2,6-Dinitro-4-n-octylphenol	Assignments
9-13 (7t)	9-10 (7t)	9-12t	9-13 (ca.5t)	9-11 ca.t	ω -Me
9-09 (7d)	9-08 (6d)	9-09d	8-69 (6 5d)	8-69s	1-Me [CH ₂] ₆ (CH ₂) ₄
8-72 ca.s 8-31m	8-74 ca.s 8-41m	8-73 ca.s 8-35m	8-74 ca.s	8-35m	2-CH ₃ +CH ₃ of 1-Et
7-41 (7g)	7-37g	7-26m	8-33m	7-28 (7.5t)	1-CH ₃ OH
2-70s	2-66s	2-04s	7-15 (6-8se)	1-87s	5-H 3-H
1-91s {	1-89s {	1-95s 1-88s	2-71s {		
			1-90s {		

* Purified from Rohm and Haas Co. DNOP via morpholine salt.

† Purified from May and Baker, Ltd., DNOP via piperidine salt.

synthetic salts was found to melt at 118° C, so that there is no discrepancy here); (iii) the identities of eluates I and II, already deduced from the amine salts melting points, are confirmed.

The process used by the various firms manufacturing fungicides based on dinitro-octylphenols therefore produces not only the *para*-substituted phenol (as would be expected) but also causes the alkyl chain to add on to the benzene ring at an unexpected position in the chain, namely, at C₃. It has hitherto been assumed that addition of a chain derived from a 2-hydroxyalkane would take place at C₂.

We thank Mr. P. J. Ayres and Dr. J. A. Elvidge for the provision of evidence by NMR spectra, and May and Baker, Ltd., Murphy Chemical Co., Ltd., n.v. Philips-Duphar, and Rohm and Haas Co. for gifts of DNOP from their respective processes. We also thank Dr. D. Woodcock, Long Ashton Research Station, Bristol, for valuable help in the reduction of the alkenylphenols, and Dr. G. G. S. Dutton for advice on routes of synthesis.

A. H. M. KIRBY
L. D. HUNTER

Plant Protective Chemistry Section,
East Malling Research Station,
Maidstone, Kent.

¹ Kirby, A. H. M., *Meded. Landbouwhogeschool. Opzoekstus Gent*, **29**, 976 (1964).

² Dutton, G. G. S., Briggs, T. I., Brown, B. R., and Powell, R. K., *Canad. J. Chem.*, **31**, 837 (1953).

³ Kirby, A. H. M., Frick, E. L., Hunter, L. D., and Tew, R. P., *Nitro Compounds, Proc. Intern. Symp., Warsaw*, 1963.

⁴ Clarke, M., and Woodcock, D., *J. Chem. Soc.*, 519 (1962).

n-Alkan-1-ols in Butter after Oxidative Deterioration

CHEMICAL mechanisms for the formation and decomposition of hydroperoxides in oxidizing lipids predict the presence of a wide range of compounds. Of these, three classes of aliphatic aldehydes (*n*-alkanals, *n*-alk-2-enals and *n*-alka-2,4-dienals) have long been isolated and identified and more recently the presence of non-conjugated unsaturated aldehydes (hex-*cis*-3-enal² and hept-*cis*-4-enal³), a vinyl ketone (oct-1-en-3-one⁴) and its corresponding alcohol (oct-1-en-3-ol^{5,6}) has also been demonstrated. The *n*-alkan-1-ols, also predicted by the mechanisms, had not hitherto been isolated and identified.

Experiments conducted in these laboratories have now shown that, in addition to oct-1-en-3-ol, *n*-alkan-1-ols are

also present in the steam distillate of oxidized but appreciable quantities (less than the *n*-alkanals but greater than the *n*-alk-2-enals) of the C_{1,2,5-8} and minor amounts of the C_{3,4} *n*-alkan-1-ols have been isolated from butter prepared from cream treated with copper and ascorbic acid.

The occurrence of *n*-alkan-1-ols in a degrading system has probably been overlooked as a result of the relative lack of flavour, their difficulty in characterization and the general acceptance that carbonyl compounds are the main contributors to the flavour of oxidized butter.

Work on the change in ratio of *n*-alkan-1-ols to aldehydes during low-temperature storage of butters will be reported in the *Journal of Dairy Research*.

W. STARK
D. A. FORSS

C.S.I.R.O.

Division of Dairy Research,
Melbourne, Australia.

¹ Lea, C. H., in *Proc. Second Symp. Foods*, 3, edit. by Schultz, H. W., E. A., and Sinnhuber, R. O. (Westport, Conn., Avi Publ., 1962).

² Hoffman, G., *J. Amer. Oil Chem. Soc.*, **38**, 1 (1961).

³ Haverkamp Begemann, P., and Koster, J. C., *Nature*, **202**, 552 (1964).

⁴ Stark, W., and Forss, D. A., *J. Dairy Res.*, **29**, 173 (1962).

⁵ Hoffmann, G., *J. Amer. Oil Chem. Soc.*, **39**, 439 (1962).

⁶ Stark, W., and Forss, D. A., *J. Dairy Res.*, **31**, 253 (1964).

Dependence of Photophosphorylation by Isolated Chloroplasts on the Oxidation-Reduction State of *N*-Methylphenazinium Methyl Sulphate (Phenazine Methosulphate)

It has been previously observed that the rate of photophosphorylation in chromatophores from photosynthetic bacteria was dependent on the oxidation-reduction state of phenazine methosulphate¹. On addition of ascorbic acid which reduces phenazine methosulphate non-enzymatically², the rate of photophosphorylation was observed to decrease markedly. However, ascorbate has been shown not to have any inhibitory effect on photophosphorylation in isolated chloroplasts catalysed by phenazine methosulphate³. Indeed, ascorbate is commonly used to stimulate photophosphorylation with phenazine methosulphate in isolated chloroplasts^{3,4}.

We have now made a systematic investigation of the effect of the oxidation-reduction state of phenazine methosulphate on photophosphorylation by isolated chloroplasts. The oxidation-reduction state of the system was controlled by using a TPNH-generating system and varying the effective reducing power by changing the concentration of added TPN. The experiments were conducted under strictly anaerobic conditions in the presence of a catalase-ethanol trap to remove traces of oxygen⁵.

Fig. 1 illustrates that phenazine methosulphate catalysed photophosphorylation is indeed dependent on the oxidation-reduction state of the electron carrier. Maximal rates of photophosphorylation were obtained in the presence of 1×10^{-5} M TPN. In the absence of TPN or in the presence of concentrations higher than 10^{-7} M the rate of photophosphorylation was markedly decreased. Measurement at 388 mμ (ref. 2) showed that the amount of oxidized phenazine methosulphate remaining after the reaction was completed was as follows: 75 per cent in the absence of TPN, 60 per cent with 1.3×10^{-5} M TPN (optimal) and 0 per cent at and beyond 1.3×10^{-5} M.

This direct demonstration of the dependence of the rate of photophosphorylation with phenazine methosulphate on the oxidation-reduction state of the system is in agreement with the conclusions of previous investigators using different experimental techniques. Thus, Jagendorf and Avron⁶ and Whatley⁷ concluded from the effects of *p*-chlorophenyl dimethylurea and oxygen that the oxidation-reduction potential of phenazine methosulphate was

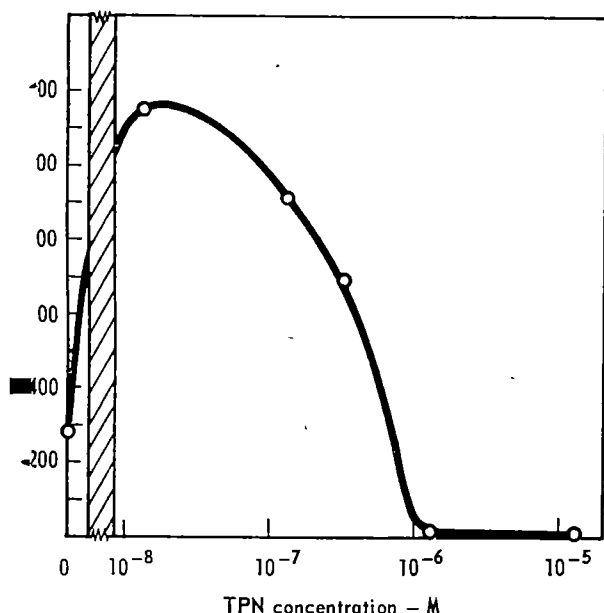


Fig. 1. Photophosphorylation by isolated chloroplasts as affected by PNH concentration. The reaction mixture included in μ moles or μ g: H_2O , 45; NaCl , 60; MgCl_2 , 12; Na_2K , phosphate, containing $\times 10^4$ c.p.m. ^{32}P , 12; ADP, pH 7.8, 4; phenazine methosulphate, 0.1; NaOH , 700; catalase (Boehringer, crystalline), 50 μ g; sodium isocitrate, 1; isocitric dehydrogenase (Sigma, Type IV), 30 μ g; TPNH-diaphorase³ in excess; TPN as indicated; once-washed chloroplasts⁴ containing 28 μ g of chlorophyll in a final volume of 3.0 ml. The reaction mixture was placed in a modified Thunberg tube fitted with a 1-cm cuvette, evacuated, 25 mm mercury with continuous tapping for 2 min, and closed. Chloroplasts were added from the side arm, and the tubes illuminated at 40°C for 2 min with 180,000 lux of white light filtered through a 'Cellophane' filter transmitting only red light (above 600 m μ). AT^{32}P formed was analysed as previously described⁴.

major importance in determining the rate of photophosphorylation by isolated chloroplasts.

GUNTER ZWEIG*
MORDHAY AVRON

Biochemistry Section,
Weizmann Institute of Science,
Rehovoth, Israel.

* Present address: Agricultural Toxicology and Residue Research Laboratory, University of California, Davis, California. Supported by an E. J. Rothschild Memorial Grant during Sabbatical leave from the University of California.

- ewton, J. W., and Kamen, M. D., *Biochim. Biophys. Acta*, **25**, 462 (1957).
agendorf, A. T., and Margulies, M., *Arch. Biochem. Biophys.*, **90**, 184 (1960).
agendorf, A. T., and Avron, M., *J. Biol. Chem.*, **231**, 277 (1958).
vron, M., *Biochim. Biophys. Acta*, **40**, 257 (1960).
ehler, A. H., *Arch. Biochem. Biophys.*, **33**, 65 (1951).
agendorf, A. T., and Avron, M., *Arch. Biochem. Biophys.*, **80**, 246 (1959).
hatley, F. R., in *Photosynthetic Mechanisms of Green Plants*, Pub. No. 1145, 243 (U.S. Nat. Acad. of Sci., 1963).
vron, M., and Jagendorf, A. T., *Arch. Biochem. Biophys.*, **72**, 17 (1957).

PHYSIOLOGY

Monocular versus Binocular Visual Acuity

THIS communication describes experiments in which the threshold contrast of gratings viewed monocularly and binocularly have been determined (Fig. 1). We have modified the technique of Schade¹ so that a grating target, generated on an oscilloscope by supplying suitable signals to the x , y and z axes. It could be continuously varied both in contrast and fineness (spatial frequency) without the mean luminance of the screen changing. The energy distribution across the grating varied sinusoidally. The grating filled a rectangular area subtending 5° by 1.3° , and was surrounded by a circular field of 12° diameter of the same luminance as the oscilloscope screen (30 cd/m²). The screen was viewed from 57 in., and in all experiments particular care was taken to correct the eye to within 0.12 diopter with spectacle lenses.

To exclude possible artefacts due to fluctuations of accommodation² and pupil size, both eyes of one subject (D. G. G.) were fully accommodated. He viewed the screen through artificial pupils (2.8 mm diam.) placed close to the cornea. Special care was taken to centre the pupils^{3,4}. The non-viewing eye was covered with a piece of glass sufficiently frosted to obscure the details of the screen while presenting a field of similar luminance to that of the unobscured screen. The subject adjusted the contrast until he was satisfied that the grating could just be resolved. At each spatial frequency, threshold measurements were made with each eye and with both eyes together. The sequence of measurements was made in a random order.

The results are shown in Fig. 2. The general shape and magnitude of the contrast threshold confirm that

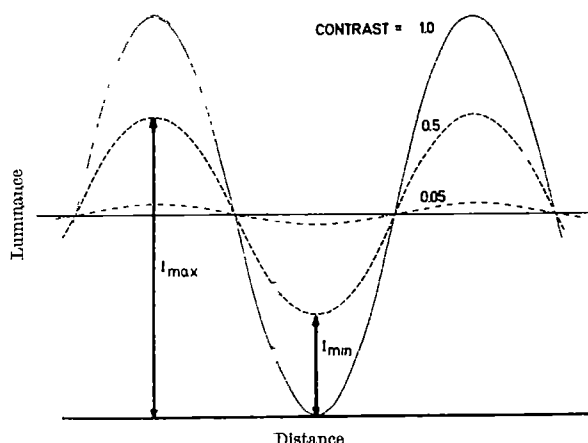


Fig. 1. The contrast of a grating the intensity of which varies with distance sinusoidally is defined as $\frac{I_{\max} - I_{\min}}{I_{\max} + I_{\min}}$. Three levels of contrast are shown: 1.0, 0.5 and 0.05. Note that as contrast varies the mean light level across the grating remains constant. The spatial frequency of a grating is the reciprocal of the angular distance between successive maxima in the sinusoidal intensity distribution. Spatial frequency is thus equivalent to visual acuity.

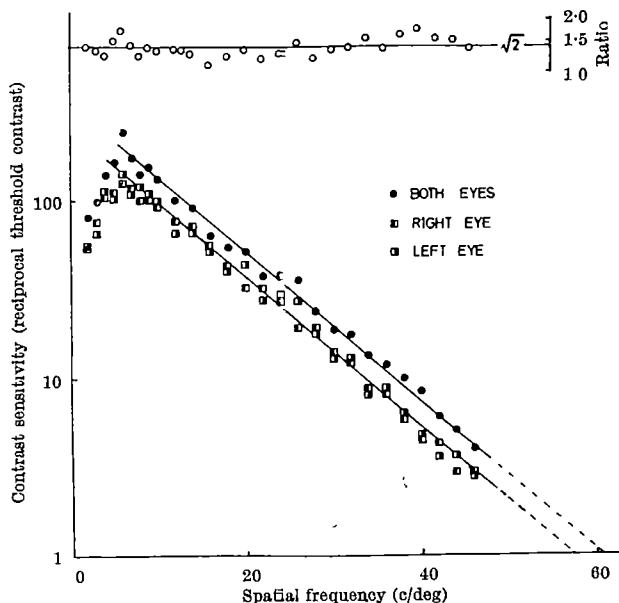


Fig. 2. The results from observer D. G. G. are plotted as contrast sensitivity on a log scale. Contrast sensitivity is defined as the reciprocal of the contrast at threshold. Contrast sensitivity = $\frac{I_{\max} + I_{\min}}{I_{\max} - I_{\min}}$. Spatial frequency in c/deg of visual angle is plotted on a linear scale. Each point is an average of two observations. The two straight lines are placed at a ratio of $\sqrt{2}$ apart in contrast and fitted to the results by eye. The interrupted lines are extrapolations to unit sensitivity (100 per cent contrast). In the upper portion of the figure the open circles represent the ratio, plotted on log scale, of binocular/mean-monocular sensitivity at each spatial frequency. The straight horizontal line corresponds to a ratio of $\sqrt{2}$.

found by Schade¹. It is clear that the threshold contrasts for the left and right eyes are very similar at all spatial frequencies. At each and every frequency the binocular threshold was found to be lower than that of the monocular threshold. That is, binocular sensitivity is greater than monocular.

Plotted along the top of Fig. 2 is the ratio of the binocular to the (arithmetic) mean monocular sensitivity at each observed frequency. A straight line has been drawn corresponding to a ratio of 1.414, that is, $\sqrt{2}$. The mean of the ratios in this experiment is 1.440 ± 0.030 S.E. The experiment was repeated on D. G. G., this time using natural pupils and accommodation, and the mean ratio was found to be 1.443 ± 0.032 S.E. For subject F. W. C., also with natural pupils and accommodation, the mean ratio obtained was 1.372 ± 0.045 S.E. The mean ratio for these three experiments, on two subjects, is 1.418 ± 0.021 S.E. None of these ratios differs significantly from $\sqrt{2}$.

The two parallel straight lines drawn through the results for frequencies higher than 6 c/deg are separated by a factor of $\sqrt{2}$ in contrast. The dotted extensions show extrapolations to unity contrast sensitivity; that is, the point where the grating is modulated from zero luminance to a peak level of twice the mean luminance. To avoid non-linearities in the responses of the oscilloscope phosphor, measurements were not made for contrasts higher than 0.4. It is estimated that at unity contrast the threshold frequency for monocular viewing would be 57.0 c/deg and for binocular viewing 61 c/deg. Thus, the calculated improvement in visual acuity for binocular viewing at high contrast would be $61/57.0 = 1.070$, that is, 7.0 per cent. Several workers have compared the visual acuity of one eye with two, using high contrast test objects, and have found values ranging from 5 to 10 per cent^{5,6}. However, they did not measure the ratio in the contrast domain, where we find improvements of the order of 42 per cent for binocular viewing at all spatial frequencies.

Our findings may be explained if it is assumed that when identical images are fed to two matched eyes their outputs are summed and that these outputs are noisy; that is, contain spurious extra components not correlated in each channel. Because the standard error of the sum of n independent measurements of a random or noisy process decreases as \sqrt{n} , an observer using two eyes can obtain two measurements which thus permit a $\sqrt{2}$ lower contrast to be detected.

It is interesting to consider the question of whether covering the other eye with a diffusing screen affects the threshold; the argument being that permitting an equal amount of light, containing no image detail, to reach the obscured eye would lower the contrast sensitivity of the viewing eye by introducing additional noise. To test the effect of the diffusing screen technique, a comparison was made with the effect of an opaque cover, using 30 c/deg as the test frequency for the viewing eye. It was found that the mean ratio of contrast sensitivity for diffuse/obscured presentation to the non-viewing eye was 1.019 ± 0.022 S.E. As this mean does not differ significantly from a ratio of 1, it suggests that it makes no difference whether no light, or a diffuse light, enters the non-viewing eye, providing the pupil size of the viewing eye is fixed.

The fact that a stimulus that should have only added noise does not raise the threshold could be interpreted as evidence against a simple hypothesis of signal-to-noise detection. However, it may be that complete addition of signal and noise occurs only when congruent images are presented to the two retinæ.

Lythgoe and Phillips⁷ and Crozier and Holway⁸ compared monocular with binocular luminance thresholds for a patch of light exposed for 0.2 sec; they similarly found that the ratio of the binocular to the average of the monocular thresholds was equal to $\sqrt{2}$. These workers

explain their results in terms of Piper's law, which states that the threshold is inversely proportional to the square root of the area of the test field. It is difficult to account for our findings in terms of an area-threshold relationship.

Pirenne⁹, who has also measured binocular and monocular absolute thresholds, finds an improvement of a factor of 1.25. As he clearly illustrates, this improvement can be explained by probability summation between independent channels without invoking the physiological summation of signals from the two eyes; a class of argument which must be distinguished from our own which requires the actual physical summation of signals from the two eyes. Recent electrophysiological evidence seems to provide a basis for such physiological summation. Furthermore, a description in terms of the detection signals in the presence of noise seems to offer a more general explanation^{11,12} which can encompass the improvement found under such widely differing binocular viewing conditions.

The effect of doubling the signal luminance and presenting it to one eye was next tested. This was done by measuring the contrast sensitivity for identical fields differing only by a factor of 2 in luminance. The field was viewed by D. G. G. through a 2.8 mm-diam. pupil with his atropinized right eye. Ten measurements of threshold were made at each luminance (40 and 80 cd/m²) for a grating of 30 c/deg. The mean ratio of the contrast sensitivity for the higher/lower luminance was found to be 1.173 ± 0.028 S.E. This mean ratio differs significantly from 1, and also from 1.440 ± 0.030 S.E. found for the same subject viewing the field with both eyes through pupils of 2.8 mm. This result indicates that we are observing at a luminance (80 cd/m²) sufficiently high to be on the 'plateau' where visual acuity is substantially independent of luminance¹³. Thus, at these luminance levels it is advantageous to use a binocular eyepiece for microscopy for contrast sensitivity is improved, even binocular viewing is achieved by splitting the light into two portions each of half the intensity.

One of us (D. G. G.) is supported by a U.S. National Science Foundation post-doctoral fellowship. This investigation was supported by a U.S. Public Health Service research grant (NB 05046-01) and a grant from the W. H. Ross Foundation (Scotland).

F. W. CAMPBELL
D. G. GREEN

The Physiological Laboratory,
University of Cambridge.

¹ Schade, O. H., *J. Opt. Soc. Amer.*, **46**, 721 (1956).

² Campbell, F. W., Robson, J. G., and Westheimer, G., *J. Physiol.*, **145**, 57 (1959).

³ Stiles, W. S., and Crawford, B. H., *Proc. Roy. Soc.*, **B**, **112**, 428 (1933).

⁴ Campbell, F. W., *J. Physiol.*, **144**, 25P (1958).

⁵ Barany, E., *Acta Ophthalm. Kbh.*, **24**, 63 (1948).

⁶ Horowitz, M. W., *J. Exp. Psychol.*, **39**, 581 (1949).

⁷ Lythgoe, R. J., and Phillips, L. R., *J. Physiol.*, **91**, 427 (1937-38).

⁸ Crozier, W. J., and Holway, A. H., *J. Gen. Physiol.*, **22**, 341 (1939).

⁹ Pirenne, M. H., *Nature*, **152**, 698 (1943).

¹⁰ Hubel, D. H., and Wiesel, T. N., *J. Physiol.*, **160**, 106 (1962).

¹¹ Barlow, H. B., *J. Opt. Soc. Amer.*, **46**, 634 (1956).

¹² Barlow, H. B., *J. Physiol.*, **141**, 337 (1958).

¹³ Schlaer, S., *J. Gen. Physiol.*, **21**, 165 (1937).

A Central Action of Hemicholinium

THE action of hemicholinium No. 3 (α - α -dimethyl ethanolamine 4,4'-bisacetophenone, HC 3) on the synaptic release of acetylcholine at the neuromuscular junction and within autonomic ganglia is well established¹. However, apart from the finding that intravenously administered HC 3 reduces the acetylcholine content of the pons and medulla of dogs², and the report that locally administered HC 3 reduces the content and synthesis of acetylcholine within the caudate nucleus of rats³, there

yet no evidence that this substance affects the output of acetylcholine from central cholinergic synaptic terminals. There is convincing evidence that acetylcholine is the excitatory transmitter released from the axon lateral endings on Renshaw cells of the feline spinal cord⁴, and the opportunity was taken to assess the action of HC 3 at these synapses.

The experiments were performed in the lumbar segments of spinal cats anaesthetized with pentobarbital sodium. Renshaw cells were activated synaptically by pulses in the appropriate segmental ventral or dorsal roots, and extracellular spike potentials (negative-positive, 100–1,000 μ V in amplitude) were recorded by the diaphragm chloride-containing centre barrel of five barrel micropipettes⁵. The overall diameter of these micropipettes was 4–6 μ , and the other barrels contained aqueous solutions of acetylcholine bromide (1 M); DL-homocysteic acid (sodium salt, 0.2 M, pH 7.5); HC 3 bromide (0.1 M); triethylcholine chloride (0.1 M) or diaphragm chloride (M), from which pharmacologically active substances could be ejected by suitably directed electrophoretic currents⁶. The orthodromically evoked spikes were counted by means of a gated electronic counter, and the responses were also photographed for accurate measurement of latencies.

Control experiments established that although there was some reduction in the number of spikes evoked by prolonged maximal ventral root stimulation at frequencies of 3–10/sec, the number was reasonably stable after the first 5–10 min for a period of at least several hours. In particular there was no appreciable increase in the latency of the first spike. During the electrophoretic administration of HC 3 (5–20 n. amp) there was an increase in the latency of this and subsequent spikes, and often a marked reduction in the total number of spikes. These alterations in firing pattern were first noticed 10–20 min after the beginning of the HC 3 administration and were progressive: in all cases the ventral root was stimulated continuously at the foregoing frequencies. Alterations in the total number of spikes were complicated by a reduction in the sensitivity of some cells to electrophoretically administered acetylcholine, and by actual excitation by the HC 3, as revealed by a facilitation of firing induced by DL-homocysteic acid with or without an actual increase in the 'spontaneous' discharge rate. Excitation by HC 3 was characterized by periodic bursts of high-frequency firing similar to those produced by gallamine and D-tubocurarine⁷; often such a burst followed the delayed first synaptic spike (Fig. 1, B, lower record). These effects, presumably due to a direct interaction of HC 3 with

postsynaptic structures¹, were usually maximal within a few minutes of the administration of this agent and could be minimized to some extent by using extremely low electrophoretic ejection currents (less than 10 n. amp).

The most marked effect of HC 3 was an increase in the central latency of the first spike evoked by a supramaximal ventral root stimulus, as illustrated in Fig. 1, and in several instances prolongation of this time beyond 2–4 msec was followed by complete block of synaptic excitation approximately 60–80 min after the beginning of the HC 3 administration. Partial recovery was observed in several instances within 5–10 min of the cessation of the HC 3 administration (Fig. 1, D). As dihydro- β -erythroidine, a powerful antagonist of the nicotinic receptors of Renshaw cells⁴, has little or no effect on the latency of the first spike evoked by such a stimulus, it is unlikely that the increase of latency produced by HC 3 is the result of a postsynaptic action, particularly as increases were observed when the actual sensitivity of Renshaw cells to acetylcholine was undiminished. Hence it may be reasonably proposed that in the presence of HC 3 the output of acetylcholine per stimulus becomes diminished, with a consequent reduction in the rate of rise of the resultant excitatory postsynaptic potential. Two factors may be involved in the time over which it was necessary to stimulate the ventral root repetitively before such a presynaptic action could be demonstrated: the presynaptic store of acetylcholine is evidently comparatively large compared with the amount released per impulse, and a maximum ventral root stimulus normally results in the release of a large amount of acetylcholine in the environment of a particular cell compared with the amount necessary to produce an above-threshold postsynaptic depolarization.

Renshaw cells may also be fired synaptically by dorsal root volleys without the involvement of motoneurons⁷. Not all cells responded to such afferent excitation, and many did not follow repetitive stimulation (3–10/sec) of the dorsal root, presumably because of the polysynaptic nature of the excitatory pathway. However, in one case, conditions were satisfactory and the responses of this cell to dorsal root stimuli at a frequency of 3.3/sec were unaltered after 60 min administration of HC 3, at which time the latency of the first ventral root-induced spike had been increased from less than 1 msec to approximately 2.8 msec. This finding confirms other pharmacological evidence that the firing of Renshaw cells by dorsal root afferent volleys may not involve cholinergic mechanisms⁷, and also suggests that the presynaptic action of HC 3 may be specific for cholinergic terminals. Additional evidence for this latter proposal was the finding that prolonged administration of HC 3 failed to influence the synaptic excitation of dorsal horn interneurons, which are presumably not innervated by cholinergic fibres⁷.

Experiments were also carried out on Renshaw cells using triethyl(2-hydroxyethyl)ammonium (triethylcholine); but the use of this agent was complicated by a marked depressant action on the sensitivity of the cells to acetylcholine, and a strong excitant action similar to that observed with HC 3. Attempts were made to influence the synaptic firing of Renshaw cells by inhibition of choline acetylase, using electrophoretically administered fluoracetate, *p*-chloromercuribenzoate or diphenyllethylacetic acid⁸. These experiments were unsuccessful, possibly because of the failure to obtain a sufficiently high concentration of the inhibitors at the site of acetylcholine synthesis. Intravenously administered HC 3 (10 mg/kg) was also ineffective, probably for the same reason.

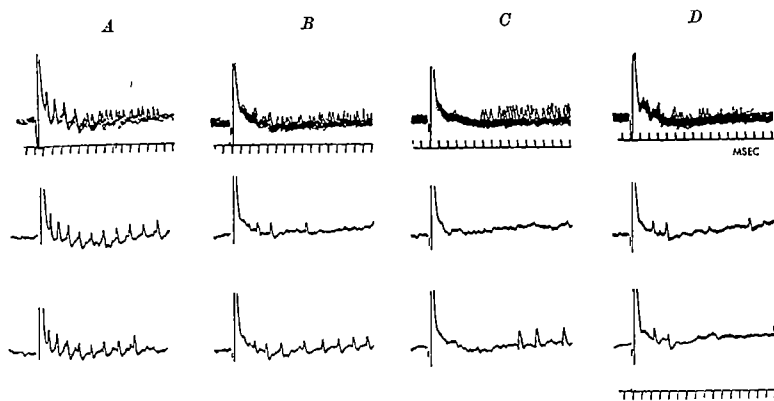


Fig. 1 Spike responses of a single Renshaw cell (upper records—approximately 20 superimposed tracings at a frequency of 5/sec; lower records—single tracings); (A) before, (B) at 31 min and (C) 61 min during, and (D) 3 min after the electrophoretic ejection of HC 3 for 69 min (10 n. amp for 59 min, 40 n. amp for 10 min). The measured latencies of the first spike are (A) 1.21 msec; (B) 2.42 msec; (C) 5.8 msec and (D) 1.9 msec from the ventral root stimulus. In many of the records of (C) the neurone failed to respond to ventral root stimulation and the latency was measured from the superimposed tracing. The sensitivity of this cell to acetylcholine and DL-homocysteic acid was enhanced by HC 3. The ventral root was stimulated supramaximally at a frequency of 5/sec throughout the series, and had been so stimulated continuously for approximately 1 h beforehand. Time, msec for all records. The spikes were approximately 300 μ V in amplitude.

In conclusion HC 3, which presumably prevents the transfer of choline into central presynaptic cholinergic terminals, thus limiting the rate of acetylcholine synthesis¹, is suitable for testing the cholinergic nature of central synapses, provided the appropriate pathway is activated sufficiently to exhaust the transmitter stored within the endings.

We thank Prof. R. Paoletti for a supply of diphenyl-ylethylacetic acid.

D. M. J. QUASTEL*
D. R. CURTIS

Department of Physiology,
Australian National University,
Canberra.

* Post-doctoral Research Fellow of the Muscular Dystrophy Association of Canada.

¹ Schueler, F. W., *Intern. Rev. Neurobiol.*, **2**, 77 (1960). MacIntosh, F. C., *Canad. J. Biochem. Physiol.*, **41**, 2555 (1963). Elmqvist, D., and Quastel, D. M. J., *J. Physiol.*, **177**, 463 (1965).

² Metz, B., *Amer. J. Physiol.*, **202**, 80 (1962).

³ Hebb, C. O., Ling, G. M., McGeer, E. G., McGeer, P. L., and Perkins, D., *Nature*, **204**, 1309 (1964).

⁴ Eccles, J. C., *The Physiology of Synapses* (Springer-Verlag, Berlin, 1964).

⁵ Curtis, D. R., and Eccles, R. M., *J. Physiol.*, **141**, 435 (1958).

⁶ Curtis, D. R., in *Physical Techniques in Biological Research*, edit. by Nastuk, W. L., 5 (Academic Press, New York, 1964).

⁷ Curtis, D. R., and Ryall, R. W. (in course of publication).

⁸ Garattini, S., Morpurgo, C., Murelli, B., Paoletti, R., and Passerini, N., *Arch. Intern. Pharmacodyn.*, **109**, 400 (1957). Garattini, S., Morpurgo, C., and Passerini, N., *Experientia*, **14**, 89 (1958).

HAEMATOTOLOGY

Kinetic Differences between Human Red Cell and Leucocyte Pyruvate Kinase

WITHIN the group of non-spherocytic haemolytic anaemias classified as 'type 11' by Selwyn and Dacie¹, Valentine, Tanaka and Miwa² found a specific erythrocyte deficiency of pyruvate kinase (PK). The leucocyte PK activity in patients with this disease is normal², suggesting that the genetic control of leucocyte PK differs from that of red cell PK. Electrophoretic differences among PK preparations from rat kidney, liver, cardiac muscle, brain and skeletal muscle were reported by Fellenberg *et al.*³. Chromatographic, electrophoretic, solubility and structural differences between the normal human leucocyte and red cell PK have been presented⁴.

Pyruvate kinase catalyses the conversion of phosphoenolpyruvate (PEP) and ADP to pyruvate and ATP. Boyer⁵ has reviewed studies of enzyme kinetics for rabbit muscle PK. He concludes that phosphoryl transfer occurs by a direct transfer of the phosphoryl group from the donor to the acceptor substrate; that there is one binding site on PK for ATP and ADP and another site for pyruvate or PEP; and that in the forward reaction non-compulsory or independent combination with ADP or PEP occurs with 'equilibrium kinetics' applying. This report describes an investigation of the kinetics of human red cell and leucocyte PK purified by methods previously described⁴. We observed properties for the human leucocyte PK identical to those described for rabbit muscle enzyme. The Michaelis constant (Fig. 1) observed for each substrate is independent of the concentration of the second substrate. The K_m values for PEP of 1.0×10^{-4} M, and for ADP of 1.78×10^{-4} M, are very similar to the values reported⁶ for the rabbit muscle preparation. The relation between the general kinetic coefficients, $\frac{\Phi_1 \times \Phi_2}{\Phi_{12}} = \Phi_0$ according to

Dalziel⁷, is compatible with a mechanism of random addition of substrates in which the combination of the enzyme with one substrate does not influence its affinity for the second substrate.

The red cell enzyme has different kinetic properties. At least ten-fold higher concentrations of PEP are required, and apparent Michaelis constants for each substrate vary with the concentration of the second substrate (Fig. 2).

Initial velocities measured in the presence of one of products, ATP, obey the general equation:

$$\frac{(E_0)}{v} = \Phi_0 + \frac{\Phi_1}{(\text{PEP})} \left[1 + \frac{(\text{ATP})}{K_{\text{ATP}}} \right] + \frac{\Phi_2}{(\text{ADP})} + \frac{\Phi_{12}}{(\text{PEP})(\text{ADP})} \left[1 + \frac{(\text{ATP})}{K_{\text{ATP}}} \right]$$

These findings suggest competitive inhibition of ATP-PEP with an inhibition constant, K_{ATP} , of 3.5×10^{-4} . Only an ordered binding of substrates will account these results. Because of the assay system used, we have not yet studied the reverse reaction, nor the effect of pyruvate on initial velocities of the forward reaction. These reasons, we cannot be more specific about the mechanism for the red cell enzyme⁸.

The demonstrated differences in chemical⁴ and kinetic properties of erythrocyte and leucocyte PK support known difference in genetic control² of this enzyme. Evaluation of kinetic differences between normal and mutant erythrocyte enzyme, however, must take into account the more complicated mechanism of action of erythrocyte PK. Waller and Lohr⁹ have reported a high Michaelis constant of red cell PK for PEP in patients with pyruvate kinase deficiency. Three unrelated patients whom we have studied the kinetics of purified red cell enzyme showed no difference from normal for both substrates.

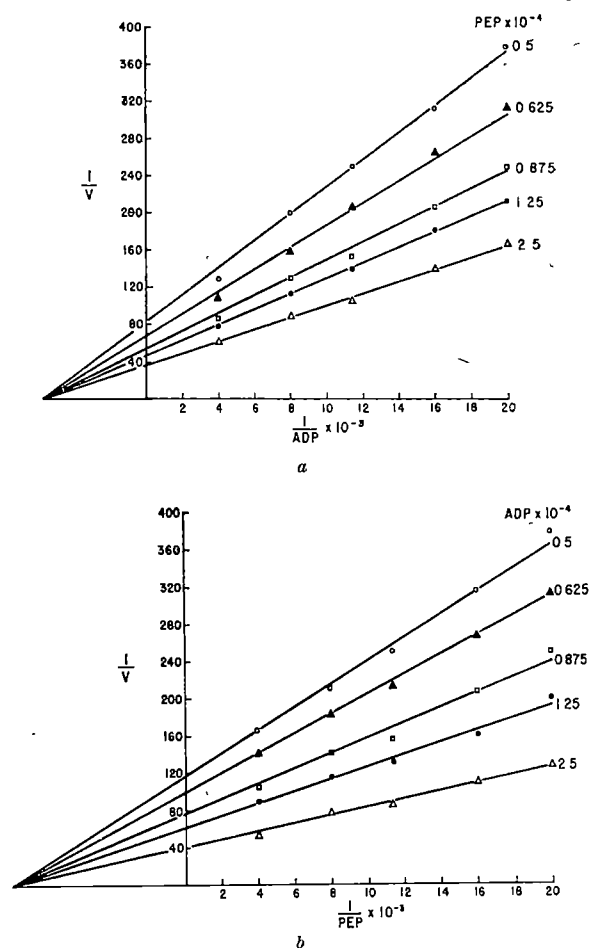


Fig. 1. (a) Effect of ADP on the velocity of the leucocyte PK reaction at various PEP concentrations. Assays were made at 37° C in a total volume of 3.0 ml. with 0.075 M K⁺; 0.016 M Mg⁺⁺; 0.008 M triethanolamine HCl at pH 7.4, and PEP at 0.5; 0.625; 0.875; 1.25 and 2.5 $\times 10^{-4}$ M. The initial velocity is in micromoles of DPNH oxidized per min (method of Bücher and Pfleiderer¹⁰). Intersection at the negative abscissa gives an estimate for K_{ADP} of 1.78×10^{-4} M. (b) Effect of PEP on the velocity of the leucocyte PK reaction at 0.5; 0.625; 0.875; 1.25 and 2.5 $\times 10^{-4}$ M ADP concentrations. Conditions were the same as above. Estimate for K_{PEP} of 1×10^{-4} M.

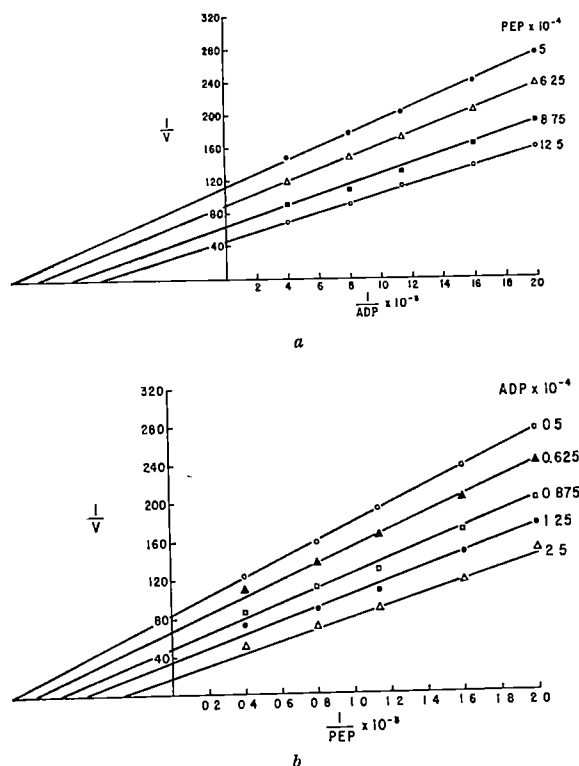


Fig. 2. (a) Effect of ADP on the velocity of the red cell PK reaction at 5; 6.25; 8.75 and 12.5×10^{-4} M PEP concentrations. Conditions were the same as in Fig. 1. (b) Effect of PEP on the velocity of the red cell PK reaction at 0.5; 0.625; 0.875; 1.25 and 2.5×10^{-3} M ADP concentrations. Conditions were the same as in Fig. 1.

strates. This would suggest a control gene mutation involved in this disease.

This work was supported in part by grant CA-07941 from the National Institute of Cancer Research and by grant AT 45-1 (581) from the Atomic Energy Commission. One of us, José Campos, has been supported by a Kellogg Foundation fellowship.

JOSÉ O. CAMPOS
ROBERT D. KOLER
ROBERT H. BIGLEY

Division of Experimental Medicine,
University of Oregon Medical School,
Portland, Oregon.

¹ Selwyn, J. G., and Dacie, J. V., *Blood*, **9**, 414 (1954).

² Valentine, W. N., Tanaka, K. R., and Miwa, S., *Trans. Assoc. Amer. Physicians*, **74**, 100 (1961).

³ Fellenberg, R. von, Richterich, R., and Aebi, H., *Enzymologia Biologica et Clinica*, **3**, 240 (1963).

⁴ Koler, R. D., Bigley, R. H., Jones, R. T., Rigas, D. A., Vanbellingham, P., and Thompson, P., *Cold Spring Harbor Symp. Quant. Biol.*, **29**, 213 (1964).

⁵ Boyer, P. D., in *The Enzymes*, **6**, 95, edit. by Boyer, P. D., Lardy, H., and Myrback, K. (Acad. Press Inc., New York, 1962).

⁶ Reynard, A. M., Hass, L. F., Jacobsen, D. D., and Boyer, P. D., *J. Biol. Chem.*, **236**, 2277 (1961).

⁷ Dalziel, K., *Acta Chem. Scand.*, **2**, 1706 (1957).

⁸ Alberty, R. A., *J. Amer. Chem. Soc.*, **80**, 1777 (1958).

⁹ Waller, H. D., and Lohr, G. W., *Abst. IX Congr. Europ. Soc. Haemat.*, Lisbon, p. 74.

¹⁰ Bucher, T., and Pfeleiderer, G., in *Methods in Enzymology*, **1**, 435, edit. by Colowick, S. P., and Kaplan, N. O. (Acad. Press Inc., New York, 1955).

Inhibition of Haemolysis by 'Cremophor' in Conserved Blood

THE biological value of conserved blood is determined by the erythrocyte survival time after re-infusion which, after storage for 3-4 weeks, is so low as to prohibit further use of the erythrocytes. The plasma, however, may be used for several other purposes if it is not too haemolytic. After a lapse of about the same time the haemolysis rate increases remarkably so that blood banks are often

obliged to discard whole-blood conserves after the date of expiration.

Claims have been made that vitamin E possesses haemolysis-inhibiting properties in blood banks¹⁻⁵. In an attempt to verify this quality of vitamin E and to elucidate its mechanism of action we added solutions of tocopherol acetate (50 mg/ml.) in a ratio of 2 per cent v/v to ACD-blood. As an emulgator of the water-insoluble vitamin E, these solutions contained 'Cremophor EL'* (manufactured by Badische Anilin- u. Sodafabrik AG., Ludwigshafen) in concentrations varying between 40 and 200 mg/ml. Appropriate controls were run with 'Cremophor EL' only. These banks were judged visually as to haemolysis after a storage time of 40-80 days. Haemoglobin determinations were carried out in the supernatant ACD-plasma after centrifugation. The results showed, much to our surprise, that 'Cremophor EL' alone has a haemolysis-inhibiting effect far exceeding a rather questionable effect of vitamin E. The plasma of ACD-blood to which solutions of 40-200 mg/ml. 'Cremophor EL' had been added (final concentration 0.8-4 mg/ml.) remained perfectly clear after a storage time of 40-80 days and contained no appreciable amounts of haemoglobin, whereas the ACD controls were severely haemolytic. Indeed, the addition of vitamin E in low concentration ranges of 'Cremophor EL' seemed to block even the 'Cremophor EL' action. It seems possible that the surface activity of 'Cremophor EL', exhausted by vitamin E in the investigations with low concentrations of 'Cremophor EL', represents the key to the mode of action. Investigation of the activity of other surface-active agents seems therefore indicated. From the technical point of view the haemolysis of ACD-blood can be virtually abolished by addition of 'Cremophor EL', up to a storage time of about 80 days (perhaps even longer).

It remains an open question whether, in the papers cited here, surface-active agents have interfered, as few data concerning controls with such substances are mentioned. However, from the data given in refs. 2, 3 and 5 it can be concluded that 'Cremophor EL' has been used as an emulgator.

K. REBER

Department of Experimental Medicine,
F. Hoffmann-La Roche and Co., Ltd.,
Basle, Switzerland.

* Trade mark.

¹ Luczak, S., *Langenbecks Arch. Klin. Chir.*, **301**, 786 (1963).

² Luczak, S., and Wolf, F., *Deutsch. med. Wschr.*, **88**, 707 (1963).

³ Meyer-Wegener, H., and Luczak, S., *Klin. Wschr.*, **39**, 754 (1961).

⁴ Weinstein, I. M., Mathies, J. C., Katzmann, R., and Forney, P. P., *Proc. Soc. Exp. Biol. (N.Y.)*, **99**, 170 (1958).

⁵ Kleine, N., *Med. Welt*, 485 (1964).

RADIOBIOLOGY

Uptake by Plants of Radiostrontium from Contaminated Soils

IN a recent report from this department¹ it was shown that the extractability of radiostrontium from contaminated soil samples was effectively reduced by heat treatment and by the addition of phosphate to the soil. It was pointed out that, under emergency conditions, heat-treatment of the contaminated soil surface and heavy phosphate application might thus reduce the uptake by plants of radiostrontium more efficiently than liming, which is only effective in soils of low calcium status. In the investigation reviewed here the influence of heat treatment and superphosphate application on the plant uptake of radiostrontium was examined in pot experiments. For comparison the effect of applying calcium carbonate to the contaminated soil surface was also determined.

Spring barley (variety 'Pallas') was grown to maturity in PVC pots, each containing 22 kg of soil. Two loamy and

Table 1. ANALYSIS OF SOIL

Locality	Soil type	pH in water	Cation-exchange capacity (m. equiv./100 g)	Exchangeable Ca ⁺⁺ (m. equiv./100 g)	Ca-saturation (%)	Organic matter (%)
Blangstedgaard	Loamy	7.6	15.2	12.6	83	2.2
Ødum	Loamy	7.0	9.2	6.1	66	1.6
St. Jyndeved	Sandy	6.5	11.1	3.9	35	2.4
Borris	Sandy	6.4	10.5	3.7	35	1.9

two sandy soils were used (Table 1). 11 kg of soil was put into each pot, and 40 µc. of carrier-free ⁸⁹Sr in 20 ml. of water was sprayed evenly over the surface before the superphosphate and calcium carbonate were added. For the heat treatment the contamination was performed by adding the carrier-free ⁸⁹Sr to 160-g samples of soil, which were then air-dried and heated to the desired temperature for 10 min. The contaminated sample was evenly distributed over the surface of the 11 kg of soil in the pot. The additional 11 kg was then added, and the seeds were sown. It was attempted with this experimental technique to imitate a surface contamination followed by treatments and ploughing. The following treatments were examined:

Control: 40 µc. ⁸⁹Sr per pot.
 T-500: 40 µc. ⁸⁹Sr and heating to 500° C
 T-800: 40 µc. ⁸⁹Sr and heating to 800° C.
 P-1: 40 µc. ⁸⁹Sr + 10 g superphosphate (equiv. to 2 tons/ha).
 P-2: 40 µc. ⁸⁹Sr + 20 g superphosphate (4 tons/ha).
 Ca-P: 40 µc. ⁸⁹Sr + 75 g CaCO₃ + 20 g superphosphate (15 and 4 tons/ha resp.).
 Ca-1: 40 µc. ⁸⁹Sr + 37.5 g CaCO₃ (7.5 tons/ha).
 Ca-2: 40 µc. ⁸⁹Sr + 75 g CaCO₃ (15 tons/ha).

The concentration of ⁸⁹Sr in grain and straw is given in Table 2.

Table 2. THE CONCENTRATION OF STRONTIUM-89 IN BARLEY GROWN IN FOUR CONTAMINATED SOIL TYPES IN POT EXPERIMENTS

Treatment	µc. ⁸⁹ Sr/g dry matter in barley grown in soils from:							
	Blangstedgaard Grain	Blangstedgaard Straw	Ødum Grain	Ødum Straw	St. Jyndeved Grain	St. Jyndeved Straw	Borris Grain	Borris Straw
Control	0.48	3.63	0.74	4.64	0.67	4.75	1.12	6.89
T-500	0.29	1.99	0.45	3.00	0.50	3.87	0.89	4.73
T-800	0.020	0.15	0.036	0.21	0.21	1.23	0.26	1.27
P-1	0.25	1.82	0.41	2.81	0.27	1.83	0.39	2.33
P-2	0.14	1.16	0.24	1.85	0.16	1.13	0.26	1.45
Ca-P	0.29	2.32	0.47	2.90	0.40	2.42	0.63	3.57
Ca-1	0.44	3.65	0.82	4.95	0.67	4.66	1.36	7.17
Ca-2	0.52	3.44	0.69	4.98	0.72	4.84	1.42	7.53

Heating the contaminated soil to 800° C reduced the uptake of radiostromium by plants from loamy soils by a factor of twenty. In the sandy soils only a four-fold decrease in the uptake was found. The difference between loamy and sandy soils is greater than expected from the extractability investigations¹; but this might be ascribed to differences in the mineral composition of the soils used. The fixation of radiostromium is probably caused by an alteration of the clay minerals at high temperatures. The results indicate that a very effective reduction in the uptake from loamy soils can be obtained by heating the contaminated soil surface; but the practical application of this method is complicated by the high temperatures needed.

Addition of superphosphate caused a marked reduction in the plant uptake of radiostromium. The largest amount—equivalent to 4,000 kg per hectare—resulted in a four-fold decrease in the strontium-89 uptake from the sandy soils; in the loamy soils the reduction factor was three. It is known from fertilizer experiments that the plants utilize only a small part of the phosphorus added in superphosphate. The remaining portion is converted into phosphate compounds of lower solubility, for example, dicalcium phosphate, octacalcium phosphate and hydroxyapatites. It seems reasonable to expect that a co-precipitation of slightly soluble calcium-strontium phosphate would occur in contaminated soils if sufficient amounts of phosphate were added. However, most of the previous experiments on the effects of phosphate application to contaminated soils²⁻⁴ have shown only a small reduction in the plant uptake of radiostromium. Although Uhler⁵ used massive amounts of ammonium phosphate

(phosphorus content equivalent to that in 28.5 tons superphosphate/ha), the uptake from a calcareous soil was only reduced by 50 per cent, and no effect was seen on acid soil. The radiostromium and phosphates were usually mixed into the entire rooting medium, and consequently the phosphate concentration may have been too low to cause precipitation. In the present investigation a high concentration of phosphate in close contact with strontium-89 was assured by applying the superphosphate directly to the contaminated soil surface. The results obtained by means of this technique could be interpreted as increased precipitation of strontium in the form of slightly soluble phosphates, and thus a reduced plant availability. Addition of calcium carbonate together with the superphosphate decreased the effect as compared with superphosphate alone.

Addition of calcium carbonate did not reduce the uptake of radiostromium from these soils. On the contrary, the results indicate a slight increase in the uptake, even from the sandy soils of relatively low calcium saturation. The high concentration of calcium carbonate in intimate contact with the contaminated soil layer has apparently caused an increased plant availability of the radiostromium. This is in agreement with the solubility characteristics reported by Schroeder *et al.*⁶ and the suggestion by Wiklander⁷ that heavy application of lime might lead to increased solubility of strontium.

Preliminary results from a similar—but long-term experiment with the same four soils in microplots in the field show the same tendencies as the results of the present experiment reported here.

A. J. ANDERSEN

Agricultural Research Department,
 Danish Atomic Energy Commission
 Research Establishment, Riso,
 Roskilde.

¹ Gregers-Hansen, B., *Nature*, **201**, 738 (1964)² Reitemeler, R. F., and Menzel, R. G., *Trans. Seventh Intern. Congr. Soil Sci.*, **3**, 35 (1961).³ Frederiksson, L., Eriksson, Å., and Haak, E., *FOA-4 Rapport A 4188-46*, Pp. 30 (Försvarets Forskningsanstalt, Stockholm, 1961).⁴ Andersen, A. J. (unpublished results).⁵ Uhler, R. L., *Hanford Biol. Res., Ann. Rep.*, **HW-65500**, 42 (1959).⁶ Schroeder, D., von Reichenbach, H. G., and Freiherr von Dem Bussche, G., *Naturwissenschaften*, **49**, 137 (1962).⁷ Wiklander, L., *Soil Sci.*, **97**, 168 (1964)

Mechanism of Uptake of Trace Elements by Plant Roots

It is well known that chemical elements can be concentrated in very large factors by plant cells, that is to say, the final concentrations in the cells may exceed those in the media by many orders of magnitude. Enrichment is often observed not only for elements that are essential to the plants, but also for elements not known to be essential and even sometimes for elements that normally do not occur to any extent in plants.

The question thus arises as to what mechanism is responsible for the elements being taken up against concentration gradients. In the case of some major nutrients accumulation is due to 'active transport', that is, the required osmotic work is done at the expense of metabolic free energy. For example, much of the uptake of potassium is due either to its own active transport or to a potential difference set up in the active transport of another ion¹. However, so far as we are aware the importance of active transport does not appear to have been demonstrated for the accumulation of 'trace elements', here to be defined as elements needed by plants only in minute quantities, or not needed at all.

The nature of the mechanism responsible for the accumulation of trace elements is of basic importance to agriculture and also to the evaluation of hazards due to

ionuclides, especially from nuclear explosions (fall-out) from nuclear reactors. Therefore, following up preliminary experiments with unicellular algae (*Chlorella*)², have studied the uptake of zinc, an essential element, roots of a crop plant.

We used barley plants that had been grown for about days in tap water. The roots were about 10 cm long; they were cut from the plants 4 h before the start of the experiments and washed several times with distilled water. Normally 1–2 g of roots were shaken with 10 ml. a solution of radiozinc (zinc-65; half-life 245 days), and aerated, for a few hours. The uptake was computed from the remaining activity of the solutions, as measured with a Geiger liquid counter after removal of the roots. For comparison, analogous experiments were performed using radiopotassium (potassium-42; half-life 5 h). In a few experiments, longer-lived rubidium-86 (3.7 days) was substituted for potassium; it is well known that rubidium and potassium behave similarly in respect of uptake³. Occasionally, experiments were repeated without severing the roots from the green parts, but the results were not very different. The enrichment factor defined as the ratio of the concentration in tissue (roots) to that in the supernatant for zinc was high—about 10^4 in the case of zinc concentrations of the order 10^{-7} M in the supernatant—and depended on experimental conditions. However, the mechanism of enrichment is clearly quite different from that for potassium.

(1) Equilibrium of zinc between roots and solution was reached within a few minutes while uptake of potassium continued for much longer periods. (2) The 'decoupling' metabolic poison dinitrophenol (DNP), in concentrations up to 10^{-2} molar (M), had no influence on the uptake of zinc, while the uptake of potassium was depressed to less than 10 per cent of the normal value; similar results were also obtained with sodium azide. (3) After the roots had been killed by treatment with alcohol for 10 min, the uptake of potassium was practically zero, while that of zinc increased by 50–100 per cent. (4) After root tissue had been homogenized by grinding with glass, the uptake of potassium was almost completely suppressed, but that of zinc again increased two-fold. (5) While the uptake of potassium was not influenced by most foreign ions, for example, sodium or magnesium, in moderate concentrations (up to 10^{-2} M), foreign ions generally competed strongly with zinc: for example, the elements lanthanum, cerium, calcium, magnesium, cobalt, manganese, strontium and barium displaced radiozinc with efficiencies decreasing in that order, namely 138–35 per cent as strongly as active zinc of equal (2×10^{-4}) molarity. (6) At least in the range 0°–20° C, and during a period of 5 h, the temperature coefficient for the uptake of radiozinc by intact roots was very much (about a factor of 10) smaller than that for the uptake of radiorubidium.

We suggest that the accumulation of zinc by the plants is due to passive processes, not dependent on metabolism or the integrity of the cells, namely, to exchange adsorption and compound formation⁴. Processes not linked with metabolism are, of course, also observed with potassium^{3,5}, but in experiments over longer periods active processes predominate.

We thank the Bundeskanzleramt-Verstaatlichte Untersuchungen (Section IV), Republic of Austria, for financial support.

G. FINDENEGG
E. BRODA

Institute of Physical Chemistry,
University of Vienna.

See Dainty, J., *Ann. Rev. Plant Physiol.*, **13**, 379 (1962).

Broda, E., Deaser, H., and Findenegg, G., *Naturwiss.*, **51**, 361 (1964).

See Epstein, E., *Ann. Rev. Plant Physiol.*, **7**, 1 (1956).

Kraus, H., and Broda, E., *Monatsh. Chem.*, **96**, 695 (1965).

See Briggs, G. E., Hope, A. B., and Robertson, R. N., *Electrolytes and Plant Cells* (Oxford, 1961).

BIOLOGY

Prolactin and the Incidence of Brood Patch Formation and Incubation Behaviour of the Two Sexes in Certain Birds with Special Reference to Phalaropes

It has been demonstrated experimentally that brood patch formation in certain passerine birds (in which typically, only the females develop brood patches and incubate in Nature) depends on oestrogen and prolactin action¹.

In the phalaropes (a sub-family of three species only, two holarctic, one Wilson's (*Steganopus tricolor*), nearctic) only males incubate and only they form brood patches. Johns and Pfeiffer² showed experimentally that the hormonal stimuli for brood patch formation in two species of phalaropes (one of which was Wilson's) are an androgen and prolactin.

One of us has made a preliminary publication³ citing data on the gonadal concentrations of the principal sex hormones of breeding Wilson's phalaropes in comparison with those of some other birds. He does not now altogether accept the interpretation given³ to these data, but the results reported show that female phalaropes are not deficient but rather 'high' in gonadal androgens and hence are unlikely to be poor androgen secretors. Therefore their failure to form brood patches might depend on a deficiency of prolactin, rather than a lack of androgen production.

This communication reports the results of experiments with: implants of the pituitaries of Wilson's phalaropes or Killdeer plovers (*Charadrius vociferus*) on the crop sac of common domestic pigeons by the technique described elsewhere¹; or with intradermal injections of pituitary extract-suspensions over the crop sac, followed in both cases by the technique of examination previously outlined by one of us⁴. Table 1 summarizes the results obtained. Although in no case did we obtain a full-blown crop sac development with up to 10% of prolactin (ovine supplied by the Endocrinology Study Section, U.S. National Institutes of Health) or with our avian materials, the data show that breeding male phalaropes produce some prolactin and females significantly less or none. Our two tests on Killdeer material permit no conclusion, but they are compatible with the view that since both sexes of this bird develop brood patches both may form prolactin.

Supporting evidence comes from the data on relative pituitary weight of breeding season Wilson's phalaropes, red-winged blackbirds (*Agelaius phoeniceus*) and of Killdeer which are summarized in Table 2. It should be noted that one of us has previously demonstrated the presence of prolactin in female (males were not tested) red-winged blackbird pituitaries⁵.

We can see no obvious *a priori* reasons for sexual differences in adeno-hypophyseal function in breeding birds of the three representative species examined, other than relative differences in prolactin production. We believe

Table 1. PIGEON CROP SAC TESTS FOR PROLACTIN, USING IMPLANTS (I) OR INTRADERMAL INJECTIONS OF SALINE OR WATER EXTRACT SUSPENSIONS (E) OF BIRD PITUITARIES APPLIED TO THE CROP SAC FOR 4 DAYS

Material applied to experimental side	Response	Material applied to control side	Response
15 M phal. pits. I	—+	Piece of phal. brain	0
14 M phal. pits. I	—+	Piece of phal. brain	0
39 M phal. pits. E	—	Prolactin 10γ	+
20 M phal. pits. E	C	Prolactin 10γ	+
17 M phal. pits. + 3 doses E	—		
of M phal. pits.	—	Nil	0
18 F phal. pits. I	C	Piece of phal. brain	0
40 F phal. pits. E*	C	Prolactin 3γ	—+
15 M Killdeer pits. E	—+	Prolactin 10γ	—+
15 F Killdeer pits. E	C	Prolactin 10γ	—+

* Pigeon died on third day of treatment.

Responses were evaluated by macroscopic and microscopic examination of the crop sacs and rated as follows: 0, no response; —+, suggestion of a positive response; +, minimal but definite positive response.

Abbreviations: M, male; F, female; phalarope pituitaries, Wilson's phalarope pituitaries.

Table 2. RELATIVE PITUITARY WEIGHT (PITUITARY WEIGHT IN MG PER 50 g BODY-WEIGHT; THE APPROXIMATE MEAN BODY-WEIGHT OF MALE WILSON'S PHALAROPES) IN BOTH SEXES OF BREEDING WILSON'S PHALAROPES AND TWO OTHER SPECIES OF BIRDS

Female Wilson's phalarope (28)	1.20 ± 0.04
Male Wilson's phalarope (60)*	1.50 ± 0.11
Female red-winged blackbird (22)*	1.81 ± 0.12
Male red-winged blackbird (20)	1.26 ± 0.16
Female Killdeer plover (29)*	0.86 ± 0.07
Male Killdeer plover (38)*	0.86 ± 0.07

Figures in parentheses indicate the number of birds examined. An asterisk denotes the sex which naturally develops a brood patch and incubates.

that, in birds in general, prolactin is the hormonal stimulus to incubation and brood patch development⁶, except in pigeons and doves where, since it controls crop sac development in members of this family, a special case is involved (see refs. 7 and 8).

It is evident from the findings given in Table 2 that in the first two species, in which only one sex naturally develops a brood patch and incubates, that sex has the higher relative pituitary weight. In the Killdeer, in which both sexes form brood patches and incubate, relative pituitary weights are similar. The unit of body-weight (50 gm), relative to which we have expressed pituitary weights in Table 2, is arbitrary but approximates the mean body-weight of the smaller of the two sexes in the first two species.

Further evidence in favour of greater prolactin production in male than in female phalaropes was obtained by a comparison of the number of acidophils per unit area of adenohypophysis in microscopic sections of this tissue from 3 males and 3 female red-necked phalaropes (*Phalaropus lobatus*), all in breeding condition. Acidophils were stained with acid fuchsin by a method previously described⁹. Mean numbers of acidophils per unit standard area (based on counts over 8 such areas for each bird, representing about one-fifth of the total area of the adenohypophysis cut at 5μ) were: males 236 ± 16.0 and females 125 ± 41.5. A paired *t* test applied to the data gave a *P* value for this difference of the means of about 0.08.

There is good evidence that prolactin is produced by fuchsinophilic acidophils in birds^{10,11}.

This work was supported by grants from the National Research Council and the American Museum of Natural History.

E. O. HÖHN
S. C. CHENG

Department of Physiology,
University of Alberta,
Edmonton.

¹ Bailey, R. B., *Condor*, 54, 121 (1952).

² Johns, J. E., and Pfeiffer, E. W., *Science*, 140, 1225 (1963).

³ Dyrenfurth, I., and Höhn, E. O., *J. Physiol.*, 169, 42P (1963). (Note The heading of Table 1 in this publication should read "Gonadal Steroid Hormone Content (μg/Individual Bird) Means for Birds Collected During 1962").

⁴ Höhn, E. O., *J. Endocrinol.*, 26, 177 (1963).

⁵ Höhn, E. O., *Nature*, 184, 2030 (1959).

⁶ Höhn, E. O., *Ibis*, 104, 418 (1962).

⁷ Riddle, O., *Animal Behav.*, 11, 419 (1963).

⁸ Eisner, E., *Animal Behav.*, 8, 155 (1960).

⁹ Höhn, E. O., and Westwood, L. A., *Nature*, 157, 484 (1946).

¹⁰ Yasuda, M., *Proc. Japan. Acad.*, 29, 586 (1953).

¹¹ Payne, F., *Anat. Record*, 86, 1 (1943).

Relation of Population Density to Sodium Availability and Sodium Selection by Microtine Rodents

A COLLECTION of 55 published records of population densities of rodents of the genus *Microtus* reveals a strong correlation between levels reached at population peaks and local soil sodium-levels as reported in soil surveys or deduced from indirect evidence. In areas with low soil sodium the highest density reported was 230 animals per acre and populations exceeded 30 individuals per acre in only 12 (19 per cent) of 63 reports. In regions of inter-

mediate soil sodium, *Microtus* populations ranged up to 400 with 16 of 22 (72 per cent) reports listing more than 30 per acre. In regions of high soil sodium there were records in excess of 1,000 animals per acre and 30 or more (91 per cent) records were more than 30. Cyclic population fluctuations seem to occur in the animals regardless of sodium-levels, but the densities reached at population peaks are characteristically many times higher in regions of high soil sodium than in regions of low soil sodium. The correlation could be due to other ions associated with sodium in the soil. However, the possibility that it reflects a functional relationship between availability of sodium and population regulation suggested a series of laboratory experiments with meadow voles (*Microtus pennsylvanicus*) from a laboratory colony established with wild animals trapped at Madison, Wisconsin.

Two experiments were conducted to test the effect of restricted and unrestricted sodium chloride diets on reproduction and population growth. Eight 3' × 6' pens, each containing two males and two females, and eight 3' × 3' pens, each containing one male and three females, were established and left undisturbed for 15 and 19 weeks respectively. Alfalfa hay and whole oats were supplied in excess to all animals. Four replicates of each sex-group were supplied *ad lib.* with a 0.5 per cent NaCl solution and with distilled water; the control groups received only distilled water. Populations supplied with the NaCl solution produced 60 young in the 2 male groups compared to 43 and 64 in the restricted sodium diet groups (*P* less than 0.05, paired *t*-test). The groups with sodium *ad lib.* maintained a higher net population over the test periods (*P* less than 0.05, paired *t*-test).

In a second group of experiments segregated sex and mixed sex groups of 5- to 8-week-old voles were tested for self-selection of NaCl at various levels of crowding. They were provided with a choice of distilled water and NaCl solution in paired 'J' tubes, which were calibrated for direct measurement of daily fluid consumption. The series of tests were conducted: (a) high-density groups (8 animals per pen) and low-density groups (4 animals per pen) of each sex were compared directly; (b) 24 animals of each sex were subjected successively to four 14-day test periods with densities of 4, 8, 24 and then back to 4 animals per pen; (c) mixed-sex groups with one male and three females per pen were compared with groups containing two males and two females. High-density animals in test series a selected about 70 per cent (males) and 90 per cent (females) more of the NaCl solution than the low-density animals (*P* less than 0.01, paired *t*-test). In test series b NaCl selection varied directly with crowding when density was changed on the same twenty-four animals. This was most striking in males, where the ratio of NaCl solution to distilled water selected increased from about 2:1 successively to 6:1, 10:1 and back to about 4:1 with the return to low density. In test series c, groups consisting of two males and two females selected a significantly higher ratio of NaCl solution to distilled water (6.2:1) than did the groups of one male and three females (2.1:1).

Increased appetites for sodium shown by adrenalectomized laboratory animals have been considered to reflect an increased physiological need for this salt¹⁻³. This suggests that the higher selection of NaCl by our animals under conditions of continued crowding was a manifestation of sodium deficiency, perhaps due to inadequate adrenocortical regulation of sodium metabolism. The behavioural and physiological mechanisms by which sodium deficiency might affect reproduction in crowded animals is beyond the scope of this report.

The field data and laboratory tests described above suggest that with restricted access to salt the growth of vole populations may be checked at relatively low levels by physiological responses associated with crowding, and that with unrestricted access to salt the suppressive effects of crowding are alleviated, allowing populations to increase.

higher levels. Implied in these deductions is the principle that sodium is the critical factor limiting vole populations in many areas. In regions with high sodium, enough salt may be ingested with the vegetation to satisfy the increased requirements of crowded animals, thereby permitting unrestricted reproduction and population growth to the point where food or some other factor becomes critical.

GLENN D. AUMANN
JOHN T. EMLEN

Department of Zoology,
University of Wisconsin,
Madison.

hater, C. P., *Endocrinology*, 24, 387 (1939).
hater, C. P., in *Essays in Biology*, 500 (Univ. Cal. Press, Berkeley, 1943)
rman, D. S., in *L'instinct dans le comportement des animaux et de l'homme*
475 (Librairie de l'Académie de Médecine, Paris, 1956)

Simple Technique for embedding and supporting Delicate Biological Specimens

MANY biological specimens are too large for treatment by normal microscopical techniques and, at the same time, too small and delicate to withstand very much manipulation. Many embedding media have been tried in the past few years, and one of the most efficient and effective is agar.

A 4 per cent aqueous (distilled water) solution of agar (Difco Laboratories) is prepared, either by free boiling in an autoclave or steamer, or by using a beaker suspended in a boiling water bath. The molten agar is cooled to about 40° C and a thin layer poured onto a solid watch-glass and allowed to set. The specimen is arranged on the surface of the agar and is then covered by a further layer of molten agar. It is important to ensure that no moisture is left on the first layer. Moisture bubbles will spread between the two layers and can cause separation after setting. This trouble is obviated by wiping the specimen and the surface of the agar with a small piece of filter paper after the specimen has been tented. When the agar has set, the lens-shaped block is eased out of the watch-glass and the part containing the specimen trimmed into a cubical block, using a sharp scalpel or razor blade. The cube (which is opaque when set) is then transferred to 70 per cent alcohol, in which it may be stored until required for clearing. For clearing, the block is gradually dehydrated by being taken through 70 per cent, 90 per cent and absolute alcohol, one day in each, and then transferred to a mixture of equal parts of absolute alcohol and benzyl alcohol for a few hours. It is then removed to pure benzyl alcohol for complete clearing. Both block and specimen will become transparent quite quickly but specimens containing a great deal of yolk may require a longer time to achieve complete transparency. In the case of amphibian eggs this is an advantage because the slight opacity gives a clearer picture of the segmentation stages.

The technique is particularly useful for eggs of cyclopoidea, teleost fishes and amphibia, coelenterate medusae, cross-sections of small molluscs and molluscan radulae too large for ordinary microscope mounts. Specimens may be stained before embedding, but they must be returned to water before processing. Blocks may be removed from the benzyl alcohol for examination under a dissecting microscope, but should not be kept in the air for a period of more than 3 h, otherwise splitting and drying of the block may occur. All material for treatment under these conditions must first be fixed and preserved as for normal storage. The blocks are tough and resistant to wear, but should it be necessary to remove a specimen from a block a careful cut across a block in one plane will enable the specimen to be removed without damage. All parings from the first trimming may be remelted and

used for more blocks. Agar cannot be recovered after treatment with alcohol.

R. H. HARRIS

Department of Zoology,
British Museum (Natural History),
London, S.W.7.

Selective Effect of Particulate Insecticides on *Simulium* among Stream Fauna

Simulium, the vector of onchocerciasis and the cause of considerable nuisance in northern latitudes, is usually controlled by killing its aquatic stages by putting insecticides in a soluble form into the streams where they occur. This kills most of the other insects; the natural predatory suppressors of the *Simulium* larvae—the larvae of some of the stone flies and caddis flies—are removed, and unrestrained recolonization by *Simulium* may occur.

The community of creatures living in fresh-water streams is highly organized and stable and constitutes many food chains, one ending in fish. The use of soluble DDT necessitates repetition and produces an entirely different organization, the maintenance of which is dependent on the maintenance of the interference. This changed environment can be regarded in many respects as biologically sterile.

Simulium larvae are particle feeders. They live only in the fast-flowing parts of the stream, and their method of feeding is different from that of the other detritus feeders such as the chironomids, which live in the slow-running and silted part of the stream. The size of particles ingested by British species of *Simulium* is about 10–12 μ (ref. 1). Since 1962, we have used DDT in the form of particles of different size in streams in North Wales. In one such experiment, 1×10^{12} particles of 4–15 μ were put into a stream in half an hour, when the stream was flowing at a rate of 0.15 cu. ft./sec. This corresponded to a dosage of 0.5 p.p.m. The next day, all *Simulium* larvae had disappeared from polythene tapes placed 150 yards below the point of dosage, and the tapes remained free of *Simulium* until a month after the dosing. During this time no other creatures in either the water or the stream bed showed any population variation other than the minor fluctuations expected from the changing seasons and rainfall. Not even net-spinning caddis fly larvae were affected by the small particles of DDT.

It is possible that a method of control developed on this principle will not disturb the predator insects which regulate the numbers of *Simulium* larvae, and it should not interfere with the tightly knit community of creatures which includes the food chain leading to fish. But we do not know whether the particulate insecticide accumulates in the sediment of the river bed, nor do we know anything of the potential effects of such accumulation, and we know nothing of the fate of that fraction which is taken up by *Simulium* larvae. These and other factors must be investigated.

We wish to thank "Shell" Research, Ltd., Woodstock Agricultural Research Centre, for providing water dispersible powders of the required particle size.

W. E. KERSHAW
T. R. WILLIAMS
S. FROST
H. B. N. HYNES*

Department of Parasitology and Entomology,
Liverpool School of Tropical Medicine and
University of Liverpool.

* Present address: Department of Biology, University of Waterloo, Ontario, Canada.

† Williams, T. R., Connolly, R. C., Hynes, H. B. N., and Kershaw, W. E., *Nature*, 189, 78 (1961).

MICROBIOLOGY

Bacterial Count and Cell Differentiation in Milk

A METHOD has been developed in this laboratory which has been found to be superior to the Breed-type smears such as are routinely used for assessing the bacteriological quality of cow's milk. The technique not only stains the bacteria, but also the somatic cells in such a way that the various cell-types may be satisfactorily differentiated and classified. Granular cytoplasmic inclusions which might be released on cell-degeneration are easily distinguished and not mistaken for the coccoid form of micro-organisms.

The use of 0.2 ml. of sample instead of 0.01 ml. (as in the Breed-method and its modifications), even distribution and low microscopic factor improves the precision of the count.

The fact that the cells are differentiated will help the investigator who is not only concerned with a total count to distinguish between pathological and normal conditions of the udder. The micro-organisms and the cells present in the milk sample are stained in a siliconed serological tube. This stained preparation is filtered, by vacuum filtration, through a membrane filter (M.F. Corp. plain white D.A.; diam., 25 mm; pore size, 0.65 μ). This filter, after being dried, is made transparent with immersion oil and the bacteria and cells can then be counted and studied with a light microscope.

The procedure is as follows: 2 ml. 'Triton X-100', 0.1 per cent solution, pH 7.0 (prepared with double distilled water and filtered through a membrane filter), is placed in a serological tube. 2-3 drops 0.5 per cent solution $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ is added. Three drops benzidine-peroxide solution is added (benzidine 0.2 g/200 ml. distilled water; to which is added 4 drops of H_2O_2 , 3 per cent). The milk sample (0.2 ml.) is added, and the mixture allowed to stand for 3 min. One drop of May-Grünwald stain (BDH standard stain in solution) is added, with 2 drops of Giemsa stain (improved Giemsa stain, Gurr R66 in solution). The tube is stood for 20 min in a water bath at 48° C, then poured into a filter funnel; two rinsings of the serological tube with 'Triton' solution at 48° C are also poured into the funnel. Vacuum is applied to pull the emulsion through the membrane filter. Rinsing is accomplished by flushing about 7 ml. 'Triton' solution (at 48° C) through the filter. The membrane filter is removed and dried on a glass slide at 35° C for about 10 min; immersion oil is added until the membrane filter becomes transparent. It is then covered with a cover slip. Another drop of immersion oil is placed on the cover slip, and the preparation is examined under the microscope.

I thank Prof. A. G. Leggatt for his advice.

C. L. DUTSCHAEVER

Department of Dairy Science,
University of Guelph,
Ontario.

Formation of Protocatechuic from Quinic Acid by Fluorescent Pseudomonads

ROGOFF¹ described two fluorescent pseudomonads he electively isolated from soil which could oxidize quinic acid with the formation of protocatechuic acid as an intermediate.

Fifty-two isolates, assignable to the genus *Pseudomonas* and producing pyoverdine in the medium of King, Ward and Raney², have been examined for their ability to aromatize quinic acid by this pathway. The strains were isolated from infected hen-eggs, surface waters, chicken carcasses, chicken droppings, beef and milk, using ordinary peptone media containing no quinate.

Protocatechuic formation by the isolates was investigated in the following medium, adapted from that of

Rogoff¹: Solution A. NH_4Cl , 0.4 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.05 g; $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.01 g; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.001 g; CaCO_3 , 0.2 g; yeast extract (Difco), 0.05 g; agar N (Oxoid), 0.05 g; distilled water, 50 ml., pH 7.4. Solution B. K_2HPO_4 , 0.1 g; quinic acid, 1.0 g; distilled water, 50 ml., pH 7.4 (adjusted with 10 per cent (w/v) NaOH). The two solutions were mixed, dispensed in 2-ml. amounts into test-tubes (100 mm \times 10 mm) and autoclaved 15 min at 15 lb. pressure. One drop from broth culture of the 52 strains was used to inoculate the quinate medium. On incubation at 25°, protocatechuic acid was readily detected by the formation of a purple colour below bacterial surface growth due to the action of ferrous ions on this compound. In most such cases, a green colour also appeared at the medium surface: this may have been due to the reaction of protocatechuic acid with ferrous ions, formed at the medium surface by oxidation of ferrous ions (Soloway and Rosen³), but since this zone fluoresces strongly it may simply have been pyoverdine produced on the surface of the medium. A drop of medium removed from the purple portion of each culture did, however, form a deep green colour on the addition of a drop of aqueous 0.5 per cent (w/v) $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$. By these tests, protocatechuic acid was discernible in the culture within 2-3 days; after a week or so, the purple colour slowly disappeared and no ferric chloride reaction could be demonstrated.

Of the 52 fluorescent pseudomonads, 49 produced protocatechuic acid. The three negative strains (from river water, chicken droppings and spoiling beef respectively) grew more sparsely on the medium, utilizing the yeast extract as a carbon source, presumably. An interesting biochemical characteristic these three strains shared was the ability to decarboxylate L-glutamic acid to γ -aminobutyric acid; one of the other strains was also able to carry out this reaction but it possessed in addition L-aspartic acid decarboxylase.

Manometric experiments with washed cell suspensions gathered after 60 h growth on the quinate medium (solidified with 1.5 per cent w/v agar) demonstrated oxygen uptake by the three strains in the presence of sodium quinate (pH 7.4, 30°), whereas representatives of the other 49 strains rapidly oxidized this substrate.

It would seem that fluorescent pseudomonads commonly possess the ability to oxidize quinic acid via protocatechuic acid but that some strains are unable to attack it by this or any other pathway. Since the formation of protocatechuic acid can be easily demonstrated in growing cultures by its reactions with ferrous and ferric ions, and since a significant number of strains do not effect production, the test should prove a useful one for inclusion in taxometric methods for the sub-grouping of *Pseudomonas*. It has been found that the use of a solid medium favours the accumulation of the protocatechuic acid formed in the region below the surface growth and prevents its too rapid oxidation.

D. J. STEWART

Department of Agricultural Bacteriology,
Ministry of Agriculture for Northern Ireland
and The Queen's University of Belfast.

¹ Rogoff, M. H., *J. Gen. Microbiol.*, 19, 330 (1958).

² King, E. O., Ward, M. K., and Raney, D. E., *J. Lab. Clin. Med.*, 54, 1 (1954).

³ Soloway, S., and Rosen, P., *Anal. Chem.*, 25, 595 (1953).

VIROLOGY

Rubella Virus Complement-fixation Test

METHODS in present use for the serological diagnosis of rubella consist of the neutralization^{1,2} and the indirect fluorescent-antibody³ techniques. The former is expensive and laborious and requires usually 7-10 days to carry out, while the latter, although it can give an answer within a matter of hours, is a difficult technique for routine

agnosis. The preparation of a complement-fixing antigen recently been described⁴, providing for a rapid and simple test of infection. The antigen, which is cell-associated, was made in the RK₁₃ line of rabbit kidney cells or in primary cultures of kidney from the African green monkey. The infected cells were processed 2 days and 7–10 days respectively after inoculation of virus. Successful antigen preparation, however, is in both cases dependent on a critically large virus inoculum; otherwise results are erratic. The present report describes a simpler and more reliable method of complement-fixing antigen preparation, involving the use of aged cultures of a chronically infected line of LLO-MK₂ cells⁵.

The chronically infected cells were grown in Roux bottles in Medium 199 containing 1 per cent inactivated horse serum and antibiotics and adjusted to pH 7.4 with sodium bicarbonate. The cultures were maintained by replacing half the medium every 3–4 days. Antigen was prepared at intervals by discarding the medium and scraping the cells from the glass into a small volume of Rönal-buffered saline. The cells were sedimented by centrifuging at 1,000 r.p.m. for 10 min, and resuspended in buffer to a final volume equivalent to 1 ml. per Roux bottle. This cell suspension was then frozen and thawed three times, or subjected to ultrasonic vibrations for 15 sec. The final product constitutes the antigen. The technique is similar to that used in preparing cytomegalovirus complement-fixing antigen⁶. The antigen can be stored at -70° C for at least 1 month without loss of potency. Activity is lost at 56° C within 0.5 h. Because of the relatively small amounts of antigen available, complement-fixation tests were carried out by the micro-technique, using 0.025-ml. Takatsy loops, micropipettes and 'Perspex' plates⁷. Each batch of antigen was examined in a chess-board titration against a positive control convalescent serum, using 2 units of complement and overnight fixation at 4° C.

Little or no antigenic activity was demonstrable in preparations from cell cultures less than 7 days old. Antigenicity first became detectable with 7–14-day-old cultures but the titres, measured as the reciprocal of the greatest dilution of antigen showing at least 3+ complement fixation with the positive serum, did not exceed 2. Maximum antigen titres of 8 were, however, reached after 4 days of incubation of the cell cultures. The material showed no or only minimal anticomplementary activity when used undiluted. While Sever *et al.*⁴ briefly mention in their paper the preparation of complement-fixing antigen from the chronically infected LLO-MK₂ cells, they apparently found no advantage with this technique and no details are given. The present findings indicate that with aged cells as described here the antigen potency is at least equivalent to that produced in RK₁₃ and primary African green monkey kidney cells. Although prolonged incubation of the chronically infected cells is required for antigen manufacture, the initial preparation of large inoculum doses of virus becomes unnecessary and, in our experience, potent antigens are obtained regularly.

Table 1 shows the results observed with a number of sera from children and adults. All sera for complement-fixation tests were inactivated by 56° C, and were titrated against 2 units of antigen. The tests were controlled with known positive and negative sera. Neutralizing antibody titres were measured in RK₁₃ cells. Serial dilutions of non-inactivated sera were mixed with equal volumes of a suspension of the Judith strain of rubella virus containing 100 TCID₅₀ per 0.1 ml., and the mixtures were incubated at 37° C for 1 h. Tissue culture tubes were then inoculated with 0.2 ml. amounts of the serum-virus mixtures, and incubated at 33° C in a roller drum. The tubes were examined for cytopathic effects over a period of 14 days. The neutralizing antibody titres were calculated as the reciprocal of the highest dilution of serum which completely suppressed the cytopathic effect of the virus.

Table 1. ANTIBODY RESPONSES IN PATIENTS WITH RUBELLA VIRUS INFECTION

Age	Time of serum collection	Antibody titres CF	Neutr.
Patients with clinical rubella			
18 yr.	First day*	<4	40
	21 days later	64	320
20 yr.	Second day*	<4	20
	24 days later	32	160
3 yr.	Third day*	<4	20
	7 days later	32	80
	23 days later	64	160
6 yr.	Third day*	<4	20
	20 days later	64	160
18 yr.	Sixth day*	16	80
	17 days later	64	80
Infants with rubella syndrome			
4 wk.†		<4	20
Mother		128	640
8 mos.‡		32	80
Mother		128	320
2.5 yr.§		<4	80
Adults with no or past history of rubella			
25 yr.		<4	160
24 yr.		<4	40
23 yr.		<4	20
21 yr.		<4	80
20 yr.		4	40

* After onset of rash.

† Virus isolated from throat and faeces.

‡ Virus isolated from urine and throat.

§ Not excreting virus.

While only a few cases of clinical rubella have so far been investigated with the complement-fixation test, it is apparent that the complement-fixing antibody develops in parallel with the neutralizing antibody but is delayed by several days. The latter antibody is already detectable in most cases by the second day after the onset of the rash^{1,2}, whereas the complement-fixing antibody probably does not appear until about the fifth day. The presence of neutralizing antibody without complement-fixing antibody in adults who give no recent history of rubella suggests that the complement-fixing antibody is transient⁴. The neutralization test will therefore still be necessary for the detection of past infection and for epidemiological studies. The three cases of rubella syndrome illustrate three different and interesting antibody situations. The first case possesses only neutralizing antibody, despite active infection and virus excretion. However, inability to make complement-fixing antibody is not uncommon in small infants in many other virus infections. The 8-month-old case shows both types of antibody, perhaps because of greater maturity and continued infection. The 2.5-year-old case has ceased to excrete virus, and no longer possesses demonstrable complement-fixing antibody. This disease differs, therefore, from cytomegalovirus and herpes virus infections, and the findings in this child appear to suggest that there is no persistent latent infection with rubella virus in children with the rubella syndrome.

The complement-fixation test provides an easy and rapid method of serological investigation of recent rubella virus infection, and should contribute to a more complete understanding of the pathogenesis and epidemiology of this common and important disease.

H. STERN

Department of Virology,
St. George's Hospital Medical School,
London, S.W.1.

¹ Parkman, P. D., Buescher, E. L., and Artenstein, M. D., *Proc. Soc. Exp. Biol. and Med.*, **111**, 225 (1962).

² McCarthy, K., Taylor-Robinson, C. H., and Furminger, I. G., *Lancet*, **ii**, 21 (1963).

³ Brown, G. C., Maassab, H. F., Veronelli, J. A., and Francis, jun., T., *Science*, **145**, 943 (1964).

⁴ Sever, J. L., Huebner, R. J., Castellano, G. A., Sarma, P. S., Fabyli, A., Schiff, G. M., and Cusumano, C. L., *Science*, **148**, 385 (1965).

⁵ Maassab, H. F., Veronelli, J. A., and Hennessy, A. V., *Bact. Proc.*, **144** (1964).

⁶ Stern, H., and Elek, S. D., *J. Hyg. (Camb.)*, **63**, 79 (1965).

⁷ Sever, J. L., Schiff, G. M., and Traub, R. G., *J. Amer. Med. Assoc.*, **182**, 663 (1962).

⁸ Plotkin, S. A., *J. Amer. Med. Assoc.*, **190**, 265 (1964).

⁹ Sever, J. L., Brody, J. A., Schiff, G. M., McAllister, R., and Cutting, R., *J. Amer. Med. Assoc.*, **191**, 624 (1965).

GENETICS

Phenocopies of the *ma-l* and *ry* Mutants of *Drosophila melanogaster*: Inhibition *in vivo* of Xanthine Dehydrogenase by 4-Hydroxypyrazolo(3,4-d)pyrimidine

PHENOCOPIES are environmentally induced abnormalities which mimic the phenotype resulting from mutant genes. Pomaies *et al.*¹ have shown that 4-hydroxypyrazolo(3,4-d)pyrimidine (HPP) is an inhibitor *in vivo* of xanthine oxidase in mice, and we have attempted to produce phenocopies of the maroon-like (*ma-l*) and rosy (*ry*) eye colour mutants of *Drosophila melanogaster* with it. Xanthine dehydrogenase is undetectable in these mutants (see Glassman² for a discussion of these loci), and as a result, there is a partial loss of the red pteridines of the eye, the eyes being a dark red-brown rather than red. In addition, the products of this enzyme's activity, isoxanthopterin and uric acid, cannot be detected, and its substrates, 2-amino-4-hydroxypteridine and hypoxanthine, accumulate. In this communication we describe the production of phenocopies of *ma-l* and *ry* by HPP in various strains of *D. melanogaster*.

The medium on which the flies were raised was modified from one described by Dr. E. B. Lewis (personal communication). 100 ml. medium was prepared by adding 1.7 g brewer's yeast, 5.7 g dextrose, 1.4 g sucrose, 9.4 g fine-ground yellow corn meal, 0.6 g agar, and 1.0 ml. food colouring (for identification) to 94 ml. water at 85° C. The mixture was heated to 95° C until it started to thicken. When it had cooled to 70° C, 1.2 ml. of 3 per cent benzyl benzoate (Eastman) in 95 per cent ethanol (to control mites), 1.0 ml. of a mixture containing 41.8 ml. propionic acid, 4.15 ml. phosphoric acid (85 per cent), and water to 100 ml. (to control mould), and the desired amount of HPP were added. The medium was then mixed in a Waring blender and 10-ml. portions were dispensed into 25 × 95 mm vials. The next day, adult flies were placed in each vial and allowed to deposit eggs on the surface of the medium for 3 to 4 days. Flies were reared at 25° ± 1° C.

Eye colours were recorded daily when the adults emerged. In order to facilitate this a strain containing the scarlet eye colour gene (*st*) at locus 44 on the third chromosome was used. The *st* mutant lacks the brown eye pigment and the eye colour is usually bright red. The double mutant, *st ry* or *ma-l*; *st*, has a yellow-orange eye colour, and this is also the eye colour of the *st* flies which are phenocopies of *ma-l* and *ry*. For comparative purposes, the Pacific wild-type strain and the *ru lxd by* strain was also used (*ru* and *by* are the symbols for roughoid eye and blister wing, and are only incidental markers). The *ru lxd by* strain has relatively low xanthine dehydrogenase activity because of *lxd* (low xanthine dehydrogenase) at locus 33 ± on the third chromosome³. Only those flies that duplicate the *ma-l* or *ry* eye colour exactly (red-brown eye colour in the case of Pacific and *lxd*, and orange eye colour for *st*) were scored as phenocopies; those showing intermediate eye colours were scored as normal.

Fig. 1 shows the percentage of flies grown on different amounts of HPP that resembled the *ry* and *ma-l* mutants. In no instance did any of these phenocopy flies transmit their abnormal eye colour to their progeny grown on regular food. The concentration of HPP required to transform 50 per cent of the flies into phenocopies varied. For *ru lxd by* it was about 0.008 per cent, for *st* it was about 0.026 per cent, and for *PAC* it was about 0.095 per cent. This difference in response might be due to the average xanthine dehydrogenase activity found in these strains; for the *ru lxd by* it was 3.8, for *st* it was 10.0 and for *PAC* it was 22.4 enzyme units per fly as measured by the method of Glassman^{3,4}. However, additional work

Table 1. THE EFFECTS OF HPP ON THE *st* STRAIN OF *Drosophila melanogaster*

Conc. of HPP	% Flies with phenocopy eye colour	Relative survival	Relative amount of isoxanthopterin*	Xanthine dehydrogenase units per fly
0.0	0	1.00	4.0	12
0.01	2.4	0.84	3.2	9.6
0.02	3.1	—	—	10
0.03	93.8	—	—	9.4
0.04	100.0	—	—	8.6
0.05	100.0	0.81	2.2	11
0.10	—	0.02	0.5	—

* Data for *st*, *PAC*, and *ru lxd by* flies are combined for this column.

† The mean of 8 replicate assays of single flies

is required to substantiate this relationship. High doses of HPP have an appreciable detrimental effect on viability (Table 1).

To test whether the other pteridines (2-amino-4-hydroxypteridine and isoxanthopterin) related to xanthine dehydrogenase were also affected, flies were chromatographed by the following modification of the method of Hadorn and Mitchell⁵. A fly was placed in boiling water for 1 min and then squashed with a glass stirring rod on the origin of a 9 in. × 11 in. sheet of Whatman No. 3 *M* filter paper. The chromatograms were developed by ascending chromatography for about 4 h in propanol 1 per cent NH_4OH (2:1). When dry, the chromatograms were observed under an ultra-violet lamp emitting mair at 365 mμ. In order to quantitate these results, we applied an arbitrary visual grading system using '0' to represent the absence of isoxanthopterin and '4' to represent the highest amounts we observed. The grading was done on coded flies to eliminate bias. The data is presented in Table 1, where it can be seen that the amount of isoxanthopterin declined as the concentration of HPP increased. In contrast, 2-amino-4-hydroxypteridine was present in higher-than-usual amounts in the flies raised on the higher concentration of HPP. This indicates that the effect of HPP is on xanthine dehydrogenase and not directly on the eye colour or on pteridines.

Furthermore, HPP has no effect on the synthesis of this enzyme. Table 1 shows that the enzyme activity was not affected in *st* flies grown on HPP even though the flies grown on 0.030, 0.040 and 0.050 per cent HPP were phenocopies. It is concluded that the synthesis of xanthine dehydrogenase is not affected by HPP.

It is clear that HPP effectively inhibits xanthine dehydrogenase activity in *D. melanogaster*, *in vivo*. Furthermore, the effectiveness of HPP seems to be relatively inversely to the amount of enzyme activity present in each strain. This phenomenon will be useful in many ways (1) by suppressing the maternal effect of the *ma-*

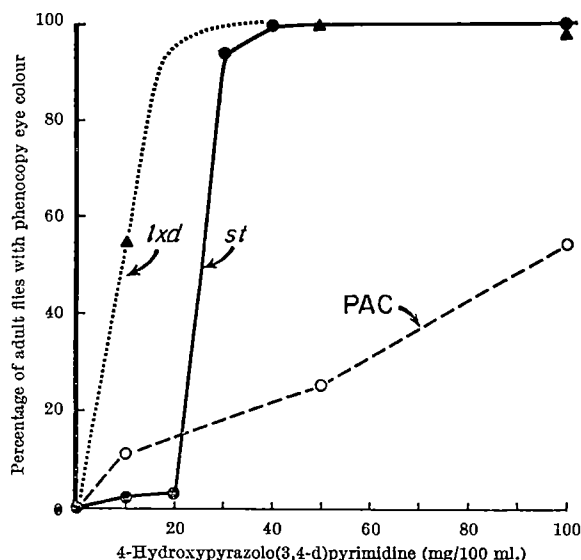


Fig. 1. The effect of HPP on phenocopy production in the *lxd*, *st*, and *PAC* strains of *Drosophila melanogaster*

10⁶,⁷ with a low concentration of HPP in the food, we expedite the genetic analysis of the *ma-l* locus; (2) by using low concentrations of HPP in food and using the usual genetic selection techniques, it might be possible to select for new mutant alleles at the *lxd* locus, or for leaky mutants at the *ma-l* and *ry* loci; (3) the use of high levels of HPP in the food may facilitate the selection of strains of flies that have very high xanthine dehydrogenase activity. These applications are now being explored.

We thank Dr. George H. Hitchings of Burroughs Wellcome and Co. for his generous gift of HPP. This work was supported by a grant (GM-08202) from the National Institutes of Health, and by a U.S. Public Health Service research career development award M-K3-14, 911-C1).

E. C. KELLER, JUN.*
EDWARD GLASSMAN

Department of Biochemistry
and
The Genetics Curriculum,
School of Medicine,
University of North Carolina,
Chapel Hill.

*U.S. Public Health Service Postdoctoral Fellow (GM-10, 296-02) present address, Department of Zoology, University of Maryland, College Park, Maryland

Omales, R., Bieber, S., Friedman, R., and Hitchings, G. H., *Biochim. Biophys. Acta*, **72**, 119 (1963)

Glassman, E., *Proc. U.S. Nat. Acad. Sci.*, **48**, 1491 (1962).

Keller, jun., E. C., and Glassman, E., *Genetics*, **49**, 663 (1964).

Glassman, E., *Science*, **142**, 40 (1962).

Radorn, E., and Mitchell, H. K., *Proc. U.S. Nat. Acad. Sci.*, **37**, 650 (1951).

Glassman, E., and McLean, J., *Proc. U.S. Nat. Acad. Sci.*, **48**, 1712 (1962).

Glassman, E., and Mitchell, H. K., *Genetics*, **44**, 547 (1959).

Induction of Crossing-over in *Drosophila* Males by Means of Ovarian Extract

THE frequency of gene recombination is known to be affected by extrinsic factors or intrinsic ones such as sex. Crossing-over is completely blocked in dipteran males, including those of *Drosophila melanogaster*, and in silkworm males. It is a puzzling problem why sex should have such an influence on crossing-over. Lack of homology between regions of the X and Y may explain the absence of crossing-over between the sex-chromosomes of the heterogametic sex, but the absence of crossing-over in the autosomes must have a different cause.

All agents that so far have been found to produce crossing-over in *Drosophila* males—irradiation and certain chemicals—cause mutations and chromosomal aberrations as well. In the work recorded here, an attempt has been made to induce crossing-over in males by means of fresh ovarian extracts. Males and females utilized in this work were heterozygous for the second-chromosome markers dumpy (*dp*), black (*b*), cinnabar (*cn*), and brown (*bw*). About 300 ovaries of fertilized females were dissected into 0.3 c.c. of 0.4 per cent saline. The ovaries were then homogenized and centrifuged twice at 3,000 r.p.m. for 15–20 min. The supernatant was filtered and/or directly taken into a microsyringe, and 0.2 μ l. was injected into the testis region of males. A portion of the same or a similarly obtained supernatant was boiled before injection. Males injected with 0.4 per cent saline and untreated males served as additional controls. 24 h after injection, each male was individually mated with three virgin females homozygous for *dp b cn bw*, and six broods of three days each were analysed for induced cross-overs in *F*₁. Presumptive cross-overs were verified by back-crosses to the multiple recessive strain.

Table 1 presents a summary of the results. It will be seen that cross-overs occurred exclusively among the progeny of a proportion of those males that had been injected with fresh ovarian extract.

Table 2 presents an analysis of the progeny from the 25 males that had yielded cross-overs. The overall fre-

Table 1			
Injected with	No. surviving males	No. <i>F</i> ₁ tested	No. cross-overs in <i>F</i> ₁
—	87	14,472	0
Saline	85	14,043	0
Boiled ovarian extract	69	11,530	0
Fresh ovarian extract	154	29,147	103*

* Among 1,901 progeny of 25 males.

Table 2. DISTRIBUTION OF CROSS-OVERS IN THE PROGENY OF 25 MALES THAT HAD BEEN TREATED WITH OVARIAN EXTRACT

Brood	Observed phenotypes				B	C	D	E	Total
	$\frac{+}{dp}$	$\frac{+}{b}$	$\frac{+}{cn}$	$\frac{+}{bw}$					
Non cross-overs					897	675	196	108	1,876
Cross-over regions									
I (<i>dp-b</i>)	$\frac{+}{dp}$	$\frac{+}{b}$	$\frac{+}{cn}$	$\frac{+}{bw}$	9	3	6	12	30
II (<i>b-cn</i>)	$\frac{+}{dp}$	$\frac{+}{b}$	$\frac{+}{cn}$	$\frac{+}{bw}$	1	3	—	—	4
III (<i>cn-bw</i>)	$\frac{+}{dp}$	$\frac{+}{b}$	$\frac{+}{cn}$	$\frac{+}{bw}$	13	5	2	—	20
	$\frac{+}{dp}$	$\frac{+}{b}$	$\frac{+}{cn}$	$\frac{+}{bw}$	2	3	—	1	6
I and II	$\frac{+}{dp}$	$\frac{+}{b}$	$\frac{+}{cn}$	$\frac{+}{bw}$	1	—	—	—	1
	$\frac{+}{dp}$	$\frac{+}{b}$	$\frac{+}{cn}$	$\frac{+}{bw}$	1	—	—	—	1
I and III	$\frac{+}{dp}$	$\frac{+}{b}$	$\frac{+}{cn}$	$\frac{+}{bw}$	7	1	—	—	8
II and III	$\frac{+}{dp}$	$\frac{+}{b}$	$\frac{+}{cn}$	$\frac{+}{bw}$	16	12	—	—	28
	$\frac{+}{dp}$	$\frac{+}{b}$	$\frac{+}{cn}$	$\frac{+}{bw}$	—	1	—	—	1
	Numbers per cent				50	28	8	13	99
Total cross-overs					5	5	4	11	5

quency of crossing-over was 5 per cent. The distribution over the 3 chromosomal regions used did not correspond to the lengths of these regions, and there were great disparities between complementary classes; these unexpected features may, however, be due to the fact that sampling errors were greatly exaggerated by the occurrence of clusters. The brood pattern shows that, as in the experiments by Reddi and Auerbach¹, some of the spermatozoa utilized in brood B must have been pre-meiotic or in early meiosis at the time of treatment. The occurrence of some clusters in this brood would suggest the former possibility but, with the few markers used, apparent clusters may equally well have been due to independent cross-overs in several spermatocytes of the same male.

While the details of the observed effect require a more elaborate analysis, the results clearly indicate that fresh ovarian extract contains a factor that induces crossing-over in male germ cells. The ineffectiveness of boiled ovarian extract suggests that this factor is a heat-labile substance.

O. S. REDDI
G. M. REDDY
M. S. RAO

Radiobiological Research Unit,
Osmania University,
Hyderabad,
India.

¹ Reddi, O. S., and Auerbach, C., *Genet. Res.*, **2**, 63 (1961).

Regression of a Phenotypic Value on the Values for the Parents and Grandparents

THE problem discussed in this communication was suggested by Dr. Donald Michie in connexion with an investigation, by himself and Mrs. Jean Hayes, of pedigree records in British Bloodstock.

Let x be some phenotypic value for some animal, and let x^P be the average of the value for its two parents, and x^G that of its four grandparents. The problem is to find the regression of x on x^P and x^G , that is to find the partial regression coefficients $b_{HP.P}$ and $b_{HG.P}$ such that:

$$E(x|x^P, x^G) = b_{HP.P}x^P + b_{HG.P}x^G.$$

Write :

$$x = w + z, x^P = w^P + z^P, x^G = w^G + z^G,$$

where the w 's are 'environmental values' and the z 's are 'genotypic values'. We make the following assumptions, the first of which implies no real loss of generality, and the remainder are natural.

$$E(w) = 0, E(w) = E(z) = 0, \text{cov}(w, w^P) = \text{cov}(w, w^G) = \text{cov}(w, z) = \text{cov}(w, z^P) = \text{cov}(w, z^G) = 0.$$

We also assume that the conditions are stationary from generation to generation, and that:

$$E(z|z^P) = E(z|z^P, z^G) = z^P.$$

This last assumption means that the breeding (genotypic) properties of the parents supersede those of the grandparents, at any rate in regard to expectations.

Let h be the correlation coefficient between x and z . It is customary to call h^2 the 'heritability' of the relevant trait. It can then be proved, by making use of standard regression theory, that:

$$b_{HP.G} = h^2(1 - \frac{1}{2}h^2)/(1 - \frac{1}{2}h^4)$$

$$b_{HG.P} = h^2(1 - h^2)/(1 - \frac{1}{2}h^4)$$

I. J. GOOD

Trinity College, Oxford, and
Atlas Computer Laboratory,
Chilton, Berkshire.

AGRICULTURE

Objective Evaluation of Quality in Poultry Meat

RECENT work in this laboratory¹⁻⁵ has indicated a relation between biochemical and quality changes occurring in chicken muscle during storage. During frozen storage the buffer-extractable nitrogen of chicken muscle decreased and the products of protein breakdown increased¹. The decrease in buffer-extractable nitrogen occurred as a result of loss of solubility of the actomyosin fraction and was accompanied by a loss of sulphydryl groups (-SH) of muscle tissue. During storage at above-freezing temperatures, extensive proteolysis occurred, but the changes in the protein extractability and -SH group content of muscle tissue were small². In meat in which the proteolytic activity had been destroyed by cooking, the -SH groups of muscle tissue decreased progressively during frozen storage without any increase in the protein-breakdown products³. The loss of -SH groups and solubility of muscle protein was accompanied by a loss of tenderness and development of dryness in frozen stored meat, while the amount of protein-breakdown products increased with the development of off-odour in meat stored either frozen or unfrozen.

Since both biochemical and quality changes depend on storage time and temperature, the possibility of employing the biochemical changes as an index of quality in stored poultry was considered. This communication presents evidence that the ratio between the -SH groups of muscle tissue, which indicate the degree of denaturation of muscle proteins, and protein-breakdown products, which indicate the degree of proteolysis, may be useful as a quality index for poultry stored frozen or unfrozen and for cooked frozen poultry. Separation and fractionation of myofibrillar proteins are lengthy procedures and cannot be used for routine quality control work, but simple analytical methods can be used to measure these parameters.

Samples of meat were obtained from male broiler-type chickens. The birds were killed in the laboratory by cutting the jugular vein and carotid arteries. They were bled for 2-3 min, scalded for 2 min at 53°-54° C, plucked by hand, eviscerated and aged for 48 h in drained, crushed ice. For frozen storage, birds were vacuum packaged in 'Cry-O-Vac' bags, frozen in an air blast (300-500 ft./min) at -30° C, stored at -18 and -10° C for the desired length of time (up to 2 years), and thawed for 2 h in running water at 18°-22° C. For storage at 0° C, birds were dipped in chlortetracycline solution (10 p.p.m.), placed in sterilized 'Cry-O-Vac' bags, flushed with nitrogen and stored under aseptic conditions for maximum storage life (up to 5 weeks). For pre-cooked frozen storage, the birds were vacuum packaged in 'Visking' bags, heated for about 3 h in boiling water to bring the internal temperature to about 85° C and stored at -10° C (up to 2 years) under nitrogen to minimize oxidation.

For analysis, breast and leg muscle were removed separately, freed from visible fat, tendon and connect tissues and minced with scissors. Minced tissue (5 g) was homogenized with sodium chloride (3 g), sand (3 g) and metaphosphoric acid (2.25 per cent, 20 ml.). The mixt was shaken, left for 5 min and filtered (Whatman No. 4). These operations were performed at 0° C on duplicate samples. The filtrate was used to estimate both -SH groups and protein-breakdown products. Sulphydryl groups were estimated in 2-4 ml. of filtrate by a colorimetric method using sodium nitroprusside reagent⁴ expressed as μg glutathione-SH per g muscle. For estimation of protein-breakdown products, 5 ml. filtrate was treated with 5 ml. 20 per cent trichloroacetic acid solution, allowed to stand at room temperature for 1 h and centrifuged for 10 min at 12,000 g . Protein breakdown products were estimated with Folin-Ciocalteu reagent⁵ 4 ml. supernatant^{1,2} and expressed as μg of tyrosine nitrogen per g muscle.

The ratio of -SH groups and tyrosine nitrogen content (suggested quality index) of both breast and leg muscle tissue decreased progressively with storage time in raw meat stored at temperatures above and below freezing and in cooked frozen meat (Fig. 1). During frozen storage the 'quality index' decreased as a result of loss of -SH groups and an increased proteolysis. During storage at 0° C for 5 weeks, changes in -SH group content of muscle tissue were small, and the 'quality index' decreased mainly as a result of proteolysis. In cooked meat in which proteolysis occurred, the 'quality index' decreased as a result of loss of -SH group content during frozen storage. The rate of decrease of 'quality index' increased rapidly with storage temperature in much the same way as the rate of quality deterioration increased in studies with uncooked meat^{5,6}. In muscle of birds from a single flock, killed, processed, and stored under identical conditions in the laboratory, the 'quality index' varied within ± 10 per cent.

The determination of the suggested objective 'quality index' should be much simpler, more accurate and reliable than the assessment of quality by sensory testing. The results may, therefore, be of interest in laboratory studies on the effect of freezing and storage procedures on poultry quality as well as in commercial freezing and storage of poultry. The effects of biological variability and processing techniques on the 'quality index' are being investigated.

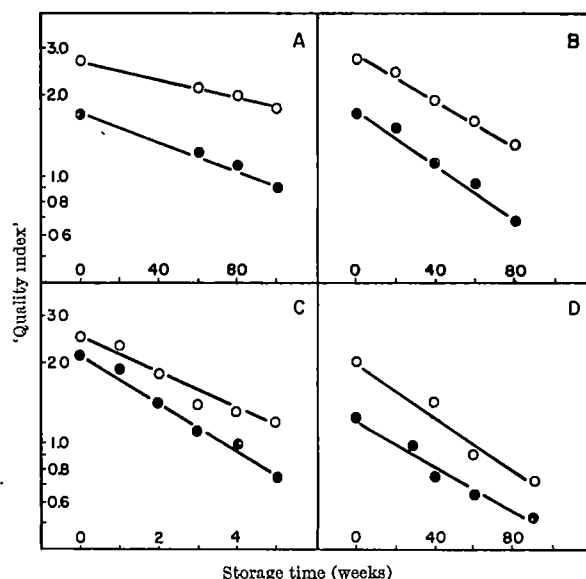


Fig. 1. Effect of storage time on suggested quality index (ratio between -SH group content and Folin-Ciocalteu reagent-positive nitrogen content of muscle tissue, plotted logarithmically). ●, Breast muscle; ○, leg muscle. A, raw poultry stored at -18° C; B, raw poultry stored at -10° C; C, raw poultry stored at 0° C; and D, cooked poultry stored at -10° C.

sed, and values of the 'quality index' corresponding to quality of meat when deterioration is first detected by sensory evaluation are being studied.

A. W. KHAN

Division of Biosciences,
National Research Council, Ottawa 2, Canada.

han, A. W., van den Berg, L., and Lentz, C. P., *J. Food Sci.*, **28**, 425 (1963).
han, A. W., and van den Berg, L., *J. Food Sci.*, **29**, 49 (1964).
han, A. W., and van den Berg, L., *J. Food Sci.*, **30**, 151 (1965).
han, A. W., *Agr. Food Chem.*, **12**, 378 (1964).
van den Berg, L., Khan, A. W., and Lentz, C. P., *Food Technol.*, **17**, 91 (1963).
Lose, A. A., Pool, F. M., Campbell, A. A., and Hanson, H. L., *Food Technol.*, **13**, 477 (1959).

ANTHROPOLOGY

Lower Palaeolithic Hominid and Pebble-industry in Hungary

In the neighbourhood of the village of Vértesszöllös, to the north-west of Budapest, a rich Lower Palaeolithic site has been found: the results of the first excavation have already been published¹.

In 1964 a great part of the site was excavated, and this resulted in some new finds, which included a rich fauna as well as several thousand primitive pebble-tools.

The pebble-industry in Hungary, called *Buda-industry*, related to the Oldowan industry in Africa, the Moukoutienian in China, the Sohan in India and to the Tactonian in England so far as implement-types is concerned. The most characteristic forms are pebble-choppers, chopping-tools and polyhedrons. On the basis of its well-determinable stratigraphical conditions (the ravertine complex bearing the finds is situated on a high, old—possibly fourth—Pleistocene terrace) and of the rich vertebrate fauna, it was possible to compare our site with the Upper Biharian (that is, Mindel 1/2 and Mindel 2).

During the 1964 excavations four separate cultural horizons were found. The two lower ones were in solid ravertine and in lime-mud, while the upper two were at the base and at the top of the overlying loess. The loess again was covered with another layer of ravertine several metres thick. It was the lowermost layer which yielded the richest material; this represented a continuous living surface at the base of a former tectarata (calcareous tuff oasis). The material consists of numerous broken bones, and some small hearth, stones and chips. Of the three upper layers, that at the base of the loess was almost as rich in material as the lowest layer, but it covered a smaller area. The tools—so far as can be stated after a preliminary investigation—do not show any noticeable changes at the different cultural horizons. However, the fauna shows a considerable change: the vertebrate (rodent) material found in the loess belongs to cold-enduring species while those in the lower layers belong to a warmer climate: in the upper layer, beside the northern type red vole (*Myodes* [= *Clethrionomys*] cf. *rutillus*), there are *Microtus*-species and *Ochotona*, etc., in great abundance, thus indicating a cold climate. On the other hand, in the lower layers, Murines (*Apodemus*, *Mus*), *Cricetus* and *Glis* accompanied by *Phomys* (dying out later), and Microtines, which belong to a relatively warmer climate, indicate more temperate climatic conditions.

During the excavation, several hundredweights of lime-mud from the cultural layer were washed out and sieved, and taken to the Hungarian National Museum for preparation. The washing process and the collecting from the washed material took several months. It is for this reason that the most significant find of the excavation only came to light early in 1965: namely, tooth-remains of a hominid from the washed material of the lowest cultural horizon. The hominid remains consist of a left deciduous canine, the fragment of a first deciduous molar and fragments of another molar belonging to a

child's jaw. Even at first glance it was obvious that the teeth are characteristic of the *Pithecanthropus-Sinanthropus* group. Dr. A. Thoma has been asked to undertake detailed examination.

From the washed material where the human teeth were contained, the following mammalian species were also determined (there are only one or two new species in this list as compared with that already published): *Citellus* sp. (*citellus*-group), *Cricetus cricetus* ssp., *Myodes* [= *Clethrionomys*] cf. *glareolus* (*Schreber*), *Phomys* sp., *Arvicola* sp., *Phaiomys* sp., *Microtus* (*Pitymys*) *arvaldens* (Kretzoi), *Microtus conjungens* Kretzoi, *Microtus arvalis-agrestis*-group, *Apodemus* sp. (*szilaticus*-group).

The decreasing numbers, from the base upwards, of the *Pitymys arvaldens* and the greatly increasing numbers of the *Microtus conjungens*—an intermediate form between *Microtus hintori-gregaloides* and *Microtus gregalis*—are a clear indication of a deteriorating climate during the laying down of the upper layers of the site.

M. KRETZOI

L. VÉRTES

Hungarian National Museum, Budapest.

¹ Kretzoi, M., and Vértés, L., *Current Anthropology*, **6**, No. 1, 74 (1965).

Cranial Capacity of the Hominine from Olduvai Bed I

PROF. P. V. TOBIAS¹ has produced a range of values for the cranial capacity of the 'pre-*Zinjanthropus*' juvenile from Bed I, at the F.L.K.N.N. I site at Olduvai Gorge, Tanganyika. This communication presents evidence which confirms Tobias's calculations.

The figures which Tobias calculated on the basis of his 'B/A' ratio, the parietal-to-total volume, were 642.7–723.6 c.c. These figures and the method of obtaining them have been criticized², and it is generally held that his figures are questionable. The main reason for this unwillingness to accept Tobias's figures rests with his method of reconstruction of a partial endocranion from the parietal fragments. Pilbeam and Simons² hold that the articulation of the parietals along the sagittal suture would make a considerable difference in the parietal volume, since a more acute angle of articulation along the suture would reduce the width at the base, thereby decreasing the volume.

Since the fragments are from both sides of the cranium, accurate reconstruction is possible by mirroring one side with the other. Thus, the reconstruction should be reasonably correct, disregarding for the present the question of articulation. Assuming also that the range of values found for partial endocranions, parietal-to-total, is within the values obtained (50.22–56.54 per cent), the question of articulation requires consideration.

Using the photographs and scale provided by Tobias¹, a full-scale drawing was made of the endocranion both as seen from above and behind. Using the maximal breadth and height as a plane, a template was constructed of the outline of the endocranion in this plane, from one side. By simply turning the template over, the mirror image could be traced. Using the most superior point in the mid-sagittal plane as a pivot point, the template was rotated a number of times, and outlines traced on fine-quality tracing paper. A 'reference' outline was made to conform with the scale of the published photograph. The maximum breadth in the coronal plane was varied from the 'reference' value in both directions. In other words, the angle of articulation at the sagittal suture was made both more and less acute. Each outline was traced, giving a total of four complete outlines: (1) at scale; (2) minus 0.5 cm each side; (3) minus 1.0 cm each side; (4) plus 0.5 cm each side. Thus, maximum breadth was varied from plus 1.0 cm to minus 2.0 cm, a range surely greater than necessary.

A planimeter was then used to obtain the areas enclosed by each of the four outlines, five readings being

taken for each outline. Each was then calculated in terms of the percentage of the 'reference' or scale area. The values resulting are as follows (per cent): (1) 'scale', 100; (2) minus 0.5 cm, 98.3; (3) minus 1.0 cm, 95.9; (4) plus 0.5 cm, 101.

It is immediately apparent that variations in the articulation angle along the suture do not radically reduce volume estimates. In varying the articulation angle, the decrease essentially affects but one dimension, that is, the lateral breadth. At the same time, the vertical dimension is increased. Since the shift is in one direction, it can be seen that by taking some small volume in the coronal plane, $A(dx)$ and integrating from anterior to posterior dimensions, one is essentially adding small volumes until total parietal volume results. What the planimeter method shows is that the loss in area from constriction of the breadth is almost compensated by the increase in depth. The area remains essentially constant, and since the length of the endocast is not affected by constriction the volume remains essentially constant also. It should be appreciated, however, that this method can only give an approximation, since the articulation in question is along a curved surface. If the entire suture were present in the fragments, it would not be possible to vary greatly the angle of articulation. Since the fragments are partial in this respect, some degree of freedom is allowed for articulation, and taking the area of but one plane of coronal orientation at the point of greatest depth would be roughly representative of a series of areas in different coronal planes.

In sum, Tobias's¹ values are probably correct, provided that: (1) the reconstruction is valid; (2) the partial endocast variation, 'B/A' ratio, is within the limits obtained.

It must be added, however, that this confirmation of Tobias's figures should in no way be taken as supporting his conclusions regarding the creation of a new species of early hominid. The parameter of cranial capacity is quite variable in the higher primates. The end-points of the greater than 1,000 c.c. variation between members of modern *Homo* are not placed in separate species, nor are gorillas, the volumes of which vary between 752 c.c. and 320 c.c. (ref. 3). Thus, it would not be unlikely that on the basis of cranial capacity, at least, the 'pre-Zinj' materials are within the Australopithecine taxon of early hominids.

RALPH L. HOLLOWAY, JUN.

Department of Anthropology,
Columbia University, New York.

¹ Tobias, P. V., *Nature*, 202, 3 (1964).

² Pilbeam, D. R., and Simons, E. L., *American Sci.*, 53, 237 (1965).

³ Schultz, A. H., *Anthropol. Anz.*, 25, 197 (1962).

PROF. HOLLOWAY has shown effectively that the partial endocast of *Homo habilis* provides a valid basis for estimating its cranial capacity, and even for a far greater range of angles along the sagittal suture than are anatomically likely. He has increased the maximum (biasterionic) breadth by as much as 10 mm and reduced it by as much as 20 mm and found a minimal effect on the cross-sectional area of the biparietal tunnel. It is of interest that the difference in biasterionic breadth between two reconstructions made in Nairobi and in Johannesburg was scarcely 2 mm. The first was made by Dr. L. S. B. Leakey and myself, with the auxiliary observational judgment of Dr. G. G. Simpson, Mrs. M. Leakey and Mrs. S. Coryndon; the second was made by A. R. Hughes and myself. The tolerance limits of our reconstructions were 2 mm; Prof. Holloway has validated the method for a range of 30 mm between the greatest and smallest biasterionic distances!

Two comments may be added on variability in higher primates:

(1) The capacity of 680 c.c. in *H. habilis* exceeds the australopithecine mean of 502 c.c. ($n=7$) by 3.79 standard

deviations. (The *S.D.* of 47.0 c.c. is an estimate of *t* population *S.D.*, based on an australopithecine sample range of 435–562 c.c. For this calculation, the adult capacity of the Taung fossil was re-computed from the newer growth data of Ashton and Spence², which show that, by the dental age of the Taung child, the cranial capacities of hominoids have reached 90–94 per cent the adult mean values. The proposed new 'adult' value for Taung of 562 c.c. replaces the 600 c.c. I formerly accepted³. Even on the former estimate, however, 680 c.c. was 3 *S.D.*'s higher than the australopithecine mean.)

(2) It is extremely hazardous to apply to a fossil group such as *Australopithecus* the *a priori* argument that variability must have been at least of the order four in living higher primates. There is much evidence that the variability of metrical characters may vary from taxon to taxon; thus, for cranial capacity, the coefficient of variation may be as low as 6.8 in chimpanzee and as high as 13.6 in gorilla ($\sigma+\varphi$), or 12.8 in modern man. Even if we separate male and female gorilla capacities, the gorilla male *V* of 10.4 is higher than that of orang-utans or chimpanzees for both sexes combined! Many factors enhance the variance and these may not operate equally in different hominoids, for example, varying degrees of sexual dimorphism, the possible effect of zoo conditions on higher primates and the proportions of zoo-reared and wild-shot animals represented in a skeletal series, the degree of polytypism and polymorphism within a taxon etc.

Not only may a single metrical character vary to different degrees from population to population, but also different taxa may show high or low overall variability. Morant⁴ proved that, in contrast with modern man, Neanderthal man was remarkably homogeneous in cranial metric characters. This was stressed again not long ago by Blanc, by Vallois and by Caspari⁵, the latter adding that "in recent man interindividual variability appears to be relatively large as compared with a number of other species". The Biometric School produced many investigations which showed wide diversity of variability among different human populations and different great apes.

We simply do not know if *Australopithecus* was a highly variable taxon in general, or whether cranial capacity in this group was of low or high variability. The estimated coefficient of variation based on the present australopithecine sample of 7 cranial capacities is 9.35 per cent which is higher than that of smaller-brained apes, but lower than that of gorilla and modern man. But the problems intrinsic in palaeontological sampling make it very likely that this is an imperfect estimate. Further specimens of australopithecines may place the value of 680 c.c. well within the mean ± 3 *S.D.*'s; but we cannot assume that this will be so, nor can we base statements on the possible features of specimens still to be discovered. On the existing sample, the estimated cranial capacity of *H. habilis* is significantly greater than the mean australopithecine capacity.

Note added in proof. Dr. J. K. Woo⁶ has meanwhile applied my method to his important new pithecanthropine cranium from Lantian, Shensi Province. From a biparietal reconstruction and endocast, he has obtained estimates of the total endocast volume of 775–783 c.c. Using one of Pearson's formulae on the complete reconstructed cranium, he has obtained a value of 778 c.c. These remarkably similar estimates agree with Woo's total morphological assessment in aligning the Lantian specimen with the small-brained Indonesian crania of *Homo erectus*.

P. V. TOBIAS

University of the Witwatersrand,
Johannesburg.

¹ Ashton, E. H., and Spence, T. F., *Proc. Zool. Soc. Lond.*, 130, 169 (1958).

² Tobias, P. V., *Nature*, 197, 743 (1963).

³ Morant, G. M., *Ann. Eugen.*, 2, 318 (1927).

⁴ Caspari, E. W., in *Social Life of Early Man* (Chicago, Aldine, 1961).

⁵ Woo, J. K., *Scientia Sinica*, 14, 1032 (1965).

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, October 11

- INSTITUTION OF MECHANICAL ENGINEERS, EDUCATION AND TRAINING GROUP (Joint Conference with the Schools Council, at 1 Birdcage Walk, Westminster, London, S.W.1), at 2.30 p.m.—Conference on "Applied Science Engineering Activities in Schools".
- SOCIETY OF CHEMICAL INDUSTRY, COLLOID AND SURFACE CHEMISTRY GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Mr. B. W. Wilson: "Surface-Active Agents in the Pharmaceutical Field".
- UNIVERSITY OF LONDON (in the Botany Lecture Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. G. Drews (University of Freiburg): "The Cell of the Photosynthetic Bacteria in Light- and Dark-Metabolism" (further lecture on October 13).
- ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 6 p.m.—"Germany: a Regional Geography" (colour film).

Tuesday, October 12

- UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Dr. H. E. Lewis: "The Islanders of Tristan da Cunha—a Medical Study of Isolation".
- ZOOLOGICAL SOCIETY OF LONDON (at the Zoological Gardens, Regent's Park, London, N.W.1), at 5 p.m.—Scientific Papers.
- INSTITUTION OF CIVIL ENGINEERS (at Great George Street, Westminster, London, S.W.1), at 5.30 p.m.—Mr. E. M. Wilson: "A Study of the Feasibility of Tidal Power from Loughs Strangford and Carlingford with Pumped Storage at Rostrevor".
- INSTITUTION OF MECHANICAL ENGINEERS, AUTOMOBILE DIVISION (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Annual Meeting. 10 p.m.—Mr. R. S. Moul: "An Engine Designer's Scrapbook" (Chairman's address).

Wednesday, October 13

- SCHOOL OF PHARMACY, UNIVERSITY OF LONDON (in the Assembly Hall, 21 Tavistock Square, London, W.C.1), at 3 p.m.—The Lord Brain, Bt., D.M., R.C.P., F.R.S.: "Drug Dependence".
- ROYAL STATISTICAL SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5 p.m.—Mr. M. Hills: "Allocation Rules and Their Error Rates".
- INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS (at 9 Bedford Square, London, W.C.1), at 6 p.m.—Dr. D. C. Cooper: "Signal Processing Using Optical Techniques".
- SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Meeting on "The Future of Food Legislation". Mr. D. D. Moor: "The Public Analyst's View"; Dr. A. M. Taylor: "The Viewpoint of the Manufacturer".
- INSTITUTE OF SCIENCE TECHNOLOGY, LONDON BRANCH (in the Electrical Engineering Department, Imperial College, Exhibition Road, London, S.W.7), at 6.30 p.m.—Prof. C. Cherry: "The Communication Explosion".

Thursday, October 14

- INSTITUTE OF PETROLEUM, ECONOMICS AND OPERATIONS GROUPS (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. R. Marsh: "The Industrial Training Act".
- INSTITUTION OF CIVIL ENGINEERS, TRANSPORTATION ENGINEERING GROUP (at Great George Street, Westminster, London, S.W.1), at 5.30 p.m.—Formal Discussion on "Motorist's View of Road Problems", introduced by Mr. E. Shipley.
- INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Avoy Place, London, W.C.2), at 5.30 p.m.—Mr. L. E. S. Mathias: "Long Wavelength Laser Generation".
- INSTITUTION OF MECHANICAL ENGINEERS, HYDRAULIC PLANT AND MACHINERY GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Dirt in Fluid Power Systems—Should it be Excluded or Tolerated?"

Friday, October 15

- ARTHUR STANLEY EDDINGTON MEMORIAL TRUST (in Room 3, Mill Lane Lecture Rooms, The University, Cambridge), at 5 p.m.—Sir John C. Eccles, F.R.S.: "The Brain and the Unity of Conscious Experience" (1965 Eddington Memorial Lecture).
- INSTITUTION OF MECHANICAL ENGINEERS, THERMODYNAMICS AND FLUID MECHANICS GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 5.30 p.m.—Discussion on "Flow Visualization".

Saturday, October 16

- INNER LONDON EDUCATION AUTHORITY (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Mr. John Okell: "Burmese Musical Instruments".

Monday, October 18

- PLASTICS INSTITUTE, PLASTICS PROPERTIES DISCUSSION CIRCLE (at the Mandeville Hotel, Mandeville Place, London, W.1), at 3 p.m.—Mr. D. A. Hill: "Impact Testing and Brittle Failure".
- UNIVERSITY OF LONDON (in the Botany Theatre, University College, Gower Street, London, W.C.1), at 5 p.m.—Prof. B. A. D. Stocker: "Salmonella Antigens—Structure and Genetics" (further lecture on October 25).
- BRITISH COAL UTILIZATION RESEARCH ASSOCIATION (in the Lecture Theatre, Institution of Civil Engineers, Great George Street, London, S.W.1), at 5.30 p.m.—Prof. D. B. Spalding: "Heat Transfer Aspects of Coal Utilization" (Fourteenth Coal Science Lecture).
- SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Dr. J. H. Hamence: "The Public Analyst's Approach to Pesticide Residues".

INSTITUTION OF MECHANICAL ENGINEERS, INTERNAL COMBUSTION ENGINES GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Whither Small V-Torn Diesel Engines?"

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Dr. Charles Swithinbank: "A Year with the Russians in Antarctica".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

POSTDOCTORAL FELLOW (cell biologist or biochemist) IN THE DEPARTMENT OF ZOOLOGY to work with Dr. L. G. E. Bell on problems associated with cell surface function—The Deputy Secretary, The University, Southampton (October 12).

LECTURER (with a good honours degree in a science subject and preferably experience of teaching chemistry in secondary schools; a knowledge of Welsh will be an additional qualification) IN EDUCATION—The Registrar, University College of North Wales, Bangor, North Wales (October 14).

LECTURERS (with a good honours degree and a keen interest in any branch of electronics and control engineering) IN THE ELECTRONICS AND CONTROL DIVISION, School of Engineering Science—The Registrar, University College of North Wales, Bangor, North Wales (October 14).

ASSISTANT LECTURER or LECTURER (preferably with a special interest in analysis) IN PURE MATHEMATICS—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (October 18).

LECTURER IN METALLURGY—The Principal, Lanchester College of Technology, Priory Street, Coventry (October 18).

ASSISTANT LECTURER (with a wide interest in genetics so as to be able to lecture to students of both departments) IN GENETICS IN THE DEPARTMENTS OF BOTANY AND ZOOLOGY—The Registrar, The University, Manchester, 13, quoting Ref. 174/65/Na (October 20).

LECTURER (Grade II) IN THEORETICAL CHEMISTRY—The Registrar, University Senate House, Bristol, 2 (October 20).

LECTURER or ASSISTANT LECTURER (with research experience in any branch of computer science and preferably an interest in programming languages, systems programming or non-numerical applications of computers) IN THE COMPUTATION LABORATORY, DEPARTMENT OF MATHEMATICS—The Deputy Secretary, The University, Southampton (October 22).

RESEARCH FELLOW (with a first- or second-class honours degree, and preferably ecological training and previous research experience) IN THE DEPARTMENT OF ZOOLOGY, to work on the general ecology of the fox for a period of three years—The Secretary, The University, Aberdeen (October 23).

SENIOR LABORATORY ADMINISTRATOR (with previous administrative experience, and preferably laboratory or medical knowledge) IN THE DEPARTMENT OF ANATOMY—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (October 23).

CHAIR OF MATHEMATICS and HEAD OF THE DEPARTMENT OF MATHEMATICS—The Academic Registrar, Northampton College of Advanced Technology, St. John Street, London, E.C.1 (October 25).

CHAIR OF NUMERICAL ANALYSIS—The Registrar, University of Strathclyde, Glasgow, C.1 (October 25).

LECTURERS IN NUMERICAL ANALYSIS; LECTURERS IN STATISTICS; and a LECTURER and an ASSISTANT LECTURER IN PURE MATHEMATICS—The Registrar, University of Strathclyde, Glasgow, C.1 (October 25).

RESEARCH ASSISTANT (male or female honours graduate in any of the biological sciences, including medicine, interested in animal cell biology, and preferably laboratory experience in culture techniques) IN TISSUE CULTURE IN THE DEPARTMENT OF ANATOMY—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (October 25).

WELLCOME CHAIR OF PHARMACOLOGY at the SCHOOL OF PHARMACY—The Academic Registrar, University of London, Senate House, London, W.C.1 (October 28).

SENIOR LECTURER IN PHYSICS and THEORETICAL PHYSICS; a SENIOR LECTURER IN HIGHWAY ENGINEERING; a SENIOR LECTURER/LECTURER IN ANTHROPOLOGY; a LECTURER or SENIOR LECTURER IN GEOPHYSICS, a LECTURER IN MICROBIOLOGY; and a SENIOR DEMONSTRATOR/DEMONSTRATOR IN ANTHROPOLOGY at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, October 29).

SENIOR LECTURERS or LECTURERS (3) IN GEOLOGY at the University of Canterbury, Christchurch, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, October 29).

ASSISTANT CURATOR (with field experience in ethnology) OF THE MUSEUM OF ARCHAEOLOGY AND ETHNOLOGY—The Secretary of the Appointments Committee, Faculty of Archaeology and Anthropology, University of Cambridge, Downing Street, Cambridge (October 30).

CHAIR OF BOTANY—The Deputy Secretary, The University, Southampton (October 30).

EXPERIMENTAL OFFICER (young graduate interested in the design and construction of electronic and electro-acoustic equipment) IN THE DEPARTMENT OF AUDIOLOGY and EDUCATION OF THE DEAF—The Registrar, The University, Manchester, 13, quoting Ref. 179/65/Na (October 30).

LECTURER IN THE DEPARTMENT OF PHARMACOLOGY—The Clerk to the Council, School of Pharmacy, University of London, 29/39 Brunswick Square, London, W.C.1 (October 30).

LECTURER IN BIOCHEMISTRY—The Registrar, King's College (University of London), Strand, London, W.C.2 (October 31).

SENIOR LECTURER or LECTURER IN AGRICULTURAL ENGINEERING at the University of Khartoum—The Registrar, University of Khartoum, c/o The Inter-University Council, 33 Bedford Place, London, W.C.1 (October 31).

LECTURER (preferably with experience in comparative and cellular physiology) IN THE DEPARTMENT OF ZOOLOGY, University of Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (November 1).

RESEARCH STUDENT IN METALLURGY for work concerned with the mass-transfer of alloying elements, especially carbon, between plain carbon and alloy steels, immersed in high purity molten sodium at temperatures up to 850 deg. C.—The Registrar, King's College (University of London), Strand, London, W.C.2 (November 1).

CHAIR OF PLANT SCIENCE; and LECTURERS IN BIOLOGICAL SCIENCE at the University College of Rhodesia and Nyasaland—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (November 4).

DEPARTMENTAL ADMINISTRATOR IN THE DEPARTMENT OF CHEMISTRY—The Deputy Secretary, The University, Southampton (November 6).

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

SENIOR LECTURER or LECTURER (medically qualified with adequate experience) in the DEPARTMENT of CHEMICAL PATHOLOGY, University of Ibadan, Nigeria—The Secretary, Senate Committee on Higher Education Overseas, University of London, Senate House, London, W.C.1 (November 8).

SENIOR LECTURER or LECTURER (chemical engineer, preferably with industrial experience of process control) in CHEMICAL PROCESS CONTROL in THE DEPARTMENT of SYSTEMS ENGINEERING—The Secretary, University of Lancaster, Bailrigg House, Lancaster (November 13).

PRINCIPAL of THE WEST of SCOTLAND AGRICULTURAL COLLEGE and PROFESSOR of AGRICULTURE in the University of Glasgow (combined appointment)—The Principal, The West of Scotland Agricultural College, 6, Blythswood Square, Glasgow, G.2 (November 15).

POST-DOCTORAL FELLOW (chemist, biologist or physicist with experience in electron microscopy, with a Ph.D. degree or postgraduate research experience of equivalent standard and duration supported by satisfactory evidence of research ability) in ELECTRON MICROSCOPY in THE DIVISION of PROTEIN CHEMISTRY, Commonwealth Scientific and Industrial Research Organization, Parkville, Melbourne—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 462/236 (November 20).

ASSISTANT BACTERIOLOGIST (preferably female, with a university degree and/or practical experience in food microbiology)—The Director, Research Station, The Fruit and Vegetable Canning and Quick Freezing Research Association, Chipping Campden, Glos.

ASSISTANT LECTURER or LECTURER (with research interests in nuclear magnetic resonance spectroscopy) in ORGANIC CHEMISTRY—Prof D. H. R. Barton, F.R.S., Department of Chemistry, Imperial College of Science and Technology, London, S.W.7.

ASSISTANT LECTURER (with appropriate qualifications in biology, mycology or botany, and preferably special qualifications in plant mycology or general mycology) in BIOLOGY—The Registrar, University of Strathclyde, Glasgow, G.1.

CHEMISTRY MASTER (well qualified) to teach up to advanced level—The Headmaster, Latymer Upper School, Hammersmith, London, W.6.

DEMONSTRATOR (honours B.Sc. biochemistry or chemistry) to ASSIST in LABORATORY and TUTORIAL INSTRUCTION in THE DEPARTMENT of BIOCHEMISTRY—The Secretary, Royal Free Hospital, School of Medicine (University of London), Hunter Street, London, W.C.1.

ELECTRON MICROSCOPE TECHNICIAN (with experience in biological and physical work and a sound knowledge of replica, embedding and sectioning techniques) to take charge of the operation and maintenance of a Siemens Elmiskop MKI and ancillary equipment—The Director of Research, Natural Rubber Producers' Research Association, 56 Tewin Road, Welwyn Garden City, Herts.

ENTOMOLOGIST (with a B.Sc. degree with honours in zoology or entomology, and preferably practical experience of control of field pests and pests of stored products) in British Honduras, to advise the Agricultural Extension Staff and farmers on pest control measures, to evaluate insecticides and undertake basic field research in pest control, to lecture on pests and pest control and to advise on legislation to control the use of certain insecticides, and pest control generally—The Appointments Officer, Ministry of Overseas Development, Room 301, Eland House, Stag Place, London, S.W.1, quoting Ref. RG 213/26/03.

EXPERIMENTAL OFFICER to work on electronic instrumentation in the field of cardiology—The House Governor, Charing Cross Hospital, London, W.C.2.

HEAD of THE NEW ZEALAND SCHOOL of PHARMACY, Central Institute of Technology—The High Commissioner for New Zealand, New Zealand House, Haymarket, London, S.W.1, or The Director of Education, Private Bag, Wellington, New Zealand.

MICROANALYST (with experience in micro-elementary and functional group analysis, and preferably familiarity with the operation and maintenance of electronic equipment) in THE ORGANIC CHEMISTRY DIVISION of the National Chemical Research Laboratory, O.S.I.R., South Africa, to take charge of the routine microanalytical operations of the laboratory—The South African Scientific Liaison Officer, Chichester House, 278 High Holborn, London, W.C.1.

POST-DOCTORAL ASSISTANT; and a TECHNICAL ASSISTANT in INORGANIC CHEMISTRY to work under the supervision of Dr. J. F. Nixon—The Secretary, Department of Chemistry, St. Salvator's College, University of St. Andrews, St. Andrews, Fife, Scotland.

PRINCIPAL GRADE BIOCHEMIST (with a higher degree in chemistry or biochemistry)—The Clerk to the Governors, St. Bartholomew's Hospital, London, E.C.1, quoting Ref. No. P/836.

RESEARCH ASSISTANT (preferably with a degree in botany, agriculture, agricultural botany or other equivalent qualification) in THE POTATO BRANCH for work on herbicides in relation to potato varieties—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge.

RESEARCH ASSISTANT (zoology graduate) in THE DEPARTMENT of ZOOLOGY and APPLIED ENTOMOLOGY, Imperial College of Science and Technology, to work at the College Field Station, Silwood Park, on chemical factors in host plant selection by phytophagous insects or on the reproductive behaviour of insects—Dr. C. T. Lewis, Silwood Park, Sunninghill, Ascot, Berks.

RESEARCH ASSISTANTS (with a degree in chemistry or equivalent and preferably interested in the kinetics of exchange reactions) in RADIOCHEMICAL STUDIES—The Senior Lecturer in Radiochemistry, Sir John Cass College, Jewry Street, London, E.C.3.

RESEARCH ASSISTANTS (with a good degree in physics or electrical engineering) for work on resonances excited by microwaves in the plasma of a discharge column and on the application of these resonances in the field of electronics—Prof. M. Potok, Royal Military College of Science, Shrivenham, near Swindon, Wiltshire.

RESEARCH ASSISTANTS (2) (with qualifications in engineering or physics and preferably some experience in research) in THE DEPARTMENT of MECHANICAL ENGINEERING, for work in the fields of (a) hydrodynamic lubrication, and (b) fluid control systems—The Registrar, The University, Sheffield, 10.

SENIOR TECHNICIAN in THE DEPARTMENT of CHEMICAL PATHOLOGY to assist in the investigation of sick children—The Chemical Pathologist, Hospital for Sick Children, Institute of Child Health, Great Ormond Street, London, W.C.1.

SENIOR TECHNICIAN (preferably with previous experience with polymer equipment) for the maintenance and operation of modern equipment in a polymer characterization laboratory—The Departmental Superintendent, Chemistry Department, Imperial College, London, S.W.7.

SENIOR TECHNICIAN (with H.N.C. or equivalent qualification in physics, chemistry or biology, and preferably experience in physical instrumentation and an interest in field work) in THE ECOLOGY SECTION of THE DEPARTMENT of FORESTRY—Dr. M. E. D. Poore, Department of Forestry, Forestry Institute, University of Oxford, Oxford.

Bulletin of the British Museum (Natural History). Entomology. Vol. No. 2: A Systematic Revision of the Ameninidae (Diptera: Calliphoridae) By R. W. Crosskey. Pp. 33-140 35s. Vol. 16, No. 3: A Revision of Nodini and a Key to the Genera of Eumolpidae of Africa (Coleoptera: Eumolpidae). By B. J. Selman Pp. 141-174. 14s. (London: British Museum (Natural History), 1965)

Water Resources Board. Logarithmic Plotting of Stage-discharge Observations. By A. I. G. S. Robertson. Pp. 36. (T.N.3.) Origin published 1962. (Reading: Water Resources Board, 1965.)

Philosophical Transactions of The Royal Society of London. Series Biological Sciences. No. 758, Vol. 249 (28 August, 1965). A Submer Late-quaternary Deposit at Roddars Port on the North-East Coast Ireland. By M. E. S. Morrison and N. Stephens. (With Appendices Mrs Margaret Jope and S. P. Dance, F. W. Anderson, F. E. Round, H. Godwin, and E. H. Willis.) Pp. 221-225. (London: The Royal Society, 1965.) 18s.; 2.70 dollars.

University of Bristol. Annual Report of the Long Ashton Agricultural and Horticultural Research Station (The National Fruit and Cider Institute for the year 1964). Pp. xi+229+5 plates. (Long Ashton: The Agricultural and Horticultural Research Station, 1965.) 15s.

Fourth Report from the Select Committee on Procedure, together with Proceedings of the Committee Relating to the Report, Minutes of Evidence and Appendices, Session 1964-65. Pp. xxiii+148. (London: H. Stationery Office, 1965.) 15s. net.

Other Countries

Republic of Zambia. Ministry of Lands and Natural Resources. Annual Report of the Department of Water Affairs for the year 1964 Pp. (Lusaka: Government Printer, 1965.) 2s.

The Vegetation of Uganda and its Bearing on Land-use. By I. Langds Brown, H. A. Osmaston and J. G. Wilson. Pp. 159+24 plates. (Entebbe: Government Printer; London: The Uganda High Commission, 1965) 30/-

Académie Royale de Belgique. Classe des Sciences—Mémoires. Tome 1: Fascicule 1: On the Summability Methods of Divergent Series. By Kan Ishiguro. Pp. 43. 60 francs. Tome 35, Fascicule 3: Schistosité et Granitisation dans le Synchronisme de Laval et ses Abords. Par Paul Fourmarier, Annik Palhate et Bernard Auvray. Pp. 60. 140 francs. Tome 35, Fascicule 4b: Étude Expérimentale et Interprétation du Spectre Infra-Rouge des Silicates et des Germanates. Application à des Problèmes Structuraux Relatifs à l'état Solide. Fascicule 1: Pp. 261. Fascicule 2: Pp. 134 (164 fig 540 francs. (Bruxelles: Académie Royale de Belgique, 1965.)

Records of the Australian Museum. Vol. 26, No. 7 (12th June, 1965). Soil and Landscape History in the Vicinity of Archaeological Sites at Gl. Davis, New South Wales. By P. H. Walker. Pp. 247-264+plates 25-27. (Vol. 26, No. 8 (19th June, 1964): Notes on the Genera *Amoria* Gray, 18 and *Zebromoria* Iredale, 1924 (Gastropoda: Volutidae), with Descriptions New Species. By Donald F. McMichael. Pp. 265-274+plate 28. 3s. Vol. 2 No. 9 (28th June, 1964): Rediscovery of the Echinoderm *Clypeaster tumid* (Tenson-Woods) and an Amended Description. By R. Endeian and Elizabeth Pope. Pp. 275-281+plates 29 and 30. 2s. 6d. Vol. 26, No. 10 (17th July 1964): New Linguloid Shells from Lower Ordovician and Middle Palaeozoic Rocks of New South Wales. By H. O. Fletcher. Pp. 289-294+plates 31 and 32. 4s. Vol. 26, No. 13 (30th December, 1964): Merostomolidae (Arthropod Trilobitomorpha) from the Australian Middle Triassic. By E. F. Riek. Pp. 327-332+plate 35. 2s. (Sydney: The Australian Museum, 1964.)

New Zealand Forest Service: Forest Research Institute. Research Note No. 41: Trials with Arboricides for Control of Coppice Growth of Indigenous Hardwoods. By A. E. Beveridge. Pp. 10. (Wellington: Government Printer, 1965)

Mutations in Quantitative Traits: Report on Symposium held on July 1 and 29, 1964. (Gamma Field Symposia, No. 3.) Pp. iii+150 (Ohmiya-machi Ibaraki-Ken, Japan: The Institute of Radiation Breeding, Ministry of Agriculture and Forestry, 1965)

Académie Royale de Belgique. Classe des Sciences—Mémoires. Tome 35, Fascicule 5: A Study of Molecular and Physical Processes in Comets. By D. Claude Arpigny. Pp. 157. (Bruxelles: Académie Royale de Belgique, 1965) 200 francs.

Western Australian Naturalists' Club. Handbook No. 8: Frogs of Southern Western Australia. By A. R. Main. Pp. 73 (5 plates). (Perth: Western Australian Naturalists' Club, 1965.) A6s.

National Hydatids Council, Wellington, New Zealand. Fifth Annual Report and Statement of Accounts for the year ended 31st March, 1965 Pp. 40. Information Bulletin No. 15: Hydatids Eradication in Iceland Pp. 11. Hydatid Disease in New Zealand: An Account of the Events leading to the Establishment of the National Hydatids Council, and the Method adopted to Eradicate the Disease. By F. S. Maclean. Pp. 48. Parasitic Animals with Special Reference to Hydatid Disease and Its Prevention. By R. M. Miller. (Reprinted from "Biology for Sixth Forms". Teach and Test Publications, Ltd., Otahuhu, New Zealand.) Pp. 36. (Wellington: The National Hydatids Council, 1965.)

Annals of the New York Academy of Sciences. Vol. 123, Article 2: Geotical Problems in Lunar Research. By J. E. M. Adler and 60 other authors Pp. 367-1257. (New York: New York Academy of Sciences, 1965.)

Editorial and Publishing Offices of "NATURE"

MACMILLAN (JOURNALS) LIMITED

4 LITTLE ESSEX STREET, LONDON, W.C.2

Telephone Number: Temple Bar 6633. Telegrams Phusis London WC 2

Annual subscription: Inland £14, Overseas £13 15s., payable in advance, postage paid to any part of the world.

Advertisements only should be addressed to

T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2

Telephone Number: Holborn 4743

Registered as a newspaper at the General Post Office

Copyright © Macmillan (Journals) Limited, October 9, 1965

NATURE

No. 5007

SATURDAY, OCTOBER 16, 1965

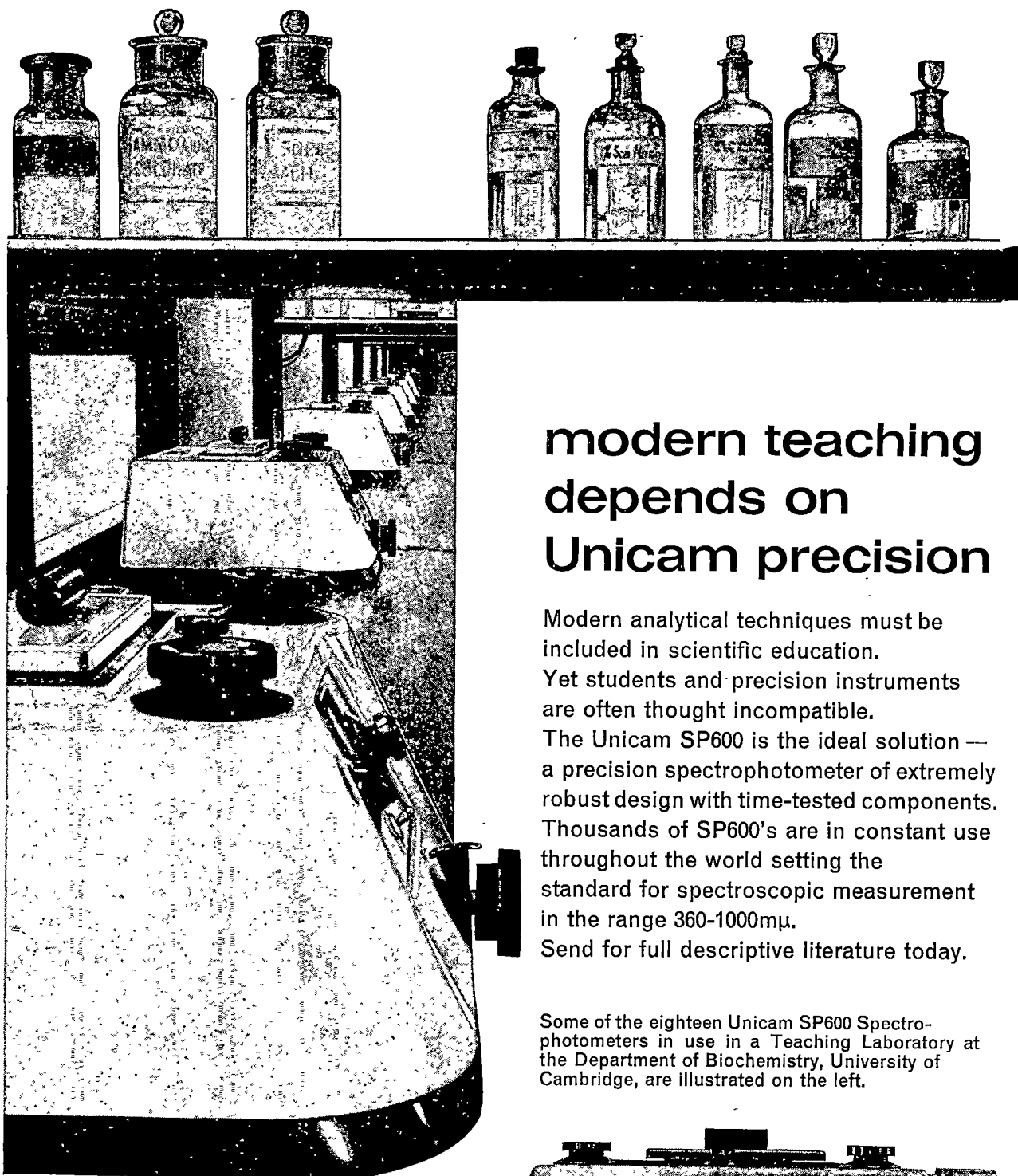
Vol. 208

Published by
MACMILLAN (JOURNALS) LTD.

CONTENTS

	Page
SCIENTIFIC AND TECHNOLOGICAL INFORMATION IN PARLIAMENT	209
THE MAMMALS. By Dr. L. Harrison Matthews, F.R.S.	210
SALT TOLERANCE IN PLANTS. By Dr. P. A. Thompson	211
RESEARCH IN PHOTOSYNTHESIS. By Dr. E. A. C. MacRobbie	212
INDIAN TIMBERS. By Dr. C. R. Metcalfe	212
TAXONOMIC AND EXPERIMENTAL BACTERIOLOGY. By Dr. D. Allan	213
SKIN AND ITS DISEASES. By Prof. C. D. Calnan	213
ATOMIC AND MOLECULAR SPECTROSCOPY. By Dr. R. F. Barrow	214
BASIC STATISTICAL TECHNIQUES. By N. E. Day	214
PACIFIC GEOLOGY. By Dr. A. S. Laughton	215
A CENTURY OF ANTHROPOLOGY. By Prof. M. Fortes	215
THE NON-GUILTY PARTY. By Prof. D. C. Marsh	216
ORIGIN AND SIGNIFICANCE OF THE POWER OF VOCAL IMITATION: WITH SPECIAL REFERENCE TO THE ANTIPHONAL SINGING OF BIRDS. By Dr. W. H. Thorpe, F.R.S., and Myles E. W. North	219
THE INTERNATIONAL BIOLOGICAL PROGRAMME. By Dr. E. B. Worthington	223
OBITUARIES:	
DR. L. H. GRAY, F.R.S. By Dr. O. C. A. Scott	226
DR. O. S. SINNATT. By Group Capt. C. E. P. Suttle, O.B.E.	227
NEWS AND VIEWS	228
THE INSTITUTE OF PHYSICS AND THE PHYSICAL SOCIETY. By S. Weintraub	232
SCIENTIFIC RESEARCH IN NEW ZEALAND	233
COMPARATIVE STUDY OF HOUSING IN BRITAIN, FRANCE AND WESTERN GERMANY	233
INSPECTION OF ALKALI, ETC., WORKS	234
INTERACTION OF CEPHALORIDINE WITH PENICILLINASE-PRODUCING GRAM-NEGATIVE BACTERIA. By Dr. J. M. T. Hamilton-Miller, Dr. J. T. Smith and Prof. R. Knox	235
THE IRON DEXTRAN COMPLEX. By Dr. C. R. Ricketts, Dr. J. S. G. Cox, C. Fitzmaurice and G. F. Moss	237
PENICILLINASE SYNTHESIS CONTROLLED BY INFECTIOUS <i>R</i> FACTORS IN ENTEROBACTERIACEAE. By Dr. Naomi Datta and Dr. Polyxeni Kontomichalou	239
DEOXYRIBONUCLEIC ACID SYNTHESIS IN CULTURED HUMAN CELLS AND ITS BEARING ON THE CONCEPTS OF ENDOREPLICATION AND POLYPOIDY. By Dr. Ursula Mittwoch, Mrs. Kusum P. Lele and W. S. Webster	242
PROTEIN SYNTHESIS IN RETICULOCYTES MATURING <i>in vivo</i> . By Dr. Peter T. Rowley	244
NUCLEO-CYTOPLASMIC INTERACTIONS IN THE RAT LIVER CELL. By E. R. Godwin, Dr. K. R. Rees, Dr. G. F. Rowland and J. S. Varcoe	246
BIOLOGY OF THE CYTOPLASM. By Dr. Ole A. Schjeide, Ruth G. McCandless and E. J. Munn	247
BREAKDOWN RATE OF SERUM γ -GLOBULINS IN MICE. By Dr. D. E. H. Tee, Dr. J. Watkins and Mildred Wang	251
LABELLING OF MOUSE THYMOCYTES <i>in vivo</i> WITH TRITIATED THYMIDINE FOR CELL TRANSFER EXPERI- MENTS. By Dr. Kirstine Borum	253
EFFECT OF A PYRIMIDOPYRIMIDINE DERIVATIVE ON THROMBUS FORMATION IN THE RABBIT. By Dr. P. R. Emmons, M. J. G. Harrison, Dr. A. J. Honour and Dr. J. R. A. Mitchell	255
A SEARCH FOR MUTATION IN CHEMICAL SYSTEMS AT STEADY STATE. By Dr. Gordon Allen	258
PLAQUE-FORMATION BY NON-IMMUNE AND X-IRRADIATED LYMPHOID CELLS ON MONOLAYERS OF MOUSE EMBRYO CELLS. By Goran Moller and Erna Moller	260
MEASUREMENT OF UNSTEADY STATE GROWTH RATES OF MICRO-ORGANISMS. By Prof. R. I. Mateles, D. Y. Ryu and T. Yasuda	263
PROTONATION EQUILIBRIA AND ALKALINE HYDROLYSIS OF GLYCINE ETHYL ESTER. By Dr. Margaret Robson	265
DIFFUSIVE TRANSPORT RATES IN STRUCTURED MEDIA. By Prof. A. B. Metzner	267
CONCENTRATION EFFECTS IN CHROMATOGRAPHIC ANALYSIS. By Prof. I. D. Rastee, D. M. Lewis and Dr. C. B. Stevens	269
GEOCHEMISTRY OF ANDESITES AND THE GROWTH OF CONTINENTS. By Prof. S. R. Taylor and Dr. A. J. R. White	271

(Continued overleaf)



modern teaching depends on Unicam precision

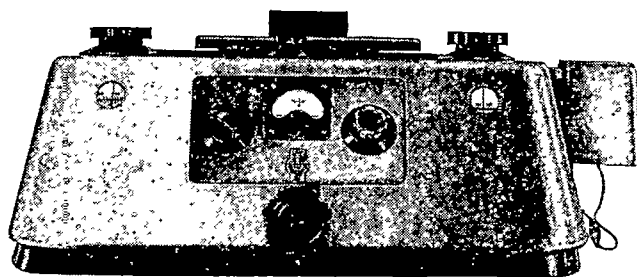
Modern analytical techniques must be included in scientific education.

Yet students and precision instruments are often thought incompatible.

The Unicam SP600 is the ideal solution — a precision spectrophotometer of extremely robust design with time-tested components. Thousands of SP600's are in constant use throughout the world setting the standard for spectroscopic measurement in the range 360-1000m μ .

Send for full descriptive literature today.

Some of the eighteen Unicam SP600 Spectrophotometers in use in a Teaching Laboratory at the Department of Biochemistry, University of Cambridge, are illustrated on the left.



UNICAM

Precision Spectrophotometers

LETTERS TO THE EDITOR

	Page		Page
ASTROPHYSICS		Use of Polyethylene Glycol in Investigations of Absorption of Fat. —Dr. H. Vavřínková and Dr. A. Krondl .	
Interstellar Obscuration.—Dr. K. Nandy .	274		293
New Limits to the Angular Sizes of Some Quasars.—R. L. Adgie, H. Gent, O. B. Slee, Prof. A. D. Frost, Dr. H. P. Palmer and Dr. B. Rowson .	275	PHARMACOLOGY	
RADIOPHYSICS		Some Effects of Drugs on the Isolated Superfused Carotid Body.—Dr. T. J. Biscoe .	
Scintillations on the Small Diameter Radio Sources.—Prof. Marshall H. Cohen .	277		294
PHYSICS		Lethal Effects of Aggregation and Electric Shock in Mice treated with Cocaine.—Harbans Lal and Dr. Richard D. Chessick .	
Combined Very High Pressure/High Temperature Calibration of the Tetrahedral Anvil Apparatus, Fusion Curves of Zinc, Aluminium, Germanium and Silicon to 60 kilobars.—J. Lees and B. H. J. Williamson .	278		295
A New Absolute Determination of the Acceleration due to Gravity at the National Physical Laboratory.—Dr. A. H. Cook .	279	Interference by the Carcinogenic 4-Nitro-quinoline N-Oxide of Tryptophan and Indole Uptake in <i>Escherichia coli</i> .—A. C. Zahalsky and Prof. S. L. Marcus .	
Deformation of Gas Bubbles and Liquid Drops in an Electrically Stressed Insulating Liquid.—Dr. K. C. Kao .	279		296
GEOPHYSICS		HAEMATOLOGY	
Variation according to Latitude of the Electron Content of the Ionosphere near the Auroral Zone.—Ludwik Liszka .	280	Plasma Co-factors in Adenosine Diphosphate-induced Aggregation of Human Platelets.—Dr. Daniel Deykin, Carole R. Pritzker and Edward M. Scolnick .	
GEOLOGY			296
Holothurian Sclerites from the Speeton Clay.—B. N. Fletcher .	281	A Modification System for Fingerprinting Haemoglobin.—Dr. G. Efremov .	
CHEMISTRY			298
Polyamide Layer Chromatography of DNP Amino-acids.—Prof. Kung-Tsung Wang and Joyce Mei-Kuo Huang .	281	PATHOLOGY	
Distribution of Plutonium in Serum Proteins following Intravenous Injection into Rats.—G. Boccock and D. S. Popplewell .	282	Blood Group Secretion Factors in Bronchogenic Carcinoma.—Prof. Crichton McNeil, Elmer F. Trentelman, J. Noel Ladle, Willa Mae Helmick and Prof. Henry P. Plenk .	
Far-infra-red Spectra of some Square-planar Ions.—Dr. D. M. Adams and D. M. Morris .	283		299
Methane-rich Perchloric Acid Flames.—Dr. G. S. Pearson .	283	Induction of Intestinal Tumours by N-Ethyl-N-nitrosourethane.—Dr. R. Schoental .	
BIOCHEMISTRY			300
Polymorphism of Lactate Dehydrogenase Isozymes in Pigeons.—Prof. Wm. H. Zinkham, L. Kupchik, A. Blanco and H. Isensee .	284	BIOLOGY	
Metabolism of 5-Chloro-2-cyclohexyl-1-oxo-6-sulphamoyl Isoindoline, Clorexolone (M and B 8430).—K. Corbett, Miss S. A. Edwards, G. E. Lee and T. L. Threlfall .	286	Influence of Hexadecane on Absorption of Carbon Dioxide by Plants.—Prof. J. M. Riedhart .	
Formation of Hydroxamates during Proteolysis by an Enzyme System from Yeast.—Dr. D. E. Briggs and Dr. D. J. Millin .	287		300
Hydrolysis of Two 17 α -Oestradiol Glycosides by Rabbit Tissue Glycosidases.—Katherine S. Whittemore and Dr. Donald S. Layne .	288	Social Companions and the Mother-Infant Relationship in Rhesus Monkeys.—Y. Spencer-Booth, Prof. R. A. Hinde and M. Bruce .	
Metabolites in Urine of Rat on Diets containing Aldrin or Dieldrin.—P. R. Datta, Dr. E. P. Laug, J. O. Watts, A. K. Klein and M. J. Nelson .	289		301
PHYSIOLOGY		ENTOMOLOGY	
Electrical Activity in Human Uterine Muscle Strips.—Dr. T. B. MacLachlan, Prof. E. E. Daniel and Prof. W. M. Paul .	290	Bilateral Asymmetry: a Larval Polymorphism in some Argid Sawflies (Hymenoptera, Symphyta).—Dr. M. F. Claridge and Dr. J. M. Edington .	
Release of Plasminogen Activator by the Isolated Perfused Dog Kidney.—Dr. Rudolf Holemans, Dr. John G. Johnston and Dr. Robert L. Reddick .	291		301
Thinning of the Human Cornea on Awakening.—Dr. R. B. Mandell and Prof. I. Fatt .	292	Feeding Mechanism of Blood-sucking Arthropods.—M. M. J. Lavoipierre .	
			302
		MICROBIOLOGY	
		Metabolism of Nucleic Acids and Protein in Starving Bacteria.—B. Brdar, E. Kos and M. Drakulić .	
			303
		VIROLOGY	
		Transmission of Guamá and Oriboca Viruses by Naturally Infected Mosquitoes.—Amazonia Toda and Robert E. Shope .	
			304
		CYTOLOGY	
		Circulation in the Cell.—Prof. Carl C. Lindegren .	
			304
		GENETICS	
		Schizophrenia as a Genetic Morphism.—Prof. A. Hoffer .	
			306
		Identical S Alleles in Different Botanical Varieties of <i>Brassica oleracea</i> .—K. F. Thompson and J. P. Taylor .	
			306
		VETERINARY SCIENCE	
		Association of Adenoviruses with Bovine Respiratory Disease.—Dr. J. H. Darbyshire, A. R. Jennings, A. R. Omar, P. S. Dawson and P. H. Lamont .	
			307
		Hepatic Tumours in Ducks fed a Low Level of Toxic Groundnut Meal.—R. B. A. Carnaghan .	
			308

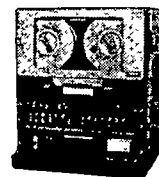
what do you do with data?



As makers of a range of rugged, reliable, economical and flexible Data and Instrumentation Recording equipment, we know a thousand and one ways of using magnetic recording for data acquisition, storage and analysis—in aeronautics, medicine, industry and university research work. Magnetic recording provides a wide range of band widths and high information packing density. Data expansion and contraction facilities are available which are not possessed by other forms of data acquisition. Thermionic equipment is the lowest priced range of digital and analogue recorders for research—we designed it on the unit principle for complete flexibility of use and we're sure it will answer your data handling problems.

You tell us!

Tell us what your particular research requirements are and we'll show you how THERMIONIC Data Recording Systems can meet them quickly, simply, cheaply. Drop a line to us today.



THERMIONICS



DATA & INSTRUMENTATION RECORDING EQUIPMENT

THERMIONIC PRODUCTS (Electronics) LTD., Hythe, Southampton. Tel: Hythe 3265-9. Cables: Technico Hythe Southampton
A MEMBER OF THE CONTROLS & COMMUNICATIONS GROUP

Lock

Gas Chromatograph Test Set & Reference Signal Source



enables the Chemist or Instrument Engineer to check quickly the operation and performance of his complete Gas Chromatograph equipment. If the equipment is not operating correctly, it will quickly localise the faulty section. No electrical knowledge is necessary to use this Test Set, which is suitable for Flame Ionisation, Thermistor type and Katharometer Gas Chromatographs.

Please write for details now:-



A. M. Lock & Co. Ltd.
Prudential Buildings,
79, Union Street, Oldham, Lancs.
Telephone: MAIn 0333

SCIENTIFIC AND TECHNOLOGICAL INFORMATION IN PARLIAMENT

At a meeting in June 1964, the Parliamentary and Scientific Committee agreed to set up a sub-committee, consisting of the Parliamentary members of its Steering Committee, to consider whether there was need for improved methods by which Members of Parliament could quickly obtain information from scientists about matters likely to be raised in Parliament. Moreover, the sub-committee was to consider what could be done to prove existing machinery to ensure that Parliament could establish more effective control over scientific and technological policy. For the purposes of this enquiry, the sub-committee co-opted Sir Harry Legge-Bourke and Dr. Jeremy Bray. An interim report entitled 'Parliament and Science', dealing solely with the means for ensuring more effective Parliamentary control over scientific and technological policy, has now been issued. This is included, together with a memorandum by Dr. Bray on 'Financial Procedure', as an appendix to the fourth report from the Select Committee on Procedure for the Session 1964-65*.

Although in the report from the Select Committee on Procedure this memorandum constitutes the chief interest of the scientist, it is not the sole feature of interest. The report as a whole and its four recommendations are of general interest, reflecting the critical problem of the mechanism of advice and informed discussion in general, but it may be as well to deal first with this report from the Parliamentary and Scientific Committee. Its sub-committee fully recognized that science and technology are both means and objects of policy, and that, besides their responsibility for implementing any policy adopted, experts could point out the probable effects of alternative choices. The sub-committee also recognized that, if Parliament decided to improve the present system of Parliamentary control of policy and administration in the scientific field, for example, by means of a 'select committee', it would have to define its order of reference so as to try to avoid becoming involved in policy decisions.

The sub-committee had before it a paper written by the chairman of the Parliamentary and Scientific Committee, Mr. Austen Albu, entitled "The Member of Parliament, the Executive and Scientific Policy", published in *Minerva* in 1963, to which reference has already been made in *Nature* (201, 955; 1964), as well as a pamphlet *Change or Decay* written by a group of Conservative Members of Parliament. However, the sub-committee decided to restrict itself to the means by which Parliament could be better informed on research and development in the civil field undertaken with public funds and in support of public policy. The considerations already noted led it to recommend the establishment of a select committee to examine, with the view of informing the House on their work and future, the annual reports of such bodies as the Research Councils, the Atomic Energy Authority, the National Research Development Corporation, and also the activities of scientific research groups and establishments in Government departments. It also recommended that this committee should, from time to time, make *ad hoc* enquiries into scientific and technological matters which

are under consideration as a basis for legislation or the formulation of policy.

The advantage of this proposal would be that Members of Parliament would hear something of the differing scientific and technical views which were under consideration in the department concerned. This would make for more informed debates and should also assist in differentiating technical from political issues. Admittedly reference to a select committee should be sparingly used, but the sub-committee hoped that a practice would develop of referring legislation involving scientific matters to this committee before or during its passage through Parliament. The sub-committee recommended that the select committee should consist of thirteen members (five to constitute a quorum), and that it should have the power to send for persons, papers and records. It did not consider that the select committee should be provided with expert staff but rather that experts should be called, when required, as witnesses. It was hoped that an expanded library and reference service, with scientific staff, would also be available to assist the proposed committee.

This latter point is strongly urged in a memorandum by the Study of Parliament Group, which is a private group of university teachers and officers of both Houses of Parliament which has been meeting since the summer of 1964, under the chairmanship of Sir Edward Fellowes, for the serious study of the working of Parliament. The memorandum concentrates on the procedure on public Bills and on the scrutiny and investigation of policy and administration; it emphasizes that Parliamentary scrutiny of the Executive is fundamental to the whole question of Parliamentary reform. The main task of the Government is to govern, and the main tasks of Parliament are to sustain the Government and to criticize its policies and actions.

The memorandum firmly asserts that Parliamentary control means influence, not direct power; advice, not command; criticism, not obstruction; scrutiny, not initiative; and publicity, not secrecy. The Group believes that strong government needs critical opposition; it can benefit from such criticism and stand up to it. Accordingly the Group suggests a greater degree of specialization by Members of Parliament and the Committees through which, increasingly, they should work. It is in this context that it points to the implications for the research and information services available to Parliament. Changes in and expansion of the services provided by the Library could be as important as any procedural changes in the basic task of creating or restoring adequate supervision by Parliament of the activities of Government. Present difficulties in this respect are, in the view of the Group, unlikely to be resolved unless Parliament is willing some time to look at the problem as a whole and consider how procedure, research and information, facilities and accommodation, form part of an integrated system so that changes in one, voluntary or involuntary, must lead to changes in others.

The essential aspect of any comprehensive proposal for enabling the House of Commons to perform its crucial role in scrutiny and investigation of policy and administration is the provision of adequate research and information facilities for both committees and individual Members.

* Fourth Report from the Select Committee on Procedure, together with the Proceedings of the Committee Relating to the Report, Minutes of Evidence and Appendices, Session 1964-65. Pp. xxiii + 148. (London: H.M.S.O., 1965.) 15s. net.

Only a properly developed Library with an expanded research department could provide access to the information necessary to Members for the informed discharge of their Parliamentary responsibilities, and the Group believes that the present research services of the Library could and should be improved. It has, in fact, prepared a report on the provision of research and information services which it hopes to submit to any committee established to consider this problem.

The Select Committee on Procedure did not, in the present report, pursue this question, but its four recommendations give substantial effect to the recommendations of the Parliamentary and Scientific Committee and to the proposals of the Study of Parliament Group for Specialist Committees of Advice and Scrutiny. Five such committees were suggested by the Group for initial experiment, covering scientific development; prevention and punishment of crime; machinery of national, regional and local government and administration; housing, building and land use; and the social services. The Select Committee on Procedure recommends the establishment, as a development of the present Estimates Committee, of a new select committee "to examine how the departments of State carry out their responsibilities and to consider their Estimates of Expenditure and Reports". The new committee should function through sub-committees specializing in the various spheres of Government activity and should be able to employ specialist assistance. There should be two clerks supervising the work of the Committee and one full-time clerk to each sub-committee. The power of select committees to adjourn from place to place should include the power to travel abroad, with the leave of the House, when investigations require it.

In making these recommendations, the Select Committee was obviously influenced by the experience gained with the Select Committee on the Nationalized Industries. It was also noticeably influenced by views expressed by the Estimates Committee, which is precluded by its terms of reference from investigating long-term proposals and prospects for expenditure in the various fields or from examining the administrative policy of Government departments apart from considerations of economy alone. It is not the wish of the Select Committee on Procedure that the specialist committees proposed should become involved in matters of political controversy. Certainly, it is well aware that it is not easy to distinguish between what are questions of policy and what are not, but it believes that the Select Committee on the Nationalized Industries has demonstrated what is possible in a field that is politically highly sensitive. The Committee was anxious also to retain the experience and method of work of the Estimates Committee, and for this reason recommended a series of sub-committees operating through a co-ordinating committee rather than a series of independent committees.

While the recommendations do not strictly follow those of the Parliamentary and Scientific Committee, running more on departmental lines than those of the Study of Parliament Group, it should still be possible for scientific matters to be examined in a context such as the examination of the annual reports of the Research Councils and other bodies would provide—as suggested by the former Committee. The idea of specialist committees has had powerful advocates in recent years, though Mr. Albu did not give it strong support in his paper on "The Member of Parliament, the Executive and Scientific Policy". The more limited proposal of the Parliamentary and Scientific Committee might yet

command wider support among those who recognize vital importance of informed discussion of scientific and technological matters in forming public policy. Curious enough, the recommendation drew no comment from Mr. Horace King in his statement before the Committee. However, he did affirm that he was one who thought that the power of the Executive was growing and ought to be diminished. For all that he wished to preserve, so far as possible, Parliament's historic and traditional features, he was concerned above all to promote its efficiency and would remove anything that impaired it.

Mr. Albu at least recognized the growing demand especially among younger Members, for a properly equipped research and information service, staffed with men and women who had been educated in a broad range of subjects, including the scientific and technological. The observations of the Study of Parliament Group on this point are among the most pertinent in the whole report and deserve further attention by the Parliamentary and Scientific Committee itself. Adequate library research and information services are indeed a key factor in informed discussion in Parliament. The Parliamentary and Scientific Committee should be able to do something to see that the observations of the Study Group, which includes such authorities as Prof. M. Beloff, Prof. J. Bromhead, Prof. A. H. Hansen, Prof. W. J. M. Mackenzie, Prof. W. A. Robson, Prof. H. Wiseman, Dr. D. N. Chest and Dr. B. Crick, are duly noted and that the committee suggested to look into this whole question is duly established. That may not be the whole solution, but it is the essential factor. Unless such a step is taken, any system of specialist committees is unlikely to work really effectively. The fourth report from the Select Committee on Procedure provides evidence which should be noted and used by all who recognize the value of informed public discussion in the formation of public policy—above all where scientific and technical factors are involved.

THE MAMMALS

Mammals of the World

By E. P. Walker, F. Warnick, K. I. Lange, H. E. Uible, S. E. Hamlet, M. A. Davis and P. F. Wright. Vol. 1, Pp. xlviii + 644. Vol. 2, Pp. viii + 1,500. Vol. 3, Classification Bibliography, Pp. xii + 769. (London: Oxford University Press; Baltimore: Johns Hopkins Press, 1964.) 300s per set.

THE mammalian fauna of the world at present consists of something between 4,000 and 4,500 living species divided into about a thousand genera—1,044 are recognized as valid in this work, which gives an illustration of all but four of them. The validity of genera as of species is however, often a matter of opinion that varies widely between the 'splitters' and the 'lumpers' among taxonomists; Walker himself speaks of the "twelve to fifteen thousand species" of mammals, a generous total even for a splitter.

Ernest P. Walker has been amassing the material for these books for more than thirty years, twenty-seven of them while he held the post of assistant director of the National Zoological Park at Washington, D.C. After his retirement the completion of the work was supported for seven years by funds from the National Institutes of Health, and the publication was sponsored by the New York Zoological Society. As the President of that Society says in the foreword, "The heroic task Mr. Walker set himself was to find and reproduce a photograph of a living representative of every genus. If he has not quite accomplished this, he has missed by the narrowest of margins and only because the genus is too obscure or the

otograph hidden where world-wide enquiries could not find it". He has had perforce to use some photographs of stuffed animals in museum collections, and occasionally reproduce drawings from original descriptions. The illustrations nevertheless form a unique and extremely valuable gallery, for they have been carefully chosen to convey a "better image of the animals than could be given by a word picture... many attractive and appealing photographs have been rejected because they did not show the characteristic form of the mammals to the best advantage". More than one picture is provided to illustrate some of the major genera, and there are many insets of important anatomical and osteological details.

The illustrations are accompanied by a concise text summarizing all that is known of the members of each genus; for many genera all recorded information is given in a few paragraphs, such is our lack of knowledge. Information, whenever available, is given about the number of species in each genus, the distribution, measurements and weights, coloration, type of body covering, structural peculiarities, habits, food, gestation period, number of young in a litter, economic importance, and the vernacular as well as scientific names. A page or two of introductory text is also given to each order and each family. A useful feature is the listing of museums holding specimens of mammals that are very rare in collections. A table showing the world distribution of the genera of recent mammals occupies twenty-four pages of introductory matter and, by means of a simple regional code and few footnotes, makes much useful information available at a glance. The text and illustrations together with a selected bibliography of about 4,500 titles occupy the first two volumes; the third volume, which is available separately for the more specialized worker contains a catholic bibliography of about 50,000 titles, containing many references of a popular character as well as those of serious scientific content. The pagination runs straight through volumes 1 and 2, and an index to the whole work appears at the end of both volumes; the end-papers carry scales for the comparison of metric and U.S. units of measurements.

The naturalist's first feeling on opening these volumes is one of surprise and delight at their completeness and comprehensiveness; his next, one of amazement that anyone should have contemplated undertaking such a staggering task, still more that he should have finished it successfully. Here are pictures and descriptions of rare and obscure mammals that hitherto had been no more than scientific names in fauna lists—often not even that—as well as pithy summaries of the biology of more familiar forms. It is of equal value to the general naturalist as a review of the mammalian fauna of the world, as it is as a reference book and guide to the literature for the specialist.

Although no one has approached the author's diligence in searching the literature, seeking information by correspondence throughout the world, in personal observation and study, and in developing a high degree of skill in photography, it would be too much to expect human endeavour to avoid occasional inaccuracies in a work of such great size. The state of Mr. Walker's health compelled him to pass some of the editorial burden to his colleagues during the last four years of the marathon, and he handsomely acknowledges their help for doing the "work that I should have done, but my declining energy prevented me from doing my part". There are, inevitably, some misprints, especially in unfamiliar geographical names, and such errors as "8 vol." for "8vo." in the bibliography. A few more serious errors have crept in, especially in the sections dealing with those orders of mammals with which the author presumably had less personal experience, so that he had to rely on others with consequent occasional misunderstanding. As examples one may mention the photograph of young seals on p. 1302, which, according to the caption, are common or harbour seals, *Phoca vitulina*, but are unmistakably the young of the harp seal

Pagophilus groenlandicus; or the photographs on p. 1129 labelled "Common or Harbor Porpoises (*Phocaena phocaena*)" which plainly represent the bottle-nosed dolphin, *Tursiops truncatus*—the error here may have arisen from the unfortunate difference between the American and English use of the names "porpoise" and "dolphin". Mr. Walker himself realizes that the devil is ever at hand to frustrate man's striving for perfection, and asks readers who find errors or who know of better photographs or additional facts to communicate with the publishers so that improvements can be made in a future edition.

It is, however, really ungenerous to point out the few blemishes in so splendid an achievement, and all naturalists, especially those who concentrate their studies on the mammals, are deeply grateful to Mr. Walker and his team for consolidating the foundations on which their work is based. The three volumes are not cheap but they are well worth their price, and considering that they contain nearly 1,800 half-tone illustrations it is surprising that they do not cost more; the typography, paper and binding are excellent.

This is one of the major contributions of the century to the study of mammalian biology. The author and his colleagues are to be congratulated on their unremitting labours, and the National Institutes of Health, the New York Zoological Society and the Johns Hopkins Press all gain the highest credit for the parts they played in bringing them to fruition.

L. HARRISON MATTHEWS

SALT TOLERANCE IN PLANTS

Physiological Basis of Salt Tolerance of Plants

(As Affected by Various Types of Salinity.) By B. P. Strogonov. Translated from the Russian by Prof. A. Poljakoff-Mayber and Dr. S. M. Mayer. Pp. 279. (Jerusalem: Israel Program for Scientific Translations; London: Oldbourne Press, 1964.) 88s.

AT a time when more and more countries are embarking on ever-increasing schemes for the irrigation of vast areas of arid land, it requires no effort of imagination to appreciate the potential usefulness and relevance of a book dealing with the physiological basis of salt tolerance in plants.

Physiological Basis of Salt Tolerance of Plants was first published in the U.S.S.R. in 1962, and we are indebted for its rapid translation into particularly lucid and easily readable English to Prof. Poljakoff-Mayber and Dr. Mayer working under the auspices of the Israel Program for Scientific Translations. In a note at the beginning it is stated that the translation was undertaken because this was the first attempt to summarize in one book the information available in this field; it contained a great deal of factual information not readily available to Western scientists, and presented a number of ideas not previously adequately stressed in work on salt tolerance. There can be little doubt that these claims are fully justified by the subject-matter of the book.

In the first three chapters, Dr. Strogonov reviews the present state of the physiology of salt tolerance, the mode of action of salts on the plant, and theoretical considerations of experimental results, and this section provides a valuable and stimulating introduction to the literature, especially that from the U.S.S.R. on this subject. In the following eight chapters a series of subjects is dealt with one by one, each directly related to the growth of plants under saline conditions. These include the effect of salinity on development and anatomical structure; water relations and adaptation; nitrogen metabolism, and effects on the soil microflora, and a contribution on practical means for increasing salt tolerance of plants. In each of these chapters an extensive review-type introduction is followed by a section in which the results of experiments performed by Dr. Strogonov with cotton are presented by means of

tables and illustrations, and finally a concise summary of these results is provided. This method of presentation is not entirely successful since it provides the reader with a very large bulk of rather indigestible experimental results, and, owing to the absence of a discussion of the results by the author, considerable effort is required to correlate the experimental results reported with the information detailed in the introductory review of the literature.

Dr. Stroganov stresses that the book is concerned not so much with observations of conditions arising from salinity as with the mechanism of the action of excess salts on plant growth, and the specific effect of the type of salinization of the substrate. In particular, the importance of distinguishing between osmotic effects and toxic effects is emphasized. It is suggested that the toxic effects are due not directly to the effects of the salts themselves but to interference with the normal metabolic processes of the plant resulting in the accumulation of toxic substances such as ammonia, hydrogen peroxide or putrescine.

The value of the book is wantonly reduced by the complete absence of any sort of index, and also by the quite appalling quality of the illustrations. Nevertheless it does provide a useful and provoking review of the present knowledge on the subject, and, to the Western scientist, an excellent bibliography of a large body of inaccessible Russian work. It should provide a considerable stimulus to a branch of plant physiology which, in view of its outstanding practical importance, has been sadly neglected in recent years.

P. A. THOMPSON

RESEARCH IN PHOTOSYNTHESIS

Photosynthetic Mechanisms of Green Plants

(Papers presented at a Symposium sponsored by the Committee on Photobiology of the National Academy of Sciences-National Research Council, October 14-18, 1963.) (Publications No. 1145.) Pp. ix + 766. (Washington, D.C.: National Academy of Sciences-National Research Council, 1963.) n.p.

THIS volume contains the papers presented at a symposium sponsored by the Committee on Photobiology of the National Academy of Sciences-National Research Council, in October 1963.

It provides a wide, if uneven, coverage of the field of photosynthesis to that date, and is divided into ten main sections, including spectroscopic and fluorescence studies; biochemical studies of electron transport paths, and of isolated electron carriers; enhancement studies; respiration, photosynthesis and hydrogen metabolism; functions of pigments; chloroplast structure, mechanism of phosphorylation; carbon metabolism.

The volume contains seventy-five papers, of ten pages average length. Not unexpectedly, these differ widely in content, in presentation and in style. Some authors make a real attempt to review work in their particular laboratories, and to synthesize a picture of the mechanism of photosynthesis from the confusion of experimental data, but others present only isolated experimental data, thinly or thickly spread. Inevitably the volume lacks cohesion, and would have been better for a more uniform presentation. Very little of it is easy reading, but one feels that to include papers in which the text, the figures, and the figure legends are each in separate sections is asking too much of any reader. Quick publication is an important aim, but one feels that the volume would have been much more valuable had more rigid instructions for presentation been enforced.

The volume includes useful discussion of the nature of the long-wave absorbing pigments, and of the trapping centre of System I, which also serves to direct attention to our ignorance of System II. Work on biochemical mutants and their contribution to our present picture of photosynthesis is well covered. The volume emphasizes

how a wide range of techniques and experimental approaches combine to give a reasonably coherent, incomplete, view of the process, and much of the discussion centres around this picture of two photo-systems acting in co-operation with one another. The nature of the biochemical events set in motion by the absorption of quanta in each photo-system, and the mechanisms of the co-operation between them, provide much of the discussion and reflect the amount of effort at present devoted to these aspects of the subject.

By contrast, there is very little discussion of new information on the processes of energy transfer from the site of the initial absorption of a quantum to the active centre from which the subsequent biochemical changes arise. Nor is there evidence presented on the transformation of excitation energy in oxidation-reduction reactions at the trapping centre—another of the major unsolved problems in the field. Thus the book, both by its content and its omissions, presents a useful picture of the present state of the subject.

There is a seven-page editorial summary, which does something to draw together the wide range of experimental results and of hypotheses, but inevitably, at such a length, this can only highlight the more important points covered, and direct attention to areas of strong interest or extreme ignorance. One would like to read much more complete, critical assessment of the papers presented. Thus as a review this book is less satisfactory than other recent reviews of the field, and its value rather as a source book. Unfortunately for this purpose an index becomes a prime necessity and this is entirely lacking—doubtless a sacrifice to rapid publication. The book is essential reading for those working in the field of photosynthesis or in related fields, but it is a book for those already familiar with the literature; it is too heavy going to be in any way an introduction to the subject even for advanced students. With these limitations, it provides a valuable collection of papers covering a range of work which is otherwise widely scattered, and will be useful as such.

E. A. C. MACROBBIE

INDIAN TIMBERS

Indian Woods

Their Identification, Properties and Uses. Vol. 2: Linaceae to Moringaceae. Pp. x + 386 + plates 31-63. (Delhi: Manager of Publications, 1963.) Rs. 32-50 n.p.; 75s. 10d. 11.70 dollars.

THIS is the second of a series of volumes (the first of which was published in 1958) describing the timbers of India which are already commercially important or which are likely to become so as timber supplies run short. In the present volume the woods of 263 species are described, and these belong to 23 families ranging from Linaceae to Moringaceae. Descriptions and editing are mainly by S. S. Ghosh, K. R. Rao and S. K. Purkayastha, who have been supported by a team of assistants and specialist colleagues. Dr. K. A. Chowdhury, the senior joint author of the first volume, has revised the drafts of twenty of the present family descriptions and has also helped to select the photomicrographs.

The present volume is in the same style as the first. The reader is told under each family the number of genera and species of which it consists, and there are notes on economic plant products. Then follows a description, based mostly on naked eye or lens characters, of the gross structure of the timber of each of the species selected for inclusion in the book. Each description of the structure is followed by notes on durability, liability to attack by insect or fungus, working qualities, availability of supplies and, finally, uses. Reference numbers of the wood specimens on which the descriptions are based are given, and at the end of each family description there is a biblio-

aphy. With some of the larger families there are keys for the identification of the genera based on wood structure.

At the end of the book there are four appendixes dealing, respectively, with mechanical properties, classification of woods according to anatomical structure, classification of woods according to uses and, finally, recent name changes. In the second of these, the genera are very usefully listed under the diagnostic characters by which they can most easily be identified. It seems a pity that the name changes in Appendix IV are not covered under "Synonymy" in the main body of the text. There are 197 photomicrographs, mostly of excellent quality, showing the end-grain appearance of the different woods, and the book closes with separate indexes to (i) scientific names, and (ii) trade and common names.

This excellent book deserves a much better cover, for it will undoubtedly have to stand up to the hard wear that frequent consultation entails. C. R. METCALFE

TAXONOMIC AND EXPERIMENTAL BACTERIOLOGY

Manual for the Identification of Medical Bacteria

by S. T. Cowan and the late K. J. Steel. Pp. x+217. Cambridge: At the University Press, 1965. 50s.

Theory and Practice in Experimental Bacteriology

by G. G. Meynell and Elinor Meynell. Pp. xii+288+4 plates. (London: Cambridge University Press, 1965.) 60s. net; 9.50 dollars.

THE demand for diagnostic tables for the common medical bacteria by these authors was so great among clinical bacteriologists and others that it was inevitable that they should produce a book on the subject. It is therefore most regrettable that K. J. Steel, who contributed much to this work, did not live to see the proofs.

The *Manual* is clear and well produced, written with authority and an insight which at times is almost embarrassing; for who has not at one time or another been tempted to create a new species rather than carry out the necessary work to put an organism into its right place in an existing species. The book is centred on the two- and sometimes three-stage tables, which are easily read and clearly understandable to the bacteriologist. These do not, however, by themselves characterize organisms, but as the authors state, they focus attention on the most valuable of taxonomic tests. The early chapters are devoted to classification and nomenclature, the importance of obtaining pure cultures, and a useful chapter is included on the much-vexed problems associated with media preparation and sterilization. There follows a chapter on characterization in which the primary and secondary tests for the first and second stage diagnostic tables are discussed. The authors then get down to describing characters of Gram-positive and Gram-negative bacteria, and include what they term "minidefinitions" which give the main characters of each organism succinctly and precisely. These should be very helpful to harassed clinical bacteriologists who wish to refresh their memories as briefly as possible. Following these chapters is one on refractory organisms of uncertain characterization; and a short chapter on taxonomic implications (for those interested) ends the discursive part of the book.

There are seven appendixes, consisting of laboratory notes covering instructions for media preparation and control, staining reagents, characterization tests including rapid methods some of which, it is claimed, are sensitive and give more clear-cut results than the standard characterization tests. Finally, there is a list of recommended strains of test organisms with conditions for their maintenance; and the book ends with a comprehensive list of references.

The *Manual* will undoubtedly be popularly received in most diagnostic laboratories, and this must be due in no

small measure to the realization by the authors of the different points of view between the taxonomist and the clinical bacteriologist, or the taxonomist and the epidemiologist. The majority of workers who will use this book will undoubtedly have ideas as to the probable causal organisms to be isolated from a known specimen. Whether their assessment is correct or not, they can with value use the progressive approach to identification indicated by the tables, and probably arrive at a correct diagnosis. Isolation of pure cultures of organisms from clinical specimens is the prime requirement, and it seems a pity that more comprehensive advice on this problem, and on the use of enrichment media, was not included within the stout covers of this admirable handbook.

Experimental microbiologists and research students will find themselves well served by the efforts of Meynell and Meynell in a book which is packed with information about measurement, both quantal and quantitative, of micro-organisms, and which does not contain one single dull or uninteresting page. This is a fascinating guide to theoretical principles underlining the significance and importance of bacteriological experimentation, and can be approached with minimal understanding of simple statistical terms.

The book commences with a chapter on measurement of bacterial mass and number, neatly describing the various methods in use and showing clearly that in estimation of the precision of viable counts the presence of sampling errors lessens the effect of technical error. The section on quantitative measurement of bacterial growth could be read with profit by undergraduate students of bacteriology.

The next four chapters deal with culture media; oxidation-reduction potential and anaerobiosis; the kinetics of sterilization, including sections on chemical disinfection and filtration; and microscopy. The penultimate chapter, dealing with quantal responses, Poisson distribution and normal and log-normal distributions, is supremely clearly written, and with well-chosen examples makes relative simplicity out of what, to a large number of biologists, are complex and difficult subjects. The book ends with a chapter on miscellaneous techniques.

Not the least contribution of this book is the inclusion of a variety of most useful tables which would be of considerable value in any microbiology laboratory.

D. ALLAN

SKIN AND ITS DISEASES

The Epidermis

Edited by William Montagna and Walter C. Lobitz, jun. Pp. xx+649. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1964.) 107s. 6d.

FOR a variety of reasons which are familiar to most scientific workers, the published proceedings of symposia rarely contain any information which is not available elsewhere. They are, however, sometimes of value in two ways. First, they may bring specialized information to the notice of a reader from an allied field of research with which he is not familiar because of its publication in journals not immediately available to him. Secondly, symposia may be used by investigators to take a broader look at current thought in their own field. Both these aspects make their appeal on reading the papers presented at a symposium on "The Epidermis" which was sponsored by the Division of Dermatology of the University of California. It was made possible by a grant from the U.S. Public Health Service, the advisory committees of which felt the need for a series of such meetings on the biology of the skin.

The volume is dedicated to the late Prof. Stephen Rothman, a pioneer dermatological investigator, whose first chapter on "Keratization in Historical Perspective"

sets the high standards to follow. The book is large but contains a wealth of basic dermatological thought and fundamental information. The chapters range widely over embryology, keratinization, ultrastructure and histochemistry, carbohydrate, nucleic acid, sterol and lipid metabolism, permeability and blister formation. Many of them are contributed by the acknowledged experts in each field whether from the United States, Europe or Australia.

Philippe Sengel (Paris) describes his elegant experiments which have thrown so much light on the differentiation of cutaneous appendages in the embryo, and Dame Honor Fell summarizes the present studies on keratinization in organ culture of skin being carried out at Strangeways Research Laboratory. Mercer (Canberra), Brody (Stockholm) and Swanbeck (Stockholm) each give excellent accounts of their own work on the ultrastructure of the components of the horny layer in normal skin, and the deviations which characterize psoriasis. Kligman's (Philadelphia) chapter on the biology of the stratum corneum is especially readable, not only because of his own distinctive style of writing but also because he has directed attention to some of the more neglected biological phenomena in the epidermis. Epidermal cells in the stratum corneum become very much more cohesive to one another than in the basal, Malpighian or granular layers, and yet this cohesion is lost at the surface in such a way that the fully keratinized cells are shed in small flakes which are not normally perceived. An understanding of the mechanism by which such a change occurs should lead to valuable knowledge of many scaly dermatoses.

The organizing committee of this symposium were fortunate to succeed in 'capturing' so many outstanding scientists actively working on the biology of the skin. Drs. Montagna and Lobitz and the Academic Press have produced a volume which is a credit to the participants. There is an author index of the references used in all the papers, and a subject index. Typographical errors are very rare indeed. "Bellberg" for "Bettley" is repeated several times. For a book of this quality it is not overpriced.

C. D. CALNAN

ATOMIC AND MOLECULAR SPECTROSCOPY

Spectroscopy and Molecular Structure

By Gerald W. King. Pp. xiv + 482. (London: Holt, Rinehart and Winston, 1964.) 86s.

EXCELLENT advanced monographs on many different aspects of atomic and molecular spectroscopy are available, and articles in, for example, *Annual Reviews of Physical Chemistry and Advances in Spectroscopy*, enable research workers to keep abreast of present development. There is, however, at a different level, a need for an introductory text-book suitable for senior undergraduate or postgraduate teaching in chemistry or in physics. Dr. King's book fills some of the gaps admirably.

A very short introduction on spectroscopic technique is followed by chapters on wave-mechanics and the hydrogen atom, many electron atoms, diatomic molecules, the symmetries, rotations and vibrations of polyatomic molecules, and concludes with a discussion of the electronic spectra of polyatomic molecules and appendixes on operators, selection rules, character tables and physical constants. Each chapter has a short bibliography, and a collection of illustrative problems, some of which are very searching. Mathematical techniques are introduced progressively throughout the book, so that the later chapters are, reasonably, much more difficult than the early ones. However, the elements of group theory and of matrix algebra, required for the treatment of polyatomic molecules, are introduced both clearly and attractively.

There has necessarily been a rather careful selection of material, so as to limit the book to a convenient length.

Microwave spectroscopy is one of the subjects to suffer. Some description of the techniques would have been welcomed by many students, and the measurement of dipole moments and of nuclear quadrupole coupling constants has provided information of such wide interest that it is surprising that these subjects have been omitted. Again, chemists interested in the properties of complex compounds will find the discussion of the energy states of transition metal atoms and ions inadequate. The treatment of dissociation energies is also very brief (it would have been helpful to have included the paper by R. Mulliken (*J. Chem. Phys.*, **33**, 247; 1960) in the bibliography at the end of Chap. 6). But, it must be stressed that some selection must be made, and by and large this is a book on the energy-levels of atoms and molecules, and as very successful it is too.

The book is well produced and easy to read. The least successful features of the production are the six half-ton reproductions of spectra. There are very few misprints or mistakes. For example, the statement (p. 75): "Spin-orbit interactions increase rapidly with atomic weight, and are especially large in excited states of heavier atoms" is misleading. These interactions certainly increase rapidly with atomic number, but for a given configuration, say np , of a particular atom or ion, the spin-orbit coupling *decreases* rapidly with principal quantum number. Again, p. 211: "The Λ -type splitting seldom exceeds a few hundredths of a wave-number" should be replaced by "The Λ -type splitting *constants* seldom exceed a few hundredths of a wave-number". In the review copy, Fig. 6, 7 (p. 206) was inverted, and there is an obvious error in the printing of problem 8.2 (p. 314).

Nearly half the book is devoted to the energy-levels and spectroscopy of polyatomic molecules, and it is this which is its real strength. This is not an easy subject, but the author has succeeded in developing an account which is both lucid and stimulating. This will be recommended reading for many mathematically minded chemists and physicists, undergraduates and postgraduate alike.

R. F. BARROW

BASIC STATISTICAL TECHNIQUES

Statistics and Experimental Design in Engineering and the Physical Sciences

Vol. 1. Pp. xiv + 523. 84s. Vol. 2. Pp. ix + 399. 87s. By Prof. Norman L. Johnson and Prof. Fred C. Leone (New York and London: John Wiley and Sons, Inc. 1964.)

THIS book is intended to give students and research workers in science and, particularly, engineering a sound understanding of and facility with basic statistical techniques.

"The material in this book should cover two or possibly three semesters of applied statistics at the upper-division undergraduate level."

These two sentences from the preface indicate the authors' intention. Despite the number of books which aim at fulfilling this function, there is still a need for a good one. The book is long, nearly a thousand pages, and is divided into two volumes. The first volume has three chapters on probability theory describing the properties of the usual distributions, and also some of the distributions which are of practical interest but are often ignored, and chapters on estimation, testing hypotheses, tests of significance, regression and correlation which are not particularly well done and appear in almost any other book of this nature. There are chapters on order statistics and non-parametric methods, both of them clearly written, a long and detailed chapter on control charts, and a balanced chapter on the utilization of prior information. The second volume has a long section, more than two hundred pages, on the analysis of variance and the design

experiments and a chapter each on sequential analysis, multivariate analysis and sampling. There is an appendix of tables which is comprehensive enough to be useful, and a bibliography of statistical journals and tables. The examples, of which there are a good number well placed in the text, are mostly credible and instructive.

The general character of the book is in line with what the authors take as their definition of statistics, the scientific study of the analysis of quantitative data. It seems to me that by concentrating almost exclusively on the description and analysis of data, the book suffers from lack of balance. Thus the aim of the chapters on probability theory is to present the distributions which are usually used to describe engineering data, and this is done well enough. But there is no account of why one could expect to use a particular distribution in one class of problems but not in another, nor is there any indication that probability theory might be useful in investigating the structure of a problem. The normal distribution is introduced as being of importance because it fits much of observed data, and is then fitted to some data. Nothing is said about why this should be so. The Poisson distribution is discussed as a useful limiting case of the binomial distribution, but as most of the examples can be described more naturally in terms of the Poisson process, it is a pity the Poisson process is not mentioned. It seems odd that the book should contain an elegant treatment of the non-central χ^2 , t and F distributions but have no mention of stochastic processes. Again, the section on the analysis of variance is primarily concerned with the analysis rather than the design of experiments, and little is said of the merits or otherwise of the many designs for which the analysis is presented. The section on multiple linear regression gives several methods for inverting the design matrix, but says nothing on choosing it.

It is difficult to see this book being used as a text-book or engineering students. Most universities in Britain only have short courses in statistics for engineers, and as an introduction to the subject this book is not to be recommended. There is nothing new in the more elementary chapters, which consist mainly of explaining techniques rather than the underlying principles or general usefulness, and a knowledge of statistics based on these chapters alone would probably do more harm than good. To statisticians who do a certain amount of engineering consulting, however, the book might prove valuable, as there are a fair number of topics discussed which many books ignore.

N. E. DAY

PACIFIC GEOLOGY

Marine Geology of the Pacific

By Prof. H. W. Menard. (International Series in the Earth Sciences.) Pp. x+271. (New York: McGraw-Hill Book Company, Inc.; Maidenhead: McGraw-Hill Publishing Company, Ltd., 1964.) 12.50 dollars; 100s.

THE Pacific Ocean comprises nearly half the total area of the surface of the Earth: it is therefore an ambitious project to describe and discuss the geology of the underlying crust in one book. That it is possible at all results from the way in which the subject of marine geology has grown owing to the necessity of conducting operations several miles above the sea-floor. The facility with which rocks can be examined at close range on land led to the growth of geology through hand specimens up to the broader concepts of tectonics and global structures. Submarine geology, on the other hand, has largely developed from the broader studies of bathymetry and geophysical surveys, such as magnetism and seismics, that are necessitated by the inaccessibility of the bottom. Hand-samples are rare, and rigorously controlled sampling is still impossible except in a few special areas.

Prof. Menard has brought together, in a very readable form, nearly all that is known about the Pacific geology

and discusses it in terms of the broader concepts of the history of the ocean basins, and continental margins. Inevitably many of the data are mentioned only by reference but this has left the author freer to discuss the relative merits of the different theories presented. As one of the leading authorities on Pacific geology, many of the data have been obtained by Prof. Menard himself and many of the hypotheses discussed have been published by him over the past two decades. However, the book contains new material, especially in the form of summary diagrams, a bathymetric map of the whole Pacific (regrettably split into 14 sections to fit page size) and an admirable physiographic diagram of the north-east Pacific in a folder at the end. This diagram follows the style of those produced by Heezen and Tharp of the Atlantic and Indian Oceans but with the addition of 500-m contours in the basins.

But more important is the development of the new idea of the Darwin rise as an old and foundered mid-ocean rise, the importance of which in the Pacific has been overshadowed by the younger East Pacific Rise. The great east-west linear faults which are such a feature of the Pacific, are described in detail in Chapter 3, but a discussion of their relevance to crustal deformation is deferred to the later chapter on oceanic rises.

This book is much more than a treatise on Pacific geology. It contains a stimulating review of the features and processes of marine geology in general. Whole chapters are devoted to volcanism, manganese nodules and turbidity currents, while elsewhere new evaluations are presented of the composition of the seismic second layer, abyssal hills, changes of sea-level and the development of continental margins.

An attractive feature of the book, so often absent in the discussion of geological phenomena, is the order-of-magnitude calculations made about such processes as transportation of sediment through submarine canyons and the origin of sea-water. In the many problems where our knowledge is least sure, Prof. Menard has frankly admitted it and given rein to imaginative speculation that is a challenge to future research.

This book is highly recommended as an up-to-date and well-presented account of marine geology both for the student and for all involved in research in this field.

A. S. LAUGHTON

A CENTURY OF ANTHROPOLOGY

A Hundred Years of Anthropology

By T. K. Penniman. With contributions by Beatrice Blackwood and Dr. J. S. Weiner. Third edition, revised. (Duckworth's 100 Years Series.) Pp. 397. (London: Gerald Duckworth and Co., Ltd., 1965.) 42s. net.

THE third edition of Penniman's survey brings up to date the story he first unfolded in 1935. Two extra chapters, by Dr. J. S. Weiner and Miss Beatrice Blackwood, review developments in physical anthropology and in Americanist studies, respectively, over the past thirty years. Readers who can look back to the first appearance of Penniman's classic will remember the admiration it evoked in anthropological circles at that time. The common opinion was that the several branches of anthropology had become too specialized for any one scholar to have competence in all. Pupil and later colleague of R. R. Marett, Dudley Buxton and Henry Balfour, whom he succeeded as curator of the Pitt-Rivers Museum, Penniman showed that it was still possible for an all-round ethnologist to keep abreast of the main trends in all branches of the subject. The extra chapters in the present edition eloquently testify to the end of that epoch.

A history as comprehensive as this could easily degenerate into a catalogue of names, dates and titles of publications—Penniman avoids this by his narrative method. Defining 'anthropology' as the generic science

of man embracing the biological, historical (and therefore archaeological), cultural and social conditions of his existence, he directs attention to the major figures, those whose discoveries and theories set the general patterns of anthropological enquiry for a period of development. Lesser figures are dealt with more summarily in relation to the topics to which they made contributions rather than the issues of principle that were uppermost in their time.

To find Darwin's, Herbert Spencer's and E. B. Tylor's work and influence discussed at length—and, be it added, with a sure sense of what was significant in this for their successors—seems natural enough. But how many of us are aware of the seminal influence exercised by Bastian (1826–1905) during his life-time? Indefatigable traveller and encyclopaedically learned, he is ranked by Penniman with Darwin and Tylor. Bastian is famous for the hypothesis of the "psychic unity of mankind", which he attributed to the universality of a limited number of "elementary ideas". Penniman's account of how he reached his theories, what he really meant by them and how they served to unite the incipiently divergent evolutionary and diffusionist trends of the period is typical of the exposition followed throughout.

Penniman divides the history of anthropology into five main periods: (1) The "Formulary Period", from the Greeks to 1835; (2) The "Convergent Period" (1835–59); (3) The "Constructive Period" (1859–1900); (4) The "Critical Period" (1900–35); (5) The "Period of Convergence and Consolidation", since 1935. For each of the modern periods, each branch of anthropology is treated under a separate rubric, but the details of authors and publications are bound together by keeping common and general tendencies to the fore.

Up to 1935 the story is told with a masterly command of the literature and admirably balanced judgment. To take an instance at random, one would have to search diligently in the anthropological literature of the 'thirties to find comments as judicious and sympathetic as Penniman's on the significance of psycho-analysis for anthropology. The part which traces more recent developments is, inevitably, more sketchy. The excellent index and the careful bibliographical documentation deserve particular commendation.

M. FORTES

THE NON-GUILTY PARTY

Prisoners and their Families

By Pauline Morris. Pp. 327. (London: George Allen and Unwin, Ltd., 1965.) 50s.

MOST of us, fortunately, are relatively law-abiding citizens and we are unlikely in the course of our daily work, and may be even during the whole of our lives, to come up against the problems which result from the imprisonment of law-breakers. Occasionally a sensational crime, such as "the great train robbery", and, even more sensationally, the escape from prison of one or more of the convicted train robbers, will attract our attention and give rise to discussion about the penal system and imprisonment as a form of punishment, but it is very doubtful whether most of us will spare a thought even then for the prisoner and his family. On the whole, we prefer to leave the problems of crime and its punishment to the police, the courts and the Home Office (Prison Department) and the few worthy citizens who are prepared to give voluntary service as prison visitors. But are we entitled to ignore so easily the social effects of imprisonment as a method of social punishment, and can we be quite sure that when an offender is punished we do not impose additional punishment on his family?

A remarkably thorough study of the problems encountered by the families of prisoners in Britain has recently been made by Mrs. Pauline Morris, and the conclusions

derived as a result of her investigations should make all think more seriously about the social effects of imprisonment. The great virtue of this book is that it records the results of a truly scientific investigation as yet it is written with warmth, insight and compassion. Despite the appalling misery which Mrs. Morris all too often has to describe, she never allows her emotions to deflect her from the path of strict objectivity and scientific analysis of the data so carefully and systematically collected.

The primary object of the research project was "to elicit facts upon which penologists and administrators might base future policies" and, curious as it may seem, this "is the first attempt in this country to look at the problems of the families of prisoners on a national scale. As a result of previous research undertaken by Mr. Morris "amongst the recidivist populations of two maximum security prisons" which showed "that a high proportion of men appeared to have severe family problems", a number of hypotheses were put forward and explored in this new investigation. For example, the "family relationships following upon conviction and imprisonment will follow a pattern set by family relationships which existed before imprisonment", and "the adjustment of the family to imprisonment will vary with the type of offence, and with the extent of previous criminal experience".

A national sample of male married prisoners (837 in all) was selected for interview and permission was sought from each prisoner for his wife (legal or otherwise) to be interviewed at home. In addition, an intensive study of the families of 100 prisoners living in the London area was undertaken. The organizational problems involved in arranging the interviews and carrying them out were enormous, but the care taken and the controls exercised in planning the project and in its execution are of the highest scientific standards. Indeed, for those who still doubt whether scientific methods can be applied to investigations of human and social phenomena, Chapter 1 of this book on "The Design of the Enquiry" should be compulsory reading.

The picture which emerges of the varieties of problems encountered by the families of prisoners makes interesting and sometimes heartbreaking reading. Mrs. Morris is to be congratulated on producing such a vivid and living account of the real life problems faced (not always squarely) by real live persons less fortunate (and on occasion less lucky) than most of us. Inevitably there were methodological difficulties which could not always be solved, as, for example, when the 'story' given by the prisoner about his family circumstances differed substantially from that given by his wife. On further investigation "it transpired that the wife shared herself between two 'husbands', both of whom happened to be in gaol at the time of our interview with her. Since this fact was unknown to the interviewer, it was not possible to tell that the information the wife gave related to a different 'husband' from the one whom we actually saw!" Fortunately, there were very few discrepancies of this kind in fact, because of the exacting standards applied by Mrs. Morris to this investigation.

This book undoubtedly makes a significant contribution to our knowledge of a social problem about which all too little was known in the past. It should also provide a firm basis for developing an enlightened penal policy in the future. Mrs. Morris and her colleagues have obviously not given us all the answers we need to solve the difficult problems of punishment, but they have produced an intensely interesting and valuable investigation which can be thoroughly recommended to those (and I hope they would be many) who have an interest in the problems of their less fortunate fellows and in the machinery used by society to inflict punishment on offenders, and, inadvertently, their families.

D. C. MARSH

Probability and Statistics

H. Freudenthal. Pp. v+139. (Amsterdam, London and New York: Elsevier Publishing Co., 1965.) 30s.

A SCIENTIST or technologist with modest mathematical skill and no fear of unfamiliar notation could gain considerable insight into statistical thought from this book. He would not advance far in learning standard statistical techniques for use in his own problems because the book does not attempt their systematic presentation. Prof. Freudenthal's strength lies in a largely intuitive approach allied to an attractively easy style. He does not retreat from difficulties, but he refuses to conceal the essential features of a proof by trappings of mathematical rigor appropriate only to more advanced texts.

Early chapters are largely concerned with probability theory, made vivid by excellent simple examples of independence and conditionality. The implication that sampling with replacement is theoretically preferable to sampling without replacement is strange, but possibly translation from the Dutch has permitted entry of a confusion between simplicity of theory and practical desirability. Prof. Freudenthal uses the central limit theorem to justify widespread practical dependence on the normal distribution. The final one-third of the book—as chapters concerned with sampling inspection (including sequential sampling), distribution-free tests, games and strategy, and population genetics. None of these topics is taken far, and all are regarded a little abstractly as playgrounds for mathematicians rather than as fields of investigation in which the statistician must make a great contribution. In the context of a book that may make an intellectual appeal to mature readers but that is not suitable as an introductory text, they play their part well. The book can be confidently recommended to those who find its form seeming to meet their needs, but disregarded as of no fundamental importance by those to whom its somewhat unusual style makes no appeal. Of few books on probability and statistics can both these statements be made!

D. J. FINNEY

ALGOL 60 Implementation

The Translation and Use of ALGOL 60 Programs on a Computer. By B. Randell and L. J. Russell. (A.P.I.C. Studies in Data Processing, No. 5.) Pp. xiv+418. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1964.) 84s.

THE introduction of automatic computer languages has opened up the use of computers to a large public by making the programming of digital computers much easier. This is due to the development of compilers: the large programmes which translate automatic languages, which are relatively easy to use, into machine languages which are much more difficult to use. Initially automatic languages and their compilers were very much empirical *ad hoc* affairs; the writing of compilers was more of a craft than a science. But recently this state of affairs has changed, an important step in this direction being the publication some five years ago of the ALGOL report. Using the notations and techniques of mathematical logic, this defines precisely a programming language suitable for scientific computations. This stimulated the systematic study of compiling methods and many papers on the subject began to appear.

This book is an excellent and welcome contribution to the literature; it will make more accessible to a large public some of the more commonly used and basic compiler techniques. The compiler described produces in one pass an object programme which is obeyed interpretively by a control routine during run time. This may not be very efficient, but many of the concepts explained in this book are relevant to other forms of compilers. In particular, repeated use is made of the concept of stack or push-down store.

Because this is among the first books on the subject, the form and contents of the book may put off the uninitiated reader, but it is well worth persevering with. An attractive aspect of the book is the large number of very short programmes illustrating the many difficulties a compiler has to deal with.

I. M. KHAZAZA

Einführung in die Atomphysik

Von Dr. Wolfgang Finkelburg. Neunte und Zehnte Ergänzte und Neubearbeitete Auflage. Pp. xii+552. (Berlin, Göttingen und Heidelberg: Springer-Verlag, 1964.) 45 D.M.

THE present issue of *Einführung in die Atomphysik*, by Prof. W. Finkelburg, is the nineteenth and twentieth reprint, revised and brought up to date by the author, who is a member of the Siemens-Schuckertwerke group in Erlangen. It is intended for physicists in the applied fields, engineers, chemists and technical personnel. Its scope is very wide, comprising, besides atomic physics, extensive chapters on quantum mechanics, nuclear physics, 'elementary' particles, molecular and solid-state physics. The author is manifestly an experienced pedagogue who leads the reader in carefully planned steps through the various phases of an argument. It is a magnificent and, on the whole, successful effort to provide a readable text of a complex scientific field.

It is, however, clear that the maximum one can achieve in the circumstances is plausibility, which is probably what the author aimed at. The question then arises if it is a good plan to incorporate a fair proportion of the wave mechanical formalism (according to Sommerfeld), without the mathematical background (see p. 360, where the formula is completely without meaning). It must be admitted that the figures of electron distribution and some wave functions are excellent.

The book, as such, comes up to the expectation one holds for a production from the publishing firm of Springer; it is quite cheap at 45 D.M., and will no doubt greatly contribute to the education of that most important part of the scientific community who apply science.

E. BRETSCHER

Physique des Semiconducteurs

Comptes Rendus du 7^e Congrès International. (Physics of Semiconductors: Proceedings of the 7th International Conference.) Pp. xxii+1368. (Paris: Dunod, 1964.) 145 francs.

PHYSIQUE des Semiconducteurs: Comptes Rendus du 7^e Congrès International contains about 200 distinct original items, five of which are reports on symposia on related topics. It will be an invaluable work of reference pointing to other and fuller papers on certain topics, and indicating the major aspects of present interest in the field. It has been remarked that this field, like women, has become more sophisticated with age, and this volume certainly gives evidence in favour of this assertion. More accurate measurements, and more detailed calculations are reported here, of quantities the broad significance of which has in many cases been known for some years. This impression is strengthened by the fact that the details of the symposia on radiative recombination, radiation damage, and solid-state plasma effects, all very active fields, have been published separately. This still leaves interesting effects within the scope of this volume—effects which were not expected two years before (during the corresponding meeting at Exeter in 1962). The relativistic correction to band structure calculations is an example. The importance of lasers is another example, for they have enhanced the interest in studies of electron transitions in solids, which may compete with radiative transitions. Papers in this volume deal with these and other important effects. It is clear that this field is con-

tinuing to throw up important new developments, and it is reasonable to expect an average of one important development per annum to be maintained until the next volume of this series (reporting the meeting in Tokyo in 1966) is reviewed in these columns.

P. T. LANDSBERG

Counterexamples in Analysis

By Prof. Bernard R. Gelbaum and Prof. John M. H. Olmsted. (The Mathesis Series.) Pp. xxiv+194. (San Francisco, London and Amsterdam: Holden-Day, Inc., 1964.) 7.95 dollars.

THE schoolboy learns that the harmonic series does not converge, although its n th term tends to zero; the undergraduate is shown Weierstrass's function, which is continuous but not differentiable; at a later stage, a mathematician's pleasing speculation may be disrupted by an instance of its falsity. The construction of counterexamples is a not unimportant part of the technique of the analyst, as, for example, in the theory of Fourier series, and this collection of some 250 items may serve for profit as well as for amusement. The authors have made a selection from number systems, functions, limits, differentiability, integration, convergence, set and measure theory in one and two dimensions, metric, topological and function spaces. Almost any dip will come up with something pleasing; a random opening provided functions f and g such that f^2 and g^2 are integrable but $(f+g)^2$ is not. But the book will be more seriously useful to the teacher of analysis, who is often in need of examples of this type to show why the conditions imposed on a theorem are necessary, and to the young research worker who may have to test heuristic ideas by hard fact.

T. A. A. BROADBENT

Radioactive Fallout, Soils, Plants, Foods, Man

Edited by E. B. Fowler. Pp. 317. (Amsterdam, London and New York: Elsevier Publishing Company, 1965.) 80s.

THE evaluation of the significance of dietary contamination with radioactive fall-out requires the co-operation of many disciplines, and our understanding of this subject has owed much to meetings at which meteorologists, health physicists, agricultural scientists and medical radiobiologists have brought together the relevant information from their various subjects.

Two particularly important meetings of this type were arranged in 1959 by the University of Minnesota and the Food and Agriculture Organization in Rome. The publications to which they led in the following year largely paved the way to the first authoritative and comprehensive assessment of mechanisms of dietary contamination which the United Nations Scientific Committee on the Effects of Atomic Radiation included in its 1962 report. Other meetings with the same general objective had meanwhile been held, among them being a symposium of the American Chemical Society, at Cleveland, Ohio, in April 1960.

The volume under review is based on the papers presented at the Ohio meeting. The editor's preface shows his consciousness of the disadvantage of delay in publishing reports of symposia on rapidly developing subjects, and an endeavour was made to revise and to 'up-date' contributions. This was notably achieved in the discussion of the "Transfer of Fallout Radionuclides from Diet to Man", by Drs. Wasserman Lengemann, Thompson and Comar, in a chapter which contains about half the references to publications dated 1962 or later which are to be found in the entire volume. Read in 1965, some of the other chapters are mainly of historical interest. Not only are a number of transfer routes of much smaller importance than was sometimes thought in 1960 but also interest in the significance of fall-out now centres primarily on the effects of weapon tests in 1961

and 1962 which were of considerably greater magnitude than the earlier series.

Piante Medicinali

Chimica Farmacologia e Terapia, Vol. 2. Da R. Benign C. Capra and P. E. Cattorini. Pp. 731-1832. (Milan: Inverni e della Beffa, 1964.) L.12,000.

THIS is the second part of a comprehensive Italian work on medicinal plants, Volume 1 having already been reviewed in these pages (*Nature*, 196, 609; 1962). Plants are arranged according to their Italian common name. Volume 1 covered the letters A-H. The present volume deals with the rest of the alphabet, representing some 120 different species. As in the case of the earlier volume, there are notes on etymology, nomenclature, habitat, parts used, active principle, therapeutical properties, pharmacology, and chemical composition. There is special emphasis on the chemistry of the plants, which is covered in some detail and the latest information incorporated. This applies particularly to plants that have recently attracted special attention, for example, *Rauwolfia serpentina* has 149 pages devoted to it and there are more than 800 references. *Strophanthus* (*Strophanthus kombé*) covers 25 pages, with 161 references.

Other well-known medicinal plants dealt with in this volume include golden seal, Iceland moss, ipecacuanha, jaborandi, liquorice, lobelia, clive, opium poppy, pepper mint, rhubarb, senna and strychnine or nux-vomica. Many of the other species treated, although well known are of little importance medicinally to-day. Nevertheless it is of value to have so much information in relation to them brought together. The common horse-chestnut (*Aesculus hippocastanum*) is not usually looked on as a medicinal plant, yet 14 pages are devoted to it along with 38 references. This may be because of the saponin it contains.

F. N. HOWES

Pollen Physiology and Fertilization

Edited by H. F. Linskens. (A Symposium held at the University of Nijmegen, The Netherlands, August 1963.) Pp. xii+257. (Amsterdam: North-Holland Publishing Company, 1964.) 80s.

IN August 1963 a symposium was organized at the University of Nijmegen to bring together a number of people interested in the study of pollen tube formation and germination, and the processes leading up to the formation of the zygote in higher plants. The papers and discussions given at this symposium are now published under the title *Pollen Physiology and Fertilization*, edited by Prof. H. F. Linskens.

The symposium was organized in seven sections each of about three or four papers followed by a tape-recorded discussion. Subjects included the physiology of the embryo sac, and the biochemistry of pollen wall formation; pollen tube metabolism; the effects of boron on growth; chemotropism and sections on controlled fertilization and incompatibility. Particular interest is derived from the high proportion of papers in which relatively modern techniques such as electron microscopy, histochemistry and *in vivo* methods of controlling fertilization are used to investigate the problems raised.

The publication of papers read at any specialized symposium inevitably results in the reappearance of a fairly high proportion of material already familiar as a result of its appearance in regular journals, and this is no exception. Nevertheless, this volume does provide a readily accessible collection in one place of contributions by a number of well-known authorities on the subject; and the convenience for comparison and survey which this provides, combined with the general high standard, or controversial interest, of the papers will make it a welcome addition to the collection of anyone interested in the physiology of pollen germination and fertilization.

P. A. THOMPSON

ORIGIN AND SIGNIFICANCE OF THE POWER OF VOCAL IMITATION: WITH SPECIAL REFERENCE TO THE ANTIPHONAL SINGING OF BIRDS

By DR. W. H. THORPE, F.R.S.

Sub-Department of Animal Behaviour, Department of Zoology, University of Cambridge

AND

MYLES E. W. NORTH

Milton Close, Muthaiga, P.O. Box 8225, Nairobi, Kenya

HERE are few abilities which separate man more clearly from other members of the animal kingdom than his powers of imitation. Even among the primates the ability to learn a new action as a result of having seen performed by another member of the species has only been established relatively recently; and convincing examples among mammals below the primate level are scarce indeed¹.

When we come to consider vocal imitation the difference between man and the other mammals is even more striking. Even with the chimpanzee a capacity for vocal imitation seems to be almost non-existent and no satisfactory scientific evidence seems yet to have been presented in behalf of claims made for the powers of vocal imitation among porpoises^{2,3}. This remarkable difference is considered by Konorski⁴, as a result of his analysis of various forms of speech disorder, as comparable with the audio-verbal aphasia which results from certain types of brain lesion in man. Lesions which have this effect are shown to have damaged either the postero-superior part of the temporal lobe, or to have severed the 'arcuate bundle' which originates in this area and runs to the lateral frontal regions. According to Konorski, it is a striking fact that there is nothing exactly comparable with this 'arcuate bundle' known in non-human mammals, and it is this absence which is thought to account for the lack of powers of vocal imitation.

When we come to compare the imitative abilities of the birds with those of the mammals, we find that while the ability of the former group to imitate bodily actions is probably even less than it is in mammals, the powers of vocal imitations are of an astonishingly high order, being even comparable in some cases with those possessed by man. The origin and significance of this difference raise, therefore, most puzzling problems. Unfortunately, on the neurological side, nothing can be said; for the investigation of the bird's central nervous system has not yet nearly reached the stage at which we can say whether or not there is any structure functionally comparable to the 'arcuate bundle' of the human brain. The only birds on which modern neurological investigations are being actively carried out are the doves; but it appears⁵ that these are incapable of imitation. We have, therefore, to come down to behavioural evidence, as this does indeed provide some interesting clues.

In the Oscines, or song birds, at least those of the temperate regions of the world, the most usual function of the full-song is to serve as a territorial proclamation by the male establishing claim to a territory and, as a substitute for fighting, defending it. Thus it serves as a signal which is sufficiently stereotyped to function as a recognition mark characteristic of the species. But it is well known (refs. in Thorpe⁶) that in many species the song is capable of sufficient individual variation within the overall specific fixity of pattern to differentiate one individual bird from another. Thus, the song comes to serve two functions—specific recognition and individual recognition.

This individual differentiation of the song is often developed by imitative learning, the bird in its first year either imitating its parents' song or, perhaps more often,

learning individual differences from other members of its species when it first comes to sing in competition with them during the process of establishing a territory during its first breeding season. Thus, chaffinches (*Fringilla coelebs*) reared in isolation produce an abnormally simple song which is mainly accounted for by the hereditary make-up. Only when the bird is reared in contact with its own kind does it develop the full species pattern and add the aforementioned individual characteristics. Thus, experience influences the selectivity for the song which will be imitated, and in early spring the bird tailors its own song to match the patterns which it has heard. There is, therefore, a functional explanation for the ability to imitate the vocalizations of one's own species even where the prime function of the song is territorial advertisement and defence.

But, as everyone knows, the imitative performance of a number of birds goes far beyond this. There are species, such as the starling (*Sternus vulgaris*), the mocking bird (*Mimus polyglottos*) and the marsh warbler (*Acrocephalus palustris*), which include notes and phrases from many other species in their own song. Even more remarkable are the 'true' talking birds, especially the members of the parrot family and the grackle, known as the 'Indian mynah' (*Gracula religiosa*), which can imitate not only human speech to astounding perfection but also a seemingly endless variety of other noises. This extreme imitative ability of certain groups poses a real evolutionary problem, since it appears not to be used at all except under conditions of domestication. In other words, it seems as if the bird is in some way 'pre-adapted' for the performance of dazzling feats of imitative virtuosity which conditions of life in the wild never require of it.

In the tropics, although there are many species of birds the song of which is doubtless just as territorial in function as is usual in the temperate regions, the ornithologist is also struck by the number of examples where song appears much less aggressive in intent and where its function is apparently as a social signal, for maintaining pair and family bonds and as part of the sexual display, rather than a territorial one. Moreover, it is perhaps significant that most of the outstanding vocal imitators are found among tropical or sub-tropical species. It is suggested that the extreme developments of the imitative ability occur where the main function of the song is to provide for social recognition and cohesion rather than for territorial defence. This seems to apply specially in the tropics.

In 1903 Waite⁷ described how two captive Australian magpies (*Gymnorhina tibicen*) learned to sing a fifteen-note melody, played to them on the flute, as an antiphonal duet. When the younger bird died the survivor resumed the performance of the whole; which it had never been heard to produce during the years when it had a companion. A rather similar case of the antiphonal division of a learned melody between a pet canary (*Serinus c. canarius*) and a captive bullfinch (*Pyrrhula pyrrhula*) was described in the same year by the famous musician Sir George Henschel⁸. Recently, Gwinner and Kneutgen⁹ described how, with two pairs of captive ravens (*Corvus corax*) and three pairs of the shama (*Copsychus mala-*

baricus), the males and females each had sounds or song elements which were principally, if not exclusively, their own private utterances and were not normally used by their mates. However, when the partners were absent, the remaining bird would use the sounds normally reserved for his partner, with the result that the said partner would return as quickly as possible as if called by name. (We have both experimental and field evidence for the same effect with *Laniarius aethiopicus major*.) Gwinner and Kneutgen find the method to be particularly effective if it is the male who is absent, since, they suggest, there is nothing more stimulating to a mated male than to hear its own repertoire repeated in its own territory. Thus, the vocalizations of these species appear to be used for securing the return of the desired partner. It seems from the work of Zur Strassen¹⁰ that, contrary to much previous supposition, parrots can also use their learnt vocalizations in a 'purposive' manner for securing food or other particular needs.

The study of the antiphonal singing of certain tropical birds, on which we have now been engaged for some years, is particularly relevant to these problems. Our investigations have been concerned with three widely separated groups of birds, one non-passerine and two passerine, namely, the barbets, *Trachyphonus* spp. (Capitonidae), fantail warblers of the genus *Cisticola* (Muscicapidae, sub-family Sylviinae) and shrikes of the genus *Laniarius* (Laniidae including Laniariidae). In all these groups one encounters species capable of a duetting in which the notes of the male and female are different—the sexes alternating to sing antiphonally but nevertheless with a very precise and exactly maintained time interval

between each contribution. One of us¹¹ has already published an account of the antiphonal singing of *Laniarius erythrogaster*, where the response time with which the female answers the male is of the order of 150 msec or less, with a standard deviation over a long series of 12.6 msec. Here it was suggested that it is the exact precision of timing of the answer of the second bird which is the most likely basis for the mutual recognition in dense foliage rather than any individual difference in tonal quality or structure of the notes—although this is not ruled out.

Since this work was published, Grimes¹² has obtained almost identical results with another species in West Africa—*Laniarius barbarus*.

However, from the point of view of the general problem already outlined here, it is the species that we are now mainly concerned with, namely, *Laniarius aethiopicus*—the tropical bou-bou shrike—which is providing most of the important results to date. Although a great deal remains to be done, a number of essential points already seem clear, and it is the object of the present article to outline the most relevant of them. In this species both male and female have a considerable vocabulary which it seems, they utilize so as to work out with practice a series of duet patterns by which, presumably, when the birds are no longer in sight of each other mutual recognition is still possible. This work is proceeding in the field in Kenya and Uganda, and in tropical aviaries at the Cambridge Sub-Department of Animal Behaviour. The following essential points seem to be clear:

(1) Although there may be periods of solitary 'practice' during the juvenile stage, the duet is worked out during

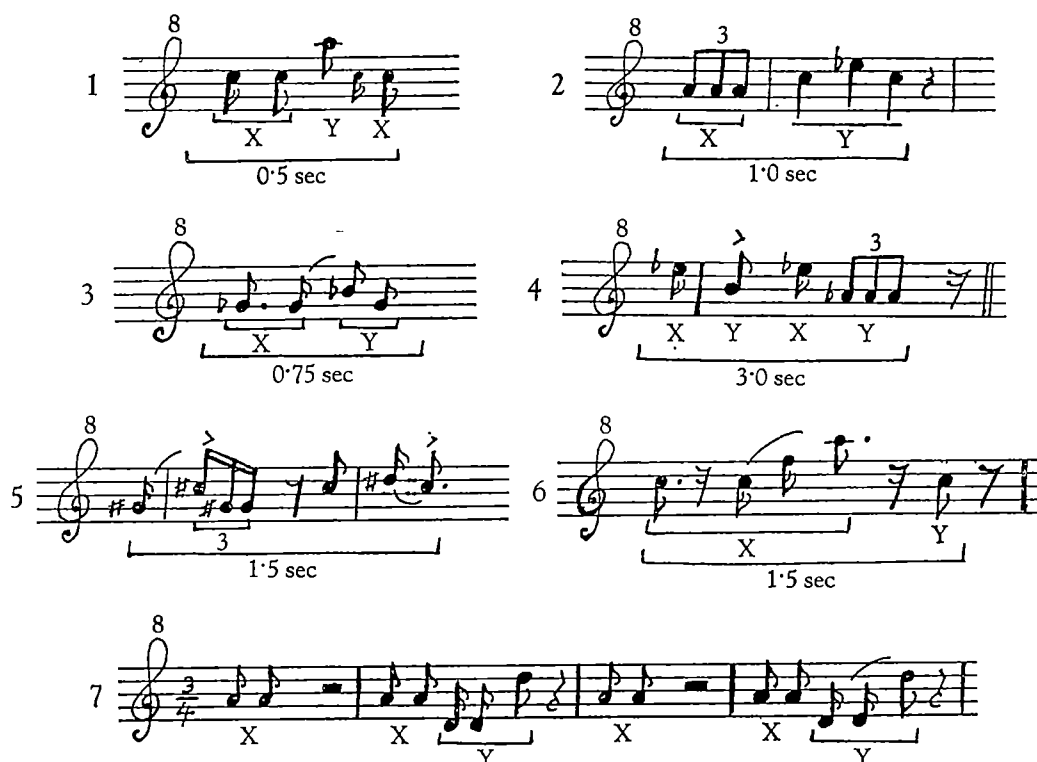


Fig. 1. *Laniarius aethiopicus sublaetis*. Vipingo, Kilifi, Kenya. December, 1954. *N.B.* All the illustrations are given at approximately scientific pitch (middle 'C' = 256 c/s). Unless otherwise stated, as here, all the figures refer to race *major*.

Fig. 2. *L. aethiopicus*. Dundori, Nakuru, Kenya, March 17, 1964

Fig. 3. *L. aethiopicus*. Kabale, Uganda, February 15, 1962

Fig. 4. *L. aethiopicus*. Meadow Point, Lake Nakuru, Kenya, March 17, 1964

Fig. 5. *L. aethiopicus*. Hippo Pool, Lake Nakuru, Kenya, March 17, 1964. Note that this is a rather more elaborate duet than the previous ones. The contribution of the two birds is not indicated in this case since it seemed to vary a good deal.

Fig. 6. *L. aethiopicus mossambicus*. San Martino, Mozambique Coast (C. Haagner). The timing in this example is very precise, but the bar length might vary between 0.75 and 1.5 sec.

Fig. 7. *L. aethiopicus mossambicus*. San Martino, Mozambique Coast (O. Haagner). This is a duet with a more complex time pattern. Bar length 1.5 sec.



Fig. 8. a-q. *L. aethiopicus*. Reed Inlet, Lake Nakuru, Kenya, September 5, 1963. The separate figures a-q show the 17 different duet patterns produced by a single pair of *L. ae. major* during the course of a single day. The last four patterns were transcribed in notation (ref. 12). All the rest were recorded directly on tape. All were presumed to be duets, but the distribution of the parts between 'X' and 'Y' is only inserted where the evidence is clear. The expression 'chatter scold' denotes a characteristic harsh pulsed sound of wide frequency distribution. The term 'snarl' is expressive of a common note, not markedly pulsed, but with the energy, though widely spread, showing a peak at the frequency indicated by the wavy line.

a long practice period (probably of many months) between two birds in their territory.

(2) These duets are composed of notes, the quality of which is determined, no doubt, by hereditary constitution; but the pitch, timing and phrasing of which can be varied very exactly by the singers as a result of this practice.

(3) For nearly every species so far examined it appears that: (a) Either sex can start and the other finish. (There is clear evidence for this in *Laniarius aethiopicus* and *L. funebris*. It is probably also true for *Cisticola hunteri* and *Cossypha heuglini*.) (b) Either bird can sing the whole pattern alone if the partner is absent (*L. aethiopicus* and *L. funebris*). (c) In *L. aethiopicus*, when the partner returns two birds can either duplicate in perfect time or (more usually) sing antiphonally again. Thus it appears that in the course of developing its elaborate duets each individual of the *L. aethiopicus* pair has, in fact, learned the contribution of the other member and its relation to the whole.

In *Laniarius aethiopicus* each pair in the wild may have a considerable number of alternative duet patterns. Consequently, while many of the simpler patterns may be very widespread in a given population, some are likely to

be peculiar to individual pairs. If this is correct, then the mate is that bird which can answer with the right pattern of notes in the right time. These vocalizations have, of course, been recorded by us and analysed by means of the sound spectrograph, but with this particular species the notes are of such a pure flute-like quality that they can be rendered satisfactorily in ordinary musical notation, and consequently this is the method we are adopting in the present report.

In those exceptional cases where, for one cause or another, tape recording was not possible, the songs were noted down by means of a specially adapted notation⁸, the pitch being determined by comparison with a chromatic pitch pipe. The illustrations very largely speak for themselves. Since it is usually impossible in the field to distinguish the sexes apart, the contributions of the two members of the pair are simply indicated as 'X' and 'Y' rather than by male and female signs. Thus, the first bird to be transcribed is labelled 'X' as the bird which, on that occasion, sings the first component of the duet pattern. The second contributor is then labelled 'Y'. Thus, if the sexes alternate their roles, 'X' will sometimes be the male and sometimes the female; and similarly

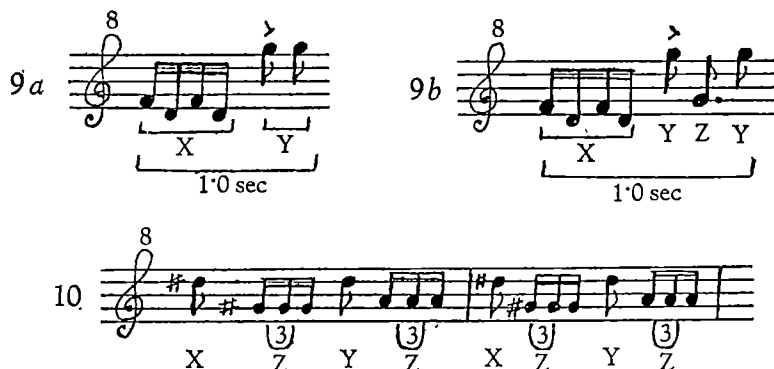


Fig. 9. *a* and *b*. *L. aethiopicus*. Lake Bunyoni, Kabale, Uganda, February 14, 1962. Fig. 9*a* represents a duet pattern heard as a very long, precisely timed series. During one considerable stretch of this series a third bird 'Z' joined in. It was far away from the others, but nevertheless inserted its single note remarkably accurately (Fig. 9*b*). It tended to intervene in every second duet of 'X' and 'Y'.

Fig. 10. *L. aethiopicus*. Dundori, Nakuru, Kenya, April 3, 1964. A remarkable trio. All three birds were in the same tree. Note that bird 'X' gave a D sharp every two seconds and bird 'Y' a D sharp every other two seconds while bird 'Z' gave a G sharp and an A natural every other two seconds in alternation.

with 'Y'. To date, rather more than 135 different duet patterns of *Laniarius aethiopicus major* have been recorded and analysed. A characteristic selection of these is here shown. Some races of *Laniarius aethiopicus* (for example, race *major*) appear to produce a greater variety of duet pattern and to be more flexible in their combinations; but this may merely be due to the fact that this is the race which it has so far been possible to examine most intensively. So far we have encountered one pair of birds in Nairobi which produced twelve different duet combinations, and another pair at Nakuru, Kenya, which produced seventeen different duet patterns in the course of a single day (Fig. 8).

Figs. 1-7 show a characteristic selection of duet patterns, proceeding from the rather simple to the more complex. Note also the way in which the total time taken by a duet can be varied considerably even by a single pair of birds more or less irrespective of the number of notes which it contains. Note, too, how the second bird may add or interpolate a single note within an already well-established pattern. Sometimes this note almost exactly duplicates one of the existing notes, in which case it can usually be distinguished only when the record is analysed by sound spectrograph. At other times it can be distinctly heard—as in Fig. 8*k*.

The 'musicality' of these songs strikes all who hear them. This is partly because of the purity of tone and the low pitch as compared with the songs of most birds. But in addition to this we find the birds using a familiar musical tonal system in a strikingly 'orthodox' manner. The implications of this are now being investigated.

With several of these birds which sing antiphonally, trio singing is sometimes observed. The meaning of this is at present quite obscure. It often recalls the well-known visiting of fulmar petrel (*Fulmarus glacialis*), the 'piping parties' of the oyster-catcher (*Haematopus ostralegus*) and the dance of the spur-winged lapwing (*Belonopterus chilensis cayennensis*) described by W. H. Hudson¹⁴. In more than one instance of trio singing in *L. aethiopicus* observed by us, besides the three birds part-taking, a fourth bird was observed standing silently by; possibly the mate of No. 3. Figs. 9 and 10 show examples of such trio singing in *L. aethiopicus major*. Trio singing is also very frequent in *Cossypha heuglini*, Heuglin's robin chat, which is also being studied in field and in aviary. An account of this will be reserved for later publication.

It is concluded that our observations so far give strong support to the suggestion put forward by Gwinner and Kneutgen, and also by Konrad Lorenz, that one of the major functions of the imitative ability of birds is to

establish and strengthen the individual pair bond. We suggest that this will be so particularly in those species where territorial aggressiveness is not very marked—as so often the case in the tropics. In *Laniarius aethiopicus* this ability enables each bird to learn the normal contribution of its mate as well as its own. It can thus use this appropriately for maintaining contact with the partner and recalling him or her when absent. If this is so, the extreme of apparently unused imitative ability in birds such as parrots and mynahs received a plausible explanation. It may well be that in the wild these birds use their powers for imitating the minute idiosyncrasy and inflexions of their partners or other members of the group. If this proved to be true, it is no longer necessary to suppose a mysterious 'pre-adaptation'.

Many, perhaps most, mammals recognize their associates primarily by smell and secondarily by sight. Voice and its recognition seem to come (with certain exceptions among them possibly the Canidae and some Ungulates) a long way behind in importance. With birds, creature of vision as they are and with the powers of olfaction greatly attenuated, audio-vocal recognition is probably paramount. But how did it come about that *Homo sapiens* so greatly outdistances his fellow Primates in his powers of using his auditory sense for social purposes?

We thank Mr. C. E. Cade, of Nairobi, for his pains taking work with hand-reared birds in the early stages of investigation. We also thank Mrs. Avril Royston, formerly of Sotik, now of Nakuru, Kenya, for her work in catching and hand-rearing birds—particularly shrikes. Without her expert help the aviary observations so essential to this work would have been impossible. We are also much indebted to Mr. Clem Haagner for allowing us to use some of his tape recordings of race *mossambicus*. Much of the cost of the work has been defrayed by grants from the Royal Society.

¹ Thorpe, W. H., *Learning and Instinct in Animals*, second ed., 464 (Methuen, London, 1963).

² Andrew, R. J., *Science*, **137**, 585 (1962).

³ Lilly, J. C., *Proc. Amer. Phil. Soc.*, **106**, 520 (1962).

⁴ Konorski, J., *Rozprawy Wydziału Nauk Medycznych* (Warsaw) **R**, 6-T, 2, 11-32 (1963) (In Polish with summaries in Russian and English).

⁵ Lade, B. I., and Thorpe, W. H., *Nature*, **202**, 366 (1964).

⁶ Thorpe, W. H., *Bird Song: The Biology of Vocal Communication and Expression in Birds* (Camb. Univ. Press, 1961).

⁷ Waite, E. R., *Nature*, **68**, 322 (1903).

⁸ Henschel, G., *Nature*, **67**, 609 (1903).

⁹ Gwinner, E., and Kneutgen, J., *Zeit. Tierpsychol.*, **19**, 692 (1962).

¹⁰ Strassen, O. Zur, *Verh. Deutsch. Zool. Ges. Freiburg* (1952).

¹¹ Thorpe, W. H., *Nature*, **197**, 774 (1963).

¹² North, Myles E. W., *Ibis*, **92**, 99 (1950).

¹³ Grimes, L., *Ibis*, **107**, 101 (1965).

¹⁴ Hudson, W. H., *The Naturalist in La Plata* (London, 1892).

THE INTERNATIONAL BIOLOGICAL PROGRAMME*

By DR. E. B. WORTHINGTON

Scientific Director (Designate)

THE International Biological Programme is now well into its first year—the first of seven or eight. It is concerned with “the biological basis of productivity and human welfare”. It is divided into two phases: Phase I, which consists mainly of design and feasibility studies, to occupy most of 1965 and 1966; Phase II, the operational programme, to commence in 1967 and carry on for about five years. Beyond these simple facts I doubt if there are many people who know much about the Programme, for we have been so busy planning it that little thought has been given to advertising it.

The International Biological Programme is a programme which, because of the effects of various factors, particularly the climate, must extend over a number of years, since it is obvious that an investigation of growth in a summer such as we have had this year would be quite different from a similar study undertaken last year. It has, however, drawn some of its inspiration from the International Geophysical Year. Indeed, if one looks at the development of the sciences during the past few decades one is struck by the high peaks of achievement reached in international collaboration and also through international competition in the physical sciences. Research on the ionosphere, the atmosphere, and the geosphere has been greatly stimulated by organized activities—the International Polar Years, the International Geophysical Year and the International Years of the Quiet Sun which have developed one from the other. Programmes are going forward strongly, too, in connexion with the hydrosphere, as arranged by the Intergovernmental Oceanographic Commission and the International Hydrological Decade. But in such investigations the biosphere has been almost completely neglected. Yet on the natural resources of this Earth—especially the renewable ones which come from biological productivity—the future of mankind depends. They are much more important to most human beings than getting to the Moon or taking close-up photographs of Mars. One could even argue that it is more important to humankind to ensure a sustained yield of fish from the North Sea than to discover and extract the oil that may lie beneath it.

What do we mean by biology within the context of the International Biological Programme? To some, biology means the life sciences as opposed to the earth sciences; to some it stimulates thoughts of the inner workings of the cell; and to some perhaps, merely the facts of human reproduction. For the purposes of an International Biological Programme, I think the best definition is the biosphere, that is the living layer around the Earth, on land, in the waters and in the air.

This word biosphere, by the way, seems to present a problem in semantics. I have heard it explained by a television astronomer as a concentric region of the solar system, comprising the orbits of Venus, the Earth and Mars, where there is some reason to believe that life as we know it exists. I use it, however, as the layer which supports life in, on and around the Earth, for the International Biological Programme is likely to have little time or opportunity to extend its activities to other planets.

Some biologists are inclined to think that the International Biological Programme is rather a waste of effort and money. They argue that opportunities for the exchange of knowledge and ideas are not lacking, but are

almost overburdening, for international unions and associations exist in every branch of biology. From spring to autumn scarcely a week passes without some international biological congress, often several running contemporaneously, even in the same country. Is this not enough?

The counter argument is that we scientists concerned with environmental biology have to take but one look at our colleagues concerned with the physical environment to appreciate what a degree of organization can do. Few would deny that studies of this kind of biology, concerned with the organism in relation to its environment, which had such a splendid start in the past century, have been sadly overtaken in this century by studies in the physical sciences. In the long run, I believe that the International Biological Programme will be looked on as a major effort—and a successful one—to redress this balance. In doing so it will provide a part of the fundamental knowledge which to-day is so much needed so that biological science can be applied more effectively to the provision of human needs.

History

Before examining the content of the International Biological Programme we should consider how the idea came into being. British scientists have taken a prominent part in this. Sir Rudolph Peters some years ago, when president of the International Council of Scientific Unions (ICSU), took a major part in the conception of the International Biological Programme. Prof. H. W. Thompson of Oxford, the current president of ICSU, did a good job as midwife during the first International Biological Programme Assembly held in Paris last year. Prof. C. H. Waddington, as president of IUBS, has been a good doctor in the pre- and post-natal clinics; while the president, officers and secretariat of the Royal Society, by priming and holding the feeding-bottle, have enormously helped the infant's early growth.

How the ideas leading up to the International Biological Programme have fluctuated and developed has been related by Prof. Montalenti, the Italian geneticist who led much of the preparatory work. Initially, it was thought that the programme should concentrate on the human organism, and especially population dynamics and genetics. Later there was a body of opinion which thought it could involve itself best on the preservation and conservation of biological systems in different parts of the world, especially those which are most endangered by economic progress. Finally, these and other approaches have been brought together in such a way as to be of interest to a rather broad spectrum of biological scientists. The objective, as defined in 1964, is to ensure a world-wide study of: (a) organic production on the land, in fresh waters, and in the seas, and the potentialities and uses of new as well as of existing natural resources; (b) human adaptability to changing conditions. The programme should not range through the entire field of biology but should be limited to basic biological studies, related to productivity and human welfare, which will benefit from international collaboration, and are urgent because of the rapid rate of the changes taking place in all environments throughout the world.

Thus, of two major problems now facing the human species on Earth—of controlling his rate of reproduction

* Substance of an Evening Discourse delivered on September 3 at the meeting in Cambridge of the British Association for the Advancement of Science.

and of increasing the biological product on which he subsists—the International Biological Programme is concerned with the second. The advances in techniques to control reproduction which have been produced in the past decade have reached a point at which there is more need for sociologists to apply them than for biologists to invent new ones. Yet the attributes and potentialities of man are by no means left out of the programme, as I will explain.

Principles

The programme is based on a number of principles. First, it is a directional and a selected programme. It is not a free-for-all, nor is all research bearing on productivity and human adaptability automatically incorporated into it. The initials IBP can be regarded, if you like, as a kind of status symbol, and to ensure that this is not misused we have a sifting arrangement for projects at the national level and later at international level before admission into the programme. The tag of IBP does not, of course, mean that the project is necessarily good science, nor that other work on biological productivity conducted outside IBP is not good science. It merely implies that those immediately concerned with IBP agree that the project fits in and is likely to advance materially the objects of the programme.

Secondly, the programme is concerned throughout with the fundamental approach to research. It starts from the premise that the applied sciences of biological production, such as agriculture, forestry, fisheries, have in recent decades gone striding ahead more quickly than the fundamental understanding of the causes of productivity. Similarly, the sciences of man, medicine and some of the applied social sciences, have in some ways out-paced fundamental understanding of mankind and the differences between one man and another. The International Biological Programme is an opportunity to restore the balance and to provide new organized knowledge as a springboard for the technologies to take further plunges into progress.

Thirdly, the International Biological Programme is urgent. This is not only because of the steady growth of the pressure of human populations on renewable resources, but also because many of the situations in the world, both biological and human, are changing so rapidly that they will soon cease to exist. The International Biological Programme is not a preservationist body, for our approach is dynamic not static, conservation rather than preservation; but the perpetuation of sample biological systems for future biologists to study is definitely among its objectives.

Fourthly, and this could perhaps almost go without saying, the International Biological Programme is limited to research which could benefit from international co-operation. There are some scientists, and great ones at that, who do not readily co-operate with others and whose special attributes lead them to penetrate deeply on a narrow front the unknown, behind locked doors so to speak, rather than, by a sharing of knowledge, to advance with others along a broad one. The International Biological Programme is clearly not designed for the isolationist researcher, although his findings might well be picked up and developed as a Programme project.

Divisions of the Programme

In order to get to grips with what inevitably is a very large content, the International Biological Programme is divided into seven sections. One of the first and most obvious divisions was into the biological communities of the land, of fresh waters, and of the sea, because terrestrial ecology, limnology, and oceanography have each developed their own discipline of research, although biological energy often flows from one to another. These three sections are known by their initials; PT for Productivity

of Terrestrial Communities; PF for Productivity of Freshwater Communities; PM for Productivity of Marine Communities.

All three have their problems of conservation, include the definition, description and management of prescribed areas or samples of living communities. The problem of conservation on the land are more extensive and generally more complex than those of the waters. They include such concepts as national parks, nature reserves and sites of special scientific interest. Therefore, there is a special section of IBP labelled CT for Conservation of Terrestrial Communities. Each of the three, moreover, presents different facets of the processes of production—photosynthesis (utilization of solar energy) and the nitrogen cycle—this section is called Production Processes (P). Each of the three may reveal new resources of value to mankind or new methods applicable to the use and conservation of resources, which require fundamental study; this will be carried out within the section named UM for Use and Management of biological resources. Finally, the human problems, which bring in another series of disciplines, notably physiology and anthropology, will be studied within the Section of HA, Human Adaptability. This makes seven: PT, PP, CT, PF, PM, HA and UM.

Each of these sections is headed by an international convenor and sectional committee, and is busy in producing a five-year programme of its own. Two of the convenors are British—Mr. Max Nicholson of CT and Prof. J. S. Weiner of HA. The seven sections are, of course, closely related to each other, but also to other programmes going on during the same period, for example PF to the International Hydrological Decade.

IBP consists of the sum of its parts, not only in the sense of containing seven different programmes, but also in the sense that the bulk of the research will be undertaken and financed nationally, not internationally. Many countries are at present busy preparing their national programmes, and of these one of the most advanced that for the United Kingdom, now published by the Royal Society.

Examples of Research

Let us take a few examples of the kind of work which IBP will contain, and think especially of the British contribution. Although a great deal has yet to be learned about our own biological communities and our own people, some of the thinking has been on the lines that we should use the knowledge and abilities of a good many scientists overseas, where ignorance about biological productivity is still very great. Thus the U.K. programme in its PP section aims to establish two bases for IBP work in the warmer regions overseas, in co-operation, of course, with the countries concerned. One of these is likely to be in a savannah country, at the Cambridge Nuffield Unit of Tropical Animal Ecology, situated in the Queen Elizabeth Park in Uganda; another at a site yet to be determined in an area of tropical rain forest. At these bases, in co-operation with local scientists, it is hoped to undertake investigations of productivity in depth—primary production of the trees, shrubs, grasses and herbs, secondary by herbivorous mammals and insects, tertiary by predators and parasites.

It is hoped to quantify as well as qualify each link in the food chain so as to obtain data to compare production in the wild with production from the tame, for example where domestic animals and plants have been established on land used for agriculture.

Passing to another section, namely CT, it is pleasing to report that something has been actually achieved. In the spring of 1964 and again in 1965, expeditions went from Great Britain to the desert lands of Jordan, and made a survey especially of the Azraq Oasis and its environs, to the east of Amman. This has resulted in several things: a useful IBP booklet entitled *An*

approach to the Rapid Description and Mapping of Biological Habitats, and more important, in detailed commendations about the creation of national parks in Jordan. Further preparatory work has been based on the Azraq Oasis where the park headquarters and garden are now established with American aid. Within a week or two, two experienced members of the Nature Conservancy's staff are going there to prepare a management plan, applying the experience in management of wild areas gained in this country. Meanwhile, in August 1964, M. The King of Jordan publicly announced that he will set up the Azraq National Park, and also that Jordan will participate in the IBP by establishing at the Park's centre an institute for biological and human investigations in desert and oasis conditions. This indeed will make a useful counterpart to the proposed IBP bases in savannah and rain forest to which I have already referred.

From the PF section I will draw two examples of British initiative: one is an intensive study of the biological productivity of a reach of the Thames, organized from the University of Reading. This is dealing with primary and secondary production and will include studies on the feeding and population dynamics of a considerable number of fish species. It will fill a major gap in our knowledge of British fresh waters, for most intensive work has been devoted to lakes and very little to rivers. The other example relates to research in tropical lakes. In terms of food supplies through fisheries, and water supplies for irrigated agriculture, they are of much greater importance to the world than the little lakes in our own country. So the programme includes the establishment, based on a grant from Britain, of a team of limnologists for work in the tropics, especially Africa. This takes advantage of the fact that we happen to have in Britain a rather particular expertise in tropical lakes.

During the present planning phase each of the seven sections has already held, or has arranged, a number of symposia consisting of selected specialists from all over the world to discuss methods of research in particular spheres and to prepare handbooks. The object here is not to 'straight-jacket' the methods in use in IBP, for field biology is at present advancing very rapidly through the development of old methods and the devising of new ones; but it is to agree on those proved methods which can be advised for general use throughout the world and can be relied on to produce comparable results. A good example was a symposium held in Aberdeen and Cambridge in September concerning research on large herbivorous mammals. In Australia, studies of kangaroos and introduced sheep suggest that the former may be more effective converters of grass into meat than the latter; if only kangaroos produced wool instead of hair, they might be the basis of Australian prosperity. In Africa there are indications that the broad spectrum of indigenous African mammals, such as giraffe, antelopes, buffalo and wart hog, may be better agents of secondary production of meat than the exotic cattle, sheep and goats which have replaced them in many areas and are apt to depress the yield of the habitat. In Scotland we have the Island of Rhum, where management of red deer by the Nature Conservancy has resulted in more venison coming off the island than mutton under the former agricultural management and at the same time it seems as though the level of primary production of the vegetation has been raised. This symposium brought experiences of this sort together and advised on methods for future work under IBP from the three points of view—the ecological, the physiological and the pathological.

Another example I want to give is one with which I am personally involved just at present, namely research on large man-made lakes. The basic reason for creating these is generally for hydroelectric power: for example, at Kariba, Volta and Kainji in Africa, Brokopondo in Surinam, and a number of large impoundments in North America. Sometimes water conservation for irrigation

is equally or more important, as in the Aswan High Dam in Egypt. But the influence of these great sheets of water will extend not only to producing power and water but also to many other human needs, and has a profound bearing on the biology of large regions. Anyone familiar with Africa, for example, will realize the fundamental influence of the great natural lakes on climate, vegetation, fisheries, water supply for man and stock, transport, sites for urban development, and the rest. Since the period of IBP happens to coincide with the period of constructing several new great impoundments and of the biological changes consequent on several of those already completed, their study will take quite a significant place in the programme. Here again, it is good to note that Britain has taken an initiative, and as I speak a group of eight scientists drawn from Liverpool and other universities in Britain and Nigeria, and supported financially by the Ministry of Overseas Development, are undertaking a preliminary investigation of the part of the River Niger where Mungo Park met his end in 1806, the reaches which, in a year or more, will form a five-hundred square mile lake impounded by the dam at Kainji. The group has now been joined by several American and African sociologists who are extending the aquatic study to the 50,000 or so people now living below the future water-level. Under the leadership of local people, assisted by scientists from outside, and with funds which we hope will be provided by the special fund of the United Nations, it is hoped to establish a co-ordinated programme of study on these great impoundments.

I have been speaking mainly about the purely biological aspects of IBP, but the section on human adaptability has so far been quite one of the most active. It brings in a lot of scientists in the spheres of medicine and of physical anthropology, and its programme is designed rather differently from some of the other sections. It is wide and comprehensive, divided into a large number of headings and subheadings. Research topics include environmental physiology, including tolerance of different human groups to cold, heat, and high altitude; fitness, growth and physique, which, incidentally, will include a survey of highly fit athletes; the genetics of populations, with international data centres to be developed on existing national ones such as the blood-group centre of the Medical Research Council in London; and lastly health, nutritional and epidemiological topics. Clearly a lot of this work will need to be done by groups of research workers travelling with their equipment to study communities in their natural environments. This involves mounting a good many quite complex expeditions to remote places. British workers under IBP/HA expect, for example, to work this autumn on the Hazda tribe around Lake Eyasi in Tanzania and the people of Bhutan in the very high Himalayas.

Organization

There is a school of scientific thought that resents all forms of direction or organization. Science is free: give us the funds and let us go unfettered in any direction where research leads, and we will show results. Good scientists should not become administrators; any who do so are so much loss to science. This is an attitude which some of us associate with the older universities, but it is quite widespread, within Government scientific service as well as outside it. I met it strongly expressed when discussing IBP recently in Uganda. Now this libertine approach to science does not face the realities of how the funds and facilities for research are to be produced, how the results are to be discussed and published, or how those results which have an element of application in them might be turned to practical use. Nevertheless, it is an attitude with which I personally, and many others who are concerned with IBP, have a good deal of sympathy. We have been influenced by it in thinking up the organization necessary for IBP, at least to the extent that we have

devised this as lightly as possible. We are trying to build a simple structure from the foundations and to make the roof no heavier than is necessary to protect those inside from the weather.

Starting from the top there is ICSU which has set up the Special Committee for the International Biological Programme (SCIBP), comparable with its scientific committees for Oceanic Research (SCOR) and Antarctic Research (SCAR). SCIBP is a democratic organization which holds a general assembly about every two years. It has a Swiss president, Prof. Jean Baer, vice-presidents from Italy, Poland, the United States and Great Britain, and a number of other members, some representing the main international unions in the biological sciences and some drawn from the different bio-geographical regions of the world. It includes also the seven international conveners of the sections of IBP. Unesco, FAO, WHO, SCOR, and SCAR have representatives on it. Each of the conveners has also his sectional committee of specialist members drawn from all over the world.

The central office for IBP is at 7 Marylebone Road, Regent's Park, London, in accommodation generously provided and furnished by the Government and the Royal Society. Each of the seven international conveners has his own office, although only four of them as yet have whole-time staff.

Publications of IBP are so far very few: a journal called *IBP News* was started in October 1964, and so far three numbers have been published. We are also starting a series of handbooks, and the first of these, being a general guide to the activities of HA section, has just gone to press.

The central funds, for running the offices and holding meetings and for publications, come from national dues paid in by each participating country. These are on a rather modest scale and in fact are at present insufficient. I am glad to say they are augmented by grants and loans from ICSU and Unesco, and a particularly generous grant recently made by the U.S. National Academy of Sciences. It is hoped that other countries will follow this excellent example.

The finance I have spoken of is concerned only with the organizational framework and, by international standards, is quite small. The cost of the actual research undertaken under the auspices of IBP in all parts of the world will naturally be quite large if the programme is to be at all worth while. As yet it is impossible to say how much it may cost, because very few national programmes have yet been prepared in detail although a number are in active preparation. If we take the British programme as a model, and if some twenty major nations have comparable programmes and twenty or thirty others prepare smaller programmes, the total is bound to run to several million pounds. The finance for national programmes will of course be provided nationally: in this first year of design and feasibility studies, the British Treasury has provided some £80,000 specially for IBP; added to the normal votes for the Royal Society and the Nature Conservancy. This will enable some appointments to be made and some research of a trial nature to be started. A number of other

countries have acted somewhat similarly. Czechoslovakia for example, in addition to financing its own planning phase, has provided from its own funds the office and staff for one of the international conveners, Prof. Malek who is based in Prague.

In order to plan and carry out national programmes many countries are setting up their own national committees, often with sub-committees equivalent to the international sectional committees of SCIBP. Thus the United Kingdom, through its committee structure which operates under the patronage of the Royal Society, brings about 150 British scientists into the active planning stage. The United States now has a national committee and Dr. Roger Revelle, Poland one under Prof. Petruszewicz, Japan not only has a national committee but has already submitted a programme. I believe that before long a major problem of SCIBP and of its Central Office will be not so much to get IBP in motion which has been a preoccupation up until now, but to prevent it running away from itself by becoming too large and unmanageable.

Conclusion

The gestation period of the Programme has been long, but recent progress is at a much greater rate than we have dared to hope a couple of years ago, for the world needs a IBP. What, then, is the philosophy behind it? What is the driving force that causes many busy people about the world to devote their time and energy to helping it on? I suggest it is a spirit of service to our fellow men and to the other animals and plants that make up the very varied living communities of the Earth. Even in these days of rather selfish approach to living, apparent in the political, commercial and even scientific spheres, I suppose there is some feeling of service in all of us, even if it is only an instinct towards conserving the race, after conserving the individual. Here in IBP is the opportunity for very many biologists, whose efforts may have been not perhaps of very high value to others, to bring their researches together to focus them and to make them more useful. Here in IBP is the opportunity of showing the world the value of field biology as well as laboratory biology. New resources will undoubtedly be discovered, and so also will be the aesthetic as well as the scientific and economic value of plants and animals in their natural and also in their man-made environments.

An important part of the service IBP hopes to render will be to ensure that at least samples of these wonderful creations of evolution, so different and yet so similar in the several bio-geographical zones, will continue and flourish for subsequent generations of humans to appreciate and use. Nor should we forget the many and diverse races of man himself, with their separate and particular attributes. Unless we understand these matters and use our understanding to plan the future, they will go down before the axe and fire, the bulldozer or the hypodermic needle. This marvellous biological differentiation on the Earth would then tend towards dull uniformity and even obliteration.

OBITUARIES

Dr. L. H. Gray, F.R.S.

DR. LOUIS HAROLD GRAY, first director of the British Empire Cancer Campaign Research Unit in Radiobiology, died suddenly on July 9, 1965. He was fifty-nine.

Hal Gray, as he was known to his friends the world over, enjoyed a unique position in his chosen subject. Radiobiology calls for a wide-ranging mind, able to grasp the fundamental concepts of physics, chemistry, biology and medicine. To the end of his life, Gray retained an

intellectual vigour which enabled him to seize the essentials of a new scientific discipline, and relate them to the existing body of knowledge.

Educated at Christ's Hospital, Horsham, to which he won a scholarship, he soon displayed an outstanding aptitude for science and mathematics. At school he was much influenced by "Chas." Browne, then head of science, who educated his pupils on the principle that it is better to do an experiment than to look up the answer in a

ok. This 'do it yourself' approach was to remain a characteristic feature of Gray's work throughout his life. From school, he won an Exhibition at Trinity College, Cambridge, and two years later took Part I of the Tripos in physics, chemistry, mathematics and mineralogy, and as awarded first-class honours. Proceeding to Part II in physics, he again achieved a first and was able to commence research at the Cavendish Laboratory during an exciting period of Rutherford's professorship. In 1930 he took his Ph.D. and received a Fellowship at Trinity.

His work at this period was concerned with the scattering and absorption of γ -rays, and in his first publication (1929) he enunciated the cavity ionization principle, which had previously been described by Bragg in 1912, although Bragg's work was unknown to Gray at the time. His theory, which is fundamental to γ -ray dosimetry, became known as the Bragg-Gray principle.

Despite his success in pure physics, he began to find the subject not completely satisfying, and he felt the need to do something of more obvious value for his fellow men. An offer to come to Mount Vernon Hospital as physicist appealed very strongly to him and he took up the position in 1933. Although his work at Mount Vernon was primarily concerned with the accurate measurement of ionizing radiation, he began to take an interest in biological problems under the guidance of that remarkable man, Dr. J. C. Mottram. Before leaving Cambridge, he had begun a fruitful collaboration with F. G. Spear, of the Strangeways Laboratory, studying the effect of neutrons on hen eggs. The neutron source available to them proved to be too weak, Gray, with the collaboration of John Read, built a highly effective neutron generator for an outlay of some £600, with which the first biological results were obtained in 1938. At this time, Read and Gray suggested a unit, the Energy Unit, which was applicable to all forms of ionizing radiation and was almost identical with the rad unit adopted by the International Commission on Radiological Units and Measurements some fifteen years later. During the Second World War, when he was working alone, he began to consider the dynamics of cellular population changes underlying the gross changes in behaviour of a bean root following irradiation, thus anticipating one of the focal points of interest in modern radiobiology.

In 1946 Gray went to the Medical Research Council Radiotherapeutic Research Unit, as senior physicist, and in 1950 was promoted to deputy director. Here, he had overall responsibility for building up several teams to carry out ambitious projects in radiation research, the construction of a cyclotron, the development of radioisotopes in research, and general radiobiology. This heavy administrative load left little time for his personal researches, but his wise choice of staff is reflected in the advances in radiobiology made by his group. The classic investigations of Howard and Pelc on the relation of DNA synthesis to the mitotic cycle, and of Miss Alper on bacteriophage, date from this period.

In 1952 Dr. Gray initiated a programme of research on the oxygen effect in radiobiology, and its relation to radiotherapy. Those who had the good fortune to work under Hal Gray at that time will always remember his boyish and generous enthusiasm, which infected all those near to him. Within six months the main outlines of the picture had been established—a picture which still inspires much of present research in radiobiology. It was characteristic of Hal's disarming modesty that his collaborators had the greatest difficulty in persuading him to be the first author of the paper which recorded this work.

In 1953 Gray resigned his position at Hammersmith, following disagreements on scientific policy. Thanks very largely to the good offices of the late Lord Horder, who placed great faith in Gray, the British Empire Cancer Campaign undertook to finance a new Unit at Mount

Vernon, where he would be director with freedom to pursue his own lines of research.

With considerable foresight, Gray planned the use of a linear accelerator for the development of pulse radiolysis methods, in collaboration with J. W. Boag, a technique which has already revolutionized radiation chemistry. At the same time, he gathered around him a small group whose basic training lay in physics, chemistry, biochemistry, biology and medicine, since he believed strongly that collaborative studies by such a group offered the best hope for advance in an inter-disciplinary field. It was also a great delight to him to return to experimental work, which he did with undiminished energy, planning experiments which made few concessions to human requirements for sleep and food.

He had no love of committees, but he bravely accepted the administrative burdens which were placed on him. His work for the International Commission on Radiological Units will be particularly remembered, and the personal effort which he put in as president of the second International Congress of Radiation Research had a lot to do with the acknowledged success of that meeting.

Gray received many honours in recognition of his work, but two he prized highly were American awards, the Judd Award in 1954 and the Bertner Foundation Award in 1964. He was elected Fellow of the Royal Society in 1961 and received an Honorary D.Sc. at Leeds in 1962.

No catalogue of publications or prizes gives a true picture of Hal Gray's contribution to radiobiology. He delighted in helping other workers, particularly the younger ones, and he never grudged the time devoted to giving help and advice. Even an anonymous referee's comment would engage the whole of his great powers of critical appraisal and constructive comment. Varied and remarkable as his contributions were, it is certain that he will be remembered by those who knew him, not only as a great scientist, but also as a great man.

O. C. A. SCOTT

Dr. O. S. Sinnatt

DR. OLIVER STURDY SINNATT, professor of aeronautical science in the Royal Air Force College, Cranwell, from 1920 until 1940, died on May 28, 1965, at the age of eighty-two. Dr. Sinnatt was born in Liverpool on September 6, 1882, and educated at the College of Technology, Manchester, and the Owens College (later University of Manchester). He graduated B.Sc. in 1904, M.Sc. in 1907, D.Sc. (London) in 1915, and was elected Associate Fellow of the Royal Aeronautical Society in October 1924. After a year as master of engineering at the Carpenters' Technical Institute, Stratford, he joined the staff of King's College, London, in 1905 as a demonstrator, becoming a senior demonstrator and then a lecturer in the Department of Mechanical Engineering.

During the early part of the First World War he held various posts with the University of London Officers' Training Corps; for a period he was in charge of the Training Camp, Perivale. He was afterwards transferred to the London Regiment and posted to France early in 1917, rising to be second in command of the 2/2 Battalion of that Regiment.

He was severely wounded at Poelcappelle in 1917 and awarded the Military Cross in 1918. He joined the Air Ministry as a technical officer (Instrument Section) in 1918. He was appointed professor of aeronautical science at the Cadet College, Cranwell, in 1920.

In 1940, Dr. Sinnatt was appointed to the School of Aeronautical Engineering, Henlow, as senior education officer, where he remained until his retirement in 1942.

In 1920 he married Marjorie Helen, the only daughter of the late W. R. Randall, by whom he had two sons, the elder of whom was killed in action in 1944.

C. E. P. SUTTLE

NEWS and VIEWS

Director of Building Research, Ministry of Technology :
Dr. Frederick Measham Lea, C.B., C.B.E.

DR. F. M. LEA relinquishes his duties as director of building research in the Ministry of Technology on December 31, having reached retirement age. During his twenty-year tenure, the work of the Building Research Station has been greatly extended in scope, now ranging from research on the use of materials to structures, soil mechanics, heating, ventilating, lighting and acoustics, mechanical plant for building, operational and economics research and studies of user needs. Although often accused of technological backwardness, the industry has, in fact, made very rapid application of much of the work of the Station, particularly in the newer fields of research on mechanical plant and on costs and economics. In addition to this national activity, Dr. Lea has taken an active part in international collaboration in building research and he has served as president or as a member of council of most of the organizations working in this field. His early work at the Station, before appointment as director, was on the chemistry of cement and concrete and he has throughout retained a special interest in this field. It has attracted to him a number of awards, culminating in the Walter C. Voss Award of the American Society for Testing Materials, in 1963. His text-book *Chemistry of Cement and Concrete* is an established source of reference. He became a Fellow of the Royal Institute of Chemistry in 1936. He was appointed O.B.E. in 1944, C.B.E. in 1952 and C.B. in 1960, elected an Honorary Associate of the Royal Institute of British Architects in 1947 and an Honorary Fellow of the Institute of Builders in 1965.

Dr. J. C. Weston

DR. J. C. WESTON has been appointed director of the Ministry of Technology's Building Research Station at Garston, Watford, Hertfordshire, in succession to Dr. Lea. The appointment takes effect from January 1, 1966. Dr. Weston joined the Building Research Station in 1947 as a principal scientific officer, and was promoted to senior principal scientific officer in 1953. His concern was with building production methods and costs. Since 1959 Dr. Weston has been, as a deputy chief scientific officer, group head of the Station's Building Operations and Economics, User Requirements and Mathematical Services Divisions. He has been on secondment to the National Building Agency as chief executive of the Operational Division since June 1964. Dr. Weston was educated at Nottingham and obtained his degree at London and his Ph.D. for work on temperature changes accompanying magnetization processes in ferromagnetic substances. During and immediately after the Second World War, Dr. Weston was with the Admiralty Scientific Service. He was engaged in work on torpedoes and mine detection. This was followed by a year at The Plessey Company, Ltd., where he was responsible for the Physics Research Laboratory.

Director of the Scottish Plant Breeding Station :
Dr. J. W. Gregor, C.B.E.

DR. J. W. GREGOR retired on September 30 from the post of director of the Scottish Plant Breeding Station, Pentlandsfield, Roslin, Midlothian. After taking his Ph.D. at the University of Edinburgh in 1925 he joined the staff of the Station as chief assistant. In 1939 he was awarded a D.Sc. and was appointed director in 1950. He was made a Fellow of the Royal Society of Edinburgh in 1957 and was awarded a C.B.E. in 1961. Internationally known for his experiments on the genetics of

wild populations of *Plantago maritima*, he endeavoured to show the agricultural significance of his genealogical studies by relating them to pasture improvement in the Scottish hills. Always interested in the nomenclature of plants, he has made a number of contributions to the literature on ecotypic differentiation and the recognition of intra-specific variation in plants. Among many consultative groups on which he served was the International Commission for the Nomenclature of Cultivated Plants which produced the *International Code of Nomenclature*. Dr. Gregor has been succeeded by Mr. N. W. Simmonds

Mr. N. W. Simmonds

MR. N. W. SIMMONDS, head of the Department of Potato Genetics at the John Innes Institute, Hertford, has been appointed to the post of director of the Scottish Plant Breeding Station, in succession to Dr. J. W. Gregor. Mr. Simmonds was educated at Whitgift and Downing College, Cambridge, and then went to the Imperial College of Tropical Agriculture, Trinidad, in 1944 under a Colonial scholarship. After holding lectureship in botany at the Imperial College of Tropical Agriculture, Trinidad, he became senior cytogeneticist in the Banana Research Scheme and Regional Research Centre: a new tetraploid variety of banana was produced by the Scheme during his tenure of the post. In more recent years he has been consultant in sugar cane breeding in the West Indies. He returned to Britain in 1959 to become head of the Department of Potato Genetics at the John Innes Institute. Mr. Simmonds's botanical and plant-breeding investigations have taken him on extensive travels in Europe, Asia, Africa and America, and, while his main interests lie in the cytogenetics and evolution of crop plants (especially of bananas and potatoes), he has also published widely in the fields of ethnobotany, systematics and phenol biochemistry.

Psychology in the University of Reading :
Prof. Magdalen D. Vernon

THE retirement of Prof. M. D. Vernon from the chair of psychology in the University of Reading constitutes a landmark not only in her own working life, but in the development of the subject in Britain. She started work in Sir Frederic Bartlett's laboratory at Cambridge in 1921 as an Industrial Health Research Board investigator at the beginning of the remarkable period of progress there between the two Wars. She remained—later as a Medical Research Council staff member—until 1946, when the late Prof. A. W. P. Wolters secured her services as a lecturer at Reading. Appointed senior lecturer in 1955 and reader in 1956, she succeeded Prof. R. C. Oldfield in the chair in that year. Her massive contributions to the study of visual perception and especially of the processes involved in reading, both in experimental papers and in books such as *Visual Perception*, had already earned her an international reputation before she went to Reading. During her twenty years there that reputation has much increased, while her work in other fields, such as the motivation of children, has widened its basis. Her unfailingly high standards in research and in teaching have been a spur to her colleagues and students alike, and if many perhaps have found them difficult to emulate, much excellent work and an acknowledged distinction have been their fruit. Prof. Vernon's astringent good sense on academic and other committees has always been effective and deeply appreciated by her colleagues. Her period at Reading has spanned a time of very considerable expansion of the subject, with a large increase

student numbers and in research activities. As elsewhere, this has entailed a long struggle with the difficulties of inadequate accommodation and staff recruitment. But her admirable management and persistent advocacy leave the Department with a greatly enhanced reputation, and she is well poised for the further developments which may confidently be expected under her successor, Dr. M. Treisman (see *Nature*, 207, 689; 1965).

Discoverer of Nickel: Axel Fredrick Cronstedt

AXEL FREDRICK CRONSTEDT, the Swedish chemist and mineralogist, who died on August 17, 1765, is remembered not only for his discovery of nickel but also for his researches and writings. Though a member of the Stockholm Academy of Sciences, he had no university training; for, as a student at Uppsala, lack of finances meant abandoning his academic career to take a post as metallurgist in the Swedish Department of Mines. Yet his proved beneficial both to him and to his country. Georg Brandt coached Cronstedt in the techniques of assaying. In 1751 Cronstedt came across a new mineral from a cobalt mine, which at first was thought to contain copper, yet which yielded none by displacement from its solutions. The mineral, now known as niccolite, or nickel arsenide, formed green crystals on weathering; it was from these that Cronstedt prepared an oxide which on reduction with charcoal gave a new metal. Only in 1754 did Cronstedt christen it nickel, and describe the silver-white and feebly magnetic regulus to the Stockholm Academy. Cronstedt noted the brown borax bead, the blue solution formed with ammonia, and the close resemblance to cobalt. Chemists in France in particular refused to accept the new metal until Torbern Bergman, in 1774, added further evidence by preparing a specimen of it free from the cobalt, arsenic and iron which had contaminated Cronstedt's first specimen. In the history of chemistry and metallurgy Cronstedt's name appears frequently in connexions other than nickel, being listed with platinum, iron ores and zinc extraction—a field in which Cronstedt collaborated with Rinman, whose name remains in 'Rinman's Green'. Cronstedt won a niche even if only for his *System of Mineralogy* translated into several languages.

Political and Economic Planning

THE annual report of Political and Economic Planning (P.E.P.) outlines the current research programme which, during 1964–65, focused on problems of planning and policy-making in economic policy, Government, management, social issues and international affairs (Pp. 12. London: Political and Economic Planning, 1965). The Social and Economic Archives Committee has entrusted to P.E.P. the establishment of a Survey Archives Centre in Britain; for an initial period of two years P.E.P. will collect and disseminate information about survey data held, and surveys planned by university departments, research institutes, market research and commercial organizations and other relevant problems. During the year, a study of the effects of imprisonment of a man on his wife and dependants was published under the title *Prisoners and their Families* (see p. 216 of this issue). A survey of East African students in Britain was also completed during the year and published under the title *New Commonwealth Students in Britain—with Special Reference to Students from East Africa*. Since 1931, P.E.P. has organized 500 broadsheets and 50 books; a list of publications issued during 1963–65 is appended to the annual report.

Energy Forecasts for Britain

MR. D. G. TIPPING in the *Westminster Bank Review* for August 1965 questions the forecast of energy demand for 1980 of 450 million coal equivalent tons as too high,

since it is based on the assumption of a 4 per cent growth in the economy. He suggests that a more realistic range of expectation for 1980 would be 400–420 million tons, but he does not think that Britain is likely to be faced with a sudden critical shortage of energy brought about by unexpected changes in demand. He considers that the output of 200 million tons of coal per annum advocated by the coal industry is unrealistic, and points out that the demand for coal from the gas industry is likely eventually to fall to zero. The one market for coal which is likely to grow considerably is for the generation of electricity, and the main uncertainties here are the proportion of future demand to be met by nuclear power and the balance to be struck between the conventional fuels. There seem to be three major ways in which the coal industry could be helped to play its part in a cheap energy policy: through capital reorganization, apportionment of social costs (which lie at the core of the problem), and through the pricing system. Mr. Tipping insists that the State and not the consumers should bear the burden of interest on over-capitalization of some parts of the industry. Social costs should be borne by society and also a more rational pricing policy should be introduced. Any rational pricing system is logically prior to a rational target. He points out that we must plan not only to minimize any error but also to introduce sufficient flexibility to overcome the effects of error. There is already considerable spare primary capacity because the coal is accessible and merely needs the men and machines to get it. We are unaware, however, of the physical and economic possibilities of mothballing pits, or of the extent to which the present surplus capacity of 40 million tons would match the capacity of loss-making pits which one would like to close.

Utopia Reviewed

THE Spring, 1965, issue of *Daedalus*, the journal of the American Academy of Arts and Sciences, consists of a series of essays on various aspects of Utopia by writers who if not American born are, with two exceptions, working in the United States. Some, like Lewis Mumford's "Utopia, the City and the Machine", Prof. F. E. Manuel's "Toward a Psychological History of Utopias", Prof. Northrop Frye's "Varieties of Literary Utopias" and Judith Shklar's "The Political Theory of Utopia", have a certain historical bias. Others, including two at least of those already mentioned, are more concerned with the social implications, for example, Prof. C. Brinton's "Utopia and Democracy", Prof. A. B. Ulam's "Socialism and Utopia", F. Bloch-Lainé's "The Utility of Utopias for Reformers", Bertrand de Jouvenal's "Utopia for Practical Purposes" and J. R. Pierce's "Communications Technology and the Future". These, and also Prof. P. B. Sears's "Utopia and the Living Landscape" and Prof. J. M. Smith's "Eugenics and Utopia", may appropriately be read in conjunction with Jacques Ellul's recent book *The Technological Society*, to the fatalism of which some of the essays apply a slight corrective. Not even the more historical essays, however, refer to Marie Louise Berneri's scholarly work *Journey Through Utopia*, published posthumously in 1950, to which Mumford himself paid a generous tribute at the time. Prof. Frye, in fact, provides no references at all and generally, in contrast to Mari Berneri's book, the bibliographical side of the essays is weak. The titles of such essays as those of de Jouvenal, Sears, Smith and Pierce indicate their topical character, and perhaps the most optimistic are those of Prof. G. Kater on "Utopia and the Good Life" and Prof. Maren Lockwood on "The Experimental Utopia in America": both of these essays, incidentally, are well provided with references. While Mumford himself has not lost the optimism that marks his well-known trilogy, in the essay in *Daedalus* he accepts much of Ellul's position as to the dominance of the machine and points out that we can no longer think of the components of technology as additive.

We must face the implications of an articulated totalitarian system of which the machine is god and must recognize that our present scientific methodology is inadequate to deal with every aspect of human experience.

Social Implications of Automation

DR. A. HOVNE, in a recent issue of *Impact of Science on Society* (15, No. 1; 1965), discusses some social implications of automation, including the reasons which are likely to be decisive for the introduction of automation and why a new technique may not be adopted. After considering the unemployment aspects, he deals with the implications for four selected aspects of social life; for work in automated enterprises; for their organization; for the organization of Government. There are also certain broad choices to be made, particularly those relating to the place of work in the life of men, mobility in employment, the content of education, and to study as work. The two Cantor Lectures given to the Royal Society of Arts last March by T. R. Thompson and now published in the *Journal of the Royal Society of Arts* for August 1965 also deal with automation. The first of these lectures considers the economic problems of introducing automation into any process and the problem of finding the people to plan automation systems. The second lecture, dealing with the social consequences of automation, considers the need for anticipating its effects and the character of the new society in which automation is widely used. Besides the planning for this future, there is the problem of ensuring that the plan is feasible and also the further problem of providing the type of labour required. Finally, Mr. Thompson stresses the need to educate people to fit them for such a future and the importance of minimizing the effects of change. He suggests that a new emphasis should be placed on the real purpose of economic change; we must ensure that automation is made to serve our deepest needs, to give more leisure and to make work more interesting, so that we find greater contentment and happiness.

Research in Human Genetics and Studies of Twins

THE World Health Organization, in recognition of the increasingly important part that genetic studies are playing in the medical sciences, has established at its headquarters in Geneva a Human Genetics Unit. The purpose of the Unit is to co-ordinate and stimulate research and training in human genetics. Dr. R. L. Kirk, who is in charge of the new Unit, was formerly reader in human genetics in the University of Western Australia, where his research was concerned mainly with population genetic studies in isolated groups in south and south-east Asia, Australia and the Western Pacific. Current activities of the new Unit in Geneva include investigations of populations of unusual genetic interest, study of various hereditary conditions such as haemoglobinopathies, the setting up of facilities to service research projects, such as international reference centres for genetic markers, and improvement and standardization of methodology in genetic studies. The Unit hopes to give increasing attention to research on the distribution and public health significance of the haemoglobinopathies, including the thalassaemias and glucose-6-phosphate dehydrogenase deficiency, as well as to stimulate interest in new methods of treatment for cases of homozygous sickle-cell disease and thalassaemia.

The use of twins in epidemiological and genetic research is being evaluated at the present time, and the World Health Organization hopes to act in future as a clearing-house for information on twin studies. It also hopes to play an important part in co-ordinating population genetic studies, particularly those concerned with populations still having a food-gathering or simple agricultural economy, or living as isolates in more highly developed communities. Future activities will be directed toward

the role of genetic factors in mental disorder, the standardization of techniques in studying chromosome aberrations associated with spontaneous abortion, the study of somatic cell genetics and the genetic consequences of exposure to novel chemicals in the human environment.

Portraits of Tasmanian Aborigines

ALTHOUGH the aborigines of Tasmania have been extinct for some decades, a number of portraits of them are in existence which are of considerable anthropological interest. An account of them, the circumstances which they were produced and passed into the hands of various collectors, together with their present whereabouts, has recently been published (*Records of the Queen Victoria Museum, Launceston. New Series, No. 1. Thomas Bock's Portraits of the Tasmanian Aborigines* By N. J. B. Plomley. Pp. 24 (10 plates). Launceston: The Queen Victoria Museum, 1965.) The portraits are in water-colour. The originals are almost certainly the work of Thomas Bock, an engraver who was transported to Tasmania in 1824, and they were made at the request of G. A. Robinson, who was engaged on expeditions of conciliation with the aborigines at that time. Duplicates were also produced by Bock as well as by his son, Alfred Bock, along with other artists, mainly for Lady Franklin, wife of the Governor General. Later, many of the pictures found their way into the hands of collectors, like Dr. Joseph Barnard Davis, a surgeon living at Shelton, Staffordshire, and Richard Cull, who was secretary of the then Ethnological Society. Now, most of the portraits form part of permanent collections at the Pitt-Rivers Museum, Oxford, the British Museum, the Royal Anthropological Institute, the Fuller Collection (London), the Tasmanian Museum, and the Queen Victoria Museum, Launceston, Tasmania. Some of them were housed in the Crystal Palace, and were destroyed by the fire in 1936. The paper contains twenty reproductions of the portraits in black-and-white. On aesthetic grounds alone they are of outstanding merit; and while it is now impossible to judge of their quality as likenesses, they are impressively life-like, constituting a record of the characteristics and facial expressions of a group of people which is compelling and unique.

The Great Barrier Reef, Australia

THE Editor has received the following communication from Dr. O. A. Jones, chairman of the Great Barrier Reef Committee, Australia: "I would like to draw the attention of your readers to the greatly expanded facilities for research on the Great Barrier Reef, Australia. The Great Barrier Reef Committee has recently spent nearly £30,000 on providing living accommodation and additional laboratory space and equipment. The facilities might be described as rather more than basic, in that they include, in addition to collecting gear and preserving materials, such items as a fume cupboard, microscopes, centrifuge, mettler balances and an incubator. There is fresh and salt water on tap, gas for laboratory heating and cooking, refrigeration (both laboratory and domestic), electric light and power, aquaria and two small boats. Scientists from anywhere in the world may live in reasonable comfort at the Station and carry out research programmes, short or lengthy, of their own choice in any of the scientific disciplines applicable to barrier reefs. The Station is admirably situated for carrying out sedimentation and beach rock studies as well as marine biology. Heron Island Reef is about twelve square miles in area; neighbouring Wistari Reef is much larger, and there are nineteen other reefs within an area of forty square miles. The fauna of Heron and Wistari Reefs is protected by law, but scientists may collect specimens for their research under Government permits. Additional information is obtainable from the honorary secretary, Dr. R. Endean, Department of Zoology, University of Queensland, Brisbane, Australia."

International Conference on Mechanisms and Machines

DURING September 27–October 1 the first international inference to be held in Bulgaria took place at Drouzhba sort, near to Varna. Delegates from Canada, the United Kingdom, Australia, Czechoslovakia, both German Republics, Hungary, Italy, Poland and the U.S.S.R. were present, and the topics under discussion ranged from the termination of the instantaneous screw axes in spatial mechanisms, and from non-linear problems of centrifugal endulum vibration absorbers to the design of electronic computers and of high-speed input–output devices. The levels of presentation and of technical content of all the papers were high; thus, together with the excellent organization of the conference, and the really beautiful situation of the hall—not 20 yards from a sandy beach and the Black Sea—made the event one which will be long remembered with pleasure by those who were present. An important outcome of the meeting has been the setting up of an informal communications system for the interchange of information in a field which is of interest and importance—but as yet not provided with a recognized journal.

'Clean Room' Technology

A CONFERENCE was held at the Technische Hochschule, Stuttgart, during September 6–7, under the chairmanship of Dr. R. Kratel. About a hundred scientists and engineers attended, most of the main German firms engaged in ventilation and air filtration being represented. A message was received from the American Association for Contamination Control. The first day was devoted to the physics of aerosols. Dr. C. N. Davies gave a general account of the deposition of airborne contamination by diffusion processes and by mechanical effects from air at rest and in laminar and turbulent flow. Natural aerosols in the atmosphere were described by Dr. K. Bullrich of Mainz University. Two papers followed on the analysis of aerosols, Dr. Gahm, of Zeiss, giving a complete analysis of limiting factors in visual microscopy, and Prof. R. Hodgkinson, of Virginia State College, discussing the performance of automatic optical dust counting instruments in the light of the Mie theory of light-scattering and recent research on the effect of varying design factors, such as angle of acceptance. During the second day, problems of immediate practical application were dealt with. Dr. D. Hasenclever, of Staubforschungsinstitut, Bonn, gave an analysis of filter performance, and J. R. Weersing, of Royco Instruments, dealt with recent findings on the performance of his firm's particle counter which was developed particularly for use in factories making components for missiles in the United States. The need for dust-free work-places is strongly felt in the electronics industry, and some typical problems in International Business Machines Corp. were explained by Dr. Lechler. The conference concluded with an address by Dr. Kratel, who considered the correct lines for industrial development and the question of standards of aerosol concentration and particle size.

American Society of Plant Physiologists : Awards

PROF. FRITS W. WENT, of the University of Nevada, has been awarded the Charles Reid Barnes Life Membership Award of the American Society of Plant Physiologists. Each year the Society confers this distinction on a senior member of the profession in recognition of his character and contributions to scientific research. Prof. Went is recognized for his broad contributions to the field of botany and for his pioneering research on the bioassay of plant hormones (auxins). His work laid the foundation for the agricultural success of synthetic weed killers, fruit drop inhibitors, and the many other practical applications of these substances. Prof. Went was also concerned with pioneering research in the quantitative study of the effect of weather on plants. This culminated in his

development of the air-conditioned greenhouse (phyton) which has led to the plant growth chambers that are now part of the equipment of every major botanical laboratory. His manifold other interests include investigations of the formation of atmospheric smog from plant volatile exudates, the interrelationships of various plant species with each other, the discovery of many of the properties of plant hormones, and the physiology of desert plants. In addition to his scientific research, Prof. Went has achieved recognition through his effective lecturing, inspiring teaching and competent administration, which have been recognized through professorships at the California Institute of Technology, and the Washington University, St. Louis, and the directorship of the Missouri Botanical Garden.

Officers

The following have been elected officers of the American Society of Plant Physiologists: *President*, Prof. A. Carl Leopold (Purdue University); *Vice-President*, Prof. Robert S. Bandurski (Michigan State University); *Secretary*, Prof. Robert S. Loomis (University of California at Davis). Prof. Kenneth V. Thimann, of the University of California at Santa Cruz, has been elected to the Executive Committee.

University News:

Birmingham

THE following appointments have been made: *Lecturers*, Dr. A. J. Biddlstone (chemical engineering); Dr. I. L. Dillamore (physical metallurgy); D. E. Evans (physics); Dr. A. W. Nicol (mining and minerals engineering); Dr. N. M. Queen (mathematical physics); Dr. K. H. Star (psychology); *Senior Research Fellow*, Dr. J. Stephen (microbiology); *Research Fellows*, B. E. P. Beeston, M. D. Hunt, M. Norman and R. J. M. Willcox (physical metallurgy); Dr. M. Cordey-Hayes (physics); Dr. R. Haque (mining and minerals engineering); M. A. S. Sweet (electronic and electrical engineering); D. R. Whitehouse (chemistry); R. C. Woodman (engineering production); Dr. A. J. Collings (medical biochemistry and pharmacology).

Announcements

DR. SIGVARD EKLUND (Sweden), director general of the International Atomic Energy Agency since December 1, 1961, was reappointed for a second four-year term of office at the Agency's General Conference in Tokyo on September 27.

A SYMPOSIUM on "Subcellular Movement", arranged by the American Society for Cell Biology, will be held in Philadelphia during November 10–12. Further information can be obtained from Professional Associates, 6520 Clayton Road, St. Louis, Missouri.

AN international symposium on "Volcanology", sponsored by the International Association of Volcanology of the International Union of Geodesy and Geophysics and organized by the New Zealand Geological Survey, will be held in Rotorua and Wellington, Auckland, during November 22–December 3. The two particular subjects proposed for the symposium are: rhyolites, ignimbrites and pumice, and associated volcanism; geothermal resources with particular reference to the origin, geology, geophysics and geochemistry of hydrothermal systems of possible economic importance. Further information can be obtained from the Secretary, International Symposium on Volcanology, P.O. Box 499, Rotorua.

ERRATUM. In the article entitled "Light-induced Stomatal Opening and the Postulated Role of Glycolic Acid", by Prof. O. V. S. Heath, Dr. T. A. Mansfield and Dr. Hans Meidner, which appeared on p. 960 of the August 28 issue of *Nature*, in column 1 line 13 from bottom on p. 961 '1.34' should read '1.34' (that is, a lower log stomatal resistance in blue than in red light).

THE INSTITUTE OF PHYSICS AND THE PHYSICAL SOCIETY

THE fifth annual report of the Council of the Institute of Physics and the Physical Society* covers the period ending on December 31, 1964, and incorporates details of the activities of the various committees, branches and groups of the Institute and Society, together with the financial statement and account for the year. The report was presented and adopted at the annual general meeting held on July 6, 1965, at the headquarters of the Institute and Society, 47 Belgrave Square, London.

During the year under review, 970 applications for election or transfer to the various grades of membership were received, compared with 1,165 in 1963. The total membership of 9,750 on December 31, 1964, consisted of 1,416 Fellows, 3,558 associates and 2,444 graduates of the Institute and 1,256 Fellows of the Physical Society, in addition to students and subscribers. Although this represents a net increase of 280, the rate of growth has declined during the past two years, and the Council in its report expresses considerable concern. It is known that many physicists qualified to become members attend the scientific meetings and conferences organized by the Institute and Society, but nevertheless do not apply for membership. Various methods to improve recruitment are under active consideration by the Membership and Education Committee. A new grade of membership, Licentiate, intended for assistants in laboratories possessing a knowledge of physics not quite so extensive as that required for graduateship, and for certain teachers in schools and technical colleges, became operative at the beginning of 1965.

Representatives of the Institute and Society visited five technical colleges, and the applications of three of these colleges for recognition or extension of recognition for the purpose of the membership regulations were approved. Sixty-two technical colleges presented 892 candidates for the Ordinary National and forty-one colleges 614 candidates for the Higher National Certificate in applied physics; 589 and 418 respectively were successful. Examinations for the Higher National Diploma in applied physics were held for the first time during 1964. Twelve of the thirteen candidates entered by two colleges were successful. The seven question papers set in Part 2, and the four compulsory papers and the general paper in Part 1 of the graduateship examination were published in the October issue of the *Bulletin* of the Institute and Society, and also as a separate booklet. Of the 97 candidates for Part 2, 27 passed (two with honours), and of the 79 for Part 1, only 12 were successful.

An enquiry conducted by the Membership and Education Committee revealed that 1,597 students were admitted at the beginning of the session 1964-65 to the 1,907 available places in the physics departments of the universities (excluding the University of Cambridge) in England and Wales. The corresponding figures for 1963 were 1,524 and 1,708 respectively. A report on the survey of salaries and emoluments received by the members of the Institute as at August 1, 1964, was published in the January 1965 issue of the *Bulletin*. This was the sixth survey carried out by the Institute and the first since the amalgamation of the Institute and the Society. A sub-committee of the Membership and Education Committee is now completing a revision of the first draft of a statement on professional conduct which deals mainly with terms and conditions of employment and consulting work. The revised draft is to be printed and submitted to members for comment and revision before adoption.

The Institute and Society were responsible for the organization of the international conference on magnetism held during September 7-11, 1964, at the University of Nottingham. More than 500 attended the conference and

some 230 papers were presented. The text of the 19 Guthrie Lecture, on the subject of magnetic processes weak and moderate fields, which was delivered by Prof. L. F. Bates on September 9 during the conference, has now been published (*Proc. Phys. Soc.*, 84, 625; Nov 1964). The lecture had been specially postponed so that Prof. Bates might deliver it in his own Department Nottingham. Many other conferences and symposia were organized and held during 1964, both by the Meeting Committee and the Nuclear Physics and Solid-state Physics Sub-committees of the Institute and Society and by the twelve branches and eleven specialist subject groups. Details of these are given in the annual report.

The 1964 Guthrie Lecture, which was delivered by Prof. M. Ryle on October 1 during the autumn conference of the Electronics Group at the University of Keele, was entitled "Radio Telescopes" and the text appears in the February 1965 issue of the *Proceedings of the Physical Society* (85, 201; 1965). Dr. P. H. Fowler, who gave the 1964 Rutherford Memorial Lecture on September 9 during the conference on "Low- and Medium-energy Nuclear Physics" at the University of Sussex, spoke on the subject of "Pi-mesons versus Cancer" (*Proc. Phys. Soc.*, 85, 1051 June 1965). Dr. A. R. Lang received the Charles Vernon Boys Prize for his development of the technique of X-ray topography, and Dr. W. Marshall the Maxwell Medal and Prize for his contributions to the theory of magnetism. The presentations were made at the annual dinner of the Institute and Society in London on May 5, 1964. Prof. J. Friedel was awarded the 1964 Holweck Medal and Prize for his work on the electronic structure of metals. The Council has decided that after 1965 the Guthrie and Rutherford Lectures will be replaced by the awards of medals and prizes.

The forty-eighth annual exhibition of scientific instruments and apparatus was held during January 6-9, 1964 in the two halls of the Royal Horticultural Society, London. The *Journal of Scientific Instruments*, in its May 1964 issue, contained several articles and a special survey devoted to equipment displayed at the exhibition. The careful selection of exhibits and the section devoted to educational instruments and experiments were commendable features. The *British Journal of Applied Physics* revised its format to that of the *Proceedings*, but retained the original colour of its cover. A reorganization of subject-matter between the two journals was made. The volume of material submitted to the Institute and Society's three journals continues to increase, but the report directs attention to the growing number of papers which in the form received are unacceptable for publication. The Council had decided to launch a new monthly journal, the *Journal of Physics Education*, principally for sixth-form teachers of physics. In co-operation with other scientific bodies the Institute and Society have urged that closer collaboration be established between editors of physics journals so that by better choice and improvement of articles and by uniformity of presentation the standing and sales of British journals may be enhanced. The *Student Monograph Series* of books, commenced in 1953 and intended for students of the Higher National Certificate in applied physics, has since been widened in scope for undergraduate and graduate students, but since there are now adequate publications of this kind it has been decided to complete the series with the publication of "Subjective Limitations on Physical Measurements" by C. A. Padgham. The volume entitled "Dimensional Analysis and Scale Factors", by R. C. Pankhurst, was published during 1964.

The financial statement refers to the gratifying increase of £13,964 in members' fees and to the increases in the exhibition receipts and in non-members' conference fees. The increase in activities and the general rise in costs

* Report of the Council of the Institute of Physics and the Physical Society for the year 1964. Pp. 25. (London: The Institute of Physics and the Physical Society, 1965.)

combined to raise the total expenditure, and the total surpluses at the end of the year of £158,673 still fell short of the aim to obtain a sum equal to the amount required for one year's working.

The report gives prominence to the proposal by the Council in July 1964, and the agreement in December 1964, to purchase the Fulmer Research Institute. The reasons for the bold venture were announced in the February issue of the *Bulletin* when the purchase was made. Not only will the staff at the Research Institute benefit from the association with the Institute and Society, but investment by the Institute and Society in sponsored

research, regarded solely as a business venture, will be evidence that the Council is of the opinion that the application of physics to industry is a paying proposition. The purchase was made possible by the generous terms of the owners' offer and by a substantial interest-free loan made to the Institute and Society by Imperial Chemical Industries, Ltd. It is intended that surplus revenue from the Fulmer Institute will be used to help to replace the income at present derived from the seven-year covenants signed by a number of industrial firms in favour of the Physics Trust Fund and which will stop in 1967 and 1968.

S. WEINTROUB

SCIENTIFIC RESEARCH IN NEW ZEALAND

THE National Research Advisory Council, New Zealand, was established by the National Research Advisory Council Act of 1963 to advise the Minister of Science on matters related to scientific research in New Zealand. Its first annual report covers the year ending March 31, 1965*.

At its first meeting in April 1964, the National Research Advisory Council decided to set up 16 working parties to review and report on research and service in various fields. The reports of these working parties cover: (1) *agriculture and forestry*, the dairy industry, field crops, forestry, horticulture, meat and wool; (2) *secondary and tertiary industry*, building and construction, manufacturing and engineering, and transport; (3) *earth and related sciences*, the atmospheric sciences, mineral resources, oceanography, limnology and fisheries and solid earth sciences; (4) *basic sciences*, training and services, atomic energy (scientific services for Government departments and agencies are appended); (5) *manpower, education and training* (this report was deliberately delayed until the working party was able to consider the recommendations of the other working parties and to obtain additional information on manpower).

Meanwhile, the Council is satisfied that the implementation of its present recommendations is feasible and would not be jeopardized by a shortage of trained manpower.

For the year ended March 31, 1965, the estimated expenditure on scientific research and services was about £7.5 million and in addition £827,000 was scheduled for expenditure on new laboratories for Government departments and research associations; the total expenditure represented about 0.48 per cent of the gross national product. Of the £7.5 millions, £6.1 millions was provided in the votes of eleven Government departments and, of this, £661,000 went in grants to other research organizations. These figures probably underestimate the total expenditure on science since no allowance is made for expansion by the Post Office, railways, the Broadcasting Corporation, or for the salaries of university staff, accom-

modation or services provided from university funds and used in part for research.

Between 1954-55 and 1964-65 expenditure on science in New Zealand increased from £2.73 millions at about 10 per cent annually, and the scientific staff in Government departments increased in the same period from 568 to about 800. The Council suggests that investment in research for an industry should depend on such criteria as the annual value of production; the national importance of the industry; the significance and number of problems affecting the industry; the chances of a solution being found to selected problems, taking into account the amount of existing scientific information required; the calibre of the staff available and the availability of suitable facilities; the application of results, bearing in mind the problem of implementation because of difficulties of liaison and information services and the time-lag in the application of research results.

The Council recommends that every effort should be made to increase the research effort directed towards New Zealand's agricultural and pastoral products, particularly those of the meat, wool and dairy industries, and that over the next five years research expenditure in this area should be increased cumulatively by £320,000 a year. Forestry research also requires expansion, and a cumulative increase in expenditure of £40,000 per year is recommended. Very high priority to research into problems of the transport industry is also recommended, including an expenditure starting at £10,000 a year and rising to £50,000 a year in five years, while a Transport Research Advisory Committee should be set up to advise the Commissioner of Transport on the operations of the proposed Research Unit. An additional £26,000 is recommended for the existing building research units, and increased support for operational research in the Department of Scientific and Industrial Research and other organizations is highly desirable. An increase in the next year of about £3,000, rising to £18,000 in about five years, is suggested, and £50,000 should be granted for implementing the second year's recommendations of the report of the Mineral Resources Committee.

COMPARATIVE STUDY OF HOUSING IN BRITAIN, FRANCE AND WESTERN GERMANY

BROADSHEET No. 490, issued by Political and Economic Planning and prepared by E. G. Howes, deals with housing in Britain, France and Western Germany*.

In Britain, building research is financed to the extent of about £900,000 per annum at the Building Research

Station, and some £100,000 is spent by the Civil Engineering Research Council; the Minister of Works and Public Building has placed research contracts to the amount of £50,000 with universities. Most building research is carried out privately, but whether this is adequate and whether the information gained is accessible is regarded as very doubtful. In Germany the Federal Government and the Regents devote considerable funds to research and the nationalization of techniques and procedure, and also

* *Planning*, 31, No. 490 (August 1965): *Housing in Britain, France, and Western Germany*. By Eric G. Howes. Pp. 215-270. (London: Political and Economic Planning, 1965.) 7s 6d.

support exhibitions, publications, films and other means of disseminating information (no figures for expenditure, however, are given in the Broadsheet).

For the rest, the Broadsheet concludes that British standards in housing are still high in comparison with those of France, but are being overtaken by those of West Germany. In numbers of dwellings built in recent years (until 1964) Britain has been lagging. Her present need is to step up new building schedules, particularly of dwellings to let, and to demolish and replace slums. Housing policy in Britain has been confused by indecision as to whether the provision of houses should be regarded as a social service, or as a commercial operation. The existence of subsidized and also non-profit housing has almost completely stopped the investment of private capital in dwellings for letting.

While it is proper that local authorities should have the major responsibilities for housing families of all income-levels, it may be argued that subsidies should be reserved for those families that, because of age, lack of earning capacity, or other handicaps, are unable to afford economic rents. In both France and Western Germany the policy is to grant housing allowances to such families and the consequences of adopting such a system in Britain should be explored. In Britain the National Assistance Board pays out rent allowances to 1.5 million families that are eligible for assistance, but the policy elsewhere is to make a clear distinction between housing allowances and public assistance. It is desirable also to diversify the means by which houses for letting at economic rents should be provided: the recently created Housing Corporation should be given every support in its encouragement of cost-rent and ownership societies. There seems to be no point in deterring the private sector from competing in this field.

Adoption of long-term planning policies would enable those engaged in producing dwellings to plan ahead and to

concentrate on their work without fear of too frequent change. It is impossible to ignore the success of German housing problem and the extent to which that attributable to long-term policies. The efforts of West Germany to influence the supply and price of building land have not always been fully rewarded, but have achieved some part of their object. Ways and means whereby the British Government could exert an adequate influence have received intensive study, and action now urgently required. If tax concessions are to be given to some form of saving, like those of Trustee Savings Banks, it is arguable that equivalent concessions should be awarded to Building Societies, the efforts of which directed exclusively to providing finance for housing. The psychological and material effects of such measures have been clearly demonstrated in Western Germany by the successful revival of the saving habit. Encouragement should be given to the house-building part of construction industry to rationalize its organization, to out restrictive practices, intensify training and make the best possible use of the skills and knowledge of its management and work people. The possibility of further curtailment of the apprenticeship period with more intensive training and training on actual work is worth considering. Consideration might also be given to whether it is possible to allow public, commercial and industrial building to outstrip the building of dwellings. Experience in Germany and France suggests that self-building societies, which in small way have done useful work, could be more vigorously encouraged in Britain.

The Broadsheet considers that the trained personnel of the British building industry are probably of as high calibre as, if not higher than, their counterpart in other countries, and to perform the task that confronts them they need only the leadership, guidance and encouragement that vigorous Government backing can provide.

INSPECTION OF ALKALI, ETC., WORKS

THE one hundred and first annual report on alkali, etc., works in England and Wales, prepared by the Chief Inspector*, covers the year 1964. It stresses the co-operation of industry with the Inspectorate without which, individually by works, and collectively through research associations, and the like, the task of the Inspectorate would be much more onerous. Certainly without this co-operation the Inspectorate would have to be multiplied several times in size and a department would be needed for specialized techniques of waste gas sampling and analysis, as well as for developing control measures for air pollution. The number of works registered under the Act at the end of the year was 1,995, involving the operation of 3,208 processes, and 11,966 visits and inspections were paid during the year, compared with 10,850 in 1963, including 123 special visits by the Chief and Deputy Chief Inspectors. Of this total, 231 were to, or in connexion with, works not registered under the Act, 46 were concerned with control of radioactive emissions and 37 were to, or in connexion with, colliery spoilbanks. During the inspections 4,563 quantitative analyses were made of gases evolved from processes in operation, compared with 3,433 in 1963, while 746 special samples, compared with 615 in 1963, were taken and submitted to the Government Chemist.

The report also emphasizes the effect of the changing face of industry on the task and responsibilities of the inspectorate; a searching and realistic appraisal of the responsibilities and day-to-day activities of the inspectorate has been made. A historical and technical review of the decline and fall of the traditional sulphuric acid

lead chamber process and one of the early days of the copper industry are included.

In the belief that most new contact sulphuric acid plants will burn sulphur of high quality as the raw material, tables have been prepared giving the stack heights for final discharge of waste gases from units in clusters of units making daily 200–2,000 tons of monohydrate. If different raw materials are used it will be a simple matter for inspectors and industry to calculate appropriate chimney heights using the information given and to apply it to the particular permitted loss of sulphur burned. Reference is again made to mist emission from certain contact sulphuric acid plants, and while our knowledge of the reasons for this is still obscure the problem receives the close attention of the Inspectorate (some progress appears to have been made).

The Chief Inspector, under "Alkali, etc., Works and Regulations (Scotland) Acts, 1906 and 1951", records that at December 31, 1964, 263 works were registered under the 1906 Act, and 361 processes were inspected within these works. 622 visits were paid by the Inspectorate on works under the Act or in the interest of preventing air pollution, of which 420 were to premises registered under the Act of 1906.

Further experimental testing of emissions of dust from registered premises was carried out on behalf of the Inspectorate by the two-man team provided on contract by the Department of Scientific and Industrial Research. Thirteen works were covered in 41 visits, and 87 tests were made mostly at the electricity power stations. In general, inspections showed that the provisions of the Act were observed in good spirit as well as with attention to the tests.

* Ministry of Housing and Local Government—Scottish Development Department. One Hundred and First Annual Report on Alkali, etc., Works by the Chief Inspectors, 1964. Pp iv+76 (London: H.M.S.O., 1965) 5s. 6d. net.

INTERACTION OF CEPHALORIDINE WITH PENICILLINASE-PRODUCING GRAM-NEGATIVE BACTERIA

By DR. J. M. T. HAMILTON-MILLER, DR J. T. SMITH and PROF. R. KNOX

Department of Bacteriology, Guy's Hospital Medical School, London

CEPHALORIDINE (7-((2-thienyl) acetamido)-3-(1-pyridylmethyl) cephalosporanic acid, 'Ceporin') has been shown to be of value in the treatment of infections caused by Gram-negative bacteria^{2,3}. Although many cephalosporins are resistant to hydrolysis by β -lactamases in Gram-positive bacteria^{1,2,4,5}, there have been several reports^{2,6-10} that they are readily hydrolysed by certain Gram-negative bacteria. This destruction has been attributed to an enzyme 'cephalosporinase', although it has seldom been shown to be enzymatic and the end-product has not been identified. In view of these reports, it was decided to investigate the action of organisms known to produce penicillinase (EC 3.5.2.6.) on cephaloridine, and the action of cephaloridine on these strains of bacteria. In addition, the relationship between 'cephalosporinase' and 'penicillinase' activities was studied. The following bacterial strains were used in the investigations: *Klebsiella aerogenes* 1, 43, 83, 366, 370, 373, 2, 407, 414, 415 and 418; *K. ozaenae* 61; *Escherichia coli* 214T, 419, K 12 (F⁻ met⁻) and TEM; *Aerobacter aerogenes* 15 and 53. All these strains produce penicillinase constitutively. *Proteus morgani* G and 185 and *Pr. tegeri* 410, which produce an inducible penicillinase, were also used. We are very grateful to Dr. Naomi Datta for supplying *E. coli* K 12 and TEM. All the other strains were isolated from clinical specimens at Guy's Hospital and are referred to by their laboratory reference numbers. Organisms were cultured in infusion broth, collected by centrifugation and disrupted by ultrasonic treatment, previously described^{11,12}. The *Proteus* strains, which gave little or no basal penicillinase activity¹³, were cultured in sub-inhibitory concentrations of benzylpenicillin, inducing agent before gathering. Such cell-free penicillinase preparations hydrolysed the β -lactam bond in certain penicillins, and it was of interest to determine whether the β -lactam bond present in cephaloridine was so susceptible to hydrolysis by these β -lactamases. The rates of destruction of penicillins and of cephaloridine, brought about by the β -lactamase preparations, were measured either acidimetrically (by the spectrophotometric method), or by the hydroxylamine method. This latter method had to be modified slightly from that previously used¹²: it was found that the hydroxamate of cephaloridine was somewhat unstable, in that it started to decompose after about 5 min; however, the hydroxamate was fully formed in 3 min, and addition of the ferric ammonium sulphate reagent at this time gave a chromogen of maximum intensity. With this modification, satisfactory results were obtained by the use of this assay both for cephaloridine and for penicillins.

Rate of hydrolysis of cephaloridine. Cell-free penicillinase preparations from the various strains were incubated in the presence of cephaloridine (initial concentration 5 mM) at 37° in 25 mM sodium phosphate buffer, pH 7.4; the rate at which cephaloridine was destroyed by each preparation was compared with the rates of hydrolysis of benzylpenicillin, ampicillin and 6-aminopenicillanic acid. Results are shown in Table 1, taking the rate of benzylpenicillin hydrolysis as 100 in each case. It can be seen that cell-free extracts of the *Klebsiella* and *Aerobacter* strains destroy cephaloridine at approximately the same rate as 6-aminopenicillanic acid, but less rapidly than ampicillin; the *E. coli* and *Proteus* preparations, however, which destroy ampicillin and 6-aminopenicillanic acid only very slowly, hydrolyse cephaloridine more rapidly than benzylpenicillin under these conditions.

The enzymes from the *Proteus* strains hydrolyse cephaloridine many times more rapidly than benzylpenicillin.

Identity of 'penicillinase' with 'cephalosporinase'. The distinction between penicillinase and cephalosporinase activity is not at all clear. Some authors^{4,10} claim that cephalosporinases and penicillinases are different enzymes whereas Ayliffe⁶ leaves the question open. The following three approaches were used in an attempt to answer this question.

(1) Cell-free extracts of *A. cloacae* 53, *E. coli* 214T and 419 and *K. aerogenes* 366 were treated with protamine sulphate to remove nucleoprotein, and the supernatant fractions were gel filtered on 'Sephadex G50'; each fraction was then assayed against both benzylpenicillin and cephaloridine. In each case, destruction of the two substrates ran in parallel in each fraction.

(2) Penicillinase preparations from *K. aerogenes* 366 and 373 were exposed to 1.31 mM iodine in 2 mM potassium iodide for 1, 2 or 3 min, at 30°, the reaction being stopped when required by the addition of an appropriate volume of 19 mM sodium thiosulphate. Aliquots were then assayed in parallel against benzylpenicillin and against cephaloridine. In each instance, reduction of the 'penicillinase' activity occurred to precisely the same extent as did reduction of the 'cephalosporinase' activity.

(3) The three *Proteus* strains were cultured in the presence of a range of concentrations of benzylpenicillin, varying from one-half to one-twentieth of the minimum inhibitory concentration. Cell-free preparations were then assayed against benzylpenicillin and against cephaloridine. In the absence of inducing agent, there was no activity against either substrate, but in cultures grown in the presence of benzylpenicillin, induction of 'penicillinase' activity ran quantitatively parallel to induction of 'cephalosporinase' activity, for each concentration of inducing agent.

Thus these data suggest that, in the strains investigated so far, penicillinase and cephalosporinase activities are manifestations of one and the same enzyme.

Affinity of substrates for enzymes. The effect on the rate of hydrolysis of varying the concentration of various

Table 1. RATES OF HYDROLYSIS OF CEPHALORIDINE, AMPICILLIN AND 6-AMINOPENICILLANIC ACID BY CELL-FREE PREPARATIONS OF PENICILLINASE-PRODUCING GRAM-NEGATIVE BACTERIA IN M/40 SODIUM PHOSPHATE BUFFER pH 7.4

Initial substrate concentration was 5 mM. The rate of destruction of benzylpenicillin is expressed in μ moles/min/10⁹ bacteria/ml. The rates of destruction for the other β -lactam antibiotics are expressed in terms of μ moles/min, taking the rate of destruction of benzylpenicillin as 100 in each case

Strain of bacteria	Absolute rate of destruction of benzylpenicillin	% Rates of destruction taking the rate of benzylpenicillin destruction as 100%		
		Cephaloridine	Ampicillin	6-Aminopenicillanic acid
<i>A. cloacae</i>	15	5.4	123	20
" "	53	430.0	105	15
<i>E. coli</i>	214T	54.4	—	—
" "	419	10.3	—	—
" "	K12	1.5	—	—
" "	TEM	1.6	—	—
<i>K. aerogenes</i>	1	10.0	210	60
" "	43	10.5	232	59
" "	83	1.7	163	41
" "	366	3,180.0	198	78
" "	370	22.0	187	60
" "	373	1,360.0	195	77
" "	402	7.6	227	68
" "	407	5.4	185	55
" "	414	3.8	151	48
" "	415	10.3	260	77
" "	418	20.6	175	55
<i>K. ozaenae</i>	61	22.3	200	68
<i>Pr. morgani</i>	G	60.0	—	—
" "	185	57.9	1,012	—
<i>Pr. tegeri</i>	410	4.8	3,800	—

—, Destruction too slow to be measured accurately (that is, < 10).

substrates was investigated, using cell-free bacterial extracts, by the use of the spectrophotometric assay. From the progress curves obtained, Michaelis constants were calculated where possible; results are shown in Table 2. Cephaloridine has a lower affinity, as can be seen, for the penicillinases from the *K. aerogenes* and *E. coli* strains than have either benzylpenicillin or ampicillin; thus, at concentrations that are obtained in the blood¹ during therapy (approximately 20 μ M), cephaloridine will be at least 17 times as stable as ampicillin to *K. aerogenes* penicillinase. In the case of the *A. cloacae* enzyme, the affinity of cephaloridine is some 8 times as high as that of ampicillin. Thus, by calculation, towards the penicillinase of this organism cephaloridine will be 3.5 times as labile as ampicillin, at concentrations attained therapeutically; this should be contrasted with the situation at 5 mM (Table 1), when cephaloridine is less labile than ampicillin.

Ease of entry into intact bacteria. Most penicillinase-producing Gram-negative bacteria studied in this laboratory¹¹ possess accessibility barriers against penicillins; the extent of the barrier for any one substrate is expressed as the 'permeability factor'¹⁴ (which is the fold increase in hydrolytic activity toward a substrate subsequent to disruption of an intact bacterial suspension). Permeability factors have been determined for benzylpenicillin, ampicillin, 6-aminopenicillanic acid and cephaloridine for 18 strains; values are tabulated in Table 3. The *Proteus* strains have been omitted because the extent of the permeability barrier depends on the nature and the concentration of the inducing agent¹³. From the results it is quite clear that cephaloridine can enter bacterial cells of every strain tested with complete freedom, whereas these same cells possess a marked accessibility barrier to benzylpenicillin and ampicillin. 6-aminopenicillanic acid, although obtaining considerably easier access than the latter two compounds, does not have complete freedom of entry in all strains. A consequence of this free access is that, whereas the crude penicillinase of *A. cloacae* 53 hydrolyses cephaloridine at 61 per cent of the rate of ampicillin (to which the intact cells have a permeability factor of 8), cephaloridine was found to be hydrolysed by intact cells about 5 times as rapidly as ampicillin. There does not seem to be an adequate explanation, in terms of molecular size, charge or the like, for this property of cephaloridine. One explanation which could be put forward to account for this phenomenon is that cephaloridine affects the cells so that the penicillinase becomes extracellular and thus there is no longer any barrier between enzyme and substrate. However, this was disproved by the findings in the following experiment.

Table 2. AFFINITY OF CELL-FREE PENICILLINASE PREPARATIONS FOR THREE SUBSTRATES

Bacterial strain	Michaelis constants expressed as μ M		
	Benzylpenicillin	Ampicillin	Cephaloridine
<i>A. cloacae</i> 53	≤ 8	541	69
<i>K. aerogenes</i> 366	≤ 68	≤ 143	1,330
<i>K. aerogenes</i> 373	≤ 68	≤ 143	1,200
<i>E. coli</i> 419	≤ 68	*	276

* No appreciable hydrolysis was detected at any substrate concentration.

Table 3. PERMEABILITY FACTORS FOR 4 DRUGS AND 18 BACTERIAL STRAINS, MEASURED IN 25 mM PHOSPHATE BUFFER pH 7.4

Strain of bacteria	Benzylpenicillin	Ampicillin	6-Aminopenicillanic acid	Cephaloridine
<i>A. cloacae</i> 15	1	1	1	1
" 53	18	8	1	1
<i>E. coli</i> 214T	10	—	—	1
" 419	4	—	—	1
" K12	1	—	—	1
" TEM	2	—	—	1
<i>K. aerogenes</i> 1	6	7	1	1
" 43	11	13	2	1
" 83	2	2	2	1
" 366	11	14	2	1
" 370	8	8	1	1
" 373	9	5	2	1
" 402	3	2	3	1
" 407	3	2	1	1
" 414	2	2	2	1
" 415	4	3	1	1
" 418	4	4	1	1
<i>K. ozaenae</i> 61	3	3	1	1

Table 4. INHIBITION OF BENZYL-PENICILLIN AND CEPHALORIDINE HYDROLYSIS BY CLOXACILLIN, METHICILLIN AND QUINACILLIN

The concentration of inhibitor required to cause 50 per cent inhibition (I_{50}) is expressed as μ M (initial substrate concentration was 2.7 mM)

Penicillinase prep.	Substrate	Cloxacillin	I_{50} values Methicillin	Quinacillin
<i>K. aerogenes</i> 366	Benzylpenicillin	431*	10*	70†
	Cephaloridine	80	0.32	12.4
<i>K. aerogenes</i> 373	Benzylpenicillin	711*	7*	161†
	Cephaloridine	90.5	0.4	25.4
<i>E. coli</i> 419	Benzylpenicillin	0.8	52.4	> 1,090
	Cephaloridine	0.1	4.8	392

* Taken from ref. 15.

† Taken from ref. 14.

Table 5. MINIMUM INHIBITORY CONCENTRATIONS (μ g/ml.) OF CEPHALORIDINE AND AMPICILLIN FOR 16 STRAINS OF PENICILLINASE-PRODUCING GRAM-NEGATIVE BACTERIA

Inoculum	G	Cephaloridine		Ampicillin	
		10 ⁷	10	10 ⁷	10
<i>Pr. morgani</i>	185	2,500	313	500	125
" "	185	1,250	39	250	31
<i>Pr. rettgeri</i>	410	2,500	5	2,000	12
<i>K. aerogenes</i>	1	125	31	2,000	31
" "	43	125	16	1,000	31
" "	370	125	4	2,000	16
" "	402	25	3	1,000	31
" "	407	32	2	1,000	16
" "	414	31	4	1,000	16
" "	415	25	3	200	13
" "	418	12	2	1,000	25
<i>A. cloacae</i>	15	100	10	125	10
" "	53	8,000	500	20,000	3,200
<i>E. coli</i>	214T	200	25	250	250
" "	419	2,000	16	2,000	2,000
" "	K12	16	2	8	2

Whole-cell suspensions were assayed against cephaloridine (initial concentration 5 mM); they were then spun down, re-suspended in fresh buffer and re-assayed against cephaloridine. It was found, allowing for small losses due to lysis and the centrifugation and re-suspending operations, that the pre-incubation with cephaloridine had no resulted in any loss of enzyme from the intact cells in the supernatant fluid.

Inhibition of penicillinase activity. The hydrolysis of benzylpenicillin by penicillinases from Gram-negative bacteria is inhibited by methicillin, certain isoxazol penicillins and quinacillin^{14,15}. I_{50} values¹⁶ were determined, using cephaloridine (2.7 mM) as substrate, for methicillin, cloxacillin and quinacillin; results are shown in Table 4 (the I_{50} values obtained previously^{14,15} using benzylpenicillin as substrate for *K. aerogenes* 366 and 373 enzymes are included for comparison); it is clear that cephaloridine hydrolysis by these penicillinases is more susceptible to inhibition than is benzylpenicillin hydrolysis. Inhibition of cephaloridine hydrolysis by methicillin appears to be competitive, as increasing the substrate concentration with a given amount of inhibitor decreases the amount of inhibition.

Antibacterial activity against penicillinase-producing Gram-negative bacteria. Minimum inhibitory concentrations were determined for cephaloridine, using the serial doubling dilution technique; two sizes of inoculum were used, about 10⁷ and 10 organisms per tube, and incubation was carried out for 16 h. Results are shown in Table 6. It can be seen that cephaloridine is approximately 10 times as effective as ampicillin against the penicillinase-producing ampicillin-resistant *K. aerogenes* strains against the other strains there seems to be little to choose between cephaloridine and ampicillin as regards antibacterial activity. In most cases there was a marked inoculum size effect¹⁶.

It is generally accepted that a direct causal relationship exists between penicillinase activity and resistance to hydrolysable penicillins. Cephaloridine was destroyed more rapidly than ampicillin by intact cells from the majority of the strains studied here and hence it would be expected that cephaloridine would be a less effective antibacterial agent than ampicillin. However, with the exception of the *Proteus* strains, the reverse was found to be true with the strains examined in this report. This apparently paradoxical situation can be explained by the discovery made here that the entry of cephaloridine into these Gram-negative bacteria is not hindered by a cellular

accessibility barrier, whereas entry of all the penicillins so hindered^{11,12}.

With eight strains of penicillinase-producing ampicillin-resistant *K. aerogenes* cephaloridine was found to be more effective than ampicillin, in terms of minimum inhibitory concentrations, against both large and small inocula. Even so, therapeutic administration of cephaloridine may not give rise to blood levels¹ sufficiently high to have an antibacterial action *in vivo* with these strains, but the levels attainable in urine¹ suggest that cephaloridine may have a place in the treatment of urinary tract infections caused by similar organisms. However, the *in vivo* performance of a drug cannot be predicted solely in terms of its effectiveness in *in vitro* sensitivity tests. Other factors, for example, absorption, excretion and possible allergism with penicillinase-resistant β -lactam antibiotics, must be considered before this antibiotic can be recommended for use in infections caused by penicillinase-producing Gram-negative bacteria.

We thank Beecham Research Laboratories, Ltd., Boots Pure Drug Co., Ltd., and Glaxo Laboratories for

gifts of penicillins and cephalosporins. We also thank Mrs. B. Heywood and Miss C. Nicholls for their assistance.

¹ Muggleton, P. W., O'Callaghan, C. H., and Stevens, W. K., *Brit. Med. J.*, ii, 1234 (1964).

² Murdoch, J. McC., Speirs, C. F., Geddes, A. M., and Wallace, E. T., *Brit. Med. J.*, ii, 1238 (1964).

³ Stewart, G. T., and Holt, R. J., *Lancet*, ii, 1305 (1964).

⁴ Newton, G. G. F., and Abraham, E. P., *Nature*, 175, 548 (1955).

⁵ Crompton, B., Jago, M., Crawford, K., Newton, G. G. F., and Abraham, E. P., *Biochem. J.*, 83, 52 (1962).

⁶ Fleming, P. C., Goldner, M., and Glass, D. G., *Lancet*, i, 1399 (1963).

⁷ Jago, M., Mighaici, A., and Abraham, E. P., *Nature*, 199, 375 (1963).

⁸ Ayliffe, G. A. J., *Nature*, 201, 1032 (1964).

⁹ Walton, R. B., *Science*, 143, 1-38 (1964).

¹⁰ Chang, T. W., and Weinstein, L., *Antimicrobial Agents and Chemotherapy*, 1963, 278 (1964).

¹¹ Smith, J. T., and Hamilton-Miller, J. M. T., *Nature*, 197, 976 (1963).

¹² Hamilton-Miller, J. M. T., Ph.D. thesis, London Univ. (1964).

¹³ Hamilton-Miller, J. M. T., *Biochem. Biophys. Res. Comm.*, 13, 43 (1963).

¹⁴ Smith, J. T., Hamilton-Miller, J. M. T., and Knox, R., *Nature*, 203, 1148 (1964).

¹⁵ Hamilton-Miller, J. M. T., and Smith, J. T., *Nature*, 201, 999 (1964).

¹⁶ Hamilton-Miller, J. M. T., Smith, J. T., and Knox, R., *Nature*, 201, 867 (1964).

THE IRON DEXTRAN COMPLEX

By DR. C. R. RICKETTS

Medical Research Council Industrial Injuries and Burns Research Unit, Birmingham Accident Hospital

AND

DR J. S. G. COX, C. FITZMAURICE and G. F. MOSS

Research Department, Fisons Pharmaceuticals, Ltd., Holmes Chapel, Cheshire

PHARMACEUTICAL preparations ('Imferon' and 'Imposil', Fisons Pharmaceuticals, Ltd. (Benger Laboratories)) containing iron and dextran are used for the treatment of iron-deficiency anaemia in man and farm animals. They are prepared by neutralizing ferric chloride in the presence of an alkali-modified dextran and heating to form a stable clear brown solution. The nature of the linkage between the iron and the dextran and the molecular weight of the product are not known. The following techniques seemed likely to throw light on the nature of the iron dextran preparation: (1) gel-filtration on 'Sephadex'; (2) ultrafiltration; (3) separation in the ultracentrifuge in comparison with coloured proteins of known molecular weight.

Separation on 'Sephadex' columns. A column of 'Sephadex G200' (2.4 cm x 100 cm) using 0.9 per cent sodium chloride for elution was available and had previously been calibrated with dextran fractions of known molecular weight distribution (Pharmacia, Ltd.) and with serum proteins. The reproducibility of chromatography had been established using a sample of dextran labelled with iodine-125 (ref. 1); on successive runs the size of each fraction was reproducible within ± 1 per cent of the whole. In an initial experiment 'Imferon' (1 ml.) was mixed with 0.1 ml. of 0 per cent iodine-125 dextran and diluted to 5 ml. with saline. A sample of 1 ml. of this diluted solution was applied to the column and eluted with saline into 5-ml. fractions. Part of the iron was adsorbed on the 'Sephadex' making the whole column brown, the remaining iron being eluted. The adsorbed iron could not be eluted with saline and was finally recovered by elution with N sulphuric acid.

The radioactivity, the brown colour (430 m μ) of iron and the dextran con-

centration of the fractions are shown in Fig. 1. The radioactivity measurements showed that the chromatography was comparable with previous calibrations. From the position of the iron peak one might infer that the molecular weight of the iron dextran complex was less than that of the labelled dextran which had \bar{M}_w about 110,000. The peak lies between the positions of peaks previously found for dextrans of \bar{M}_w 75,000 and \bar{M}_w 40,000 and is closer to the lower value. The dextran peak is adjacent to the peak due to inorganic iodide ions and is to the low molecular weight side of a peak previously obtained with a dextran fraction of \bar{M}_w 9,400; its position is thus consistent with a molecular weight of about 5,000. However, the shape of this peak

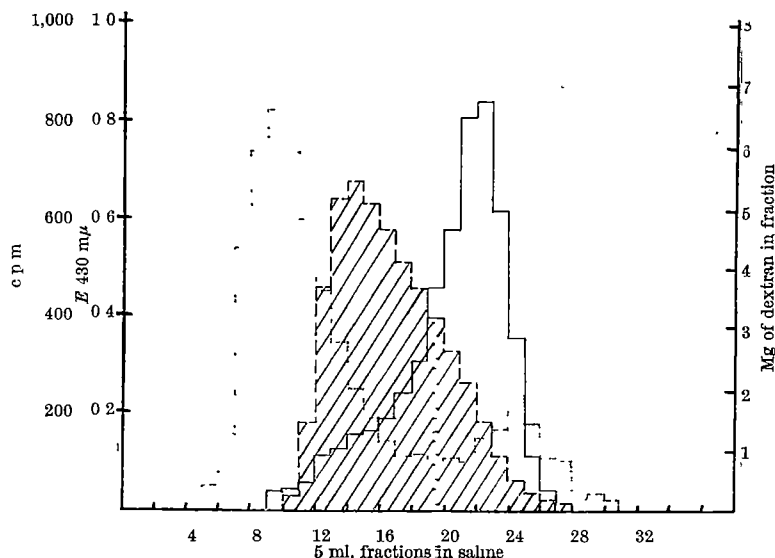


Fig. 1. Gel-filtration of 'Imferon' on a column of 'Sephadex G200' in 0.9 per cent sodium chloride solution. The dotted line shows the distribution of radioactivity from ¹²⁵I dextran of \bar{M}_w 110,000 used as a reference substance. The dashed line and shaded area denote the distribution of iron and the solid line denotes the distribution of dextran from the 'Imferon'.

is worth considering. Dextrans of \bar{M}_w 40,000 and below give a symmetrical peak on columns of 'Sephadex G200'. Dextrans of higher molecular weight give an unsymmetrical peak, sharpened on the high molecular weight side, that is, the tail toward the low molecular weight side. The dextran peak in Fig. 1 has a tail toward the high molecular side, suggesting that the iron is carrying some of the dextran through the column ahead of most of the dextran. This is an indication of the existence of a linkage between the iron and the dextran existing under the physiological conditions of isotonic saline and a measured pH of 6.8. However, adding up the material in the fractions, 101 per cent of the radioactivity, 97.7 per cent of the dextran but only 32 per cent of the iron were recovered. Evidently 68 per cent of the iron had been separated from dextran under these mild conditions, possibly through competition for the iron between the dextran and 'Sephadex', which is a cross-linked and insoluble form of dextran.

This initial experiment suggested that 'Sephadex G75' might separate the iron-dextran complex from free dextran. Molar sodium sulphate solution was chosen in an attempt to minimize adsorption of iron on to 'Sephadex'.

A sample of 0.5 ml. of 1 in 5 dilution of 'Imferon' was applied to a column of 'Sephadex G75' (1.4 cm \times 48.5 cm) and eluted into 2 ml. fractions with M sodium sulphate. The separation of the iron complex from free dextran is shown in Fig. 2. Recovery of the iron was 87.4 per cent and of dextran was 95.5 per cent. Of the recovered dextran 61.4 per cent was free and the remainder was associated with iron. The iron-dextran ratio of fractions 9, 10 and 11 was 0.536, 0.533 and 0.470 respectively, that is, about two parts of dextran to one part of iron.

It was found that under the same conditions the dextran fraction used in the preparation of 'Imferon' was eluted in the same volume as the second dextran peak of Fig. 2. This would indicate that the second peak was either dextran liberated by breakdown of the complex, free dextran which was present in 'Imferon' but not complexed with iron or a combination of these.

Later experiments using a 'Sephadex G75' column showed that between 75-85 per cent of the iron could be recovered in the iron-dextran peak by elution with 0.9 per cent sodium chloride. These suggest that it may not have been an effect of ionic strength which was governing the adsorption of the iron on to 'Sephadex', but the length of time that the 'Imferon' was in contact

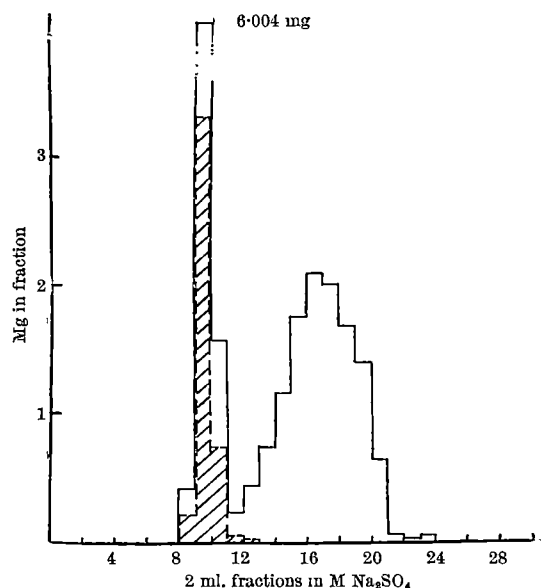


Fig. 2. Gel filtration of 'Imferon' on a column of 'Sephadex G75' in M sodium sulphate solution. The hatched area denotes the distribution of iron and the solid line denotes the distribution of dextran. —, Mg dextran in fraction; ---, mg iron in fraction

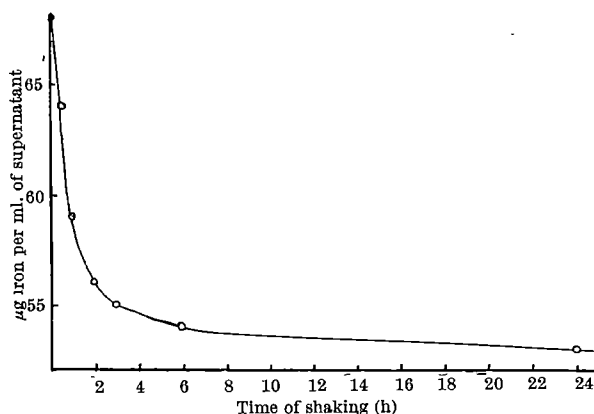


Fig. 3. The adsorption of iron on to 'Sephadex G75' in 0.9 per cent sodium chloride solution.

with the dextran gel. This was confirmed by examining the effect of flow-rate on the adsorption of iron on 'Sephadex G75' column. By elution at a flow-rate of 100 ml./h 70 per cent of the iron was recovered, whereas at 5 ml./h only 20 per cent of the iron was recovered.

There were no differences in the relative peak positions for the two fractionations but the amount of dextran associated with the iron in the first peak varied according to the flow-rate so that the iron to dextran ratio was 0.33 : 1 with a short elution time and 0.5 : 1 with a long elution time.

In order to examine further the effect of time on the adsorption of iron on to 'Sephadex' a 1-ml. aliquot of 1 in 20 dilution of 'Imferon' was added to 1 gm of 'Sephadex G75' in 50 ml. of 0.9 per cent sodium chloride. The mixture was shaken for 5 min and a 2-ml. aliquot removed, centrifuged and the iron content estimated on the clear supernatant using bathophenanthroline reagent². The mixture of 'Imferon' and 'Sephadex G75' was shaken for 24 h, 2-ml. samples being removed at intervals, centrifuged and the iron contents of the supernatants estimated. Fig. 3 shows the effect of plotting iron content of the supernatant against the time the 'Imferon' was in contact with 'Sephadex'. By 24 h, when an equilibrium appeared to have been established, 22 per cent of the added iron had been adsorbed by the 'Sephadex'. During the first 2-3 h there was a very rapid fall in the iron content of the supernatant while during the next 20 h the rate of adsorption of the iron was very much slower.

When the amount of added 'Imferon' was reduced to one-tenth of that in the previous experiment a similar result was obtained with 30 per cent of the iron being bound to the 'Sephadex'. Using a polyacrylamide gel in place of 'Sephadex' gave a similar result.

Ultrafiltration. The 'Sephadex' gel fractionation suggested that 'Imferon' might contain uncombined dextran and it was thought that this could be more easily separated using ultrafiltration.

'Imferon' was not retained by a membrane with a porosity of 10 μ , but cellulose ('Visking') film retained the iron-dextran complex while allowing uncombined dextran to pass through extremely slowly. In order to increase the pore size of the filter the membrane was treated with 60 per cent zinc chloride solution for 10 min. Membranes treated in such a way completely retained bovine haemoglobin, which has a diameter of 2.7 μ . A solution of 10 ml. of 'Imferon' diluted to 40 ml. with water was subjected to filtration through such a membrane at 80 lb./in.² pressure. At intervals the volume of the 'Imferon' solution was restored by adding quantities of water equal to the volume of fluid which had passed through the filter. After a period of three weeks a total of 160 ml. had filtered. Table 1 shows the analysis of the solutions.

Zone ultracentrifugation in a density gradient. Density gradient tubes were prepared³ containing carbon tetra-

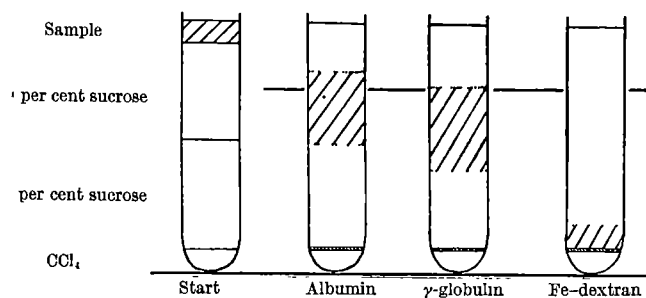


Fig. 4. Ultracentrifugation, in a sucrose density gradient, of 'Imferon' and for comparison serum proteins coupled with 'Procion' dyes

loride (0.3 ml.), which has a density of 1.59, 30 per cent crose solution (2.3 ml.) of density 1.26 and 10 per cent crose (2.3 ml.) of density 1.07. The sample (0.2 ml.), having a density of less than 1.07, was carefully layered on top and the tubes were centrifuged at 35,000 r.p.m. for 16 h. Samples used were a 1 in 20 dilution of 'Imferon', a 4 per cent solution of albumin coupled with 'Procion' red dye (Imperial Chemical Industries, Ltd.) and a 4 per cent solution of γ -globulin coupled with 'Procion' blue dye (Imperial Chemical Industries, Ltd.). After centrifuging, the tubes presented the appearance shown in Fig. 4. Traces of insoluble protein had collected at the carbon tetrachloride interface. The iron colour was close and at the interface, indicating a much higher molecular weight than albumin 69,000 or γ -globulin 156,000.

Discussion

In the initial 'Sephadex' gel filtration experiment the position of the eluted iron peak lay between the positions previously found for dextrans of \bar{M}_w 75,000 and 40,000.

Table 1. ANALYSIS OF SOLUTIONS OBTAINED BY ULTRAFILTRATION OF 'IMFERON'

	Iron concentration	Dextran concentration	Iron dextran ratio
Original 'Imferon' solution	1.22%	5.42%	0.224
Final 'Imferon' solution	2.8%	6.7%	0.418
Ret 10 ml. filtrate	50 p.p.m.	2.3%	—
Ret 10 ml. filtrate	1 p.p.m.	0.14%	—

However, it seems very likely that the iron peak was retarded in its progress through the column by adsorption so that its position did not truly indicate molecular size. Adsorption of iron in this way would increase the proportion of free dextran detected.

The ultracentrifugation experiment showed that the iron-dextran complex has a molecular or particle size much greater than that of γ -globulin. Muir and Golberg⁴ showed by electron microscopy the presence of electron-dense particles 2–3 μ in diameter, and the particulate nature of the complex was confirmed by an electron micrograph of one of the fractions (fraction 10, Fig. 2) which showed particles approximately 3–4 μ in diameter.

The ultrafiltration experiments showed conclusively that a large proportion of uncombined dextran (42.2 per cent) was present. By this means it was possible to remove the latter, leaving what was presumably a solution containing only iron-dextran complex. The ratio of iron to dextran in this solution was 0.418:1, which is slightly higher than that calculated for one iron atom per anhydroglucose unit, in which the ratio is 0.346:1. The ratio corresponds with 2.4 parts of dextran to 1 part of iron.

The molecular weight of the dextran from which the preparation is made is little more than 5,000. In order to have a molecular weight greatly exceeding 150,000 and a particle size of 2–3 μ there must be considerable cross-linking or association of molecules to form the complex. Further investigations are being carried out on this aspect.

Electron micrographs of fraction 10 were kindly prepared and interpreted by Dr. A. T. Charles, Medical Research Council Unit for Research on the Experimental Pathology of the Skin, University of Birmingham.

¹ Brooks, S. A., Davis, J. W. L., Graber, I. G., and Ricketts, C. R., *Nature*, **188**, 675 (1960).

² Webster, D., *J. Clin. Path.*, **13**, 246 (1960).

³ Stanworth, D. R., Squire, J. R., and James, K., *Anal. Biochem.*, **2**, 324 (1961).

⁴ Muir, A. R., and Golberg, L., *Quart. J. Exp. Physiol.*, **46**, 290 (1961).

PENICILLINASE SYNTHESIS CONTROLLED BY INFECTIOUS R FACTORS IN ENTEROBACTERIACEAE

By DR. NAOMI DATTA

Department of Bacteriology, Postgraduate Medical School, London

AND

DR. POLYXENI KONTOMICHALOU

Department of Bacteriology, Clinic of Therapeutics, University of Athens

RESISTANCE to antibacterial drugs may be transferred from one bacterium to another among the Enterobacteriaceae by direct cell-to-cell contact (conjugation). This transferable resistance was discovered in Japan¹⁻³ and later reported in Britain⁴ and in Germany⁵. The infectious agents which mediate the transfer are known as resistance factors (*R* factors) and have been shown to be extra-chromosomal genetic elements, or plasmids, consisting of deoxyribonucleic acid^{3,6}. *R* factors are transferred by conjugation between cells of many genera—all the Enterobacteriaceae⁷ as well as other Gram-negative bacilli such as *Vibrio*⁸, *Serratia*⁹ and *Pasteurella*¹⁰. The resistance conferred by the *R* factors first described was against the four drugs streptomycin, tetracycline, chloramphenicol and sulphonamides, usually of all four together, but sometimes against three, two or one of them. Lebek⁵ described an *R* factor which conferred resistance to neomycin and kanamycin, as well as to the original four drugs.

That genetic information for resistance to penicillins, including ampicillin, could be carried on *R* factors was found independently in Britain¹¹, in Greece (Kontomichalou, unpublished data) and in Switzerland (Lebek, personal communication). Preliminary experiments¹¹ suggested that infectious resistance to penicillins was dependent on the production of a penicillin-destroying enzyme. We now report that this enzyme is penicillinase (*EC* 3.5.2.6), since on incubation with benzylpenicillin it liberates penicilloic acid. We therefore present evidence that the genetic information for the biosynthesis of penicillinase is carried on the *R* factor.

R factors and host bacteria. The *R* factors we have studied are listed in Table 1. The host bacteria in which penicillin resistance was studied were *Escherichia coli* K12, *E. coli* strain TEM the *R* factor of which had been eliminated by acriflavine treatment¹², and *Salmonella typhi* strain 152, a drug-sensitive strain isolated in Greece in 1962.

Table 1

R factor	Species in which isolated	Year of isolation	Place of isolation	Pattern of infective resistance
R _{TEM}	<i>Escherichia coli</i> , strain TEM	1963	Athens	A S
R ₁₅₁₅	<i>Salmonella typhimurium</i> (ref. 11) phage type 1a	1962	Brighton	A S T Su
R ₇₂₆₈	<i>Salmonella paratyphi</i> B	1963	London	A C Su K

Species resistant to: A, ampicillin; C, chloramphenicol; S, streptomycin; Su, sulphonamides; T, tetracycline; K, kanamycin.

The R factors were transferred from their original hosts to the bacterial strains used experimentally by growing resistant and sensitive cultures together in broth, and plating on medium selective for the recipient organisms, but incorporating ampicillin. For example, in transferring resistance from *Salmonella* to *E. coli* the mixtures were plated on minimal agar with lactose as sole C source and with ampicillin, 25 µg/ml.

Stability of R factors in their bacterial hosts. Cultures carrying R factors contain a variable proportion of drug-sensitive cells from which the factor has been entirely lost^{1,2}. The rate of loss varies greatly between different R factors and between different hosts. The rate of loss of all the 3 R factors we describe here was 1:1,000 cell divisions or less, in the original hosts as well as in *E. coli* K12, *E. coli* TEM and *S. typhi* 152.

'Training' to ampicillin resistance. The sensitive host bacteria, *E. coli* K12 and *E. coli* TEM, were 'trained' to become resistant to ampicillin by passage on nutrient agar gradient plates containing increasing concentrations of ampicillin. These laboratory-trained resistant cultures were compared with R factor infected cultures in the experiments described later (see Table 2).

Extraction of enzymes. No penicillinase was found in supernatant fluid from our cultures carrying R factors. Highest yields of enzyme were from suspensions broken ultrasonically with a Mullard drill. Fast centrifugation (105,000g for 2 h) of the ultrasonicates left all activity in the supernatant. Methods of extracting enzyme without disrupting the cells, such as by toluene treatment, ethylenediamine tetraacetic acid (EDTA) at pH 8 (ref. 13) or EDTA + 20 per cent (w/v) sucrose at pH 8 (ref. 14), liberated up to 35 per cent of the penicillinase activity found in ultrasonicated preparations. Extraction of the enzyme by these methods, which leave the cell membrane intact, indicates that, like some other degradative enzymes in *E. coli*¹⁴, these penicillinases are 'periplasmic' enzymes.

Penicillinase assays. The cultures with and without R factors (R+ and R-) were assayed iodometrically for

hydrolysis of benzylpenicillin, ampicillin and cephaloridine the latter two drugs being chosen because of their clinical importance in treatment of infection caused by Gram-negative bacilli. 6-Amino penicillanic acid (6-APA) also used, because it had been found¹⁵ to be a highly effective antibiotic against at least one strain of penicillinase-producing Gram-negative bacilli.

Bacteria from exponential phase-aerated broth cultures were collected by centrifugation and the bacteria resuspended in distilled water, the volume being chosen according to the expected enzyme activity of the preparation. Assays were made using these suspensions untreated and after ultrasonic disruption. The method of assay was that of Perret¹⁶ as modified by Nevick¹⁷. Hydrolysis of cephaloridine was measured similarly to that of benzylpenicillin and ampicillin and 6-APA, except that the amount destroyed was calculated on a basis of 1 mole hydrolysed cephaloridine being equivalent to 4 equivalents of iodine¹⁸. The enzyme activities of the preparations were expressed in the units defined by Pollock and Torriani¹⁹: 1 unit equivalent to 1 µmole penicillin hydrolysed/h at 30° at pH 7.0.

Levels of resistance to penicillin, ampicillin, cephaloridine and 6-APA. The presence of R factors in the cultures was recognized by their greatly increased resistance to ampicillin. We measured the levels of resistance of our cultures against the four drugs used as substrates in the enzyme assays. Aerated broth cultures in the exponential phase were diluted to contain approximately 10⁷ organisms/ml and 10⁸ organisms/ml. These suspensions were dropped from calibrated pipettes on nutrient agar plates incorporating serial dilutions of the drugs under test. The heavy suspension gave confluent growth on drug-free control plates; the light suspension yielded isolated colonies. The minimal inhibitory concentration (M.I.C.) recorded was the lowest which prevented visible growth.

The M.I.C.s and the results of enzyme assays are summarized in Table 2.

From Table 2 the following observations may be made:

(1) The host *E. coli* cultures, both K12 and TEM without R factors, were drug-sensitive and had very low but just measurable, hydrolytic activity against benzylpenicillin and cephaloridine, but none against ampicillin or 6-APA. *S. typhi* 152 was considerably more sensitive to benzylpenicillin and ampicillin than our *E. coli* strains. No penicillinase was detected in *S. typhi* 152 without R factors, but it may, nevertheless, possess very low activity since we did not assay highly concentrated suspensions

Table 2. LEVELS OF RESISTANCE AND PENICILLINASE ACTIVITY CONFERRED BY R FACTORS

R factor	Host bacteria	Minimal inhibitory concentration (μg/ml medium)					Enzyme activity (units/h/mg dry weight)				
		Inoculum (No bacteria/ml.)	Benzylpenicillin	Ampicillin	Cephaloridine	6-APA		Benzylpenicillin	Ampicillin	Cephaloridine	6-APA
	<i>E. coli</i> K12	10 ⁸	15	1	1	15	Intact	—	—	0.2	—
	<i>E. coli</i> TEM 'cured'	10 ⁷	30	2	2	30	Broken	0.1	—	0.3	—
		10 ⁸	15	2	1	15	Intact	—	—	0.1	—
		10 ⁷	30	2	2	30	Broken	—	—	0.2	—
	<i>S. typhi</i> 152	10 ⁸	2	< 0.5	1	15	Intact	—	—	—	—
		10 ⁷	4	< 0.5	1	30	Broken	—	—	—	—
	K12 ampicillin 'trained'	10 ⁸	1,000	125	15	15	Intact	0.05	—	0.2	—
	TEM 'cured' ampicillin 'trained'	10 ⁷	1,000	250	15	30	Broken	0.10	—	0.3	—
		10 ⁸	500	125	30	15	Intact	0.05	—	0.3	—
		10 ⁷	500	125	30	30	Broken	0.05	—	0.3	—
TEM	K12	10 ⁸	1,000	1,000	6	2,000	Intact	1.7	6	170	98
	TEM	10 ⁷	4,000	4,000	60	4,000	Broken	125	180	210	175
		10 ⁸	1,000	1,000	6	2,000	Intact	1.2	3.2	106	51
		10 ⁷	> 4,000	4,000	60	4,000	Broken	80	120	140	106
		<i>S. typhi</i> 152	10 ⁸	1,000	1,000	6	2,000	Intact	14	28	215
		10 ⁷	4,000	4,000	60	4,000	Broken	200	320	330	370
1818	K12	10 ⁸	30	30	2	30	Intact	0.5	0.5	1.4	1
	TEM 'cured'	10 ⁷	250	250	4	60	Broken	2.4	2.2	1.9	1.6
		10 ⁸	30	30	2	30	Intact	0.4	0.8	1.8	1.4
		10 ⁷	200	200	4	60	Broken	2.5	2.8	2.4	1.7
7268	K12	10 ⁸	250	250	4	1,000	Intact	0.5	2	20	12
	TEM 'cured'	10 ⁷	1,000	1,000	8	2,000	Broken	22	22	32	19
		10 ⁸	250	250	4	1,000	Intact	0.3	2	18	13
		10 ⁷	1,000	1,000	8	2,000	Broken	18	17	31	22

—, No detectable activity. Lowest detectable activity: in *E. coli*, 0.05 units/h/mg dry wt; in *S. typhi*, 0.5 units/h/mg dry wt.

is organism, as we did for the non-pathogenic ones. 'raining' of our *E. coli* cultures on ampicillin did not lead to their developing appreciably higher hydrolytic activity against any of the four substrates, and there was still no detectable hydrolysis of ampicillin or 6-APA.

(2) Acquisition of *R* factors by the cultures resulted in greatly increased resistance to benzylpenicillin, ampicillin and 6-APA, and greatly increased hydrolytic activity against all four drugs tested. The levels of drug-resistance obtained by the *R* + bacteria were different for each of the three *R* factors studied. The levels of resistance were early related to penicillinase activity, the most resistant having highest penicillinase activity and the least resistant, the least.

(3) Different host bacteria carrying the same *R* factor showed rather similar penicillinase activities. With all three factors, *E. coli* K12 showed slightly but consistently higher activity than *E. coli* TEM and, where tested, *S. typhi* 152 showed even higher activity, but these differences were not accompanied by differences in the levels of drug-resistance.

(4) Whole *R* + cell suspensions, assayed for hydrolysis of penicillin and ampicillin, gave low levels of activity, indicating that the penicillinase was inaccessible to these substrates unless liberated by ultrasonic disruption (or any of the other methods already discussed). For cephaloridine and 6-APA, on the contrary, the difference in the rate of hydrolysis by whole cell suspensions and by ultrasonicated ones was small, indicating that for these substrates the permeability barrier excluding penicillin and ampicillin was not effective. Table 2 also shows that the *R* + cultures, although their hydrolytic activity against cephaloridine is raised to the same degree as that against benzylpenicillin and ampicillin, have not acquired nearly such a high degree of resistance to cephaloridine as to the penicillins.

(5) The *R* - cultures were more sensitive to ampicillin than to benzylpenicillin but the *R* + cultures were equally resistant to both.

Substrate profiles. The penicillinase activity of some of our cultures carrying *R* factors was assayed against a wider range of substrates than shown in Table 2 (see Table 3). The penicillinase produced by three different hosts infected by one *R* factor (*R*TEM) all showed similar substrate profiles. But when the penicillinases from one host (*E. coli* K12), infected with three different *R* factors, were compared they showed interesting differences in substrate profiles. Penicillinase from *E. coli* K12 with factor *R*7258 had a similar substrate profile to that with *R*TEM, but with *R*1818 the profile was quite different; with *R*1818 cloxacillin was hydrolysed as rapidly as benzylpenicillin and phenoxymethyl- and phenoxypentyl-penicillin considerably more rapidly still.

Induction. Tests for inducibility of penicillinase have so far been limited to the examination of *E. coli* K12 carrying factor *R*1818. When methicillin was added to a growing culture no increase in penicillinase activity was observed.

Discussion. The synthesis of penicillinase by our cultures carrying *R* factors appears to be under the direct genetic control of the *R* factors. It might be argued that,

since the host cultures without *R* factors themselves produce some penicillinase, the *R* factors act only to remove a repressor mechanism and so increase penicillinase activity; extracts of our *R* + cultures differ, however, from those of our *R* - cultures not only in their greatly increased penicillinase activity, but also in having high activity against ampicillin and 6-APA. We believe, therefore, that the *R* + cultures synthesize penicillinases which are not present in the *R* - cultures. Penicillinases from our *R* - *E. coli* strains resemble in their substrate range those of *E. coli* strains described by Smith and Hamilton-Miller^{20,24}, while the same *E. coli* strains, carrying *R* factors, synthesize penicillinases with very different substrate profiles.

The penicillinases synthesized by *E. coli* K12 carrying factors *R*TEM and *R*7258 resemble one another in their substrate profiles, although not in their levels of activity. Their substrate profiles resemble those of extracts of *Aerobacter* (*Klebsiella*) *cerogenes* described by Smith and Hamilton-Miller²⁰. We do not yet know whether each enzyme is specific to its *R* factor or whether these two factors both determine the synthesis of the same enzyme, each at a characteristic rate. The penicillinase of factor *R*1818 differs markedly from the others, and from all penicillinases so far described, in hydrolysing cloxacillin as rapidly as benzylpenicillin and in hydrolysing phenoxymethyl and phenoxypentyl penicillins considerably faster still.

The parallelism between the levels of drug-resistance and the penicillinase activities conferred by the different *R* factors strongly suggests that the resistance was the direct result of the presence of penicillinase in the cells. The increase of drug-resistance with drug-hydrolysis was much less with cephaloridine than with the penicillins; thus the penetration of a drug into the cell may increase the antibacterial effectiveness of the drug, as indicated by Hamilton-Miller¹⁶; however, in spite of the permeability of the cells to 6-APA, the latter was not a highly effective antibiotic against our strains.

It is especially interesting that penicillinase biosynthesis can be controlled by infective extrachromosomal elements in the Enterobacteriaceae, since it is known that penicillinase in staphylococci is controlled by comparable, though non-infective, plasmids²¹⁻²³.

We thank Mrs. Roma Pride for her assistance and Dr. M. H. Richmond for his advice.

This work was partly supported by a grant from the Medical Research Council of Great Britain to one of us (N. D.) and by a grant from the International Cell Research Organization to the other (P. K.).

¹ Mitsuhashi, S., Harada, K., and Hashimoto, H., *Jap. J. Exp. Med.*, **30**, 179 (1960).

² Watanabe, T., and Fukasawa, T., *J. Bact.*, **81**, 669 (1961).

³ Watanabe, T., *Bact. Rev.*, **27**, 87 (1963).

⁴ Datta, N., *J. Hyg., Camb.*, **69**, 301 (1962).

⁵ Lebek, G., *Zbl. Bakt., I Abt. Orig.*, **188**, 494 (1963).

⁶ Falkow, S., Wohlhüter, J. A., Citarella, R., and Baron, L. S., *Bact. Proc.*, **31** (1963).

⁷ Harada, K., Suzuki, M., Kameda, M., and Mitsuhashi, S., *Jap. J. Exp. Med.*, **30**, 289 (1960).

⁸ Baron, L. S., and Falkow, S., *Genetics*, **46**, 849 (1961).

⁹ Falkow, S., Marmur, J., Carey, W. F., Spilman, W. M., and Baron, L. S., *Genetics*, **46**, 703 (1961).

¹⁰ Ginoza, H. S., and Matney, T. S., *J. Bact.*, **85**, 1177 (1963).

¹¹ Anderson, E. S., and Datta, N., *Lancet*, **1**, 407 (1965).

¹² Mitsuhashi, S., Harada, K., and Kameda, M., *Nature*, **189**, 947 (1961).

¹³ Repaske, R., *Biochim. Biophys. Acta*, **30**, 225 (1958).

¹⁴ Neu, H. C., and Heppel, L. A., *Biochem. Biophys. Res. Commun.*, **17**, 215 (1964).

¹⁵ Hamilton-Miller, J. M. T., *Biochem. J.*, **87**, 209 (1963).

¹⁶ Perret, C. J., *Nature*, **174**, 10-2 (1954).

¹⁷ Novick, R. P., *Biochem. J.*, **83**, 229 (1962).

¹⁸ Allieo, J. F., *Anal. Chem.*, **33**, 648 (1961).

¹⁹ Pollock, M. R., and Torriani, A.-M., *C.R. Acad. Sci., Paris*, **237**, 276 (1953).

²⁰ Smith, J. T., and Hamilton-Miller, J. M. T., *Nature*, **197**, 976 (1963).

²¹ Novick, R. P., *J. Gen. Microbiol.*, **33**, 121 (1963).

²² Harmon, S. A., and Baldwin, J. N., *J. Bact.*, **87**, 593 (1964).

²³ Novick, R. P., and Richmond, M. H., *J. Bact.* (in the press).

²⁴ Hamilton-Miller, J. M. T., Smith, J. T., and Knox, R., p. 235 of this issue of *Nature*.

Table 3. RELATIVE RATES OF HYDROLYSIS OF VARIOUS SUBSTRATES BY ULTRASONICALLY DISRUPTED PREPARATION OF *R* + CULTURES

<i>R</i> factor	<i>R</i> TEM	<i>R</i> TEM	<i>R</i> TEM	<i>R</i> 1818	<i>R</i> 1818
Host bacteria	<i>S. typhi</i> 152	<i>E. coli</i> TEM	<i>E. coli</i> K12	<i>E. coli</i> K12	<i>E. coli</i> K12
Substrate					
Benzylpenicillin	100	100	100	100	100
Ampicillin	160	150	145	91	100
6-APA	165	142	132	73	86
Methicillin	<0.1	0.6	<0.1	21	<0.1
Cloxacillin	1.5	2.3	1.8	126	1.5
Cephaloridine	180	175	168	86	145
Cephalothin	16	19	15	46	17
Phenoxymethylpenicillin	61	57	69	92	50
Phenoxyethylpenicillin			24	180	24
Phenoxypropylpenicillin			8.5	240	7

DEOXYRIBONUCLEIC ACID SYNTHESIS IN CULTURED HUMAN CELLS AND ITS BEARING ON THE CONCEPTS OF ENDOREDUPPLICATION AND POLYPLOIDY

By DR. URSULA MITTWOCH, MRS. KUSUM P. LELE and W. S. WEBSTER

Galton Laboratory, University College, London

THE synthesis of deoxyribonucleic acid (DNA) is a prerequisite for mitosis, but it is now known that the two processes are essentially separate^{1,2}. If DNA synthesis occurs in excess of requirements for the production of new cells, some of the existing cells will become polyploid. We wish to present some data showing that this happens in human fibroblast-like cells grown in tissue culture.

The cells originated from skin biopsies which were cultured according to the technique of Harnden³. Culture *C*, which had been growing for five weeks and had been sub-cultured 4 times, had an apparently normal female karyotype, although the cells originated from a male; culture *D*, grown also for 5 weeks and sub-cultured three times, had two X-chromosomes and a structural abnormality of a small autosome, which will be described by Crawford and Lele⁴. After trypsinization, the cells were allowed to grow on cover slips for about 25 h, when they were fixed in 95 per cent ethanol (0.5 h), hydrolysed in N hydrochloric acid at 60° C (10 min) and stained with Feulgen (1 h). The cover slips were mounted on glass slides. The light absorbed by individual nuclei was measured with an integrating microdensitometer (Barr and Stroud). This instrument was designed by Deeley⁵. The amount of light absorbed by Feulgen-stained nuclei may be regarded as proportional to the amount of DNA present. For diagrammatic representation, the DNA values were converted into logarithms to the base 2, thus separating any value from double its value by one unit. The lengths of the two main nuclear axes were measured with an eyepiece micrometer and the areas were converted into μ^2 with the help of a stage micrometer. From each culture, 1,000 nuclei were measured.

The DNA values, in arbitrary units, of interphase nuclei are shown in Fig. 1. The number of nuclei scored as in mitosis was 87 in culture *C* and 83 in culture *D*. But although the mitotic rates were similar in both cultures, the distributions of DNA values are clearly very different. The approximate number of cells belonging to each DNA class are given in Table 1. Whereas in culture *C* more than two-thirds of the nuclei fall into the diploid class, only about one-third of the nuclei in culture *D* are diploid, and two-thirds are either in the process of DNA synthesis or, having completed this, are polyploid. Although the size of the nuclei varies even within the same DNA class, as is evident from the standard deviations, it will be seen that the mean nuclear areas increase step-wise with increased amounts of DNA. As the nuclei are very flat, their areas may be regarded as proportional to their volumes. The figures in Table 1 show that the increase of about 12 per cent in the average nuclear area of culture *D* compared with culture *C* can be accounted for by a higher incidence of polyploid cells.

It must be emphasized that a high degree of polyploidy is characteristic of the nuclei in interphase only. The

incidence of polyploid mitoses was only about 2–3 per cent in both cultures. Wolman *et al.*⁶ reported a similar incidence of tetraploid mitoses in tissue cultures during the period of rapid cell division; the incidence of tetraploid mitoses increased with the decline of cell divisions in the culture and following infection with simian virus 40. Polyploid mitoses are common in many tumours, as was shown by Levan⁷, and Atkin⁸ concluded that for most human tumours the ploidy indicated by DNA values in interphase is in agreement with that obtained from chromosome counts in mitosis. It appears likely, however, that this relationship does not apply to normal dividing tissues, in which the incidence of polyploidy among cells in interphase may be much higher than in mitotic cells.

There can be little doubt that polyploid interphase cells occur also *in vivo*. Thus Swift found DNA values of different multiples in various tissues of the mouse⁹ as well as in plants¹⁰; Swartz¹¹ reported different DNA

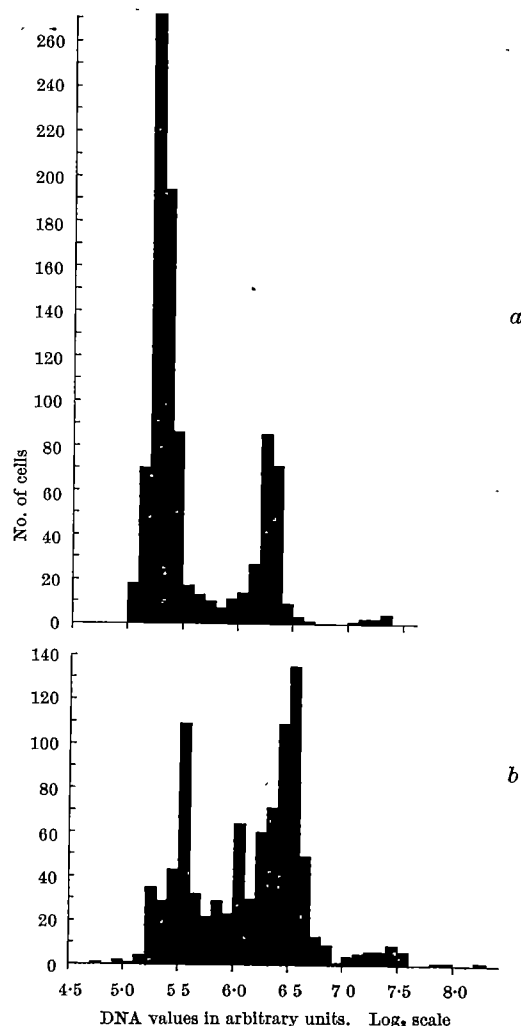


Fig. 1. DNA values in arbitrary units, log scale, of fibroblast-like cells. (a) Culture *C*, (b) culture *D*

Table 1. MEAN NUCLEAR AREAS IN CELLS WITH DIFFERENT DNA VALUES

	Culture <i>C</i>			Culture <i>D</i>		
DNA value	No. of cells	Mean nuclear area (μ^2)	Standard deviation	No. of cells	Mean nuclear area (μ^2)	Standard deviation
Diploid	659	86	28.1	278	74	31.4
Intermediate	55	100	34.7	206	106	31.3
Tetraploid	194	140	45.5	390	125	38.4
Octoploid	9	263	81.6	39	259	85.9
Total	917	100		913	112	

asses in cells of human liver, and Klinger and Schwarzer¹² in human amnion epithelium and foetal liver.

It was shown by Howard and Pelc¹³ that in rapidly dividing cells of the bean, *Vicia faba*, the interphase between two mitoses consisted of three stages: (1) a rest growth period, *G*₁; (2) the period of DNA synthesis, *S*; (3) a second growth period, *G*₂, leading to mitosis. It is becoming clear, however, that mitosis is not a necessary consequence of *G*₂. Thus, Gelfant¹⁴ found that epidermal cells of mice fell into two classes, those in which *G*₂ was followed by mitosis and others which appeared to be blocked in the *G*₂ stage and stayed there for a long time; and Patau and Das¹ have presented evidence that cells of the tobacco plant which have synthesized DNA are ambivalent in the sense that, according to circumstances, they may either enter mitosis or go through another DNA synthesis.

It is clear that DNA synthesis unaccompanied by mitosis is synonymous with the process which has been described as 'endomitosis' or 'endoreduplication'. The term 'endomitosis' is due to Geitler¹⁵, who observed that in the nuclei of insects of the class Heteroptera, chromosome-like structures appeared which apparently underwent an abortive mitosis. Afterwards such nuclei became polyploid. The most highly polyploid nuclei were those of the salivary gland cells, which became 64 ploid during the first instar and at least 1,024 ploid in the imago. It should be emphasized that the concept of endomitosis arose at a time when it was not yet recognized that the process of chromosome replication is essentially separate from the mitotic process. Consequently, Geitler¹⁶ distinguishes between those cases of endopolyploidy in which the nuclei show some resemblance to nuclei in mitosis and others in which polyploidy arises without any visible symptoms apparently at a 'sub-microscopical level'. A similar distinction was made by Levan and Hauschka¹⁷, who limited the term 'endomitosis' to nuclei which seemed to show conspicuous activity inside the nuclear membrane and gave the name "endoreduplication" to the process of chromosome replication which went on concealed during the despiralized stage. It would appear that with our present state of knowledge this distinction has lost its importance. Furthermore, the realization that mitosis does not result in chromosome replication has made the term 'endomitosis' self-contradictory, as has already been suggested¹. The term 'endoreduplication', therefore, seems preferable to describe the process of chromosomal replication which, in the absence of mitosis, leads to polyploidy. The term 'endopolyploidy' is useful to distinguish polyploidy which has arisen in this way from polyploidy originating by nuclear fusion.

If nuclei which have undergone at least two series of replications afterwards undergo mitosis, the duplicated chromosomes characteristically occur in pairs (Fig. 2). Polyploid mitoses with paired chromosomes were first demonstrated by Levan¹⁸ in plant cells which had become polyploid following treatment with growth hormones, and Levan and Hauschka found an abundance of such figures in ascites tumours of the mouse. Polyploid mitoses with paired chromosomes in human tissue culture cells derived from skin were first described by Fraccaro *et al.*¹⁹. However, the incidence of such cells in tissue culture tends to be low. As pointed out above, the incidence of all polyploid mitoses in rapidly dividing cultures is only a few per cent, and only a small percentage of polyploid mitoses have paired chromosomes. The significance of such cells is clearly that they represent the first mitotic division following endoreduplication; thus the twice-duplicated chromosomes are still more or less *in situ*, having not yet become dispersed by the movements of cell division.

The difference in the incidence of polyploidy in interphase and mitotic nuclei directs attention to the problem of nomenclature. Some authors have tried to evade the concept of polyploidy in interphase by ascribing to cells which have duplicated their DNA a certain *C* value¹⁰. Thus a diploid cell which has completed DNA synthesis would be 4*C*. This nomenclature appears to be unsatisfactory for a number of reasons. The symbol *C* in this context does not stand for any definite term. Patau and Swift²⁰ wrote: "It is also generally agreed upon that these multiples, usually terms of the series *C*, 2*C*, 4*C*, show a close relation to the chromosome or rather chromatid number. *C* is apparently always found after the second meiotic division". There is, however, as yet no way of counting either chromosomes, chromatids or centromeres in interphase nuclei. As regards the apparent ambivalence of cells which have undergone DNA synthesis, there can be no logical objection to the statement that a cell becomes tetraploid prior to its giving rise to two diploid ones; while it is becoming clear that a substantial proportion of cells with duplicated DNA contents do not undergo mitosis but remain as interphase cells of larger size. Large interphase nuclei have been recognized as polyploid for a long time. There appears to be no reason to change this concept now that it is possible to obtain an accurate assessment of the degree of polyploidy by measuring their DNA contents.

Geitler¹⁶ wrote that, owing to the belief in the constancy of chromosome numbers, the phenomenon of endopolyploidy was neglected. It now appears that the adherents of both apparently conflicting views were right: while



Fig. 2. Tetraploid mitoses in human fibroblast-like cells. (a) Usual type; (b) with paired chromosomes, as is characteristic for the first mitosis following endoreduplication. (By courtesy of Dr. Joy Delhanty)

dividing cells have a strong tendency to retain the diploid chromosome number, a substantial proportion of cells become polyploid in interphase.

We thank Dr. Joy Delhanty for contributing Figs. 2a and b. The integrating microdensitometer was provided by the Central Research Fund, University of London. The work was also partly supported by grant HD-00500-04 from the U.S. Public Health Service.

¹ Patau, K., and Das, N. K., *Chromosoma*, **11**, 553 (1961).

² Firkin, H., *Arch. Biol.*, **69**, 1 (1957).

³ Harnden, D. G., *Brit. J. Exp. Path.*, **41**, 11 (1960).

⁴ Crawford, M. d'A., and Lele, K. P., *Ann. Hum. Genet.*, **29** (in the press).

⁵ Deeley, E. M., *J. Sci. Instrum.*, **32**, 263 (1955).

⁶ Wolman, S. R., Hirschhorn, K., and Todaro, G. J., *Cytogenetics*, **3**, 45 (1964).

⁷ Levan, A., *Exp. Cell Res.*, **11**, 613 (1956).

⁸ Atkin, N. B., *Cytogenetics*, **1**, 113 (1962).

⁹ Swift, H. H., *Physiol. Zool.*, **23**, 169 (1950).

¹⁰ Swift, H. H., *Proc. U.S. Nat. Acad. Sci.*, **36**, 643 (1950).

¹¹ Swartz, F. J., *Chromosoma*, **8**, 53 (1956).

¹² Klinger, H. P., and Schwarzscher, H. G., *J. Biophys. Biochem. Cytol.*, **345** (1960).

¹³ Howard, A., and Pelc, S. R., *Heredity*, Supp., **6**, 261 (1953).

¹⁴ Gelfant, S., in *Cell Growth and Cell Division*, edit. by Harris, R. J. C., (Academic Press, 1963).

¹⁵ Geitler, L., *Chromosoma*, **1**, 1 (1939).

¹⁶ Geitler, L., *Endomitose und Endomitotische Polyploidisierung (Proplasmalogie)*, **6**, 1; (1953).

¹⁷ Levan, A., and Hauschka, T. S., *J. Nat. Cancer Inst.*, **14**, 1 (1953).

¹⁸ Levan, A., *Heredity*, **25**, 87 (1939).

¹⁹ Fraenkel, M., Kaiser, K., and Lindsten, J., *Ann. Hum. Genet.*, **24**, 45 (1960).

²⁰ Patau, K., and Swift, H. H., *Chromosoma Berl.*, **6**, 149 (1953).

PROTEIN SYNTHESIS IN RETICULOCYTES MATURING IN VIVO

By DR. PETER T. ROWLEY

Department of Medicine, Stanford University School of Medicine, and Medical Service, Veterans Administration Hospital, Palo Alto, California

THE transition from mammalian reticulocyte to mature erythrocyte is marked by the loss, not only of the stainable reticular material, but also of the ability to synthesize protein. Recently, the site of protein synthesis, not only in reticulocytes but also in a wide variety of cells, has been shown to be aggregates of ribosomes called polyribosomes¹⁻³. The present search for the cause of the declining rate of protein synthesis in the maturing reticulocyte was undertaken in the hope that the explanation might contribute to a general understanding of the rate determinants of mammalian protein synthesis.

Two methods have been previously used to examine reticulocyte maturation⁴. One is the comparison of cells from an animal at various times following an erythropoietic stimulus; a cell population so derived, however, is far from homogeneous. The second is the investigation of cells aged under controlled conditions *in vitro*. Using the latter method, Bertles and Beck⁵ showed that maturation *in vitro* is associated with a conversion of RNA into products of low molecular weight, which are released from the cell. Marks *et al.*⁶ by sucrose gradient analysis, and Rifkind *et al.*⁷ by electron microscopy, showed that maturation *in vitro* is associated with a progressive disaggregation of polyribosomes to smaller clusters and single ribosomes.

The present investigation of reticulocyte maturation was prompted by the report of a method to separate red cells by age using density gradient centrifugation⁸. This method provided a means of determining whether the above conclusions about cells matured *in vitro* also applied to cells of different age removed from the same animal at the same time.

Specifically, answers were sought to two questions: (1) how does the rate of protein synthesis in the maturing reticulocyte vary with age, and (2) which component of the protein synthetic apparatus fails? Protein synthetic rates were determined for young and old reticulocytes (a) as whole cells, (b) in cell-free systems derived from each, and (c) in cell-free systems in which young and old fractions were interchanged. Evidence is presented that the decline in protein synthesis in the maturing reticulocyte correlates primarily with the decline in the quantity of polyribosomes per cell.

The density of the red cell increases with age. This fact enabled Leif and Vinograd⁸ to separate erythrocytes according to age on a linear density gradient of bovine serum albumin. They verified the method for rabbit erythrocytes by the serial analysis of cells obtained from a rabbit injected with ⁵⁹FeCl₃.

Using position in such a gradient as a measure of age, protein synthesis in rabbit reticulocytes was determined

as a function of age, first in whole cells. The experiment shown in Fig. 1 indicates that, as the cells get denser and occupy a lower position in the gradient, they are also less active in protein synthesis. In this experiment, the top sixth of the cell population was 240 times as active as the bottom sixth. What accounts for this difference between old and young reticulocytes?

To determine this, 'young' and 'old' reticulocytes were separately pooled from the top and bottom halves respectively, of an albumin-density gradient. The samples contained 97 and 94 per cent reticulocytes respectively, the mature red cells from the initial sample (60 per cent reticulocytes) having formed a pellet at the bottom of the gradient tube. Both whole-cell and cell-free syntheses were examined. As whole cells, the young cells were 2.5 times more active than the old cells (Fig. 2)

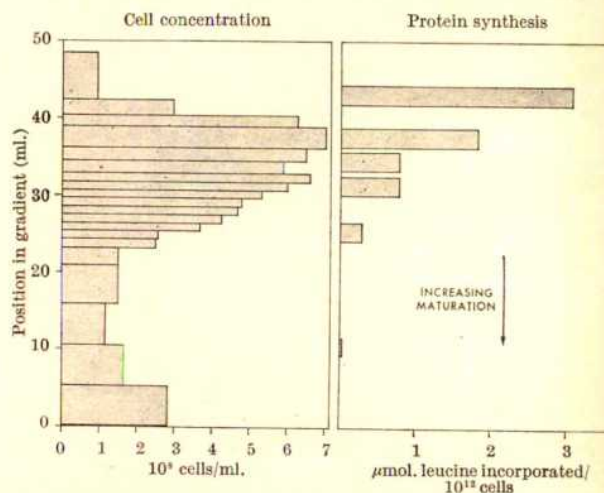


Fig. 1. Distribution and protein synthetic activity of reticulocytes separated by age on a density gradient. A New Zealand albino rabbit was injected subcutaneously with phenylhydrazine HCl (12.5 mg/kg the first day, then 10 mg/kg for four days) and bled on the sixth day. Washed, packed cells 2.5 ml., diluted to 5 ml. with saline, were layered on to a 50-ml. linear density gradient (20-35 per cent, w/w) of bovine serum albumin in saline (ref. 8) and centrifuged at 20,000 r.p.m. in a rotor type 25-2 in the Spinco Model 'L-2' ultracentrifuge for 30 min. Six fractions were collected and the cells recovered by dilution with saline and centrifugation at 2,000g for 5 min. From each fraction, 0.05 ml. packed cells were incubated with L-leucine-¹⁴C (3.56 × 10⁶ c.p.m./μmol.) 9 μmol., other amino-acids in the ratio of Lingrel *et al.* (ref. 11) 96 μg. glucose 0.88 μmol., sodium citrate 0.18 μmol., Fe(NH₄)(SO₄)₂ 2 μmol., NaCl 16.8 μmol., MgCl₂ 0.31 μmol., NaHCO₃ 0.26 μmol., 0.04 ml. of the same rabbit's plasma and tris 3.6 μmol. at pH 7.4 in a total volume of 0.23 ml. at 37° C for 1.5 min. The cells were then washed and lysed by the addition of 0.45 ml. of 0.01 M KCl, 0.003 M MgCl₂, 0.01 M Tris-Cl, pH 7.4, containing 100 units of heparin per ml., and the membranes removed by centrifugation at 10,000g for 15 min. The acid-precipitable radioactivity of the membrane-free lysate was determined, after washing on 'Millipore' filters, using a liquid scintillation counter.

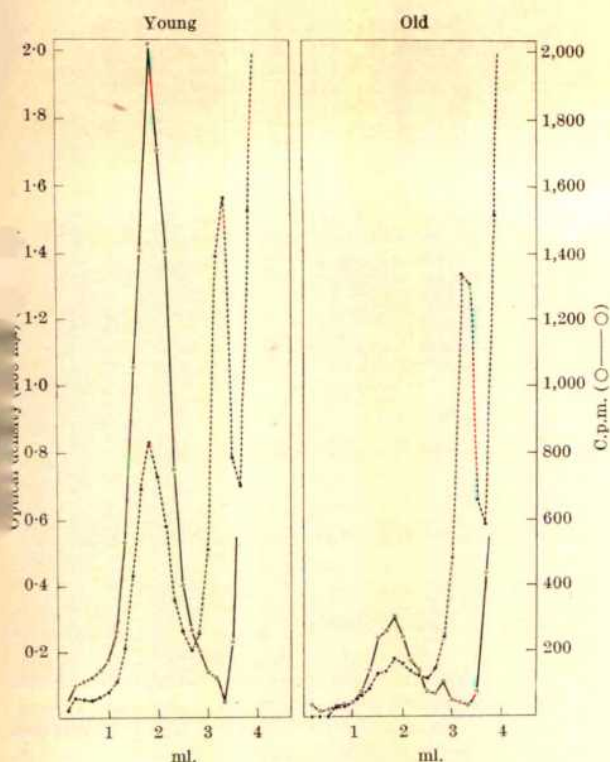


Fig. 2. The membrane-free lysates from the experiment described in Fig. 1 were used. Of the six fractions, only the analyses of fractions 2 ('old') and 6 ('young') are reproduced. Membrane-free lysate, 0.2 ml., was layered on to a 4.8-ml. gradient of sucrose, 10–30 per cent in 0.01 M KCl, 0.003 M $MgCl_2$, 0.01 M $tris\text{-}Cl$, pH 7.4, containing 100 units of heparin per ml. and centrifuged at 40,000 r.p.m. in a 'SW-39' rotor in a Model L-2 Spino ultracentrifuge for 1 h. Fractions, 0.25 ml., were analysed for absorbance at 260 $m\mu$ using a Beckman 'DU' monochromator and a Gilford 'Model 220' photometer, and for radioactivity using a liquid scintillation counter after addition of 10 ml. Bray's solution (ref. 12).

Protein synthesis by fractions from young and old reticulocytes are compared in Table 1 (incubations 1 and 2). The fractions from young cells were also several (3.8) times more active 'per cell' (see footnote) than fractions from old cells. The relative contribution of ribosomes and supernatant was determined by interchanging young and old fractions (incubations 3 and 4). The mixed incubation containing young ribosomes (No. 3) was 70 per cent as active per cell as the incubation of the wholly young fractions (No. 2), whereas the mixed incubation containing old ribosomes (No. 4) was scarcely more active than the incubation of wholly old fractions (No. 1). Hence the ribosomes were largely the rate-determining fraction.

The young cells contained more ribosomal material than the old cells. Expressed as optical density units at 260 $m\mu$, the young cell averaged 6.24×10^{-9} units and the old cell 2.49×10^{-9} units. The greater activity of the ribosomal fraction from the young cells is due, however, not solely to their greater quantity of ribosomal material per cell, but also to a more active type of ribosomal material, as shown by a higher rate of protein synthesis per O.D. unit of ribosomes (Table 1).

Since protein is synthesized chiefly on polyribosomes, one might suppose that the principal difference between the ribosomal fractions of young and old cells would be in the quantity of polysomes. To test this hypothesis, young and old cells were incubated whole with radioactive amino-acids, and their lysates analysed on sucrose gradients. Young and old whole-cell fractions, separated in the experiment described in Fig. 1 and differing 10-fold in their whole-cell synthetic rate, were used. The results are shown graphically in Fig. 2 and quantitatively in Table 2. Cell for cell, the two striking differences are in the quantity of polyribosomes (old = 20 per cent of the young) and in the radioactivity in the polyribosomes

(old = 14 per cent of the young). Since the quantity and radioactivity of polyribosomes are comparably reduced, their specific activity ($\mu\text{mol. leucine bound per optical density unit of polysome}$) showed only a modest decrease (old = 66 per cent young). The quantity of single ribosomes (Table 2) showed only modest reduction. The significance of the increase in average release time is not known.

Maturation of the reticulocyte is then marked by two processes, a decrease in the total quantity of ribosomes and a disproportionate decrease in the quantity of polyribosomes, as described by Rifkind⁷ for maturation *in vitro* and in a peritoneal diffusion chamber. The cause of neither of these processes is known. Investigations of ribonuclease activity in lysates of young and old cells are in progress.

Maximal recovery of polysomes requires minimizing ribonuclease action during isolation. In controlled experiments, the use of heparin, a known inhibitor of ribonuclease⁹, in the lysing solution and in the sucrose gradient increased polysome yields. Lysis by brief osmotic shock, described by Marks *et al.*¹ as specific for red cells, may also have been of value, since white cells have much more ribonuclease than red cells¹⁰.

In summary, the most striking change in the maturing reticulocyte as haemoglobin synthesis decreases is a decline in the quantity of polyribosomes per cell. This conclusion has been previously reached for cells maturing *in vitro* or in a peritoneal diffusion chamber. It has now been documented for cells of different age removed from the same animal at the same time.

Since this account was prepared, a report has appeared by Glowacki and Millette (*J. Mol. Biol.*, 11, 116; 1965)

Table 1. PROTEIN SYNTHESIS BY FRACTIONS FROM YOUNG AND OLD RETICULOCYTES

Source of fractions:	1	2	3	4
Ribosomes	Old	Young	Young	Old
Supernatant	Old	Young	Old	Young
Incorporation into protein:				
$\mu\text{mol. leucine}$	155	486	342	172
$\mu\text{mol./}10^6$ cells*	166	639	450	185
$\mu\text{mol./O.D.}_{260}$ ribosomes	53	82	57	59

* The calculation of $\mu\text{mol./}10^6$ cells involved the number of cells which provided the ribosomes for the incubation.

Blood (30 ml.) from a New Zealand albino rabbit (2.8 kg), treated with phenylhydrazine HCl (10 mg/kg) on the preceding 5 days, was passed through a tightly packed column of 'Deponol'-treated nylon fibres (3 g) at room temperature to remove granulocytes (ref. 13) and centrifuged (2,000g, 5 min). Packed cells (2.5 ml.) were separated according to age by density gradient centrifugation (Fig. 1) and the gradient divided into two parts, an upper ('young') and lower ('old'), containing an equal volume (1 ml.) of cells after packing. Packed cells (0.8 ml.), young (4.57×10^6 cells) or old (5.6×10^6 cells), were separately lysed by the addition of 3.2 ml. 0.005 M $MgCl_2$, followed in 30 sec by 0.8 ml. 1.5 M sucrose–0.15 M KCl (ref. 1). Fractions were prepared and cell-free synthesis carried out in a manner similar to that of Allen and Schweet (ref. 14). Lysates were centrifuged at 15,000g, 10 min. The supernatants were re-centrifuged at 8,000g, 1 h. Pellets were rinsed with 0.05 ml. 0.25 M sucrose, 0.0175 M KH_2PO_4 , 0.002 M $MgCl_2$, homogenized in 2 ml. of the same, and ribosomes and supernatants were re-centrifuged as before. The final pellets were homogenized in 0.3 ml. 0.25 M sucrose in glass homogenizers by hand, the suspensions re-centrifuged at 15,000g, 5 min, and the final supernatants used as 'ribosomes'. Each incubation (0.15 ml.) contained: in μmoles , $tris\text{-}HCl$, pH 7.5, 56; KCl, 50; glutathione, 20; creatine phosphate, 10; ATP, 1; GTP, 0.25; $MgCl_2$, 5; $^{14}C\text{-L-leucine}$, 3.55×10^3 c.p.m./ $\mu\text{mol.}$, 0.005; 18 other L-amino-acids, 2.1 μg total, in the ratio of Lingrel *et al.* (ref. 11); creatine kinase, 0.12 mg; supernatant, 0.04 ml.; and ribosomes, 0.05 ml. After 20 min at 37°, cold water was added and the material insoluble in 5 per cent trichloroacetic acid was washed and counted on 'Millipore' filters in a liquid scintillation counter. A control incubation of young fractions to which $^{14}C\text{-L-leucine}$ was added after incubation incorporated a total of 80 c.p.m. Optical density of ribosome suspensions at 260 $m\mu$ was determined in 0.01 M NaOH.

Table 2. POLYSOMES FROM YOUNG AND OLD RETICULOCYTES

	Per 10^6 cells		
	Young	Old	Old/Young
Whole cell synthetic rate $\mu\text{mol. leucine}$ incorporated/1.5 min	305	27.7	9.1%
O.D. ₂₆₀ in polysomes	5.08	1.04	20%
O.D. ₂₆₀ in single ribosomes	5.60	4.00	71%
Polysome-bound leucine, $\mu\text{mol.}$	31.8	4.3	14%
Specific activity of polysomes: $\mu\text{mol.}$			
leucine bound/O.D. ₂₆₀ $m\mu$	6.25	4.14	66%
Average release time, min*	0.156	0.233	149%

* Average release time is the average time radioactive amino-acid is bound to polysomes before release into supernatant: (leucine in polysomes/total acid-insoluble leucine) \times 1.5 min. The data in this table were calculated from the data in Fig. 2.

describing experiments similar to some of those reported here. They also incubated intact rabbit reticulocytes, separated by age using density gradient centrifugation, with radioactive amino-acids and analysed the lysates on sucrose gradients. Despite several differences in technique, both their results and ours indicate that the decline in protein synthesis with maturation is accompanied by, cell for cell, a decrease in total ribosomal material, a decrease in the fraction present as polyribosomes without a decrease in the apparent size of the aggregate, and some decrease in the specific activity of the polyribosomes. From available data, the last mentioned could be due to either a decrease in activity of all polysomes or to a growing fraction of inactive polysomes.

I thank Josephine Morris, Michael Johnson, Hilary Cheadle and Myrna Sproul for their assistance, and Dr. Paul Berg for advice.

This work was supported by grants from the National Institutes of Health (AM 7469), the Veterans Administration, and the American Cancer Society (IN-32E).

- ¹ Marks, P. A., Burka, E. R., and Schlessinger, D., *Proc. U.S. Nat. Acad. Sci.*, **48**, 2163 (1962).
- ² Warner, J. R., Knopf, P. M., and Rich, A., *Proc. U.S. Nat. Acad. Sci.*, **122** (1963).
- ³ Gierer, A., *J. Mol. Biol.*, **6**, 148 (1963).
- ⁴ Lowenstein, L. M., *Intern. Rev. Cytol.*, **8**, 135 (1959).
- ⁵ Bertles, J. F., and Beck, W. S., *J. Biol. Chem.*, **237**, 3770 (1962).
- ⁶ Marks, P. A., Rifkind, R. A., and Danon, D., *Proc. U.S. Nat. Acad. Sci.*, **336** (1963).
- ⁷ Rifkind, R. A., Danon, D., and Marks, P. A., *J. Cell Biol.*, **22**, 599 (1964).
- ⁸ Leif, R. C., and Vinograd, J., *Proc. U.S. Nat. Acad. Sci.*, **51**, 520 (1964).
- ⁹ Zollner, N., and Fellig, J., *Amer. J. Physiol.*, **173**, 223 (1953).
- ¹⁰ Gupta, S., and Herriott, R. M., *Arch. Biochem. Biophys.*, **101**, 88 (1963).
- ¹¹ Lingrel, J. B., and Borsook, H., *Biochemistry*, **2**, 309 (1963).
- ¹² Bray, G. A., *Anal. Biochem.*, **1**, 279 (1960).
- ¹³ Greenwalt, T. J., Gajewski, M., and McKenna, J. L., *Transfusion*, **2**, (1962).
- ¹⁴ Allen, E. H., and Schweet, R. S., *J. Biol. Chem.*, **237**, 760 (1962).

NUCLEO-CYTOPLASMIC INTERACTIONS IN THE RAT LIVER CELL

By E. R. GODWIN, DR. K. R. REES, DR. G. F. ROWLAND and J. S. VARCOE

Department of Chemical Pathology, University College Hospital Medical School, London, W.C.1

ONE aspect of the investigation of nucleo-cytoplasmic interaction in the liver cell is the study of the intracellular movement of proteins. This requires the application of chase-labelling techniques to an *in vitro* system using a sufficiently large quantity of tissue to permit subcellular fractionation at the end of the experiment.

Whole-liver cell preparations would be ideal for these experiments, but preparations that have been described have a low yield of cells and these often have impaired metabolic activity. A metabolically active liver cell preparation such as the slice has a disadvantage in that only a limited number of slices can be prepared from each liver. For these reasons it was decided to explore the 'tissue chop' technique as a method of producing a large amount of a cellular preparation of liver.

McIlwaine and Buddle¹ designed a mechanical tissue chopper and showed that the respiration of tissue 'chops' of cerebral cortex was within 3 per cent of the respiration of ordinary hand-cut slices. They also demonstrated that such a chopper would produce tissue chops from small quantities of a whole range of tissues such as heart, liver, spleen and kidney.

In the present investigation it was necessary to be able to handle quickly at least 7 g of liver and often more. A tissue chopper was made, based on that of McIlwaine and Buddle, modified, however, to take larger quantities of tissues by increasing the size of the chopping platform and the blade. For each experiment two male rats weighing 150 g were killed by cervical fracture and the livers rapidly removed into ice-cold Krebs-Ringer-phosphate, pH 7.3 (Umbreit, Burris and Stauffer²), containing 0.1 per cent glucose. The lobes were then carefully trimmed to give slices of liver 2–5 mm. thick and chopped in two directions. The device was set to chop at intervals of 0.3 mm, thus producing minute columns of liver. The columns from 10 to 15 g liver were suspended in 30–50 ml. cold Krebs-Ringer-phosphate and allowed to sediment. The supernatant fluid containing broken cell debris was decanted and the chopped liver preparations washed three more times with cold Krebs-Ringer-phosphate before being finally suspended in Krebs-Ringer-phosphate plus glucose (30 ml. per 6–8 g chopped liver) and transferred to 100 ml. conical flasks for incubation. 10 μ c. of ¹⁴C-leucine was added to the flasks, which were then sealed with vaccine caps through which were passed syringe needles to allow for gas exchange. The chopped liver preparations were incubated for various times as required at 37° C under oxygen. After incubation

the liver suspensions were cooled in crushed ice and washed by decantation and resuspension twice in a solution 0.5 per cent w/v non-radioactive leucine in 0.25 M sucrose and then twice in 0.25 M sucrose. The liver preparations were finally resuspended in 0.25 M sucrose and the suspensions divided in two. One half was homogenized in Potter-Elvehjem homogenizer for preparation of mitochondria, microsomes and cell sap as described by Schneider³. To the other half, calcium chloride was added to a final concentration of 5 mM and the suspension homogenized in the hand-operated homogenizer for preparation of nuclei as described previously by Rees and Rowland⁴.

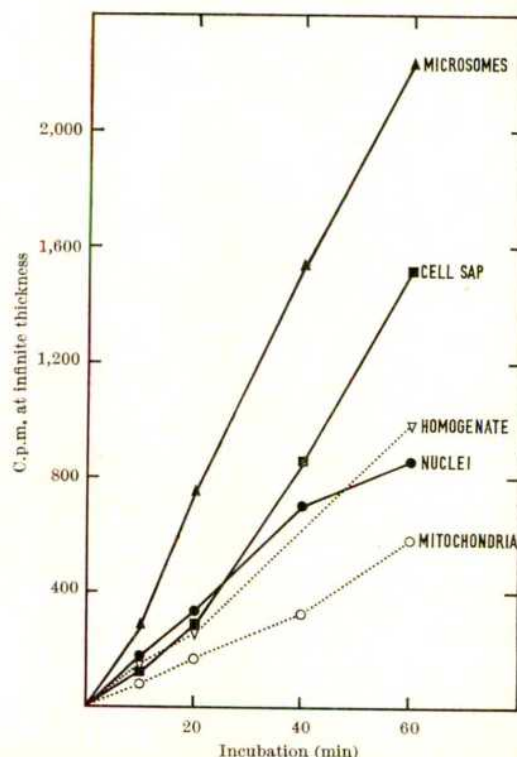


Fig. 1. Time-course of incorporation *in vitro* of 1-¹⁴C DL-leucine into proteins of subcellular fractions during incubation of chopped liver preparation. Results are the means from three experiments.

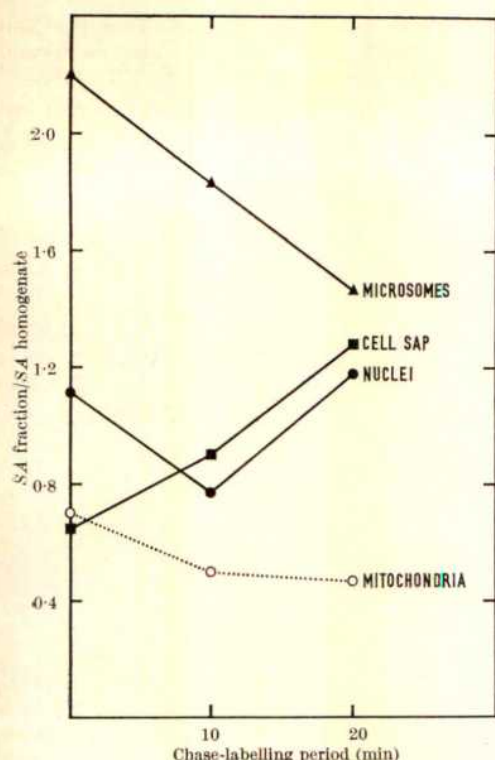


Fig. 2. Specific activities (SA) of sub-cellular fractions relative to specific activity of homogenate during the 'chase-labelling' period of the experiments shown in Table 1. The results are the means from four experiments.

By incubating a suspension of chopped liver in a Krebs–phosphate the time-course of ^{14}C leucine incorporation into the various subcellular fractions was examined. Fig. 1 shows the result of such an experiment. It is of particular interest to note that whereas at 40 and 60 min the cell sap proteins are more highly labelled than nuclear proteins, at earlier times this is not the case.

Several experiments of the 'chase-labelling' type were carried out in the following manner. The chopped liver preparation was washed free of broken cells in Krebs–phosphate solution and divided into three roughly equal portions. The portions were all incubated at 37°C with ^{14}C leucine for 10 min. At this stage one portion was cooled in crushed ice while 100-fold excess of non-radioactive leucine was added to the other portions, which were allowed to continue incubating for a further 10 and 20 min, respectively, before cooling in ice also. The cooled liver suspensions were washed free of isotope and broken cells as already described and then homogenized and fractionated to yield the various subcellular components. Table 1 shows the results of a typical experiment carried out in this way. To correct for

variations in the degree of overall labelling in the three portions the results have also been expressed as a ratio of the specific activity of each subcellular fraction to the specific activity of the homogenate from which they were isolated, and the means of the ratios from all the experiments are given in Fig. 2. It may be seen from these results that there is an overall fall in the radioactivity of both the microsomal and mitochondrial protein during the 20 min 'chase' period, and there is a considerable rise in the radioactivity of cell sap protein during this time. The nuclear protein shows a considerable drop in radioactivity after the first 10 min of 'chase-labelling', but during the second half of the 'chase' period the radioactivity returns to its former level.

Table 1. INCORPORATION OF $1\text{-}^{14}\text{C}$ DL-LEUCINE BY SUSPENSIONS OF CHOPPED LIVER FOLLOWED BY 100-FOLD EXCESS UNLABELLED LEUCINE IN ORDER TO FOLLOW MOVEMENT OF LABELLED PROTEIN FROM SITES OF PROTEIN SYNTHESIS WITHIN THE LIVER CELL

Incubation time with ^{14}C -leucine	10 min	10 min	10 min
Incubation time with ^{14}C -leucine	+ 0 min	+ 10 min	+ 20 min
Total time	10 min	20 min	30 min
Tissue fraction	c.p.m. at infinite thickness	c.p.m. at infinite thickness	c.p.m. at infinite thickness
Homogenate	109	124	142
Nuclei	105	85	140
Mitochondria	77	57	65
Microsomes	232	195	208
Cell sap	68	112	188

Results are those of a representative experiment of a group of 4 similar experiments.

These findings are in agreement with those of Goldstein⁵ and Prescott⁶, whose autoradiographic investigations have shown similar movements of protein to and from the nucleus of *Amoeba proteus*. Goldstein has examined two types of nuclear proteins: proteins that migrate from the nucleus to the cytoplasm and those that do not migrate. He concluded that both types are synthesized in the cytoplasm, but his experiments do not entirely rule out the existence of a species of non-migratory proteins synthesized within the nucleus. There is at present no indication as to the function of these migratory proteins in the cell.

One possible interpretation of these results is that there may be a movement of labelled protein out of the nucleus into the cytoplasm during the early stages of incubation followed by a movement of protein in the reverse direction—from cytoplasm into the nucleus.

This work was supported by a grant from the British Empire Cancer Campaign for Research. One of us (G. F. R.) is a Beit Memorial Research Fellow.

¹ McIlwaine, H., and Buddle, H. L., *Biochem. J.*, **53**, 412 (1953).

² Umbreit, W. W., Burris, R. H., and Stauffer, J. F., *Manometric Techniques*, third ed., 149 (Burgess Publishing Co., Minneapolis, 1959).

³ Schneider, W. C., *J. Biol. Chem.*, **176**, 259 (1948).

⁴ Rees, K. R., and Rowland, G. F., *Biochem. J.*, **83**, 523 (1962).

⁵ Goldstein, L., in *Cell Growth and Cell Division*, edit. by Harris, R. J. C., 129 (Academic Press, N.Y., 1963).

⁶ Prescott, D. M., in *Cell Growth and Cell Division*, edit. by Harris, R. J. C., 111 (Academic Press, N.Y., 1963).

BIOLOGY OF THE CYTOPLASM

By DR. OLE A. SCHJEIDE, RUTH G. McCANDLESS and R. J. MUNN

Laboratory of Nuclear Medicine and Radiation Biology, Department of Biophysics and Nuclear Medicine, School of Medicine, University of California, Los Angeles

Cytoplasmic Nucleic Acids

ACCORDING to the scheme initially proposed by Gierer¹ and Noll *et al.*², messenger RNAs leave the cell nucleus to become individually associated with one or more ribosomes in the cytoplasm. Thereafter, the ribosomes assemble a specific protein by moving along the strand of messenger RNA, exposing by 3-base increments the codes for individual amino-acids which are linearly arranged thereon.

This concept has received abundant experimental verification from the work of Rich *et al.*³ and others⁴, and is entirely convincing for certain systems. However, many investigators have held that the foregoing mechanism constitutes only a fragment of the total protein synthesizing machinery of most of the cells of higher animals⁵.

Electron micrographs of rapidly metabolizing cells offer a unique insight to this problem, and in the past we

have exploited the oocytes, follicular cells and developing organ systems of the chicken in this regard. In a recent communication⁶ we reported the following observations which are pertinent to the question of how nuclear materials reach the cytoplasm and how they function therein.

(a) Nucleolar nucleic acid is often present in the form of small vesicles (300–400 Å diam.) possibly containing protein as well as nucleic acid. Considerable numbers of these vesicles are sometimes seen arrayed in double helical 'stacks' within the body of the nucleolus. The phenomenon may be peculiar to a given brief phase of activity or it may be that these bodies are commonly so dispersed but that fixation of nuclear materials proceeds too slowly to preserve the orientation in most cases.

(b) Strings of such vesicles are often seen outside the nucleolus, in the karyoplasm, one end of the string usually being in intimate association with the nuclear membrane.

(c) The vesicles closest to a nuclear 'pore' are observed to be markedly reduced in size.

(d) It appears that such smaller vesicles (microvesicles) emerge from the site of a nuclear 'pore' at the periphery of that pore—which is characterized by so-called 'annular rods'. The 'annular rods' appear to radiate into both nucleus and cytoplasm.

(e) It may be that the 'rods' consist of rows of very small vesicles which are continuously moving into the cytoplasm.

(f) In any event, the microvesicles assemble in the cytoplasm to form larger oval bodies which resemble the 'kinetosomes' of *Paramecium*⁷. At the magnifications afforded by the electron microscope we were using at the time, these bodies (1000–1500 Å smaller diam., up to 4000 Å larger diam.) appear to consist of a relatively dense membrane with regularly spaced outwardly projecting bristles (200–250 Å in length—200–250 Å apart) and a centrally located dark streak.

(g) Bristle-membraned bodies ('kinetosomes') were also seen to arise from the plasma membrane. We have so far not been able to distinguish these from those elaborated at the nuclear 'pores'. In the literature such have often been referred to as pinocytosis—or absorption—vesicles.

(h) Although we have found no way of distinguishing between the bristle-membraned bodies of differing origin once they have entered the cytoplasm proper, some such bodies in the cytoplasm were observed to be in various stages of condensation into—or synthesis of—denser granules. The most advanced appeared to be in transition from vesicles to ribosome clusters.

(i) Many of the bristle-membraned bodies were seen to be participating in the formation of yolk granules in the oocyte, and vesicles of various sizes were conspicuous among other developing cell organelles, with and without transformation into ribosomes or polyribosomes.

Since we reported the foregoing findings, an electron microscope of higher resolution (Siemens 1A) has been installed in our laboratory and we have examined numerous additional samples of these avian tissues with this more powerful instrument. Thinner sections (400 Å) have been made with a diamond knife mounted on a Porter-Blum MT2 microtome. As was done previously, eggs and embryos were fixed by exposure to 1–2 per cent osmic acid (pH 7.8) for 2 h, were taken up through 50, 75, 95 and 100 per cent ethyl alcohol steps to propylene oxide and were then embedded in 'Epon 812'. Copper grids without support were used and staining with lead was carried out according to method B of Karnofsky⁸.

One of the more gratifying results of this new investigation was the confirmation of something we had suspected earlier, namely, that the bristle-membraned bodies (or 'kinetosome-like' bodies) are largely constituted of small vesicular units of between 150 and 250 Å outside

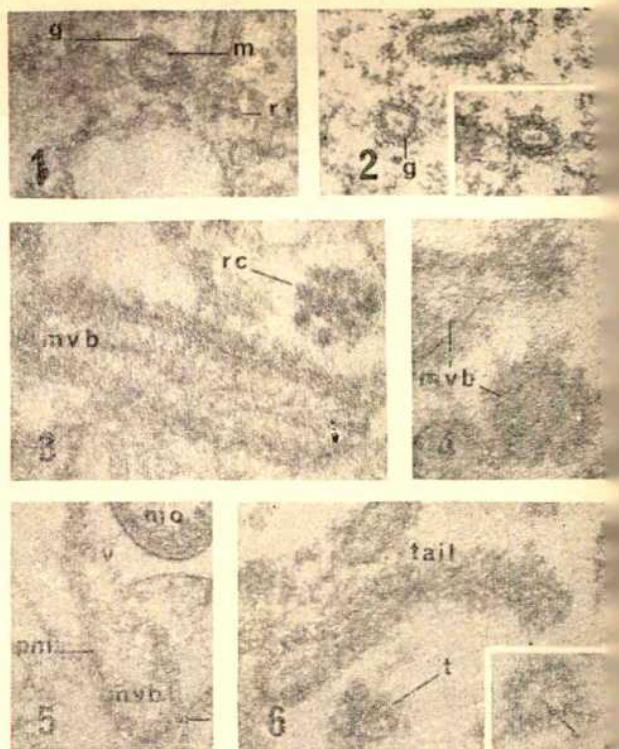


Fig. 1. Multivesicular body within cytoplasm of 4 mm hen's egg showing outer sheet of vesicles with small internal granules (*g*), membrane (*m*) between outer sheet and inner closely packed sheet of vesicles, 'honey-comb'-like interior with separations composed of small granules or microvesicles and central streak consisting of either granules or microvesicles. A cluster of ribosomes appears to be associated with this body and may be derived from it. ($\times 56,000$)

Fig. 2. Multivesicular bodies from cytoplasm of 1.8 mm hen's egg showing relative sizes of vesicles in shells separated by dense membrane. Note that vesicles of internal sheet also contain very small granules (*g*). ($\times 50,000$)

Fig. 3. Elongated multivesicular body (*nvb*) and ribosome cluster (*rc*) from 6-mm egg. Note that ribosomes are enmeshed in mass of less dense material and are about the same size as larger surface vesicles of multivesicular body (*nvb*). ($\times 120,000$)

Fig. 4. Multivesicular body (*nvb*) with 'tail' which may be condensing into ribosomes (top) and multivesicular body (*nvb*) sectioned through surface sheet of vesicles (bottom). Note dense granules marking centres of vesicles. 6 mm egg ($\times 120,000$)

Fig. 5. Multivesicular body apparently budding off from vesicle (*v*) carrying 'mitochondrion-like' organelle (*mo*) into 6 mm hen's egg. The plasma membrane (*pm*) appears to be homologous to the membrane of the multivesicular body (arrow). ($\times 80,000$)

Fig. 6. Multivesicular body in 6-mm hen's egg with elaborated 'tail'. This suggests that the body may either be (*a*) duplicating itself, (*b*) in an early stage of forming ribosomes or (*c*) both. A section through the surface layer of a multivesicular body is shown (*t*) which clearly reveals the vesicular orientations of this sheet and the central location of the granules. The arrow (insert) points to microvesicles which sometimes appear to comprise the central streaks of these bodies. ($\times 80,000$)

diameter (Figs. 1 and 2). The 'bristles' then, seen at lower resolution, have proved to be the sites at which the sides of adjacent microvesicles are pressed together. It is, however, not clear whether material in addition to that contained in the two membranes may be present at these junctions.

The so-called limiting membrane of the multivesicular body lies between two layers of tightly packed vesicles. In Figs. 1 and 2 it can be seen that the internal layer is made up of somewhat smaller vesicles (about 150 Å) as opposed to those of the external layer (about 200 Å). Figs. 4 and 6 show a cut through the surface layer of one of these bodies and confirm the notion of the regular size and disposition of the microvesicles. Note the presence of small 'granules' in the microvesicles. In Figs. 1 and 2 it can be seen that the 'granules' are present inside the microvesicles of both surface layers. These specks are so small (about 40 Å) that they do not fulfil the

tial requirements for a nucleic acid molecule of any respectable size. At the most they can represent only a nucleotides and, indeed, they might consist of some together different substance. The so-called 'limiting membrane' of the multivesicular body stands out quite distinctly as can be seen in Figs. 1-4. This does not seem to be due to reinforcement due to the pressing together the sides of adjacent microvesicles since, as is seen in Fig. 5 (arrow), the 'limiting membrane' appears to be the usual plasma membrane in those cases where the multivesicular bodies arise from the surface of the cell. The 'limiting membrane' affords a stronger contrast and appears thicker in the vertical regions between the microvesicles and seems somewhat granular. It may be a major site of nucleic acid concentration in these bodies. As we have pointed out before⁶, these bodies display a considerable contrast with the background following destaining with hydrogen peroxide—indicating the presence of a dense material such as nucleic acid. They also stand out in sharp contrast following application of periodic acid and silver methionine as do other nucleic acid-containing structures.

In Figs. 1-3 the impression is obtained that microvesicles organized into somewhat larger wedge-shaped structures 'combed' the interiors of the multivesicular bodies. The dense central streaks in these bodies sometimes appear to be a collection of very small microvesicles (Fig. 6, row). The relative thickness of their walls could account for the dense streak but they may also be condensed into annules.

Conversion of some multivesicular bodies to individual dense objects morphologically indistinguishable from ribosomes and to clusters of ribosomes is strongly suggested by Figs. 7a, b, c and d, and 8. In Fig. 7a several ribosomes are seen extending from a partially condensed, but not the less clearly recognizable, multivesicular body. The 'stalks' appear to consist of very small microvesicles. Fig. 7b shows another such disposition of ribosomes but the multivesicular body is itself beginning to display solid dense nodes. A cut through the surface layer of microvesicles of another multivesicular body (Fig. 7c) shows that many of the microvesicles seem to be filled in with dense material. Three nearly completed ribosome clusters are seen in Fig. 7d. Note that the cluster at the upper right still retains the orientation imposed by the original multivesicular body. In Fig. 8 it appears that entire ribosome clusters may arise from multivesicular bodies without disintegration of these bodies. It is possible that multivesicular bodies even duplicate themselves (body with the long 'tail' in Fig. 6) and these bodies could in turn give rise to ribosomes and ribosome clusters.

Still another piece of evidence for the conversion of multivesicular bodies—or at least cytoplasmic vesicles—into ribosomes is the observation that cytoplasmic

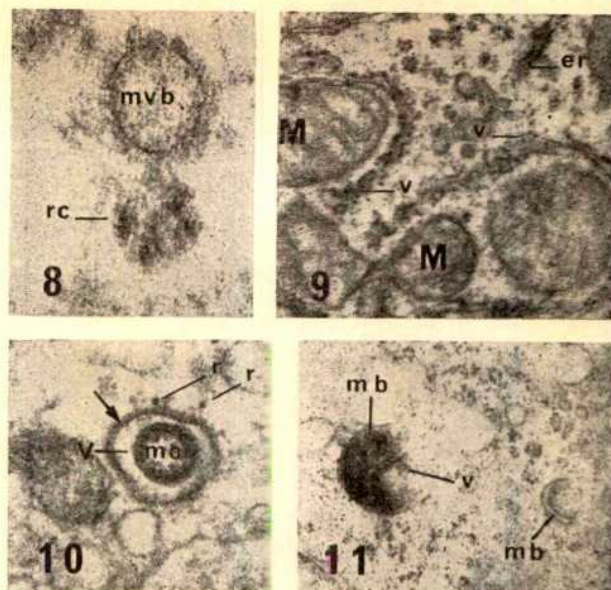


Fig. 8. The connexion between this ribosome cluster (rc) and the multivesicular body (m.v.b.) suggests that one was derived from the other. It seems likely that a 'tail' of more or less organized vesicles was elaborated as in Fig. 6 and that this tail then condensed to give a ribosome cluster. Note vesicular nature of ribosomes and vesicular mass in which they are embedded. ($\times 138,000$)

Fig. 9. Endoplasmic reticulum (er) forming around mitochondria (M) in liver of 6-day-old chicken embryo. Note apparent joining of ribosome-studded vesicles (V). ($\times 25,000$)

Fig. 10. Ribosomes (r) apparently forming from vesiculated surface (arrow) of invaginated pocket of plasma membrane (V) carrying 'mitochondrion-like' organelle into cytoplasm of 6 mm hen's egg. ($\times 42,000$)

Fig. 11. A general darkening in a mitochondrial bud (mb) and the accumulation of vesicles (v) are associated with the maturation and increase in size of a mitochondrion in somatic tissue of a 1-day-old chicken embryo. ($\times 26,000$)

vesicles in embryonic cells sometimes appear to fuse or elaborate into endoplasmic reticulum. This characteristically occurs at a more-or-less regular distance from a newly developed mitochondrion. Not only is the resulting endoplasmic reticulum studded with ribosomes but dense granules sometimes appear in the migrating vesicles prior to complete incorporation into the reticular system (Fig. 9).

For a time we thought that multivesicular bodies arising from the plasma membrane, although thus far morphologically indistinguishable from such bodies elaborated from the nuclear pores, might not display the apparent capacity for producing ribosomes¹⁵. However, further investigations in the oocyte have revealed that the plasma membrane often is converted in localized regions into a layer (or layers) of microvesicles (Figs. 5 and 10). It is from these regions that the multivesicular bodies of plasma membrane origin appear to be elaborated inward into the cytoplasm. As can be seen in Fig. 10, individual ribosomes are sometimes connected to the vesicular surface of the plasma membrane by thin, probably vesicular, strands. Because of the similarity of this orientation to that observed in those multivesicular bodies undergoing the first stages of differentiation into ribosome clusters (Fig. 7a), we entertain the possibilities: (a) that they may be elaborated from materials within the plasma membrane or (b) synthesized under the direction of—or in association with—this vesicular membrane.

We have no unequivocal proof that the multivesicular bodies specifically arising from the nuclear pores also give rise to ribosomes and ribosome clusters. However, incompletely developed ribosome clusters have been seen associated with nuclear forces. Moreover, in view of our theory that nucleolar nucleic acid is exported from the nucleus to the cytoplasm in this physical form and from the evidence presented by Edström⁹ that nucleolar RNA has

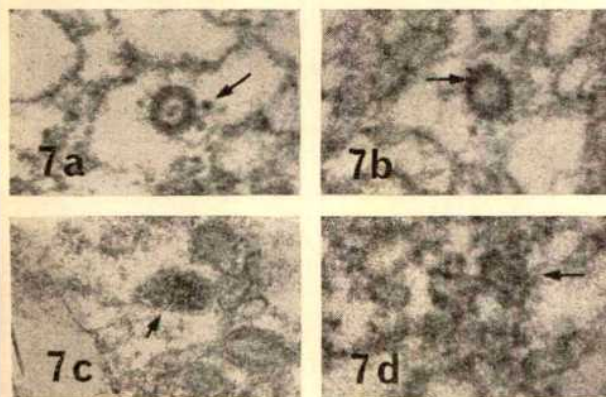


Fig. 7 a, b, c, d. This sequence suggests one mode of evolution of ribosomes and ribosome clusters from multivesicular bodies. ($\times 46,000$)

a similar base ratio to ribosomal RNA, this seems likely. Also, the extreme similarity in morphology between multivesicular bodies arising from nucleus and plasma membrane suggests the existence of this degree of similarity of function.

It is, to say the least, challenging to attempt correlation of the foregoing observations with recently proposed schemes for nucleic acid participation in protein synthesis by Gierer¹, Noll *et al.*² and Rich *et al.*³, in which the individual ribosomes are considered as single manufacturing units transcribing information for amino-acid sequence by movement from one end of an mRNA strand to the other. The nature of the evolution of the ribosome clusters in avian material suggests that the connexions from one ribosome to the other is derived from material originally present in the multivesicular precursor or at least from materials elaborated *in situ*. Although this connecting material may be mRNA already incorporated into the multivesicular body, the presence of as many as 20 ribosomes in such a cluster, the apparent linking of many ribosomes in more than two directions (indeed, by a mass of material in some cases, Figs. 3 and 8) and the presence of a centrally located ribosome in the cluster as well as encapsulating membrane material in some cases (Figs. 3 and 8), would seem to preclude the possibility of this unit acting in the manner proposed by the foregoing authors, namely, the simple movement of ribosomes in a single direction along a strand of mRNA.

Perhaps the ribosome clusters as such are not actively participating in protein synthesis. We have seen many examples in which individual ribosomes are attached by the thinnest of threads to the main mass of a multivesicular body. These may detach from the main body and thus become available to associate with a strand of messenger RNA forming a 'true' polyribosome. Following an essentially similar principle the entire ribosome clusters might disintegrate, again releasing individual ribosomes.

On the other hand, the ribosome-mRNA read-off-concept is difficult to apply to protein synthesis as it takes place in the plasma membrane, in growing mitochondria (Fig. 11) and in endoplasmic reticulum. Few, if any, such classical orientations of ribosomes and mRNA can be seen in these structures although it might be assumed that the ribosomes on the outside surface of the endoplasmic reticulum are reading off a code from mRNA in, or applied to, the membrane. Nisman and Pelmont⁵ have pointed out that careful analysis of several systems forces the conclusion that a complete *de novo* synthesis of protein does not occur in material lacking membrane or lipoprotein and that ribosomes are usually associated with such systems. Henshaw *et al.*¹⁰ have demonstrated that the major active unit for amino-acid incorporation in microsomal materials is, however, much larger than a ribosome cluster. In the livers of roosters that have received pharmacodynamic doses of oestrogens and are consequently synthesizing large amounts of phosphoproteins, we see relatively few ribosome clusters but many more ribosomes associated with a hypertrophied endoplasmic reticulum.

The apparent participation of multivesicular bodies and smaller vesicular structures which may be derivatives of multivesicular bodies in formation of yolk granules, Golgi complex, mitochondria and plasma membrane⁶ as well as cilia⁷, implies that guidance of protein incorporation into definite structure (and, probably, synthesis of protein) can be accomplished by some property of certain vesicular bodies and does not directly require the presence of ribosomes. Messenger RNA may already be present in multivesicular bodies or may associate with them in the cytoplasm and consequently may also be present in or about the structures into which the vesicles are incorporated. In these circumstances, the mRNA may be transcribed by some process other than the movement of granular ribosomes. Beljanski *et al.*¹¹ have demonstrated

the presence of an RNA in *Alcaligenes faecalis*, which accepts amino-acids directly in a ratio of one amino-acid per 6 nucleotides. Existence of DNA as well as RNA has been demonstrated in plasma membrane¹² mitochondria and *E. coli* spheroplast membranes⁵. Labelling with actinomycin D has revealed the presence in the cytoplasm of developing frog's eggs of considerable amounts of DNA largely contributed from the surrounding follicular cells. These findings, taken together with the foregoing observations, lead to the conclusion that although a variety of protein-synthesizing mechanisms may obtain in the same cell, multivesicular bodies, the ultimate origins of which would be expected to be nuclear, either that existing in the present cell, one contained in a contributing cell, one which existed in a relatively close ancestor, may of paramount importance. By means of such bodily elements necessary for protein synthesis, disbursement both, may possibly be sent, stored, duplicated, transcribed into structure and, perhaps, often re-transcribed in such mobile units, according to the demands of the immediate environment.

Balbani Ring Particles in the Cytoplasm?

In the cytoplasm of oocytes which have attained size of about 1.5–2.0 mm but not in eggs larger than 4 mm diameter, we have consistently observed the presence of largish (about 300 Å) particles. These occur singly

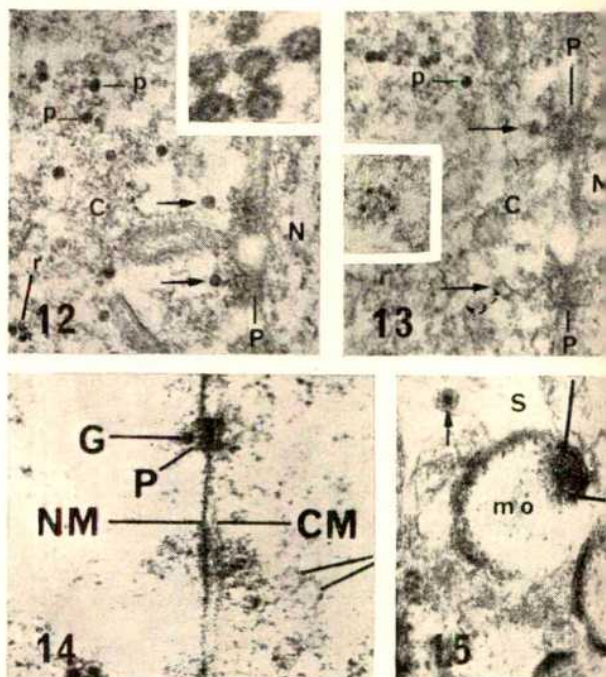


Fig. 12. The 300-Å particles (*p*) seen in the cytoplasm (*C*) of this 2-mm egg appear to have emerged from the nuclear pores (*P*) (see arrows for 2 examples), but the particles usually observed in the centres of these pores are always smaller (see tangential cuts through such pores in insert). The size, appearance and apparent origin of the larger particles suggest that they may be Balbani ring granules containing messenger RNA. *r* = ribosomes. *N* = nucleus. ($\times 50,000$)

Fig. 13. These two examples of 300-Å particles apparently having just passed through the nuclear pores (arrows) indicate either that the particles re-form from several smaller particles after entering the cytoplasm or that they sometimes unravel in the cytoplasm. It will be noted that even in the condensed form they appear to consist of several dense subunits. The insert shows the relative size of ribosomes, here arranged in a cluster. ($\times 50,000$)

Fig. 14. A presumed Balbani ring granule (*G*) is depicted here, apparently in the process of entering an annular, or nuclear, pore. *NM* = nuclear membrane. *CM* = cytoplasmic membrane. ($\times 55,000$)

Fig. 15. Many such 300-Å particles (enclosed in a matrix or membrane sheath, arrow) are seen in the intercellular spaces (*S*) between follicular cells at the time of rapid synthesis of 'mitochondrion-like' organelles (*mo*) by these cells. ($\times 50,000$)

most part and appear to be slightly more dense in their centres and along their peripheries (Figs. 12 and 13). In some cases they appear to be condensed into several nested sub-granules. Although detectable in the cytoplasm, they are present there in much less concentration than in the cytoplasm, a situation which may be due to relatively poor fixation of the interior of the nucleus. In many cases (Figs. 12 and 13) they are associated with the nuclear pores that they give the impression of having emerged from these. In Fig. 14 similarly sized particles are observed on the nuclear side of the nuclear pores. Although it is possible (perhaps even probable) that they are essentially identical with the granules observed to be present in most of the apertures of the pores (Fig. 12, insert), few instances have been seen in which the granules in the pores are as large as the large such particles in the cytoplasm. Perhaps, as is suggested in Fig. 13 (arrows) an at least partial unravelling of the granule occurs during passage through an annular pore. In morphological appearance, mode of distribution and size, these granules closely resemble the particles that originate at the sites of the Balbiani puffs, or rings, on chromosomes¹⁴. Because of the site of their origin and base ratios of the RNA associated with the Balbiani puffs⁹ the so-called Balbiani ring particles are thought to consist largely of messenger RNA. Thus, the observed particles in the cytoplasm of oocytes of this stage may represent messenger RNA.

If these particles are indeed Balbiani ring particles, as may be the first observed instance of such bodies appearing in compact form in cytoplasm. Beermann and others have shown them in the process of passing through the nuclear 'pores' of giant cells in Diptera, but in this case they appear to assume a more dispersed or thread-like form immediately on entry into the cytoplasm¹⁴.

As mentioned earlier, we have seen these granules in the cytoplasm of the oocyte at only one stage of maturation (1.5–4.0 mm egg size). It is possible that they are en route to cytoplasmic or plasma membrane sites where they can be incorporated for future utilization. On the other hand, they may be participating in some function that is peculiar to, or being originated at, this stage of development. A most conspicuous such process is the formation of 'mitochondrion-like' organelles¹⁶. Although these are synthesized at the surfaces of the follicular cells, it is suspected that the 'messages' for such syntheses originate in the oocyte. Their ultimate destinations are, indeed, the yolk granules of the developing egg. We have

long been puzzled by the occasional presence of dense granules (about 300 Å) enclosed in what appear to be envelopes of plasma membrane, in the intercellular spaces (Fig. 15). Is it possible that these granules are Balbiani ring particles carrying information to the surfaces of the follicular cells from the oocyte?

If this be so, a logical explanation is forthcoming for the occurrence of Balbiani ring granules in a condensed form (resistant to RNases) in the cytoplasm of the small oocyte. The condensed form of the messenger might provide an efficient 'capsule' for the transport of information through a region where not only is it not required but where it might also be detrimental. Inherent in this concept is the principle that condensed mRNA is recognized by, or recognizes, the site where it is to unfold and carry out its function.

This work was supported by contract AT (04-1) GEN-12 between the U.S. Atomic Energy Commission and the University of California.

¹ Gierer, A., *J. Mol. Biol.*, **6**, 148 (1963).

² Noll, H., Staehelin, T., and Wettstein, F. O., *Nature*, **198**, 632 (1963).

³ Warner, J. R., Knopf, P. M., and Rich, A., *Proc. U.S. Nat. Acad. Sci.*, **49**, 122 (1963). Slayter, H. S., Warner, J. R., Rich, A., and Hall, C. E., *J. Mol. Biol.*, **7**, 652 (1963). Kiho, Y., and Rich, A., *Proc. U.S. Nat. Acad. Sci.*, **51**, 111 (1964).

⁴ Gros, F., Naono, S., Attardi, G., Dubert, J. M., and Jacob, F., *Proc. Intern. Symp. Mechanisms of Regulation of Cellular Activities in Microorganisms*, C.N.R.S., Marseille (1963).

⁵ Nisman, B., and Pelmont, J., *Prog. Nucleic Acid Res., and Mol. Biol.*, **3**, 235 (1964).

⁶ Schjeide, O. A., McCandless, R. G., and Munn, R., *Nature*, **205**, 156 (1965).

⁷ Ehret, C. F., and de Haller, G., *J. Ultrastructure Res.*, **6**, Supp. (1963).

⁸ Karnofsky, M. J., *J. Biophys. and Biochem. Cytol.*, **11**, 729 (1961).

⁹ Edström, J. E., and Beermann, W., *J. Cell. Biol.*, **14**, 371 (1962).

¹⁰ Henshaw, E. C., Bojarski, T. B., and Hiatt, H. H., *J. Mol. Biol.*, **7**, 122 (1963).

¹¹ Beljanski, M., Fischer, C., and Beljanski, M., *C.R. Acad. Sci., Paris*, **257**, 547 (1963).

¹² Wachsmann, J. T., Fukuhara, H., and Nisman, B., *Biochim. Biophys. Acta*, **42**, 388 (1960).

¹³ Nass, S., and Nass, M. M. K., *J. Cell Biol.*, **19**, 613 (1963).

¹⁴ Beermann, W., and Clever, U., *Sci. Amer.*, **210**, 50 (1964).

¹⁵ Roth, T. F., and Porter, K. E., *Fifth Intern. Cong. Electron Microscopy* (1962), suggest that the movement of multivesicular bodies (their 'specialized sites on the cell surface for protein uptake') from the cell surface to the developing yolk granules constitutes circumstantial evidence that this is the form in which yolk proteins are carried from the circulation to the yolk granule. In this connexion we have noted that the surface sheet of vesicles of the multivesicular body are usually stripped off before this body joins the yolk granule. However, the vesicular nature of the internal sheet and the centrally located granules (Figs. 1, 2) suggest that at least this layer is also not made up of yolk proteins. It is possible that a major function of the stripped multivesicular bodies is to provide nucleic acids to the yolk granules (these are known to be present therein) but this question (and the question of yolk protein transport by these bodies) is yet to be resolved.

¹⁶ Schjeide, O. A., McCandless, R. G., and Munn, R. J., *Growth*, **27**, 111 (1963).

BREAKDOWN RATE OF SERUM 7S γ -GLOBULINS IN MICE

By DR. D. E. H. TEE, DR. J. WATKINS and MILDRED WANG

Department of Pathology, King's College Hospital Medical School, London, S.E.5

THE turnover rate of γ -globulin has been determined in a number of animal species by means of radioisotopically labelled protein. The rate of γ -globulin catabolism can be influenced by several factors. An investigation by Fahey *et al.*¹ on mice indicates that these factors include the metabolic rate of host, specific features of the protein molecule, the serum γ -globulin level and, indirectly, the rate of synthesis of γ -globulin. The production of antibodies might also be expected to confer a different catabolic rate on the γ -globulin fraction containing the antibody.

Preliminary work has shown that the antibody response in mice to hyperimmunization with homologous transplantable tumours is confined to the 7S γ -globulin fraction². Ultracentrifuge investigations have revealed appreciable increases in molecular heterogeneity of 7S

γ -globulin samples isolated from mice immunized with homologous transplantable tumours, as compared with 7S γ -globulin samples from non-immunized mice of the same strain³.

In view of the wide range of electrophoretic mobilities of 7S γ -globulin of mice and other species, it is difficult to believe that all the 7S γ -globulin molecules arise from the same site or are broken down in an identical fashion. It is therefore of interest to know whether there are differences in the breakdown rates of normal and hyperimmune serum 7S γ -globulins and whether fractions of these 7S γ -globulins, with different mean electrophoretic mobility on starch gel, also show differences.

Four series of experiments were carried out and details of strain and sex of the mice are tabulated in Table 1. Batches of about 50 normal mice of each strain were bled

Table 1. MEAN BIOLOGICAL HALF-LIFE VALUES OF MOUSE SERUM γ -GLOBULINS AS DETERMINED BY DIFFERENT METHODS

Exp. No.	Strain	Sex	No. of mice	Body count method	Serum method	Starch-gel method
Normal mice						
1	Albino	M	6	—	4.6 ± 0.37	4.4 ± 0.29
2	Albino	F	6	4.5 ± 0.16	4.3 ± 0.15	4.2 ± 0.17
3	129	3 M, 3 F	6	4.4 ± 0.29	—	3.9 ± 0.25
Immune mice						
4	129	4 M, 4 F	8	5.0 ± 0.21	5.6 ± 0.35	5.1 ± 0.23

from the tail vein to provide normal sera. Immune serum was obtained from a batch of 50 mice 129 strain immunized with a transplantable mouse sarcoma BP8. The immunizing tumour BP8 was kindly supplied by Dr. J. R. Batchelor, of Guy's Hospital Medical School, London, and this was maintained in this laboratory in strain C57BL mice. The mice received three subcutaneous injections over a period of about 3 months of the freshly homogenized tumour, collected from the C57BL mice. The sera of these mice showed considerable agglutinating activity against C57BL red cells at the end of this period.

7S γ -globulin was isolated from the normal and the immune sera by chromatography on Whatman DEAE-cellulose³. These preparations appeared to be free from other serum proteins as shown by starch gel-electrophoresis, immunoelectrophoresis (using rabbit anti-mouse serum antiserum) and by sedimentation velocity analysis³. Autoradiography after immunoelectrophoresis of preparations labelled with iodine-131 revealed some contamination with β -globulin. However, only labelled γ -globulin could be detected by this technique in serum taken from the mice 5 days after injection of the labelled γ -globulin.

The γ -globulin preparations (10 mg/ml.) were labelled with iodine-131 according to the method of McFarlane⁴, without preoxidation, and diluted with isologous mouse serum to produce solutions with activities of 100–200 μ c./ml. 0.2–0.5 ml. of these solutions were injected intraperitoneally into normal mice of the isologous strain. Before and during the experiment, the mice were given drinking-water containing sodium iodide. The total amount of γ -globulin, labelled and unlabelled, injected into each mouse was of the order 2–10 mg. This additional γ -globulin would not be expected to have any significant effect on the rate of γ -globulin catabolism.

Radiation counts were made using an annular sample arrangement with a 15-mm cylindrical sodium iodide crystal and a Panax 'Dekatron' scaler type D 657. C. The total decay of the labelled protein was followed in three ways. These were: (a) total-body counts; (b) serum counts; (c) counts of the γ -globulin fraction of the serum after separation by starch-gel electrophoresis. The latter is referred to as the starch-gel method in this article.

For method (a) each mouse was lightly anaesthetized with ether and introduced into a Paxolin tube symmetrically situated between four integrated Geiger-Müller probes. For methods (b) and (c) 0.2 ml. of blood was obtained from each mouse by bleeding from the tail vein⁵ at suitable intervals of time. Serum from each mouse was separated individually and 0.02 ml. volumes were diluted with 10 ml. normal saline for counting. Further 0.02 ml. serum samples were electrophoresed on starch gel. After electrophoresis the starch gel was sliced horizontally and both halves stained with amido black. Two fractions of the γ -globulin zone were cut out from each half of the gel. One fraction, designated S_{γ} , was cut out between the loading origin on the gel and the stained $S\alpha_2$ globulin band. The other fraction, designated F_{γ} , was the remainder of the γ -globulin up to, but excluding, the β -globulin bands. Measurements showed that the radioactivity was almost exclusively confined to the stained γ -globulin zone. Not more than 1 per cent of the overall activity was associated with the β -globulin bands and some of this was probably due to γ -globulin contamination. The gel fractions were each dissolved in 10 ml. N sodium hydroxide and counted.

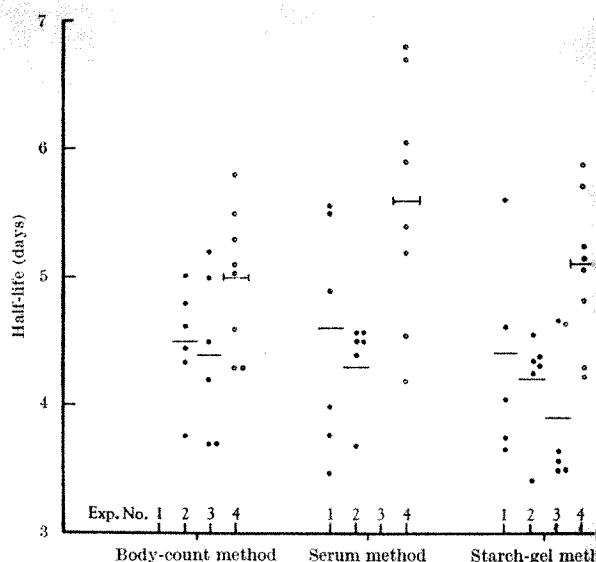


Fig. 1. Biological half-life values of the serum 7S γ -globulins of individual mice as determined by different methods. ●, Normal mice; ○, immune mice; —, mean values of half-life for normal mice; —, mean values of half-life for immune mice

The radioactivity counts obtained by each method were corrected for normal isotopic decay. Activity measurements were made 4 or 5 days after injection of the dose and were continued for up to 22 days. Biological half-life values were computed graphically for individual mice from plots of \log_{10} radioactivity against time. These half-life values were averaged and the standard error evaluated for each experiment and for each method (Table 1). The individual values are shown in Fig. 1.

These experiments suggested that there was no difference in the rate of γ -globulin turnover between the two sexes. In order to verify this, 15 male and 15 female mice strain 129 were injected with labelled normal γ -globulin and the rate of breakdown followed by the body-count method. Average half-life values of 4.6 ± 0.11 and 4.9 ± 0.17 days were obtained respectively for male and female mice. The difference between these values is not statistically significant.

The values calculated from body and serum counts were in close agreement with the starch-gel values. The starch-gel method provided three half-life values for γ -globulin, corresponding to the turnover rates of the total 7S γ -globulin (T_{γ}), and S_{γ} and the F_{γ} fractions. Table 2 shows the average half-life values obtained by the starch-gel method. The difference in rate of turnover between the S_{γ} and F_{γ} globulin fractions of normal γ -globulin was statistically highly significant.

In normal iodine-131-labelled γ -globulin preparation the initial ratio of the radioactivity in the two fractions expressed as S_{γ}/F_{γ} , was about 1.5–2. However, with labelled immune preparations, produced from two different strains of mice immunized with different transplantable tumours, ratios of about 6–8 were observed. Similar relative increases in concentration of a slow electrophoretic fraction of the γ -globulin have been observed semi-quantitatively in the immune response of the mouse to other antigens, and agglutinating antibody was largely confined to this slow fraction⁶. The extremely low level of radioactivity associated with the F_{γ} fraction of the immune γ -globulin preparation made it difficult experimentally to obtain a value of the half-life of this fraction with any degree of accuracy.

Table 2. MEAN BIOLOGICAL HALF-LIFE VALUES OF NORMAL MOUSE SERUM 7S γ -GLOBULIN FRACTIONS

	Half-life (days)
Total 7S γ -globulin (T_{γ})	4.2 ± 0.12
Slow fraction (S_{γ})	3.6 ± 0.14
Fast fraction (F_{γ})	4.6 ± 0.16

The immune γ -globulin preparation had a longer half-life in normal mice than the corresponding normal globulin preparation. This difference was highly significant statistically. This effect might have been produced by the presence of long-lived impurities in the immune preparations which were absent in the normal preparation. Similar consideration could, of course, apply to the α and $F\gamma$ fractions. There was no evidence, however, to indicate that the immune γ -globulin was significantly less pure than the normal γ -globulin. Since β -globulins had not been detected as minor impurities in both preparations by autoradiography, it was possible that the differences in catabolism rate might be correlated with small differences in the level of this impurity. Mouse β -globulins were separated from serum using DEAE-'Sephadex' chromatography. Preliminary experiments showed that the rate of turnover of β -globulin in the normal mouse was very similar to that of 7S γ -globulin, indicating that β -globulins are not responsible for any of the observed differences. It was found necessary to use freshly isolated γ -globulins from fresh sera or sera stored at -20°C for only a short time. γ -Globulin isolated from serum which had been stored at -20°C for 18 months behaved normally in starch-gel electrophoresis, immunoelectrophoresis and sedimentation velocity analysis. However, when labelled and re-introduced into mice, it was found to have a half-life of only two days. The fact that the decay of this globulin was constant for some 20 days shows that this is not denatured protein in the usual sense. It seems probable that this γ -globulin had undergone some degree of limited aggregation. Such aggregation phenomena in purified γ -globulin preparations have been reported by other workers^{8,9}. Limited aggregation of the molecules, *in vivo* or *in vitro*, may be responsible for the observed differences between the $S\gamma$ and $F\gamma$ fractions.

In summary, these studies indicate a mean biological half-life of about 4.2 days for normal mouse γ -globulin. The turnover rate did not appear to be influenced by either the sex or the strain of the mouse. The use of starch-gel electrophoresis for separating the labelled γ -globulin has made it possible to examine the rates of turnover of two fractions of the γ -globulin. These fractions, corresponding to molecules migrating with different mean mobilities when electrophoresed, had mean half-lives of 3.6 days ($S\gamma$) and 4.6 days ($F\gamma$) respectively. The fractions are probably identical with those identified by Fahey on the basis of immunological differences and on their electrophoretic mobility on starch-gel.

Immunization of 129-strain mice with transplantable tumour type BP8 produced hyperimmune γ -globulin which had a longer half-life than the corresponding normal γ -globulin preparation when re-introduced into normal 129-strain mice. The reasons for this are unknown, but it appears that this effect is not produced by long-lived impurities in the immune γ -globulin preparation. The considerable increase in the radioactivity ratio, $S\gamma/F\gamma$, of immune γ -globulins as compared with normal γ -globulins may indicate a specific response to immunization in some fraction of the 7S γ -globulin and not in the 7S γ -globulin as a whole.

¹ Fahey, J. L., and Robinson, A. G., *J. Exp. Med.*, **118**, 845 (1963).

² Tee, D. E. H., Watkins, J., Wang, M., Gammack, D. B., *Nature*, **204**, 391 (1964).

³ Tee, D. E. H., Watkins, J., Wang, M., Gammack, D. B., and Raper, J. H., *Immunology*, **9**, 85 (1965).

⁴ McFarlane, A. S., *Nature*, **132**, 53 (1958).

⁵ Adams, K. M., *J. Clin. Path.*, **13**, 265 (1960).

⁶ Parish, W. E. (personal communication).

⁷ Watkins, J., and Tee, D. E. H. (unpublished work).

⁸ James, K., Henney, C. S., and Stanworth, D. R., *Nature*, **202**, 563 (1964).

⁹ Pain, R. H., *Biochim. Biophys. Acta*, **94**, 183 (1965).

LABELLING OF MOUSE THYMOCYTES *IN VIVO* WITH TRITIATED THYMIDINE FOR CELL TRANSFER EXPERIMENTS

By DR. KIRSTINE BORUM*

National Bacteriological Laboratory, Stockholm, Sweden

N experiments designed for the tracing of tritiated thymidine-labelled donor thymocytes after injection to a recipient animal it is of importance that the donor cell suspension should have a high percentage of labelled cells. In autoradiographic detection of transfused cells, reasonably high grain count per cell is also desirable.

After a single injection of tritiated thymidine 15–20 per cent¹ and 6.1 per cent² of mouse thymocytes are reported to have been labelled, and 7–17 per cent³ of guinea-pig thymocytes.

In vitro culturing of thymocytes in a medium containing tritiated thymidine has also been applied. With this method 9 per cent of rat thymocytes⁴, 6.8 per cent⁵ and 7–12 per cent⁶ of guinea-pig thymocytes, and 6.7 per cent of mouse thymocytes⁷ were found to be labelled.

Repeated injections of tritiated thymidine *in vivo* have given a higher yield of labelled cells in the thymus. Everett *et al.*⁸, in rats, obtained a proportion of labelled thymocytes of 50 per cent after injections every 4 h for 6 h, and Murray *et al.*⁷ report a proportion of 50 per cent after three intraperitoneal injections. In young mice Sims⁹ found a maximum count of 54 per cent after six injections every fifth hour.

In the present study, inbred CBA male mice were used. Tritiated thymidine (Schwarz) with a specific

activity of 3 c./mmole was diluted in nine volumes of sterile saline to a concentration of 0.1 mc./ml., and 1 or 2 $\mu\text{c.}$ per g body-weight was injected intraperitoneally at 12 h intervals (Table 1).

From $\frac{1}{2}$ to $3\frac{1}{2}$ h after the last thymidine injection the mice were killed in ether and the thymuses removed immediately. One half the organ was fixed in 4 per cent neutral buffered formal and embedded in paraffin. Histological sections were Feulgen stained and, after being thoroughly rinsed in distilled water, were coated with 'NTB₂' (Kodak) photographic emulsion in the dark room. Autoradiographs were exposed at 4°C for five weeks and developed, fixed and rinsed in tap water for half an hour, and thereafter in distilled water. The other

Table 1. LABELLING PERCENTAGE OF THYMUS CELLS AFTER INTRAPERITONEAL INJECTIONS OF TRITIATED THYMIDINE INTO CBA MALE MICE

Mouse No.	Age at 1st inj., days	No. of injections	Dose $\mu\text{c.}/\text{g}$	Killed, hours after 1st inj.	Last inj.	Labelling percentage
1	51	5	1	50	2	45
2	51	5	2	50	2	53
3	51	6	1	63 $\frac{1}{2}$	3 $\frac{1}{2}$	46
4	51	6	2	63 $\frac{1}{2}$	3 $\frac{1}{2}$	54
5	51	7	1	75 $\frac{1}{2}$	3 $\frac{1}{2}$	60
6	51	7	2	75 $\frac{1}{2}$	3 $\frac{1}{2}$	65
7–9*	11	8	2	84 $\frac{1}{2}$	$\frac{1}{2}$	88
10–15*	48	8	2	85	1	84

* The thymus glands of mice Nos. 7–9 and 10–15 were pooled.

* Present address: Department of Rheumatology, University Hospital, Lund, Sweden.

half of the thymus was made into a cell suspension. The organ was cut with fine scissors and gently pressed through a stainless-steel mesh into a small volume of Tyrode's solution. For the preparation of smears one part of the cell suspension was mixed with an equal volume of normal mouse serum. A small drop of this mixture was placed on a glass slide which immediately before had been smeared with a solution of 20 per cent mouse serum in 0.25 M sucrose. As the sample of the cell suspension came into contact with the still moist area on the glass slide, it would spread evenly when the slide was tilted slowly. It is believed that this technique for making smears will cause little damage to fragile cells. After drying in cool air, the smears were fixed in absolute methanol for one hour and prepared for autoradiography as described above for the sections. After processing, rinsing and drying, the smears were stained through the 'NTB₃' film with Giemsa stain by the method of Gude *et al.*⁹ (Fig. 3). The percentage of labelled cells was determined by counting 1,000 cells. A density of four grains over a cell area corresponding to that of a small lymphocyte was considered as significant labelling. The labelling percentages will be seen in Table 1.

The size of the thymus in the donor animal is also of some importance in the preparation of labelled thymocyte suspensions. To study the changes in size with age, male and female mice were killed at intervals from the day of birth to the age of one year and their thymuses weighed. In male *CBA* mice, the thymus reaches its greatest weight at the age from 20 to 30 days, in the females at the age from 25 to 35 days. The weight of the thymus in relation to body-weight as a function of age

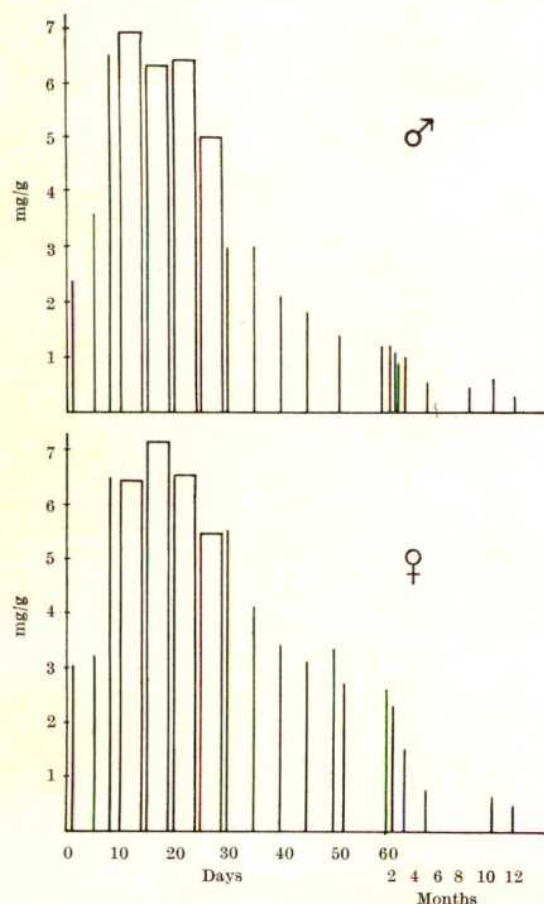


Fig. 1. Weight of thymus in relation to body-weight for male and female mice as a function of age. Each column represents the mean of values from several animals with ages within the interval indicated: days 10-14 from seven mice, days 15-19 from seven mice, days 20-24 from four mice, and days 25-29 from four mice. The single lines represent values for one or the average of two mice from the same day.

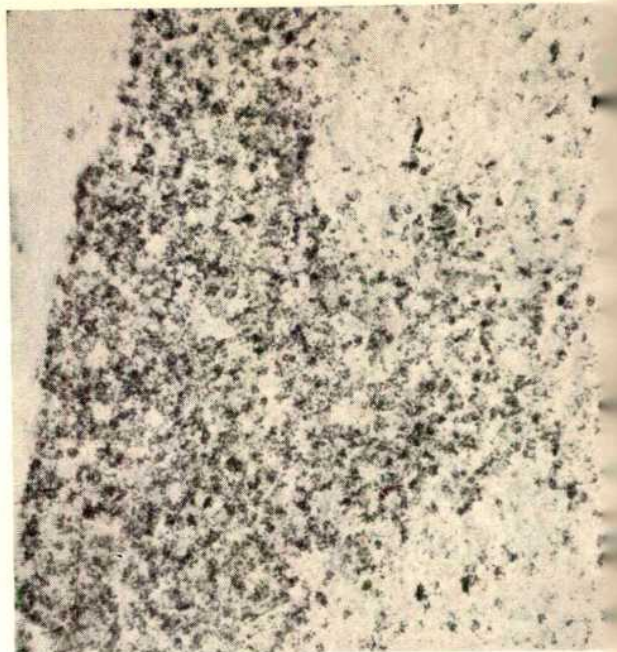


Fig. 2. Autoradiograph of Feulgen-stained section of mouse thymus. The mouse was, at the age of seven weeks, given seven injections of tritiated thymidine intraperitoneally at 12-h intervals. Virtually all cells in the thymic cortex are labelled. ($\times 250$)

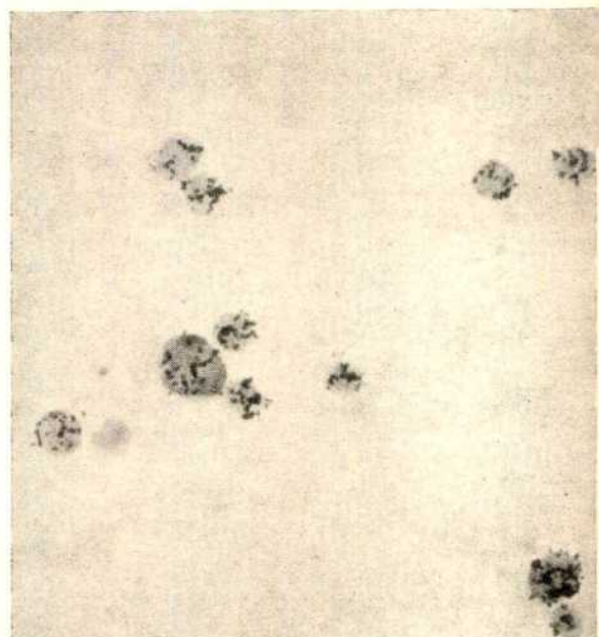


Fig. 3. Autoradiograph of Giemsa-stained smear of a thymus cell suspension. From the eleventh to the fifteenth day of age the mice received eight intraperitoneal injections of tritiated thymidine. All cells except a small lymphocyte carry label. ($\times 1,000$)

is shown in Fig. 1. It appears that the relative size reaches a maximum between 10 and 15 days of age in male mice and between 15 and 20 days of age in female mice. As the highest labelling percentage in our study 88 per cent, was also obtained at the age interval from 11 to 15 days in the males, this age seems most favourable from the point of view of isotope economy in labelling the thymus with tritiated thymidine for the purpose of making cell suspensions.

The high labelling percentage after several injections of tritiated thymidine every twelfth hour is due to the fact that virtually all cells in the thymic cortex carry the label (Fig. 2). In the not so densely populated thymi

hulla the labelled cells are scarce. From autoradiographic studies of cellular kinetics in mouse thymus¹⁰ there is evidence showing that the population of lymphocytes in the thymic cortex is renewed every fourth day. Therefore, for purposes of labelling it seems to be less to continue injections of tritiated thymidine for more than four days because of the continuous loss of label from the thymus, probably due to emigration of small thymocytes. Augmenting the dose of tritiated thymidine from 1 to 2 μ c. per g body-weight appears to give a higher labelling percentage. It is for the investigator to decide whether the possible radiation damage from the higher dose will be acceptable in his experimental set-up.

Preliminary results from experiments where the donor lymphocyte suspension pooled from mice Nos. 7-9 was injected intraperitoneally into isologous mice showed that at 1 and 4 days after the injection labelled lymphocytes

were found in the spleen and lymph-nodes, and more diffuse labelling was noted in the liver. No labelling was detected in the thymus.

This study was supported by a grant from the Swedish Medical Research Council, project F-0143-B-A.

¹ Schooley, J. C., Bryant, B. J., and Kelly, L. S., in *The Kinetics of Cellular Proliferation*, edit. by Stohman, F., 208 (Grune and Stratton, New York, 1959).

² Hill, M., *Exp. Cell Res.*, **28**, 21 (1962).

³ Yoffey, J. M., Reinhardt, W. O., and Everett, N. B., *J. Anat.*, **95**, 293 (1961).

⁴ Everett, N. B., Rieke, W. O., Reinhardt, W. D., and Yoffey, J. M., in *Ciba Found. Symp. Haemopoiesis*, edit. by Wolstenholme, G. E. W., and O'Connor, Maeve, 43 (Churchill, London, 1960).

⁵ Diderholm, H., *Acta Path. et Microbiol. Scand.*, **51** (Suppl. 146) (1961).

⁶ Everett, N. B., Caffrey, R. W., and Rieke, W. O., *Ann. New York Acad. Sci.*, **113**, 887 (1964).

⁷ Murray, R. G., and Murray, A., *Blood*, **18**, 737 (1961).

⁸ Mims, C. A., *Brit. J. Exp. Path.*, **43**, 639 (1962).

⁹ Gude, W. D., Upton, A. C., and Odell, T. T., *Stain Technol.*, **30**, 161 (1955).

¹⁰ Borum, Kirstine, *Tenth Congr. Intern. Soc. Haematol.*, H: 20 (1964) (Abstr.).

EFFECT OF A PYRIMIDOPYRIMIDINE DERIVATIVE ON THROMBUS FORMATION IN THE RABBIT

By DR. P. R. EMMONS, M. J. G. HARRISON, DR. A. J. HONOUR and DR. J. R. A. MITCHELL

Department of the Regius Professor of Medicine, Radcliffe Infirmary, Oxford

BORN, Honour and Mitchell¹ showed that the systemic administration of adenosine and 2-chloradenosine modified the behaviour of platelet thrombi in injured arteries in the rabbit. They suggested that if these substances proved to be free from toxic effects, they might be of value in the prevention and treatment of human arterial thrombosis. Adenosine is a powerful vasodilator², and produces a marked fall in arterial blood-pressure; moreover, it is rapidly broken down in the body by deamination. 2-Chloradenosine is more resistant to deamination³, but produced respiratory arrest in two of the rabbits examined by Born, Honour and Mitchell¹. When unag *et al.*⁴ reported that a pyrimidopyrimidine derivative (RA8-Persantin; Boehringer-Ingelheim, Ltd.) retarded the rate of disappearance of exogenous adenosine from whole blood *in vitro*, it seemed possible that this compound might be used to potentiate exogenous adenosine *in vivo*, and permit the anti-thrombotic effect observed by Born, Honour and Mitchell¹ to be obtained with lower doses. A detailed examination of the properties of the compound was therefore undertaken⁵. In concentrations of 10^{-4} M, RA8 was found to inhibit the platelet aggregation produced *in vitro* by adenosine diphosphate (ADP); no exogenous adenosine had to be added to produce this effect. It therefore seemed profitable to examine the effects of RA8 on thrombus formation *in vivo*, and this article reports the results of these observations.

Grey chinchilla rabbits of either sex (2.9-4.6 kg) were used. Each rabbit was anaesthetized with urethane (6.0 ml. per kg of a 25 percent solution in 0.9 percent sodium chloride injected intraperitoneally) and a tracheotomy tube was inserted. Arterial blood-pressure was recorded kymographically from a cannula in the femoral artery and intravenous injections were given through a polythene cannula tied into the left external jugular vein, the catheter being perfused with normal saline at a rate of 0.1 ml./min between injections. Blood samples for platelet clumping were withdrawn from a polythene cannula passed down the left common carotid artery into the aorta. Between samples the cannula was occluded with a wire stilette, and the samples were collected into all-glass syringes, lubricated with silicone fluid (MS 550; Hopkin and Williams). Platelet-rich citrated plasma was then prepared, and its clumping activity was determined by the Born

optical density method⁶, the details of the techniques used being set out fully elsewhere⁷.

The right cerebral cortex was exposed and the vessels on its surface observed as described by Honour and Ross Russell⁸. Two degrees of arterial injury⁹ were used to assess the effects of RA8.

(a) *Major injury.* An artery was pinched firmly with needle-pointed ophthalmic forceps, causing bleeding and the formation of a haemostatic plug outside the vessel. Inside the vessel a steady succession of white platelet masses or 'white bodies' formed at the site of injury and embolized. By observing the size of the emboli and by timing their rate and duration of production, the activity of the site could be assessed.

(b) *Minor injury.* An artery was gripped gently with the forceps. This did not produce bleeding and no white bodies formed spontaneously. When solutions of ADP, adenosine triphosphate (ATP) or 5-hydroxytryptamine were applied to the external surface of the injury, platelet masses formed in the lumen and embolized for as long as the application was continued. By applying the agents in a standard way (1 drop/sec for 1 min) and by testing progressively lower concentrations, the lowest concentration of each agent which produced white bodies was determined.

(A) *RA8 given after major injury.* Table 1 summarizes the results obtained in 7 rabbits which were given a single intravenous injection of RA8. In each case, a major injury had been inflicted at least 30 min before the injection, and the steady production of white bodies had been

Table 1. EFFECT OF INTRAVENOUS INJECTIONS OF RA8 ON THE PRODUCTION AND EMBOLIZATION OF WHITE BODIES AT MAJOR INJURY SITES

Rabbit	RA8 dose (mg)(mg/kg)	Effect	Interval from injection to effect (min)	Duration of effect
I	40 10.3	White body formation stopped	1	Still present at 5 h
II	10 3.0	" "	9	Present at 4 h
III	10 2.7	" "	2	Present at 3 h
IV	10 2.4	" "	3	Present at 3 h
V	5 1.5	" "	10	Present at 3 h
VI	1 0.3	Small, fragmentary white bodies formed, which washed away as they developed	8	20 min, but masses still abnormal at 4 h
VII	0.25 0.06	Small white bodies which did not stick at bifurcations	5	11 min

observed and timed. When more than 1.5 mg/kg of RA8 was given, white body formation was completely abolished, and did not start again during a prolonged period of observation. In these rabbits, a qualitative change in the character of the white bodies was noted in the interval between the injection of RA8 and the cessation of activity: the masses became smaller, were pink, floccular and less well defined. They streamed away rapidly from the site and did not become impacted at bifurcations. These changes were similar to those observed by Born *et al.*¹ when adenosine and 2-chloradenosine were given. The lower doses (rabbits VI and VII) did not abolish white body production, but profoundly modified the activity of the site. The white bodies produced were very small, in comparison with those seen in the control period, and for a period of 20 min and 11 min respectively in these two rabbits, the platelet masses consisted merely of swirling fragments which did not stick at bifurcations.

(B) *Behaviour of major injuries inflicted after RA8 injections.* Major injuries were inflicted on two rabbits, and observed for 15 min, during which period a steady succession of dense white bodies formed and embolized. When these masses left the injury site they stuck at bifurcations. Each rabbit was given 10 mg RA8 intravenously and in 2 and 3 min respectively the initial injury sites were inactive. Major injuries were then inflicted at intervals after the RA8 injection, new arterial systems being used for each injury. Table 2 shows that the duration of white body formation at each new injury site increased with the passage of time after RA8 administration. The nature of the masses produced also showed a graded response with time: injuries inflicted immediately after RA8 could only produce a faint hazy mass, while at longer intervals after the injection the masses became larger, more persistent and more dense. None of the injuries produced an unusual amount of bleeding, and firm external haemostatic plugs formed on the outside of the injured vessels in the usual way. Even the injuries inflicted immediately after the RA8 had been given showed a normal haemostatic response, although these sites could only produce feeble, transient white bodies. Born *et al.*¹ found a similar dissociation between haemostasis and thrombosis when adenosine and 2-chloradenosine were given, and this holds out the hope that effective antithrombotic drugs may be found which do not prejudice haemostasis. The dissociation between external and internal platelet plug formation may mean that the stimulus to platelet aggregation in blood shed from a vessel is different from the mechanism which operates within the lumen, or that the mechanism is the same, but that the initial response of the injury site is so intense that it cannot be blocked by any of the agents so far tested.

(C) *Effect of RA8 on minor injuries.* Table 3 shows the effect of RA8, 10 mg given intravenously, on the lowest concentrations of the three clumping agents which were able to produce white bodies when they were applied to a minor injury site. Eighteen min after RA8 had been given, the response to 5HT had been abolished, while the end-point levels for ADP and ATP had only changed by a factor of two and four respectively. However, the character of the ADP and ATP response had been profoundly changed. Under control conditions, increasing the concentration of the locally applied substances above the

Table 3. EFFECT OF INTRAVENOUS RA8 (10 MG, 2.6 MG/KG) ON THE MIN EFFECTIVE CONCENTRATIONS OF ADP, ATP AND 5HT WHICH WERE CAPABLE OF PRODUCING WHITE BODIES AT A MINOR INJURY SITE

Substance	Before RA8	Minimal effective concentration (μg/ml.)		
		18 min after RA8	At 30-min intervals after RA8	5 h after RA8
ADP	8	16	Not tested	8
ATP	2	8	Not tested	2
5HT	2	No effect from 250	No effect from 250	No effect from 250

Table 4. EFFECT OF A SERIES OF INJECTIONS OF RA8 ON THE MIN CONCENTRATION OF 5HT WHICH WAS CAPABLE OF PRODUCING WHITE BODIES AT A MINOR INJURY SITE

(mg)	RA8 dose (mg/kg)	Minimal effective concentration of 5HT (μg at specified intervals after RA8 injection)			
		0	0.5	0.5	0.5
0.25	0.09	+6 min	2.0	+60 min	2.0
0.5	0.17	+3 min	8.0	+37 min	8.0
1.0	0.34	+2 min	64.0	+60 min	4.0

end-point produces progressively larger, denser and more persistent bodies; after RA8 this graded response was lost and ADP and ATP at concentrations of up to 250 μg/ml. produced the same faint, transient masses which the end-point concentrations evoked. 5 h after the RA8 injection the site still did not respond to 5HT, 250 μg/ml., where it had regained its responsiveness to ADP and ATP. The end-points having returned to their pre-RA8 level and the normal gradation of response being restored.

Table 4 shows that the effect of RA8 on the 5HT sensitivity of a minor injury site is closely related to the dose used, 0.25 mg giving a four-fold decrease in sensitivity, 0.5 mg a sixteen-fold reduction and 1.0 mg reducing it a factor of 1/128. In rabbit X (Table 3), which received 10 mg RA8, the change in 5HT response persisted more than 5 h, whereas in rabbit XI, which received or 1.75 mg, spread over 97 min, the 5HT responsiveness was beginning to return some 60 min after the last RA8 injection.

(D) *Effect of local application of RA8.* A major injury was inflicted, and was observed to produce dense, adherent white bodies for 23 min. RA8, 500 μg/ml. in physiological saline, was then applied to the external surface of the injury at 1 ml./min for 5 min. As soon as the application began, the white bodies became smaller (only grew half-way across the lumen before embolizing, where they had previously been filling the whole lumen) and were less dense, appearing to wash away as rapidly as they were forming. Twenty-one min after the beginning of the application, the site became inactive and did not regain its activity in a 200-min period of observation. 107 min after the application, the responsiveness of the site to ADP, ATP and 5HT was tested. ADP and ATP both produced characteristic white bodies (end-point 2 μg/ml. for each agent with a normal gradation of response to higher concentrations) while 32 μg/ml. of 5HT was needed for white body production. Higher concentrations of 5HT produced white bodies similar in size, density and duration to those seen with the end-point concentration. A second major injury was then inflicted, and after it had produced white bodies for 15 min, RA8, 500 μg/ml., was applied as already described here. Once again, there was immediate reduction in the size of the white bodies, and 20 min after the application, the site became inactive.

A major injury was inflicted on another rabbit. The site produced huge, tenacious white masses for 26 min. RA8, 100 μg/ml. in saline, was then applied, and within 1 min the white mass present at the site began to break up and wash away. Small, fragmentary white bodies continued to form, but 37 min after the start of the application the site became quiescent and remained so for the remaining 2 h in which the preparation was observed.

(E) *Effect of RA8 on circulating platelets.* The inhibitory effect of RA8 applied locally to injured arteries shows that the agent can act directly on the injury site, but in view of the reduction of *in vitro* platelet clumping activity observed by Emmons *et al.*⁵ when RA8 was added to platelet-rich plasma systems, we measured the clumping

Table 2. DURATION OF WHITE BODY FORMATION AT MAJOR INJURIES INFLECTED AT VARIOUS INTERVALS AFTER A SINGLE INTRAVENOUS INJECTION OF RA8

Rabbit	RA8 dose (mg)	Interval between RA8 injection and injury (min)	Duration of white body formation (min)
VIII	10	2.6	2
			15
			4
			7
IX	10	2.7	18
			3
			10
			15
			35
			37

vity of rabbit platelet-rich plasma before and at intervals after an injection of *RA8*. Two samples of arterial blood were obtained, and *RA8*, 10 mg, was then given intravenously. Further arterial blood samples were obtained 2, 10–19, 20–40 and 60–90 min after the injection. Platelet-rich citrated plasma was prepared, and clumping response to ADP 1.5 µg/ml. was measured. For each sample the maximum fall in optical density observed after the addition of ADP was expressed as a percentage of the fall in optical density produced by high-speed centrifugation. The mean percentage fall in optical density for the two control samples was then taken as unity, and the clumping activity at the various times after *RA8* was expressed as a ratio of this control level. The *RA8* content of platelet-poor plasma was measured spectrofluorimetrically as described by Beisenherz *et al.*¹⁰. The results are shown in Table 5. There was a marked reduction in ADP-induced clumping 2 min after the injection of *RA8*; in the next sampling period this inhibition was less marked, and thereafter platelet-clumping activity had returned to its control-level.

Table 5. MEAN CLUMPING ACTIVITY WITH ADP 1.5 µg/ML. EXPRESSED AS A RATIO OF CLUMPING ACTIVITY IN CONTROL PERIOD, AND MEAN PLASMA *RA8* LEVELS, IN 6 RABBITS GIVEN 10 MG *RA8* INTRAVENOUSLY

Time (min) after <i>RA8</i> injection	Clumping ratio	Plasma <i>RA8</i> level (µg/ml.)
2	0.76	3.05
10–19	0.88	0.82
20–39	0.99	0.48
60–89	0.95	—

Our results show that *RA8* profoundly modifies thrombus formation in injured arteries. The efficacy of topical applications, and our inability to demonstrate anything more than a transient change in the clumping activity of the circulating platelets, suggest that the agent is acting at the site of injury rather than on the circulating blood. Honour and Mitchell⁹ showed that thrombus formation in damaged vessels is brought about by compounds similar in properties to ADP and ATP. These are formed in the injured tissues, and are released into the lumen. *RA8* must interfere with this mechanism, either by cutting off the supply of thrombus-producing material or by producing an inhibitory substance.

RA8 has been shown to possess many interesting properties; it has marked vasodilator activity, produces some reduction in blood pressure, increases the rate and depth of respiration and produces bronchodilatation¹¹. The sites remain responsive to infused catecholamines, so simple sympathetic blockage cannot account for these changes, and Ungvárny *et al.*¹² have produced an alternative mechanism by showing that the agent produces a reduction in circulating catecholamines. *RA8* has other more fundamental actions, for it increases glucose consumption and reduces lactic acid formation in the hypoxic heart¹³. This finding may relate to the work of Frimmer *et al.*¹⁴, who showed that *RA8* was fixed by mitochondria and that it reduced the critical oxygen pressure in tissue culture studies. In ischaemic heart muscle, *RA8* increases the adenosine content; this increase results from decreased adenosine breakdown, rather than increased formation¹⁵. *RA8* might therefore interfere with white body formation in our injured arteries by allowing adenosine to accumulate at the injury site. However, topical and systemic *RA8* produce immediate inhibition, whereas there is a lag of some 30 min after systemic adenosine administration before an inhibitory effect is seen, while topical adenosine is inactive. It therefore seems probable that *RA8* interferes directly with the formation by the injury site of the white-body producing substance. Alternatively, it may affect the way in which the thrombus-forming message is propagated through the growing platelet mass. After injury, the first layer of platelets to adhere to the injury site must be capable of transmitting information to oncoming cells, and this process continues until the mass completely blocks the lumen. *RA8* might be acting on the circulating cells, and rendering them incapable of

propagating the injury message. The methods of studying platelets described here may not be capable of detecting this change. It would be of interest to know whether a major injury site rendered inactive with *RA8* is free from platelets, because the site is no longer producing the injury substance, or whether it is covered with a thin layer of platelets which are not propagating the injury message. We hope that electron micrograph investigation will help to resolve this question.

As *RA8* exerts such a powerful anti-thrombotic effect in injured arteries, could it be of value in the management of human vascular thrombotic disease? Mitchell¹⁶ has suggested that there are two alternative models of thrombus formation. In the first, hitherto normal circulating cells encounter an abnormal arterial segment. This is the situation in the injured rabbit cortical arteries which we have used to investigate *RA8*. In the second system, the circulating cells are already abnormal and are ready to adhere together; the location of the thrombus is determined, not by a message from an abnormal vessel wall, but by critical flow situations which permit cell collision and adhesion. We do not know which of these model situations is relevant to human disease, but would point out that our studies on the efficacy of *RA8* only pertain to thrombus formation in injured vessels, and we need further information on the action of *RA8* in man.

We have examined the effect of intravenous and oral *RA8* on the behaviour of human blood platelets⁵, and have shown that spontaneous platelet aggregation is reduced. Further examination of the clinical effects of *RA8* in thrombotic disease is clearly called for; unlike adenosine and 2-chloroadenosine, which would need detailed toxicity investigations before they could be used therapeutically, there is already a substantial corpus of information available about *RA8*. It appears to be remarkably non-toxic for a drug with such varied and powerful actions. Kadatz¹¹ showed that in mice the acute *LD*₅₀ for intravenous *RA8* was 150 mg/kg, while for oral administration it was 2,150 mg/kg. In long-term investigations, dogs were given 20 mg/kg/day, and rats received 100 mg/kg/day, without ill effect. We have used 600 mg/day by mouth in man⁵ obtaining plasma *RA8*-levels of up to 5 µg/ml. (10⁻⁵ M) without ill effect. One undesirable property of *RA8* might be its hypotensive effect; but in our anaesthetized rabbits we did not observe a reduction in pressure of more than 30 mm mercury. This is in accordance with the experience of Kadatz and Beisenherz¹⁷, who found a similar fall in blood pressure in dogs given 1.0 mg/kg (18.5 mm pressure reduction) to those given 4.0 mg/kg (23.8 mm pressure fall). We would therefore suggest that, in addition to the contribution made by *RA8* to the investigation of experimental thrombus formation, its lack of toxicity makes it a suitable material for clinical investigations in man.

¹ Born, G. V. R., Honour, A. J., and Mitchell, J. R. A., *Nature*, **202**, 761 (1964).

² Born, G. V. R., Haslam, R. L., Goldman, M., and Lowe, R. D., *Nature*, **205**, 678 (1965).

³ Born, G. V. R., *Nature*, **202**, 95 (1964).

⁴ Banag, R. D., Douglas, C. E., Imai, S., and Berne, R. M., *Circ. Res.*, **15**, 83 (1964).

⁵ Emmons, P. R., Harrison, M. J. G., Honour, A. J., and Mitchell, J. R. A., *Lancet* (in the press).

⁶ Born, G. V. R., *J. Physiol.*, **162**, 87P (1962).

⁷ Emmons, P. R., and Mitchell, J. R. A., *Lancet*, **i**, 71 (1965).

⁸ Honour, A. J., and Ross Russell, R. W., *Brit. J. Exp. Path.*, **43**, 350 (1962).

⁹ Honour, A. J., and Mitchell, J. R. A., *Brit. J. Exp. Path.*, **45**, 75 (1964).

¹⁰ Beisenherz, G., Koss, F. W., Schüle, A., Gebauer, I., Bärtsch, R., and Fröde, R., *Arzneimittel Forschung*, **10**, 307 (1960).

¹¹ Kadatz, R., *Arzneimittel Forschung*, **9**, 39 (1959).

¹² Ungvárny, L., Hovanyi, M., and Farkas, F., *Z. Kreislaufforschung*, **51**, 1096 (1962).

¹³ Kunz, W., Schmid, W., and Siess, M., *Arzneimittel Forschung*, **12**, 1098 (1962).

¹⁴ Frimmer, M., Hegner, D., and Winkelmann, W., *Klin. Wschr.*, **41**, 715 (1963).

¹⁵ Gerlach, E., and Deuticke, E., *Arzneimittel Forschung*, **13**, 48 (1963).

¹⁶ Mitchell, J. R. A., in *Biological Aspects of Occlusive Vascular Diseases*, edit. by Chalmers, D. G., and Gresham, G. A. (Cambridge, 1964).

¹⁷ Kadatz, R., and Beisenherz, G. (unpublished results).

A SEARCH FOR MUTATION IN CHEMICAL SYSTEMS AT STEADY STATE

By DR. GORDON ALLEN

National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland

IN a steady state, a transient condition is maintained by continuous repetition. A complex system (for example, an organism) may be essentially in a steady state with respect to all major components, while minor components continue to change. A chemical mixture containing active reagents and continually replaced at a slow rate with freshly mixed solution approaches a steady state gradually. In such an 'open' system, systematic changes will presumably become smaller and smaller until they reach the level of random molecular events. A late, small systematic change and even a single molecular event might be detectable if amplified in some way.

Catalysis and especially autocatalysis seem to offer promise as amplifiers. In an open chemical system, a late-appearing minor product which inhibited or catalysed some early and major reaction might alter the number or proportions of the recognized components. Such a catalyst, interesting in itself, could be inferred from its effects on the system even if it was below the minimum concentration for direct assay. Thus, if one cannot deliberately use catalysis to detect late changes in an open system, one may investigate late changes in the hope of detecting interesting examples of catalysis.

With respect to molecular species having individually small probabilities of occurrence, the mixture would continue to change indefinitely. The more probable molecules would appear early and frequently; the least probable molecules, only singly and at long intervals. One of these rare molecules, even if it were a powerful catalyst, could not exert enough effect on other reactions to be detected during its lifetime. However, such a molecule might catalyse the formation of one of its own precursors. If in its lifetime the first molecule of catalyst increased the amount of precursor enough to make probable the formerly improbable synthesis of catalyst molecules, an autocatalytic system would result. The precursor and all derivatives including the catalyst would tend to increase until side reactions or the reverse of the catalysed reaction imposed a new steady state. If the reactants in the catalysed step were sufficiently abundant, one or more of the derivatives could reach detectable levels so that the new mixture would be recognizably different from the original.

This phenomenon, a late change due to the origin of a self-catalysing system, will be referred to as mutation in the common non-technical sense of the word and also in analogy with genetic mutations in organisms. Such a mutation can be defined operationally as a permanent change of composition in an established steady state such that the new composition can be induced in another similar mixture by a small inoculum from the original. It is not important whether the catalyst was present at very low concentration in the first steady state, or whether it appeared after an interval as a single, improbable molecule; these conditions are interchangeable with changes in volume and molar content of the system.

The general interest attached to homogeneous catalysis by organic molecules and particularly to simple self-reproducing systems¹⁻³ prompted the search described here. Despite negative results, it is reported as an investigation of evolution in a chemical system⁴, distinct from the already frequent and successful investigations of processes which could initiate chemical evolution⁵. Since there would be little reason for others to repeat the negative experiments, the findings and methods will not be described in complete detail. It is appropriate, how-

ever, to report the number and diversity of experiments and the analytical effort which will have to be improved if the demonstration of mutations in chemical systems is to succeed.

To maximize the number and rate of reactions and the likelihood of catalytic effects, it seems appropriate to use highly reactive compounds at moderately elevated temperatures and in maximal concentration consistent with a homogeneous solution. An approximate steady state can be maintained by periodic renewal of part of the mixture. Recognition of late changes which yielded new products would require only standardized analysis for detection, not identification of components. One more new spot appearing during chromatography after a solution had apparently stabilized, or a sharp change in relative intensity of certain spots, would direct attention to possible catalysis by a late product. A mutation would then be indicated if the old and new chromatographic results could be produced side-by-side in simultaneous analyses of two freshly-started solutions, one of which had been 'seeded' with the old solution. Even if no change had been detected in the original steady state, a reproducible difference between seeded and unseeded solutions would demonstrate an effect of inoculation that might represent catalysis.

The experiments were conducted in 100-ml. samples in side-arm flasks half immersed in oil at 90° C. The flasks were connected through reflux condensers to a slow-flowing stream of nitrogen under slight positive pressure. Most of the seeding tests were conducted in smaller samples in side-arm test-tubes with cold-finger condensers. The basic reagents, formaldehyde and cyanide, were selected as the most reactive one-carbon compound readily obtainable in a high state of purity. Ammoniacal allyl alcohol, diacetyl and pyruvic acid were added singly or in combination to some solutions, and in two solutions cyanide was omitted. Each of nine combinations was replicated in solutions buffered at pH 7 and pH 9 with sodium, chloride and phosphate ions. Table 1 shows the main reagents in the eighteen experiments.

Experiments 17 and 18 were initiated just two months before the end of the project, but the other experiments ran for between 14 and 20 months. The initial renewal schedule was 10 per cent daily, and after 2-4 months the interval was lengthened to 20 per cent semi-weekly or 10 per cent weekly in different experiments. A number of the solutions were eventually modified by addition of minerals in catalytic quantities: MgSO₄, CaCl₂, CuSO₄, FeSO₄, MnCl₂, ZnCl₂, KI, I₂, and reduced sulphur as cysteine. Continuous ultra-violet radiation⁶ was supplied in experiments 17 and 18 and in the last four months of experiments 13-16 by means of pencil-shaped mercury vapour lamps mounted in the side-arm stoppers and dipping under the surface of the solutions.

Samples were analysed by evaporation to dryness under nitrogen at 40° C, and the initial distillate was collected

Table 1. MOLAR CONCENTRATIONS OF PRINCIPAL REAGENTS
Odd-numbered solutions were buffered at pH 9; even-numbered solutions at pH 7

	Exp. (solutions)																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Sodium cyanide	0.1	0.1	0.1	0.1	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.1	0	0	0
Formaldehyde	0.1	0.1	0.1	0.1	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.1	0.1	0.1	0.1
Ammonium hydroxide					0.1		0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.1	0.1	0.1	0.1	0.1
Allyl alcohol									0.1						0.1	0.1	0.1	0.1
Diacetyl											0.1				0.1	0.1	0.1	0.1
Pyruvic acid													0.1	0.1	0.1	0.1	0.1	0.1

or gas chromatographic analysis. The residue after distillation was extracted in turn with methyl acetate and ethanol and was finally re-dissolved in water. The three resulting extracts were chromatographed in two dimensions on thin layers of silica gel. Three pairs of solvent mixtures were devised to give good separations of each extract in the early experiments (Fig. 1), but for the more complicated solutions these systems were supplemented with one-dimensional chromatography in other solvents (Fig. 2). The dry chromatograms were in most cases sprayed with alkaline KMnO_4 , yellow spots on the pink background were marked to indicate their order of appearance, and the results were recorded as negative photographic prints before they began to fade. In most of the seeding tests, carbon-14-labelled formaldehyde and/or cyanide were used and autoradiograms were made⁶. Some chromatograms, particularly in the seeding tests, were examined by means of more sensitive or more specific location reagents or were viewed under ultra-violet light.

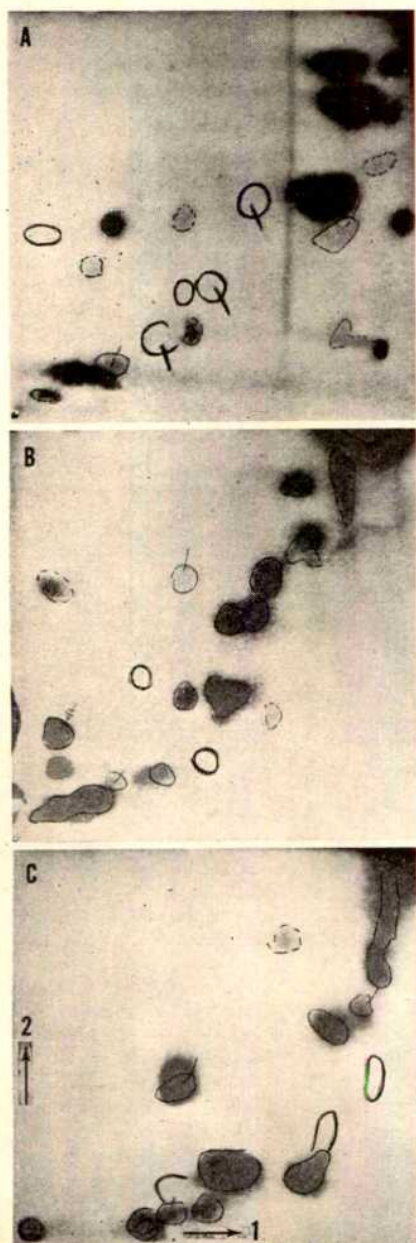


Fig. 1. Typical thin-layer chromatograms of solution 3 in the steady state. A, Methyl acetate extract; B, methanol extract; C, extracted residue. Each fraction was chromatographed with a different, appropriate pair of solvents, but there is some duplication of separated compounds in successive chromatograms

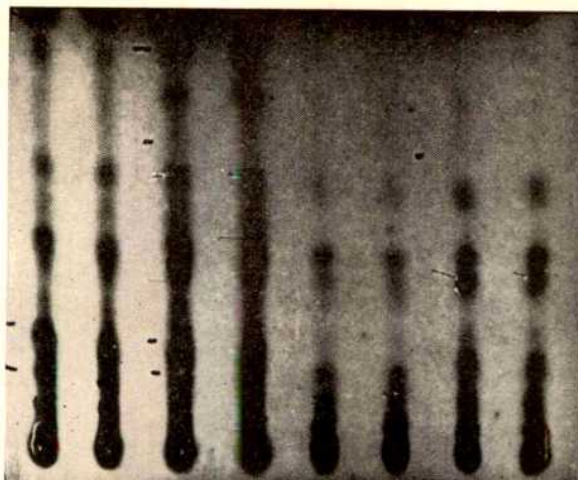


Fig. 2. Methyl acetate extracts chromatographed in a water-free system to separate compounds that coalesced near the front in the usual two-dimensional analysis (compare with Fig. 1 A). Extracts of four pairs of solutions obtained in seeding tests of experiments 15, 16, 17, 18; the left member of each pair had been seeded

Hydrogen cyanide in aqueous ammonia is known to produce a large variety of biologically interesting compounds spontaneously. Oro⁷ identified seven compounds including adenine. At least 75 ninhydrin-reacting compounds have been distinguished after hydrolysis of the products and at least fifty compounds absorb or fluoresce in ultra-violet light⁸. The reactions of alkaline cyanide with formaldehyde also proceed rapidly and yield a variety of compounds⁹. In the experiments recorded here, cyanide and formaldehyde yielded 27 reproducible spots on chromatograms of the entirely non-overlapping aqueous and methyl acetate extracts when permanganate was the location reagent. Autoradiography revealed as many as 48, and many additional spots could be detected by fluorescence. Four strong spots were ninhydrin positive and many others revealed nitrogenous content by reacting with sodium hypochlorite or tertiary butyl hypochlorite. A few spots gave one or more positive tests for double bonds, phenols, esters and aldehydes. Gas-liquid chromatography of the formaldehyde-cyanide mixtures in a steady state revealed from three to five products; but the method would not have detected highly polar compounds.

Addition of other reagents resulted in more numerous products. Thus, gas chromatograms of solutions 15 through 18 all had at least 19 indicated peaks in addition to those obtained with the reagent blanks (Fig. 3). Solutions containing diacetyl acquired a surface film and a variable amount of brown turbidity or sediment. Changes in renewal schedule and addition of minerals had surprisingly little effect on most of the chromatograms. Ultra-violet light had little effect in the turbid solutions, but changed solutions 13 and 14 rather extensively. In addition to the regularly appearing spots, others occurred in only a few analyses or were poorly differentiated. Some which appeared late in the investigation could be attributed to improvements in technique or to changes in the experiments. While the less-reliable spots could not be taken as evidence of mutation in themselves, they could be examined to advantage in the seeding tests, in which seeded and control solutions were analysed simultaneously and compared in fine detail (Fig. 2).

The reactions proceeded rapidly in all solutions, yielding yellow or red-brown colour in the first hour and going on to nearly black in many of the mixtures. Nearly all spots which appear on chromatograms could be found at 24 h, and after four days there were only minor changes. In experiments 1-8, control solutions were maintained without renewal for several weeks; they yielded pro-

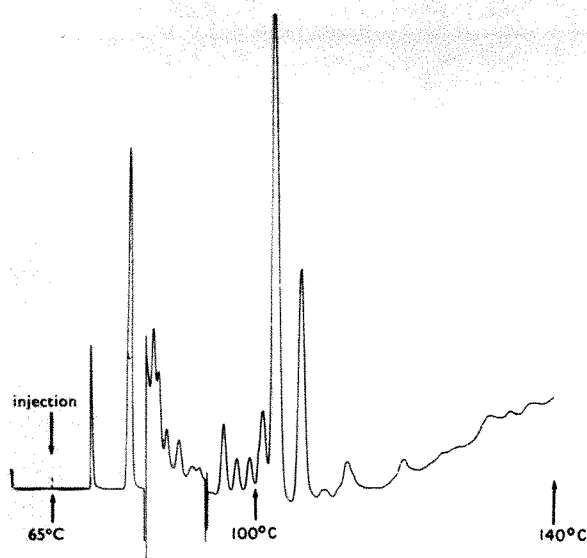


Fig. 3. Gas-liquid chromatogram of volatile fraction, solution 17. The two deflexions below the base-line correspond to increases in sensitivity by factors of 200 and 2, respectively. The second peak coincides with that of one reagent, allyl alcohol. Column used: 'Castorwax' on 'Chromosorb W' (HMDs) (Wilkens Instrument Co.), 2 meters

gressively simpler chromatograms, and alkaline solutions 1 and 5 lost colour.

No good evidence of mutation was found. A few differences between seeded and control solutions were observed, but these were either not reproducible or could be attributed to late reaction products in the seeding solutions, detectable at the effective dilution of about 1:20. The only observed late change in a stable mixture was seen in the physical characteristics of solution 1. After about five months this solution became relatively resistant to evaporation in the analytical procedure. This change did not coincide with any detectable change in the chromatograms; it was not perpetuated in seeding tests and disappeared from the flask within three weeks.

Failure to demonstrate mutations in these experiments does not, of course, prove that mutations cannot occur. The variety of reagents and conditions was limited and detection of products was not exhaustive even at concentrations of more than 10^{16} molecules in 100 ml. How-

ever, the total number of molecular species produced an given an opportunity to catalyse ongoing synthesis of their own precursors was probably many hundred. In addition, contamination by micro-organisms must have added innumerable compounds of known biological activity. The reaction conditions were intentionally inimical to reproduction by whole organisms and would have quickly degraded their enzymes, but some native compounds and many modified ones would have been stable. If any of these contaminating compounds could have been produced in one or more of the solutions, they had an opportunity to catalyse their own synthesis or that of their precursors.

It is possible that similar experiments with different reagents might yield detectable mutations; in that event the required catalysis may depend on rather specific reagents and conditions, and is not a frequent phenomenon at low levels of molecular complexity. It is also possible that some of the molecules occurring in these experiments did catalyse steps in their own synthesis, but that the inefficiency of the catalysis or slow intervening steps prevented detectable change. Several such mutations would have to accumulate and provide a more or less complete 'metabolic' system¹ before any catalysts could reach concentrations that would alter the major components. If such a low level of efficiency is characteristic, mutation can be detected only in more prolonged experiments, by more sensitive analytic techniques, or by the application of quantitative and statistical methods.

These experiments were initiated at the Geophysical Laboratory, Carnegie Institution of Washington, under the sponsorship of Dr. P. H. Abelson. I thank Dan F. Bradley of the National Institutes of Health, for his advice during a training period, and Frank Eisenberg, jun., Seymour L. Friess, Thomas C. Hoering, Patrick L. Parker and Hans Weil-Malherbe for further advice.

¹ Ycas, M., *Proc. U.S. Nat. Acad. Sci.*, **41**, 714 (1955).

² Allen, G., *Amer. Naturalist*, **96**, 65 (1957).

³ Anker, H. S., *Perspectives Biol. Med.*, **5**, 86 (1961).

⁴ Pirie, N. W., *I.C.S.U. Rev.*, **1**, 40 (1959).

⁵ Oro, J., *Ann. N.Y. Acad. Sci.*, **108**, 464 (1963).

⁶ Allen, G., in *Year Book, Carnegie Inst. Washington*, No. 62, 236 (Carnegie Institution of Washington, Washington, D.C., 1963).

⁷ Oro, J., *Nature*, **190**, 389 (1961).

⁸ Lowe, C. U., Rees, M. W., and Markham, R., *Nature*, **199**, 219 (1963).

⁹ Abelson, P. H., in *Year Book, Carnegie Inst. Washington*, No. 56, 179 (Carnegie Institution of Washington, Washington, D.C., 1957).

PLAQUE-FORMATION BY NON-IMMUNE AND X-IRRADIATED LYMPHOID CELLS ON MONOLAYERS OF MOUSE EMBRYO CELLS

By GÖRAN MÖLLER and ERNA MÖLLER

Department of Tumour Biology, Karolinska Institutet Medical School, Stockholm

THE demonstration that immune lymphoid cells are capable of killing histo-incompatible target cells *in vitro* has greatly facilitated the examination of the mechanism of cellular immunity in tissue transplantation^{1,2}. Various characteristics of the cytotoxic effect of humoral antibodies and sensitized cells on different targets appear to justify the conclusion that they act by different mechanisms. Thus, cytotoxicity caused by cellular immunity does not require the participation of complement in contrast to the action of humoral isoantibodies. Furthermore, target cell death was detected after 24–48 h, subsequent to treatment with immune lymphoid cells, whereas humoral antibodies killed within 1 h in the presence of complement³. As a matter of fact, an antagonistic relationship exists between cellular and humoral immunity in tissue culture and *in vivo* as well^{3,4}: pretreatment of neoplastic target cells with humoral isoantibodies inhibited the cytotoxic effect of immune

lymphoid cells partly or completely, presumably by competition for the same antigenic determinants.

A fundamental difference was revealed between cellular and humoral immunity by the finding that target cell death subsequent to treatment with immune lymphoid cells could be ascribed to close contact with the foreign histocompatibility antigens carried by the lymphoid cells². It could be shown that normal allogeneic lymphoid cells were as efficient in killing target tumour cells *in vitro* as immune cells, provided that they were aggregated to the targets by phytohaemagglutinin or heterologous antibodies; aggregation of isogenic lymphoid cells had no detectable effect. The cytotoxic action of non-immune lymphoid cells was not caused by the induction of a primary immune response *in vitro* since semi-isogenic F_1 hybrid lymphoid cells killed the parental target cells efficiently, although they were genetically incompetent to respond immunologically². The

results suggested that immune lymphoid cells were equipped with a specific receptor making them capable of becoming attached to the target cells; target cell death was not due to an immunological reaction, however, but appeared to be related to a non-immunological recognition process of antigenic and/or structural incompatibility, leading to cell death by so far unknown reactions. This phenomenon has been termed *antigenic recognition*.

In the work recorded here a plaque-technique was used to evaluate the effect of normal lymphoid cells aggregated to monolayers of embryonic target cells. Particular interest has been focused on the importance of genetic disparity between the non-immune lymphoid cells and the embryonic targets.

Monolayers of mouse embryo cells were prepared by treating fragments of embryos (14–19 days old) with 0.25 per cent trypsin for 1 h at room temperature. The cells were washed and suspended in lactalbumin in Earle's solution supplemented with 10 per cent foetal calf serum. 10^7 Trypan blue unstained cells were added to each 10 cm diameter Petri dish and 3×10^6 cells to each dish with 5 cm diameter. After 24–48 h inoculation the medium was replaced with Parker's Medium 199 supplemented with 10 per cent calf serum and containing phytohaemagglutinin diluted 1/50–1/100. One hour later 0.1 ml. of a lymphoid cell suspension containing $5-10 \times 10^6$ cells was carefully dropped on to a limited area of the monolayer culture. The lymphoid cells usually occupied an area with a diameter of 1–2 cm, but did not spread further and became firmly fixed to the monolayer culture due to the presence of phytohaemagglutinin. Each Petri dish received lymphoid cells at 3–4 different spots, one suspension always containing isogenic cells.

In some experiments the lymphoid cells were irradiated with 1,500 r. 1–2 h prior to the addition on to the monolayers. Irradiation was carried out with cell suspensions in Petri dishes. The dose of irradiation was sufficient to prevent the division of the lymphoid cells completely as shown by their failure to incorporate thymidine labelled with carbon-14 subsequent to stimulation with phytohaemagglutinin. In contrast a marked stimulation of isotope incorporation was observed with non-irradiated cells after 24 h.

The effect of aggregation of non-immune lymphoid cells on embryonic target cells was evaluated by the appearance of plaques in the monolayer culture at the site of lymphoid cell introduction (Fig. 1). An analogous technique has been described by Granger and Weiser⁵ for the detection of cytotoxic effects mediated by immune macrophages on monolayer cultures of neoplastic cells. In our system the plaques were seen macroscopically as a clear circular area in the monolayer (Fig. 1). Microscopically the plaques were found to contain a few scattered embryo cells, usually surrounded by aggregated lymphoid cells (Fig. 2). In cases where no plaques developed the monolayer cells were intact and surrounded by dense aggregates of lymphoid cells.

The addition of allogenic or semi-isologous F_1 hybrid spleen or lymph node cells on to monolayers of parental embryo cells in several different strain combinations led to the appearance of plaques, whereas isogenic lymphoid cells had no detectable effect (Table 1, Exps. 1–7). The plaques appeared at the same time in each experiment, but the time needed for detectable plaque formation varied between 1 and 6 days in different experiments. In the majority of the experiments the cultures were observed for 4–7 days after the first appearance of the plaques, but no further plaques were found to appear. It cannot be stated with certainty that plaque formation was caused by target cell death, but the results are in agreement with the earlier findings, demonstrating that both F_1 and allogenic cells were capable of killing parental neoplastic target cells *in vitro*. The previous investigations led to the conclusion that close contact with foreign histocompatibility antigens resulted in the target cell

death. It was of considerable interest, therefore, to study the effect of parental lymphoid cells on F_1 hybrid targets, since the parental lymphoid cells are not expected to contain any antigens foreign to the target cells.

Non-immune parental lymph node or spleen cells were found to be fully competent to induce the appearance of plaques in the majority of cases studied (Table 1, Exps. 8–13). Thus, parental lymphoid cells behaved as allogenic cells in this respect. However, exceptional results were obtained with some parental lymphoid cells. Thus, CBA, C3H and DBA/2 cells failed to induce plaques on hybrids with strain A as the other partner, although both non-immune A parental lymphoid cells and allogenic cells were efficient. It is not possible by the technique to differentiate between a complete non-reactivity and a decreased efficiency of the lymphoid cells. However, recent findings⁵ obtained with a quantitative test system described previously^{2,3} showed that non-immune parental lymphoid cells of the genotypes mentioned killed efficiently semi-isogenic F_1 hybrid targets. It seems possible, therefore, that the present results are due to quantitative factors. The failure of CBA, C3H and DBA cells to affect the hybrid targets was not limited to hybrids with strain A as the other partner, but was also with (A.SW \times CBA) F_1 hybrids and with allogenic (5M) target cells. A discrepancy between the effect of two parental cells on hybrid targets was also noticed in one experiment with A and C57BL

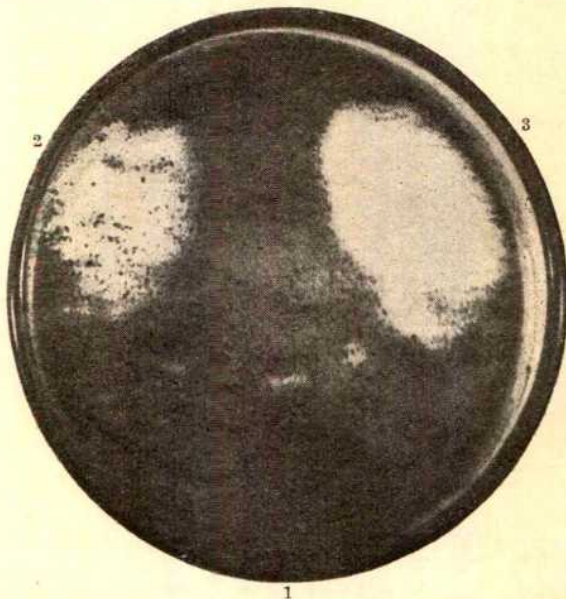


Fig. 1. Macroscopic appearance of plaques on (A \times C57BL) F_1 mouse embryo cells. At the indicated sites were added (A \times C57BL) F_1 (1), A (2) and A.CA (3) X-irradiated (1,500 r.) lymphoid cells.

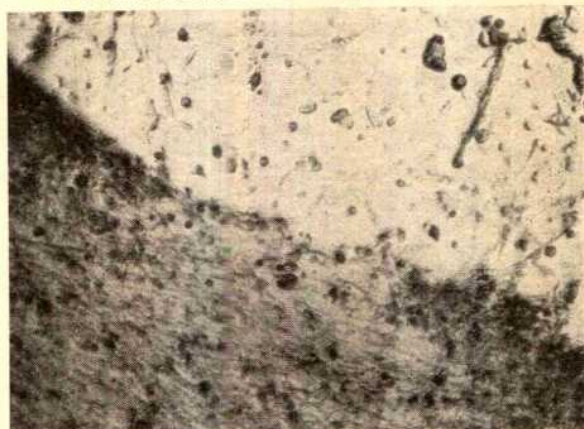


Fig. 2. Microscopic appearance of plaques on (A \times C57BL) F_1 mouse embryo cells subsequent to addition of irradiated A lymphoid cells. The destroyed monolayer is seen in the upper part of the figure, whereas the uninterrupted monolayer is seen in the lower part.

cells on F_1 embryo cells: only the A lymphoid cells induced plaques. However, in another experiment the $C57BL$ cells were efficient, again emphasizing the importance of quantitative factors in the formation of plaques.

The findings that parental lymphoid cells are capable of killing hybrid targets as a rule require a modification of the concept that *in vitro* cytotoxicity is caused by target cell contact with foreign histocompatibility antigens, since the parental lymphoid cells do not contain any foreign isoantigens. The experimental findings might be explained by two alternative possibilities: (1) Target cell death is the consequence of close contact with a structurally different but antigenetically not necessarily incompatible cell surface. Although the parental lymphoid cells do not contain isoantigens foreign to the hybrid targets, the cell surface structures are necessarily different. (2) The lymphoid cells are killed primarily by the contact with the foreign antigens on the target cells and their death affects the viability of the target cells secondarily. The correct alternative is not yet known.

Since F_1 lymphoid cells are efficient against parental embryonic target cells the possibility that plaque-formation can be ascribed to a primary immune response is unlikely. However, in the experiment with allogenic and parental lymphoid cells the necessary requirements for an immune response are present. Although the tissue culture conditions and the speed of the reaction make

this possibility less likely, experiments were performed to examine the effect of heavily irradiated lymphoid cells on the monolayer cultures. The cells were given 1,500 r, which completely inhibited the phytohaem agglutinin-induced stimulation of DNA synthesis. It was found that X-irradiation did not detectably inhibit the ability of hybrid lymphoid cells to cause plaques on parental embryo cells (Table 1, Exps. 5 and 7). Neither did X-irradiation affect the plaque-forming efficiency of allogenic and parental lymphoid cells (Table 1, Exps. 5, 7, 10). Thus, lymphoid cell division is not a necessary prerequisite for the effect.

A few experiments were performed to study the ability of erythrocytes to induce plaques on monolayer cultures. Addition of allogenic or parental erythrocytes on to embryonic targets did not result in plaques, whereas the same number of lymphoid cells were efficient in the same Petri dishes (Table 1, Exps. 1 and 4). Since red cells are known to contain considerably fewer $H-2$ isoantigenic determinants than lymphoid cells⁶ it cannot be excluded that quantitative factors regarding antigenicity were responsible for the discrepancy.

The results reported substantiate the earlier conclusion that a non-immunological recognition process is initiated by the close contact between antigenically and/or structurally incompatible lymphoid cells and target neoplastic and embryonic cells, which results in damage or death

Table 1. PLAQUE-FORMATION BY NON-IMMUNE AND IRRADIATED LYMPHOID CELLS AND ERYTHROCYTES ON MONOLAYER CULTURES OF MOUSE EMBRYO CELLS

Exp. No.	Target genotype	Added cells			No. of plaques studied	Plaque formation	Latency period (days)
		Genotype	Type	Treatment			
1	$A.SW$	$A.SW$	lgl (1)	—	1	—	6
		$C57BL$	lgl (1)	—	1	+	6
		$A.SW$	Red cells	—	1	—	—
2	A	$C57BL$	Red cells	—	1	—	—
		A	lgl	—	1	—	—
		$A.SW$	lgl	—	1	+	1.5
3	$5M$	A	lgl	—	1	+	2
		$(A \times C57BL)F_1$	lgl	—	1	+	2
		$(A \times C57L)F_1$	lgl	—	1	+	2
4	$A.SW$	DEA	lgl	—	1	—	—
		$C3H$	lgl	—	1	—	—
		$A.SW$	lgl	—	1	—	—
5	$A.SW$	$A.CA$	lgl	—	4	—	—
		A	lgl	—	2	+	4
		$A.SW$	lgl	—	2	+	4
6	$A.SW$	$A.CA$	Spleen	—	3	+	4
		A	Spleen	—	2	+	4
		$A.SW$	Spleen	—	2	+	4
7	$A.SW$	A	Red cells	—	1	—	—
		$A.CA$	Red cells	—	2	—	—
		A	Red cells	—	2	—	—
8	$A.SW$	A	lgl	1,500 r.	1	—	—
		$A.CA$	lgl	1,500 r.	1	+	3
		$(A.SW \times A.CA)F_1$	lgl	1,500 r.	1	+	3
9	$C3H$	$C3H$	lgl	—	1	—	—
		CBA	lgl	—	1	—	—
		A	lgl	—	1	+	3
10	$5M$	$5M$	lgl	—	1	+	3
		$(A \times C3H)F_1$	lgl	—	1	+	3
		A	lgl	—	1	+	3
11	A	$C57BL$	lgl	1,500 r.	6	—	—
		$(A \times A.CA)F_1$	lgl	1,500 r.	3	—	—
		$(A \times C57BL)F_1$	lgl	1,500 r.	3	—	—
12	$(A \times C57BL)F_1$	$(A \times C57BL)F_1$	lgl	1,500 r.	3	+	4
		A	lgl	—	4	+	3
		$C57BL$	lgl	—	4	+	3
13	$(A \times C57BL)F_1$	$A.SW$	lgl	—	2	+	3
		A	lgl	—	2	+	3
		$A.CA$	lgl	—	2	+	3
14	$(A \times C57BL)F_1$	$(A \times C57BL)F_1$	lgl	—	2	+	2
		A	Spleen	—	2	+	2
		$A.CA$	Spleen	—	2	+	2
15	$(A \times C57BL)F_1$	A	Spleen	—	2	+	2
		$C57BL$	lgl	1,500 r.	6	—	—
		A	lgl	1,500 r.	4	+	5
16	$(A \times DBA)F_1$	$C57BL$	lgl	1,500 r.	4	+	5
		$A.CA$	lgl	1,500 r.	4	+	5
		$(A \times DBA)F_1$	lgl	—	1	+	1
17	$(A \times DBA)F_1$	A	lgl	—	1	+	1
		DBA	lgl	—	1	+	1
		$A.SW$	lgl	—	1	+	1
18	$(A \times CBA)F_1$	$(A \times CBA)F_1$	lgl	—	1	+	1
		A	lgl	—	1	+	1
		CBA	lgl	—	1	+	1.5
19	$(A \times CBA)F_1$	$A.SW$	lgl	—	1	+	1.5
		$(CBA \times A.SW)F_1$	lgl	—	1	+	1.5
		CBA	lgl	—	1	—	—
20	$(CBA \times A.SW)F_1$	$A.SW$	lgl	—	1	+	2
		A	lgl	—	1	+	2
		$5M$	lgl	—	1	+	2

lgl = lymph node cells.

the target cells. Lymphoid cell division is not involved in the process. It appears likely that the target cells are led as a consequence of contact with incompatible tigens or surface structures carried by the normal lymphoid cells and not by immunological reactions of conventional types. According to this concept cellular immunity might be the expression of a more general biological phenomenon, which may function as a cell-to-cell homeostatic mechanism tending to eliminate cells with altered cell surface structures and which may be, therefore, important as a control mechanism against neoplasia and other aberrations of cell differentiation.

This work was supported by grants from the Swedish Cancer Society, the Swedish Medical Research Council, the Knut and Alice Wallenberg's Foundation and the Damon Runyon Memorial Fund.

¹ Govaerts, A. J., *J. Immunol.*, **85**, 516 (1960). Rosenau, W., and Moon, H. D., *J. Nat. Cancer Inst.*, **27**, 471 (1961). Wilson, D. D., *J. Cell Comp. Physiol.*, **62**, 273 (1963). Brondz, B. D., *Folia Biol. Praha*, **10**, 164 (1964).

² Moller, E., *Science*, **147**, 873 (1965).

³ Moller, E., *J. Exp. Med.*, **122**, 11 (1965).

⁴ Moller, G., *J. Nat. Cancer Inst.*, **30**, 1205 (1963).

⁵ Granger, G. A., and Weiser, R. S., *Science*, **145**, 1427 (1964).

⁶ Moller, E. (data to be published).

⁷ Moller, G., *J. Immunol.*, **86**, 56 (1961).

MEASUREMENT OF UNSTEADY STATE GROWTH RATES OF MICRO-ORGANISMS

By PROF. R. I. MATELES, D. Y. RYU and T. YASUDA

Department of Nutrition and Food Science, Massachusetts Institute of Technology

THE measurement of growth rates of micro-organisms by determining the rate of change of the concentration of a cellular component such as protein, DNA, or RNA is well established¹, as is the use of dry weight or optical density measurements on the growing culture to approximate the dry weight². Most frequently these techniques are applied during the phase of growth known as 'log phase', and the growth rate is determined simply by plotting the logarithm of the optical density (or dry weight, protein concentration, etc.) versus time. Because the growth rate remains constant for at least several generations under most conditions of culture, the accuracy of the estimation is increased by plotting a number of points and determining the best straight line, the slope of which is used to calculate the growth rate.

For studies of growth rates under conditions where the growth rate is changing with time, these techniques are unsatisfactory because a 'best straight line' does not exist, and estimation of the slope of a curved line is not reliable. We have found that a technique combining continuous culture with continuous indirect measurement of the cell mass concentration permits accurate estimation of unsteady-state growth rates.

The method is based on the principle of measuring continuously the concentration of a single nitrogen source in the medium on which a micro-organism is grown in continuous culture. Typically, the nitrogen source is an ammonium salt, although the use of an amino-acid or other nitrogen source would be equally satisfactory, provided that an assay exists suitable for use with a continuous analyser such as the Technicon 'AutoAnalyzer'.

By writing material balances on the growth vessel, the following equations are obtained³:

$$\frac{dN_c}{dt} = \mu N_c - DN_c \quad (1)$$

and:

$$\frac{dN}{dt} = DN_0 - DN - \mu N_c \quad (2)$$

where N represents the concentration of source nitrogen in the growth vessel, N_c represents the concentration of cellular nitrogen in the growth vessel, N_0 represents the concentration of source nitrogen in the incoming medium, μ represents the specific growth or nitrogen assimilation rate, D represents the dilution rate or the ratio of medium flow rate to medium volume contained in the growth vessel, and t represents time. In the absence of excretion of nitrogen compounds and significant changes in intracellular amino-acid pools, the nitrogen assimilation rate will correspond to the growth rate⁴.

The growth rate determined in this manner would correspond to a growth rate measured by following the increase in Kjeldahl nitrogen incorporated by the cells. For the case in which the nitrogen source is made the growth-rate-limiting nutrient, the following equation⁵ also applies.

$$\mu = \frac{\mu_{\max} N}{K_s + N} \quad (3)$$

in which μ_{\max} represents the maximum growth rate of the cells in the presence of an excess of limiting nutrient, and K_s is a constant. For nutrient-limited conditions in a continuous culture, the concentration of limiting nutrient in the culture vessel at steady-state is independent of its concentration in the incoming medium.

When N_0 is held constant, equation (2) can be rearranged to yield the growth rate in terms of the other variables:

$$\mu = D - \frac{dN}{dt} / (N_0 - N) \quad (4)$$

Thus, in order to calculate the instantaneous growth rate, one need only determine from the recorder-chart the value of N at some time t , estimate the value of dN/dt by numerical methods, and knowing D and N_0 , calculate the growth rate, μ .

The sensitivity of this technique resides in the fact that changes in N are easier to observe and measure accurately than changes in N_c , as may be seen from Table 1.

As Table 1 shows, during the first minute after the change in growth rate, the concentration of ammonium nitrogen in the medium more than doubles while the cell nitrogen concentration falls by less than 2 per cent. The change in ammonia concentration is readily measurable with a Technicon 'AutoAnalyzer', while it would require unusually precise measurements to detect a difference in cell dry weight or optical density of 2 per cent.

We have applied this method to the problem of determining the behaviour of a microbial culture during a 'shift up'¹. In this case the shift up was obtained by increasing the dilution rate of the culture, rather than by shifting to a richer medium capable of supporting faster growth. The organism used was *Escherichia coli* B, growing at 37° C in a well-stirred growth vessel holding 200 ml. of *M*-63 medium⁶ containing glycerol as a carbon source and ammonium sulphate (63 mg of ammonium nitrogen/l.) as a nitrogen source. The medium was fed from a reservoir by a 'Sigmamotor' pump. A sample of broth and cells from the growth vessel was taken continuously by a Technicon 'AutoAnalyzer', and the concentration of dissolved ammonia was determined using an alkaline phenol-hypochlorite technique⁷. Glucose in the medium interfered with this assay and therefore glycerol was used as carbon source.

Table 1. CHANGES IN THE CONCENTRATION OF CELLULAR NITROGEN (N_c) AND MEDIUM NITROGEN (N) IN A CONTINUOUS CULTURE AFTER THE MICRO-ORGANISM'S GROWTH RATE SUDDENLY FALLS TO ZERO

Time (min)	N (mg/l.)	N_c (mg/l.)
0	1	99
1	2.6	97.4
2	4.2	95.8
3	5.8	94.2

Calculations made for a continuous culture with, $D = 1.0 \text{ h}^{-1}$; $N_0 = 100 \text{ mg/l.}$

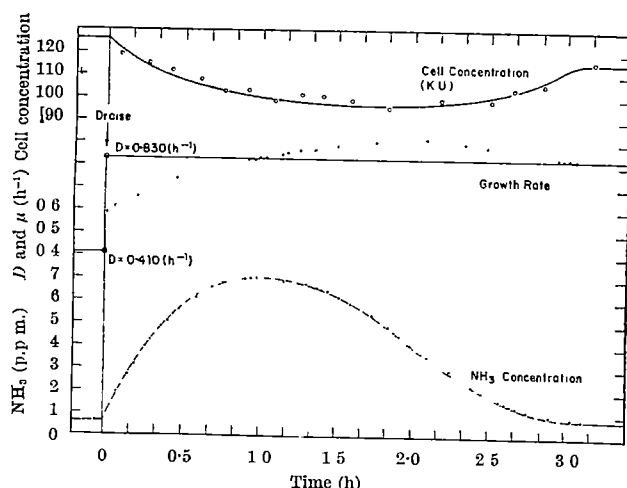


Fig. 1. Results of an unsteady-state experiment in which ammonium nitrogen was the limiting nutrient

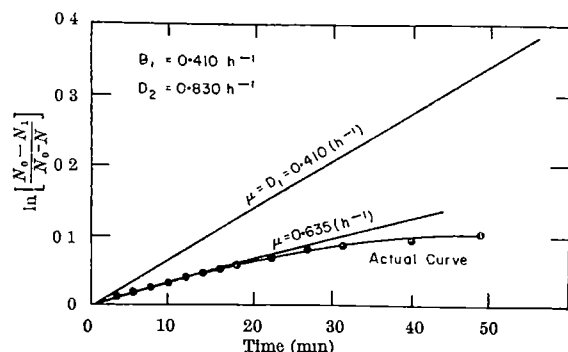


Fig. 2. Evidence that the jump in growth rate (see Fig. 1) is not an artefact. N_1 represents the steady-state concentration of ammonium nitrogen in the growth vessel prior to the increase in dilution rate

Prior to the unsteady-state growth rate measurements, a steady state was maintained for at least 15 h. Then an increase in dilution rate was made, the concentration of ammonium nitrogen determined as a function of time after the step change in dilution rate, and the instantaneous growth rate calculated from data obtained from the plot of N versus time. The calculations were carried out on an IBM 7094 digital computer because they involved the manipulation of considerable data.

The results of a typical run are shown in Fig. 1, for which $D_1 = 0.41 \text{ h}^{-1}$ and $D_2 = 0.83 \text{ h}^{-1}$. It appears that the growth rate rose about 0.2 h^{-1} immediately after the dilution rate change and then slowly rose further, overshooting a new steady-state growth rate, and then settling back into a steady state. That the instantaneous rise is real, and not an artefact introduced through numerical differentiation, is shown in Fig. 2, in which $\ln(N_0 - N_1)/(N_0 - N)$ versus time is plotted. The slope of the curve is equal to $D - \mu$ (ref. 8). The solid line indicates the results that would be obtained if the growth rate remained at the value it had prior to the dilution rate change. Since the actual line is not tangent to the solid line, it indicates that a real increase in growth rate occurred at the time of the dilution rate increase, and since the line has a constant slope for about 15 min, that the growth rate stayed constant at 0.63 h^{-1} for about 15 min before increasing further over the course of the next few hours.

Table 2 shows the results of a series of experiments made at a number of dilution rates and dilution rate changes. For small dilution rate changes, the perturbation in ammonium nitrogen concentration was absent or small, indicating that the growth rate accommodated the dilution rate change, whereas when the dilution rate change increased, the ability of the cells to increase their growth rate was not sufficient and a large perturbation in ammonium nitrogen was observed.

Table 2. RESULTS OF STEP RESPONSE ON NITROGEN-LIMITED CULTURE

	1	2	3	4	5	6	7
$4D \text{ (h}^{-1}\text{)}$	0.18	0.19	0.19	0.29	0.42	0.47	0.48
$D_1 \text{ (h}^{-1}\text{)}$	0.286	0.316	0.525	0.492	0.410	0.377	0.38
$D_2 \text{ (h}^{-1}\text{)}$	0.466	0.503	0.710	0.777	0.830	0.845	0.871
Maximum deflexion of $N \text{ (mg/l.)}$	—	—	—	1.7	7.0	11.5	23.2
Time at which N reaches max (min)	—	—	—	35	60	70	130
Time to reach new steady state (h)	—	—	—	1.25	3.0	4.6	12.5
$\mu_{\text{max}} \text{ (h}^{-1}\text{)}$	—	—	—	1.0	0.91	0.93	1.0
Comment	No deflexion			Very small deflexion			

In order to determine whether Monod's equation (equation (3)) could be used to represent unsteady-state growth rates, equations (1), (2) and (3) were solved by numerical method using the digital computer. Regardless of the magnitudes of the constants μ_{max} and K_s or the parameters N_0 and D , the transient growth rate calculated on the assumption that Monod's equation is applicable always lacks a maximum and is a smooth transition between the two steady states⁹. Clearly the experimental curve (Fig. 2) departs significantly from Monod's model. Thus, during transient operation of a continuous culture Monod's equation may not be used to relate growth rates and concentrations of limiting nutrient.

In contrast to the results of Kjeldgaard, Maaløe and Schaechter¹, we have found that the cell can increase its rate of protein synthesis immediately after a shift up, and that this small increase is then followed by a prolonged gradual increase. The discrepancy between the present findings and the earlier ones may be ascribed to the relative insensitivity of the direct measurement of the growth rate by following changes in optical density, or protein concentration as practised by the earlier workers.

The possibility that the immediate increase in the nitrogen assimilation rate, here interpreted as the growth

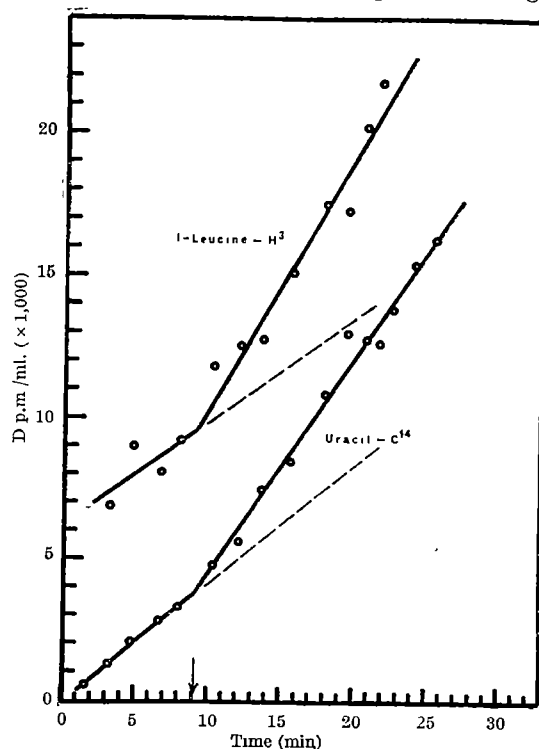


Fig. 3. Incorporation of uracil- ^{14}C and l-leucine- ^3H by *E. coli* B before and after change in dilution rate. The medium contained 10 mg of l-leucine and 10 mg of uracil per litre in addition to the ingredients contained in M-63 medium used in the previous experiment. Ten μC of l-leucine- ^3H and 10 μC of uracil- ^{14}C were added to the culture 9.1 min prior to the change in dilution rate. At the time indicated by the arrow, the dilution rate was increased from 0.45 h^{-1} to 1.16 h^{-1} . Samples were taken approximately at 1-min intervals, added to an equal volume of ice-cold 5 per cent TCA, and at the conclusion of the experiment filtered through a Millipore filter, washed, and counted by a liquid scintillation counter. The counts were corrected for dilution of the radioactive tracer in the culture vessels by incoming medium containing only unlabelled l-leucine and uracil, and for the cell concentration

ate, represented a preferential increase in the rate of RNA synthesis alone rather than an increase in the rate of both protein and RNA synthesis can be excluded on the basis of the experimental results depicted in Fig. 3. The results clearly indicate that at the time of the change in dilution rate, the rate of incorporation of both L-leucine- ^3H and uracil- ^{14}C increased sharply, implying that the rates of protein and RNA synthesis increased.

The results recorded here further indicate that protein and RNA biosynthetic activities in the cell are not at the maximum levels of their capacities under the given conditions, since the rate of nitrogen assimilation is increased immediately when the feed rate of limiting nutrient is increased.

In the experiments described here, it was found that all the nitrogen in the medium could be accounted for as free ammonium nitrogen; the cells did not excrete any intermediary products of nitrogen metabolism. This might not always be true, particularly under conditions in which the carbon source is the limiting nutrient, and it would be desirable to make certain that the nitrogen in the medium is all in the form which is being assayed. This can be done by collecting on ice a portion of effluent, centrifuging to remove cells, and comparing the ammonium nitrogen analysis of the cell-free medium with a total nitrogen analysis.

These results clearly indicate that, contrary to the views of Herbert¹⁰, a significant lag in adjustment of growth rate to dilution rate does exist, and that this lag must be taken into account in experimenting with single or multi-stage continuous culture systems. For example, determining the apparent maximum growth rate (μ_{max}) from the slope of a plot of $\ln x$ versus t may underestimate the true μ_{max} .

It should be noted that it is not necessary for the nitrogen source to be the limiting nutrient in order to use this technique for studying unsteady-state growth rates. All that is required is for the concentration of the nitrogen

source in the growth vessel to be low relative to its concentration in the reservoir. This could be accomplished, for example, by having the carbon source as limiting nutrient and using a concentration of nitrogen source in the medium sufficient to leave five or ten parts per million unused under steady-state condition. For most cases, this concentration will be in excess, that is, the growth rate of the organism will not be affected by small changes in the concentration of nitrogen source. Using this technique it would be possible to study the effects on growth rates of concentrations of inhibitory substances too low to study by standard techniques, because they would not stop growth entirely but only reduce the growth rate slightly for short periods of time.

The calculations were performed on an IBM 7094 digital computer at the Computation Center of this Institute.

This work was supported in part by grants from the U.S. National Science Foundation (G-21389), the U.S. National Aeronautics and Space Administration (NSG-496), and the Edanros Research Foundation, and was reported in part at the 145th and 148th meetings of the American Chemical Society.

¹ Kjeldgaard, N. O., Maaløe, O., and Schaechter, M., *J. Gen. Microbiol.*, **19**, 607 (1958).

² *Manual of Microbiological Methods*, Soc. Amer. Bacteriologists (McGraw-Hill Book Co., New York, 1957).

³ Herbert, D., Elsworth, R., and Telling, R. C., *J. Gen. Microbiol.*, **14**, 601 (1956).

⁴ Mandelstam, J., *Biochem. J.*, **69**, 103 (1958). Moyed, H. S., and Umbarger, H. E., *Physiol. Revs.*, **42**, 444 (1962).

⁵ Monod, J., *Ann. Inst. Pasteur*, **79**, 390 (1950). *Récherches sur la Croissance des Cultures Bactériennes* (Herman and Cie, Paris, 1942).

⁶ Pardee, A. B., Jacob, F., and Monod, J., *J. Mol. Biol.*, **1**, 165 (1959).

⁷ *Technicon AutoAnalyzer Manual* (Technicon Instrument Co., Chauncey, New York, 1961). Van Glyke, D. D., and Hiller, A., *J. Biol. Chem.*, **102**, 499 (1933). Borsook, H., *J. Biol. Chem.*, **110**, 481 (1935). Russell, J. A., *J. Biol. Chem.*, **156**, 457 (1944).

⁸ Yasuda, T., thesis, Mass. Inst. Technol. (1964).

⁹ Sinclair, J., thesis, Mass. Inst. Technol. (1964).

¹⁰ Herbert, D., in *Continuous Cultivation of Micro-organisms*, Proc. of the Second Symposium, Prague, June 18-23, 1962, edit. by Malek, I., Beran, K., and Hospodka, J. (Czechoslovak Acad. Sci., Prague, 1964).

PROTONATION EQUILIBRIA AND ALKALINE HYDROLYSIS OF GLYCINE ETHYL ESTER

By DR. MARGARET ROBSON*

Department of Chemistry (University of St. Andrews), Queen's College, Dundee

IN the past decade, interest has developed in the interaction of metal ions with biologically important compounds, for example, amino-acid esters and peptides. The complexing tendencies of such molecules, the stabilities and reactivities of the resulting complexes have been investigated¹⁻³, but very little systematic work has been carried out. The following account describes preliminary work towards such an investigation.

The proton ionization equilibria of glycine ethyl ester and glycine, and the alkaline hydrolysis of the ester, have been examined prior to a detailed analysis of the metal ion complexing and catalysis.

The ionization constants were measured at $25^\circ \pm 0.05^\circ \text{C}$ and $0^\circ \pm 0.05^\circ \text{C}$; and at three differing ionic strengths in the case of the ester. Values for the pK_a of glycine⁴ have been reported and temperature coefficient data⁵ are available. The glycine work was therefore carried out mainly as a check on the accuracy of the ester measurements. pH titrations were carried out using a Radiometer pH-stat titrator, coupled with a Radiometer pH scale expander. Alkali was added from an all-glass micrometer syringe. The pH assembly was operated manually. Where necessary, the ionic strength was brought to the required value by addition of potassium bromide solution. pK_{a1} at 25°C for glycine was estimated by decreasing the

pH of the initial solution to approximately 2.5 by addition of hydrochloric acid. pK_a values were calculated from selected points (on the average, sixteen points were chosen) along the titration curve using conventional methods⁶. An average of five titrations were performed for each ionic strength. pH values were converted to concentrations using the Davies equation⁷, and equilibrium constants thus calculated (Table 1) are concentration quotients. For the ester, drifting values, which were a result of hydrolysis, were obtained at high pH.

pK_a values independent of ionic strength are expected in the ionization of glycine ethyl ester ($\text{H}^+\text{E} \rightarrow \text{H}^+ + \text{E}$), and this is confirmed by the results. The scatter in values at 25°C is much higher than at 0°C . This is expected as a consequence of the increased rate of hydrolysis at 25°C . The average value at 25°C , 7.72, agrees well with 7.73 reported by Emerson and Kirk⁸, and with an estimated value of 7.76 (ref. 9) based on a ΔpK ($\text{NH}_3\text{CH}_2\text{COO}^-$

Table 1

Acid	Temp.	Ionic strength	pK_{a1}	pK_{a2}
Glycine ethyl ester hydrochloride	25°C	0.05	7.71 ± 0.01	—
		0.075	7.74 ± 0.01	—
		0.100	7.76 ± 0.02	—
		0.01	8.36 ± 0.00	—
		0.10	8.46 ± 0.01	—
Glycine	25°C	0.1	2.38 ± 0.02	9.61 ± 0.01
		0.1	—	10.30 ± 0.00
	0°C	0.1	—	—

* Now Dr. Margaret Robson Wright.

$\text{NH}_3\text{CH}_2\text{COOEt}$) of 2.02. It is also close to the value for the methyl ester, 7.68, found by Edsall¹⁰.

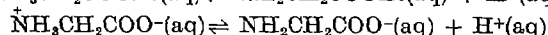
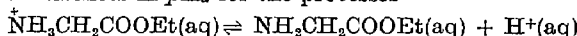
pK_a values strongly dependent on ionic strength are expected for the second ionization of glycine ($\text{H}^+\text{G} \rightleftharpoons \text{H}^+ + \text{G}^-$), and approximately independent of ionic strength for the first ionization ($\text{H}^+\text{G} \rightleftharpoons \text{H}^+ + \text{G} + \text{H}^+$). Thus for comparative purposes a thermodynamic pK_{a2} , 9.84₃, was calculated from the second ionization using the equation:

$$\log K_{T,D} = \log K_c - \frac{2Az^2\sqrt{I}}{1 + \sqrt{I}} + 0.2z^2I$$

This value compares favourably with that of 9.78₀ (ref. 5), as does the value reported here, 2.33₈, with that listed⁵, 2.35₀, for the first ionization.

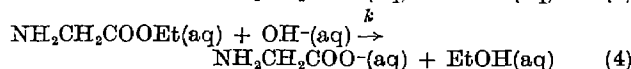
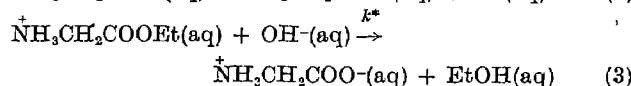
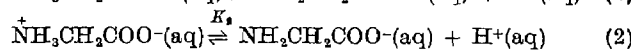
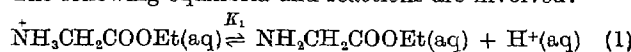
Table 2 lists ΔG° , ΔH° , ΔS° and $T\Delta S^\circ$ values for the ionizations, assuming ΔH° independent of T from 0° C to 25° C, along with analogous values calculated from Edsall's¹⁰ data on glycine methyl ester over the smaller temperature range (20°–30° C), and from tabulated data⁵ for the second ionization of glycine.

Differences in pK_a for the processes



are predominantly due to a difference in ΔS° for the ionization process. Qualitatively a process, electrically neutral species \rightarrow 2 ions, would be expected to have a more negative ΔS° than the process ion \rightarrow ion. This is, in fact, observed, while no great difference in ΔH° is found. Values of ΔS° for the ionization of carboxylic acids¹¹ (for example, -22 cal mole⁻¹ deg.⁻¹ for acetic acid) are considerably more negative than that for the second ionization of glycine. The low value of $|\Delta S|$ for glycine seems related to the existence of the dipolar zwitterion. Comparable ΔS° values to that of the ester are found in monobasic amine acids¹¹.

The alkaline hydrolysis of glycine ethyl ester has been investigated at an ionic strength of 0.1, and at 25° C and 0° C over a wide pH range. The kinetics were followed by a pH-stat device which enables the pH to remain constant by automatic and recorded addition of alkali. The accuracy of the results is limited by the accuracy with which the machine can maintain a constant pH. The following equilibria and reactions are involved:



It follows that the total alkali uptake, assuming the reaction to go to completion, is given by the expression:

$$V_\infty = [\text{ester}]_{\text{initially}} \left\{ 1 + \frac{1}{1 + \frac{K_1}{[\text{H}^+]}} - \frac{1}{1 + \frac{K_2}{[\text{H}^+]}} \right\}$$

All pseudo first-order rate plots were calculated on this basis. At high pH, where the runs were taken to completion, good agreement with the observed V_∞ was obtained. Second-order rate constants were estimated from the pseudo first-order rate constants by division by

Table 2
Glycine ethyl ester
hydrochloride

		Glycine ethyl ester hydrochloride	Glycine second ionization
ΔG°_{298} kcal mole ⁻¹	+10.45	+10.50*	+13.06
ΔH° kcal mole ⁻¹	+9.7	+12.10*	+10.6
$T\Delta S^\circ$ cal mole ⁻¹ °K ⁻¹	-2.7	+5.6*	-8.6
$T\Delta S^\circ_{298}$ kcal mole ⁻¹	-0.8	+1.7*	-2.5

* Glycine methyl ester—Edsall (ref. 10)

† Robinson and Stokes: *Electrolyte Solutions*

ΔG° calculated from $-RT \ln K$.

Table 3		
Temp.	Protonated ester	Non-protonated ester
0° C	$k^* = 230$	$k = 8.5$
25° C	$k^* = 1,130$	$k = 35.6$
	$E_A^* = 10.6$	$E_A = 9.4$
	$A^* = 7.4 \times 10^{10}$	$A = 3 \times 10^9$
	$\Delta S^\circ_{298} = -12$	$\Delta S^\circ_{298} = -23$

Rate constant in units: 1 mole⁻¹ min⁻¹.
Activation energy in units: kcal mole⁻¹.
Entropy of activation: cal mole⁻¹ °K⁻¹.

the hydroxide ion concentration calculated from the pH of the run (or average pH if drifting with time occurred, and the Davies equation⁷). Second-order rate constants were found to vary with hydrogen ion concentration. The pH range extended from 9.6 to 11.7. Fig. 1 gives the results for some 50 runs at 25° C.

There is a large scatter of points, about the line drawn, but a 'deviations plot' shows the scatter to be random, in marked contrast to the situation where no variation with concentration is assumed.

If the kinetics follow the scheme outlined previously and corrections have been made for the protonation equilibria involved:

$$k_{\text{obs}} a = k^*[\text{H}^+\text{E}] + k[\text{E}]$$

where a represents the initial total ester concentration (that is, $[\text{H}^+\text{E}]_0 + [\text{E}]_0$). If, to a first approximation, $[\text{E}] = a$, then:

$$k_{\text{obs}} = k + \frac{(k^* - k)[\text{H}^+]}{K_1}$$

A higher approximation would give:

$$k_{\text{obs}} = k + (k^* - k) \frac{[\text{H}^+]}{K_1} \left\{ 1 - \frac{[\text{H}^+]}{K_1} \right\}$$

Such a correction is well within the experimental errors and, therefore, pointless.

Thus an observed rate constant varying with $[\text{H}^+]$ should be found, and a plot of $k_{\text{obs}} V [\text{H}^+]$ should give k as intercept, and k^* calculable from the gradient. Table 3 summarizes the data for experiments at 25° C and for a few experiments at 0° C. Provisional estimates of E_A , A , ΔS° are given.

The Bjerrum¹² type equation representing the effects of electrostatic interactions between a charged substituent and the reaction site in the transition state is:

$$\frac{k^*}{k} = \exp \left(\frac{-z_1 z_2 e^2}{DkTR} \right) \quad (5)$$

where k^* and k are the rate constants for reactions involving the charged molecule and uncharged molecule, respectively, $z_1 e$ is the charge on the initial molecule, and $z_2 e$ is the charge developed at the reaction site. R is the distance between the charges.

Thus:

$$E^* - E = \frac{Ne^2 z_1 z_2}{DR} \left[1 + \frac{T}{D} \frac{dD}{dT} \right] \quad (6)$$

where E^* and E are the activation energies for reactions involving charged and uncharged molecules, respectively. N is Avogadro's number.

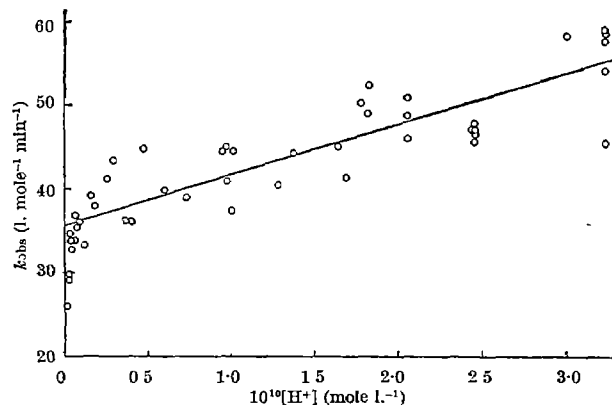


Fig. 1. Variation of observed rate constant with hydrogen ion concentration at 25° C

As:

$$\left(1 + \frac{T}{D} \frac{dD}{dT}\right) < 0$$

or aqueous solutions, $E^* > \text{or} < E$ depending on whether z_i and z_j are of unlike or like sign, respectively. For the system reported here $z_i z_j < 0$, and thus $E^* > E$. The preliminary results indicate that this may be so. This effect has been noted previously for $z_i z_j < 0$ (ref. 3) and $z_i z_j > 0$ (ref. 14). The present case, however, provides a much more direct test of effects of charges in so far as the two molecules being compared ($\text{NH}_2\text{CH}_2\text{COOEt}$, $\text{NH}_2\text{CH}_2\text{COOEt}$) differ, apart from charge, only in respect of the hydrogen. With substances investigated previously the comparison made was of the type $\text{SO}_3^-(\text{CH}_2)_n\text{COOEt}$ with $\text{CH}_3(\text{CH}_2)_n\text{COOEt}$.

From equation† (5) a value of R , 1.8 Å, is found assuming D to be the macroscopic dielectric constant. This can, in turn, be used to find $E^* - E$ equal to approximately 1.0 kcal mole⁻¹. Further refinements using a Kirkwood-Westheimer¹⁵-Tanford⁸ procedure indicate a value of approximately 1.6 kcal mole⁻¹ ($D_E = 14$, $R = 4.85$ Å).

The effect of charge on rate should be apparent in the pre-exponential terms. For reaction involving the charged species, formation of the transition state involves a decrease in net charge and thence an increase in entropy, due to release of water molecules of solvation on formation of the transition state. A less negative ΔS^\ddagger , relative to that for the uncharged species, is expected. This is tantamount to saying that there is an enhanced probability of reaction (relative to a neutral molecule-ion reaction) when two ions of opposite sign approach each other.

The alkaline hydrolysis of glycine esters has been examined previously, but no variation with pH has been noted. Bell and Coller¹⁶ investigated the hydrolysis over the pH range 9.70–11.55, but found no pH dependence. Other authors did not carry out the reaction at varying pH values. Reported values of the rate constant for the hydrolysis at 25°C are: (a) Bell and Coller¹⁶, 50.4 l.mole⁻¹ min⁻¹; (b) Gustafsson¹⁷, 36 l.mole⁻¹ min⁻¹; (c) White, Manning and Li², 44 l.mole⁻¹ min⁻¹; (d) Gelles and Robson¹⁸ (for methyl ester), 39 l.mole⁻¹ min⁻¹.

Conditions for values (b) and (c) are not reported, but from the experimental technique it seems safe to conclude that the pH was probably greater than 10. These data

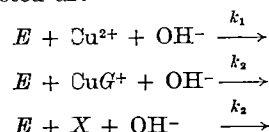
† Values of the rate constants at infinite dilution were calculated, prior to substitution into equation (5).

are thus consistent with the present investigation and agree well with work^{2,8} on the methyl ester.

It can be seen that the effect of temperature on the overall observed rate, and on the gradient of the k_{obs}/V $[\text{H}^+]$, is complex; depending on the temperature coefficients of k , k^* and K_1 . Knowing these coefficients it is possible to predict that the pH dependence will be larger (and consequently more easy to measure) at temperatures $> 25^\circ\text{C}$, and work is in progress to extend the measurements.

Preliminary work has been carried out on the cupric ion catalysis. Titration curves for the ester in the presence of varying quantities of cupric ion suggest the presence of complexes such as CuE , CuE_2 .

Preliminary kinetic work shows that in addition to the hydroxide ion catalysed hydrolysis, a series of consecutive pseudo first-order reactions are occurring which can be initially interpreted as:



where CuG^+ is copper monoglycinate, and X is some, as yet, undetermined reactant. This is analogous to previous work¹⁸ where a maximum in the rate was obtained at pH 7–8 for k_1 , k_2 and k_3 .

I thank Dr. E. Gelles for suggesting this topic, and Dr. P. G. Wright for helpful discussion. This work was carried out during the tenure of the Edward A. Deeds junior fellowship of Queen's College, Dundee.

¹ Kroll, H., *J. Amer. Chem. Soc.*, **74**, 2034, 2036 (1952).

² White, J., Manning, R., and Li, N., *J. Amer. Chem. Soc.*, **78**, 2367 (1956).

³ Bender, M., and Turnquest, B., *J. Amer. Chem. Soc.*, **79**, 1889 (1957).

⁴ *Chem. Soc. Spec. Pub.*, part 1 (1957).

⁵ Robinson, R. A., and Stokes, R. H., *Electrolyte Solutions*, second ed., 519 (Butterworths, London, 1959).

⁶ Glasstone, S., *Introduction to Electrochemistry*, Chapter 9 (Van Nostrand Co. Inc., New York, 1942).

⁷ Davies, C., *J. Chem. Soc.*, 2993 (1938).

⁸ Emerson, O., and Kirk, P., *J. Biol. Chem.*, **87**, 597 (1930).

⁹ Tanford, C., *J. Amer. Chem. Soc.*, **79**, 5348 (1957).

¹⁰ Edsall, J., and Blanchard, D., *J. Amer. Chem. Soc.*, **55**, 2337 (1933).

¹¹ Bell, R. P., *Proton in Chemistry*, Chapter 5 (Cornell Univ. Press, 1959).

¹² Bjerrum, N., *Z. Phys. Chem.*, **106**, 219 (1923).

¹³ Bell, R. P., and Lindars, F., *J. Chem. Soc.*, 4601 (1954).

¹⁴ Bell, R. P., and Wright, G., *Trans. Farad. Soc.*, **57**, 1377 (1961).

¹⁵ Kirkwood, J., and Westheimer, F., *J. Phys. Chem.*, **6**, 503, 513 (1938).

¹⁶ Bell, R. P., and Coller, B., *Trans. Farad. Soc.*, **60**, 1087 (1964).

¹⁷ Gustafsson, C., *Ann. Acad. Sci. Fennicae*, A II (Chem), No. 15 (1945).

¹⁸ Gelles, E., and Robson, M. (unpublished results).

DIFFUSIVE TRANSPORT RATES IN STRUCTURED MEDIA

By PROF. A. B. METZNER

Department of Chemical Engineering, University of Delaware, Newark, Delaware

THE purpose of this short article is to call attention to the importance and potential applications of the abnormal diffusive transport rates recently observed in suspensions and in polymeric solutions and to offer a tentative interpretation of these phenomena.

The experimental results obtained for a variety of systems are given in Table 1; it is not the purpose of this article to present a complete review of all available data and additional results are given both in the references quoted in Table 1 and in other related papers^{1,2}. The important point to be made is that not infrequently the addition of either suspended solids or polymeric material to a homogeneous liquid increases the diffusive transport rates of some third species in this medium. This is not the case for all systems or at all concentration levels, but when it does occur the changes may be appreciable (up to 40 per cent in the case of the data of Table 1, and significantly larger effects have been noted³). Presumably,

even greater effects would be possible by optimizing the relevant factors involved, if these were understood, since the data given in Table 1 represent only measurements on systems chosen more or less at random rather than with the view of finding maximal effects of this kind. As the diffusivity increases are obtainable using either suspended solids or dissolved macromolecules, the total effects obtainable in biological media containing both kinds of constituents could conceivably be very appreciable and significantly affect the rates of respiratory and cellular ingestive processes of living organisms or the operability of machines designed to provide such functions. Thus the problem under discussion may be of interest in rather broad areas of biophysics and bioengineering as well as in polymer and colloid science.

Students of the diffusive behaviour of polymeric molecules in solution have observed or predicted effects which may have a common molecular origin, although, except

Table 1. MEASURED DIFFUSIVITIES

Diffusing solute	Medium used	Temp. (°C)	Diffusivity, (cm ² /sec × 10 ⁶)	Investigators
Ethylene	Water	18	~1.10	Astarita ^{15,16}
Ethylene	0.5% CMC*	18	1.06	Astarita
Ethylene	1.0% CMC	18	1.08	Astarita
Ethylene	2.0% CMC	18	1.29	Astarita
Carbon dioxide	Water	18	1.72	Astarita
Carbon dioxide	0.122% Carbopol†	18	1.82	Astarita
Carbon dioxide	0.132% Carbopol	18	1.82	Astarita
Carbon dioxide	0.172% Carbopol	18	1.98	Astarita
Carbon dioxide	0.222% Carbopol	18	2.16	Astarita
Carbon dioxide	0.375% CMC	18	1.72	Astarita
Carbon dioxide	0.75% CMC	18	2.06	Astarita
Carbon dioxide	1.50% CMC	18	2.44	Astarita
Carbon dioxide	3.80% Bentonitic clay in water	18	1.91	Astarita
Carbon dioxide	5.01% Bentonitic clay in water	18	2.02	Astarita
Carbon dioxide	5.10% Bentonitic clay in water	18	2.40	Astarita
Benzoic acid	Cyclohexane	25	1.18	Hopper ¹⁹
Benzoic acid	2% PIB‡	25	1.35	Hopper
Benzoic acid	4% PIB	25	0.92	Hopper
Acetic acid	Water	20	1.24	Perry ¹⁷
Acetic acid	4% CMC	20	1.63	Heertjes et al. ¹⁸

* CMC denotes carboxymethylcellulose (wt. per cent given) dissolved in water.

† A water soluble synthetic polymer; solution concentrations studied as indicated.

‡ Two wt. per cent of polyisobutylene dissolved in cyclohexane.

in the case of a small number of lucid publications⁴⁻⁶, the actual phenomenon has frequently been obscured by the dedicated devotion of the authors in this area of science to the reporting of phenomena in terms of empirical coefficients of obscure origin and value, as well as by the fact that the macromolecular transport rates are, at best, frequently quite low. The transport characteristics of systems in which the diffusivity increases with increasing concentration of the polymeric molecules involved have been shown to be closely related to the second virial coefficient of the solution and hence, among other variables, to the partial molal volume of the polymeric solute⁴⁻⁷. However, the extension of these considerations to make it possible to predict the behaviour of an ordinary (that is, not macromolecular) solute, in a polymer solution or a colloidal suspension, would appear to require additional considerations.

Such considerations appear to be embodied in a recent analysis of diffusion in liquids⁸ which enables the prediction of diffusivity in terms of the molecular geometry (size) of both the solute and the solvent constituents and the intermolecular force fields of each. If, as indicated by the high values of the second virial coefficient of the polymeric solutions of interest, the molecular 'packing' of the solvent molecules in the neighbourhood of a macromolecule is lower than in the bulk of the solution, a region of low resistance to mass transport will have been provided. In fact, since the term reflecting such decreased intermolecular packing appears in an exponential⁸, small changes in intermolecular spacing would be predicted to have major effects on the diffusivity. Similarly, in the case of diffusion in a slurry of colloidal particles, the intermolecular packing at the particle surface would be expected to be less than in the bulk of the fluid just from the usual geometric considerations of molecular packing at all planar surfaces. The visualization of the transport process therefore becomes one in which the addition of certain polymeric materials or colloidal solids to a continuous fluid may lead to the creation of a structured medium in which the packing of the fluid molecules in the immediate neighbourhood of the added macromolecule or particle is lower than in the continuum itself; these regions of low packing may represent regions of very high 'conductivity' for the diffusing species, depending in turn on their size and on the intermolecular force fields of the several species involved. The macromolecular or colloidal particles, of course, may also block the path of a diffusing species when the low-density 'short circuit' around the particles does not exist, as discussed elsewhere¹, and either mechanism could predominate, depending on

the molecular force fields involved. The magnitude of the diffusivity in a 'high conductivity' environment (the added species may in principle be predicted by extensions of the previous analysis of diffusion in liquids) although in practice several difficult steps remain to be overcome before the extension is a quantitative one.

The effect of regions of abnormally high or low diffusivity on the macroscopic diffusivity of the medium as a whole may be computed from the analogous analysis of conduction of electricity or heat in structured media⁹⁻¹², providing the distribution of these regions is random and the particle asymmetry may be considered adequately. A random distribution of these regions would appear to be reasonable in an undeformed fluid, but imposition of rapid rates of deformation of the fluid could be expected to lead to asymmetric mass transport properties. This problem has been investigated in steady laminar shearing flow fields over rather broad ranges of shear rate, using both suspensions and polymeric solutions, and no measurable effects have been found^{3,13}. The implications of this apparent inconsistency are not known at present.

Finally, it should be noted that the conductivity of the medium may become independent of that of the high-conductivity phases or regions if the ratio of conductances is sufficiently great¹¹ and, if this is presumed to be the case, some predictions of the diffusivity characteristics of the structured medium may be made without detailed knowledge of the actual diffusivity in the regions in the immediate neighbourhood of the dispersed matter.

In summary, it is seen that diffusive transport rates in liquids may be increased appreciably by creation of structured media through the solution of macromolecular species in the fluid or by the suspension of colloidal particles in it. Presumably, even much greater effects than those noted so far could be obtained by optimizing the relevant parameters. These effects would appear to have potentially important implications in those areas of physics and engineering involving biological or polymeric materials. A tentative hypothesis, which assumes the creation of a structured fluid medium having regions of high diffusivity in the immediate vicinity of the added particles or molecules, is advanced as a possible explanation of this phenomenon. Methods of developing this hypothesis, which proposes a mechanism analogous to that of conduction of heat or of electricity in disperse systems or of diffusion in crystalline solids¹⁴, are discussed.

This work has been supported by the National Science Foundation. I thank Prof. Astarita and Dr. Secor for letting me see their papers prior to publication, and Prof. Astarita for many stimulating discussions of the problem.

¹ Clough, S. B., Read, H. E., Metzner, A. B., and Behn, V. C., *Amer. Inst. Chem. Eng. J.*, **8**, 346 (1962).

² Nishijima, Y., and Oster, G., *J. Poly. Sci.*, **19**, 337 (1958).

³ Behn, V. C. (personal communication, 1963).

⁴ Flory, P. J., *Principles of Polymer Chemistry*, ch. 7 and 12 (Cornell University Press, Ithaca, N.Y., 1953).

⁵ Secor, R. M., *Amer. Inst. Chem. Eng. J.*, **11**, 452 (1965).

⁶ Tsvetkov, V. N., and Klenin, S. I., *Tech. Physics U.S.S.R.*, English translation, **4**, 1283 (1960), and *Zhur. Tek. Fiz.*, **29**, 1393 (1959).

⁷ Mandelkern, L., and Flory, P. J., *J. Chem. Phys.*, **19**, 984 (1951).

⁸ Gainer, J. L., and Metzner, A. B., Paper preprinted for joint meeting, Institution of Chemical Engineers—American Institute of Chemical Engineers (1965).

⁹ Jefferson, T. B., Witzell, O. W., and Sibbitt, W. L., *Indust. Eng. Chem.*, **50**, 1589 (1958).

¹⁰ Maxwell, J. C., *Electricity and Magnetism* (Clarendon Press, Oxford, 1873).

¹¹ Metzner, A. B. in *Advances in Heat Transfer*, **2**, edit. by Hartnett, J. P., and Irvine, jun., T. F. (Academic Press, 1965). See also in *Advances in Chem. Eng.*, **1**, edit. by Drew, T. B., and Hoopes, J. W. (Academic Press, 1956).

¹² Thomas, D. G., *Indust. Eng. Chem.*, **55**, No. 11, 18 (1963).

¹³ Hopper, J. R., M.Ch.E. thesis, University of Delaware, Newark, Delaware (1964).

¹⁴ Shewmon, P. G., *Diffusion in Solids* (McGraw-Hill, N.Y., 1963).

¹⁵ Astarita, G., *Indust. Eng. Chem. Fundamentals*, **4**, 236 (1965).

¹⁶ Astarita, G. (personal communication, 1964).

¹⁷ Perry, J. H., *Chemical Engineers Handbook*, section 14, fourth ed. (McGraw-Hill, N.Y., 1963).

¹⁸ Heertjes, P. M., van Mens, M. H., and Butaye, M., *Chem. Eng. Science*, **10**, 47 (1949).

CONCENTRATION EFFECTS IN CHROMATOGRAPHIC ANALYSIS

By Prof. I. D. RATTEE, D. M. LEWIS and Dr. C. B. STEVENS

Department of Colour Chemistry, University of Leeds

DURING an investigation of the highly complex reaction of ω -chloroacetyl amino dyes with wool, thin-layer and paper chromatography have been widely used for the detection and identification of the reaction products. From time to time it was noticed that with the dyes used the R_F values, relative to one another, varied considerably, although the final order of separation with a given mixture using a particular solvent was the same. R_F values were observed to be dependent on the concentration of material initially loaded on to the paper. A number of workers have referred to such concentration effects. Åkerfeldt¹ found that the final area of the spot was directly proportional to the load of material applied. Fowler², using sucrose, found a linear relationship between the logarithm of the spot length and the logarithm of the load initially applied. He also observed that at low concentrations the plot of spot length against the logarithm of the load applied was linear. Fisher, Parsons and Morrison³, discussing quantitative paper chromatography, also found a linear relationship between spot length, or spot area, and the logarithm of the spot content when using certain amino acids and the sugars xylose and arabinose. Miyaki⁴ found that the logarithm of the difference between the initial and final lengths of the spot was a linear function of the concentration of material initially present in the spot. The effect we have observed is, however, not so much an increase in the length of the spot but a definite increase in R_F with increase in the load applied.

Giddings and Keller⁵ developed an equation relating a given number of molecules Nx in a spot, the distance moved, and the mean diffusion coefficient of the particular material in the support. We have sought to explain in rather different terms the concentration effects we have observed using an equation based on present-day theories of the adsorption of dyes by textile substrates.

It is considered that the effects arise from a Freundlich type of adsorption of the dyes by the support medium. The literature contains references to the desirability of avoiding the use of supports towards which the compounds under examination show strong substantivity, but no account seems to have been taken of the possibility of changes in the pattern of chromatographic behaviour arising from relatively weak substantivity effects.

If it is assumed that the distribution of substance between the eluent and the support obeys a Freundlich isotherm, and if D_F denotes the concentration of adsorbed material in equilibrium with D_S , the concentration of the substance in the eluant, then:

$$D_F = K_1 D_S^x$$

where K_1 is a constant and $x < 1$.

If it is further assumed that the velocity with which the substance under examination moves relative to the solvent front is proportional to the concentration not adsorbed, then:

$$R_F = K_2 D_S$$

Since the total concentration of the substance in the spot \bar{D} is equal to $D_F + D_S$, then:

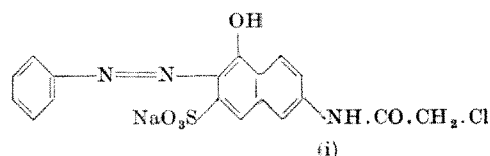
$$\begin{aligned} \bar{D} - D_S &= K_1 D_S^x \\ &= \frac{K_1}{K_2^x} R_F^x \end{aligned}$$

and if $\bar{D} \gg D_S$, that is, quite a high degree of adsorption occurs, then:

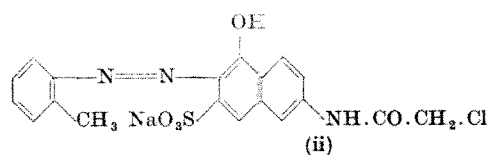
$$\log \bar{D} = \log \frac{K_1}{K_2^x} + x \log R_F$$

Thus a linear relationship should exist between the logarithm of the R_F value and the logarithm of the load, if the substantivity for the support medium of the substance loaded is sufficiently high. The accuracy of this prediction will vary according to the scale of the error introduced by assuming $\bar{D} \gg D_S$. With the low solvent/support ratios obtained in chromatography this should, in fact, even hold with compounds which show only moderate substantivity to the particular support medium. Certainly using azo dyes of the type described here and paper as the support, \bar{D} will in most circumstances exceed D_S quite considerably.

Two orange dyes were used:



Mol. wt. 442



Mol. wt. 456

Chromatograms were run using 'Whatman 3MM' paper as support, *n*-butyl alcohol/acetic acid/water (4 : 2 : 1) as the eluant and enclosing the chromatogram in an all-glass Shandon tank, containing, in the bottom, sufficient eluent to saturate the atmosphere in the tank. All the runs were continued for 21 h at 20° C. For each dye spots of equal area were formed on the starting line. The distance between the starting point and the maximum concentration point of each spot was measured. An EEL densitometer was used to determine the maximum concentration points. Since the behaviour of both dyes at each concentration was determined at the same time under identical conditions, the distances moved were equivalent to the R_F values. The results are shown in Table 1.

Fig. 1 shows the plot of the logarithm of the distance against the logarithm of the load. In both cases the predicted linear relation was obtained up to an initial load of about 0.1200 mg.

Further evidence that substantivity plays a major part in determining the movement of the spots was obtained by examining the effect of different eluents. Using *n*-butyl alcohol/pyridine/water (1 : 1 : 1), under which conditions

Concentration of original dye solution (mg)	Distance (L) moved (cm)	
	Dye (i)	Dye (ii)
0.0125	16.80	16.10
0.0250	17.50	16.75
0.0375	18.20	17.20
0.0500	18.50	17.50
0.0625	19.40	17.80
0.0750	19.10	18.00
0.0875	19.60	18.20
0.1000	19.10	18.40
0.1125	19.80	18.40
0.1250	19.80	18.50
0.1375	19.75	18.50
0.1620	20.00	18.50
0.1750	20.10	18.60
0.1870	20.00	18.60

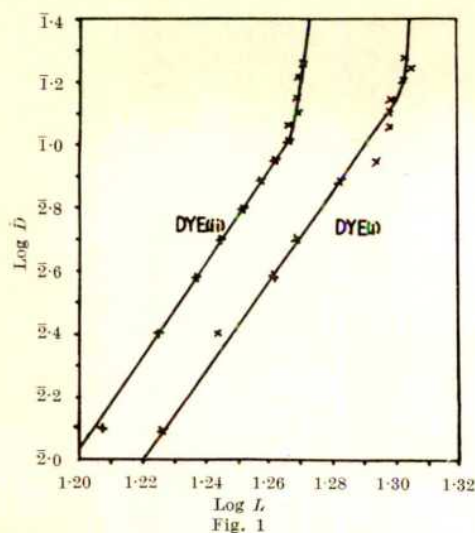
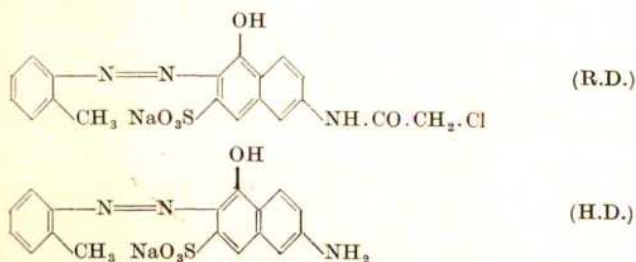


Fig. 1

hydrogen bonding of the dye to the substrate, and hence dye-substrate sensitivity, is reduced, the spots were far more compact, but the distances travelled were still dependent on concentration, though to a lesser extent.

The concentration of dye in the load has also been found to influence the chromatographic behaviour of mixtures of dyes:

Descending chromatograms were run for 21 h in *n*-butyl alcohol/acetic acid/water (4:1:2) under the same conditions as before. The dyes used in these experiments were:



Spots of equal area containing these dyes in different proportions were produced as follows:

- (1) [R.D.] constant
[H.D.] increasing (from right to left)

The resulting chromatogram is shown in Fig. 2.

It will be seen that the expected increased movement of dye H.D. with increasing concentration occurred. Dye R.D. behaved in exactly the same way even though it was applied in constant concentration. (Note: dye H.D. is the spot with the lower R_F value.)

- (2) [R.D.] increasing (right to left)
[H.D.] constant

The resulting chromatogram is shown in Fig. 3. In this case no obvious mixture effects were observed except that a slight retarding of the movement of dye R.D. is observed when this result is compared with that shown in Fig. 4 (a).

- (3) [R.D.] increasing (left to right)
[H.D.] increasing (right to left)

The resulting chromatogram is shown in Fig. 5. The position of the spots (on the extreme left) containing most dye H.D. and least dye R.D. shows that dye R.D. is held back. Particularly marked separation of the spot containing most dye R.D. and least dye H.D. occurred (on extreme right of Fig. 5).

It is obvious that the chromatographic behaviour of mixtures of dyes is affected by differences in the substantivity of each dye for the support. That this is the case with the adsorption of direct dyes on cellulose has

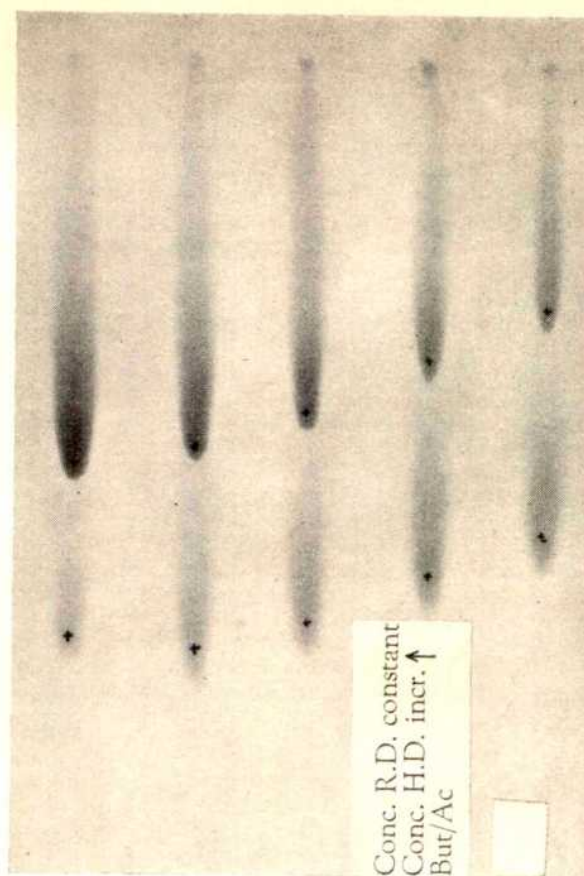


Fig. 2

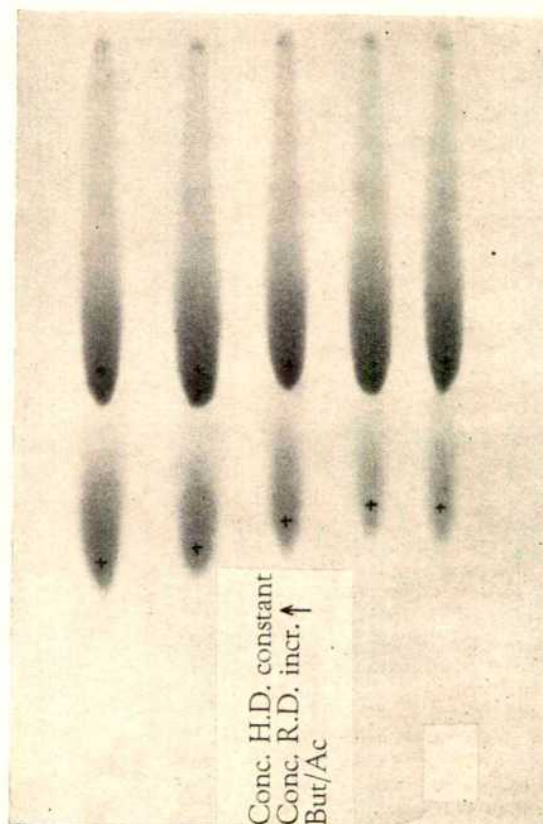


Fig. 3

en shown by several workers. It has been observed at the equilibrium adsorption of a particular dye from solution containing a mixture of dyes is lower than from solution containing this dye alone (Boulton, Delph, Fothergill and Morton⁶; also Neale and Stringfellow⁷). Such behaviour is considered to be due to the following causes: (1) Competition between the different dye anions for the available surface sites. (2) The effect of each dye contributing to the surface charge on the fibre, and to the sodium ion concentration in it. (3) Interactions between the dyes in solutions.

In chromatography (1) is especially important since, under the conditions used, the support will be approaching saturation with dye. Applying all these factors to paper

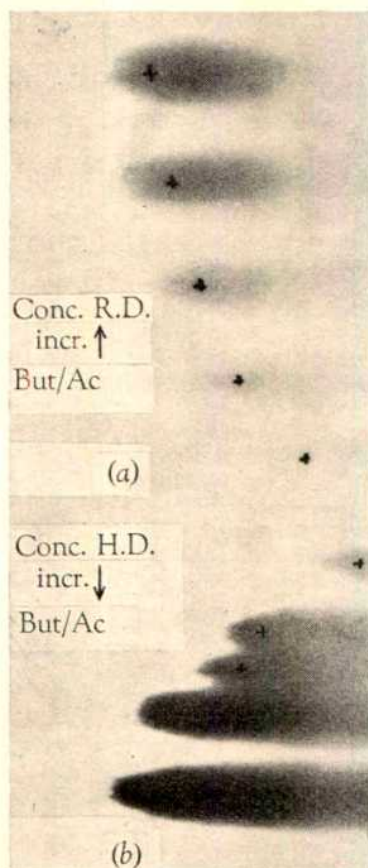


Fig. 4

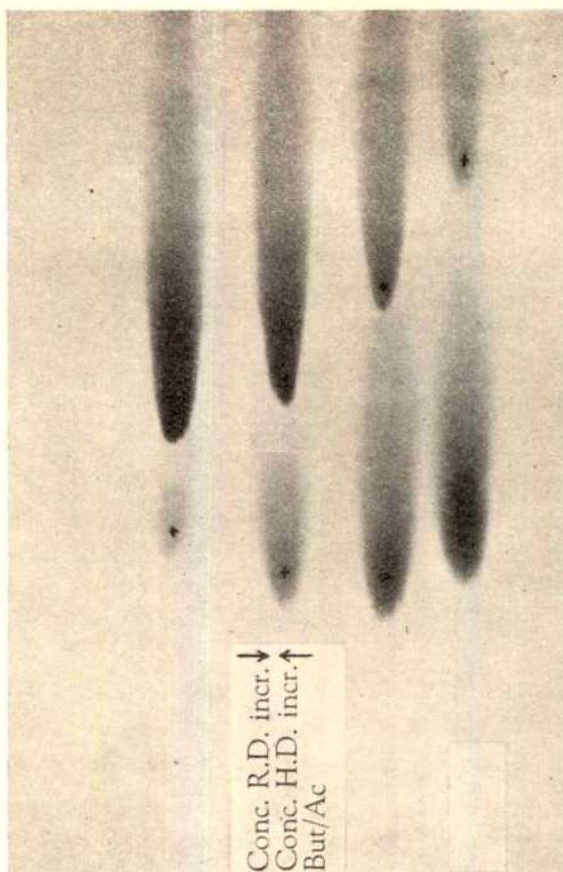


Fig. 5

chromatography, one would expect the behaviour of a dye in mixtures to differ from that when it alone is present, especially in the case of dyes which are substantive to the support. It would therefore appear to be important that this possibility should be taken into account, when using paper chromatography, in order to detect and separate dyes in mixtures.

¹ Åkerfeldt, S., *Acta Chem. Scand.*, **8**, 521 (1954).

² Fowler, H. D., *Nature*, **168**, 1123 (1951).

³ Fisher, R. D., Parsons, D. S. and Morrison, G. A., *Nature*, **161**, 764 (1948).

⁴ Miyaki *et al.*, *J. Pharm. Soc. Japan*, **71**, 249 (1951).

⁵ Giddings, J. C., and Keller, E. A., *J. Chromat.*, **2**, 626 (1959).

⁶ Boulton, J., Delph, A. E., Fothergill, F., and Morton, T. H., *J. Text. Inst.*, **24**, 113 (1933).

⁷ Neale, S. M., and Stringfellow, W. A., *J. Soc. Dyers' Col.*, **59**, 241 (1943).

GEOCHEMISTRY OF ANDESITES AND THE GROWTH OF CONTINENTS

By PROF. S. R. TAYLOR and DR. A. J. R. WHITE

Department of Geophysics and Geochemistry, and Department of Geology,
Australian National University, Canberra

THE growth of continents is one of the basic problems in geology. The rate of denudation is sufficient to erode continents to very low levels in periods brief ($10\text{--}50 \times 10^6$ years) compared with the extent of geological time¹. Accordingly, it is necessary to have a process by which material is added to the continents, not only to keep pace with the denudation, but also to allow for some growth. The chemical differences between continental crust and either oceanic crust or mantle demand that a chemical fractionation process operates as well as a simple addition of material.

Of the observable geological processes, basalt igneous activity is seen to add material from the mantle to the

crust in both oceanic and continental regions. Granitic rocks and their associates seem to be derived from within the deep crust. In orogenic regions eruption of andesitic lavas and ash is the dominant volcanic activity and adds material to the surface at rates of perhaps 1 km^3 per year². These orogenic zones are here interpreted as areas of active continental growth, as suggested by Wilson³.

The andesites possess the following properties:

(1) They appear to be restricted to orogenic belts and are absent from oceanic areas.

(2) They are the most voluminous eruptive rock in these belts. Other rocks have occasionally been referred to as andesites (Table 1) but are excluded from this study.

Table 1. NOMENCLATURE OF ANDESITES AND ANALOGOUS ROCKS

	Some past usages	Present nomenclature
Cale-alkaline volcanic series	Andesite	Andesite
Alkali volcanic series	Orogenic andesite	Hawaiite (ref. 3)
	Andesine andesite	Mugearite (ref. 3)
	Oligoclase basalt	Benmoreite (ref. 4)
	Trachyandesite (soda-rich)	Tristanite (ref. 4)
	Trachyandesite (potash-rich)	
Tholeiitic volcanic series	Andesite	
	Pigeonite andesite	Icelandite (ref. 5)
	Tholeiitic andesite	

They are developed in minor amounts in both continental and oceanic environments by fractional crystallization of basaltic magma³⁻⁵ and are different petrographically and chemically from the true andesites.

(3) There is a common, but not universal association of more silica-rich rhyolites, ignimbrites and dacites.

(4) True basalts are often absent, or present in minor amounts; only occasionally are they abundant (for example, Japan).

(5) The andesites frequently contain glass, are typically porphyritic and the plagioclase exhibits reverse and oscillatory zoning indicating non-equilibrium conditions during crystallization. Present data suggest that they are characterized by hypersthene in the groundmass.

(6) They are broadly uniform in composition, although commonly associated with minor more basic andesites, containing large crystals of olivine and pyroxene.

(7) Their composition bears a general resemblance to previous estimates of that of the continental crust.

In Table 2, the average composition is given of well-described andesites from New Zealand^{6,7}, Saipan⁸, California^{9,10} and Japan^{11,12}. Two recent estimates^{13,14} of the composition of the continental crust are also given. The average composition of the common igneous rocks, granite and basalt is also given. Fig. 1 shows a comparison of the composition of the continental crust, averages of granite and basalt, and the range in composition of andesites (filled rectangles). The unfilled rectangles represent the composition of rarer basic andesites, possibly resulting from accumulation of olivine and pyroxene crystals.

It is apparent from Table 2 and Fig. 1 that the composition of andesites bears a general resemblance to that of the continents. The major anomaly is the potassium content, which is too low by a factor of two. Possible explanations of this will be discussed later. Because of the importance of the andesites, a programme of geochemical research has been started, and determinations of major and trace elements, and Sr isotopes has been carried out on samples from New Zealand and Japan.

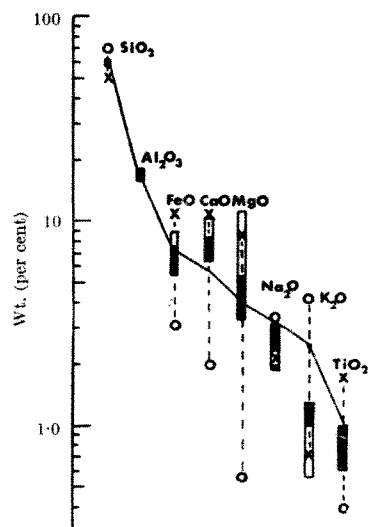


Fig. 1. Comparison of the major element composition of andesites, basalt, granite and continental crust. Filled rectangles, andesite; unfilled rectangles, basic andesite; x, basalt; —, continental crust

Work is planned on samples from New Guinea and Solomon Islands, Saipan in the Marianas arc, and Cascade Province in western United States, in order sample widely the circum-Pacific belt. Full details samples, analytical methods and individual determinations will be given elsewhere.

The major and trace element data already available permit some tentative conclusions to be drawn. Data for the strontium isotopes will be presented separately. The major elements were determined by conventional wet chemical methods, and the other elements by a combination of X-ray fluorescence, spectrochemical¹⁹ and source mass spectrographic²⁰ techniques.

The trace element data are shown in Fig. 2. Average basalt and granite abundances are shown, with the estimation for the continental crust, based on a 1:1 mix of acid and basic rocks. Although there is a general similarity between the andesite composition and that of this average the concentrations of many elements in andesites are lower than this crustal average. These include barium, rubidium, nickel, yttrium, lithium, thorium, caesium, uranium, tin and the rare earth elements. Except for nickel, these elements are all typically strongly concentrated upwards in the Earth since their large size, valency, makes it difficult for them to enter the principal crystal phases in the mantle^{21,22}. They are all typically concentrated in the last stages of fractionation, as resemble potassium among the major elements in this respect. K/Rb ratios for the common New Zealand andesites are 240, and 330 for the Japanese. The most basic andesites have ratios up to 340 for the New Zealand rocks, and 510 for the Japanese. The rare earth elements although apparently lower in absolute concentration show similar patterns to those of well-mixed crustal sediments, when normalized to lanthanum = 1.00. This shown in Fig. 3 for the odd-numbered rare earths.

The trace element data provide limitations to the various theories of origin of andesites. These theories fall into three main categories: (1) Fractionation of basic magma under oxidizing conditions. (2) Assimilation, contamination or mixing of acid and basic material. (3) Primary andesite magma developed by partial or complete melting of deep crustal or upper mantle material²³.

Volume relationships are generally not in favour of an origin by fractionation of basic magma. It is possible that the nickel content is too low for such an origin, but the trace element data do not exclude this possibility. The second alternative seems to be excluded by the present data. The low content (in andesites) of elements such as potassium, rubidium, uranium, thorium, rare earths, tin and lithium on the basis of any reasonable proportions of admixed granite rule out this mechanism. Preliminary results for strontium isotopic ratios support this conclusion²³.

Because of the serious limitations to the first process or geological grounds and to the second on geochemical arguments, the third process merits serious consideration. The relatively thin crust of about 30 km thickness present in typical orogenic areas²⁴ raises difficulties for an origin by fusion or partial fusion of deep crustal material. Temperatures to be expected at these depths are appropriate to the formation of granitic rocks²⁵, but may not be sufficient to form melts of andesitic composition. Experimental data bearing on this problem are lacking.

Table 2. AVERAGE COMPOSITIONS OF ANDESITE, CONTINENTAL CRUST AND COMMON ROCK TYPES

	Average andesite A (refs. 6-12)	Continental crust B (ref. 13)	C (ref. 14)	Average granite D (refs. 15-18)	Average basalt E (refs. 15, 17)
SiO ₂	60.1	59.4	60.3	71.2	48.9
Al ₂ O ₃	17.2	15.6	15.6	14.7	15.7
FeO	6.1	7.1	7.2	3.2	10.7
MgO	3.5	4.2	3.9	0.6	8.7
CaO	7.1	6.6	5.8	2.0	10.8
Na ₂ O	3.3	3.1	3.2	3.5	2.3
K ₂ O	1.3	2.3	2.5	4.2	0.7
TiO ₂	0.7	1.2	1.0	0.5	1.8

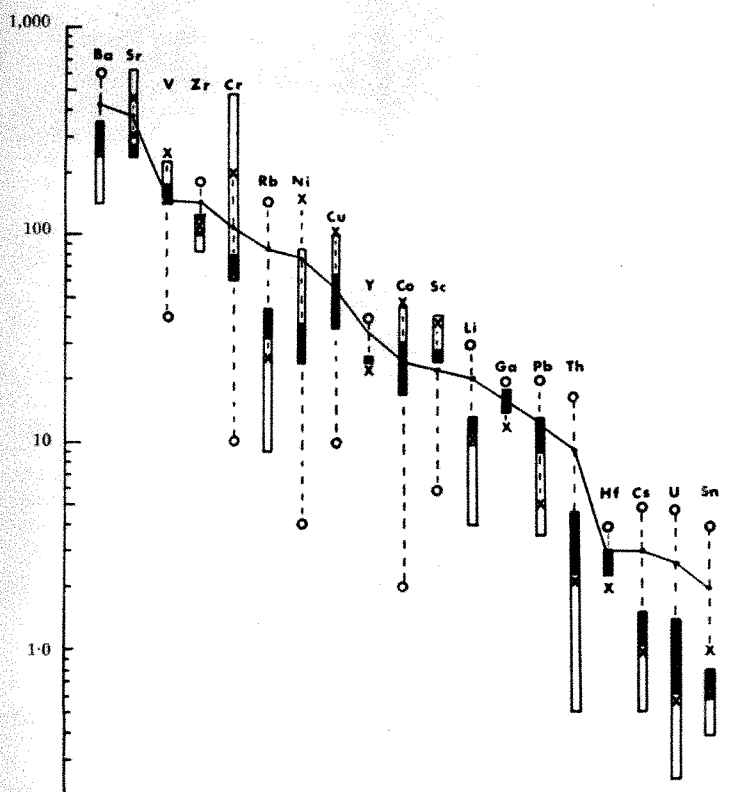


Fig. 2. Comparison of trace element composition of andesites, basalt, granite and continental crust. Filled rectangles, andesite; unfilled rectangles, basic andesite; O, granite; x, basalt; —, continental crust

zones is characterized by an anomalously low velocity region for compressional (P) waves. This is interpreted as a region of lower than normal density for the mantle²⁶ immediately beneath orogenic regions. There is thus a correlation between andesite volcanism and an anomalous low-density upper mantle. If andesites are derived from this low-density zone by partial melting, then they could make a major contribution to the growth of continents.

This hypothesis encounters the difficulty referred to earlier of a deficiency of potassium and related trace elements in andesites compared with present estimates of crustal composition. The following possibilities might explain this anomaly:

(1) Potassium and the related elements which are typically concentrated by fractionation processes might be overestimated in the continental crust. These elements could be concentrated in the upper and depleted in the lower crust so that sampling is overweighted in their favour. A lesser concentration of these elements in the crust would remove some difficulties for a chondritic Earth model²¹. Hence the composition of andesites may represent a more satisfactory overall average for the composition of the continental crust than previous estimates.

(2) The additional potassium, and other elements, could be provided by the acid volcanic rocks of rhyolitic composition commonly associated with andesites.

(3) Although we ourselves favour a philosophy based on the uniformitarian approach to geology²⁷, it is likely that the mantle has become depleted in potassium and related elements with time. Many of these elements should appear in the first products of partial melting. Thus recent andesites, studied here, could contain less of these elements than those of remote geological epochs. The composition of the continental crust would then represent the average of andesites erupted over a period of at least 3×10^9 years. Work is proceeding to test these several alternatives.

¹ Gilluly, J., *Geol. Soc. Amer. Spec. Paper*, **62**, 7 (1955).

² Sapper, K., *Z. Vulk.*, **9** (1923).

³ Macdonald, G. A., *J. Petrol.*, **1**, 172 (1960).

⁴ Tilley, C. E., and Muir, I. D., *Geol. Fören. Stockholm Förh.*, **85**, 434 (1964).

⁵ Carmichael, I. S. E., *J. Petrol.*, **5**, 435 (1964).

⁶ Gregg, D. R., *N.Z. Geol. Surv. Bull.*, **40** (1960).

⁷ Steiner, A., *N.Z. J. Geol. Geophys.*, **1**, 325 (1958).

⁸ Schmidt, R. G., *U.S. Geol. Surv. Prof. Paper*, **280**, B (1957).

⁹ Fiske, R. S., Hopson, C. A., and Waters, A. C., *U.S. Geol. Surv. Prof. Paper*, **444** (1953).

¹⁰ Coombs, H. A., *Bull. Geol. Soc. Amer.*, **50**, 1493 (1939).

¹¹ Kuno, H., *Volcanoes and Volcanic Rocks*, Iwanami and Co., Tokyo (1954).

¹² Aramaki, S., *J. Fac. Sci. Univ. Tokyo, Sec. II*, **14**, 229 (1963).

¹³ Poldervaart, A., *Geol. Soc. Amer. Spec. Paper*, **62**, 119 (1955).

¹⁴ Taylor, S. R., *Geochim. Cosmochim. Acta*, **28**, 1273 (1964).

¹⁵ Daly, R. A., *Igneous Rocks and the Depths of the Earth* (McGraw-Hill, 1933).

¹⁶ Nockolds, S. R., *Bull. Geol. Soc. Amer.*, **65**, 1007 (1954).

¹⁷ Turekian, K. K., and Wedepohl, W. H., *Bull. Geol. Soc. Amer.*, **72**, 175 (1961).

¹⁸ Kolbe, P., Ph.D. thesis, Australian National Univ. (1965).

¹⁹ Ahrens, L. H., and Taylor, S. R., *Spectrochemical Analysis*, second ed. (Addison-Wesley, Cambridge, Mass., 1961).

²⁰ Taylor, S. R., *Nature*, **205**, 34 (1965).

²¹ Taylor, S. R., *Geochim. Cosmochim. Acta*, **28**, 1989 (1964).

²² Ringwood, A. E., *J. Geophys. Res.*, **67**, 4005 (1962).

²³ Compston, W. (unpublished data).

²⁴ Thompson, G. A., and Talwani, M., *J. Geophys. Res.*, **69**, 4813 (1964); *Science*, **146**, 1539 (1964).

²⁵ Tuttle, O. F., and Bowen, H. L., *Geol. Soc. Amer. Memoir*, **47** (1958).

²⁶ Nuttli, O., *Rev. Geophys.*, **1**, 51 (1963).

²⁷ Lyell, C., *Principles of Geology*, 1 (John Murray, London, 1830).

²⁸ Wilson, J. T., *Trans. Amer. Geophys. Union*, **33**, 444 (1952).

²⁹ O'Hara, M. J., *Scottish J. Geol.*, **1**, 19 (1965).

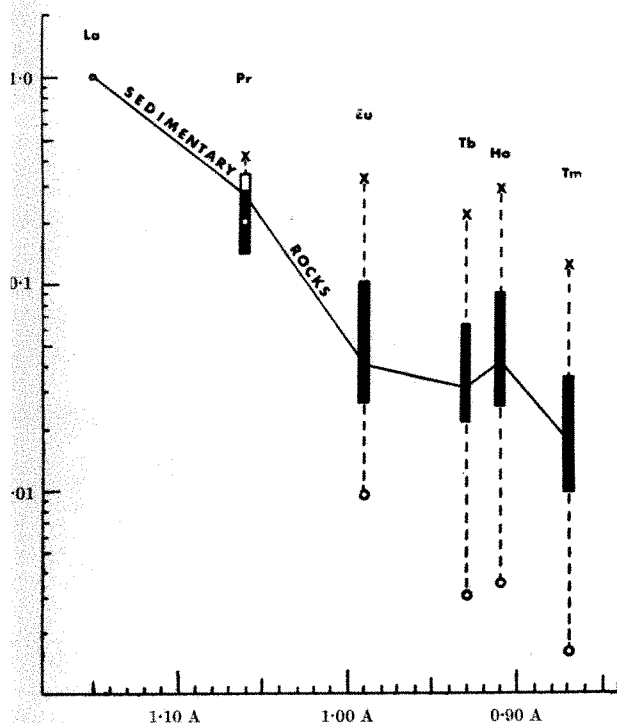


Fig. 3. Comparison of the odd-numbered rare earth abundance patterns, normalized to lanthanum, for andesites, basalt, granite and sedimentary rocks. Filled rectangles, andesite; unfilled rectangles, basic andesite; O, granite; x, basalt

If andesites are derived from the mantle, then the mantle beneath orogenic zones must differ significantly from that beneath continental shields or true oceanic areas, from which basalts are erupted. Recent workers in seismology^{24, 26} are agreed that the upper mantle beneath orogenic

LETTERS TO THE EDITOR

ASTROPHYSICS

Interstellar Obscuration

AN accurate extinction curve with a standard error of $\pm 0.008^m$ which has been derived for a region in the direction of Cygnus (looking along the spiral arm) from spectrophotometric observations at the Royal Observatory, Edinburgh¹, shows a pronounced discontinuity in slope at 4300 Å. This feature as well as recent rocket observations² rule out the possibility that the interstellar dust is made up of iron or ice particles. Hoyle and Wickramasinghe³ have suggested that graphite particles, formed on the surface of carbon stars, could be ejected into interstellar space by radiation pressure, and Wickramasinghe⁴ finds that such carbon particles may become covered with ice mantles. The wave-length 4300 Å is close to that at which the refractive index of graphite particles begins to change, the extinction of graphite being remarkably close to a $1/\lambda$ law in the region $0.8 \leq \lambda^{-1} \leq 2.3$ and thereafter deviating from such a law more or less according to particle size⁵. Nandy and Wickramasinghe⁶ have shown that the observed extinction curve for Cygnus can be well reproduced by the assumption of graphite cores with radii less than $\sim 0.06\mu$ covered with ice mantles.

Similar observations have been made at this Observatory in the direction of Perseus (across the spiral arm)⁷. The extinction curve for Perseus shows that the change of slope occurs at the same wave-length, 4300 Å, as found for Cygnus stars; but that the slope of the ultra-violet part of the extinction curve relative to the blue part decreases by 30 per cent as compared with that in Cygnus (Fig. 1). The Perseus curve can be well represented by a Gaussian distribution of graphite particles with a dispersion of $\pm 0.01\mu$ centred at 0.06μ (ref. 6).

The difference between the Cygnus and Perseus extinction laws could be explained if the interstellar matter were made up of particles covered with metallic impurities and aligned in a magnetic field⁸. The alignment properties of the particles would be different in Cygnus from those in Perseus because of the different angles of viewing, and the ratio of the slope of the ultra-violet part of the extinction curve to that in the blue would be expected to depend on the angle of viewing and the polarization of starlight per unit obscuration⁹. However, the work of Underhill and

Walker¹⁰, who examined the obscuration of stars in southern sky, and of Johnson¹¹ shows that the variation of the law of obscuration is not dependent on the angle of viewing.

More light can be thrown on this matter by examining the obscuration of the star HD 23512 in the young Pleiades cluster. This relatively nearby cluster is in the Cygnus arm and is embedded in nebulosity. The polarization per unit obscuration of the star HD 23512 is the same as the average value in Perseus and about four times that in Cygnus.

This star has now been examined both here and Mendoza¹². The Edinburgh results agree well with those of Mendoza (Fig. 2). The extinction curve of the Pleiades star is found to be almost the same as that for stars in the Cygnus region, but significantly different from that in Perseus.

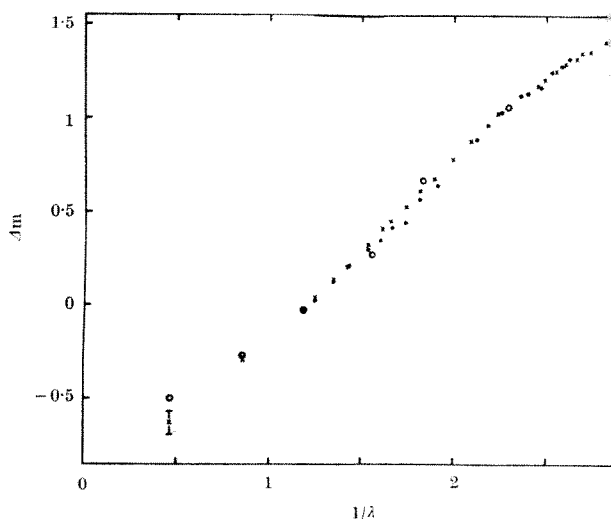


Fig. 2. Pleiades extinction curve as compared with that for Cygnus. The vertical line denotes the uncertainty of the point. ●, Pleiades (Edinburgh); ○, Pleiades (Mendoza); ×, Cygnus

The theoretical model which fits the Cygnus curve gives the value of the ratio of total to selective absorptivity, $R = 3.1$. The extrapolation of infra-red measurements of Mendoza for the Pleiades star gives $R = 3.6$. An indirect measurement of R from measures of the line $H\gamma$ in the Pleiades stars gives $R = 3.1$ (ref. 13).

The extinction of the Pleiades star does not show any correlation of the slope of the ultra-violet part of the extinction curve relative to the blue part with the polarization per unit obscuration. The variation in the extinction law cannot be explained therefore by differences in the alignment of the particles.

The space distribution of stars observed for the examination of extinction is illustrated in Fig. 3, which shows schematically the three spiral arms of the Galaxy. It is of interest to note that the Cygnus extinction curve is representative of the majority of stars in the Cygnus arm. In the direction of Perseus, the spiral arm can be easily reached, and the stars situated in a small region in the Perseus arm exhibit an extinction law different from that in Cygnus. Whether or not the Perseus law is representative of the Perseus arm in general has yet to be investigated. Few data exist as yet for stars in the inner arm. The difference between the Cygnus and Perseus extinction laws can be explained if it is assumed that the extinction in Perseus is due to graphite grains only, and that in

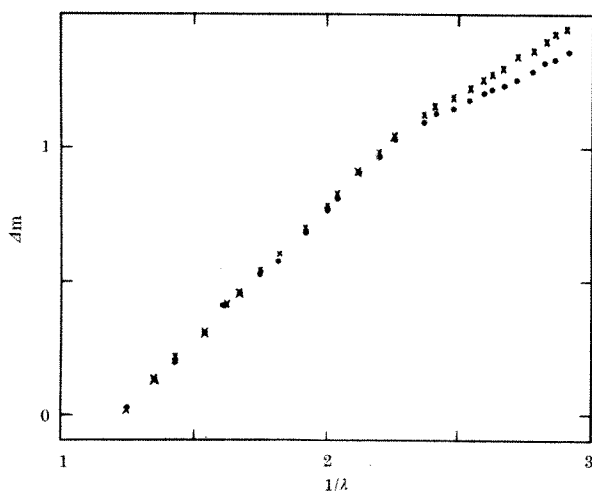


Fig. 1. Interstellar extinction curve in the direction of Cygnus and Perseus. Crosses denote results for Cygnus and filled circles results for Perseus

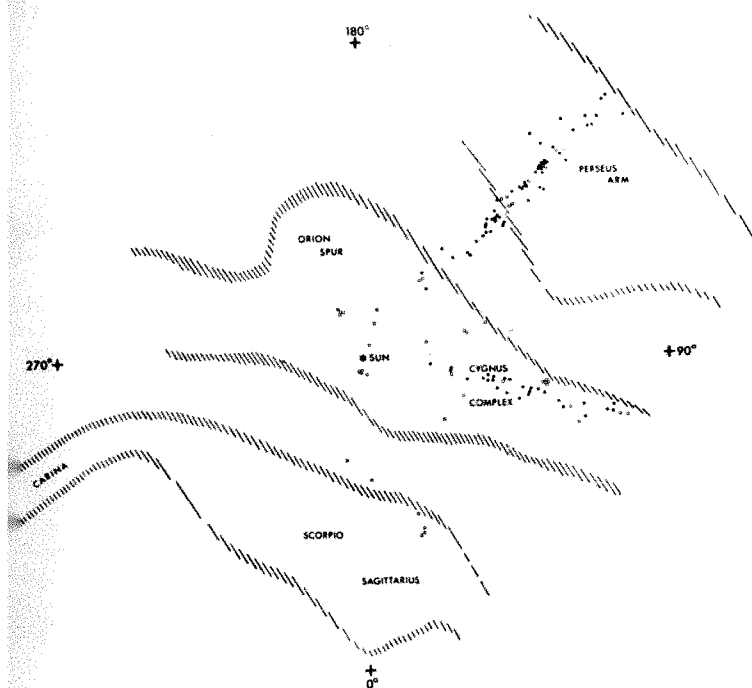


Fig. 3. Projection on the galactic plane of all stars observed for the study of interstellar extinction. Open circles refer to photoelectric measures, closed circles to photographic measures. The hatched lines represent the distribution of OB clusters, associations, and III regions, and crosses represent directions of new galactic longitude on a circle 6 kiloparsecs in diameter.

gnus to graphite cores with ice mantles. It appears therefore that there exists a regional variation in the composition of the interstellar dust particles which may be either a difference between the Cygnus and Perseus arm or a local variation within the Cygnus arm. The difference could be due to different physical conditions or to actual regional variations in chemical abundances. Further tests could come from the investigation of extinction in the Cassiopeia and Cepheus regions, which is now in progress here, and also from rocket observations.

I thank Mr. I. Nicolson and Mr. David Sugden for assistance with the reduction of the Pleiades plates.

K. NANDY

Royal Observatory,
Edinburgh, 9.

- Nandy, K., *Publ. Roy. Obs. Edin.*, **3**, 142 (1964).
 Boggess, A., and Borgman, J., *Astrophys. J.*, **140**, 1636 (1964).
 Hoyle, F., and Wickramasinghe, N. C., *Mon. Not. Roy. Astro. Soc.*, **124**, 417 (1962).
 Wickramasinghe, N. C., *Mon. Not. Roy. Astro. Soc.*, **129** (in the press).
 Wickramasinghe, N. C., and Guillaume, C., *Nature*, **207**, 366 (1965).
 Nandy, K., and Wickramasinghe, N. C., *Publ. Roy. Obs. Edin.*, **5** (in the press).
 Nandy, K., *Publ. Roy. Obs. Edin.*, **5** (in the press).
 Greenberg, J. M., and Meltzer, A. S., *Astrophys. J.*, **132**, 667 (1960).
 Wilson, R., *Mon. Not. Roy. Astro. Soc.*, **120**, 51 (1960).
 Underhill, A. B., and Walker, G. H., *Mon. Not. Roy. Astro. Soc.* (in the press).
 Johnson, H. L., *Astrophys. J.*, **141**, 923 (1965).
 Mendoza, V. E. E., *Tonantzintla y Tacubaya*, **4**, No. 26 (1965).
 Guthrie, B. N. G. (personal communication).

New Limits to the Angular Sizes of Some Quasars

In previous experiments at Jodrell Bank the angular sizes of discrete radio sources have been investigated by means of long baseline interferometers^{1,2}. The highest resolving power used previously was obtained during observations at a wave-length $\lambda = 0.73$ m with telescopes 180,000 wave-lengths apart (134 km). Four quasi-stellar and one unidentified source were found to be unresolved in those observations. Their angular sizes

were thus shown to be smaller than 0.4 sec of arc. In a further attempt to resolve these sources another experiment has been carried out using the Mark I 250-ft. radio telescope at Jodrell Bank, and one of the 82-ft. radio telescopes operated by the Royal Radar Establishment, Malvern. The separation of these telescopes is 127 km, in a direction which is close to north-south. This interferometer worked on a wave-length $\lambda = 0.21$ m, so that the maximum resolving power was more than three times greater than had been obtained in the previous observations. The effective resolving power changes as sources are observed in different directions, but its maximum value is greater than 600,000 wave-lengths for most sources. With this baseline the output fringe frequency produced as the radio source moves through the lobes of the interferometer pattern varies with hour angle from 0 to as much as 40 c/s. An almost identical frequency was produced continuously by a digital 'fringe speed machine' and this was subtracted from the interferometer output, so that the fringe patterns displayed on the chart recorder were normally slower than 0.5 cycles/min. A microwave link system was established between the two observatories, via two repeater stations. A new very-high-frequency phase-locking system was also developed, with equipment at each site.

During these observations, the five sources which had not been resolved previously (3C 119, 286, 287, CTA 21 and 102) all gave clear fringe patterns over a wide range of hour angles, though for only one source did the amplitude remain approximately constant. These sources are listed in column 1 of Table 1. Column 2 shows their catalogued³ flux density, S , at $\lambda = 0.21$ m. The observed fringe amplitudes were normalized by daily observations of the source 3C 147, which was found to give clear fringe patterns at all hour angles. Corrections arising from various instrumental effects have been applied to these normalized values of fringe amplitude. It was found that when the sources were observed at elevations less than 15° these corrections were frequently greater than 20 per cent, and such observations have not been used. The corrected values of fringe amplitude have been calibrated by considering the maximum value observed for each source, which is shown in column 3 of Table 1. It was found that these maximum values are in an almost constant ratio to the total flux from each source, as shown in column 4, the mean value of these ratios being 14.1 ± 0.9 .

A constant ratio in column 4 would be obtained if the minimum linear dimensions of all these sources were similar, and they were at comparable distances, and were partially resolved to the same extent by effective baselines between $500,000\lambda$ and $600,000\lambda$. This is an improbable situation, and it seems more likely, as we assume, that each source was unresolved by the effective baseline at which the maximum value of fringe amplitude was

Table 1. RADIO SOURCES SMALLER THAN 0.1 SEC OF ARC IN AT LEAST ONE DIMENSION

Source	Flux S at $\lambda = 0.21$ m (flux units)*	Max. fringe amplitude, A , observed with tracking interferometer of aerial spacing 605,000 λ (arbitrary units)	A/S
3C 119	3.5 ± 0.8	125 ± 10	14.7 ± 1.8
3C 286	15.1 ± 0.4	245 ± 20	15.2 ± 1.5
3C 287	7.6 ± 1.5	95 ± 5	12.5 ± 2.5
CTA 21	8.0 ± 0.8	115 ± 10	14.4 ± 2.0
CTA 102	5.6 ± 1.6	95 ± 10	14.4 ± 2.0
Mean value			14.1 ± 0.9

* One flux unit = $10^{-26} \text{ W m}^{-2} (\text{c.s.})^{-1}$.

Table 2. DATA ON 3C 273 AT 0.21 M

Total flux density (1962-3)	39.8 ± 2 flux units
Flux ratio component B : A (1962-9)	1.40
Therefore component A was then 16.5 and B,	23.3 flux units
Long baseline interferometer (1965-5)	
Two parts of one component gave	30 ± 2 flux units
Total power measurements (1965-6) A + B	46 ± 1.5 flux units
Provisional interpretation (1965-7)	

Component A is still 16.6 flux units, and component B is now 30 flux units.
Flux ratio B : A now 1.84.

that, on the assumption that the radio galaxies have remained constant, the value of total flux from 3C : in 1965-6 was 46 ± 1.5 flux units. This is compatible with the other results if it is assumed that the interferometer observations refer to the component B, and that the flux of this component has now increased to flux units, that is, by approximately 30 per cent in the years. It is not known which part of component B it

Table 3. CURRENT INTERPRETATIONS OF THE RADIO MEASUREMENTS OF THE ANGULAR AND LINEAR DIMENSIONS OF SOME QUASARS

Source	Redshift z	Remarks on individual sources	Approximate linear dimensions (parsecs)	
			If redshifts cosmological*	If sources local at about 10 Mpc
3C 273	0.158	Source has two components 19.5 sec apart in position angle 044° <i>Component B</i> , coincident with optical quasi-stellar object, has two parts, each smaller than 0.1 sec, separation probably 0.4 sec. At $\lambda = 0.21$ m flux of component B has increased by approx. 30 per cent in 2.7 yr <i>Component A</i> has dimensions 5×1.5 sec flux probably unchanged At $\lambda = 0.73$ m, elliptical, 0.4 by < 0.3 sec†‡§ At $\lambda = 0.21$ m, this source has two components (ref. 9) Separation 62 sec Each component ≤ 10 sec, but little structure ≤ 2 sec¶ At $\lambda = 0.73$ m, elliptical 0.6 by < 0.4 sec†‡§ At $\lambda = 1.89$ m, ≈ 6 sec. No structure measurements at any wave-length	Separation of A and B 32,000 Each part smaller than 170 separation probably 700 8,500 × 2,500 1,100 × ≤ 900 $\geq 180,000$ $< 30,000$ but $> 6,000$ 2,000 × $\leq 1,300$ $\approx 21,000$ 420 × < 350 $> 41,000$ < 360 500 × < 360 $> 32,000$	950 5 20 240 × 70 20 × ≤ 15 $\geq 3,000$ < 500 but > 100 30 × ≤ 20 ≈ 300 6 × < 5 > 600 < 5 7.0 × < 5 > 450
3C 48	0.367			
3C 47	0.425			
3C 147	0.545			
3C 254	0.734			
3C 286	0.86			
3C 245	1.029			
CTA 102	1.037			
3C 287	1.055			
3C 9	2.012			

* These linear dimensions have been calculated for model universes (ref. 4) in which the acceleration parameter $q_0 = +1$. If $q_0 = 0$, these values must be multiplied by a factor $(1 + 0.5z)$ which for these sources lies in the range 1.2–2.01.

† Results (ref. 7) of detailed analysis of observations at $\lambda = 0.73$ m.

‡ None of the available evidence suggests that the source contains fine structure within this elliptical component, but further analyses or observations even higher resolution could conceivably reveal a more complex structure, within these overall dimensions, as in the case of 3C 273B.

§ Fringe patterns corresponding to partial resolution of this source were recorded during the observations at $\lambda = 0.21$ m described above. They have not yet been analysed in sufficient detail to improve significantly the earlier interpretation of the angular structure of this source.

¶ A weak source at $\lambda = 0.73$ and $\lambda = 0.21$ m. Even if it were unresolved, the signal-to-noise ratio would be poor under optimum conditions, and the fringe pattern might not have been recognized during those observations which were attempted.

observed. This means that each of these sources is smaller than 0.1 sec of arc in at least one direction. The source CTA 102 did not appear to be resolved at any hour angle when its elevation was greater than 15° , so that its angular size is shown to be less than 0.1 sec of arc in all position angles. Each of the other sources was partially resolved at some hour angle, and the corresponding maximum angular dimensions (assuming gaussian source models) were in the range 0.12–0.16 sec of arc, as shown in column 5 of Table 1.

The quasar 3C 273 was also observed and was found to give clear fringe patterns of unexpectedly large amplitude with one well-marked minimum near meridian transit. Observations of lunar occultations of this source show that it consists of two components, A and B, the centres of which are 19.5 sec apart in position angle 044° . If both these components had contributed to the fringe patterns observed at this baseline, a pronounced minimum would have been observed every 10–15 min at most hour angles. As these frequent minima were not observed, it follows that only one component of the source is small enough to give fringe patterns at this baseline, and that it, in turn, probably consists of two parts, which are each smaller than 0.1 sec of arc.

As only one minimum was observed, the angular separation of these parts and the position angle cannot, by inspection, be determined uniquely, though they may be obtained in due course by more detailed analyses. If it is assumed that this position angle is also 044° , the separation is of the order 0.4 sec of arc.

This interpretation of our observations, when calibrated with the factor derived from the sources discussed earlier, shows that the two parts of one component of 3C 273 are now giving a flux density not less than 30 ± 2 flux units. As may be seen in Table 2, this is significantly greater than the value of 23.3 flux units derived for the brighter component, B, from the ratio given by the occultation observations of 1962-9. The total flux from this source was therefore re-measured recently, with the Mark I telescope at Jodrell Bank, and compared with the radio galaxies 3C 348 and 353. These measurements show

increased. This conclusion may be compared with the measurements reported by Dent³ which show that a 8,000 Mc/s, where almost all the radiation comes from component B, the flux has increased by 40 per cent in 2.5 years.

We have summarized in Table 3 our current interpretations, based on these and earlier measurements, of the angular dimensions of the ten quasi-stellar sources the redshifts of which have been published^{1,2}. Possible values of the linear dimensions of the radio-emitting regions of these sources are shown in columns 4 and 5. Those in column 4 have been calculated on the hypothesis that the redshifts of these sources arise from the general expansion of the Universe, and so correspond to distances greater than 600 Mpc. The values given in column 5 are calculated on the hypothesis suggested by Hoyle and Burbidge⁶, that the emitting regions are at distances of order 10 Mpc, and their redshifts arise from very high intrinsic velocities of recession of the individual objects

R. L. ADGIE
H. GENT
O. B. SLEE*

Royal Radar Establishment,
Great Malvern,

A. D. FROST†
H. P. PALMER
B. ROWSON

University of Manchester,
Nuffield Radio Astronomy Laboratories,
Jodrell Bank.

* On leave from C.S.I.R.O., Sydney, Australia.

† On leave from University of New Hampshire.

¹ Allen, L. R., Anderson, B., Conway, R. G., Palmer, H. P., Reddish, V. C., and Rowson, B., *Mon. Not. Roy. Astro. Soc.*, **124**, 477 (1962).

² Anderson, B., Donaldson, W., Palmer, H. P., and Rowson, B., *Nature*, **205**, 375 (1965).

³ Dent, W. A., *Science*, **148**, 1458 (1965).

⁴ Schmidt, M., *Astrophys. J.*, **141**, 1295 (1965).

⁵ Shklovsky, I. S., *Astro. Circ.*, U.S.S.R., No. 250 (1963).

⁶ Hoyle, F., and Burbidge, G. R. (personal communication).

⁷ Anderson, B., Ph.D. thesis, Univ. Manch. (1965).

⁸ Conway, R. G., Kellerman, K. I., and Long, R. J., *Mon. Not. Roy. Astro. Soc.*, **125**, 261 (1963).

⁹ Ryle, M., Elsmore, B., and Neville, Ann C., *Nature*, **207**, 1024 (1965).

RADIOPHYSICS

Scintillations on the Small Diameter Radio Sources

IRREGULARITIES in the solar wind appear to be responsible for the rapid scintillations on the radiation from small diameter radio sources¹, and for some of the fine structure of Jovian decametric bursts². At the Arecibo Ionospheric Observatory the irregularities as well as the sources are being examined by their scintillations. Early results of this programme have been reported by Cohen and Drake³. The purpose of this communication is merely to report that the scintillation phenomenon has been seen over an extended range of frequencies and positions, and that it is very strong and persistent.

Mc/s records of 3C-298 and 3C-317, taken on May 22, 1965, when 3C-298 was about 11 h east of the Sun. The two sources have approximately the same strength, as shown by the records; 3C-298 scintillates, but 3C-317 does not. The effect of scintillations shows up strongly in Figs. 2 and 3. Fig. 2 shows the unnormalized autocorrelation functions obtained from 5-min samples of the records, sampled 10 times per sec. The 3C-317 autocorrelation function has essentially the same strength and shape as those functions obtained when tracking the cold sky; it is an exponential with e^{-1} width equal to the output time constant of the radiometer. The autocorrelation function at zero lag is proportional to the variance of the fluctuating output signal, and so the root mean square fluctuation level for 3C-298 is twice that which would have been expected in the absence of scintillations. The scintillation index for 3C-298 is 0.041. (Index is defined as excess root mean square fluctuations divided by antenna temperature.)

Fig. 3 shows the power spectra of the radiometer output signals, obtained by computing the approximate Fourier transforms of the autocorrelation functions. The 3C-298 spectrum has excess energy only at low frequencies. This excess is the scintillation spectrum; it is roughly gaussian in shape, with an e^{-1} width of 0.55 ± 0.05 c/s. The peaks at 3.3 c/s are apparently due to aliasing.

A short radiometer time constant is required for scintillation observations, since the fluctuations may have periods of 1 sec or less. This results in a poor signal-to-

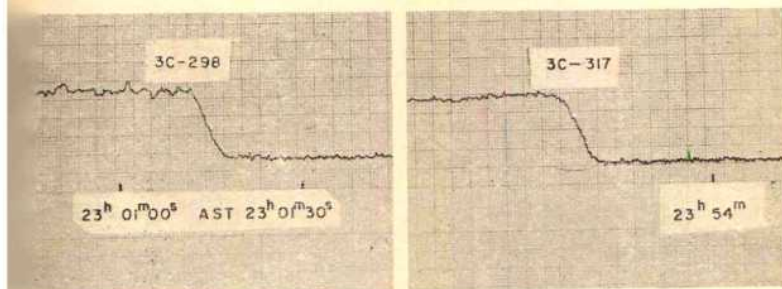


Fig. 1. 195 Mc/s records of 3C-298 and 3C-317, May 22, 1965. Successive on-off records are shown. Time constant 0.1

The scintillations are very strong when the source is very narrow and close to the Sun. For example, on May 23, 1965, CTA-21 was about 11° from the Sun. At 430 Mc/s the scintillations were very strong, with minima about 0.2 the mean value. As another example, on June 13, 1965, 3C-138 was about 7° from the Sun. Strong scintillations were observed on 195, 430 and 611 Mc/s. On all these frequencies the minima were about half the mean value. These observations were made with the 1,000-ft. spherical reflector, using point feeds giving beam-widths of 33', 16' and 14' at 195, 430 and 611 Mc/s, respectively. Small diameter sources, such as CTA-21 and 3C-138 (ref. 4), can readily be found by their very strong scintillations, if they are close to the Sun.

The very narrow sources, furthermore, always scintillate, even when they are far from the Sun. Fig. 1 shows 195

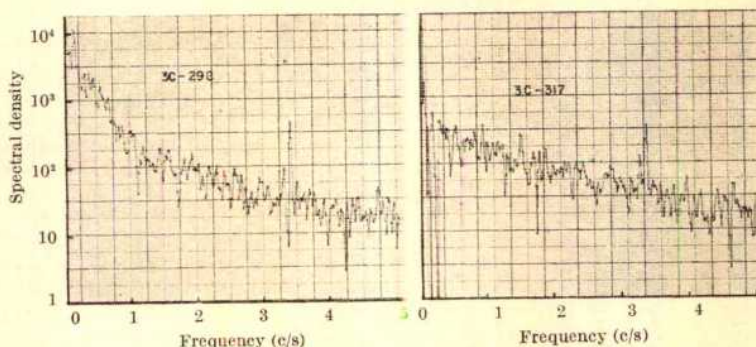


Fig. 3. Power spectra (in arbitrary units) for 3C-298 (scintillating) and 3C-317 (non-scintillating)

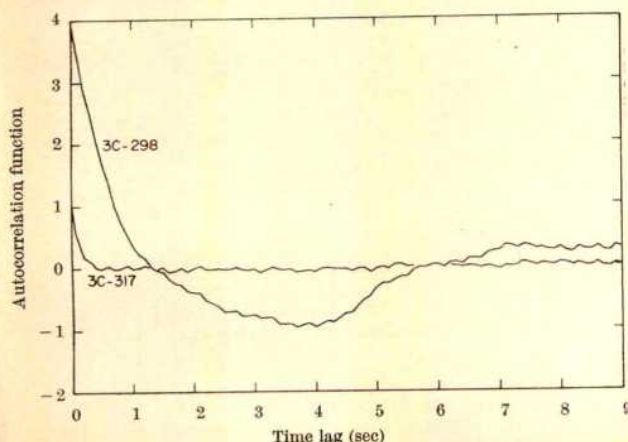


Fig. 2. Autocorrelation functions (in arbitrary units) for 3C-298 (scintillating) and 3C-317 (non-scintillating). Samples were taken 10 times per sec for 5 min; 90 lags are shown

noise ratio for weak sources. Digital processing, however, can greatly increase the visibility of the scintillations.

I thank E. E. Salpeter for his advice, and Mrs. N. Earle and Mrs. S. Jack for assistance with the calculations. The Arecibo Ionospheric Observatory is operated by Cornell University with the support of the Advanced Research Projects Agency under a research contract with the Air Force Office of Scientific Research.

MARSHALL H. COHEN

Center for Radiophysics and Space Research,
Cornell University,
Ithaca,
New York.

¹ Hewish, A., Scott, P. F., and Wills, D., *Nature*, **203**, 1214 (1964).

² Douglas, J. N., *I.E.E.E. Trans. Mil. Elec.*, **MIL-8**, 173 (1964).

³ Cohen, M. H., and Drake, F. D., U.R.S.I. Spring Meeting, Washington (April, 1965).

⁴ Anderson, B., Donaldson, W., Palmer, H. P., and Rowson, B., *Nature*, **205**, 375 (1965).

PHYSICS

Combined Very High Pressure/High Temperature Calibration of the Tetrahedral Anvil Apparatus, Fusion Curves of Zinc, Aluminium, Germanium and Silicon to 60 kilobars

In apparatus of the tetrahedral anvil or of the 'belt' type¹, where a solid-pressure transmitting medium such as pyrophyllite is used, the relation between load and pressure is fairly complicated². The pressure calibration at room temperature is frequently made in terms of the usual resistance transitions in bismuth, thallium and barium¹. There is no guarantee that such a calibration will be valid for the high-temperature range up to more than 1,000° C in which such apparatus is often used.

To check on this point, the tetrahedral anvil apparatus has been used to determine the melting points of zinc, aluminium, germanium and silicon as a function of the pressure inferred from a room temperature calibration. These results can then be compared with previous determinations³⁻⁶ using piston and cylinder apparatus for which the temperature dependence of the load versus pressure calibration would be expected to be relatively small.

The NBS-type tetrahedral anvil apparatus used had 1.90-cm edge cemented (6 per cent cobalt) carbide anvils, and the 2.38-cm edge tetrahedra were made from pyrophyllite ('Alsil' grade). The experimental arrangement is shown in Fig. 1; the two alternative thermocouple positions shown gave identical results. The thermocouples used were chromel/alumel, and were of the sheathed 'Thermocoax' type with a sheath diameter of 0.5 mm: these were carried directly into the tetrahedron through an edge. No correction was applied for the effect of pressure on the thermal e.m.f. Melting was detected by simple thermal analysis, with the thermal e.m.f. displayed against a constant time base on a fast-acting XY recorder, since the cooling rate of the tetrahedral apparatus is rapid (400°/sec from 1,000° C).

The materials used in these experiments were 99.99 per cent pure or better and, to minimize possible contamination of the sample by its container, the following procedure was adopted. At a given anvil load, temperature was first raised slowly to ~0.7 of the expected melting point. Temperature was then increased rapidly (using the fast thermal response of the apparatus) by switching a suitable resistance from the primary of the heater power supply transformer. When melting was indicated by the appropriate 'arrest' on the rise curve, the heater supply was disconnected and the cooling curve taken. This ensured that the sample was heated in contact with its

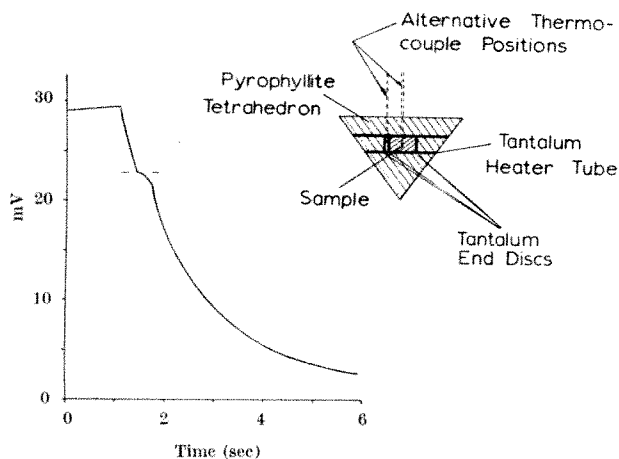


Fig. 1. Cooling curve of zinc specimen cooled from 720° C at 32 kbar, showing 'arrest' due to freezing. Inset shows tetrahedron arrangement

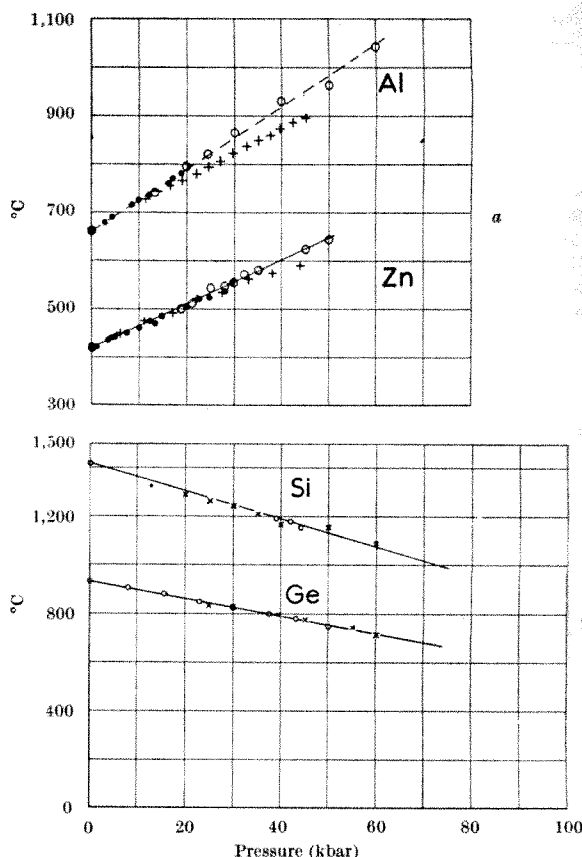


Fig. 2. Fusion temperatures as a function of pressure. (a) Zinc and aluminium: (b) germanium and silicon. \circ , ref. 3; +, refs. 4 and 5; \times , ref. 6; \bigcirc , present work, pressures inferred from room temperature calibration

surroundings for a few seconds only, and X-ray microprobe analysis of selected specimens confirmed that no significant contamination had occurred during the heating cycle for any of the materials in tantalum containers. In view of previous experience³, suggesting that molybdenum is a suitable container for aluminium, it is interesting to note that we have found evidence of considerable reaction between these metals at high pressures, especially at temperatures approaching 1,000° C, where depression of the freezing point by as much as 50° C have been observed. X-ray microprobe analysis confirmed that a reaction had occurred in these cases with the formation of an intermetallic compound, probably MoAl_2 .

A typical arrest corresponding to solidification for a zinc specimen cooled from 730° C is shown in Fig. 1. In general, well-defined 'arrests' of this type were obtained during these experiments, and there was no evidence of supercooling despite the high cooling rates. Quite frequently 'arrests' began with a region of finite slope rather than an accurate horizontal; in these cases the point of inflexion was taken to indicate freezing. The maximum error of measurement in these experiments is about $\pm 5^\circ \text{C}$.

Fig. 2 shows the fusion curves to 60 kbar, pressures being inferred from a subsidiary room pressure calibration with a similarly sized test sample. All measurements were made during the loading cycle and the repeatability of pressures is in the range ± 1.5 kbar. These results are compared with two previous investigations: (1) Butuzov³ used relatively large samples pressurized either in argon or pentane so that truly hydrostatic conditions were obtained. Melting temperature was determined by differential thermal analysis, and pressures by a manganin gauge with an accuracy of 0.1 kbar. (2) Using piston and cylinder apparatus with tale as the pressure-transmitting medium, Kennedy and Newton⁴ have determined the fusion curve of zinc, Jayaraman, Klement, Newton and Kennedy⁵ that of

minium, and Jayaraman, Klement and Kennedy⁶ that germanium and of silicon. Pressure was inferred from force and area, with a correction for piston friction, giving an accuracy of 1.0 kbar.

Fig. 2 shows a very satisfactory agreement between the curves obtained in the present work and a linear extrapolation of those of Butuzov³ for zinc and aluminium: that, in the range up to 60 kbar and 1,100° C, a reasonable agreement is also obtained with the curves obtained in refs. 4 and 6 for zinc, silicon and germanium. Considering that the various determinations were made in widely differing types of apparatus, and in particular that Butuzov's conditions were truly hydrostatic, it is a reasonable conclusion that the effect of temperature on the dP/pressure calibration curve for the tetrahedral apparatus is small, certainly less than ± 3 kbar at 50 kbar and 1,100° C.

There is a significant difference, amounting to 8 kbar (50° C) at 40 kbar and 900° C, between the present results for aluminium and those of ref. 5. It is possible that at least some of this effect is due to the reaction discussed above between molten aluminium and the molybdenum containers used in the latter work.

In a recent determination of the phase diagrams of silicon and germanium, Bundy⁷ used a modified 'belt' type apparatus to produce pressures up to about 200 kbar and 1,000° C. With this apparatus a large pressure rise due to adiabatic heating at the specimen was inferred, which was roughly proportional to the product of the initial pressure and the temperature rise, and would amount to about 3 kbar at 50 kbar and 1,000° C. The compatibility of the present results with those of other workers implies that such a large effect is not encountered with the tetrahedral apparatus as used in this work.

We thank Miss A. Thake, who carried out many of the experiments, and L. Hailes for the microprobe analysis of specimens; we also thank C. H. L. Goodman for discussion.

J. LEES

B. H. J. WILLIAMSON

Standard Telecommunication Laboratories, Ltd.,
Harlow, Essex.

Modern Very High Pressure Techniques, edit. by Wentorf, R. H., 1 (Butterworths, London, 1962).

Lees, J., *Nature*, **203**, 965 (1964).

Butuzov, V. P., *Sov. Phys. Crystallogr.*, **2**, 533 (1957).

Kennedy, G. C., and Newton, R. C., in *Solids Under Pressure*, edit. by Paul, W., and Warschauer, D. M., 163 (McGraw-Hill, New York, 1963).

Jayaraman, A., Klement, W., Newton, R. C., and Kennedy, G. C., *Intern. J. Phys. Chem. Solids*, **24**, 7 (1963).

Jayaraman, A., Klement, W., and Kennedy, G. C., *Phys. Rev.*, **130**, 540 (1963).

Bundy, F. P., *J. Chem. Phys.*, **41**, 3809 (1964).

A New Absolute Determination of the Acceleration due to Gravity at the National Physical Laboratory

An absolute determination of the acceleration due to gravity at a site in the National Physical Laboratory has recently been completed. It is the first to be made by timing the symmetrical up-and-down motion of a body moving freely under gravity, and the uncertainty of the result is considerably less than that of any previous measurement.

If a body is timed as it crosses two horizontal planes with a vertical separation H , and if the time interval between crossings of the lower plane is T_1 and of the upper plane T_2 , then:

$$g = \frac{8H}{T_1^2 - T_2^2}$$

A correction has to be applied for the decrease of gravity with height.

In the present experiment the planes were defined optically by pairs of horizontal slits and the moving object was a glass ball which focused one slit of a pair on the other when it was symmetrically between them. One

slit was illuminated and a photomultiplier behind the other recorded the flash of light as the ball passed. The pairs of slits were incorporated in composite blocks of fused silica, the vertical distance (1 m) between the blocks at the upper and lower positions being measured by means of an interferometer.

The advantages of this method have been discussed recently¹ and it is sufficient to say that they have been fully borne out; in particular the measured acceleration is independent of air resistance.

The measured value of gravity, reduced to the British Fundamental Station in the National Physical Laboratory², is 981 181.77 mgal, with a standard deviation of 0.13 mgal (1 mgal = 10^{-3} m/sec²).

The uncertainty comes mainly from microseismic disturbances. An earlier determination at the National Physical Laboratory with a reversible pendulum³ gave a value of 981 183.2, s.d. 0.7 mgal at the British Fundamental Station. The difference of 1.4 mgal from the new result is just twice the standard deviation of Clark's value. Comparisons with other determinations will be discussed in the full account of the measurements which will be published later.

A. H. COOK

Standards Division,
National Physical Laboratory,
Teddington, Middlesex.

¹ Cook, A. H., *Metrologia*, **1**, 84 (1965).

² The British Fundamental Gravity Station, *Nature*, **173**, 794 (1954).

³ Clark, J. S., *Phil. Trans. Roy. Soc.*, **238**, 65 (1939); Jeffreys, H., *Mon. Not. Roy. Astron. Soc., Geophys. Suppl.*, **5**, 398 (1949).

Deformation of Gas Bubbles and Liquid Drops in an Electrically Stressed Insulating Liquid

THE recent paper by Garton and Krasucki¹ is of considerable interest and reminded me of some of the work I undertook about five years ago². I observed a similar phenomenon under different conditions. This brief note is to bring to notice some of my results obtained at that time, which serve as further evidence that gas bubbles and liquid drops always tend to elongate in the direction of the applied field in an insulating liquid, irrespective of whether the permittivity of the bubble or drop is larger or smaller than that of the surrounding liquid.

A glass test cell 5 in. \times 3 in. \times 2 in. was used, containing a pair of parallel brass plane electrodes of size 4 in. \times 2 in. with a separation of 0.5 in. aligned horizontally. The test cell was fully filled with transformer oil, and an air bubble or a water drop could be introduced into the cell through two small holes in the top and the bottom of the cell. The pressure on the liquid was kept at atmospheric pressure and the temperature 20° C. The air bubble was injected through the bottom hole and it moved up through the centre of the liquid gap by buoyancy force, while the water drop was injected through the top hole and it moved down through the centre of the liquid gap by gravitation force. The 50-c/s a.c. field was first applied and a photograph was taken when the bubble reached the centre of the liquid gap. This method was different from that adopted by Garton and Krasucki, who deliberately eliminated the buoyancy force by rotating the test cell to maintain the bubble in a fixed position. However, their results and mine agree qualitatively. Fig. 1 shows an air bubble elongated at 22.5 kV and 28.5 kV though they were slightly distorted. Fig. 2 shows a water drop elongated and a large drop bursting into many droplets at 6 kV. The bubbles and drops in (a), (b) and (c) of Figs. 1 and 2 were not originally identical in size, since the device could not control their sizes accurately. But it can be seen that the greater the difference in permittivity between the bubble (or drop) and the insulating liquid, and the larger the original size of the bubble (or drop), the smaller is the electrical field required to cause elongation—as predicted by theory^{1,2}.

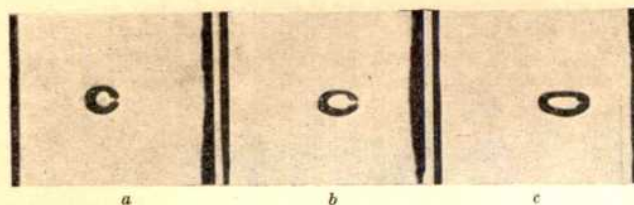


Fig. 1. An air-bubble elongated in the direction of the applied field in transformer oil. The 50-c/s a.c. voltage (root mean square) across a liquid gap of 0.5 in.: (a) $V=0$; (b) $V=22.5$ kV; (c) $V=28.5$ kV

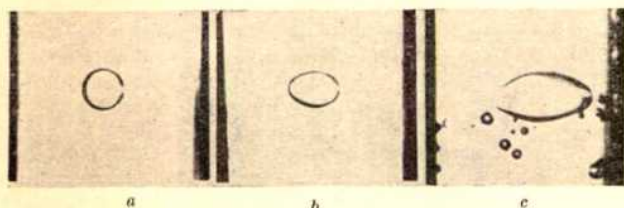


Fig. 2. A water drop elongated in the direction of the applied field in transformer oil. The 50-c/s a.c. voltage (root mean square) across a liquid gap of 0.5 in.: (a) $V=0$; (b) $V=6$ kV; (c) burst of a large water drop at $V=6$ kV

It should be noted that the experimental conditions were somewhat different from the assumed conditions on which the theory was based. For example, the bubble size under experimental investigation was not so small compared with the liquid gap that the charge distribution on the electrodes before and after the introduction of a bubble was identical with the case for a field of fixed source distribution. This point must be borne in mind when comparing experimental results with theory. Furthermore, free charges inside or outside the bubble may also influence the deformation. Allan and Mason³ have reported that under certain conditions the fluid drops were flattened into oblate spheroids in the field. This phenomenon may possibly be due to the attractive force between positive and negative charges accumulated on the bubble surface, which, so far, has been ignored in the theory. More work, both theoretical and experimental, is necessary in order to clarify many points which are still not understood.

K. C. KAO

Department of Electrical Engineering,
Royal College of Advanced Technology,
Salford.

¹ Garton, C. G., and Krasucki, Z., *Proc. Roy. Soc., A*, **280**, 211 (1964).

² Kao, K. C., *Brit. J. App. Phys.*, **12**, 629 (1961).

³ Allan, R. S., and Mason, S. G., *Proc. Roy. Soc., A*, **267**, 45 (1962).

GEOPHYSICS

Variation according to Latitude of the Electron Content of the Ionosphere near the Auroral Zone

SOME evidence has been obtained from single-frequency Faraday measurements using the satellite 1963-49C, reported by Lyszka and Taylor¹, that the sub-satellite electron content, n_t , of the ionosphere at high latitudes may consist of two components. The first component, which decreases gradually with increasing latitude, is probably produced by ultra-violet radiation from the Sun; the second, irregular, component seems to dominate at latitudes greater than 65° N. It was suggested that the latter could either be produced by low-energy corpuscular radiation, or that it could be a manifestation of transport processes. Some more accurate measurements of the electron content at high latitudes, obtained from differential Faraday observations of the multifrequency beacon satellite S-66 (1964-64A), now show that the first explanation of the irregular component seems more plausible.

The electron contents have been calculated using data from 40 and 41 Mc/s records of S-66 made at Kiruna, Sweden (67.8° N., 20.4° E.), during the first two months of the satellite's life. The method was the same as that used for the reduction of two-frequency Moon radar data; n_t was calculated at each Faraday null time and plotted as a function of sub-ionospheric geocentric latitude. Some of the resulting curves are shown in Figs. 1 and 2 and those corresponding to a time of day shortly before local noon are given in Fig. 1 and those to a time shortly after local midnight in Fig. 2. All these results refer to the second half of November 1964. It can be seen that near noon n_t decreases with latitude; small irregularities often occur at latitudes of about 65° N. Night-time curves, particularly those from 03-04 local time, show a gradual decrease of n_t up to about 60° N., followed by an increase up to values at least as great as those observed at 50° N.

It may also be noted that the electron contents shown here are, on average, lower than those obtained from single-frequency records by assuming the effective thickness of ionosphere to be 350 km (ref. 1). Equivalent slab thicknesses were calculated using simultaneous values of maximum electron density, N_{\max} , obtained from the equation:

$$N_{\max}(\text{e/cm}^3) = 1.24 \times 10^4 (f_o F2)^2$$

where $f_o F2$ is the ordinary wave critical frequency in Mc/s. For this purpose $f_o F2$ values from six Scandinavian stations, covering an interval of 10 degrees of latitude were used. The results show that at high latitudes the effective thickness is much less than 350 km during the daytime, and sometimes less than 200 km. However, when the second component of n_t was present, effective thicknesses a few times larger than 350 km were obtained. As an example, effective thicknesses obtained for one of the transits shown in Fig. 2, transit No. 658, are given in Table 1 (Nov. 27, 1964, 0200 local time).

Table 1

Station	Geographic latitude ($^\circ$ N.)	$f_o F2$ (Mc/s)	Effective thickness (km)
Sodankylä	67.4	2.0	650
Lycksele	64.7	1.9	413
Uppsala	59.8	2.0	222

An examination of $f_o F2$ values shows that in many cases during the night-time, N_{\max} changes with latitude in the same way as n_t , that is, it shows a definite minimum value. As an example, N_{\max} values from 03 to 04 local time of November 22 are shown in Fig. 3. This phenomenon may also be found during the early morning hours on averaged world contours of $f_o F2$ published by Martyn².

Any transport of ionization is almost certainly directed along the magnetic field lines, that is, nearly vertically, and it seems unlikely that the large increase of the

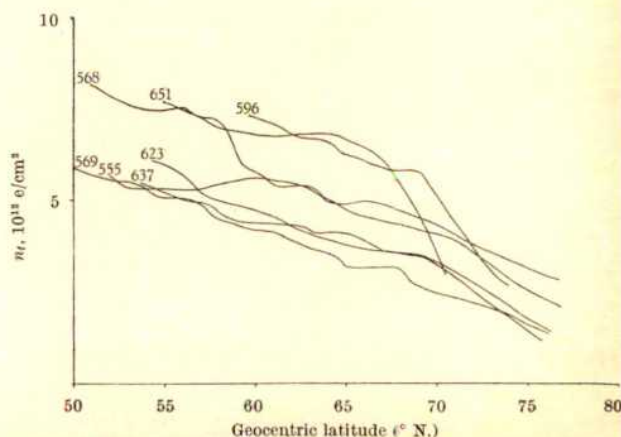


Fig. 1. Electron contents for a number of satellite transits shortly before local noon during the second half of November 1964 plotted as a function of geocentric latitude.

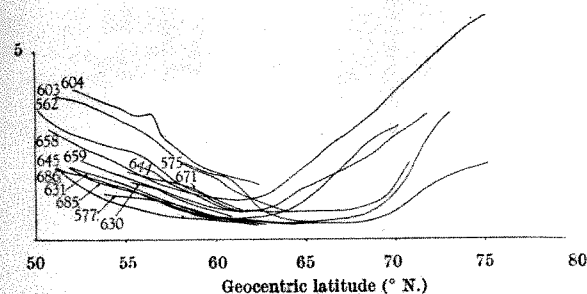


Fig. 2. Electron contents for a number of satellite transits shortly after local midnight during the second half of November 1964 plotted as a function of geocentric latitude

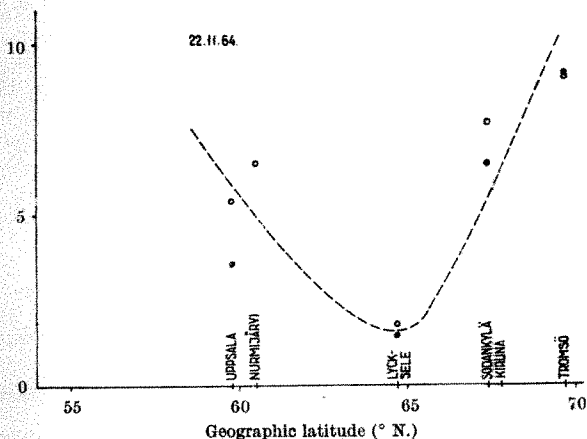


Fig. 3. Electron densities at the maximum of the F-layer, N_{max} , from 03 (O) to 04 (●) local time on November 22, 1964, plotted as a function of the geographic latitudes of the ionosonde stations

electron content up to the satellite altitude (1,000 km) can be due to this cause. It would mean that there are at least as many electrons above 1,000 km as below; electrons which can, moreover, in certain conditions move downwards. There is at present no evidence for the existence of such a reservoir of ionization in the upper F-region, though there are few relevant high-latitude observations. Thus it seems more reasonable to attribute the increase of n_e to low energy corpuscular radiation, possibly electrons, producing additional ionization in the ionosphere at auroral latitudes. Their energy must be less than 1 keV since electrons with higher energy reach altitudes below 180 km without ionizing the F-layer⁴.

Further investigation of electron contents at auroral latitudes is needed before any definite conclusions can be drawn. It is already clear that the S-66 satellite is an excellent instrument in this type of investigation.

This work was supported by the Cambridge Research Laboratories, OAR, through the European Office of Aerospace Research, U.S. Air Force (contract AF 61(052)-678).

LUDWIK LISZKA

Kiruna Geophysical Observatory,
Kiruna C, Sweden.

¹ Liszka, L., and Taylor, G. N., *J. Atom. Terr. Phys.* (in the press).

² Taylor, G. N., in *Lunar Radar Studies of the Earth's Atmosphere. Radio-astronomical and Satellite Studies of the Atmosphere*, edit. by Aarons, J., 135 (North-Holland Pub. Co., 1963).

³ Martyn, D. F., *Phys. Soc. Rep. Ionosphere Conf.*, 260 (Physical Society, 1955).

Rees, M. H., *Planet. Space Sci.*, 11, 1209 (1963).

GEOLOGY

Holothurian Sclerites from the Speeton Clay

DURING an examination of the Foraminifera from the Lower Cretaceous clays of Speeton, Yorkshire, a number of samples were found to contain holothurian sclerites. This was an unexpected occurrence, as Frizzell and Exline¹

have pointed out that virtually no work has been published on sclerites from strata of Cretaceous age. The only previous records of Cretaceous holothurian sclerites are *Calchigula* (?) *huckei* from the Gault of Pomerania, *Theelia rotula* (Egger) from Germany and *Hemisphaeranthos franki* (Müller) from the Turonian of Germany. 'Chirodota' from the Hauterivian of France were noted by Deprat², and Wetzel³ obtained sclerites from a Baltic flint which Deflandre-Rigaud⁴ identified as *Myriotrochites elegans* (Schlumberger), *Chiridotites atavus* (Waagen) and *Chiridotites cf. ingens* (Joshua). More recently Kemper⁵ recorded 'Holothurienreste' from the Hauterivian of Germany, though he did not describe them.

A systematic search for holothurian spicules in more than two hundred micropalaeontological residues of the Speeton clay, mainly from the type locality but also from borehole material, revealed several horizons which contained a large number of holothurian remains. All were obtained from strata of Hauterivian and Barremian age.

The sclerites from Speeton belong to four families, Achistridae, Theeliidae, Stichopitidae and Priscopedatidae; all are well preserved and free from matrix and secondary growth. Of particular interest in the Barremian clays is the occurrence of the Family Achistridae, which is represented by the species *Achistrum* (*Cancellrum*) *monochordata* Hodson, Harris and Lawson. This is a sclerite in the form of a hook, with a terminal loop which is crossed by a single cross-bar. Forty-one examples of this species have so far been found. With this record of *Achistrum* (*Cancellrum*) *monochordata* the range of the Family Achistridae, previously regarded as being from the Devonian (?) to the Jurassic, can now be extended to include the Lower Cretaceous.

It is interesting to note that *A. monochordata* is a common form species in the Oxfordian^{6,7}, though the specimens from the Barremian are generally smaller than those from the Jurassic. The widths of the terminal loop in the specimens from Speeton range from 0.090 mm to 0.144 mm, while those from the Oxfordian range from 0.162 mm to 0.324 mm.

So far as I am aware, holothurian sclerites have not previously been recorded from the Cretaceous of Britain.

B. N. FLETCHER

Department of Geology,
University of Hull.

¹ Frizzell, D. L., and Exline, H., *Univ. Missouri School Mines and Met. Bull.*, 89 (1955).

² Deprat, J. F. G., *Bull. Soc. d'Hist. Nat. du Doubs*, 1, 21 (1900).

³ Wetzel, O., *Palaont. Zeit.*, 27, 37 (1953).

⁴ Deflandre-Rigaud, M., in *Traité de Pal.*, edit. by Piveteau, J., 3, 951 (1953).

⁵ Kemper, E., *Geol. Jb.*, 80, 456 (1963).

⁶ Fletcher, B. N., *Geol. Mag.*, 93, 4, 322 (1962).

⁷ Hodson, F., Harris, B., and Lawson, L., *Geol. Mag.*, 93, 4, 336 (1956).

CHEMISTRY

Polyamide Layer Chromatography of DNP Amino-acids

METHODS for the identification of DNP amino-acids by paper or thin-layer chromatography were used to determine the N-terminal group in polypeptide structures. Recent developments in silica-gel thin-layer chromatography have made possible the rapid identification of amino-acids in biological mixtures¹.

The separation of various DNP amino-acids on a polyamide column was developed by Steuerle and Hille² and by Grassmann *et al.*³, but no practical method for separating micro samples by polyamide thin-layer chromatography has been developed.

The polyamide layer (Chen-Hsin-Tang Chemicals Co., No. 75, Section I, HanKou St., Taipei, Taiwan), according to Wang⁴, gave better results and durable polyamide layer. We have applied this polyamide layer to several

Table 1

	A 4 h	B 1.5 h	C 1.5 h	D 1.5 h	E 1 h
ϵ -DNP-Lys-HCl	0.72	0.14	0.56	0.91	0.95
DNP-DL-Ser	0.37	0.41	0.22	0.33	0.63
DNP-DL-Leu	0.80	0.81	0.55	0.33	0.77
DNP-L-Asp	0.27	0.31	0.17	0.25	0.61
DNP-DL-Met	0.59	0.72	0.37	0.21	0.69
DNP-Ala	0.58	0.63	0.35	0.34	0.70
bis-DNP-His	0.60	0.12	Tailing	Tailing	0.79
DNP-DL-Val	0.80	0.74	0.56	0.36	0.76
bis-DNP-Lys	0.29	0.69	0.18	Tailing	0.44
DNP-Thr	0.50	0.51	0.32	0.34	0.70
DNP-Isoleu	0.85	0.82	0.57	0.28	0.77

A, *n*-Butanol-acetic acid (9 : 1); B, 2-butanone-chloroform-formic acid (3 : 7 : 1); C, ethanol-water (63 : 37); D, dimethylformamide-acetic acid-water-ethanol (5 : 10 : 30 : 20); E, methanol-formic acid (9 : 1).

DNP amino-acids. Five solvent systems: solvent A, *n*-butanol-acetic acid (9 : 1); B, 2-butanone-chloroform-formic acid (3 : 7 : 1); C, ethanol-water (63 : 37); D, dimethylformamide-acetic acid-water-ethanol (5 : 10 : 30 : 20); E, methanol-formic acid (9 : 1), were excellent for one-dimension chromatography. The R_F values and the time required for plate development are shown in Table 1, and Fig. 1a shows a typical ultra-violet contact photograph of these chromatograms.

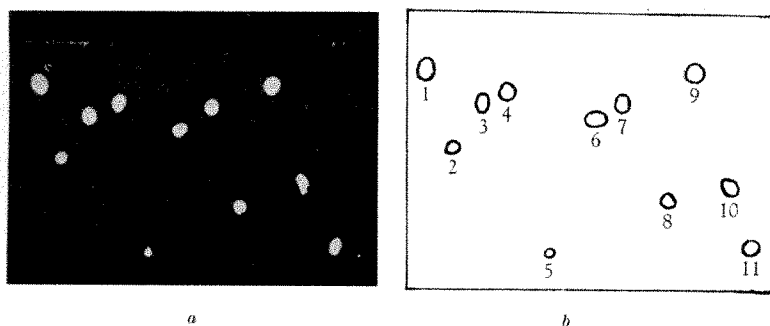


Fig. 1. Plate: 15 cm \times 15 cm; solvent system: 2-butanone- CHCl_3 - HCOOH (3 : 7 : 1) 13 cm. Key: 1, DNP-Isoleu; 2, DNP-Thr; 3, bis-DNP-Lys; 4, DNP-DL-Val; 5, bis-DNP-His; 6, DNP-Ala; 7, DNP-DL-Met; 8, DNP-H-Asp; 9, DNP-DL-Leu; 10, DNP-DL-Ser; 11, ϵ -DNP-Lys-HCl

In both solvent systems C and D, the times required for development are faster than solvent system A, but some of the DNP amino-acids give tailing spots. Solvent B exhibits a completely different tendency in R_F values. The similar effect of chlorinated hydrocarbon was also observed in phenols⁴. The solvent system D is the same as that used by Grassmann *et al.*³ for column chromatography.

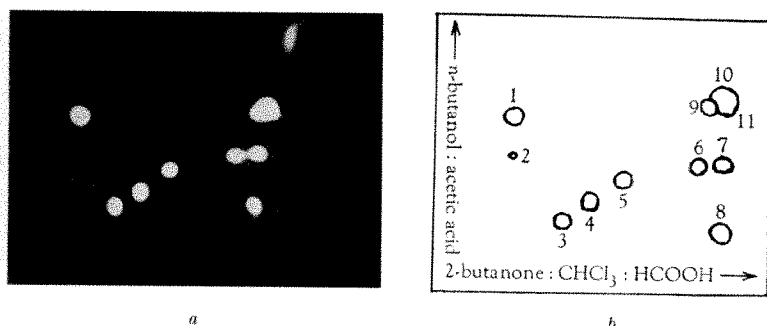


Fig. 2. Plate: 15 cm \times 15 cm; solvent system: first, BuOH-AcOH (9 : 1) 12 cm; second, 2-butanone- CHCl_3 - HCOOH (3 : 7 : 1) 13 cm. Key: 1, ϵ -DNP-Lys-HCl; 2, bis-DNP-His; 3, DNP-H-Asp; 4, DNP-DL-Ser; 5, DNP-Thr; 6, DNP-Ala; 7, DNP-DL-Met; 8, bis-DNP-Lys; 9, DNP-DL-Val; 10, DNP-Isoleu; 11, DNP-DL-Leu

The combination of solvent systems A and B in two-dimensional development was also tried. This is shown in Fig. 2a and b. Leucine and isoleucine cannot be separated clearly by these systems because these two are too closely related in structure.

The polyamide layer gives less diffused spots and better separation than silica-gel thin-layer chromatography.

This durable polyamide layer with high resolution might be a promising method for the identification of DNP amino-acids.

KUNG-TSUNG WANG
JOYCE MEI-KUO HUANG

Department of Chemistry,
National Taiwan University,
Taipei, Taiwan.

¹ Walz, D., Fahmy, A. R., Pataki, G., Niederwieser, A., and Brenner, M. *Experientia*, **19**, 213 (1963).

² Steurle, H., and Hille, H., *Biochem. Z.*, **331**, 220 (1959).

³ Grassmann, W., Hermann, H., and Portatius, H. v., *Hoppe-Seyler's Physiol. Chem.*, **321**, 120 (1960).

⁴ Wang, K.-T., *J. Chinese Chem. Soc.*, **8**, 241 (1961).

Distribution of Plutonium in Serum Proteins following Intravenous Injection into Rats

PREVIOUS workers have shown that sera of various mammals are capable of binding plutonium. Hunts and Barron¹ prepared serum from dogs which had been intravenously injected with a hexavalent plutonium citrate complex. Electrophoresis of the serum in a Tiselius type of apparatus showed that the β -globulin fraction combines with the plutonium. Beliaev², using paper electrophoretic methods, showed that plutonium is bound principally to the globulin fractions. The serum used in this latter piece of work was obtained from rats 24 h after intraperitoneal injection of a tetravalent plutonium citrate complex. The results obtained by Beliaev were not very reproducible, due possibly to buffer salt interactions and the influence of the electric field. Accordingly we have measured the distribution of intravenously injected tetravalent plutonium in the blood serum of the rat. The results presented here show that in blood obtained from the rat within 0.5 h of injection, the plutonium is bound to the transferrin proteins.

The serum used for this work was prepared by Dr. D. M. Taylor of the Institute of Cancer Research, Sutton, Surrey. The plutonium stock was $\text{Pu}(\text{NO}_3)_4$ in nitric acid. Immediately before injection of about 0.1 $\mu\text{c.}$, the sample was diluted with water. The elapsed time between dilution of the sample and its injection into the rat was short. Consequently the principal plutonium species injected into the rat was the tetravalent one. The rat was killed within 30 min of the injection. The blood was allowed to clot at room temperature for 2 h, stood overnight at 4°C, and then centrifuged. The supernate was stored at -20°C until required.

The blood proteins were separated into three fractions by the relatively mild method of gel-filtration, and further resolved by ion exchange as described by Gelotte, Flodin and Killander³. The distribution of the serum proteins in the eluates was determined spectrophotometrically at 254 m μ . The relative plutonium content of protein samples was determined by counting the delayed neutron emission following neutron irradiation. Delayed neutron emission induced by thermal neutron irradiation of fissile nuclides is a highly specific property, and has been used by Amiel⁴ as an analytical method for the determination of uranium-235. The plutonium supplied by the Radiochemical Centre, Amersham, is free from other fissile elements, hence Amiel's method was used as a simple and rapid method of analysis.

Plutonium was found to be present in the albumin/transferrin fraction only, following protein resolution by

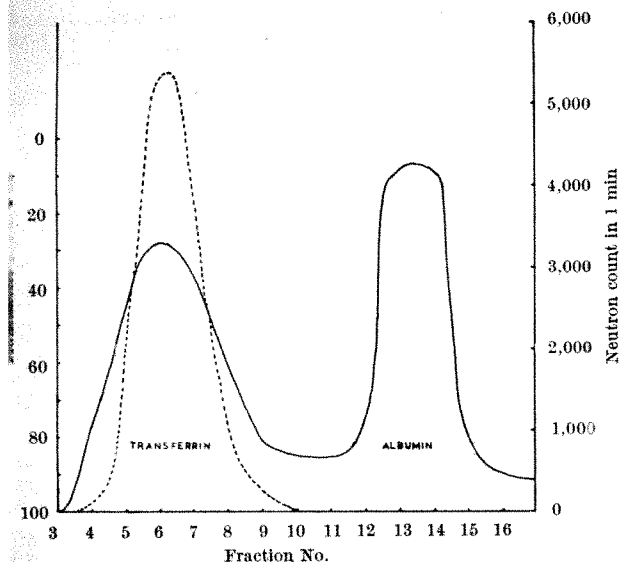


Fig. 1. Chromatography of the transferrin/albumin fraction on a column of DEAE-Sephadex A-50¹. Elution was started with 0.1 M 2-amino-2-hydroxymethylpropane 1:3 diol (*tris*) and its hydrochloride in 0.1 M sodium chloride, pH 8.0, followed by a gradient of 0.1 M–2.0 M NaCl in 0.1 M *tris*-HCl. The fraction volumes were about 3.5 ml. —, Transmission; ----, radioactivity

Sephadex G-200² gel-filtration. Resolution of the albumin/transferrin fraction into its components by DEAE-Sephadex A-50¹ and subsequent delayed neutron counting showed plutonium to be present in the transferrin fraction only as depicted in Fig. 1. Additionally the protein fractions were characterized by electrophoresis on cellulose acetate strips, using veronal buffer at pH 8.6 and staining with Ponceau S (ref. 5). Autoradiography of the unstained electrophoresis strips demonstrated that the plutonium migrates at the same rate as the transferrin.

G. BOOCOCK
D. S. POPPLEWELL

Atomic Weapons Research Establishment,
Aldermaston, Berkshire.

Hunts, J. A., and Barron, E. S. G., *U.S. Atomic Energy Comm. Rep. M.D.D.C. 1268*, Argonne National Laboratory (1947).

¹ Bellayev, Yu. A., *Meditsinskaya Radiologiya*, **4**, 45 (1959); in English, *U.S. Atomic Energy Comm. Rep. AEC-tr-3878*, Argonne National Laboratory (1959).

² Gelotte, B., Flodin, P., and Killander, J., *Arch. Biochem. Biophys.*, **Supp.** **1**, 319 (1962).

³ Amiel, S., *Anal. Chem.*, **34**, 1683 (1962).

⁴ Smith, I., in *Chromatographic and Electrophoretic Techniques*, 56 (William Heinemann Medical Books, Ltd., London, 1960).

Far-infra-red Spectra of some Square-planar Ions

THERE are three different assignments of the infra-red-active modes of K_2PtCl_4 in the literature¹⁻³. One was based on a low-resolution spectrum¹ and is thus misleading. The other two authors differed in their assignment of ν_2 and ν_7 , but their data are in good agreement except that Mathieu *et al.*³ reported a band at 160 cm⁻¹ which was not found by either Sacconi *et al.*² or by ourselves. We have observed the spectrum of K_2PtCl_4 to 40 cm⁻¹ and support Sacconi's results and assignment for the following reasons.

(1) The site symmetry of $[PtCl_4]^{2-}$ in the potassium salt is the same as that of the free ion. It is thus difficult to account for the triplet nature attributed to ν_7 by Mathieu *et al.* (2) The band near 110 cm⁻¹ assigned by Mathieu *et al.* to ν_2 is absent in the spectrum of Magnus's green salt, and can be assigned to a translational lattice mode of the potassium ions, three of which should be infra-red-active. We find additional lattice modes at 87.5 and 59 cm⁻¹, which may also be associated with the potassium ions.

We have made the first observations of ν_2 and ν_7 for K_2PdX_4 ($X = Cl$ and Br), of ν_6 for $KAuI_4$, and of the

complete far-infra-red spectra of $[M(NH_3)_4] [M'Cl_4]$ [$M, M' = Pd, Pt$]. Our assignments for the complex ions in these compounds are supported by the band width considerations: those bands assigned to a_{2u} modes are sharp and narrow, while those assigned to e_u modes are at least twice as broad. A similar band width effect is seen in $\nu(CO)$ bands for complexes of the type $M(CO)_2$ (π -arene).

Table 1. INFRA-RED-ACTIVE FREQUENCIES (CM⁻¹) OF SOME SQUARE-PLANAR IONS

	$\nu_2(a_{2u})$	$\nu_6(e_u)$	$\nu_7(e_u)$	Other bands
K_2PtCl_4	174.5	326	194	108.5, 87.5, 59
K_2PdCl_4	168	332	190.5	111, 90
K_2PdBr_4	106	254	136	80.5
$[Pt(NH_3)_4]^{2+}$	141.5	—	234	266, 82.5, 74
$[PtCl_4]^{2-}$	175	310	198	—
$[Pd(NH_3)_4]^{2+}$	144	—	226	263, 93, 80.5
$[PtCl_4]^{2-}$	174	321	187.5	—
$[Pt(NH_3)_4]^{2+}$	136.5	—	245	364, 288, 93, 83
$[PdCl_4]^{2-}$	163.5	332.5	224	—
$KAuI_4$	—	190.5	—	—

All the ions in the complexes $[M(NH_3)_4] [M'Cl_4]$ have site symmetry C_4 . There should not therefore be any splitting of existing degeneracies, but the Raman-active ν_1 in the free ions should become infra-red-active. We have not observed it, but we do find a weak-medium band at 266 cm⁻¹ ($M = M' = Pt$), 263 cm⁻¹ ($M = Pd, M' = Pt$) or 288 cm⁻¹ ($M = Pt, M' = Pd$). This is very low for ν_1 for any of the complex ions and we tentatively assign it to a combination of ν_7 of the ammine complex ions with a lattice mode. There is no evidence that the metal-metal interaction in Magnus's green salt has any significant effect on the infra-red spectra of the complex ions, as compared with K_2PtCl_4 and $[Pt(NH_3)_4]Cl_2$. In particular, the a_{2u} out-of-plane deformation bands of $[PtCl_4]^{2-}$ are constant in both frequency and intensity.

D. M. ADAMS
D. M. MORRIS

Department of Chemistry,
University of Leicester.

Adams, D. M., and Gebbie, E. A., *Spectrochim. Acta*, **19**, 925 (1963).

² Sabatini, A., Sacconi, L., and Schettino, V., *Inorg. Chem.*, **3**, 1775 (1964).

³ Poulet, H., Delorme, P., and Mathieu, J. P., *Spectrochim. Acta*, **20**, 1855 (1964).

Methane-rich Perchloric Acid Flames

THE study of flames supported by the vapour of 72 per cent perchloric acid^{1,2} has been continued by an investigation into methane-rich premixed flames of perchloric acid. Perchloric acid flames have already been shown to have no effective lower flammability limit since a decomposition flame of the acid vapour alone can be stabilized². It has now been found that mixtures containing up to at least 84 per cent methane in perchloric acid are flammable; this corresponds to 20 times as much methane as in a stoichiometric mixture, whereas methane-oxygen mixtures are only flammable up to 61 per cent methane³, corresponding to 3.1 times as much methane as in a stoichiometric mixture.

The methane-perchloric acid flames were stabilized on cylindrical 'Pyrex' burners of internal diameter of 1.13–25.4 mm surrounded by a 65 mm jacket tube so that the flames were burning in an atmosphere of argon. The apparatus was preheated to 210° C by nichrome heating wire in order to maintain the perchloric acid in the vapour phase. All the flames were at atmospheric pressure.

Burning velocities, temperatures and burnt gas compositions were measured for flames containing 2–18 times as much methane as is required for a stoichiometric mixture. No carbon formation, as revealed by luminous yellow streaks or zones, was found with any of these flames. Burnt gas concentrations for methane, hydrogen, carbon monoxide, carbon dioxide and water in molecules per 100 molecules of initial methane are presented as a function of the mixture ratio, λ , in Fig. 1 (the diluent

water is not included). λ is defined as the fuel/oxidizer ratio in the mixture divided by the fuel/oxidizer ratio in a stoichiometric mixture. The other major product was hydrogen chloride and minor products were methyl chloride, chlorine and C_2 hydrocarbons.

The large amount of methane in the burnt gas is remarkable. It is possible to calculate an effective mixture ratio, ϕ , where ϕ is the fuel reacted/oxidizer ratio in the mixture divided by the fuel/oxidizer ratio in a stoichiometric mixture. The value of ϕ so calculated is 2 for any value of $\lambda > 2$. In other words, for flames containing more than twice the methane required for a stoichiometric mixture, the additional methane does not react and acts solely as a diluent. The flame temperatures measured with 0.002 in. Pt/13 per cent Rh-Pt thermocouples were compared with theoretical equilibrium temperatures calculated by (a) assuming that all the methane reacts, and by (b) assuming that only two moles react (that is, $\lambda = 2$) and that the rest of the methane acts solely as a diluent. The experimental temperatures confirm that methane-rich perchloric acid flames are in fact $\lambda = 2$ flames diluted with methane.

Carbon formation would not be likely in a $\lambda = 2$ flame (O/C ratio 2.0) and it is suggested that it does not occur in the richer flames because the flame temperature is insufficient. Addition of oxygen to methane-rich perchloric acid flames raises the flame temperature and carbon formation ensues. This observation supports the idea that carbon formation is not observed in the methane-acid flames because the flame temperature is too low.

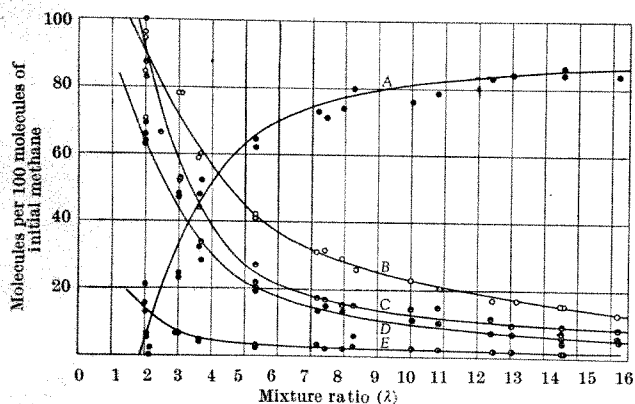
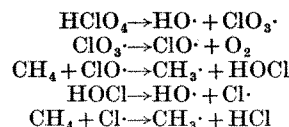


Fig. 1. A, Methane; B, water; C, hydrogen; D, carbon monoxide; E, carbon dioxide

The finding that only two molecules of methane react per molecule of perchloric acid is interesting. It cannot be directly compared to the situation with methane-rich oxygen flames since there is a paucity of sampling data. The only comprehensive study is that made by Townend in 1927 into the explosion of methane-oxygen mixtures at pressures of 6–150 atmospheres⁴. If it is assumed that the carbon produced in his experiments came from the pyrolysis of unreacted methane during the cooling of the post-flame gases, it is possible to calculate an effective mixture ratio, ϕ , in the same way as has been done for perchloric acid flames. For mixtures with $\lambda = 4$ and 5 the value of ϕ is close to 2. Further discussion is unwarranted since it is not certain that carbon is formed in this way. It would be interesting to sample premixed methane-rich oxygen flames under increased pressure to determine whether methane-oxygen flames are also essentially $\lambda = 2$ flames.

A mechanism can be proposed for the basic steps in a methane-perchloric flame. These must be different from those in a methane-oxygen flame since: (a) methane-perchloric acid flames have burning velocities about three times faster than the corresponding methane-oxygen flame⁵; (b) addition of oxygen to methane-rich perchloric

acid flames results in the production of a second flame zone downstream of the acid zone¹. Consequently, it is suggested that initial attack of the methane is by reaction with chlorine-oxygen radical or a chlorine atom.



While ClO has been written as the chlorine-oxygen radical, since it has been suggested that it will be an efficient chain carrier⁶, other chlorine-oxygen species could equally well be written in its place.

I thank Dr. A. R. Hall for his advice.

G. S. PEARSON

Ministry of Aviation,
Rocket Propulsion Establishment,
Westcott, Aylesbury, Bucks.

¹ Cummings, G. A. McD., and Hall, A. R., *Tenth Intern. Symp. Combustion* 1365 (The Combustion Institute, 1965).

² Cummings, G. A. McD., and Pearson, G. S., *Combustion Flame*, 8, No. 199 (1964).

³ Lewis, B., and von Elbe, G., *Combustion, Flames and Explosions of Gases* (Academic Press, 1960).

⁴ Townend, D. T. A., *Proc. Roy. Soc., A*, 116, 637 (1927).

⁵ Edgecombe, F. H. C., Norrish, R. G. W., and Thrush, B. A., *Chem. Soc. Special Pub.*, 9, 121 (1957).

BIOCHEMISTRY

Polymorphism of Lactate Dehydrogenase Isozymes in Pigeons

RECOGNITION of the molecular heterogeneity of enzyme in single organisms has offered a new dimension for studying the genetic control of enzyme synthesis. In the case of lactate dehydrogenase (LDH) five distinct type (isozymes) can be identified in most mammalian and avian tissues by the method of starch-gel electrophoresis¹⁻³. Each isozyme is a tetramer formed by the association of two monomers, A and B (ref. 4), so that the polypeptide composition of LDH-1 through LDH-5 respectively may be written as follows: A_4B_0 , A_3B_1 , A_2B_2 , A_1B_3 and A_0B_4 . Genetic investigations⁵⁻⁷ have shown that the synthesis of the A and B sub-units is under the control of two different structural genes, a and b. Hence the isozyme composition of each tissue depends on the relative activity of the genes at these loci.

The finding of unusual isozymes, 'band X' isozymes, in testes from sexually matured animals provided new information on the genetic control of LDH synthesis. The 'band X' isozymes are present only in mature testes, their appearance coincides with initiation of spermatogenesis, and they comprise the predominant isozymic forms of LDH in mature sperm⁸⁻¹⁰. These observations suggested that a third genetic locus was responsible for the synthesis of yet another distinct kind of LDH sub-unit, a C-polypeptide, in mature testis. Genetic evidence for the existence of this locus was the discovery of two allelic forms of the c gene in wild pigeons¹¹.

Therefore, at least three structural genes are involved in LDH synthesis. In the pigeon, assuming homozygosity for the a and b genes, three genotypes are possible: $\frac{abc}{abc}$, $\frac{abc'}{abc}$, and $\frac{abc'}{abc'}$. We now present evidence for allelism at the b locus, and thus have identified the genotypes that will be required for a future analysis of linkage relationships among the genes controlling LDH synthesis.

Racing homer pigeons, wild park pigeons, 'White Carneau', 'White King', and 'Silver King' pigeons were used in this investigation. Most of the racing homer and pure-bred pigeons were males, whereas the wild population comprised approximately equal numbers of males and females. Methods for preparation and electrophoresis of tissue homogenates and haemolysates, localization of

Table 1. DISTRIBUTION OF CLASSES I, II, AND III IN THREE DIFFERENT PIGEON POPULATIONS

Population	No. of pigeons in each class			Allele frequencies	
				L^B	$L^{B'}$
d ring homer white King white Carneaux ver King	Obs. 864	56	1	0.97	0.03
	Exp. 863	57	1		
	207	0	0	1.0	0
	215	0	0	1.0	0

LDH isozymes in starch gel, dissociation and recombination of LDH polypeptide sub-units, and determination of total LDH activity were similar to those previously described¹⁰.

A survey of the isozyme composition of tissues from proximately 1,000 wild pigeons showed that in terms of its isozyme phenotype each bird could be placed into one of three major classes which arbitrarily have been designated I, II and III. Examples of each class of pattern for breast muscle are shown in Fig. 1. The class I pattern exhibits five areas of enzyme activity, LDH-5, -4, -3, -2 and -1, LDH-5, -4 and -3 being the darkest bands. The staining of LDH-1, -2 and -3 in the class II pattern of breast muscle markedly diminished. Only three areas of activity are present in the class III pattern. The darkest band has the same electrophoretic mobility as LDH-5, and the other two bands migrate more slowly than LDH-4 and LDH-3.

Heart zymograms of the same pigeons from which the breast muscle patterns were obtained are also presented in Fig. 1. The class I pattern exhibits most of its activity

in the LDH-1 area, and less in LDH-2. Two minor bands can be seen on the anodal side of LDH-1. Five isozymes are present in the class II pattern, and the distribution of activity among the isozymes appears to be in the proportions of 1:4:6:4:1. The pattern of the class III heart, except for intensity of isozyme staining, is identical to that of class III breast muscle. Several other tissues from each pigeon were examined. In general, the differences in patterns of tissues with predominantly B polypeptides, for example,izzard and kidney, paralleled the changes exhibited by heart. The characteristics of zymograms for tissues having mostly A polypeptides or equal amounts of each polypeptide were similar to those described for breast muscle.

In most mammalian and avian species adult heart muscle is rich in LDH-1 and LDH-2. Thus the presence of a major isozyme in the class III heart with an electrophoretic mobility identical to that of LDH-5 led to an investigation of some of its other physicochemical properties. When tissue homogenates from pigeons with class I patterns were heated at 70° C for 15 min, isozymes with two or more A polypeptides, that is, LDH-3, -4 and -5, were inactivated. Inactivation of the B polypeptides did not occur until homogenates were heated at 80° C for 15 min. The heat inactivation properties of the isozymes in homogenates of the class II and III heart were identical to those observed for the class I hearts. Inhibition investigations with para-amino-salicylic acid showed that concentrations of inhibitor which decreased LDH-5 activity in class I tissues did not alter the activity of the isozymes in homogenates of class I, II or III hearts.

These experimental results suggested that the major isozyme of the class III heart does not contain A polypeptides, but that it represents polymerization of a B variant, B', the electrophoretic mobility of which is identical to that of the A polypeptide. Likewise the class II heart pattern can be explained by assuming that equal amounts of the normal and variant B polypeptides are being synthesized. If the polypeptide compositions of the major isozymes in the class I and III heart patterns are designated B₁ and B', respectively, then the isozymic complement of class II heart would be as follows: B₁B', B₁B'₁, B₁B'₂, B₁B'₃, B₁B'₄. In order to determine if the B and B' polypeptides could combine to form functional enzyme units, a mixture of equal amounts of class I and III heart homogenates was treated with 0.5 M sodium chloride and 0.1 M phosphate and frozen for 24 h prior to electrophoresis¹². As shown in Fig. 1, dissociation and re-association of the polypeptides resulted in the appearance of two new isozymes, and the pattern resembled the zymogram of a class II heart.

The nature of the two minor bands on the anodal side of the major isozyme in class III tissues is unknown. Since the electrophoretic mobilities of the A and B' polypeptides are identical, then only one isozyme should be visible in the class III patterns. Two minor bands are also present on the anodal side of LDH-1 in the pattern of class I heart. Perhaps these minor components are related in some way to the synthesis of the B and B' polypeptides.

Evidence for the genetic control of the three classes of isozyme patterns was obtained by measuring their relative frequency in different populations of pigeons. The data listed in Table 1 show that the distribution of the three classes in the wild-type population agrees with that expected according to the Hardy-Weinberg law for a single pair of alleles. All the racing homer and pure-bred pigeons exhibited class I patterns.

A conclusive demonstration of the mode of inheritance of the different class patterns was accomplished by carrying out appropriate breeding experiments. Adult wild pigeons were classified by electrophoresing haemolysates of blood obtained from a wing vein, and only class II pigeons were selected for the breeding colony. Twenty-

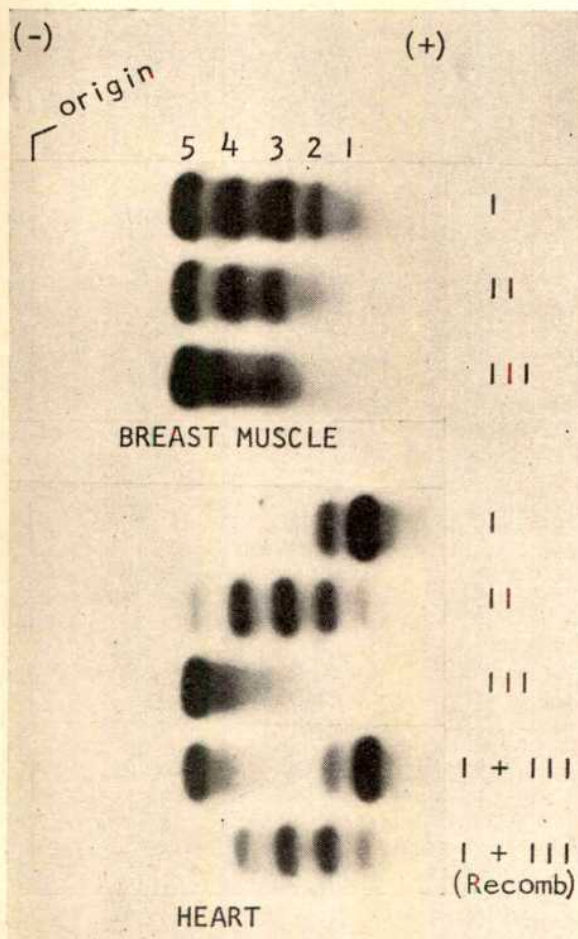


Fig. 1. Lactate dehydrogenase isozymes in breast muscle and heart from classes I, II and III pigeons. The homogenates were electrophoresed simultaneously in the same starch gel, and the conditions for identifying LDH were identical. The patterns of a mixture of equal parts of class I and class III hearts before and after treatment with sodium chloride and phosphate and freezing are designated I + III and I + III (Recomb) respectively.

one offspring have been obtained from 11 matings, and the ratio of phenotypes (class I—*BB*: class II—*BB'*: class III—*B'B'*) is 4:11:6.

These findings unequivocally show that there is allelism at the *b* locus in wild pigeons. The biological significance of the three classes of patterns is unknown. The *B'* polypeptide has never been observed in tissues from racing homer or pure-bred pigeons, and only one of the 921 trapped wild pigeons was *B'B'*. This pigeon appeared healthy, and the total LDH activities of heart, thigh muscle, breast muscle and testis were normal. The successful production of *B'B'* pigeons in the breeding colony will facilitate more extensive studies on the selective advantage or disadvantage of the *B'* variant.

This work was aided by grant HD 00994-01 from the National Institutes of Health. We thank Dr. C. L. Markert for his advice.

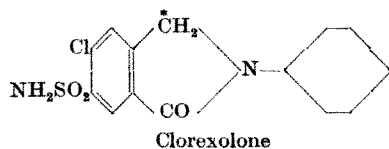
WM. H. ZINKHAM
L. KUPCHYK
A. BLANCO
H. ISENSEE

Department of Pediatrics,
Johns Hopkins Hospital,
Baltimore 5, Maryland.

- ¹ Markert, C. L., and Moller, F., *Proc. U.S. Nat. Acad. Sci.*, **6**, 753 (1959).
- ² Plagemann, P. G., Gregory, K. F., and Wroblewski, F., *J. Biol. Chem.*, **235**, 2288 (1960).
- ³ Cahn, R. D., Kaplan, N. O., Levine, L., and Zwilling, E., *Science*, **136**, 962 (1962).
- ⁴ Markert, C. L., in *Hereditary, Developmental, and Immunologic Aspects of Kidney Disease*, edit. by Metcalf, J., 54 (Northwestern Univ. Press, Evanston, Ill., 1962).
- ⁵ Shaw, C. R., and Barto, E., *Proc. U.S. Nat. Acad. Sci.*, **50**, 211 (1963).
- ⁶ Kraus, A. P., and Neely, jun., C. L., *Science*, **145**, 595 (1964).
- ⁷ Vesell, E. S., *Science*, **148**, 1103 (1965).
- ⁸ Blanco, A., and Zinkham, W. H., *Science*, **139**, 601 (1963).
- ⁹ Goldberg, E., *Science*, **139**, 602 (1963).
- ¹⁰ Zinkham, W. H., Blanco, A., and Clowry, jun., L. J., *Ann. N.Y. Acad. Sci.*, **121**, 571 (1964).
- ¹¹ Zinkham, W. H., Blanco, A., and Kupchik, L., *Science*, **144**, 1353 (1964).
- ¹² Markert, C. L., *Science*, **140**, 1329 (1963).

Metabolism of 5-Chloro-2-cyclohexyl-1-oxo-6-sulphamoyl Isoindoline, Clorexolone (M and B 8430)

CLOREXOLONE (M and B 8430) is a powerful oral diuretic¹ with pronounced antihypertensive properties even when administered in sub-diuretic doses². It has certain structural features in common with chlorothiazide and with chlorthalidone, but is unique in containing the cyclohexane-isoindoline structure.



Although the metabolism in animals of cyclohexane and its simple derivatives, for example, methylcyclohexanes, hexanols and hexanones, has been studied in detail by Williams *et al.*³⁻⁵, there is little information about more complex derivatives. We have, therefore, examined the fate of clorexolone in experimental animals, and to a lesser degree in man, with two objects in mind. We wished, first, to establish that the compound was not stored in the body, and, secondly, we required information about the principal metabolic pathways operating in man and in the most closely related—in this biochemical context—animal species.

Preliminary investigations showed that colorimetric and spectrophotometric methods of estimating clorexolone and its congeners were insufficiently sensitive to permit quantitative experiments to be carried out with doses that approached those used therapeutically. They were, however, adequate to permit the conclusion that, in

contradistinction to the thiazide diuretics, insignificant amounts of the parent compound were to be found unchanged in, for example, rat urine. We were also able to demonstrate, by thin-layer chromatography and infrared investigations, that the principal metabolite appeared to be monohydroxylated, possibly in the cyclohexane nucleus. It was clearly undesirable, however, to rely solely on techniques which depended on excessive dosages—absorption, excretion and metabolic patterns may be atypical in such circumstances—and most of our preliminary work was therefore carried out with clorexolone labelled with carbon-14 in the isoindoline methylene group (see formula).

It will be seen from the tabulated results of the three experiments, in rats, one beagle dog and one whippet that there is no significant accumulation of carbon after periods ranging from 48 to 72 h.

Recovery of ¹⁴C-labelled clorexolone after administration to two Wistar rats. Two Wistar rats, each weighing 220 g received an oral dose of ¹⁴C-labelled clorexolone (0 mg/kg) which was six times the dose necessary to produce a three-fold increase in sodium excretion. Each animal received 0.38 µc. of carbon-14

Table 1. PERCENTAGE RECOVERY OF ¹⁴C-LABELLED DRUG

Sample	Time (h)			
	0-24	24-48	48-72	0-72
Urine	40.1	5.5	0.2	45.8
Faeces	31.0	18.2	0.0	49.2
Total	71.1	23.7	0.2	95.0

Recovery of ¹⁴C-labelled clorexolone after administration to a beagle dog. A beagle weighing 12.5 kg received an oral dose of ¹⁴C-labelled clorexolone (0.34 mg/kg), which was approximately three times the dose necessary to produce a three-fold increase in sodium excretion. The total amount of radioactive compound administered was equivalent to 5.0 µc. of carbon-14.

Table 2. PERCENTAGE RECOVERY OF ¹⁴C-LABELLED DRUG

Sample	Time (h)			
	0-18	18-24	24-42	42-72
Urine	58.5	5.0	0.0	63.5
Faeces	—*	—*	27.5	27.7
Total	58.5	5.0	27.5	91.2

* The animal failed to produce any faeces in the first 24 h.

Recovery of ¹⁴C-labelled clorexolone after administration to a whippet. A whippet weighing 5.7 kg received an oral dose of ¹⁴C-labelled clorexolone (0.83 mg/kg), which was approximately eight times the dose necessary to produce a three-fold increase in sodium excretion. The total amount of radioactive compound administered was equivalent to 12.7 µc. of carbon-14.

Table 3. PERCENTAGE RECOVERY OF ¹⁴C-LABELLED DRUG

Sample	Time (h)			
	0-24	24-48	48-72	0-72
Urine	95.4	3.2	0.0	98.6
Faeces	—*	4.0	2.2	6.2
Expired CO ₂	0.0	0.0	0.0	0.0
Total	95.4	7.2	2.2	104.8

* The animal failed to produce any faeces in the first 24 h.

In the experiments involving the rats and the beagle the carbon-14 content of the samples was determined by means of an end-window Geiger counter, whereas in the experiment with the whippet a liquid scintillation technique was used.

We wished to confirm that the main metabolic change produced in the molecule involved the introduction of a single hydroxyl group and that the pattern of metabolism in the dog conformed closely to that operating in man. Preliminary investigations with the urine of volunteer had suggested that there was a great similarity between metabolic routes in man and dog.

Early experiments in which dogs received very large doses of non-radioactive clorexolone suggested the presence of three metabolites in the urine. After separation on silica-gel chromatoplates, using acetone as the developing solvent, these three compounds were visible as fluorescent spots when viewed in ultra-violet light or

7 μ g. Examination of the urine from the whippet which had received the radioactive compound, in a dose approximating to the therapeutic dose, confirmed that a whole of the administered carbon-14 was excreted in the form of these three metabolites, which will hereafter be referred to as compounds A_1 , A_2 and B . These experiments also established that there were no significant differences in the metabolic patterns of large or small doses.

Having established this fact, it was then possible to administer daily doses of unlabelled clorexolone at 1 g/kg a dog for 3 weeks so that enough urine could be collected to permit the isolation of these metabolites. Urine was collected daily and the three compounds were separated by extraction with ethyl acetate, thin-layer chromatography on silica and fractional crystallization.

Direct comparison, by chromatography and by infrared and mass spectrometry, of metabolite A_2 with an authentic specimen of 5-chloro-2-(3'-hydroxy-cyclohexyl)-1-oxo-6-sulphamoyl isindoline showed that the two compounds were identical. Complete identification of the major metabolite, B , has not yet been established. Infrared and nuclear magnetic resonance examinations suggest that it contains an equatorial hydroxyl group. The position of this group will be restricted to the 2' or 3' position, since A_2 is the equatorial isomer of the 3' position, and the 1' position has been eliminated by the nuclear magnetic resonance investigations. The chromatographic and infrared characteristics of metabolite A_1 suggest that it also is monohydroxylated in the cyclohexyl nucleus.

Three metabolites which had chromatographic and infrared characteristics identical with those obtained from dog urine have been similarly isolated from human urine. Thirty litres of urine were collected over a 2-day period from a patient who was receiving 50 mg of clorexolone orally each day. Metabolite B was obtained crystalline, but the other two compounds were more difficult to purify and their identification depended on physical characteristics in solution.

It is evident that neither clorexolone nor its metabolites are stored in body tissues and that, although the compound itself is not excreted unchanged, metabolism and elimination occur solely and completely by way of oxidative monohydroxylations of the cyclohexane nucleus.

We thank Miss H. N. Grant for preparing the ^{14}C -labelled material, and Drs. R. I. Reed and H. C. Hill for the mass spectrometric examinations.

K. CORBETT
S. A. EDWARDS
G. E. LEE
T. L. THRELFALL

Research Laboratories,
May and Baker, Ltd.,
Dagenham,
Essex.

Cornish, E. J., Lee, G. E., and Wragg, W. R., *Nature*, **197**, 1296 (1963).

Simpson, F. O., *Curr. Ther. Res.*, **6**, 21 (1964).

Elliott, T. H., Parke, D. V., and Williams, R. T., *Biochem. J.*, **72**, 193 (1959).

Elliott, T. H., Tao, R. C. C., and Williams, R. T., *Biochem. J.*, **95**, 59 (1965).

Elliott, T. H., Tao, R. C. C., and Williams, R. T., *Biochem. J.*, **95**, 70 (1965).

Formation of Hydroxamates during Proteolysis by an Enzyme System from Yeast

DURING re-investigation of an enzyme system from yeast previously reported to form 'activated' peptides¹ extensive proteolysis was noted and enzyme-catalysed hydroxylaminolysis of protein was suspected as the source of hydroxamate formation². Acyl-enzyme complexes were visualized as intermediates rather than some form of 'activated' peptide originally suggested by Cooper *et al.*¹. The main evidence for the latter hypothesis had been the appearance, after paper electrophoresis of the products of the reaction in the absence of hydroxylamine,

of a band which gave a positive reaction to the hydroxylamine ferric chloride test. This test, however, may be misleading when conducted on paper³. The formation of hydroxamates from protein substrates by other proteolytic enzymes was also demonstrated² and is presumably analogous to the well known transamidation reactions recorded by many authors⁴⁻⁸. Such reactions have only been unequivocally demonstrated using simple substrates but have been invoked to explain the formation of plasteins^{9,10} and energy-independent incorporation of amino-acids into mitochondrial protein¹¹.

Some further characteristics of the yeast enzyme system are reported here. The crude yeast enzyme was prepared in essentially the manner described by Cooper *et al.*¹ and further purified by absorption on to a hydroxyapatite-cellulose column¹² in 0.01 M phosphate buffer, pH 6.8, and subsequent elution with 0.5 M buffer of the same pH. After extensive dialysis the protein was freeze dried. Enzyme reactions were carried out at 37° in 0.035 M Tris buffer, pH 7.5, in the presence of 'low salt' hydroxylamine¹³ (2.5–4.0 μ mole) in a final volume of 3.3 ml. Additions of amino-acids, etc., were made in the same final volume after the respective solutions had been adjusted to pH 7.5. Reactions were terminated by the addition of 21.3 per cent (w/v) trichloroacetic acid in 0.43 N hydrochloric acid (2 ml.). After centrifugation the extinction of the supernatant at 280 μ was observed. The supernatant (3.0 ml.) was also mixed with 6.6 per cent $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in 0.1 N hydrochloric acid (0.5 ml.) and the extinction at 540 μ observed after 5 min. Owing to the extreme sensitivity of the colour yield to minor changes in pH (ref. 14), the pH of the final mixture was observed and, before interpolating the hydroxamate concentration, the colour yield was corrected to pH 1.0, using graph of colour yield expressed as percentage of that at pH 1.0 plotted against pH. Glycine hydroxamate was used as a standard. Each determination was also corrected using a blank of identical composition to which trichloroacetic acid was added prior to addition of enzyme.

The formation of hydroxamates closely paralleled proteolysis in autolytic experiments (Fig. 1) and when additional protein substrate was present. Both activities are heat labile and reversibly inhibited by *p*-chloromercuribenzoic acid, thus resembling the more stable of the two enzymes obtained from yeast by Lenney¹⁵. Contrary to the earlier report¹, no evidence was found for any energy-dependent hydroxamate formation except

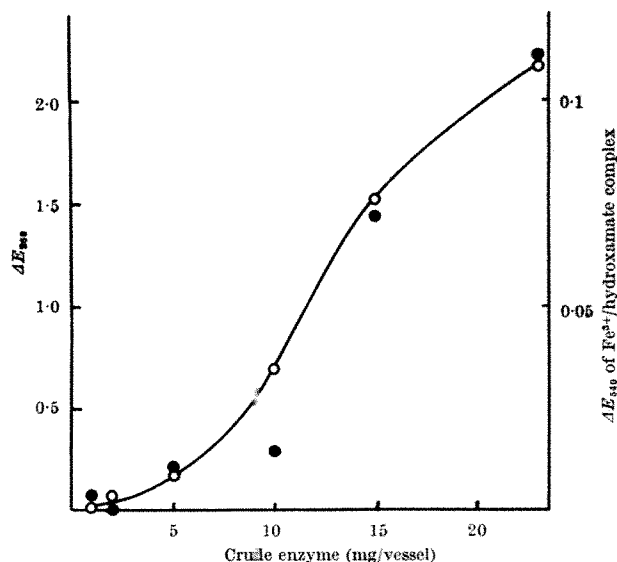


Fig. 1. Hydroxamate formation during autolysis of crude yeast enzyme in the presence of 2.7 mmole NH_2OH . Incubation time: 18 h. Increase in E_{280} μ (○); increase in E_{540} μ of Fe^{3+} /hydroxamate complex (●).

Table 1. EFFECT OF ATP ON HYDROXAMATE FORMATION DURING AUTOLYSIS OF YEAST ENZYMES

	Hydroxamate formed in 75 min (μ mole)	
Crude enzyme (15 mg), NH_4OH (3.7 mmole)	1.55	1.75
" " plus ATP (50 μ mole), MgCl_2 (33 μ mole)	1.24	1.43
" " plus ATP, MgCl_2 and amino-acid mixture*	1.15	1.24
" " plus amino-acid mixture only	1.00	0.85

* 8.3 μ mole glutamic acid and tyrosine and 20.6 μ mole methionine, phenylalanine and tryptophan in tris buffer, pH 7.5.

in the presence of amino-acids (Table 1). The highest yield of hydroxamate was, however, invariably observed in the absence of any additions. It is of interest to note that, in comparable experiments (Table 1), this system can produce up to 60 per cent of the hydroxamate observed in an apparently ATP-dependent system which was obtained by Schuur's *et al.*¹⁸ by a somewhat different procedure, from baker's yeast. The Dutch authors recognized that their system was proteolytic in nature and, most significantly, that added peptides could not be activated¹⁷.

It appears to be particularly difficult to interpret results based on the formation of hydroxamic acids when using crude enzyme systems which may contain proteases. This is especially true if trying to demonstrate peptide activation. Proof of such activation apparently requires a defined substrate and unequivocal identification of the reaction products¹⁸.

We thank Dr. A. H. Cook for his advice.

D. E. BRIGGS*
D. J. MILLIN

Brewing Industry Research Foundation,
Nutfield, Redhill,
Surrey.

* Present address: Department of Biochemistry, University of Birmingham.

¹ Cooper, A. H., Harris, G., Neal, G. E., and Wiseman, A., *Biochim. Biophys. Acta*, **68**, 68 (1963).

² Briggs, D. E., and Millin, D. J., *Biochem. J.*, **92**, 62P (1964).

³ Briggs, D. E., Millin, D. J., and Saltmarsh-Andrew, M. J., *Nature*, **204**, 286 (1964).

⁴ Fruton, J. S., *Yale J. Biol. Med.*, **22**, 263 (1950).

⁵ Johnston, R. B., Mycek, M. J., and Fruton, J. S., *J. Biol. Chem.*, **185**, 629 (1950).

⁶ Durrell, J., and Fruton, J. S., *J. Biol. Chem.*, **207**, 487 (1954).

⁷ Mettrione, R. M., and Johnston, R. B., *Biochemistry*, **3**, 482 (1964).

⁸ Epand, R. M., and Wilson, I. B., *J. Biol. Chem.*, **238**, 1718 (1963); **240**, 1104 (1965).

⁹ Horowitz, J., and Haurowitz, F., *Biochim. Biophys. Acta*, **33**, 231 (1959).

¹⁰ Determann, H., Bonhard, K., Köhler, R., and Wieland, T., *Helv. Chim. Acta*, **46**, 2498 (1963).

¹¹ Suttle, J. W., *Biochem. J.*, **84**, 382 (1962).

¹² Millin, D. J., and Smith, M. H., *Biochim. Biophys. Acta*, **62**, 450 (1962).

¹³ Meigh, D. F., in *Modern Methods of Plant Analysis*, edit. by Paech, K., and Tracey, M. V., **2**, 441 (Springer-Verlag, Berlin, 1955).

¹⁴ Raacke, I. D., *Biochim. Biophys. Acta*, **27**, 413 (1958).

¹⁵ Lenney, J. F., *J. Biol. Chem.*, **221**, 919 (1956).

¹⁶ Schuur's, A. H. W. M., de Kloet, S. R., and Koningsberger, V. V., *Biochem. Biophys. Res. Comm.*, **3**, 300 (1960).

¹⁷ Schuur's, A. H. W. M., thesis: Carboxyl-activated Peptides and Carboxyl Activation of Peptides in Yeast (State University, Utrecht, 1961).

¹⁸ Tuboi, S., and Huzino, A., *Arch. Biochem. Biophys.*, **86**, 309 (1960).

Hydrolysis of Two 17 α -Oestradiol Glycosides by Rabbit Tissue Glycosidases

It has recently been shown^{1,2} that the rabbit excretes 17 α -oestradiol as the 3-glucuronoside-17 α -N-acetylglucosaminide. To the best of our knowledge this is the first N-acetylglucosaminide of a non-sugar to be found in animals, although β -N-acetylglucosaminidase is widely distributed in animal tissues³. It was the object of the work recorded here to determine whether the steroid N-acetylglucosaminide was effectively hydrolysed by the tissue enzyme in rabbits, and whether the enzyme distribution, when measured with this naturally occurring substrate, was the same as that found by previous investigators^{3,4} who used synthetic substrates. We also wished to compare the relative ability of different tissues to remove the glucuronic acid and the N-acetylglucosamine from the oestradiol molecule.

Four rabbits were injected subcutaneously with 50 μ g day of oestrone benzoate in sesame oil. On the second an intravenous injection of 50 μ c. of 17 β -oestradiol-6,7 (sp. act. 150 μ c./ μ g) was also given. Oestradiol glucuronoside-17 α -N-acetylglucosaminide was isolated from the urine as previously described². A sample of di-glycoside was incubated in 0.5 M acetate buffer pH 5.0 with β -glucuronidase ('Ketodase', Warner-Cott). Oestradiol-17 α -N-acetylglucosaminide was extracted from the buffer with ethyl acetate and purified by countercurrent distribution¹. After crystallization from aqueous methanol, the product had a specific activity of 156 μ c./mmole. Another sample of the glycoside was incubated in 0.5 M citrate buffer at pH 4.2 with almond emulsin in the presence of 10 mg/ml. saccharo-1,4-lactone. The buffer was extracted exhaustively with ethyl acetate and the extract distributed through 99 countercurrent transfers in ethyl acetate-butanol-water, 3:1:4. A single radioactive product with a *K* value of 5.2 was located. This material was dissolved in water and, on the addition of a few drops of chloroform, yielded a semi-crystalline product of specific activity 167 μ c./mmole. The infra-red spectrum of this compound confirmed its structure as an oestrogen glucuronoside, a treatment of the material with β -glucuronidase yielded 17 α -oestradiol.

Oestradiol-17 α -N-acetylglucosaminide has an extremely low partition coefficient between chloroform and water. That of the 3-glucuronoside is even lower. A convenient method of measuring the hydrolysis of these glycosides by tissue enzymes was therefore to extract the released steroid from the incubation medium and assay the radioactivity in the extract by liquid scintillation spectrometry. In our hands this method has given accurate and reproducible results, and chromatography of the extract has shown that the chloroform-soluble radioactive material liberated from both glycosides by tissue enzymes is unchanged 17 α -oestradiol.

Organs were dissected from rabbits immediately after killing. Aliquots of the tissue were homogenized in acetate buffer at pH 4.5 and in citrate buffer at pH 4.2. A 10 per cent w/v ratio of tissue to buffer was used in each case. Aliquots of homogenate were diluted to 10 ml. with the appropriate buffer, and the radioactive glycosides (about 100 μ g/g tissue) were added in 50 μ l of ethanol. β -Glucuronidase activity was assayed in acetate buffer using oestradiol-3-glucuronoside as substrate, and β -N-acetylglucosaminidase was assayed using oestradiol-17 α -N-acetylglucosaminide in citrate buffer⁵.

At buffer concentrations of 0.5 M, the β -glucuronidase of both liver and kidney displayed the two pH optima at 4.5 and 5.2, observed by previous workers⁶, with that at pH 4.5 predominant. If lower buffer concentrations were used, effects such as those obtained by Fishman were observed, in that several optima appeared, at rather variable, but usually quite acid, pH values. A similar problem was encountered when attempts were made to assay β -N-acetylglucosaminidase at low buffer concentrations, and a smooth pH curve with an optimum at 4.2-4.4 was only obtained at a citrate concentration of 0.5 M. Accordingly, the assays of both enzymes were carried out in 0.5 M buffers.

A comparison of the ability of rabbit tissues to hydrolyse the steroid glucuronoside and the N-acetylglucosaminide is shown in Table 1. The overall distribution of both enzymes is broadly similar to that reported by previous workers^{3,4} for the rat and mouse. Female rabbit liver shows a much higher glucuronidase activity toward the steroid substrate than any other tissue. The activity is nearly three times as high as that of male liver. In the rabbit, as in other species³, the epididymis has a very high level of β -N-acetylglucosaminidase, and this tissue has the lowest ratio of β -glucuronidase/ β -N-acetylglucosaminidase activity. The spleen of both sexes is remarkably active in hydrolysing both oestradiol glycosides—the female spleen

Table 1. HYDROLYSIS OF 17 α -OESTRADIOL GLYCOSIDES BY RABBIT TISSUES *in vitro*

Results are expressed as μ g of substrate hydrolysed per g of tissue at 37°. Incubations with liver tissue were for 4 h; with all other tissues the incubation time was 2 h.

Tissue (male; F, female)	Oestradiol-17 α -N-acetylglucosaminide	17 α -Oestradiol glucuronide	Ratio of β -glucuronidase to β -N-acetylglucosaminidase activity
Liver M	4.64	248.0	53.4
Liver F	6.52	696.0	105.7
Kidney M	38.64	51.87	1.34
Kidney F	48.93	69.72	1.42
Testis M	128.73	378.0	2.94
Testis F	191.10	372.96	1.95
Spleen M	2.94	7.14	2.43
Spleen F	2.73	9.24	3.38
Small intestine F	38.01	67.20	1.77
Large intestine F	30.45	90.72	2.98
Stomach	14.28	25.83	1.81
Uterus	16.59	54.81	3.30
Adipose	157.29	55.86	0.36
Salivary glands	13.86	45.57	3.29

showing the highest β -N-acetylglucosaminidase activity, on a wet weight basis, of any tissue tested.

The results indicate that oestradiol-17 α -N-acetylglucosaminide is cleaved, under *in vitro* conditions, by the β -N-acetylglucosaminidase present in rabbit tissues. Whether this reaction has any significance *in vivo* is being investigated.

KATHERINE S. WHITEMORE
DONALD S. LAYNE

Worcester Foundation for Experimental
Biology,
Shrewsbury, Massachusetts.

Layne, D. S., Sheth, N. A., and Kirdani, R. Y., *J. Biol. Chem.*, **239**, 3221 (1964).

Layne, D. S., *Endocrinology*, **76**, 600 (1965).

Jonchick, J., Findlay, J., and Levy, G. A., *Biochem. J.*, **71**, 318 (1959).

Woolen, J. W., Walker, P. G., and Heyworth, R., *Biochem. J.*, **79**, 294 (1961).

Walker, P. G., Woolen, J. W., and Heyworth, R., *Biochem. J.*, **79**, 288 (1961).

Levy, G. A., and Marsh, C. A., *Adv. Carbohydr. Chem.*, **14**, 381 (1959).

Wakabayashi, M., and Fishman, W. H., *J. Biol. Chem.*, **236**, 996 (1961).

Metabolites in Urine of Rats on Diets containing Aldrin or Dieldrin

Using a fly bioassay technique, Kunze and Laug¹ reported the presence of toxicant in liver, kidney, abdominal fat and urine of rats fed dieldrin or aldrin. They observed that the toxicant in kidney and urine occurred mainly in males, with traces only in females. This toxicant in a male kidney and urine differed from either dieldrin or aldrin because it could be readily destroyed by treatment with 2 per cent alcoholic KOH, a property not shared by the parent substances. Since these observations, a number of investigators²⁻⁶ have reported findings which indicate the presence of derivations of dieldrin and aldrin in tissues and urine. These are assumed to be metabolites and can be most readily distinguished from dieldrin and aldrin by their increased hydrophilic properties.

It is of interest to investigate these metabolic derivatives further by the use of electron-capture gas chromatography, a tool of great sensitivity in the detection and isolation of chlorinated insecticides.

Mature male and female Sprague-Dawley rats within a weight range of 250-350 g were fed diets containing dieldrin or aldrin for 120 days. Two levels of each insecticide were fed, namely 1 p.p.m. and 25 p.p.m. These corresponded respectively to average daily intakes of 0.015 mg and 0.375 mg per rat. A control group was maintained on a standard 'Purina' laboratory chow. Analyses showed this diet to contain no detectable aldrin or dieldrin within the limits of detection in a 100-g sample, but traces of DDT and related derivatives were found.

During the experiment, urine samples were collected at intervals and at termination a final sample together with liver, kidney and abdominal fat tissues. All specimens were extracted with ethyl ether, the extracts made to volume and suitable aliquots prepared for gas chromatography. Careful consideration was given to the possibility

that the 25 p.p.m. levels could be grossly toxic, or sufficiently embarrassing to the metabolic machinery to cause qualitative differences in response between high and low dosages.

The gas chromatographic analyses were carried out in a Barber-Colman 'Model 10' gas chromatograph, utilizing a 4-ft. U-shaped glass column. The column consisted of a 2.5 per cent coating of SF-96 on 'gas Chrom P', 100-120 mesh. The temperatures of the column and the electron-capture detector were 170° and 200° C, respectively. The detector cell applied voltage was set at 18 V for optimum detection of chlorinated hydrocarbons. The system was able to detect readily 15×10^{-6} μ g of aldrin, 20×10^{-6} μ g of isodrin and 40×10^{-6} μ g of dieldrin or endrin.

Illustrative tracings shown in Fig. 1 are representative of gas chromatographic determinations made on urines

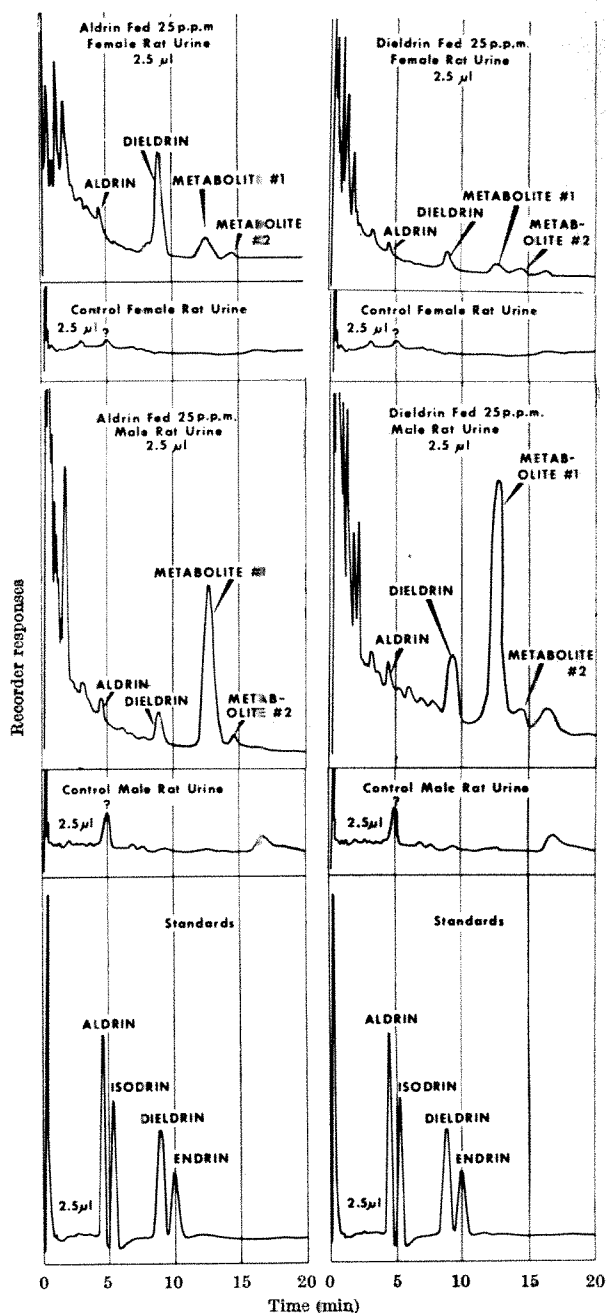


Fig. 1. Representative gas chromatograms of the ether extracts of the urine from control and experimental rats. In all cases 2.5 μ l. solution injected represented 15 μ l. urine. Standard solution: 2.5 μ l. represents 2.25×10^{-4} μ g aldrin, 2.5×10^{-4} μ g isodrin, 2.25×10^{-4} μ g dieldrin, 3×10^{-4} μ g endrin, respectively.

from animals exposed to 25 p.p.m. levels of dieldrin or aldrin. It can be seen that relatively large quantities (approximately 5:1) of a metabolite designated No. 1, having a retention time longer than any of the drin standards, appears predominantly in male urine, and only small amounts in female urine. Smaller quantities of a second metabolite No. 2, with even longer retention time, can be observed in about equal quantities in male and female urine. The latter peaks are not to be confused with two of unknown origin that appear in the control urines of both sexes. It should be especially noted that aldrin and dieldrin produce the same metabolites No. 1 and No. 2. From the type of column used, the longer retention times for both metabolites indicate that they are more polar than the parent substances. Furthermore, on hydrolysis of the urine extracts with alcoholic KOH, both metabolites disappear. This is in contrast to dieldrin and aldrin, which are alkali-stable. The epoxidation of aldrin to dieldrin both *in vitro* and *in vivo* has been well established⁷ and can be readily confirmed in our tracings. What is of interest, however, is that the animals which were fed dieldrin also appear to excrete small amounts of aldrin in the urine. These results cannot be explained on the basis of aldrin contamination of dieldrin, within the range of detectability of aldrin stated earlier. Preliminary results, notably on kidney extracts, show that the profile of metabolites in this tissue is similar to that in urine, with preponderance of metabolite No. 1 in the male kidney and sensitivity to alkaline hydrolysis.

No gross toxic effects at the 25 p.p.m. levels of dieldrin and aldrin were observed. Further, the gas chromatographic tracings made on extracts from the urine of animals exposed to 1 p.p.m. did not differ from those exposed to the higher levels except quantitatively. Shorter oral exposure to aldrin or dieldrin of rats indicates a similar profile of metabolites. It is of interest to note, however, that in some preliminary intravenous trials only minimal amounts of metabolites were observed in urine.

P. R. DATTA
E. P. LAUG
J. O. WATTS
A. K. KLEIN
M. J. NELSON

Food and Drug Administration,
Washington, D.C.

¹ Kunze, F. M., and Laug, E. P., *Fed. Proc.*, **12**, Abstr. No. 1117, 339 (1953).

² Korte, F., Ludwig, G., and Vogel, J., *Liebigs Ann. Chem.*, **656**, 135 (1962).

³ Ludwig, G., Weis, J., and Korte, F., *Life Sciences*, **123**, 3 (1964).

⁴ Cueto, jun., C., and Hayes, jun., W. J., *J. Agric. Food Chem.*, **10**, 366 (1962).

⁵ Heath, D. F., *Radioisotopes and Radiation in Entomology*, 63 (Intern. Atomic Energy Agency, Vienna, 1962).

⁶ Morsdorf, K., Ludwig, G., Vogel, J., and Korte, F., *Medicina Experimentalis*, **8**, 90 (1963).

⁷ Bann, J. M., DeCino, T. J., Earle, N. W., and Sun, Y. P., *J. Agric. Food Chem.*, **4**, 937 (1956).

PHYSIOLOGY

Electrical Activity in Human Uterine Muscle Strips

In spite of increasing interest in functional disorders of the human uterus and clinical investigation, the obstetrician has little control apart from stimulation of uterine contractions. Premature labour leading to prematurity is at the present time the greatest single preventable cause of neonatal morbidity and mortality¹⁻³. Much of the present-day investigation attempts empirically to use smooth muscle relaxants to prevent premature activity, without a basic understanding of the mechanism of normal uterine muscle control. It is not known, for example, how oxytocin exerts its stimulatory action on the gravid uterus.

We have succeeded in recording action potentials from human uterine muscle strips. The amplitude and speed of propagation of the potentials have been correlated with

the mechanical action in non-pregnant and pregnant human myometrial strips.

In the non-pregnant patients the muscle strips were carefully cut from the anterior fundal wall immediately following hysterectomy. Care was taken to keep the serosal surface intact. The strip was trimmed to approximately $45 \times 5 \times 2$ mm and immediately placed in a solution of Krebs solution, previously saturated with 95 per cent and 5 per cent CO_2 . Time elapsing from removal to muscle bath was approximately 5 min. In the pregnant cases the strips were taken from the incision site, which was made in the low transverse cervical region. Strips were occasionally removed from the anterior fundal region during sterilization procedures 2-4 days post-partum.

The muscle was then tied, serosal side up, to two clamps. One clamp was fixed while the other was connected to a Grass strain gauge. The muscle was then immersed in a bath of Krebs solution, maintained at 37°C with 95 per cent O_2 and 5 per cent CO_2 bubbling. The strain gauge was connected to one channel of a Beckman-Offner multichannel recorder. Two blunt glass-pore electrodes were then separately placed on the surface of the muscle (on the intact serosal surface) a measured distance, 5-10 mm apart. Each electrode was then connected to separate channels of the Beckman-Offner recorder. The level of the bath solution was adjusted to cover the muscle surface 1-5-10 mm to prevent drying and to maintain adequate nutrition and temperature control (Figs. 1 and 2).

In the pregnant strips, the initial action potentials recorded immediately before the onset of the contractile (Fig. 3). They have a characteristic configuration: an initial positive deflexion followed by a rapid negative deflexion, a rapid return to base-line with a slight overshoot to the positive side, then a slow decay to base-line.

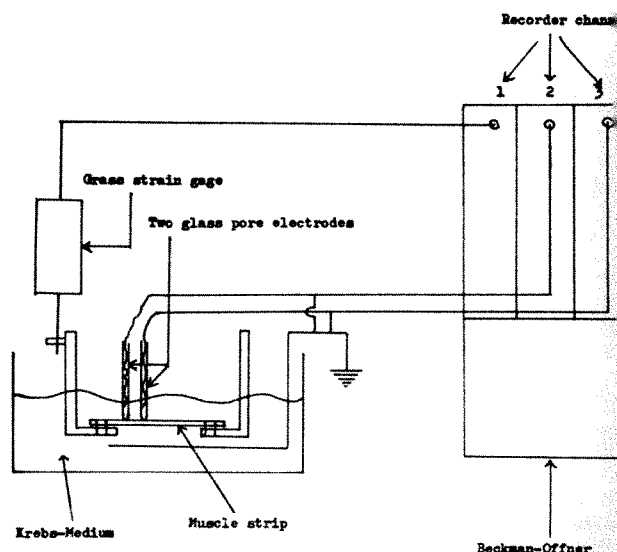


Fig. 1. Schematic illustration of technique used to record both mechanical activity and action potentials

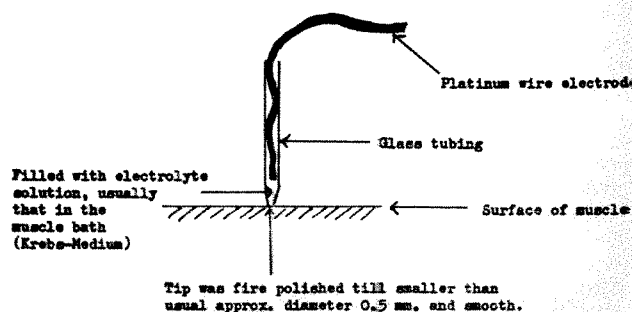


Fig. 2. Smooth glass-pore electrode used for recording action potentials

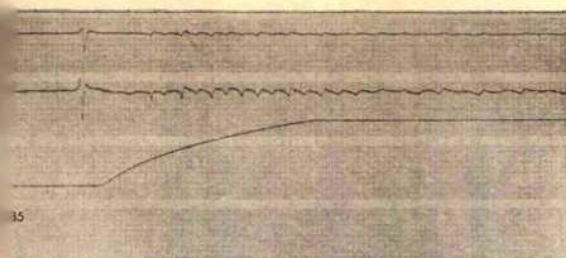


Fig. 3. Electrical and mechanical activity recorded from a pregnant human uterine muscle strip. Compare the interval between the initial action potentials to that from the non-pregnant muscle. Paper speed 30 mm/sec or 6 squares/sec

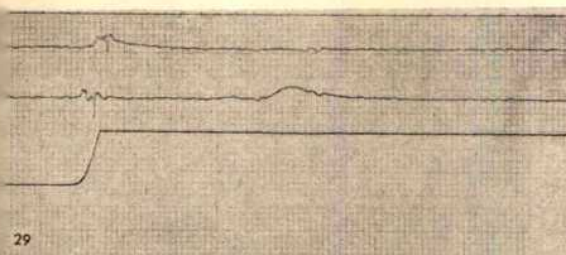


Fig. 4. Electrical and mechanical activity recorded from a non-pregnant human uterine muscle strip. Note the onset of the tension prior to the recording of the action potentials, also the distance separating action potentials from the two recording electrodes. Paper speed 15 mm/sec or 3 squares/sec

subsequent potentials are of lesser amplitude and do not show the initial positive deflexion.

In the non-pregnant strips the initial potentials are recorded after the contraction has begun (Figs. 3 and 4). The initial action potentials from a representative non-pregnant muscle averaged 0.189 mV and were propagated at an average of 0.69 cm/sec. The comparable figures for the initial action potential from a typical pregnant muscle were 0.35 mV and 13.3 cm/sec. This wide difference between voltages and conduction velocities of action potentials has also been found to occur in non-pregnant and pregnant rabbit uterine strips⁴.

If we assume that only an action potential can initiate uterine contraction, then in the non-pregnant muscle the recording of appreciable tension change before the first recorded action potential must result from the slow propagation of electrical activity. Calculations based on the maximum time required for propagation of the action potential from either end of the muscle to the electrode indicated that the slow conduction velocity could have explained the measured delay in the appearance of the action potential following the onset of contraction. Further studies will be required to establish that slow propagation is indeed the explanation for the delayed onset of action potentials in non-pregnant uterine strips. If the electrodes are randomly placed on the muscle surface and action potentials originate at random spots, we would expect on occasion to record the action potentials before, or earlier in, the contraction. To date there has been no evidence to suggest a specialized pacemaker in the uterus, though pregnant strips always propagated action potentials from the fundal area distally in the instances when the spatial origins of the strip were known.

The action potentials, following the initial one which were recorded throughout the contraction in the pregnant strips, appeared to be isolated and non-propagated. The absence of the initial positive deflexion also suggested that they are not produced by a wave of depolarization from an adjacent area. It is tempting to assume that the difference in the electrical activity in pregnant and non-pregnant muscle is due to the great difference in hormone balance present, and work is in progress on this aspect

of the problem. This work was supported by the Canadian Medical Research Council (grant MA 1656).

T. B. MACLACHLAN*

E. E. DANIEL

W. M. PAUL†

Departments of Pharmacology and Obstetrics,
University of Alberta, Edmonton, Canada.

* Present address: Department of Obstetrics and Gynecology, University Hospital, University of Saskatchewan.

† Present address: Department of Obstetrics and Gynecology, Toronto General Hospital, University of Toronto.

¹ Nelson, W. E., *Textbook of Pediatrics*, 7th ed., 306 (1959).

² Bruns, P. D., and Cooper, W. E., *Clin. Obst. and Gynec.*, **4**, 341 (1961).

³ Hardy, Janet B., *Clin. Obst. and Gynec.*, **5**, 14 (1962).

⁴ Daniel, E. E., *Canad. J. Biochem. Physiol.*, **38**, 1327 (1960).

Release of Plasminogen Activator by the Isolated Perfused Dog Kidney

Blood contains a proenzyme, plasminogen (profibrinolysin), which is converted to the proteolytic enzyme plasmin (fibrinolysin) by plasminogen activator. The level of plasminogen activator in the blood is increased in conditions of stress and after injection of epinephrine or nicotinic acid^{1,2}. The origin of blood plasminogen activator has not been established with certainty although it has been suggested that it may be derived from the plasminogen activator activity found in association with the endothelial lining of vessel walls³⁻⁵. In dogs, blood fibrinolytic activity can be increased by intravenous injection of a number of vasoactive drugs^{6,7}, but vasodilative drugs (for example, histamine) appear to be more effective than vasoconstrictive ones⁸.

We have studied the effect of histamine on the release of plasminogen activator by the isolated perfused dog kidney. A total of seven experiments were performed, using healthy mongrel dogs anaesthetized with 'Nembutal' (25 mg/kg). One kidney was removed to serve as control. The contralateral kidney of the dog was carefully freed from surrounding tissues, and the renal artery and the renal vein were ligated at their junction with the aorta and vena cava, respectively. A polyethylene catheter was inserted into the renal artery and another into the renal vein and the kidney perfused *in situ* with unoxygenated Locke's solution (pH 7.3) at 37° C until the effluent fluid had become completely clear and virtually free of red blood cells. Clearance of blood required from 180 to 480 ml. of Locke's solution. The effluent fluid during this initial perfusion period was collected in 100-ml. flasks. The kidney was then further perfused with Locke's solution and the perfusate collected as serial 5-ml. aliquots. At intervals histamine phosphate (Burroughs Wellcome and Co., Tuckahoe, N.Y.) was injected through the arterial catheter. The histamine was dissolved in Locke's solution at a final concentration of 1 mg base per ml. Each dose was 100 µg of histamine base per kg body-weight of the intact dog. The rate of perfusion was maintained at about 33 ml. per min throughout the whole experiment. Occasional red blood cells in samples of perfusate were removed by centrifugation at 4° C. The fibrinolytic activity of the perfusate was determined on unheated⁹ and heated¹⁰ bovine fibrin plates prepared as described previously¹¹. The protein content of the perfusate was measured by reading the optical density at 280 mµ and was expressed in terms of µg tyrosine per ml. Glutamic oxalacetic transaminase levels were measured according to the method recommended by the Sigma Chemical Co. (St. Louis, Missouri) and using their reagents.

For the histological study of plasminogen activator in kidney tissue, a modified Todd's technique¹² was used. Fibrin slides were prepared by clotting 0.6 ml. of a mixture of equal volumes of bovine fibrinogen solution (Bovine Fibrinogen, Behringwerke, Marburg/Lahn, Germany), 2 g per cent, and calcium chloride solution (0.05 M), with 0.1

ml. thrombin solution (Bovine Thrombin Topical, Parke, Davis and Co., Detroit, Michigan), 20 N.I.H. units per ml., on a 5 cm × 2 cm area of a standard microscopic glass slide. Veronal-hydrochloric acid buffer according to Owren¹³ was used to dissolve fibrinogen and thrombin. A block of tissue including a pyramid and its overlying cortex was excised in a transverse plane from the centre of the kidney. It was frozen at -20° C and sections 10 μ thick were cut with a freezing microtome. These sections were placed on the fibrin slides and incubated in a moist chamber at 37° C. After different intervals, slides were removed from the incubator and fixed in formaldehyde vapour and 10 per cent formol-saline solution. Afterwards, the slides were stained with Harris's haematoxylin and lithium carbonate and mounted in glycerine jelly.

The perfusate obtained before injection of histamine had low fibrinolytic activity and sometimes had no activity at all. In each experiment the injection of a first dose of histamine through the arterial catheter was followed immediately by the appearance of increased amounts of plasminogen activator in the perfusate. The perfusate had no measurable lytic activity on heated bovine fibrin plates, indicating that the active material was plasminogen activator. Injection of histamine was not followed by a significant change in the level of glutamic oxalacetic transaminase or in the protein content of the perfusate. Intra-arterial injection of a second dose of histamine after a short interval had no effect on the release of plasminogen activator into the perfusate. The results of a typical kidney perfusion experiment are given in Fig. 1.

The plasminogen activator content of frozen sections of the perfused, histamine-treated kidney and the contralateral control kidney was compared in each experiment; in 4 of the 7 experiments the histamine-treated kidney showed considerable decrease of the plasminogen activator activity normally found in association with the vasa recta¹⁴ of the subcortical zone. Fig. 2 demonstrates the decreased amount of plasminogen activator activity in a perfused kidney as compared with the normal, contralateral kidney of the same dog.

The presented data provide evidence that, in an isolated perfused organ, histamine stimulates the release of plasminogen activator into the perfusing fluid. The considerable depletion of plasminogen activator in the subcortical zone of four perfused kidneys suggests that the wall of the vasa recta is a source of releasable plasminogen activator. The fact that the level of glutamic oxalacetic transaminase, a common intracellular enzyme, does not increase suggests that plasminogen activator release is not accompanied by a significant degree of cellular necrosis.

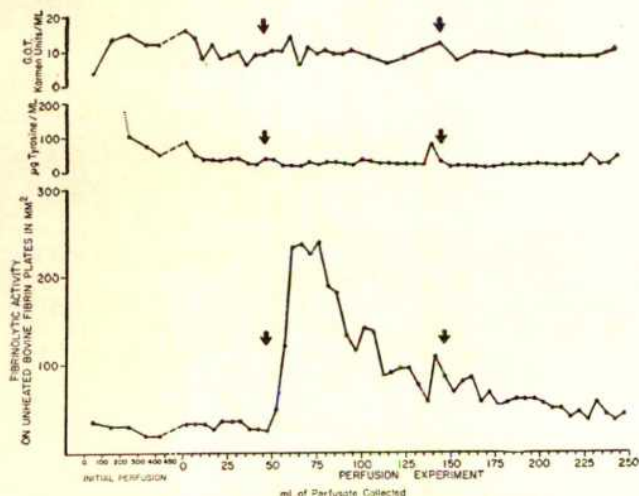
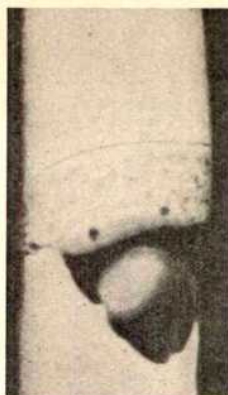


Fig. 1. Fibrinolytic activity, glutamic oxalacetic transaminase level and protein content of perfusate of a kidney perfusion experiment. Histamine, 100 μg per kg body-weight of the intact dog, was injected through the arterial catheter at the times indicated by the arrows.

Control kidney



Perfused kidney



Fig. 2. Unstained sections of a control and perfused, histamine-treated kidney of a dog. Both sections were incubated at 37° C on unheated fibrin slides for 130 min. The dark areas indicate lysis of the fibrin layer. It can be seen that there is complete lysis over the subcortical zone in the control kidney whereas the section of the perfused kidney shows little lytic activity.

Tissular hypoxaemia may have played a part in the histamine-induced release of plasminogen activator since these experiments were performed on anoxaemic organ. It cannot be ruled out that histamine releases plasminogen activator by a direct effect on endothelial cells of small vessels or capillaries, although it has been impossible to demonstrate such an effect on walls of large veins. Injection of histamine into an isolated venous segment of the dog or incubation of an everted jugular vein of the dog in histamine solution does not decrease plasminogen activator content of the vessel wall^{8,15}.

A more likely explanation is that the release of plasminogen activator by histamine is attributable to the vasoactive effects of this drug. Assuming that the amount of plasminogen activator released is directly proportional to the area of perfused inner blood vessel surface, the opening up of a large number of small blood vessels by histamine in the perfused organ can be expected to increase plasminogen activator release.

We thank Dr. G. D. Penick for advice and Mrs. C. K. Taylor for assistance in this work, which was supported by grants from the National Institutes of Health (HE-06350) and from the American Cancer Society.

RUDOLF HOLEMANS
JOHN G. JOHNSTON
ROBERT L. REDDICK

Department of Pathology,
University of North Carolina,
Chapel Hill, North Carolina.

- ¹ Sherry, S., Fletcher, A. P., and Alkjaersig, N., *Physiol. Rev.*, **39**, 343 (1959).
- ² Fletcher, A. P., Biederman, O., Moore, D., Alkjaersig, N., and Sherry, S., *J. Clin. Invest.*, **43**, 681 (1964).
- ³ Warren, B. A., *Brit. Med. Bull.*, **20**, 213 (1964).
- ⁴ Messer, D. L., Celander, D. R., and Guest, M. M., *Circ. Res.*, **11**, 832 (1962).
- ⁵ Chakrabarti, R., Birks, P. M., and Fearnley, G. R., *Lancet*, **i**, 1288 (1963).
- ⁶ Holemans, R., *Med. Exper.*, **9**, 5 (1963).
- ⁷ Holemans, R., and Langdell, R. D., *Proc. Soc. Exp. Biol. and Med.*, **115**, 584 (1964).
- ⁸ Holemans, R., *Amer. J. Physiol.*, **208**, 511 (1965).
- ⁹ Astrup, T., and Mullertz, S., *Arch. Biochem. Biophys.*, **40**, 346 (1952).
- ¹⁰ Lassen, M., *Acta Physiol. Scand.*, **27**, 371 (1953).
- ¹¹ Holemans, R., *J. App. Physiol.*, **18**, 1123 (1963).
- ¹² Todd, A. S., *J. Path. Bact.*, **78**, 281 (1959).
- ¹³ Owren, P. A., *Acta Med. Scand.*, Suppl. **194**, 125 (1947).
- ¹⁴ Trueta, J., Barclay, A. E., Daniel, P. M., Franklin, K. J., and Prichard, M. M. L., *Studies on the Renal Circulation* (Blackwell Scientific Publications, Oxford, 1947).
- ¹⁵ Holemans, R., and Johnston, J. G. (unpublished observations).

Thinning of the Human Cornea on Awakening

MUCH remains unknown about the mechanism whereby the cornea maintains its shape, thickness, and transparency. Maurice¹ points out that the observed negative water pressure inside the cornea and the positive

pressure in the aqueous humor and tears bathing the cornea should lead to continuous swelling and, therefore, corneal cloudiness. Some process, yet unknown, maintains the *in vivo* cornea at constant thickness and transparency. Mishima and Maurice² showed that whatever process operates to maintain constant corneal thickness, the process can be disturbed by closing the eyes for an hour or more. They showed that the rabbit cornea thinned by four per cent over a period of two hours after the eye was opened. The thinning was attributed to evaporation of solvent (water) from the tears to produce a hypertonic tear film which withdrew water from the cornea by an osmotic effect. Conversely, when the eye was closed and was bathed in isotonic tears, the cornea swelled. They considered the other two environmental factors which change when the eye is closed, namely temperature and oxygen tension, to be ineffective in changing thickness. The corneal temperature of the closed eye is about 4° C above that of the open eye. The oxygen tension of the epithelial surface of the closed eye must be about 50 mm mercury (the average between venous and arterial blood) whereas the open eye in air is exposed to 155 mm mercury oxygen tension.

The slow blinking rate of the rabbit (once every 10 min, compared with the human rate of 12 blinks per min) suggests that evaporation from the human eye may not be the same as from the rabbit eye. We have, therefore, measured the thinning of the *in vivo* human cornea after the eye is opened on awakening³.

The subject, a Caucasian female 20 years of age, was instructed to tape her left eye closed immediately on awakening in the morning, usually after 6-8 h of sleep. The tape was removed in the laboratory about 30 min later and corneal thickness measured as a function of time for several hours. Normal blinking was allowed at all times. Corneal thickness measurements were made by using a Vickers optical beam splitter fitted into one tube of a binocular microscope on a Thorpe-type slit-lamp⁴. The slit-lamp projects a vertical light beam about 1 mm wide and 1 cm long. In traversing the cornea it illuminates, by scattered light, the cross-section of the cornea that is observed in the microscope and beam splitter. By adjusting the beam splitter to give first a coincident image and then side-by-side images, the thickness can be obtained through a calibration which uses five corneal contact lenses of known thickness and refractive index. The calibration curve is shown in the insert of Fig. 1. The theory of such *in vivo* corneal thickness measurements has been reviewed by von Bahr⁵.

Because of the large spacial variation in the thickness of the human cornea, a critical feature of this experi-

ment was repeating the measurements at a constant corneal position. All parts of the slit-lamp assembly were rigidly fixed by clamps. The subject was held in a reproducible position by a dental-mould mouthpiece and head-bar which were fixed to a sturdy mounting on a weighted table. The standard fixation light on the slit-lamp was covered with a disk about 3 cm in diameter with a 1-mm hole in the centre. This provided a small fixation light to reduce eye movements. Only when the biomicroscope was properly aligned was a bright image produced by specular reflexion from the endothelium, and tear surface.

Measurements were made on four separate days. About ten settings of the beam splitter were made in each 5-min period for the first 80 min and then in 5-min periods separated by 15-min intervals for the remaining 80 min.

Fig. 1 shows a corneal thickness as a function of time. The cornea on awakening appears to be about 3.6 per cent thicker than normal. The normal-thickness base line was obtained by continuing the measurements, in some cases, to 6 h. The thickened cornea returns to normal in about 1 h.

The maximum corneal swelling of about 4 per cent is the same for rabbit and man. However, the rabbit cornea returns to normal thickness in 0.5 h (ref. 2) whereas the human cornea requires about 1 h. This can be explained by the lower blink rate of the rabbit. At this lower rate the tears concentrated by evaporation are replaced only infrequently, whereas in man there is frequent replacement of tears and, therefore, less-concentrated tears. Corneal thinning is probably caused by extraction of water by the osmotic pressure of hypertonic tears. A lower hypertonicity, accompanying the higher blinking rate in man, would give slower thinning.

We thank Miss Mary Linda Morrison for being the subject. The Vickers beam splitter was loaned to us by the Corneal Center, New York. This work was supported in part by U.S. Public Health Service research grants NB 04810 to R.B.M. and HE 06796 to I.F.

R. B. MANDELL

School of Optometry,
University of California.

I. FATT

College of Engineering,
University of California,
Berkeley.

¹ Maurice, D. M. in *The Eye*, edit. by Davson, H., 289 (Academic Press, New York, 1962).

² Mishima, S., and Maurice, D. M., *Exp. Eye Res.*, **1**, 46 (1961).

³ Mishima, S., *Arch. Ophthalmol.*, **73**, 233 (1965), has reported thinning of the human cornea on opening the eye (Fig. 7B) but did not give his experimental method nor the conditions under which the measurements were made. Our results are in good agreement with Mishima's.

⁴ Vickers beam splitter from Cooke, Troughton and Simms, Inc. Thorpe slit-lamp from Bausch and Lomb, Rochester, New York.

⁵ von Bahr, G., *Amer. J. Ophthalmol.*, **42**, 251 (1956).

Use of Polyethylene Glycol in Investigations of Absorption of Fat

IN 1957 Borgström *et al.*¹ introduced the use of unabsorbable polyethylene glycol (PEG) for quantitative investigations of absorption of fat, protein and carbohydrate from the intestine. Stimulated by their communication, a number of authors²⁻⁴ used PEG to examine absorption. (After the administration of PEG and a food mixture of known composition in the withdrawn specimen of the intestinal contents the PEG concentration and investigated nutrients are estimated. From the two values the percentage ratio of the absorbed nutrient is calculated.)

Wiggins and Dawson⁵, however, directed attention to the fact that in the stomach a dissociation of PEG and the fat from the food mixture takes place; PEG remains mainly in the aqueous phase, which passes more rapidly into the intestine than the lipid phase. In a preliminary

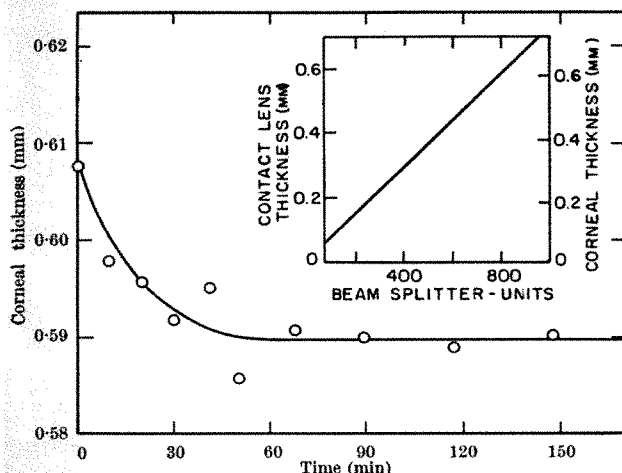


Fig. 1. Time course of corneal thinning after opening eye on awakening from 6 h sleep. Insert shows calibration curve of beam splitter with contact lenses of various thicknesses. Corneal thickness is 0.92 times contact lens thickness at a given beam splitter reading for the optical system used here.

Table 1. INFLUENCE OF PEG ON THE LIPOLYTIC ACTIVITY OF HUMAN DUODENAL JUICE

Final conc. of PEG/ml.	Lipolytic activity as percentage of activity of control specimen Juice 1 (2.5 per cent l.)	Juice 2 (3.5 per cent l.)	Juice 3 (11 per cent l.)*
0.5	100	88.5	104
1.0	68	100	104
2.5	—	73.5	90
4.0	76.5	85.5	100
5.0	50.0	73.5	101

* Percentage of lipolysis in control specimens after incubation for 30 min. Composition of incubation medium: 0.2 ml. olive oil; 0.2 ml. phosphate buffer pH 6.5; 0.5 ml. PEG solution (1, 2, 5, 8, 10 mg/1 ml. duodenal juice).

experiment we investigated the stability of the food mixture (1 part olive oil, 4 parts dried milk, 1 part dextrose, 8 parts water) during incubation with human gastric juice, 1:1, at 37° C. It was revealed that the presence of PEG in a concentration of 5 mg/ml. distinctly promotes the breakdown of the food mixture.

The adverse effect of PEG on the stability of the emulsion system of the mixture brings up the question of how this substance participates in the breakdown of fats by pancreatic lipase. So far as we know, reports on this problem are lacking; we investigated therefore the effect of PEG on lipolysis in a model experiment *in vitro*.

As a source of lipase we used an aqueous 0.1 per cent extract of acetone powder of hog pancreas and fresh human duodenal juice. Olive oil served as substrate. The final concentration of PEG (molecular weight, 4,000) varied between 0.5 and 5 mg/ml. (In the experiment three different preparations of PEG were used. We thank Prof. B. Borgström and Prof. A. M. Dawson for kindly supplying specimens of PEG.) The exact composition of the incubation medium is given in the legend of Table 1 and Fig. 1. The degree of lipolysis was assessed from the difference of free fatty acids (NEFA) estimated in the incubation medium by Dole's method⁵ at the onset of and after the incubation. The results are expressed as the percentage activity of the control specimen which did not contain PEG.

In the experiment with pancreas extract it was found that in the presence of PEG the lipolytic activity was inhibited, depending on the concentration of PEG. As is apparent from Fig. 1, the inhibition is in general the greater, the smaller the amount of lipase in the incubation medium.

In the subsequent experiment we replaced the synthetic digestive mixture by human duodenal juice. It was revealed that also in this system PEG exerts an inhibitory effect (Table 1). Contrary to the first experiment, the effect of PEG rather varied; it attained a decrease up

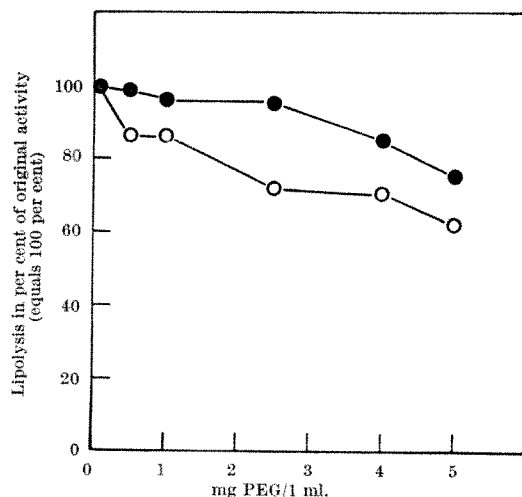


Fig. 1. Influence of PEG on the breakdown of olive oil by an extract of hog pancreas. Composition of incubation medium: 0.1 ml. olive oil; 0.1 ml. 0.2 M CaCl₂; 0.2 ml. *tris* 0.2 M, pH 8.0; 0.2 ml. lipase extract; 0.25 ml. PEG solution 2, 4, 10, 16, 20 mg/1 ml.; 0.1 ml. H₂O. Incubation period 30 min. x axis, final concentration of PEG; y axis, percentage lipolytic activity of control specimen (without addition of PEG). ●, 0.1 per cent lipase extract; ○, 0.02 per cent lipase extract

to 50 per cent, however, not observed with low lipase activity. The inhibitory effect of PEG may be explained perhaps by its adverse action on the stability of the food mixture. This assumption requires, however, further confirmation. In any event, our finding supports the view⁶ that PEG is not very suitable for the accurate quantitative investigation of absorption of fat. A satisfactory reference substance should have the following properties: (a) it must not be absorbed; (b) it must not influence gastric emptying; (c) it must be soluble in the same medium as the substrate; (d) it must not affect the digestion and absorption of the investigated substance.

From experiments of Wiggins and Dawson as well as ours presented in this communication, it is obvious that PEG does not meet the latter two conditions, so far lipids are concerned.

Experiments *in vitro* revealed that PEG inhibits lipase from hog pancreas and human duodenal juice; it is therefore not very suitable for use as a reference substance for investigating absorption of fat, at least in conditions with low lipase activity.

H. VAVŘINKOVÁ
A. KRONDL

Institute of Human Nutrition,
Prague.

¹ Dahlquist, A., Lundh, G., and Sjövall, J., *J. Clin. Invest.*, **36**, 1521 (1957).

² Lundh, G., *Acta Chir. Scand.*, Supp. 231 (1958).

³ Borgström, B., *J. Clin. Invest.*, **39**, 809 (1960).

⁴ Lindquist, B., and Lundh, G., *Amer. J. Dis. Child.*, **101**, 454 (1961).

⁵ Wiggins, H. S., and Dawson, A. M., *Gut*, **2**, 373 (1961).

⁶ Dole, V. P., *J. Clin. Invest.*, **35**, 150 (1956).

PHARMACOLOGY

Some Effects of Drugs on the Isolated Superfused Carotid Body

THE isolated superfused carotid body preparation described by Eyzaguirre and Lewin¹, and used by them to investigate some properties of the carotid body, is a useful method for investigating chemoreceptor activity without need for concern about changes in the blood supply. This communication reports the results of an examination of the effects of a number of compounds on the activity of chemoreceptors in such a preparation. Some of these drugs would normally be expected to modify the blood flow through the carotid body and hence indirectly to affect the chemoreceptor activity. The changes in activity which they induce thus, here, represent the direct actions of drugs on the chemoreceptors. In addition, the effect of changes in the calcium ion concentration of the superfusate has been investigated because there is evidence²⁻⁴ that transmitter release at a number of sites may be related to calcium ion concentration.

The experiments were performed on the carotid body and sinus nerve removed from 18 cats lightly anaesthetized with sodium pentobarbitone. The superfusion technique was similar to that described by Eyzaguirre and Lewin¹ and the bathing fluid was Krebs's solution equilibrated with 95 per cent oxygen, 5 per cent carbon dioxide, maintaining the pH at 7.4. This was also confirmed by direct measurement. Five reservoirs were available so that different drug mixtures could be applied.

The sinus nerve was laid on a stainless steel back plate and dissected under liquid paraffin floating on the Krebs's solution in which the carotid body was suspended. Action potentials were recorded through a bipolar platinum wire electrode and the nerve impulses could be photographed from an oscilloscope and counted on a ratemeter with a chart recorder giving a permanent record.

The effects of a number of drugs were tested on the chemoreceptor activity when the rate of flow of the superfusate was increased so that the discharge fell to a minimum, and when a maximum hypoxic response was produced by raising the carotid body into the liquid paraffin.

former could be held constant over long periods and latter was repeatable.

acetylcholine (ACh) in a concentration of 10^{-4} – 10^{-5} M. always caused slight excitation, though adrenaline, noradrenaline (NA) and isopropylnoradrenaline (IP) added in concentrations of 10^{-5} g/ml. Lower concentrations were without effect. In two experiments, hexathonium bromide (C6) 10^{-5} g/ml. blocked the ACh excitation and was without effect on the hypoxic response, one the converse was the case and in the other this concentration was without effect. C6 alone did not change the resting rate.

The most consistent blocking effects were seen with thalidomide which, in concentrations of 10^{-5} g/ml., invariably depressed the resting discharge and depressed the hypoxic response. In addition, it blocked ACh and IP excitation. The results with dichloro-isopropylnoradrenaline (DCI) were more variable, but either it had no effect or depressed the resting rate and hypoxic response at 10^{-5} g/ml. Phenoxybenzamine in concentrations of 10^{-5} g/ml. increased the discharge rate at maximum flow, an effect which was slow in onset and long-lasting. In one test it blocked the excitation previously produced by NA. peridyl-methyl benzodioxane (933F) in low doses, 10^{-6} – 10^{-5} g/ml., similarly caused excitation. Neither drug had constant effects on the maximum hypoxic discharge rate, which was little changed.

Bretylium tosylate at a concentration of 10^{-5} g/ml. depressed the resting discharge and maximum hypoxic frequency and blocked ACh excitation. Its administration was also usually associated with depression of action potential amplitude.

The effect of reserpine could not be adequately tested, or it usually precipitated out in the superfusate.

In four experiments the effect of changing the calcium ion concentration was investigated. Superfusion with Krebs's solution containing no calcium chloride caused an increase in the spontaneous activity at maximum flow rate, while raising the calcium concentration by two or four times depressed this activity.

The experiments have also provided the opportunity for examining the statistical properties of the nerve impulse pattern. In three experiments single unit chemoreceptor afferent preparations were made and the distribution of intervals between action potentials was analysed in the manner described by Biscoe and Taylor⁵. This analysis showed that the mean interval and the standard deviation of twenty successive intervals were similar for mean intervals larger than approximately 300 msec, but that the S.D. became progressively smaller than the mean for intervals shorter than this. This result is similar to that previously obtained with the *in vivo* perfused carotid body⁶. An equality of mean and standard deviation is a property of a random interval distribution⁶.

It seems clear that ACh, NA, A and IP can all cause excitation of carotid body chemoreceptor afferents, presumably in this preparation by an effect other than on the vascular bed. High concentrations are required, and it is not necessary to suppose that ACh, for example, is acting here as a chemical transmitter, for its excitant effects on nerve endings are well known^{7,8}. The results with hexamethonium are equivocal, and on the basis of this evidence there can be no serious disagreement with Douglas⁹, or with the criticisms of the notion that ACh is a transmitter at this site^{10,11}. One of the more interesting results is the effect of nethalide, a β -adrenergic blocking drug, for its effect was consistently obtainable and unlike bretylium, for example, it did not depress the action potential height, an effect which may be related to the local anaesthetic action of bretylium^{12,13}. According to the theory expounded by Douglas and Rubin², and Douglas and Poisner³, and the evidence of Philippu and Schumann⁴, on release of catecholamine from the adrenal gland, the changes in activity produced by calcium should be the converse of those seen here if transmitter release were

calcium dependent. But the changes in activity with the calcium ion concentration are explicable in terms of direct effects on nerve endings¹⁴, and do not yield any information about transmitter release, if indeed a transmitter exists.

I thank Mr. J. O. Yates for his assistance.

T. J. BISCOE*

Agricultural Research Council Institute
of Animal Physiology,
Babraham, Cambridge.

* Present address: Department of Physiology, Australian National University, Canberra.

¹ Eyzaguirre, C., and Lewin, J., *J. Physiol. (Lond.)*, **159**, 238 (1961).

² Douglas, W. W., and Rubin, R. P., *J. Physiol. (Lond.)*, **159**, 40 (1961).

³ Douglas, W. W., and Poisner, A. M., *J. Physiol. (Lond.)*, **165**, 528 (1963).

⁴ Philippu, A., and Schumann, M. J., *Experientia*, **18**, 138 (1962).

⁵ Biscoe, T. J., and Taylor, A., *J. Physiol. (Lond.)*, **168**, 332 (1963).

⁶ Parratt, L. G., *Probability and Experimental Errors in Science* (J. Wiley and Sons, N.Y., 1961).

⁷ Coon, J. H., and Rothman, S., *J. Pharmacol.*, **68**, 301 (1940).

⁸ Brown, G. L., and Gray, J. A. B., *J. Physiol. (Lond.)*, **107**, 306 (1948).

⁹ Douglas, W. W., *J. Physiol. (Lond.)*, **118**, 373 (1952).

¹⁰ Douglas, W. W., *Pharmacol. Rev.*, **6**, 81 (1954).

¹¹ Daly, M. de B., *Pharmacol. Rev.*, **6**, 79 (1954).

¹² Boura, A. L. A., and Green, A. F., *Brit. J. Pharmacol.*, **14**, 536 (1959).

¹³ Boyd, H., Chang, V., and Rand, M. J., *Arch. Int. Pharmacodyn.*, **131**, 10 (1961).

¹⁴ Frankenhauser, B., and Hodgkin, A. L., *J. Physiol. (Lond.)*, **137**, 218 (1957).

Lethal Effects of Aggregation and Electric Shock in Mice treated with Cocaine

SENSORY stimuli provided by aggregation¹ or sub-maximal electric shock² become incompatible with life in mice treated with low doses of amphetamine. Effects of aggregation^{3,4} or electric shock⁵ on brain levels of norepinephrine are also modified by amphetamine. Amphetamine inhibits the uptake of norepinephrine by tissues *in vitro*^{6,7} and *in vivo*⁸. Termination of the biological actions of norepinephrine to a large extent depends on the tissue uptake or 'recapturing' of the free norepinephrine⁹. It is therefore postulated that toxicity of sensory stimuli in treated mice is partly due to the interference by amphetamine of the 'recapturing' of norepinephrine liberated from sympathetic nerves due to stimulation.

Cocaine, like amphetamine, also blocks the tissue uptake of norepinephrine *in vitro*^{10,11} and *in vivo*^{12,14}. The present report describes the lethal properties of sensory stimuli in the mice treated with cocaine.

Swiss albino random-bred male mice weighing 25–28 g were placed in shock apparatus or aggregation cages immediately after intramuscular injection of cocaine. All other drugs were injected intraperitoneally. The shock apparatus was similar to one described by Weiss *et al.*², while aggregation was according to Cohen and Lal¹. In isolation, only one mouse was placed in each cage, while 10 mice in a cage constituted aggregation. Shocks were provided for 4 h. There were no deaths with chlorpromazine or reserpine when given alone and the mice were aggregated. Reserpine produced some deaths among shocked mice.

Data given in Table 1 suggest that aggregation or shock became lethal to mice treated with sub-lethal doses of cocaine. As is seen from Table 2, the enhanced lethality of aggregation was completely prevented by pretreatment with adequate doses of chlorpromazine or reserpine.

Table 1. TOXICITY OF SENSORY STIMULI IN MICE TREATED WITH COCAINE

Cocaine, mg/kg	Experimental conditions	4-h mortality	
		Dead/injected	%
0*	Isolated	0/30	0
	Aggregated	0/30	0
	Shocked	0/20	0
50	Isolated	0/14	0
	Aggregated	1/20	5
	Shocked	5/24	20
100	Isolated	1/10	10
	Aggregated	13/20	65
	Shocked	15/24	63

* Mice were injected with 90 mg of sodium chloride per kg of body-weight.

Table 2. EFFECT OF CHLORPROMAZINE AND RESERPINE ON STIMULATED MICE TREATED WITH COCAINE*

Drug	Dose (mg/kg)	Experimental conditions	4-h mortality Dead/injected	%
Sodium chloride	90	Aggregated	13/20	65
Chlorpromazine	5	Aggregated	9/10	90
Chlorpromazine	20	Aggregated	0/10	0
Reserpine	5	Aggregated	1/10	10
Sodium chloride	90	Shocked	15/24	63
Chlorpromazine	5	Shocked	6/10	60
Chlorpromazine	20	Shocked	1/10	10
Reserpine	5	Shocked	4/10	40

* 100 mg of cocaine per kg of body-wt. was injected 1 h after chlorpromazine or 4 h after reserpine into all the mice.

Chlorpromazine also protected shocked mice. Effect of reserpine in shocked mice was inconclusive since in shocked mice reserpine exhibited some toxicity of its own.

Recently several investigations have demonstrated rapid and specific uptake of circulating norepinephrine by tissues^{13,15,16}, sympathetic nerve endings¹⁷, and isolated granules from nerve endings¹⁸. In isolated perfused heart, more norepinephrine is lost by tissue uptake than by enzymatic metabolism¹⁷. Denervation¹⁹, which depresses tissue uptake of norepinephrine, produces supersensitivity to injected norepinephrine.

On the basis of these observations it has been suggested that intensity or duration of biological action of circulating or locally released norepinephrine is limited to a large extent by 'recapturing mechanisms'. Interference with these mechanisms by drugs or other procedures may result in increased and prolonged pharmacological effects of exogenously or endogenously released norepinephrine. Present studies of effects of sensory stimuli in presence of cocaine or amphetamine provide *in vivo* evidence of an exaggerated sympathetic syndrome. Excessive sensory stimuli liberate norepinephrine through central and peripheral sympathetic discharge. Inactivation of liberated norepinephrine by 'recapturing mechanisms' is prevented by cocaine or amphetamine, resulting in prolonged and intense action of norepinephrine on tissue, causing tissue damage and death. Pretreatment with normal noradrenergic blocking agents may provide protection against the exaggerated sympathetic syndrome.

HARBANS LAL*

RICHARD D. CHESSICK

Psychiatry Service,
Veterans Administration Research Hospital,
Chicago, Illinois.

*Present address: University of Kansas School of Pharmacy, Lawrence, Kansas.

- ¹ Cohen, M., and Lal, H., *Nature*, **201**, 1037 (1964).
- ² Weiss, B., Lates, V. G., and Blanton, F. L., *J. Pharmacol.*, **132**, 366 (1961).
- ³ Lal, H., and Chessick, R., *Life Sciences*, **3**, 381 (1964).
- ⁴ Moore, K. E., *J. Pharmacol.*, **142**, 6 (1963).
- ⁵ Moore, K. E., and Lariviere, E. W., *Biochem. Pharmacol.*, **13**, 1098 (1964).
- ⁶ Iversen, L. L., *J. Pharmacol.*, **16**, 435 (1964).
- ⁷ Potter, L. T., and Axelrod, J., *J. Pharmacol.*, **140**, 199 (1963).
- ⁸ Axelrod, J., and Tomchick, R., *J. Pharmacol.*, **130**, 367 (1960).
- ⁹ Kopin, I. J., *Pharmacol. Rev.*, **16**, 179 (1964).
- ¹⁰ Dengler, H. J., Michaelson, I. A., Spiegel, H. E., and Titus, E. O., *Intern. J. Neuropharmacol.*, **1**, 23 (1962).
- ¹¹ Dengler, H. J., Spiegel, H. E., and Titus, E. O., *Nature*, **191**, 816 (1961).
- ¹² MacMillan, W. H., *Brit. J. Pharmacol.*, **14**, 385 (1959).
- ¹³ Muschol, E., *Brit. J. Pharmacol.*, **16**, 352 (1961).
- ¹⁴ Whitby, L. G., Hertting, G., and Axelrod, J., *Nature*, **187**, 604 (1960).
- ¹⁵ Iversen, L. L., and Whitby, L. G., *Brit. J. Pharmacol.*, **19**, 35 (1962).
- ¹⁶ Kopin, I. J., Hertting, G., and Gordon, E. K., *J. Pharmacol.*, **138**, 34 (1962).
- ¹⁷ Wolfe, D. E., Potter, L. T., Richardson, K. C., and Axelrod, J., *Science*, **138**, 440 (1962).
- ¹⁸ Stjärne, L., *Acta Physiol. Scand.*, **62**, Suppl. 228 (1964).
- ¹⁹ Hertting, G., Axelrod, J., and Kopin, I. J., *Nature*, **189**, 66 (1961).

Interference by the Carcinogenic 4-Nitroquinoline N-Oxide of Tryptophan and Indole Uptake in *Escherichia coli*

THE potent water-soluble carcinogen 4-nitroquinoline N-oxide (NQO) is remarkably versatile and thus represents a good means of discerning pre-requisites for carcinogenesis. NQO condenses readily with sulphhydryl groups; is mutagenic to bacteria, tobacco mosaic virus,

and *Aspergillus niger*¹; inhibits incorporation of phosphorus-32 into nucleic acids of Ehrlich ascites carcinoma cells²; induces nucleolar 'caps' in Chang liver cells³; inhibits growth of the flagellates *Ochromonas dan* *Euglena gracilis*, of *Corynebacterium bovis*, and of photosynthetic purple bacterium *Rhodospseudomonas palustris*. These growth inhibitions are competitively annulled by L-tryptophan; D-tryptophan is also effective. The competitive annulment ratio is 0.2 mg/ml. tryptophan NQO 2 µg/ml.

This growth competition suggests that NQO uptake mediated by a tryptophan transport mechanism⁴. Investigate this possibility, two tryptophan-requiring mutant strains of *E. coli*, T3 and T24 (cultures obtained from Dr. C. Yanofsky), both utilizing tryptophan indole were investigated. NQO was tested for interference with the inducible tryptophan permease of both strains by a modified Burrows and DeMoss⁵ technique. 24 mg (dry weight) log-phase cells were collected on 'Millipore' cellulose filter (0.05 µ pore size) and washed 80 sec with isotonic C- and N-free medium and the filtrate discarded. C- and N-free medium + tryptophan (indole) at equimolar concentrations was then washed over the cells for 40 sec and this filtrate collected. Absorbance of the medium + tryptophan or indole was measured before and after exposure to NQO by means of a Beckman DU spectrophotometer set at 280 mµ.

Table 1. INTERFERENCE BY 4-NITROQUINOLINE N-OXIDE OF L-TRYPTOPHAN AND INDOLE UPTAKE IN *E. coli* (Readings as absorbance at 280 mµ)

Additions	Readings before exposure to cells	Control (after 40 sec washing)	Cells exposed to 4 × 10 ⁻⁴ M NQO for 5 min (reading taken after 40 sec wash with C- and N-free medium)
NQO 4 × 10 ⁻⁴ M	0.002	0.0019	0.003
L-Tryptophan 0.5 × 10 ⁻⁴ M	0.43	0.30	0.42
Indole 0.5 × 10 ⁻⁴ M	0.195	0.04	0.15

Introduction of NQO at 4 × 10⁻⁴ M (shown by viability investigations to be non-inhibitory in a culture of the density) into the growing culture completely inhibited uptake of L-tryptophan and inhibited indole uptake by 80 per cent within 5 min (Table 1). Both strains behave alike.

These results are construed as supporting our earlier idea that ability to deceive tryptophan transport system may underlie the carcinogenic specificity of polycyclic carcinogens.

This work was supported by grant 827 from the Damon Runyon Memorial Fund for Cancer Research.

A. C. ZAHALSKY

S. L. MARCUS

Haskins Laboratories,
305 East 43 Street, New York.

¹ Okabayashi, T., Yoshimoto, A., and Ide, M., *Chem. Pharm. Bull.*, **12**, 257 (1964).

² Fukuoka, F., Ono, T., Ohashi, M., and Nishimura, S., *Cann.*, **50**, 23 (1959).

³ Reynolds, R. C., Montgomery, P., and Karney, D. H., *Cancer*, **23**, 535 (1963).

⁴ Zahalsky, A. C., Keane, M. M., Hutner, S. H., Lubart, K. J., Kittrell, M., and Amsterdam, D. J., *J. Protozool.*, **10**, 421 (1963).

⁵ Burrows, S. E., and DeMoss, R. D., *Biochim. Biophys. Acta*, **73**, 623 (1963).

HAEMATOLOGY

Plasma Co-factors in Adenosine Diphosphate-induced Aggregation of Human Platelets

THE aggregation of washed human platelets *in vitro* by adenosine diphosphate (ADP) requires calcium and is stimulated by the addition of platelet-free plasma¹. The identity of the plasma activity has been the subject of recent conflicting reports. Hellem and Owren² have suggested that the plasma factor is that entity missing in von Willebrand's disease; but others^{3,4} have found that ADP-induced platelet aggregation proceeds normally in plasma from patients with established von Willebrand's disease. McLean *et al.*⁵ and Cross⁴ have indicated that fibrinogen may be the plasma co-factor, and Caen⁶ has

ently suggested that both fibrinogen and the anti-von Willebrand factor participate in ADP-induced platelet aggregation.

The experiments described here were undertaken in an effort to clarify the nature of the stimulatory activity of plasma on ADP-induced aggregation of washed human platelets. Platelets were collected and processed with ionized equipment from normal subjects and from a patient with established von Willebrand's disease, using an acid-citrate anticoagulant of Aster and Jandl¹⁷. Platelet-rich plasma was prepared by centrifugation at 5g for 15 min at 23° C. Platelet pellets were obtained by further centrifugation at 2,500g for 5 min at 4° C. The platelets were resuspended with a Pasteur pipette in McIntire's platelet buffer⁸, modified in that the final glucose concentration was 2 g/l. and the final pH was 7.5. The platelets were washed twice in the same buffer and were finally resuspended in the same medium at a final concentration of 7×10^8 /ml. The suspended platelets could be kept in this medium at 4° C for as long as 4 h with no evidence of spontaneous aggregation and with no diminution of sensitivity to ADP. Platelet aggregation was measured by turbidimetric methods similar to those of Born and Cross¹ and O'Brien⁹. To 1 ml. of platelet suspension were added saline, the plasma or serum fraction to be examined, and calcium chloride. The pH of the mixture was then titrated to 8.3 by the addition of 0.1 N sodium hydroxide, and the reaction was initiated by the addition of ADP. The final volume of the reaction mixture was 2.5 ml. The final calcium chloride concentration was 7×10^{-3} M. Final concentration of ADP was 10^{-4} M. The optical density of the suspension was determined at 60 mμ in a spectrophotometer placed over a rotating magnet. The platelets were continuously stirred by a 'teflon'-coated bar placed in the cuvette. The optical density contributed by the platelets was determined by adjusting to zero the absorbance of an identical reaction mixture in which platelet buffer replaced the platelet suspension. The optical density of the platelets was determined immediately and at 60 sec following the addition of ADP. The decrease in absorbance was expressed as the percentage of initial optical density:

$$\frac{\Delta \text{ optical density in 60 sec}}{\text{initial optical density}} \times 100$$

The stimulation of ADP-induced platelet aggregation by saline, by citrated human plasma heated to 56° for 10 min (from which the precipitated fibrinogen had been removed by centrifugation), by thrombin-free human serum heated at 56° for 10 min, and by human fibrinogen fraction I-0 is shown in Table 1. In the presence of saline alone some aggregation of platelets occurred. There was little additional aggregation when heated plasma or heated serum was added individually. Whole plasma produced a marked increment of platelet aggregation. Fibrinogen alone stimulated platelet aggregation, but not to the same extent as whole plasma. In contrast, the addition of heated plasma and fibrinogen together, or of heated serum and fibrinogen together, reconstituted the entire activity of the whole plasma. Identical results were obtained using whole plasma, heated plasma or serum drawn from the patient with von Willebrand's disease (Table 1). Heated plasma dialysed extensively against 0.154 M saline was as effective as non-dialysed plasma when added to fibrinogen. The entire activity of the heated plasma could be recovered in the fraction precipitated by ammonium sulphate at 45 per cent saturation. In other experiments purified human fibrinogen (94 per cent clottable protein) was used instead of fraction I-0 (70 per cent clottable protein). No significant differences were noted when the more purified preparation was used.

The concentration dependence of the ADP-stimulated reaction on whole plasma, on heated plasma in the presence of non-limiting amounts of fibrinogen, and on

Table 1. INFLUENCE OF WHOLE PLASMA, HEATED PLASMA, HEATED SERUM, AND FIBRINOGEN ON ADP-INDUCED AGGREGATION OF WASHED HUMAN PLATELETS*

Addition (ml.)	Δ Absorbance (per cent)	
	Normal †	von Willebrand ‡
Whole plasma, 0.8	29.1	36.5
Fibrinogen (5.5 mg/ml.), 0.3	8.9	8.9
Heated plasma, 0.8	2.9	4.1
Heated serum, 0.8	7.8	5.5
Heated plasma, 0.8 + fibrinogen, 0.3	27.9	37.0
Heated serum, 0.8 + fibrinogen, 0.3	29.9	32.5

* Stimulation of ADP-induced aggregation above that seen in the presence of saline alone. The average Δ absorbance of 5 experiments with saline alone was 9.3 per cent.

† Average of 4 experiments.

‡ In these experiments washed normal platelets were used. No significant differences were observed when von Willebrand platelets were used with either normal or von Willebrand plasma or serum.

fibrinogen in the presence of non-limiting amounts of heated plasma is shown in Figs. 1 and 2. The percentage decrease in optical density due to saline alone has been subtracted from each point plotted. The shape of the whole-plasma curve is complex. A plateau of activity was repeatedly observed between 0.05 and 0.12 ml. of plasma, suggesting the interaction of more than one plasma component. No plateau was evident in the heated plasma plus fibrinogen curve which demonstrated consistently greater activity than that of the corresponding concentration of whole plasma until maximum activity was reached. Similarly, no plateau was observed in the fibrinogen curve, which reached maximal activity at a final fibrinogen concentration of 0.34 mg/ml. Similar curves were obtained with the von Willebrand's plasma.

It is apparent that the heat-stable protein is not consumed in coagulation, since heated serum was as effective as heated plasma. It is also evident that the heat-stable plasma factor required for ADP-induced platelet aggregation is not missing in the plasma of a patient with classical von Willebrand's disease. The mechanism whereby fibrinogen stimulates the ADP-induced aggregation is not immediately apparent. It is clear, however, that the participation of fibrinogen is not dependent on a thrombin catalysed conversion to fibrin, since the aggregation proceeds fully in the presence of heparin. In addition, electron micrographs of ADP-induced platelet aggregates show no evidence of fibrin formation. These findings are similar to those reported by Caen⁶.

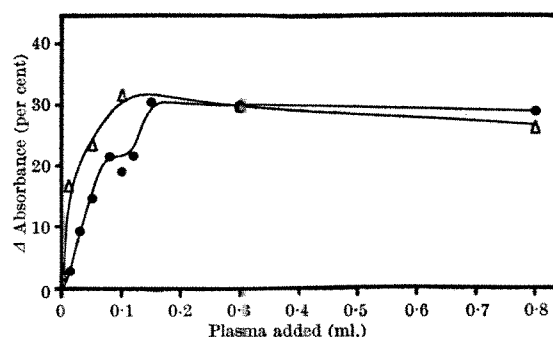


Fig. 1. Influence of plasma concentration on ADP-induced platelet aggregation. Solid circles indicate effect of increments of whole plasma. Open triangles indicate effect of increments of heated plasma in the presence of non-limiting fibrinogen concentration.

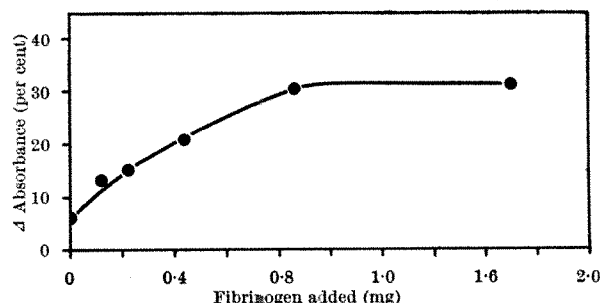


Fig. 2. Influence of fibrinogen concentration on ADP-induced platelet aggregation, in the presence of non-limiting heated plasma concentration.

These experiments demonstrate that at least two plasma factors, fibrinogen and a heat-stable plasma protein, distinct from the anti-von Willebrand factor, participate in ADP-induced platelet aggregation in the presence of human plasma.

This work was supported by U.S. Public Health grant HE-06316. We thank Dr. Herbert S. Strauss, Children's Medical Center, Boston, Mass., for making available to us the patient with von Willebrand's disease. We also thank Dr. Alan Johnson, New York University School of Medicine, for providing us with the purified fibrinogen.

One of us (D. D.) is the recipient of a career development award from the U.S. National Heart Institute.

DANIEL DEYKIN
CAROLE R. PRITZKER
EDWARD M. SCOLNICK

Beth Israel Hospital,
Boston, Massachusetts.

¹ Born, G. V. R., and Cross, M. J., *J. Physiol.*, **170**, 397 (1964).

² Hellem, A., and Owren, P. A., *Acta Haemat.*, **31**, 230 (1964).

³ Salzman, E. W., and Chambers, D. A., *Nature*, **204**, 698 (1964).

⁴ Cross, M. J., *Thromb. Diath. Haem.*, **12**, 524 (1964).

⁵ McLean, J. R., Maxwell, R. E., and Hertler, D., *Nature*, **202**, 605 (1964).

⁶ Caen, J. P., *Nature*, **205**, 1120 (1965).

⁷ Aster, R. H., and Jandl, J. H., *J. Clin. Invest.*, **43**, 843 (1964).

⁸ Gaintner, J. R., Jackson, D. P., and Maynert, E. W., *Bull. Johns Hopkins Hospital*, **111**, 185 (1962).

⁹ O'Brien, J. R., *J. Clin. Path.*, **15**, 446 (1962).

A Modification System for Fingerprinting Haemoglobin

THE technique of 'fingerprinting' developed by Ingram¹ has proved to be of great value in the molecular analysis of haemoglobins. In principle, it is a combined paper electrophoresis and chromatography of the peptides released by enzymatic digestion of haemoglobin. This enables the location of a probably occurring difference to a particular peptide. The method has proved its importance especially in the detection of amino-acid differences in normal and abnormal haemoglobins. This communication describes a modified system for fingerprinting haemoglobins.

In Ingram's procedure the first stage, paper electrophoresis, is performed in Miehl's volatile buffer²: pyridine-glacial acetic acid-water (20:0.4:20 by vol.) pH 6.4. Another volatile buffer, pyridine-glacial acetic acid-water (1:10:20 by vol.) pH 3.6, is preferred when only one-dimensional electrophoretic separation is desired. Ascending chromatography (stage 2) using the solvent *n*-butanol-glacial acetic acid-water (30:10:10 by vol.) was used by Ingram in his first investigation. Baglioni³ has obtained improved separations with a solvent made up of pyridine-isoamylalcohol-water (35:35:30 by vol.).

The solvent butanol-acetic acid-pyridine-water (50:72:15:60 by vol.), as proposed by Hill *et al.*⁴, has also been used with success. The disadvantage of a system with the strongly smelling pyridine is that it requires special conditions.

In our work on animal haemoglobins we have found that buffer made from ammonia-formic acid, and a solvent made from 2,6-lutidine-water, are very satisfactory for fingerprinting of haemoglobins. Equal amounts of 1 M ammonia and 0.5 N formic acid are mixed (final pH 6.5). If one-dimensional electrophoretic separation is desired, ammonia (0.5 M)-formic acid (1 N) buffer of pH 3.6 is preferred. The buffers can be used several times, but the pH should be checked between each running.

Whatman paper 3 MM, dimension 48 cm × 38 cm, corresponding to the size of the apparatus used in our work (AB Analysteknik, Vallentuna, Sweden), is dipped into buffer and excess liquid removed by blotting firmly between sheets of dry filter paper. The paper is then placed on a horizontal siliconized glass plate, which is cooled with water. After application of the sample the moist paper is covered with a sheet of polyethylene, and a weight is placed on the glass plate. Since the apparatus consists of two electrophoresis cells two papers could be run at the same time. A voltage of 30 V/cm is applied for 2.5 h. The papers were dried at 60° in a current of air for not less than 1 h.

Descending chromatography in 2,6-lutidine-water (122:60 by vol.) was carried out for about 20 h. Since the solvent is running rather fast (3.5 cm/h) the bottom edge of the paper is serrated with scissors as this assists even flow of the solvent off the end and helps to straighten any irregular solvent front. The paper is again dried in a current of air at 60°. The peptide spots were revealed by dipping the paper into 0.2 per cent ninhydrin in acetone, and allowing development to take place at room temperature or in a warm place not more than 40°. When a specifically staining test was needed, the ninhydrin colour was blached by dipping the paper in a solution of 1 N hydrochloric acid-acetone (1:4)⁵ followed by development with the reagents for sulphur⁶, tryptophan⁷ and histidine⁷. Alternatively, the sequence ninhydrin-sulphur and tyrosine reagent⁸ was used.

In Fig. 1 are shown the fingerprints of tryptic digest of cattle haemoglobin *F* and *B* obtained with this system. More than 20 peptides can be seen. The details of the result will be given elsewhere⁹.

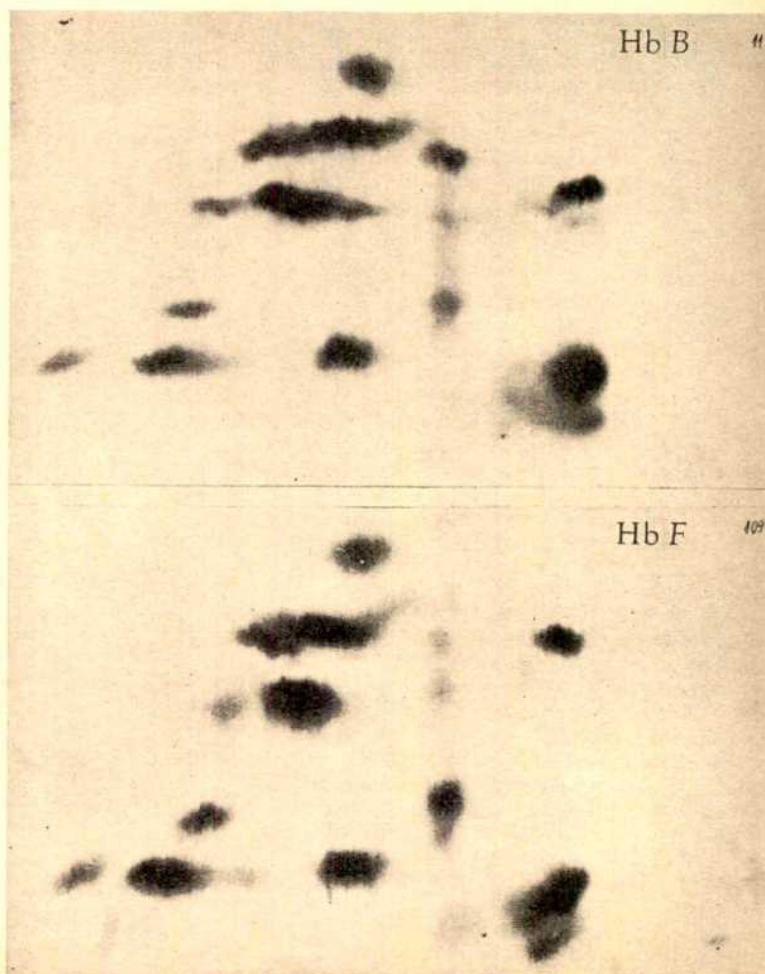


Fig. 1. Photograph of fingerprinting of cattle haemoglobin *B* and *F* in ammonia-formate buffer, pH 6.5, and 2,6-lutidine-water solvent.

his work was supported by grants from the Agricultural Research Council of Norway and Niels Thorshaugs d. I thank Prof. H. Stormorken, head of the Institute of Thrombosis Research, University of Oslo, for providing working facilities, and Mr. A. Bernhardsen for advice.

G. EFREMOV*

Department of Medicine,
Veterinary College of Norway,
Oslo.

Present address: Department of Physiology, Faculty of Agriculture
University of Skopje, Skopje, Yugoslavia.

gram, V. M., *Biochim. Biophys. Acta*, **28**, 539 (1958).
ohl, H., *Monatsch. Chem.*, **82**, 489 (1951).
glioni, C., *Biochim. Biophys. Acta*, **48**, 392 (1961).
I, R. L., Swenson, R. T., and Schwartz, H. C., *Blood*, **19**, 573 (1962).
nnies, G., and Kolb, J. J., *Anal. Chem.*, **23**, 189 (1951).
ith, I., *Nature*, **171**, 43 (1953).
ldridge, R. C., and Lewis, H. B., *J. Biol. Chem.*, **202**, 169 (1953).
her, R., and Crocker, C., *Biochim. Biophys. Acta*, **9**, 704 (1952).
remov, G., Brænd, M., and Osterhoff, D. R. (in preparation).

PATHOLOGY

Blood Group Secretion Factors in Bronchogenic Carcinoma

As a continuing study of our original report which dealt with investigations of salivary blood group secretion in metastatic cancer patients¹, we undertook an analysis of the frequencies of secretion (Se) and non-secretion (sese) related to particular cancer sites. This approach has been used by Desai and Creger² in gastric cancer and by several investigators in benign disease conditions³⁻⁵. The original premise, as extensively used in blood types and disease analyses⁶, is that these genetic factors will remain stable and not fluctuate during a disease process.

As the numbers of cancer cases increased in the various categories of organ sites, our attention was drawn to the high secretion (Se) rate among the bronchogenic cancer patients. This selection of secretors did not appear in the other high-incidence tumours such as colon and breast cancers. We thoroughly reviewed, therefore, all the bronchogenic tumours in this series to eliminate any possible metastatic lesions and found 88 primary bronchogenic carcinomata falling into the three main histological divisions of squamous, undifferentiated and adenocarcinoma. Whether metastases had occurred or not did not concern us in this investigation since our observation was designed only to record ABO blood groups and the saliva secretor or non-secretor factors (Se or sese respectively).

Table 1 shows the secretor status of patients with the three histological types of bronchogenic tumours. According to the usual distribution of secretion of ABH in the saliva, 20-2 of these 88 patients should have been non-secretors, whereas only five appeared. One of the five non-secretors had a bronchogenic adenoma of questionable malignancy, but was included in order not to show any bias. The scarcity of non-secretion in each histological group shows that there is no selection for one type. A χ^2 test of significance reveals a high value when compared with our control series and indicates a probability inconsistent with a chance occurrence. The number of tumours in each histological group is comparable with a large national series⁷, and, therefore, supports the assumption that our case distribution is within reasonable bounds of experience gained from other studies.

Table 2 gives the ABO blood groups of lung cancer and control cases. Although an excess of group A individuals and fewer group O individuals were afflicted with bronchogenic carcinoma compared with controls, the χ^2 test does not reveal a significant deviation from the controls. Similar results have already been recorded by McConnell

Table 1. SECRETION FACTORS IN HISTOLOGICALLY PROVED PRIMARY BRONCHOGENIC CARCINOMA

Source	Squamous		Undifferentiated		Adenocarcinoma		Total
	Se	sese	Se	sese	Se	sese	
Holy Cross	10	1	13	0	9	1	34
St. Mark's	23	2	23	1	5	0	54
Total	33	3	36	1	14	1	88

Se, secretor, sese, non-secretor.

	χ^2 Bronchogenic cancer		Control*
	Secretors	Non-secretors	
	83	5	721
			223

$\chi^2 = 13.10$ or $P = 0.001$.

* 944 normal controls as published (ref. 1).

Table 2. COMPARISON OF BLOOD GROUPS OF CANCER OF LUNG AND CONTROLS

	Cancer		Control	
	No.	Percentage	No.	Percentage
O	34	39	265	46
A	42	48	222	38
B	7	8	67	12
AB	4	5	24	4
Total	87	100	578	100

$\chi^2 = 1.68$, $P = 0.1$.

et al.⁸, but their excess of A was only noted in cases of undifferentiated bronchogenic carcinoma.

This finding of excess secretion of blood group substances among patients with bronchogenic carcinoma seems to parallel to some extent the findings of Desai² in patients with stomach cancer. Both mucosal surfaces are potent secretors of blood group substances whereas, in contrast, we do not obtain a significant deviation in colon cancer where the mucosa is low in blood group substance. Two possible explanations immediately occur: (1) that water-soluble blood group substances attract or alter carcinogens, favouring neoplasia or, perhaps, (2) that Lewis substance, the material produced in greater quantity by non-secretors, might reject, because of its chemical configuration, a carcinogen making it less effective.

Another interesting possibility lies in the known content of sialic acids in blood group substances. This component of mucopolysaccharides adheres closely to cell surfaces and is known to affect the 'stickiness' of cells⁹. A combination of sialic-acid-containing mucoids, which are known to be elevated in lung cancer⁸, and a carcinogen could conceivably alter the mucosal cell surface by prolonged stimulation bringing about dysplastic and neoplastic changes. Whatever the actual mechanism involved, this finding offers a valuable lead in the future investigation of bronchogenic carcinoma.

This work was supported by research grant C 5965 from the National Cancer Institute, Public Health Service, Department of Health, Education and Welfare, Bethesda, Maryland.

CRICHTON MCNEIL
ELMER F. TRENTLMAN
J. NOEL LADLE
WILLA MAE HELMICK
HENRY P. PLENK

Holy Cross Hospital Research Foundation,
Holy Cross Hospital,
and
Department of Pathology,
University of Utah College of Medicine,
Salt Lake City.

¹ McNeil, C., Trentelman, E. F., Ladle, J. N., and Plenk, H. P., *Amer. J. Clin. Path.*, **42**, 498 (1964).

² Desai, R. G., and Creger, W. P., *Transfusion*, **4**, 188 (1964).

³ Cameron, C., Graham, F., Densford, I., Sickles, G., Macpherson, C. R., Cahan, A., Sanger, R., and Race, R. R., *Brit. Med. J.*, **ii**, 29 (1959).

⁴ Clarke, C. A., Evans, D. A. E., McConnell, R. B., and Sheppard, P. M., *Brit. Med. J.*, **i**, 603 (1959).

⁵ Freiesleben, E., Kissmeyer-Nielsen, F., Christensen, J., Jensen, K. G., and Knudsen, E. E., *Vox Sang.*, **6**, 304 (1961).

⁶ McConnell, R. B., Clarke, C. A., and Downton, F., *Brit. Med. J.*, **ii**, 323 (1954).

⁷ Herman, D. L., and Critenden, M., *J. Nat. Cancer Inst.*, **27**, 1227 (1961).

⁸ Gasie, G., and Gasie, T., *Proc. U.S. Nat. Acad. Sci.*, **48**, 1172 (1962).

⁹ Parrella, M., DiPerna, A., and Carratio, L., *Arch. Tiscol.*, **19**, 73 (1964).

Induction of Intestinal Tumours by *N*-Ethyl-*N*-nitrosourethane

INTESTINAL tumours, though very rare in laboratory rodents, have been induced by several types of carcinogenic agents, including polycyclic aromatic hydrocarbons, 4-dimethylaminostilbene, 2-acetyl-aminofluorene and radiation^{1,2}. However, so far, no such tumours have been induced by alkyl nitroso-compounds, which are able to induce tumours in many organs³. Attempts to induce tumours of the colon by repeated rectal application of diethylnitrosamine led to liver, but not to local, tumours⁴.

It is of interest, therefore, that when *N*-ethyl-*N*-nitrosourethane (ENU) in 50 per cent aqueous ethanol was given to rats, males and females, by intraperitoneal injections, 3 out of 6 rats which survived more than a year developed adenoma and adenocarcinoma of the mucosa of the ileum. The tumours were multiple and were found in two male and one female rat, killed 16.5, 20 and 18 months after the first and 6, 9.5 and 7.5 months, respectively, after the last of four doses of ENU, about 20 mg per rat, *in toto*. The tumours formed large cauliflower-type nodules, up to 1.5 cm diameter, obstructing the lumen, and caused enormous distension of this part of the intestine. Some of the nodules penetrated into the muscle layer of the intestinal wall. *N*-Methyl-*N*-nitrosourethane, the methyl-homologue, is only now being tested by the intraperitoneal route in rodents.

R. SCHOENTAL

Medical Research Council Laboratories,
Toxicology Research Unit,
Woodmansterne Road,
Carshalton, Surrey.

¹ Fischer, W., and Kühn, I., *Geschwülste der Laboratoriumsnagetiere* (T. Steinkopff, Dresden, 1958).

² Schoental, R., and Bensted, J. P. M., *Brit. J. Cancer*, **17**, 242 (1963).

³ Magee, P. N., and Schoental, R., *Brit. Med. Bull.*, **20**, 102 (1964).

⁴ Schmähli, D., Thomas, C., and König, K., *Z. Krebsforsch.*, **65**, 529 (1963).

BIOLOGY

Influence of Hexadecane on Absorption of Carbon Dioxide by Plants

RESULTS of recent investigations concerning the influence of hydrocarbons and a petroleum oil on apparent photosynthesis in *Citrus sinensis*¹ and *Bauhinia blakeana*² have focused my attention on the significance of stomatal distribution in a plant's response to the application of hydrocarbons. For example, using infra-red carbon dioxide analysis with sequential multi-point sampling³, it has been shown that an application of a paraffinic hydrocarbon to either the upper or lower surface of the leaves of

Bauhinia blakeana results in a decrease of absorption carbon dioxide while a similar application to the leaf of *Citrus sinensis* results in a decrease of absorption carbon dioxide only when applied to the lower surface of the leaves. Since these results are corollary to the distribution of stomata in these species they suggest penetration only through stomata with consequential interference absorption. The present investigation was initiated in order to clarify further the role of stomatal distribution.

With the following exceptions, infra-red carbon dioxide analysis as previously described³ was used. Leaves *Heliconia humilis* were enclosed in water-cooled plastic chambers which permitted sequential sampling for 5-min periods from either the lower or upper surfaces. The leaves were sufficiently illuminated from above to obtain maximum apparent photosynthesis (carbon dioxide absorptivity from both surfaces). After a pattern of carbon dioxide absorption was established for both surfaces, applications of hexadecane were made using a settling tower lead from an aerosol container. The amounts of hexadecane were determined gravimetrically.

The result of an initial application of 79 $\mu\text{g}/\text{cm}^2$ on the upper surface is shown in Fig. 1a, while the result of initial application of 126 $\mu\text{g}/\text{cm}^2$ on the lower surface is shown in Fig. 1b. For clarification the dark period (respiration or carbon dioxide evolution) have been omitted. Corrections have been made for minor fluctuations carbon dioxide in air.

Initially, and as anticipated from the distribution of stomata in this species, approximately 20 times as much carbon dioxide is removed from the air stream passing over the lower surface as on the upper, a greater amount carbon dioxide is removed from the air stream passing over the lower surface⁴. With the application of hexadecane to either surface a depression of carbon dioxide absorption obtained. It should be noted that the application to the upper surface did not interfere with absorption from the lower surface; and, conversely, the application to the lower surface did not interfere with absorption from the upper surface. These results suggest the lack of complete hydrocarbon penetration at the levels used.

Recovery from the application appears to correlate with the dissipation of the hydrocarbon. Complete recovery from the application applied to the lower surface occurred in about 6 h, while complete recovery from the application to the upper surface was not apparent until after stomata opening the following morning. The variance between the time of complete evaporation of the hydrocarbon from the aluminium paper strips and complete recovery of carbon dioxide absorption can undoubtedly be attributed to the 'threshold levels' that result from sorption.

The results unequivocally demonstrate the significance of stomatal distribution in a plant's response to the

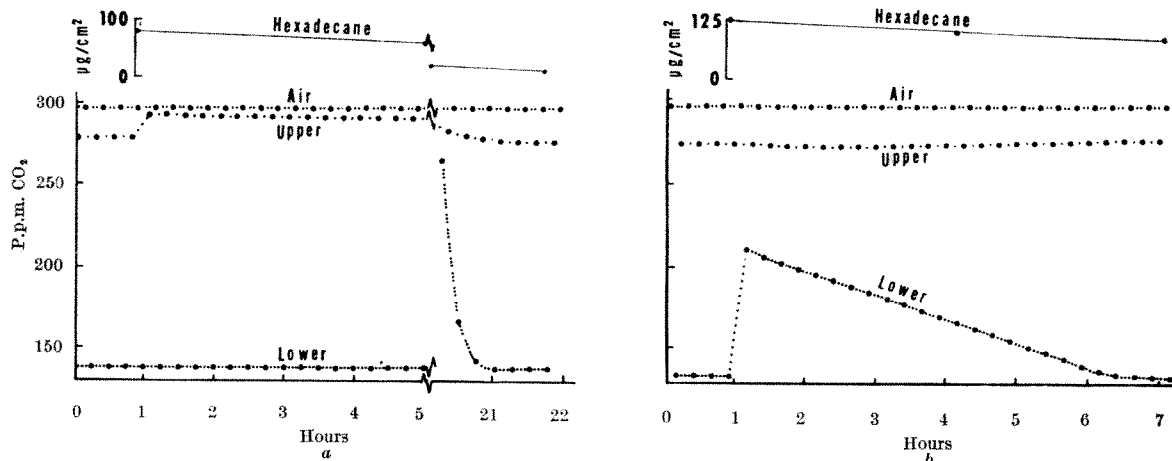


Fig. 1. Concentration, p.p.m. of carbon dioxide in air stream from 3 channels sampled sequentially. Each large dot represents one 5-min sampling period. Solid line represents evaporation of hexadecane from aluminium paper strips, $\mu\text{g}/\text{cm}^2$. a, Application to upper surface; b, application to lower surface. Dark periods not shown.

lication of hydrocarbons along with the nature and the amount of hydrocarbon applied. They also suggest, since these experiments no visual injury was apparent, a more ical definition of 'phytotoxicity'.

This work was supported by the Petroleum Research and administered by the American Chemical Society.

J. M. RIEDHART

Department of Botany,
University of Miami,
Coral Gables, Florida.

Millan, R. T., and Riedhart, J. M., *Proc. Fla. State Hort. Soc.* (in the press).

Riedhart, J. M., *Proc. Amer. Soc. Hort. Sci.* (in the press).

Riedhart, J. M., *Agric. Chem.*, **19**, 26 (1964).

Run, W. A., *Plant Physiol.*, **36**, 399 (1961).

Social Companions and the Mother-Infant Relationship in Rhesus Monkeys

THE development of behaviour in a young primate is influenced by both the physical and social characteristics of its environment. Paramount among the latter is the mother¹, while later other infants are important as play companions². Previous investigations^{3,4} of group-living rhesus monkeys suggested that adult females, other than the infant's own mother, might also play an important part, both by themselves showing maternal, social, play, aggressive and other types of behaviour towards the infant and by influencing the nature of the mother-infant relationship. For example, one adolescent female which, with the male's support, frequently interacted with the two infants in the same group, made their mothers extremely restrictive. In consequence, the infants lived confined lives: while seven other group-living infants were recorded more than 2 ft. from their mothers in a mean of 37 per cent of our observation periods when 7-18 weeks old, these two practically never went this far from their mothers up to this age.

In the experiment recorded here we attempted to assess the extent to which such a factor operates in other group-living infants where the effect is not so obvious. Nine mother-infant pairs each living in groups of a male, three or four females, and their young, were compared with four mother-infant pairs living alone. In both cases the cages were 18 ft. x 10 ft. x 8 ft., communicating with an indoor room 6 ft. x 6 ft. x 8 ft. (ref. 5). Routine watches were made between 0900 h and 1300 h G.M.T. or U.S.T. for at least 6 h a fortnight until the infants were a year old. Data were recorded on check sheets by 0.5 min periods. The levels of significance are based on the Wilcoxon matched-pairs signed-ranks test, 1-tailed, unless otherwise stated.

Differences between the group-living and the isolated mother-infant pairs were of two main types. First, the isolate infants ranged more freely. They spent less time on the nipple, less time on their mothers but off the nipple, more time off their mothers (Fig. 1), went to a distance of 2 ft. from their mothers more often, and spent longer bouts off their mothers (all $P < 0.005$). An increase in distance between mother and infant from less than 2 ft. to more than 2 ft. was more often due to the mother moving away in the isolates than in the group-living pairs, and a decrease was less often due to the mother (both $P < 0.005$).

A second group of differences between isolate and group-living mother-infant pairs we ascribe to the absence of other social companions for the former. Thus, although the isolate infants went to a distance from their mothers more frequently than the group-living ones, the number of 0.5 min periods spent wholly more than 2 ft. from their mothers during the second half year was less. Similarly, the frequency with which the 2-ft. radius around the mother was crossed was greater for the isolates (both $P < 0.005$). The isolate infants thus returned to the proximity of their mothers more often. The proportion

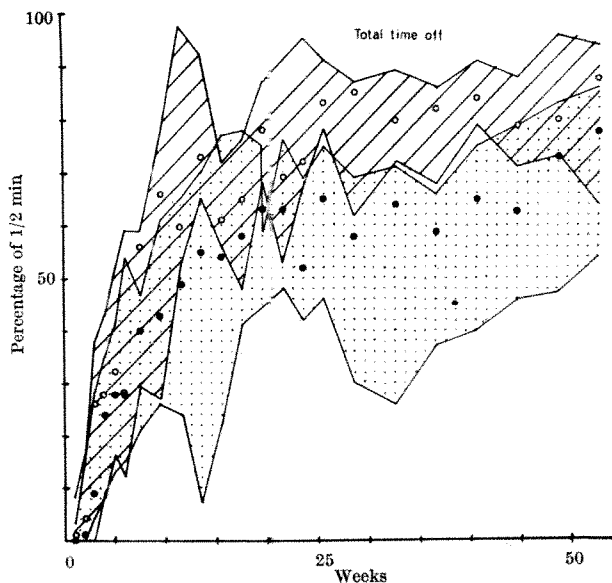


Fig. 1

of attempts made by the infants to attach to the nipple which led to acceptance by the mother was lower for the isolates ($P < 0.04$, 2-tailed sign test). Furthermore, grooming of both infant by mother ($P < 0.005$) and mother by infant (not significant) was higher for the isolates.

The results thus indicate that the relationship between rhesus mother-infant pairs is influenced in at least two ways by other members of the group. The presence of other adult or adolescent females results in the young infant ranging less freely from its mother, the effect being mediated largely through the mother's behaviour. The presence of social companions results in the infant returning to its mother less often than it would in their absence, and in consequence being rejected by her less often.

Y. SPENCER-BOOTH
R. A. HINDE
M. BRUCE

Sub-Department of Animal Behaviour,
Madingley, Cambridge.

¹ Harlow, H. F., and Harlow, M. K., *Bull. Menninger Clinic*, **26**, 213 (1962).

² Harlow, H. F., in *Research Approaches to Psychiatric Problems*, by Tourlentes, T. T., Pollack, S. L., and Heimlich, H. E. (1962).

³ Hinde, R. A., Rowell, T. E., and Spencer-Booth, Y., *Proc. Zool. Soc. Lond.*, **143**, 609 (1964).

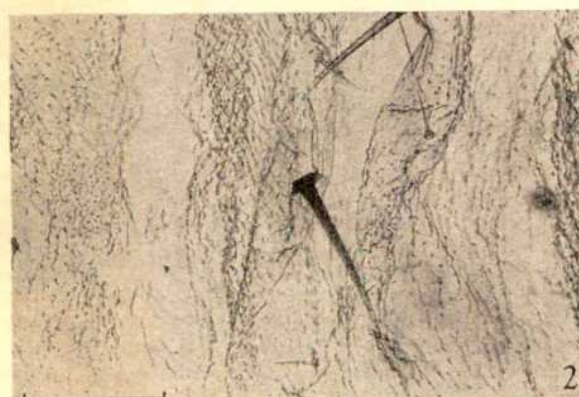
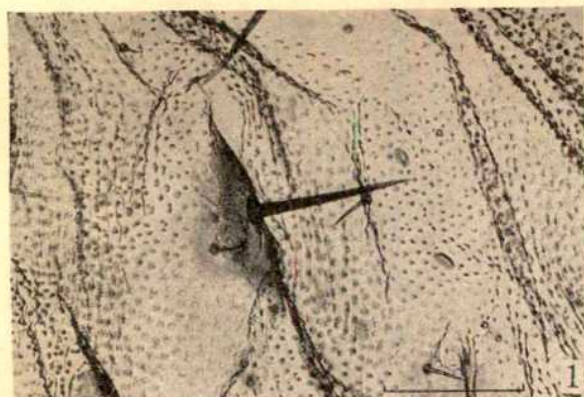
⁴ Rowell, T. E., Hinde, R. A., and Spencer-Booth, Y., *Anim. Behav.*, **12**, 219 (1964).

⁵ Hinde, R. A., and Rowell, T. E., *Proc. Zool. Soc. Lond.*, **138**, 1 (1962).

ENTOMOLOGY

Bilateral Asymmetry: a Larval Polymorphism in some Argid Sawflies (Hymenoptera, Symphyta)

BILATERAL asymmetry is virtually unknown among insects, other than as a rare aberration. Pasteels¹ has briefly noted the occurrence of colour asymmetry in larvae of *Arga ustulata* (L.) in Belgium and the Tyrol. Intensive collecting in south-eastern England and north-western Scotland has shown British populations of *A. fuscipes* (Fallén) and *A. ustulata* feeding on birches (*Betula pendula* Roth. and *B. pubescens* Ehrh.) to consist of symmetrical and asymmetrical individuals. Both forms appear to be present in proportions too large to be attributable to recurrent mutation alone. Unfortunately, however, samples from particular localities have so far been too small to allow accurate frequency determinations. Nevertheless, assuming genetic control,



Figs. 1 and 2. Flat mount in 'Euparal' of cast cuticle of asymmetrical last instar larva of *A. fuscipes* showing detail of cuticle of first abdominal segment. Fig. 1, part of darker left side. Fig. 2, corresponding part of right side. Scale, 0.2 mm.

this phenomenon would appear to constitute a true polymorphism (Ford²).

The symmetrical larvae of both *A. fuscipes* and *A. ustulata* are pale green on both sides, while the asymmetrical forms have one side distinctly darker green than the other. In all British examples so far examined the left has been the darker side, contrasting with the condition described by Pasteels¹ from continental examples in which the right was always the darker. The asymmetry seems to be a property of the cuticle alone. In our material the deposition of melanin pigment was heavy in the cuticle of the left side but negligible on the right side (Figs. 1 and 2). On the dark side the pigment is concentrated into small, closely scattered, spots which appear to have coalesced to form larger areas at the bases of the major bristles (Fig. 1). This distribution of melanin has the effect of darkening the outward appearance of the underlying green pigment.

The larvae of *A. fuscipes* and *A. ustulata* are leaf edge-feeders, particularly on birches in Britain. Asymmetrical larvae have always been found in the field feeding with their darker left sides uppermost, and they will readopt this attitude after being disturbed. The food-plant leaves are considerably darker green above than below so that an asymmetrical larva correctly oriented is remarkably cryptic to the human eye when viewed from either side. In view of the selective advantage this type of asymmetry would appear to confer on edge-feeding larvae, it is remarkable that it is such a rare phenomenon. Even in *Arge* larvae presumably there must be some associated disadvantages which serve to maintain the polymorphism (Ford³).

The situation is further complicated by taxonomic difficulties. *A. fuscipes* and *A. ustulata* are very closely allied forms and their larvae cannot at present be separated with certainty. Preliminary breeding experiments suggest that the two 'species' may be only seasonal forms

of a single species. It is hoped to carry out larger-scale breeding work with material from different areas in order to solve the taxonomic problem. Genetic analysis means of large-scale rearings is also urgently needed. It should be noticed that the adult sawflies show no obvious asymmetry whatever their larval type.

M. F. CLARIDGE
J. M. EDINGTON

Department of Zoology,
University College,
Cardiff.

¹ Pasteels, J., *Bull. Ann. Soc. Ent. Belg.*, **88**, 54 (1952).

² Ford, E. B., 'Polymorphism and Taxonomy' in *The New Systematics*, ed. by Huxley, J. (Oxford University Press, 1940).

³ Ford, E. B., *Ecological Genetics* (Methuen, 1964).

Feeding Mechanism of Blood-sucking Arthropods

It now appears to be established¹⁻⁶ that blood-sucking arthropods feed, in the main, either from the lumen of a blood vessel or from a blood pool resulting from the laceration of blood vessels. Occasionally an insect which is a vessel feeder will take part of its nourishment from a blood pool; but, except for the mosquito, this appears to be of unusual occurrence. *In vivo* observations on no sensitized hosts have shown that a considerable number of argasid and ixodid ticks, at least one horsefly and the tsetse fly are pool feeders, while several fleas, three species of triatomine bugs and the bed-bug are vessel feeders. The present consensus of opinion is that the mosquito is largely a vessel feeder, although Gordon *et al.*¹ give reasons for believing that a proportion, at least, of the insect blood meal is derived from haemorrhages formed in the tissues.

While the list of arthropods on which investigations of this nature have been carried out may look imposing, there are, nevertheless, some significant gaps in our knowledge. Hitherto, for example, no information has been available for such medically important insects as the stable-fly, biting midges, simuliid flies and lice. It is in order to provide some of the answers that the investigations on the stable-fly (*Stomoxys calcitrans*), the valley black-gnat (*Leptoconops torrens*) and the hog-louse (*Haematopinus suis*), summarized in this communication, have been undertaken.

These investigations have shown that both the stable-fly and the valley black-gnat, on laboratory white mice, are true pool feeders. The stable-fly possesses a proboscis which is not unlike that of the tsetse-fly but the labellar teeth are much coarser. The proboscis is introduced into the tissues somewhat like a cylindrical drill, the stout labellar teeth producing a haematoma from which the fly feeds. On the other hand, the feeding mechanism of the valley black-gnat generally resembles that of the horse-fly, *Haematopota pluvialis*. The mandibles cut the tissues with a scissor-like movement while the maxillae move rapidly to and fro.

Observations on *Haematopinus suis* were carried out on the ear of a laboratory white mouse. Following the introduction of the proboscis into the skin, the haustellum is everted and the strikingly flexible stylet bundle probes the tissues until a suitable blood vessel is located. This work has shown unequivocally that the hog-louse is a true vessel feeder.

Arthropods which feed from small vessels have hitherto been known as 'capillary feeders'. The term 'capillary feeder' cannot, however, be regarded as strictly correct if the capillary is defined as a small vessel of approximately 7 μ in diameter⁷, since observations on feeding carried out over the past few years have shown that feeding is not generally from capillaries but usually from venules or small veins. The term 'vessel feeding' should replace 'capillary feeding' before usage fossilizes the expression.

Finally, attention needs to be directed to the difficulty experienced in translating succinctly into certain foreign languages the expressions 'pool feeder' and 'vessel feeder'; neither the term must be directly borrowed from English, which is not always satisfactory, or else requires cumbersome translating. The introduction of two terms derived from Greek roots is therefore proposed for more universal application, 'solenophage' (from the Greek $\sigma\omega\lambda\eta\nu$ = pipe and $\phi\alpha\gamma\omicron$ = eating) for vessel feeder, and 'telmophage' (from the Greek $\tau\acute{\epsilon}\lambda\mu\alpha$ = pool and $\phi\alpha\gamma\omicron$ = eating) for pool feeder.

This work was supported by U.S. Public Health Service research grant A1 04189-02 from the International Center for Medical Research and Training Program, Office of International Research, National Institutes of Health.

I thank Prof. James Douglas, professor of parasitology in the University of California School of Veterinary Medicine, Davis, California, where this work was carried out, for the provision of facilities.

M. M. J. LAVOPIERRE

University of California International Center for Medical Research and Training (Cooper Foundation), c/o Department of Zoology, University of Singapore.

Lavoipierre, M. M. J., Dickerson, G., and Gordon, R. M., *Ann. Trop. Med. Parasitol.*, **53**, 235 (1959).

Dickerson, G., and Lavoipierre, M. M. J., *Ann. Trop. Med. Parasitol.*, **53**, 347 (1959).

Dickerson, G., and Lavoipierre, M. M. J., *Ann. Med. Parasitol.*, **53**, 465 (1959).

Lavoipierre, M. M. J., and Hamachi, M., *Nature*, **192**, 998 (1962).

Gordon, J. D., *Acta Trop.*, **Basel**, **17**, 48 (1960).

Sutton, E., and Arthur, D. R., *Symp. Zool. Soc., Lond.*, **6**, 223 (1962).

Zweifach, B. W., *Sci. Amer.*, **200**, 54 (1959).

MICROBIOLOGY

Metabolism of Nucleic Acids and Protein in Starving Bacteria

DURING an investigation of metabolic processes in starving bacteria we observed that a significant net synthesis of deoxyribonucleic acid (DNA) took place in *hem*¹; no increase in the amount of ribonucleic acid (RNA) and protein could be detected (unpublished results).

The procedure in the foregoing experiments was as follows. Log-phase cultures of *Escherichia coli* B grown in a minimal medium were collected by centrifugation at an optical density of about 0.200, washed and re-incubated in acetate buffer (pH 7) at 37° C with aeration. Samples for biochemical analysis were taken immediately after resuspension and at the time intervals indicated in Fig. 1.

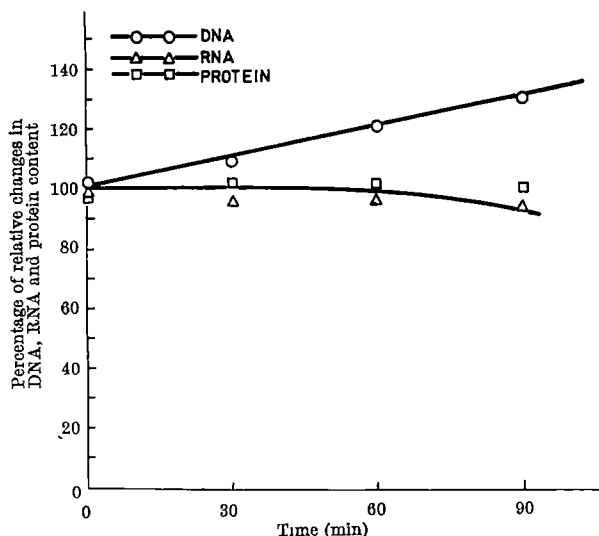


Fig. 1. DNA, RNA and protein content in *Escherichia coli* B incubated in acetate buffer. The content of these macromolecules in the bacteria at 0 min is taken as 100 per cent

Chemical fractionation of the bacteria, as well as the determination of DNA, was carried out by Burton's method², RNA was determined according to Mejbaum³, and protein as described by Lowry *et al.*⁴.

As seen in Fig. 1, the amount of DNA increased during 90 min incubation by about 30 per cent, while no biosynthesis of RNA or protein could be observed. Thus we postulated that the nucleic acid's precursor pool must be in this case preferentially utilized for DNA synthesis which is, consequently, favoured in some way in the starving bacteria.

To verify this hypothesis on a similar but more specific system, we used a 'uracil-less' mutant of *E. coli* B, which is capable of synthesizing only about 5 per cent of RNA and protein if no uracil is added to the growth medium.

E. coli B U- was incubated in '3 × D'-medium⁵, to which 30 µg/ml. uracil was added. At an optical density of about 0.150, 0.02 µc./ml. of ¹⁴C-uracil (specific activity 6.5 µc./µmole) was added to the suspension, and the incubation was continued for a further 2 min. The suspension was then quickly chilled, centrifuged in the cold, and washed twice by cold physiological saline. The bacteria were resuspended at the same optical density in cold '3 × D'-medium containing no added uracil, and re-incubated at 37° C with aeration. Samples for analysis were taken immediately after resuspension and after 10, 20 and 30 min incubation.

Chemical fractionation was carried out by a somewhat modified form of the Schmidt-Tannhauser method⁶. The radioactivity of the acid-soluble, as well as of the DNA- and of the RNA-containing fractions, was counted in a gas flow counter on planchets, after having dried on them 200 µl. of the corresponding extracts.

The results are summarized in Table 1. It is evident that there is a steady increase in the radioactivity of the DNA-containing fraction, while the activity of the acid-soluble fraction decreases rapidly. There is also a temporary increase of the radioactivity of the RNA-containing fraction, which, however, is not very significant if the initial high activity of RNA is taken into account (see data under B in Table 1).

Table 1. PERCENTAGE OF RADIOACTIVITY FROM ¹⁴C-URACIL IN THE DNA, RNA AND ACID-SOLUBLE FRACTIONS

Fraction	0 min		10 min		20 min		30 min	
	A (%)	B	A (%)	B	A (%)	B	A (%)	B
DNA	5.1	100	9.5	186	12.4	243	13.9	272
RNA	80.7	100	88.0	109	84.3	104.5	84.0	104
Acid-soluble	14.2	100	2.5	17.6	3.3	23.2	2.1	14.8

A, Percentage of total radioactivity from all three fractions.

B, Relative change in radioactivity, compared with counts at 0 min

Table 2

Fraction	0 min		20 min	
	A*	B	A	B
DNA	8.4	100	16.8	200
RNA	72.0	100	80.7	113
Acid-soluble	19.6	100	2.5	13

*A and B as in Table 1.

Data comparable with those in Table 1, but obtained in another experiment of the same type, are shown in Table 2. In this experiment DNA was isolated after 20 min incubation of the bacterial culture, by Spirin's method⁷, and hydrolysed by 72 per cent perchloric acid, the bases being isolated and separated by paper chromatography⁸.

After elution of the ultra-violet-absorbing spots, measurable radioactivity was found only in the spots with the R_F corresponding to those of cytosine and thymine, the specific activities (c.p.m./µmole) being: for cytosine, 0.929; for thymine, 0.790.

The presence in thymine of radioactivity comparable with that of cytosine indicates that the radioactivity from ¹⁴C-uracil was indeed incorporated into DNA molecules. This incorporation proceeds in spite of the very limited endogenous pool of uracil and goes on even when incorporation into RNA stops altogether.

These data seem to support our assumption as to the preferential utilization of nucleic acid precursors for DNA

synthesis in bacteria, incubated under starvation conditions or in a deficient medium.

The starving bacteria, incubated in acetate buffer, contain also an amino-acid pool which is, however, not utilized for protein synthesis, as can be seen in Fig. 1. This observation may point to the possibility that the limited potentiality of utilization of energy for anabolic processes in the starving cells is also oriented in the first place toward DNA synthesis. If this assumption was also shown to be correct, it would indicate that in our systems the kinetics of biochemical reactions on the precursor's level are such that processes leading to DNA synthesis are favoured over those leading to the synthesis of other macromolecules.

This work was performed with the financial support of the Federal Foundation for Scientific Research.

B. BRDAR
E. KOS
M. DRAKULIĆ

Laboratory of Cellular Biochemistry,
Institute Ruder Bošković, Zagreb, Yugoslavia.

¹ Drakulić, M., and Kos, E., *Radiation Research*, **19**, 429 (1963).

² Burton, K., *Biochem. J.*, **62**, 315 (1956).

³ Meybaum, W., *Z. Physiol. Chemie*, **258**, 117 (1939).

⁴ Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, J. R., *J. Biol. Chem.*, **193**, 265 (1951).

⁵ Fraser, D., and Jerrel, E. A., *J. Biol. Chem.*, **205**, 291 (1953). Goldstein, A., and Brown, B. J., *Biochim. Biophys. Acta*, **53**, 19 (1961).

⁶ Schmidt, G., and Tannhauser, S. J., *J. Biol. Chem.*, **161**, 83 (1945).

⁷ Spirin, A. S., and Belozerski, A. N., *Biokhimiya*, **21**, 768 (1956).

⁸ Kirby, K. S., *Biochim. Biophys. Acta*, **18**, 575 (1955).

VIROLOGY

Transmission of Guamá and Oriboca Viruses by Naturally Infected Mosquitoes

Most of the present knowledge about the ability of mosquitoes to serve as natural hosts for arthropod-borne viruses is based on two methods. The first consists of triturating the wild-caught mosquito and inoculating it into a laboratory animal; subsequent virus isolation proves natural infection of the mosquito but not ability to transmit. The second consists of infecting the mosquito on a viraemic laboratory host and demonstrating transmission by bite to another laboratory host. This method demonstrated the ability to transmit, but may not reflect what happens under natural conditions. The technique described here makes possible a demonstration of transmission by naturally infected arthropods.

Mosquitoes captured during the first half of 1964 in mouse- and chicken-baited traps in the Instituto Agronomico do Norte forest near Belém, Brazil, were identified while alive and liberated in screened holding cages (100 cm × 100 cm × 115 cm) in the forest. A separate cage was set up for each mosquito species examined. Families of 3-day-old Swiss mice with the mother mouse were placed in the cages to provide a blood meal. These mice were observed for subsequent illness on the chance that the wild-caught mosquitoes might have been naturally infected with arthropod-borne viruses and have transmitted them to the mice.

Between February 24 and June 30, 2,846 *Culex (Melanoconion) taenopus* females were released in their cage and 68 families of mice exposed, each for a 24-h period. Guamá virus was isolated from the blood of a mother mouse exposed on April 14.

Between January 30 and June 30, 2,860 *Culex (Melanoconion)* females, of a species morphologically similar to 'Culex No. 9' of Trinidad, were released in another cage and 109 families of mice exposed. Oriboca virus was isolated from a baby mouse exposed on May 9.

The transmission of Guamá and Oriboca viruses to Swiss mice by naturally infected *Culex (Melanoconion)* has thus been demonstrated. This same technique was used by Sérié *et al.*² to transmit yellow fever to laboratory

mice by the bite of naturally infected *Aedes simpsoni* in Ethiopia.

Belém Virus Laboratory,
Instituto Evandro Chagas,
Belém, Pará, Brazil.

AMAZONIA TODA
ROBERT E. SHOPE

¹ Aitken, T. H. G., Jonkers, A. H., and Worth, C. B., *Ann. Microbiol. (de J.)*, **11**, Part A, 67 (1963).

² Sérié, C., Andral, L., Lindrec, A., and Nerl, P., *Bull. World Hlth. Org.*, **299** (1964).

CYTOLOGY

Circulation in the Cell

THE rapidity with which cells respond to the presence of solutes or suspensions in their immediate vicinity has made the problem of the permeation of the cell matter of major interest, but the question of how substances are distributed once they have entered the cell has not commanded nearly so much attention. I shall propose that the rapid distribution of nutrients and reactants within the microcosm of the protoplast (that is inside the plasma membrane) is effected by an active circulatory apparatus, by which fluid is forced through pores by hydrostatic pressure, somewhat analogous to the apparatus by which a corresponding circulatory distribution is effected in the macrocosm of the animal body. The concept of the cell-circulation is based almost exclusively on work with the yeast cell; but there are many indications which suggest that the conclusions are generally applicable. Many cellular membranes, which were previously thought to be intact, have been shown by electron microscopy to possess systems of pores. Pores which have been first observed by electron microscopy have been afterwards observed by direct light microscopy.

The circulation-hypothesis explains the rapid distribution of cell-substances which is inconsistent with the diffusion hypothesis. The fact that cells continuously change in their ability to take up substances from the external milieu is indisputable. It seems unrealistic, however, to try to account for such changes by changes in the composition of the membrane because a minor change, which might accommodate one substance, would certainly alter the behaviour of the membrane to a variety of other substances. It seems essential that any hypothesis should require that the structural integrity of the membrane-systems should remain unaltered throughout the life of the cell. It is interesting in this connexion that Lehninger¹ has suggested: (a) that membranes are usually assembled *in situ*; (b) that protein synthesis is concerted with phospholipid synthesis in such a manner that the two syntheses are mutually dependent; (c) that membrane synthesis occurs by a process in which the lipid and protein components of the membrane serve as 'structural templates' for each other, to form a thermodynamically stabilized end-product; (d) that each membranous structure may produce new membrane from proteins and lipids present in the protoplasm by using the pre-existing membrane as a template.

Although genes might change by mutation to produce enzymes which might be able to synthesize new membrane-components which might be able to alter the selective permeability of the membrane, it seems unlikely that changes in permeability are primarily due to such a mechanism. It seems much more likely that genes controlling the entry of a substance into the cell do so by eliciting the production of an enzyme which converts the substance into a form which can pass through the cellular membrane. According to the receptor hypothesis², genes act only in response to the contact stimulus of an inducer. As a general corollary to this hypothesis, a large molecule which might not be able to pass through the intact barrier of the plasma membrane and which could not enter any 'normal' membrane in metabolically

ificant amounts (that is, in amounts detectable by changes in oxygen consumption and carbon dioxide production) might, nevertheless, be assumed to act as an indicator of enzyme activity at the molecular level. Minute amounts might be able to enter the cell-circulation by imperfections (due to pinocytotic pores or fissures) which would act as inductors of enzymes by making contact with the gene-receptor in amounts which might not be detectable by metabolic measurements.

In the circulation theory the permeability of each of the cell membranes is assumed to remain virtually unchanged throughout the metabolic processes, and no enzymes are assumed to be elicited which change the permeability of the membrane *per se*. This theory does not require that permeation be purely by diffusion nor does it include the possibility that permeation may be facilitated by specific energetic mechanisms. It is assumed that adaptive permeation in the yeast cell is usually effected by contact of large molecules with hydrolytic enzymes which have been induced by a few molecules of the inductor and that the enzymes are afterwards expelled to the cell wall, or the nucleoprotein layer. In the nucleoprotein layer³ the extra-protoplasmic enzymes, which were induced in the cell by adaptive processes, hydrolyse large molecules into smaller ones which move much more rapidly through the plasma membrane, possibly by facilitated transport. The nucleoprotein layer performs the same function for the yeast cell that the small intestine and the lymphatic system perform for a vertebrate organism.

After the plasma membrane has been passed and the substance has entered the cytoplasm, the cell circulation rapidly distributes the materials through the cytoplasm and inside the nuclear apparatus. The 'pump' controlling the cell circulation is located in the nuclear apparatus and establishes a flow from the cytoplasm into the interior of the nucleus that ensures a relatively rapid movement of materials from the external *milieu* to the surface of the chromosomes. The motive power for the pump is furnished by the 'pumping units' of Oestergren⁴, who proposed that the spindle is coated with units which carry on a 'pumping activity' by which the chromosomes are moved to the poles. Oestergren *et al.*⁴ have suggested that substances are transported on and within the spindle by a pumping activity which drives materials from the equator in the two opposite directions while the spindle substance remains stationary. The pumping activity carries both chromosomes and acentric bodies to opposite poles of the cell much as sailboats are driven by the wind. I have assumed⁵ that the pumping units ('spindle-micellae') are located in the spindle reservoir of the yeast cell (which corresponds to the resting telophase nucleus) enclosed within the nuclear membrane and oriented to pump cytoplasm through the nucleus over the surface of the diffuse chromosomes. In the yeast cell an exit port leads from the spindle reservoir into the nuclear vacuole and hydrostatic pressure inflates the vacuole to an almost perfect sphere. Two exit (extruder) ports in the vacuolar membrane permit the slow discharge of a ribbon of endoplasmic reticulum, the attached ribosomes (carrying messenger RNA) and other solid materials from the nuclear vacuole into the cytoplasm.⁶ But the solutes, especially the methylated nucleotides of the nucleolus, from which the transfer RNA is synthesized in the cytoplasm (on the surface of the mitochondria) pass rapidly through the membrane of the nuclear vacuole into the cytoplasm on the stream which enters the cytoplasm through this thin, readily permeable barrier. The membrane of the spindle reservoir is impermeable to nucleotides.

Fig. 1 shows the 'flow sheet' of the cell. (1) The cell wall is composed largely of polymers of hexoses or hexose derivatives. (2) The nucleoprotein layer is confined between the coarse network of the cell wall and the much less permeable plasma membrane and contains an abundance of a variety of enzymes which were produced inside

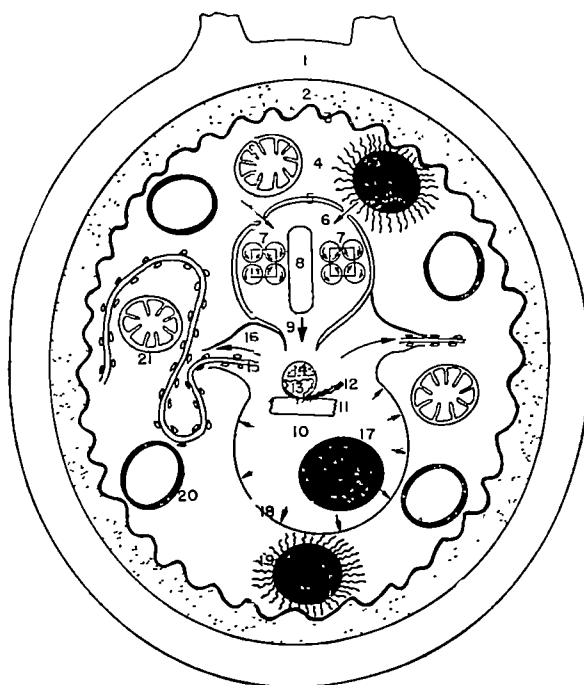


Fig. 1

the cell. (3) The plasma membrane is a typical, protein-phospholipid, biological membrane, impermeable to most sugars larger than hexoses. (4) The cytoplasm is a limpid fluid in which the cellular organelles are suspended. (5) A thick, relatively impermeable, protein-phospholipid membrane containing both entry (6) and exit (9) ports surrounds the spindle reservoir. The spindle reservoir contains the diffuse chromosomes (8) and the pumping units (7). The diffuse chromosomes are expelled into the nuclear vacuole (10) from the spindle reservoir where they become condensed chromosomes. Each particulate gene carried on the histone backbone (11) of the chromosome contains a proteinaceous receptor (12) and a double loop of structural DNA (13) on which messenger RNA (14) is synthesized. (18) The membrane surrounding the nuclear vacuole is very thin, easily permeable and quickly destroyed by most cytological fixatives. In life, it is maintained in a condition of turgor by the hydrostatic pressure of the nuclear pump. The endoplasmic reticulum and its attached ribosomes (15) are extruded into the cytoplasm through a port (16) in the vacuolar membrane. The nuclear vacuole contains the nucleolus (17) in which the nucleotides from which transfer RNA is later synthesized are collected and methylated. The cytoplasm contains mitochondria in three different states: transfer RNA is synthesized from nucleolar nucleotides on the surface of the mitochondrion (19). In this state, the mitochondrion absorbs ultra-violet and stains with uranyl acetate. (20) The mitochondrion is the site of fat synthesis and in this condition is highly refractile in the light microscope and electron transparent. (21) The cristate mitochondrion, closely associated with the endoplasmic reticulum, is the cytoplasmic source of ATP.

This work was supported by U.S. Public Health Service grant GM 12266-06.

CARL C. LINDEGREN

Biological Research Laboratory,
Southern Illinois University,
Carbondale, Illinois.

¹ Lehninger, A. L., *The Mitochondrion*, 263 (W. A. Benjamin, Inc., N.Y., 1964).

² Lindgren, C. C., *J. Theor. Biol.*, **5**, 192 (1963).

³ Lindgren, C. C., *Nature*, **198**, 1325 (1963).

⁴ Oestergren, G., Mole-Bajer, J., and Bajer, A., *Ann. N.Y. Acad. Sci.*, **90**, 381 (1960).

⁵ Lindgren, C. C., *Canad. J. Genetics and Cytol.*, **4**, 426 (1962).

⁶ Lindgren, C. C., *Nature*, **195**, 1225 (1962).

GENETICS

Schizophrenia as a Genetic Morphism

Huxley, Mayr, Osmond and Hoffer recently proposed¹ the hypothesis that schizophrenia was a genetic morphism. This was based on three main streams of evidence: (1) genetic; (2) clinical; (3) biochemical. Baldessarini and Snyder² criticized this hypothesis for being premature. But every hypothesis is premature, for once the basic data are well established hypotheses are not required.

As the biochemical-clinical member of our group, I will refer to certain errors in the Baldessarini critique.

The arguments on which the adrenochrome hypothesis was based depend not only on the presence of adrenochrome in tissues, but also on a very comprehensive number of facts recently reviewed by me³. The evidence is both indirect and direct, that is, based on the reports of many independent scientists that adrenochrome or adrenolutin was found by them in body fluids. These works have not been referred to by the two critics. Nor have they referred to work by Axelrod⁴ at the U.S. National Institute of Mental Health, who demonstrated the conversion of adrenaline into adrenochrome in salivary tissue.

They further state, "It is thus quite unlikely that adrenochrome or adrenolutin are unique biochemical factors in schizophrenia". We at no time claimed these chemicals were "unique to schizophrenia", but instead attempted to show that adrenochrome was present in normal people and was diverted in greater concentration to adrenolutin in schizophrenics.

They further suggested that the mauve factor first reported by Irvine⁵ and Hoffer and Mahon⁶ could be related to dietary factors. Had they read Irvine it would have been apparent this was one of the remotest possibilities, since dietary factors were investigated and excluded by Irvine. The Irvine and the Hoffer and Mahon factors are essentially alike although the Irvine factor may contain more components. Both methods were cross-validated against each other. The mauve factor test has been used routinely in three psychiatric centres in Saskatchewan for up to five years, and has consistently been related only to severity of the psychiatric condition. It will, therefore, follow from the critics' position that a main variable in production of these illnesses is dietary—a novel idea.

A. HOFFER

Psychiatric Research,
Department of Public Health,
University Hospital,
Saskatoon,
Canada.

¹ Huxley, Sir J., Mayr, E., Osmond, H., and Hoffer, A., *Nature*, **204**, 220 (1964).

² Baldessarini, R. J., and Snyder, S. H., *Nature*, **206**, 1111 (1965).

³ Hoffer, A., *Dis. Nerv. Syst.*, **25**, 173 (1964).

⁴ Axelrod, J., *Biochim. Biophys. Acta*, **85**, 247 (1964).

⁵ Irvine, D., *J. Neuropsychiat.*, **2**, 292 (1961).

⁶ Hoffer, A., and Mahon, M., *J. Neuropsychiat.*, **2**, 331 (1961).

Identical *S* Alleles in Different Botanical Varieties of *Brassica oleracea*

SELF-COMPATIBILITY is rare in marrow-stem kale (*Brassica oleracea* var. *acephala*) and usually occurs only in plants which have *S* alleles low in the dominance series. In such plants, genes, independent of the *S* alleles, determine self-compatibility¹. In other botanical varieties of *B. oleracea* self-compatibility is common and this suggests that these varieties might have the *S* alleles of low dominance found in marrow-stem kale.

Five inbred plants of marrow-stem kale, each homozygous for a different *S* allele, four of which had been identified in self-compatible plants, were crossed as males

to Brussels sprouts (*B. oleracea* var. *gemmifera*), to a plants of dwarf curly kale (var. *acephala*), savoy cabb (var. *sabauda*), purple-sprouting broccoli (var. *italica*) to one wild cabbage plant. Each of the five *S* alleles identified in at least one of the botanical varieties. The allele *S*₂ was found in all the botanical varieties tested; it was active in 19 of the 48 plants examined (Table 1). Another allele, *S*₁₅, occurred in three of these varieties and the five *S* alleles accounted for more than one third the total number of *S* alleles present in these plants (out of 96).

Table 1. NUMBERS OF PLANTS IN WHICH THE *S* ALLELE WAS ACTIVE IN STIGMA

Botanical variety	No. of plants examined	<i>S</i> alleles			
		2	5	15	26
Brussels sprouts	30	10	6	2	0
Dwarf curled kale	9	2	0	2	2
Purple-sprouting broccoli	5	4	0	1	0
Savoy cabbage	3	2	0	0	0
Wild cabbage	1	1	0	0	0
Totals	48	19	6	5	2

In the stigmas of plants with unidentified *S* alleles, can always be recognized because it is active in combination with each of the fifteen different *S* alleles from marrow-stem kale, with which it has been tested. In kale, *S*₂ is recessive in the pollen with thirteen *S* alleles, incompletely recessive with one, and active with two others very low in the dominance series. Hence, the proportion of plants with *S*₂ active in the pollen to those with *S*₂ active in the stigma indicates the dominance of the other alleles in a sample. Of the ten plants of Brussels sprouts, which *S*₂ was active in the stigma, *S*₂ was also active in the pollen in six. This confirms that there is a higher proportion of *S* alleles low in the dominance series in Brussels sprouts than in marrow-stem kale. Of the four purple-sprouting broccoli plants which had *S*₂ active in the stigma, only one had this allele active in the pollen.

These results suggest that horticultural varieties of *Brassica oleracea* may differ from agricultural kales in having a smaller number of *S* alleles, more of which are low in the dominance series and which therefore allow a higher proportion of self-compatible plants. Such differences could be a direct consequence of the number of plants selected to produce basic stocks. A commercial variety of marrow-stem kale may be based on several hundred mass-selected plants, whereas a variety of Brussels sprouts may be derived from a few rigorously selected plants. Selection of a few plants would reduce the number of *S* alleles, and subsequent seed multiplication would favour the retention of partially self-compatible plants with *S* alleles low in the dominance series. Thus, Sampson² found in Calabrese green-sprouting broccoli (var. *italica*) that four plants from a population had only four different *S* alleles, and he observed that "this represents a remarkably high concentration of *S* alleles but may be due to sampling rather than to any intrinsic feature of broccoli population structure". The present results suggest that Sampson's sample may be representative of the broccoli population.

The high proportion of plants in the different botanical varieties, which have allele *S*₂, is surprising. It is unlikely that it has spread during the last century by cross-pollination between breeders' distinct basic stocks of cabbage, Brussels sprouts and curly kale. *S* alleles are remarkably stable (Lewis³ found no new alleles for self-incompatibility in 22 million gametes of *Oenothera*) so that independent mutation to allele *S*₂ in different botanical varieties of *B. oleracea* can be ruled out. In potatoes, also, *S* alleles appear to be very stable; Dodds⁴ found the same *S* alleles in diploid cultivated species and in polyploids of autotetraploid 'Andigena' potatoes. It is therefore probable that allele *S*₂ occurred in the ancestral *B. oleracea*, from which the present botanical varieties have evolved. In addition, because relatively small numbers of plants are selected to maintain basic stocks of these

etities, it is probable that S_2 must have some inherent selective advantage.

K. F. THOMPSON
J. P. TAYLOR

at Breeding Institute,
Trumpington,
Cambridge.

mpson, K. F., Plant Breeding Institute, Cambridge, Ann. Rep., 41
1959-1960).

mpson, D. R., *Genetics*, 42, 253 (1957).

ewis, D., *Heredity*, 5, 399 (1951).

dds, K. S., John Innes Institute, Ann. Rep., 52, 22 (1961).

VETERINARY SCIENCE

Association of Adenoviruses with Bovine Respiratory Disease

A STRAIN (*WBR 1*) of bovine adenovirus (BA) was isolated in primary cultures of bovine kidney (BK) and found to be antigenically distinct from BA type 1 and type 2 (ref. 1). The *WBR 1* strain has therefore been proposed as the prototype strain of BA type 3.

To assess its pathogenicity for cattle, 8 colostrum-fed calves were inoculated intranasally on the day of birth with 10 ml. of BK culture fluid containing *WBR 1* strain and with a further 10 ml. intratracheally 24 h later. The inocula had a mean titre of $10^{4.0}$ TCID₅₀/ml. The calves were kept individually in isolation and then killed 4 days (3 calves), 7 days (3 calves) or 3 months (2 calves) after inoculation.

During the week following inoculation, the calves showed clinical evidence of disease manifested by pyrexia and a mucoid nasal or conjunctival discharge which lasted 4 days. One calf was dyspnoeic and two other calves died 3 and 6 days post-infection respectively. Five of the calves also developed a diarrhoea during the time of pyrexia. Virus was recovered either from the faeces or from nasal and conjunctival secretions for up to 11 days after inoculation; on one occasion, virus was recovered from the nose of a calf at 3 weeks. At necropsy, areas of consolidation, collapse and emphysema were evident in varying degree in the lungs of each calf. In calves killed at 4 days, the lung lesions were present mostly in the hilar region, whereas in those killed at 7 days, they extended to the periphery of most lobes. There was lymphadenitis of the lymph nodes draining the pneumonic lungs. In the calves killed 3 months after infection the gross pulmonary lesions were small and discrete.

Histological examinations indicated that the prime lesion was a proliferative bronchiolitis associated with necrosis and desquamation of epithelium. These changes resulted in occlusion of the bronchioles with consequent alveolar collapse. Typical adenovirus intranuclear inclusions, in varying stages of development, were present in the lungs, tonsils and tracheal mucosa, as well as in the retropharyngeal, mediastinal and bronchial lymph nodes. In the calves killed at 3 months both the gross and histological lesions were minimal and consisted of some degree of lymphoreticular hyperplasia accompanied by patchy areas of scar tissue.

Two control calves inoculated with uninfected BK culture fluid were killed for examination after 4 and 7 days respectively. Both calves remained normal and free of virus throughout and no macroscopic or histological evidence of infection was noted in either animal.

Two further calves were killed 7 days after inoculation with a comparable dose of *WBR 1* virus which had been pre-treated with trypsin to remove possible 'toxic' factors of the type associated with human adenovirus type 5 (ref. 2). There was a similar clinical response but, at necropsy, the gross lung lesions were minimal and comparable to those seen in the calves killed at 3 months after infection with untreated virus. Cells with inclusions were again present in the respiratory tract but were difficult to find. This finding suggests that the trypsin-sensitive factor associated with the virus assists in the production of the pneumonic lesions observed previously.

Virus was recovered *post mortem* from various tissues from all the calves inoculated with virus, including the lungs of the calves killed at 4 and 7 days or those which died, but not from the lungs of those killed at 3 months or from those inoculated with trypsin-treated virus (Table 1). Immunodiffusion tests on the sera of all the calves showed that adenovirus group-reactive precipitating antibody developed in 3-4 weeks after infection and could persist thereafter for at least 2 months.

These experiments indicate that the *WBR 1* strain of BA type 3 is pathogenic for calves, and the results will be reported in detail elsewhere³. Serological evidence has been adduced previously that adenoviruses are associated with bovine respiratory disease in the field⁴. In further investigations with paired serum samples from affected or in-contact cattle, significant increases in adenovirus group-precipitating antibody have been observed in approximately 30 per cent of 150 disease outbreaks over the past 2 years⁵. The majority of such outbreaks attributed to adenovirus infection have occurred during the winter months, with peak incidence during the earlier

Table 1. RECOVERY OF *WBR 1* STRAIN OF BOVINE ADENOVIRUS TYPE 3 FROM TISSUES OF CALVES *post mortem*

Tissues	Untreated virus inoculum										Treated virus inoculum		Controls	
	4 days			7 days			3 months		Died		7 days		1	2
	1	2	3	1	2	3	1	2	1	2	1	2		
Lung lobes:														
R. apical	+	+	-	+	+	+	-	-	-	-	-	-	-	-
R. cardiac	+	+	-	+	+	-	-	-	+	+	-	-	-	-
R. diaphragmatic	+	+	-	+	+	-	-	-	+	-	-	-	-	-
Intermediate	+	+	+	+	+	+	-	-	+	-	-	-	-	-
L. apical	+	+	-	+	+	-	-	-	+	-	-	-	-	-
L. cardiac	+	+	-	+	+	-	-	-	-	+	-	-	-	-
L. diaphragmatic	+	+	-	+	+	-	-	-	+	-	-	-	-	-
Conjunctiva	-	-	-	+	+	-	-	-	+	-	-	-	NT	NT
Nasal m/m	-	-	-	+	+	-	-	-	-	+	-	-	NT	NT
Retropharyngeal LN	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tonsil	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Larynx	-	-	NT	-	+	-	-	-	NT	-	-	-	NT	NT
Tracheal m/m	+	+	+	+	+	-	-	-	-	+	-	+	-	-
Bronchial LN	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Mediastinal LN	-	-	-	+	-	-	+	-	-	-	+	-	NT	NT
Cardiac muscle	NT	-	NT	-	NT	-	-	-	NT	-	-	-	NT	NT
Oesophagus	-	-	-	-	-	-	-	-	NT	-	-	-	NT	NT
Abomasum	-	-	-	-	-	-	-	-	NT	-	-	-	NT	NT
Jejunum	-	-	-	-	-	-	-	+	NT	-	-	-	NT	NT
Colon	-	-	-	-	-	-	-	-	NT	-	-	-	NT	NT

LN, lymph node. m/m, mucous membrane. +, virus recovered. -, virus not recovered. NT, not tested.

months of the year. Available evidence suggests that significant increases in neutralizing antibodies to BA types 1, 2 and 3 occur in the sera of cattle involved in respiratory disease outbreaks in Britain⁶ and that it is also possible that further, undefined serotypes of bovine adenoviruses may be involved.

J. H. DARBYSHIRE

Central Veterinary Laboratory,
Weybridge.

A. R. JENNINGS
A. R. OMAR

School of Veterinary Medicine,
University of Cambridge.

P. S. DAWSON
P. H. LAMONT

Central Veterinary Laboratory,
Weybridge.

¹ Darbyshire, J. H., Dawson, P. S., Lamont, P. H., Ostler, D. G., and Pereira, H. G., *J. Comp. Path. Ther.*, **75**, 327 (1965).

² Pereira, H. G., *Virology*, **6**, 601 (1958).

³ Darbyshire, J. H., Jennings, A. R., Dawson, P. S., Lamont, P. H., and Omar, A. R., *Res. Vet. Sci.* (in the press).

⁴ Darbyshire, J. H., and Pereira, H. G., *Nature*, **201**, 895 (1964).

⁵ Darbyshire, J. H. (unpublished results).

⁶ Darbyshire, J. H., and Pereira, H. G. (unpublished results).

Hepatic Tumours in Ducks fed a Low Level of Toxic Groundnut Meal

THE toxicity of certain samples of groundnut meal has been shown to be due to fungal metabolites, collectively known as aflatoxin, produced by strains of *Aspergillus flavus*¹. The carcinogenic effect of such meals for rats, a species relatively resistant to the acute effect of aflatoxin, has been demonstrated²⁻⁴ and it has since been shown that crystalline aflatoxin is carcinogenic for these animals^{5,6}.

Young ducklings are the most susceptible animals to the acute effect of aflatoxin⁷. This communication describes the change found in ducks fed for a prolonged period on a ration containing a low level of toxic groundnut meal.

Thirty-seven 7-days-old Khaki Campbell ducklings (group A) were fed a commercial poultry ration free of aflatoxin to which was added 0.5 per cent toxic Brazilian groundnut meal which contained approximately 7 p.p.m. aflatoxin (assayed as aflatoxin B₁). Sixteen control ducklings (group B) of the same age and breed were fed the commercial ration without added groundnut meal. All birds received tap-water *ad lib*. They were brooded for the first three weeks in electrical tier brooders and thereafter housed in arks on pasture. Weights were recorded weekly for the first 14 weeks of the experiment. Deaths in both groups were recorded and tissues retained for histological examination. The survivors in both groups were killed fourteen months after commencement of the feeding trial.

Tissues were fixed in formal-saline, embedded in paraffin wax, sectioned at 7 μ and stained with haematoxylin and eosin. Liver sections were, in addition, stained with Masson's trichrome stain and frozen sections were prepared and stained with oil red O.

Plotting the mean weight of the birds in the two groups against time showed no significant differences in the slope of the two regression lines, but the differences between means for the groups were significant at week 3 (group A 11.6 oz., group B 15.2 oz. ($P < 0.05$)), week 4 (group A 16.0 oz., group B 22.3 oz. ($P < 0.01$)) and week 5 (group A 23.5 oz., group B 27.9 oz. ($P < 0.05$)) but not at any other time to week 11. From weeks 11 to 14 there was no longer a linear weight increase in either group; the controls remained at a higher mean level than the treated birds and this difference was highly significant ($P < 0.01$) on mean values alone.

During the first four weeks of the experimental period nineteen birds in group A died. On histological examination lesions consistent with poisoning due to aflatoxin were present in the liver of all of them although in the birds death was ascribed to other causes. In the following 7 months a further seven birds in group A died, or were killed, and in each of these hepatic lesions consistent with aflatoxicosis were present.

In group B four ducks died due to bacterial disease and two were killed because of severe trauma inflicted by their pen-mates. On histological examinations of livers from these birds there was no evidence of aflatoxicosis.

Fourteen months after commencement the survivors in both groups, consisting of eleven in group A and ten in group B, were killed. Macroscopic lesions were confined to the livers; those from birds in group A were atrophied, putty coloured and contained numerous yellowish focal lesions 1-2 mm diameter. In four of the six drakes there were, in addition, solid yellow-coloured nodules varying in size from 1 to 3 cm diameter. In four of the five ducks in addition to the small focal lesions, there were nodules in the livers between 0.5 cm and 2.0 cm in diameter. Group B, comprising six drakes and four ducks, one drake had a number of small irregularly-shaped necrotic lesions in the liver. Apart from this there were no other macroscopic lesions in the organs of the birds in this group.

The small focal lesions present in the livers of all birds in group A were lymphoid foci composed mainly of mature lymphocytes surrounding degenerating hyperplastic bile duct epithelium. There were no mitotic figures but, peripherally, there was infiltration, in some cases extensive, of lymphocytes between the hepatic cords. The larger nodules were liver tumours of two types: thickly encapsulated, vacuolated hepatomata composed of hypertrophic ballooned cells occasionally containing giant vesicular nuclei with very prominent nucleoli, and cholangiomata composed of dense masses of well-differentiated bile ducts (the cells of which showed very few mitotic figures) surrounded by a thin fibrous capsule. This variation in cell type is seen in hepatic tumours of rats fed aflatoxin and other carcinogens⁸. In the liver of one duck tumours of both types were present, in the drakes and two drakes there were hepatomata, and in two of the other drakes cholangiomata occurred.

After 14 months eight of eleven birds fed a ration containing 0.5 per cent toxic groundnut meal developed hepatic tumours whereas none occurred in ten birds fed the same ration without groundnut meal. The aflatoxin B₁ content of the tumour-inducing ration for ducks was approximately 0.03 p.p.m. compared with 0.4 p.p.m. required to produce hepatic tumours in rats within 18 months⁹. The single oral LD₅₀ dose of aflatoxin for male rats is 7.2 mg/kg⁸ compared with 0.4 mg/kg for male ducklings⁹. The relative susceptibility of ducks and rats to both the acute and hepato-carcinogenic effect of aflatoxin is therefore comparable.

I thank Dr. J. G. Campbell of the British Empire Cancer Campaign for Research, Edinburgh, for his advice and Miss C. N. Hebert of this Laboratory for statistical analysis.

R. B. A. CARNAGHAN

Central Veterinary Laboratory,
Weybridge.

¹ Sargeant, K., Sheridan, A., O'Kelly, J., and Carnaghan, R. B. A., *Nature*, **192**, 1096 (1961).

² Lancaster, M. C., Jenkins, F. P., and Philp, J. McL., *Nature*, **192**, 1099 (1961).

³ Schoental, R., *Brit. J. Cancer*, **15**, 812 (1961).

⁴ Le Breton, E., Frayssinet, C., and Boy, J., *C.R. Acad. Sci., Paris*, **225**, 78 (1962).

⁵ Butler, W. H., and Barnes, J. M., *Brit. J. Cancer*, **17**, 699 (1963).

⁶ Dickins, F., and Jones, H. E. H., *Brit. J. Cancer*, **17**, 691 (1963).

⁷ Asplin, F. D., and Carnaghan, R. B. A., *Vet. Rec.*, **73**, 1215 (1961).

⁸ Butler, W. H., *Brit. J. Cancer*, **18**, 756 (1964).

⁹ Carnaghan, R. B. A., Hartley, E. D., and O'Kelly, J., *Nature*, **200**, 110 (1963).

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, October 18

ASTICS INSTITUTE, PLASTICS PROPERTIES DISCUSSION CIRCLE (at the Mandeville Hotel, Mandeville Place, London, W.1), at 3 p.m.—Mr. D. A. "Impact Testing and Brittle Failure".

UNIVERSITY OF LONDON (in the Botany Theatre, University College, Gower St., London, W.C.1), at 5 p.m.—Prof. B. A. D. Stocker: "Salmonella genes—Structure and Genetics" (further lecture on October 25).*

BRITISH COAL UTILIZATION RESEARCH ASSOCIATION (in the Lecture Theatre, Institution of Civil Engineers, Great George Street, London, S.W.1), at 3.30 p.m.—Prof. B. D. Spalding: "Heat Transfer Aspects of Coal Utilization" (Fourteenth Coal Science Lecture).

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Dr. J. H. Hamence: "The Public's Approach to Pesticide Residues".

INSTITUTION OF MECHANICAL ENGINEERS, INTERNAL COMBUSTION ENGINES GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Whither Small V-form Diesel Engines?"

ROYAL INSTITUTE OF CHEMISTRY (at Enfield Technical College, Queensway, Enfield, at 7 p.m.—Mr. W. G. Busbridge: "Isotopes in Industry".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Dr. Charles Swithinbank: "A Year with the Russians in Antarctica".

Tuesday, October 19

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Dr. D. McNally: "Are Stars Visible?"*

UNIVERSITY OF LONDON (at Imperial College of Science and Technology, London, S.W.7), at 1.30 p.m.—Mr. E. F. Schumacher: "Sources of Understanding. Is Observation Enough?"*

UNIVERSITY OF LONDON (at the Institute of Education, Malet Street, London, W.C.1), at 5.15 p.m.—Mr. John Maddox: "The Nuffield Foundation Science Teaching Project".*

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, London, S.W.1), at 5.30 p.m.—Mr. C. D. Brown: "Design and Construction of the George VI Bridge Over the River Usk, at Newport, Monmouthshire".

RESEARCH DEFENCE SOCIETY (in the Physiology Lecture Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. D. D. Reid: "Life and Men in Medical Research" (Thirty-fourth Stephen Paget Memorial Lecture), followed by the Annual General Meeting.

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 5.30 p.m.—of R. King: "Energy". (Afternoon lecture for Sixth Form Boys and Girls in Schools in London and the Home Counties. To be repeated on October 26 and 27.)

UNIVERSITY OF LONDON (at Imperial College of Science and Technology, London, S.W.7), at 5.30 p.m.—Prof. C. B. Alcock: "Problems with Solutions: Chemical View of High Temperature Materials" (Inaugural Lecture).*

SOCIETY OF CHEMICAL INDUSTRY (joint meeting of the Heavy Organic Chemicals Group and the Plastics and Polymer Group, at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Mr. J. H. Briston: "The Use of Chemical Products in Packaging".

PLASTICS INSTITUTE, LONDON SECTION ENGINEERING SUB-GROUP (at the Wellcome Building, Euston Road, London, N.W.1), at 6.30 p.m.—Dr. C. Hendrick: "Quality Control in the Mass Production of Plastics Mouldings".

Wednesday, October 20

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, 11 Cane Road, London, W.12), at 2 p.m.—Dr. B. W. Meade: "Laboratory Aspects of Drug Overdose".*

INSTITUTION OF CHEMICAL ENGINEERS (at the Institution of Civil Engineers, Great George Street, London, S.W.1), at 5.30 p.m.—Dr. A. J. V. Underwood, J.B.E.: "Chemical Engineering—Reflections and Recollections".

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Discussion on "Computer/Instrument Interfaces".

INSTITUTION OF MECHANICAL ENGINEERS, STEAM PLANT GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Mr. R. Carstairs: "Six Years' Operation of Cyclone Boilers at Thornton Power Station".

SOCIETY OF ENVIRONMENTAL ENGINEERS (in the Mechanical Engineering Department, Imperial College, Exhibition Road, London, S.W.7) at 6 p.m.—Mr. D. J. Lewis and Mr. A. S. Evans: "Radio Frequency Interference Control".

OIL AND COLOUR CHEMISTS' ASSOCIATION (in the Small Physics Lecture Theatre, Imperial College of Science and Technology, London, S.W.7), at 7 p.m.—Mr. P. Fink-Jensen (Denmark): "Rheological Properties of Paint and Two New Instruments for Measuring Them" (European Co-operation Lecture).

UNIVERSITY OF LONDON (at the Institute of Neurology, National Hospital, Queen Square, London, W.C.1), at 7 p.m.—Prof. N. S. Sutherland: "Attention and Learning in Animals".*

ROYAL INSTITUTE OF CHEMISTRY (joint meeting with the South-East Essex Technical College and School of Art Chemical Society, at Longbridge Road, Dagenham, Essex), at 7.30 p.m.—Meeting on "Computer Applications in Chemistry".

Thursday, October 21

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Mr. M. J. Brown: "Do We Need Cities?"*

UNIVERSITY OF LONDON (at Imperial College of Science and Technology, London, S.W.7), at 1.30 p.m.—Mr. Patric Dickinson: "Computer versus Creator".*

CHEMICAL SOCIETY (in the Large Chemistry Lecture Theatre, Imperial College, London, S.W.7), at 2 p.m.—Symposium on "Organic Chemical Approaches to Biosynthesis".

INSTITUTION OF CIVIL ENGINEERS, ENGINEERING MANAGEMENT GROUP (at Great George Street, London, S.W.1), at 5.30 p.m.—Informal Discussion on "Management Training for Engineers" introduced by Mr. D. L. Marples.

UNIVERSITY OF LONDON (in the Beveridge Hall, Senate House, London, W.C.1), at 5.30 p.m.—Sir George Dodder: "Scientific Progress and the Pattern of Medical Care". (First of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation).*

INSTITUTE OF REFRIGERATION (at the National College for Heating, Ventilating, Refrigeration and Fan Engineering, Southwark Bridge Road, London, S.E.1), at 6 p.m.—Mr. E. Woodcock: "Cooling Coil Evaluation".

SOCIETY FOR ANALYTICAL CHEMISTRY, Biological Methods Group (at "The Feathers", Tudor Street, London, E.C.4), at 6.30 p.m.—Discussion Meeting on "Use and Abuse of Statistics in Biological Assays" opened by Mr. K. L. Smith.

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (at Manson House, 20 Portland Place, London, W.1), at 7.30 p.m.—Prof. G. Macdonald: "On the Scientific Basis of Tropical Hygiene" (Presidential Address).

UNIVERSITY OF LONDON (at Wye College, Wye, Ashford, Kent), at 8.15 p.m.—The Rt. Hon. Lord Northbourne: "Religion and Science".*

Thursday, October 21—Friday, October 22

POWDER METALLURGY JOINT GROUP OF THE IRON AND STEEL INSTITUTE AND THE INSTITUTE OF METALS, in association with the BRITISH METAL SINTERING ASSOCIATION (in the Hoare Memorial Hall, Church House, Great Smith Street, London, S.W.1), at 11 a.m. on Thursday and 10 a.m. on Friday—Annual Meeting.

Friday, October 22

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Dr. B. D. Shaw: "Explosives".

Saturday, October 23

INNER LONDON EDUCATION AUTHORITY (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Mr. Poul Rovsing Olsen (Copenhagen): "The Greenland Eskimos and Their Music".*

Monday, October 25

PLASTICS INSTITUTE, LONDON SECTION REINFORCED PLASTICS SUB-GROUP (at the Wellcome Building, Euston Road, London, N.W.1), at 3 p.m.—Symposium on "The Changing Face of Reinforced Plastics".

PLASTICS INSTITUTE, PLASTICS PROPERTIES DISCUSSION CIRCLE (at the Mandeville Hotel, Mandeville Place, London, W.1), at 3 p.m.—Mr. E. B. Atkinson: "Flow Behaviour of Molten Polymers".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 5 p.m.—Prof. E. G. Bowen: "The Welsh Colony in Patagonia".

BRITISH SOCIETY FOR THE HISTORY OF SCIENCE (in the Council Room of the Science Museum, Exhibition Road, London, S.W.7), at 5.30 p.m.—Dr. F. W. Gibbs: "Gunpowder Priestley and Dictionary Johnson".

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, London, S.W.1), at 5.30 p.m.—Sir John Cockcroft, O.M., F.R.S.: "Competitive Nuclear Power".

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, JOINT I.E.E.—I.E.R.E. COMPUTER GROUPS (at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 5.30 p.m.—Prof. W. J. Poppelbaum: "Opto-Electronics".

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, W.C.2), at 6.30 p.m.—Mr. J. T. Bull: "A.C. Traction Power Supplies" (Chairman's Address).

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT LECTURER or LECTURER (preferably with a special interest in analysis) in PURE MATHEMATICS—The Registrar, University College of South Wales and Monmouthshire, Cardiff (October 20).

ASSISTANT LECTURER or COLLEGE LECTURER (with an honours degree in agricultural science, chemistry or biochemistry with suitable postgraduate experience) in AGRICULTURAL CHEMISTRY—The Secretary and Bursar, University College, Dublin, Republic of Ireland (October 22).

ASSISTANT LECTURER (with a good honours degree or equivalent and preferably some research or industrial experience) in BOTANY; and an ASSISTANT LECTURER (with a good honours degree or equivalent and preferably some research or industrial experience) in ZOOLOGY—The Academic Registrar, Brunel College, Woodlands Avenue, London, W.3 (October 22).

COLLEGE LECTURER IN GENETICS in the AGRICULTURAL BIOLOGY DEPARTMENT, FACULTY OF GENERAL AGRICULTURE—The Secretary and Bursar, University College, Dublin, Republic of Ireland (October 22).

SENIOR LECTURER in MATHEMATICS—The Registrar, Loughborough Training College, Loughborough, Leicestershire (October 22).

COMPUTER OFFICER (with a suitable university degree and experience in computer programming and operation) at THE TIDAL INSTITUTE AND OBSERVATORY—The Registrar, The University, Liverpool, quoting Ref. CV/296/N (October 23).

KEEPER (with a good honours degree in botany and/or the Museums Association Diploma; an interest in economic botany, museum experience of fieldwork, and preferably a working knowledge of at least one modern foreign language) of BOTANY—The Director, City of Liverpool Museums, William Brown Street, Liverpool, 3 (October 23).

PHYSICS LABORATORY TECHNICIAN for a GROUP ISOTOPE LABORATORY—The House Governor (A), Charing Cross Hospital, London, W.C.2 (October 23).

CHIEF STOREKEEPER (with experience in the supply and maintenance of scientific or technical laboratories) in THE DEPARTMENT OF CHEMISTRY—Prof. Stanley Peat, Department of Chemistry, University College of North Wales, Bangor, Caernarvonshire, North Wales (October 25).

LECTURERS IN ENGINEERING MATHEMATICS—The Registrar, The University, Newcastle upon Tyne 2 (October 27).

CHAIR OF THERMODYNAMICS AND FLUID MECHANICS IN THE DEPARTMENT OF MECHANICAL ENGINEERING—The Secretary, The Queen's University, Belfast, Northern Ireland (October 30).

LECTURER (registered medical practitioner holding the D.P.M., with broad experience of different aspects of psychiatry) IN PSYCHIATRY—The Registrar, The University, Leeds, 2 (October 30).

SENIOR BIOCHEMIST to take charge of a routine CHEMICAL PATHOLOGY DEPARTMENT—The Secretary, Queen Mary's Hospital, Roehampton, London, S.W.15 (October 30).

LECTURER IN BIOCHEMISTRY—The Registrar, King's College (University of London), Strand, London, W.C.2 (October 31).

RESEARCH FELLOW (with an honours degree in a relevant subject and a minimum of two years postgraduate experience or training) IN RADAR AND SIGNAL PROCESSING IN THE DEPARTMENT OF ELECTRONIC AND ELECTRICAL ENGINEERING—The Assistant Registrar (Science and Engineering), The University of Birmingham, Birmingham, 15 (November 1).

LECTURER IN EDUCATION, and a LECTURER IN SOCIOLOGY/SOCIAL ANTHROPOLOGY AT THE UNIVERSITY COLLEGE OF RHODESIA AND NYASALAND—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (November 2).

MYCOLOGIST (with an honours university degree in science or equivalent, and preferably suitable research experience) IN THE SOIL BIOLOGY DEPARTMENT—The Director, An Foras Taluntais (The Agricultural Institute), 33 Merrion Road, Dublin 4, Republic of Ireland (November 4).

SENIOR DEMONSTRATOR (with a degree in psychology and preferably some research or teaching or professional experience) IN PSYCHOLOGY at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, November 5).

SENIOR LECTURER or LECTURER (with an honours degree in zoology) IN ZOOLOGY at the University of Canterbury, Christchurch, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, November 5).

CHAIR OF FLUID MECHANICS AND HYDRAULIC ENGINEERING at Imperial College of Science and Technology—The Academic Registrar, University of London, Senate House, London, W.C.1 (November 12).

HEAD OF THE DEPARTMENT OF MATHEMATICS, South Australian Institute of Technology—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1, or The Registrar, South Australian Institute of Technology, North Terrace, Adelaide, South Australia (Australia, November 12).

CHAIR OF PHYSICS at Fourah Bay College, The University College of Sierra Leone—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (November 15).

LECTURER or SENIOR LECTURER in the School of Botany, University of Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 15).

LECTURERS (with a good honours degree and a keen research interest in any branch of electronics and control engineering) IN THE ELECTRONICS AND CONTROL DIVISION, School of Engineering Science—The Registrar, University College of North Wales, Bangor, North Wales (November 30).

MASTER TO TEACH GEOGRAPHY AND MATHEMATICS—The Master, Dulwich College, London, S.E.21.

MASTERS TO TEACH (a) PHYSICS and (b) MATHEMATICS (sixth form work)—The Headmaster, Forest School, Snaresbrook, London, E.17.

POSTDOCTORAL FELLOW (preferably with some experience in X-ray crystallography) IN THE DEPARTMENT OF CHEMISTRY, to collaborate with Dr. B. Stevens in a study of the photochemistry and radiation chemistry of crystalline aromatic compounds—The Registrar, The University, Sheffield.

POSTDOCTORAL RESEARCH BIOCHEMIST to join a group engaged in studying the control of protein biosynthesis in animal cells—Prof. H. R. V. Arnstein, Department of Biochemistry, University of London, King's College, Strand, London, W.C.2.

PROFESSOR IN PHYSICAL CHEMISTRY—Prof. Harry E. Gunning, Head, Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada.

RESEARCH ASSISTANT (Academic) IN THE DEPARTMENT OF PHYSICS to assist in an investigation into laser-induced surface damage under the direction of Prof. S. Tolansky, F.R.S.—The Secretary, Royal Holloway College (University of London), Englefield Green, Surrey.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Design Method Symposium, University of Aston in Birmingham Designate (College of Advanced Technology), September 21, 22, and 23, 1965—Preprints. (Organized by the Design and Innovation Group.) Pp. 322. (London: Butterworths, 1965.) [149]

Jacksons' Millboard and Fibre Co., Ltd. Directors Report and Accounts, 1965. Pp. 12. (London: Jacksons' Millboard and Fibre Co., Ltd., 1965.) [149]

Royal Observatory Bulletins. No. 97: The Absolute Magnitudes of RR Lyrae Variable Stars. By Sir Richard Woolley, G. A. Harding, Annella I. Cassells and Jennifer Saunders. Pp. E1-E30. 4s. 6d. net. No. 98: Proper Motions and Radial Velocities of Hyades Stars. By F. A. Wayman, L. S. T. Symms and K. C. Blackwell. Pp. E31-E62. 4s. 6d. net. (London: H.M. Stationery Office, 1965.) [149]

The Committee of Vice-Chancellors and Principals of the Universities of the United Kingdom. A Compendium of University Entrance Requirements for First Degree Courses in the United Kingdom (excluding part-time and external degree courses). Third edition. Pp. 219. (London: The Association of Commonwealth Universities, 1965.) 11s. 6d. [149]

University of Reading. National Institute for Research in Dairying—Report 1964. Pp. 164. (Shinfield, Berkshire: National Institute for Research in Dairying, 1965.) 7s. 6d. [149]

Anti-Locust Bulletin No. 42: The Abdomen of the Female Desert Locust (*Schistocerca gregaria* Forskal), with special reference to the Sense Organs. By Joan G. Thomas. Pp. 20+45 figures. (London: Anti-Locust Research Centre, 1965.) 8s. 6d. [149]

City of Liverpool College of Technology. Prospectus of Full-Time Courses, Session 1965-1966. Pp. 67. (Liverpool: City of Liverpool College of Technology, 1965.) [149]

Road Research Laboratory: Ministry of Transport. Road Research Technical Paper No. 74: Traffic Signals for High-Speed Roads. By Dr. F. V. Webster and P. B. Ellison. Pp. v+49+2 plates. (London: H.M. Stationery Office, 1965.) 4s. 6d. net. [149]

Other Countries

Conseil National de la Politique Scientifique, Bruxelles. Recherche Industrielle dans ses Relations avec la Croissance Économique. Pp. (Bruxelles: Conseil National de la Politique Scientifique, 1965.) [1]

World Health Organization. Public Health Papers, No. 27: Trends in Study of Morbidity and Mortality. By Dario Curiel and 13 other authors. Pp. 196. (Geneva: World Health Organization; London: H.M. Stationery Office, 1965.) 8 Sw. francs; 13s. 4d.; 2.75 dollars. [1]

Council on Research in Bibliography, Inc. Mental Health Book Review Index, Vol. 10, 1965. Compiled by the Editorial Committee and Contributing Librarians. Pp. xx+78. Annual subscription: 5 dollars. (New York: Council on Research in Bibliography, Inc., c/o Research Centre for Mental Health, New York University, 1965. Subscriptions etc. to Miss Lois Affler Paul Klapper Library, Queens College, Flushing, New York, 11367.) [1]

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Index to Reports of Geological Survey of Canada from 1955. Compiled by J. F. Wright. Pp. xli+379. (Ottawa: Queen's Printer, 1965.) 10 dollars. [1]

National Museum of Canada. Bulletin No. 201 (Anthropological Series No. 68): Folk Ballads and Songs of the Lower Labrador Coast. By Edward Leach. Pp. viii+332. (Ottawa: Queen's Printer, 1965.) 5 dollars. [1]

Government of Madras. Report of the King Institute of Preventive Medicine, Gundy, for the period from 1 April 1960 to 31 March 1961. Dr. S. Govindarajan. Pp. 53+2. (Guindy, Madras: King Institute of Preventive Medicine, 1963.) [1]

Geological Survey of New South Wales. Mineral Resources No. 40: Geological Survey of the Coonabarabran-Gunnedah District with special reference to the Occurrence of Sub-Surface Water. By E. J. Kenny. Pp. 77+5. (Sydney: Department of Mines, 1965.) 12s. 6d. [1]

F. and M. Scientific Corporation, Avondale, Pennsylvania. Meth Bulletin No. 117: Gas Chromatography Methods for Biochemistry Medicine: Qualitative and Quantitative Lipid Analysis by Gas Chromatography. Pp. 14. (Avondale, Pennsylvania: F. and M. Scientific Corporation; London: F. and M. Scientific Europa, N.V., 1965.) [1]

National Academy of Sciences—National Research Council. Publications 1284: Laboratory Animals. 4: Graduate Education in Laboratory Animal Medicine, Proceedings of a Workshop, March 26-27, 1964, Washington, D.C. (A Report of the Institute of Laboratory Animal Resources.) Pp. vii+35. 1.25 dollars. Publication 1285: Laboratory Animals. 5: Workshop in Animal Technician Training, October 2, 1964, Washington, D.C. Report of the Institute of Laboratory Animal Resources. Pp. v+57. 1.25 dollars. (Washington, D.C.: National Academy of Sciences—National Research Council, 1965.) [1]

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Paper 64-37: Manganese Occurrences in Canada (Preliminary Reports and Metallogenic Map). By A. G. Johnston and W. D. McCarty. Pp. iv+63. 75 cents. Paper 64-47: Lower Palaeozoic Sediments of Northwestern Baffin Island, District of Franklin. By H. P. Trettin. Pp. vi+75 cents. Paper 64-52: Flat River, Glacier Lake, and Wrigley Lake, District of Mackenzie and Yukon Territory. By H. Gabrielse, J. A. Roddick and S. Blusson. Pp. 30+3 maps. 75 cents. Paper 65-11: Mineral Industry of Northwest Territories. By E. A. Schiller. Pp. v+62. 75 cents. Paper 65-14: Investigation of Eakers for Mineral Exploration; (2) Buried Valleys in Kirkland Lake, Ontario. By Hubert A. Lee. Pp. iii+20. 75 cents. Paper 65-20: Notes on Moraines and Radiocarbon Dates in Northwest Baffin Island, Melville Peninsula, and Northeast District of Keewatin. By B. Craig. Pp. 7. 75 cents. (Ottawa: Queen's Printer, 1965.) [1]

Cerebrovascular Accidents as a Public Health Problem—Selected Research Abstracts, II. Pp. 64. (Baltimore, Md.: Research in Public Health Administration Project, School of Hygiene and Public Health, The Johns Hopkins University, 1965.) [1]

United States Department of the Interior: Geological Survey. Geophysical Abstracts, No. 223 (August, 1965). By James W. Clarke, Dorothy Vittalano, Virginia S. Neuschel, and others. Pp. iii+606-693. 35 cents. Primer of Water Quality. By H. A. Swenson and H. L. Baldwin. Pp. iii+80 cents. (Washington, D.C.: Government Printing Office, 1965.) [1]

New Zealand Forest Service: Forest Research Institute. Research Note No. 38: Point Density in Stems per Acre. By G. S. Brown. Pp. 11. No. 4: The Mineral Nutrition of *Pinus radiata* Seedlings. By W. W. G. Trave. Pp. 10. (Wellington: Government Printer, 1965.) [1]

Institut Royal Météorologique de Belgique. Bulletin Mensuel. Observations Ionosphériques. Juin 1965. Pp. 26. Juillet 1965. Pp. 26. (Ucc Bruxelles: Institut Royal Météorologique de Belgique, 1965.) [1]

East African Common Services Organization. Annual Report of the East African Freshwater Fisheries Research Organization for 1964. Pp. iii+77. (Jinja, Uganda: East African Freshwater Fisheries Research Organization, 1965.) 6s. [1]

Indian National Scientific Documentation Centre. Directory of Indian Scientific Periodicals 1964. Compiled by G. K. Arora, S. Dutta, D. N. Gupta and H. N. Rangachar. Pp. 133. (Delhi: Indian National Scientific Documentation Centre, 1965.) [1]

Annals of the New York Academy of Sciences. Vol. 120, Article 2: Home Medication and the Public Welfare. By T. H. Alphin and 24 other authors. Pp. 807-1024. (New York: New York Academy of Sciences, 1965.) 1.50 dollars. [1]

New Zealand. Report of the Department of Scientific and Industrial Research for the year ended 31 March 1965. Pp. 88. (H. 34.) (Wellington: Government Printer, 1965.) 3s. [1]

Editorial and Publishing Offices of "NATURE"

MACMILLAN (JOURNALS) LIMITED

4 LITTLE ESSEX STREET, LONDON, W.C.2

Telephone Number: Temple Bar 6633. Telegrams: Phusis London W.C.2

Annual subscription: Inland £14, Overseas £13 15s., payable in advance, postage paid to any part of the world.

Advertisements only should be addressed to

T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2.

Telephone Number: Holborn 4743

Registered as a newspaper at the General Post Office

Copyright © Macmillan (Journals) Limited, October 16, 1965

NATURE

No. 5008

SATURDAY, OCTOBER 23, 1965

Vol. 208

Published by
MACMILLAN (JOURNALS) LTD.

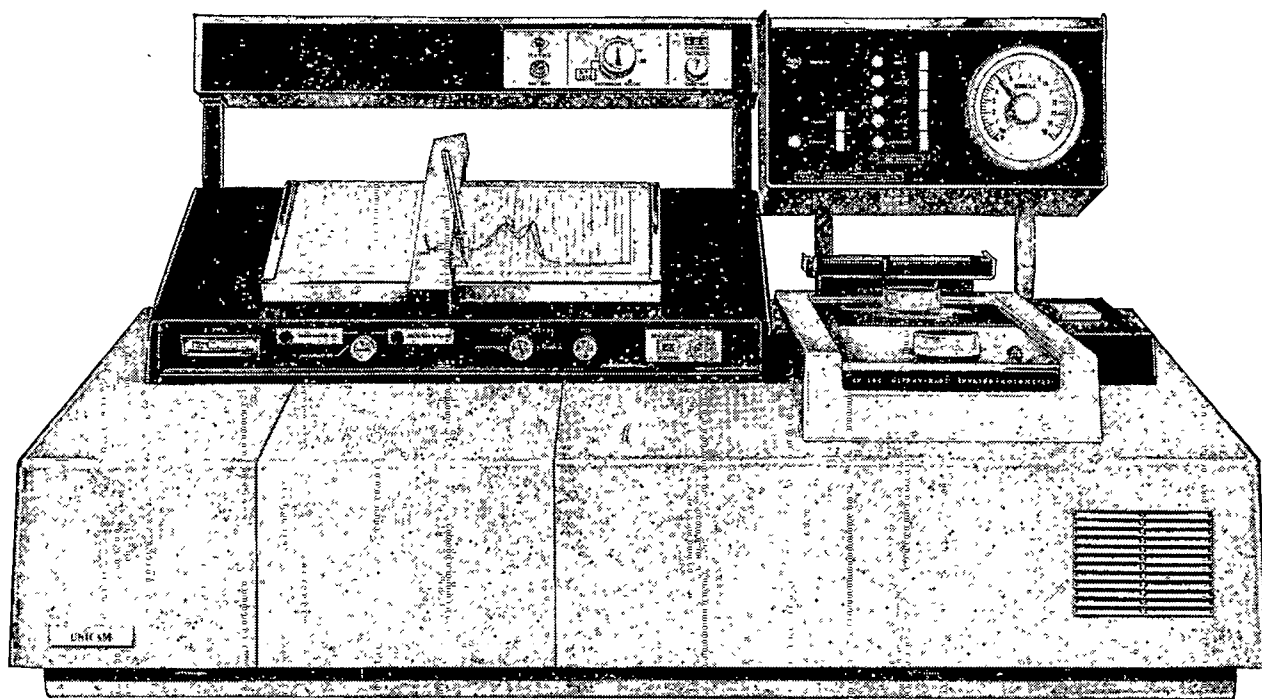
CONTENTS

	<i>Page</i>
IMPROVING THE CIVIL SERVICE IMAGE	311
HIGHER EDUCATION AND POLITICS. By Sir James Cook, F.R.S.	313
FRONTIERS OF CERTAINTY. By F. I. G. Rawlins, C.B.E.	314
ASPECTS OF LEARNING. By A. D. C. Peterson, O.B.E.	314
COSMIC RAY PHYSICS. By Dr. J. J. Quenby	315
SEDIMENTARY STRUCTURES. By J. R. L. Allen	315
A JOURNEY THROUGH MODERN BIOCHEMISTRY. By Dr. D. T. Elmore	316
ENGLER REVISED. By Prof. T. G. Tutin	316
PERISHABLE RICHES. By Dr. G. R. Wadsworth	317
THE SMITHSONIAN INSTITUTION, WASHINGTON, D.C.	320
JAMES SMITHSON: PATHFINDER IN SCIENCE AND PHILANTHROPY. By Dr. Leonard Carmichael	321
SCHOLARLY SESSIONS AT THE SMITHSONIAN BICENTENNIAL CELEBRATIONS	324
JAMES SMITHSON MEDAL: ROYAL SOCIETY OF LONDON	324
EVOLUTION IN THE BIOSPHERE. By Prof. Harold F. Blum	324
NEWS AND VIEWS	326
SECOND INTERNATIONAL CONFERENCE ON PROTOZOOLOGY. By Prof. P. C. C. Garnham, C.M.G., F.R.S.	330
TYPOGRAPHY OF BACTERIA	332
ANGLO-AFRICAN JOINT RESEARCH SCHEMES AND RECRUITMENT	333
MODERN TECHNIQUE IN RESTORATION OF ROMAN MOSAICS	334
A PROPOSAL ON NATURE'S TIME-SCALE. By Prof. Neil G. McCormick	334
SUBCUTANEOUS SARCOMATA IN HAMSTERS INDUCED BY ROUS SARCOMA VIRUS (BRYAN). By Prof. M. L. Eidinoff, B. Bates, M. Steinglass and Prof. J. R. Haddad	336
IMPLICATIONS OF THE IMMUNOLOGICAL THEORY OF CANCER TO MULTIPLE CAUSES. By Dr. Mary R. Anderson and Prof. H. N. Green	338
SELF-ADAPTIVE CONTROL AND THE RESPIRATORY SYSTEM. By Dr. I. P. Priban and Dr. W. F. Fincham	339
REACTION OF HUMAN LYMPHOCYTES IN CULTURE TO COMPONENTS OF THE MEDIUM. By G. J. Johnson and Prof. P. S. Russell	343
AFLATOXIN: EFFECT ON CULTURED HETEROPOID HUMAN EMBRYONIC LUNG CELLS. By Dr. Marvin S. Legator, Saverio M. Zuffante and Anthony R. Harp	345
NICOTINAMIDE MONONUCLEOTIDE TYROPHOSPHORYLASE ACTIVITY IN ASCITES CELL EXTRACTS. By Prof. L. S. Dietrich, L. Fuller, I. Leonor Yero and Laura Martinez	347
MATURATION <i>in vitro</i> OF MOUSE, SHEEP, COW, PIG, RHESUS MONKEY AND HUMAN OVARIAN OOCYTES. By Dr. R. G. Edwards	349
PROCESSES OF EXCITATION AND INHIBITION IN SINGLE MECHANORECEPTORS (PACINIAN CORPUSCLES). By Dr. O. B. Ilyinsky	351
A NEW FLUORESCENT ANTIBODY METHOD: MIXED ANTIGLOBULIN IMMUNOFLUORESCENCE OR LABELLED ANTIGEN INDIRECT IMMUNOFLUORESCENCE STAINING. By Prof. E. H. Beutner, Dr. E. J. Holborow and G. D. Johnson	353
EFFECT OF A LIPID FRACTION FROM RAT THYMUS ON DELAYED HYPERSENSITIVITY REACTIONS OF NEONATALLY THYMECTOMIZED RATS. By Prof. Branislav D. Janković, Dr. Katarina Isaković and Jozef Horvat	356
PRECIPITIN ACTIVITY IN THE SERA OF PATIENTS WITH DEFICIENCY OF β -LIPOPROTEINS (ACANTHOCTOSIS). By Dr. R. F. Murray, jun., and Prof. B. S. Blumberg	357
DOUBLE CHROMOSOME REDUCTION IN A TETRAPLOID <i>Solanum</i> . By G. E. Marks, Dr. R. K. McKee and Dr. J. B. Harborne	359
RETICULOCYTE RIBOSOME FRACTION WITH AN EXCEPTIONAL CAPACITY FOR POLYPHENYL-ALANINE SYNTHESIS. By Dr. John O. Bishop	361
INTERMEDIATE STATES IN THE PRECIPITATION OF HYDROXYAPATITE. By Prof. E. D. Eanes, Dr. I. H. Gillessen and Prof. A. S. Posner	365
EFFECTS OF SEEDING ON THE CONDENSATION OF ATMOSPHERIC MOISTURE IN NOZZLES. By Dr. E. R. Buckle and Dr. A. A. Pouring	367
METAL CONCENTRATION PROFILES IN OXIDE SCALES ON NICKEL-10.9 PER CENT COBALT ALLOY. By Dr. G. C. Wood and J. M. Ferguson	369
STRUCTURE OF THE QUASI-STELLAR RADIO SOURCE 3C 273 B. By M. J. Rees and Dr. D. W. Sciama	371

(Continued overleaf)

UNICAM**SP.800**

spectrophotometer
for routine U.V. analysis
with automation accessories



Save operator time, increase speed and efficiency with Unicam automation for:

ENZYME REACTION RATES

LIQUID COLUMN CHROMATOGRAPHY

**ALL ROUTINE ULTRAVIOLET AND
VISIBLE SPECTROSCOPY**

Please send for descriptive brochure

The brilliant new Unicam SP.800 Spectrophotometer is setting new standards in ultraviolet and visible spectroscopy. The widely acclaimed flat bed presentation, the beam balance smoothing cam and the unique second sample position are among the features which establish it as *the* outstanding U.V. spectrophotometer. Additionally Unicam now offers automation equipment which provides:

AUTOMATIC interchange of up to 4 sample and 4 reference cells

AUTOMATIC re-cycling over any preselected wavelength range

AUTOMATIC recordings at predetermined time intervals

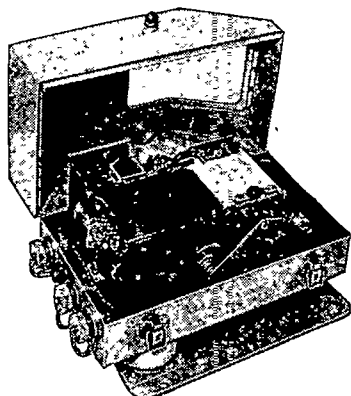
Unicam Precision Spectrophotometers

Unicam Instruments Ltd, York Street • Cambridge • Telephone Cambridge 61631 • Telegrams: Unicam Cambridge • Telex 81215

LETTERS TO THE EDITOR

	Page		Page
ASTROPHYSICS		Evidence for a Poisson Distribution of Miniature End-plate Potentials and some Implications.—Dr. P. W. Gage and Dr. J. I. Hubbard	
Rotation Period of the Planet Mercury.—W. E. McGovern, Dr. S. H. Gross and Dr. S. I. Rasool	375		395
Radial De-spin of Planets and Satellites.—Dr. Peter Goldreich	375	PHARMACOLOGY	
PHYSICS		Anticoccidial Activity of Nicotinamide Antagonists.—Dr. S. J. Ball, E. W. Warren and Dr. E. W. Parnell	
Laboratory Observations of Plasma Instabilities in a Dipole Magnetic Field.—Prof. Robert G. Quinn	376		397
Sub-millimetre Wave Spectra of Polar Liquids.—Dr. G. W. Chantry and Dr. H. A. Gebbie	378	PATHOLOGY	
METALLURGY		Induction of Malignant Lymphomas by Urethane in Adult Mice bearing Syngeneic Thymus Grafts.—Dr. L. Fiore-Donati, L. Chieco-Bianchi, G. Tridente and L. Mazzarella	
Effect of Temperature on the Discontinuity in the S/N Fatigue Curve of a Stainless Steel.—T. R. G. Williams and C. R. Shurmer	379		398
CHEMISTRY		RADIOBIOLOGY	
Correlation of the Third Excited State of Polycyclic Aromatic Hydrocarbons with the Ionization Potential.—Dr. M. A. Slifkin	380	Bone Marrow Allografts in Thymectomized X-irradiated Dogs.—I. L. Chertkov, A. S. Maximenko, M. N. Novikova and M. O. Raushenbach	399
Labelling of Beta-lipoprotein with Tritium.—T. Gosztanyi, J. Márton and A. Kovács	381	An Unusually Radioactive Fossil Fish from Thurso, Scotland.—W. R. Diggle and J. Saxon	400
Pure Quadrupole Resonance Frequency Shift and Internal Stress in Gamma-ray Irradiated Sodium Chlorate.—Dr. Hayati Budak	382	Continuous Irradiation of HeLa Cells at -196°C .—A. H. W. Nias and Dr. L. G. Lajtha	400
Acid Phosphatases.—Dr. C. A. Vernon, J. Gaudie, Jennifer M. Hanson, J. M. Humphreys, P. E. Smith, A. J. Lawrence and Dr. Barbara E. C. Banks	382	BIOLOGY	
Structure of Phosphobenzene.—Dr. J. J. Daly and Dr. Ludwig Maier	383	Hatching Time of Turtle Eggs.—Sister Marie Therese Dimond	401
BIOPHYSICS		Development of the Oocyte in a Species of <i>Cissus</i> .—N. C. Nair	402
Intercellular Matrix of the Intervertebral Disk in Ageing and in Prolapse.—Dr. T. K. F. Taylor and Dr K. Little	384	Marsupial Spermatozoa Pairing in the Epididymis of American Forms.—Dr. J. D. Biggers and Dr. E. D. DeLamater	402
BIOCHEMISTRY		Record of the Bivalve Gastropod <i>Berthelinia limax</i> (Kawaguti and Baba 1959) from the Indian Ocean.—K. Prabhakara Rao	404
Enzymatic Synthesis of the Skin-lightening Agent, Melatonin, in Amphibians.—Dr. Julius Axelrod, Prof. Wilbur B. Quay and Peter C. Baker	386	Spore Discharge in <i>Lepista konradii</i> .—Dr. M. H. Zoberi	405
Agar-gel Electrophoresis of Soluble Lens Proteins in Galactose-fed Rats.—Eva Wittgenstein and Dr. Kenneth W. Rowe, jun.	386	FORESTRY	
Freeing of Amino-acids from Proteins.—Dr. L. J. Hastewell and R. N. Priestland	387	Induction of Tension Wood with the Anti-auxin 2,3,5-Tri-iodobenzoic acid.—Prof. R. W. Kennedy and Prof. J. L. Farrar	406
Red Pigment in Leaf Galls of <i>Salix fragilis</i> L.—Gerald Blunden and Dr. Stephen B. Challen	388	Leucoanthocyanins of White Pine in relation to Weevil Attack.—Frank S. Santamour, jun.	407
Isolation of a Lymphoid-cell Protein with Relation to Delayed Hypersensitivity.—Ulf Rothman and Sture Lidén	389	MICROBIOLOGY	
Formation of Complexes between Basic Proteins of Leucocytes and Plasma Globulins.—Dr. Weldon B. Jolley and Henry W. Allen	390	Apparent Suppression of Mutation Rates in Bacteria by Spermine.—Dr. H. G. Johnson and Dr. M. K. Bach	408
PHYSIOLOGY		VIROLOGY	
Effect of Altered Perfusion Rates on the Retention of Noradrenaline by the Spleen.—Dr. D. M. Paton and Prof. C. N. Gillis	391	Derivatives of 2-Aminoethyl-guanidine and of a Eugenol as Virus Inhibitors.—Sándor Pécsa, Olga H. Hankovszky and Kálmán Hideg	409
Role of Portal Blood Supply in Liver Regeneration.—Dr. R. Y. Thomson and A. M. Clarke	392	GENETICS	
Chemotopic Organization in the Bulbar Gustatory Relay of the Rat.—Bruce P. Halpern	393	Puffing of Salivary Gland Chromosomes after Treatment with Carbon Dioxide.—Prof. Walter J. Burdette and Ruth Anderson	409
		Evidence for a Second 'Structural' Locus determining Human Phosphoglucosmutase.—Dr. D. A. Hopkinson and Prof. Harry Harris	410
		ANTHROPOLOGY	
		Hair Colour of the Population of Tristan da Cunha.—Dr. E. Sunderland	412

HENDREY SPARK RECORDER Type 1487A



Compact and weatherproof, this single channel recorder is light in weight and easily portable. The Perspex window gives a clear view of the heat-sensitive chart for approximately 6 in. of its length.

The instrument can also be used mounted on bench, wall or panel and is supplied with mounting plate incorporating three anti-vibration mountings which render the instrument shock-proof and vibration-proof to service standards.

An electro-magnetically operated stylus, operated by a separate switch not supplied with the recorder, provides transverse markings near the edge of the chart. Provision is made for an external alarm circuit which can be pre-set to operate at any deflection from zero to full scale by means of a control on the case.

Sensitivity—50 micro-amps. Full scale. Supply—12 Volt, D.C.

For full details and
specification ask for
Catalogue Sheet

A4

Hendrey Relays

HENDREY RELAYS LTD.

390-394 BATH RD., SLOUGH, BUCKS.

Telephone: Burnham 609/612 Grams: Hendrelay, Slough

MANUFACTURING ELECTRICAL ENGINEERS
CONTROL AND LABORATORY APPARATUS
A.R.B. & M.O.A. Approved Design & Inspection

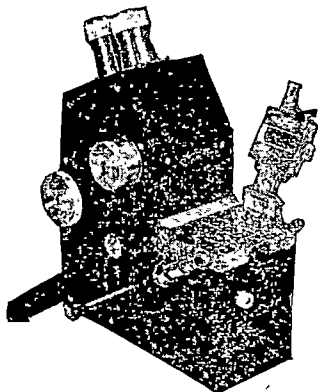


PRECISION INSTRUMENTS

Abbe 60

Refractometer—with Sugar Scale

0-50% DIVIDED TO 0.2%
50-95% DIVIDED TO 0.1%



We are now able to offer this popular laboratory instrument with a sugar scale only, expanded to the maximum limit and sub-divided at intervals of 0.2% up to 50% and thence to 95% at intervals of 0.1%.

Other features remain the same—back illumination for dealing with dark solutions, fixed horizontal prism box and totally enclosed glass arc with etched graduations.

SPECIAL INSTRUMENT — STANDARD PRICE

Send for details to Dept N

SOLE DISTRIBUTORS IN U.K. OF SCHOTT INTERFERENCE FILTERS

Bellingham & Stanley Limited

71 HORNSEY RISE, LONDON, N.19. PHONE: ARCHWAY 2270

IMPROVING THE CIVIL SERVICE IMAGE

ALTHOUGH the Civil Service Commission and recruitment to the Civil Service have been mentioned in recent books and pamphlets dealing with Civil Service reform, such reference has been mainly incidental and it has been left for the Estimates Committee in its sixth report for the session 1964-65* to examine recruitment to the Civil Service in any thorough fashion. Even so, the Estimates Committee was indebted to the Acton Society Trust for financing a survey of the attitudes towards an administrative career in the Civil Service of students at a civic university. As the Estimates Committee observes, this underlines the recommendation in its fifth report for this Session that it should be authorized to appoint temporary outside advisers and to obtain assistance from outside—any expenditure involved being borne on the Vote of the House of Commons. Much of the value of the report, in fact, derives not from the Committee's examination of witnesses but from the memoranda which were submitted in evidence by the Acton Society Trust and others—without whose co-operation the scope of the enquiry would have been severely restricted and its authority much diminished.

The enquiry was concerned both with the work of the Civil Service Commission and with the role of the Treasury in recruiting and with direct recruitment by other Departments. The Commission's responsibilities are now limited to the selection and certification of people for permanent posts, under regulations made by the Commissioners and approved by the Treasury. Unestablished posts are now filled by arrangements made by Departments, sometimes assisted by the Commission, and subject also to the approval of the Treasury. The Estimates Committee's recommendations are few, but the implications of the report are far-reaching: the difficulty which the Civil Service has consistently experienced since the Second World War, especially in graduate and professional recruitment, and in obtaining as many recruits as it needs, is set in the perspective of to-day's needs generally. The right questions are asked not simply about recruitment itself but about the opportunities open to recruits and the use that is made of them.

The report deals first with the pattern of recruitment, and here the figures quoted show that since 1954, while needing more recruits, the administrative class only maintained the number of assistant principals recruited and failed to maintain its share of a rapidly increasing field of possible candidates. Moreover, at a time when the fields of university recruitment were widening, the proportion of administrative class recruits from these wider fields was falling, and although until 1963 little difficulty was experienced in recruitment to the executive class, vacancies in some departmental executive classes were not being filled. In the 1964 report of the Commission, however, reference is also made to a shortage in the general executive class—the class which is broadly responsible for the wide range of managerial and administrative functions in the Civil Service and which has traditionally recruited from school-leavers with at least two passes in the Advanced Level Examinations for the General Certificate of Education.

In many respects, recruitment to the specialized classes of the Civil Service, which include the Scientific Civil Service and a range of technical and professional classes, is a lighter problem than that of recruitment to the administrative or executive classes—recruiting procedures being faster and more flexible and often undertaken by the departments. Even so, the Civil Service is at a disadvantage in the competition with other employers, even when the lengthy process of open competition, interview and certification is not needed for temporary posts. Direct appointment by establishments is not possible and Civil Service pay structures do not permit any recognition of the really first-class man on entry. Finally, scientific and technical work in the Civil Service is necessarily more organized than in the universities, which since the Second World War have been one of the main competitors for the best graduates. Certainly, there is not the same freedom of publication, though the rules concerning publication have recently been liberalized.

Of interest in this connexion are some observations on factors influencing the choice of employment in a memorandum from the Civil Service Commission. It is admitted that salaries are much more favourable to the universities than to the Civil Service compared with before the Second World War, but it is considered that attraction to the administrative class lies other than in the cash rewards. In the scientific officer class, the Civil Service has much to offer young graduates in the way of interesting work in their own field with unusually good experimental facilities and reasonable stability in the programme of work, and in general the Scientific Civil Service probably ranks second to a university appointment in the minds of good young scientists. There is, however, a tendency for the young scientist to try a period in industry. To meet this, the Service has in recent years resorted to temporary recruitment direct by departments with subsequent confirmation by the Commission when both sides have made up their minds. Nevertheless, the Commission admits that the Service does not attract as many of the really outstanding young scientists as it would like and that it is possible that this is due in part to the failure of the system of pay on entry to allow recognition of the really first-class man.

In the same context may be noticed some remarks in a memorandum from the Acton Society Trust on possible reasons why graduates from universities other than Oxford and Cambridge do not choose careers in the Civil Service. It is unlikely that the differences can be attributed to different sources of social origin in students, although this may partly explain the popularity of school teaching as a career. The nature of the recruitment process may be a hindrance, and an Acton Society Trust report on the arts graduate in industry pointed to a higher wastage among those who had been recruited into general training schemes than among those who had been recruited for specific posts. Moreover, an applicant for a career in the administrative class is really required to take on the job for the whole of his working life. This may well be a deterrent, as is also the picture of the administrative class as 'the talented amateur', overdrawn though this has always been. Nor has this yet been remedied by the reforms which have followed the Plowden Report, and the Civil Service continues to under-play the need for formal training for members of the administrative class.

* Sixth Report from the Estimates Committee together with the Minutes of the Evidence taken before Sub-Committee E and Appendices, Session 1964-65—Recruitment to the Civil Service. Pp. xxxviii+269. (London: H.M.S.O., 1965.) 26s. net.

It is at this point that the memorandum touches on the Civil Service Commission itself, which, it suggests, contributes to the present unsatisfactory image of the Civil Service through the rather negative way it handles its recruitment programme for the administrative class. Before examining that criticism of the Commission, some further points in the memorandum on the attitude of students may be noted. The exploratory enquiry conducted by the Trust indicated that some 60 per cent of science students at the University of Hull had no particular careers in mind. Most of the students thought that university teaching and school teaching were the careers most likely to offer a high degree of personal freedom in carrying out one's job, private industry and commerce getting a middle rating, followed by the Scientific Civil Service with the administrative class last. After university teaching, the Scientific Civil Service was regarded as the career most likely to offer opportunity of using the knowledge acquired in reading for a degree, and the administrative class again came last—though it was rated better as regards opportunity for easy transfer of jobs in mid-career. Moreover, except for the nationalized industries, where the opportunity was regarded as equal, between 60 and 85 per cent of the Hull students thought they stood less chance than graduates from Oxford and Cambridge, especially for the administrative class, closely followed by university teaching. More than half the students regarded working in London for the duration of their careers as a definite disadvantage; almost a third were indifferent, and only 12 per cent thought it an advantage. By far the most attractive feature of the administrative class to the students seemed to be its security of tenure, but this and other attractive features did not offset the fact that they gave it a low rating on those attributes which they held to be important in comparison with other jobs.

The extent of the knowledge of the students about the Administrative Civil Service was clearly small. The image of the Administrative Civil Servant which is based on that knowledge is a definite obstacle to recruitment and one that must be rectified. For this the present publicity techniques are inadequate and a new and more direct approach seems necessary. However, it should be noted that the Acton Society Trust thought that the facts about Civil Service recruitment were still extremely scarce and that a full-scale investigation into undergraduates' attitudes towards the Civil Service as a career should be undertaken on behalf of the Civil Service Commission as an essential pre-requisite to discussion. Meanwhile, the shortage of talented graduates and the efficiency of the Civil Service compel the Civil Service Commission to continue and increase its efforts to broaden the appeal of the administrative class. Whatever changes may be made inside the Service itself, the Commission must pursue a more aggressive recruitment policy for this class and do so imaginatively. The criticism is levelled also at the publicity of the Commission and at the university appointments boards. The latter are not so efficient as they might be; they are understaffed, and while they are often ill-equipped to give vocational guidance, for most of them, in quantitative terms, the administrative class is unimportant.

This criticism of the Civil Service Commission appears to have been accepted by the Estimates Committee. In a long memorandum on recruitment to the Civil Service, in which he also suggested that difficulties in recruitment and the like were due mainly to lack of experienced research staff and of money and could be remedied by closer collaboration between the universities, the Civil

Service and a Social Science Research Council, Prof. W. J. M. Mackenzie expressed the opinion, based on extensive personal experience, that the Civil Service Commission acts impeccably within its terms of reference. Witnesses before the Estimates Committee both from the Commission and from the Treasury conceded that change in the range of the Commission's functions had occurred in recent years, which was not envisaged in the original Order in Council, nor provided for in the Order of Council of 1956, from which the Commission derives its authority. The Estimates Committee agrees that there are strong arguments for keeping the related functions of recruitment and selection together within one agency, and believes that little would be gained by the Commission's attempting to restrict itself to the functions contained in the original Orders in Council. It did not pursue the wider question whether the final responsibility for recruiting policy and management should remain with the Treasury or whether the functions of the Commission itself should be extended, as Mr. S. Brittan suggested in his recent book*, and merely note with apparent approval Prof. Mackenzie's comment: "The problem is whether the Commission's terms of reference are right".

Prof. Mackenzie wrote his memorandum before the publication in the winter issue of *Minerva* (3, No. 2, 172/1965) of Prof. R. J. Stone's article "A Model of the Educational System", which illustrates the type of serious research and analysis which he regards as necessary in answering the questions from the Estimates Committee. He suggests, however, that two vital modern functions are now lost to view in the Victorian tradition which still appears to underlie the work of the Commission. The notion that numbers of corrupt persons are trying to insinuate themselves and their friends into the public service is archaic: in the modern world the Commission is the recruitment section of the personnel division of a large organization. If it cannot find its recruits, it must first look in a sophisticated way at the employment market and at what the organization offers to the market. If candidates of the right type cannot be found, it must report back fearlessly and constructively to the higher level. Secondly, the Civil Service is perhaps unique among lay organizations in having no public relations department, and Prof. Mackenzie suggests that the Commission cannot possibly do its job to-day unless it is able to face its competitors squarely. In his view, its function must be that of a recruitment and public relations department for the Treasury Department, which is now responsible for the personnel policy and general efficiency of the Civil Service. He regards the Commission as a well-run body, but considers that a symbolic break with the Victorian tradition might be an advantage. In this view he differs from the Fabian Group, which in 1964 proposed that personnel management should be transferred from the Treasury to a reformed Civil Service Commission strengthened by new appointments and by staff from the Treasury.

In this long memorandum, Prof. Mackenzie makes many pertinent observations and comments, of which only a few can be noted here. He pays tribute to the outstanding success of the British Civil Service in the organization and management of routine clerical work, but he does not see how its strong executive structure can be maintained except by upgrading the status and salaries of the executive class and its related departmental classes. Challenging the idea of the amateur, he points to some five respects in which the task of the administrator calls

* *The Treasury under the Tories, 1951-1964* By Samuel Brittan (Pelican Book A 722.) Pp. 375. (Penguin Books, Ltd., London, 1964.) 6s.

different types of special or professional knowledge and skill. After reminding us that the pool of really good general graduates increases much more slowly than the total output of graduates, he points to five weaknesses in present intake from the universities. The proportion of candidates with a solid first-class academic record appears to be declining; the net is not flung wide enough; while the proportion of social scientists, particularly economists, is rising, there are very few natural scientists and virtually no technologists; entrants come straight from university to service in a Department and lack training and practical experience of the world; there are few chances of varying the pattern of a career between ordinary and distinguished success, even of unofficial meeting and argument with contemporaries.

Prof. Mackenzie's comments on the scientific and professional classes offer some constructive guidance to the Commission in pursuing a realistic policy which takes account of the variations between different fields; if these comments are heeded, they could help to correct the attitude which in the mind of the Estimates Committee is responsible, as much as the general image of the Civil Service, for the poor response in the civic universities.

It appeared to the Estimates Committee that the Commission's procedures still concentrate on keeping people out rather than on attracting them. The many advantages which the Civil Service has to offer are not being communicated to the relevant audience, and the Commission has not always translated its understanding of the need for effective public relations into the most effective public relations.

So far as the Civil Service Commission is concerned, the Estimates Committee makes four specific recommendations. Alone, or in conjunction with the Treasury, the Central Office of Information or an outside agency, the Commission should examine the effectiveness of its methods of advertising and publicity. With the Treasury, the Commission should undertake a follow-up survey of executive officers, if necessary on a sampling basis, to determine the efficiency of selection methods and to isolate particular reasons for wastage in the early years of service. The Commission should also seek to quantify the categories of graduates from which entrants to the Civil Service are drawn, and the two posts at principal level recently sanctioned by the Treasury to improve contacts with teaching staff at universities should be used to improve contacts with civic universities as well as Oxford and Cambridge.

Apart from criticizing the approach of the Civil Service Commission to the whole problem of recruitment, the Estimates Committee comments at some length on its advertising and publicity, particularly in relation to the impact of recruitment for the Civil Service on the manpower situation generally—an aspect which incidentally is emphasized in the subsequent White Paper outlining the National Plan. The Committee is also critical of the liaison with both the schools and the universities, especially of the reliance on the appointments boards, the efficiency of which has been questioned both by the Acton Society Trust in its memorandum and earlier by the Heyworth Committee of the University Grants Committee which examined the work of the Boards. It is also critical of delays in selection procedure, the responsibility for which rests as much on the Civil Service as on the Commission itself. The staff side considered that the clerical class was at present losing the best potential recruits because of the time taken to recruit. Mr. B. J. Holloway, in a memorandum, pleaded for the exercise of greater imagination

and less rigidity by the Commission. He also credited the Scientific Civil Service with being the only section of the Civil Service that had really faced the problems of competitive recruitment, and pointed out that firms like Imperial Chemical Industries, Ltd., and Unilever could reach a decision in an urgent case on a first-class man in ten days from start to finish. Certainly he felt that the Civil Service could not expect to keep its candidates if it kept them waiting to the present extent.

The Estimates Committee's enquiry involves both short-term and long-term considerations, and in the latter especially are involved the whole structure of the Service and the desirability or not of radical changes in that structure. These issues are raised to some extent in Prof. Mackenzie's memorandum and even more so in one from Mr. D. N. Chester, the Warden of Nuffield College, Oxford. However, although these memoranda are interlocked with some of the questions considered here they are more conveniently considered together with related issues later. Meanwhile, it should suffice to add that, in spite of its specific criticism, the Estimates Committee is confident that the Civil Service's fine tradition of ability and integrity is still very much alive to-day. Moreover, while its structure and its public image may well need review in the light of modern needs, the Civil Service would emerge from such enquiry strengthened in public esteem and in its ability to discharge its responsibilities.

HIGHER EDUCATION AND POLITICS

Eighteen Plus

Unity and Diversity in Higher Education. Edited by Marjorie Reeves. Pp. 226. (London: Faber and Faber, Ltd., 1965.) 25s. net.

NO short account could do justice to the many thoughts expressed in this series of essays on the problems which confront the world of higher education in the post-Robbins age. In one sector of this world we are witnessing a crisis in the university more acute than that about which Sir Walter Moberly wrote. Time alone will show the extent to which this crisis can be overcome. It was perhaps one of the inevitable consequences of the Robbins philosophy that the dichotomy between institutions of higher education should be sharpened. This is diametrically opposed to what the Robbins Committee set out to achieve, namely, the integration of higher education. The removal of the colleges of advanced technology from the control first of the local authorities and then of the Department of Education and Science has put these last-named organizations on the defensive—a defensive which is now beginning to take the form of attack.

Mr. A. Crosland, the Secretary of State, in a surprisingly candid speech at the celebration of the seventy-fifth anniversary of the foundation of Woolwich Polytechnic, in April this year, has given a clear indication of Government policy, in which the thinking of the Department of Education and Science is no doubt reflected. In this speech, the text of which has been given very wide circulation, a clear distinction is drawn between the public sector of higher education, "under social control and directly responsive to social needs", and the autonomous sector, by implication oblivious to social needs. This is a harsh judgment on what the universities have accomplished in the years since the Second World War. Mr. Crosland indicated also that the Council for National Academic Awards is to be an instrument of policy in building up the public sector of higher education parallel with the development of the autonomous sector. In my view, there can be only one outcome of the attitude of mind displayed in these philosophies. We shall see, with

the passage of time, growing indignation at the iniquities of selection at eighteen plus and a growing clamour for the autonomous sector to be integrated with the public sector and brought under 'social control'. As Dr. Brian Wilson says in his chapter on the needs of students, in *Eighteen Plus: Unity and Diversity in Higher Education*, "the real issue of the times is not whether we shall have more university education but whether we shall maintain university education at all".

The sixteen contributors to this symposium write mostly on topics which have already been extensively discussed. Most of the papers were, in fact, first given at a conference in September 1963, that is, before publication of the Robbins Report. They deal with a variety of topics within the field of higher education and express many different points of view. These are linked and co-ordinated by a series of interesting commentaries by Miss Marjorie Reeves, Fellow of St. Anne's College, Oxford, who also contributes a chapter on "Prestige". This unsolved problem of the relative esteem to be accorded to different institutions and courses probably does more damage to the cause of higher education than any other single factor.

Dr. G. Templeman describes the considerations which have led to increasing concentration on single-subject degree courses and makes a plea for the enlargement of our notion of university duty so as to cover wider educational responsibilities. "Do students need an ivory tower?", asks Miss Reeves in introducing Dr. Wilson's chapter on student needs. He paints a gloomy picture of threats to university values and refers to the temptation to project an image as a substitute for building a reputation; to dilution by new types of discipline; to the increasingly professionalized attitudes of academic staff; to pressure by clientele with its demand for a qualification and a good time; and to public demand, characterized by growth, status inflation and eventual devaluation. Prof. W. R. Niblett discusses further the influence of expansion on traditional values. Prof. H. Butterfield, in a chapter on the "Springs of Intellectual Vitality", describes the infectiously stimulating atmosphere of the Cavendish Laboratory of the 1920's and what flowed from this. Several contributors refer to the present gulf between the generations which is apparently less acute in the sixth form than at the university. In her chapter on sixth-form studies, Dame Joyce Bishop deplores the stultifying influence of pressure from the universities.

Mr. J. Maitland-Edwards describes the role of technical colleges in higher education, and Miss Monica Wingate the place of the training colleges (now renamed colleges of education). Mr. B. Gowenlock deprecates the "academic rat-race" of the university of to-day. Perhaps the most significant comment is by Mr. D. Jenkins, who points out that education, like war, has now become too important to be left to the experts. This is a stimulating and challenging book which merits study by all who are interested in higher education.

JAMES COOK

FRONTIERS OF CERTAINTY

Beyond the Edge of Certainty

Essays in Contemporary Science and Philosophy. Edited by Robert G. Colodny. (University of Pittsburgh Series in the Philosophy of Science, Vol. 2.) Pp. vii+287. (Englewood Cliffs, N.J., and London: Prentice-Hall, Inc., 1965.) 70s.

BYOND the *Edge of Certainty* is a collection of essays typical of much present-day thought in regions common to philosophy and science. The reader, however, will not be wholly at ease until he recollects what in fact happened to pure mathematics in the nineteenth century. During those years the modern concept of proof was born. To some it may have seemed like an almost endless proliferation of detail: nevertheless, such a view would be

wrong. What was emerging was a deeper grasp of fundamentals, and a severe tightening of the screw as to what constitutes validity. To-day, we are witnessing something of the same kind in the realm of physics, a ruthless quest for greater rigour. The search is probably more difficult than before, if only because at some stage Nature will present her bundle of brute facts, and as we try to untie it a number of intractable items always fall out. This where the strain is greatest between those who, like Dirac, take beauty as the criterion (and indeed this is the more inspiring attitude), and those who maintain the agreement with experiment—although subject to change as technique advances—is at any moment of history the final court of appeal.

One thing is fairly clear: the progressive refinement of laboratory processes and instrumentation will not itself resolve the dilemma, since the burden of proof lies in the nature of physical law, and the way in which we tend to interpret it. Yet the whole movement is historically conditioned: cosmic physics would be nowhere without Newton and Laplace. Equally, the quantum rises majestically from the matrixes of Hamilton, and the frustration of those who clung to the pattern of continuity to explain the behaviour of radiation. As the editor of this book observes, the pressure is now for 'fine grain' and the right to question everything.

The various authors react as follows: "Newton's First Law" (Hanson) is discussed as a means of entry into natural philosophy; it is not axiomatic in the sense of being self-evident. It needs, as it were, the constant vigil of experience to support its status as a principle.

"The Origin and Nature of Newton's Laws of Motion" (Ellis) reveals their probable Cartesian origin. They can scarcely, therefore, be claimed as a product of induction from experiment: indeed, we have some latitude of choice as is shown by the possibility of constructing different principles of natural motion. In "A Response to Ellis' Conception of Newton's First Law" (Hanson) comment is made on the contrast between kinetics and dynamics—a distinction concerned with how bodies actually move compared with how they would move in certain circumstances. "A Philosopher looks at Quantum Mechanics" (Putnam) finds no satisfactory interpretation as we know the subject to-day. It may still be necessary to go on searching for hidden variables. "The Thermodynamics of Purpose" (Hawkins) is a study in natural teleology; this includes an interesting reconsideration of the green planet Earth, envisaged as a unique heat engine. "The Physics of the Large" (Morrison) looks for a compact theory of the universe, and what constitutes the units of terrestrial life. "Problems of Empiricism" (Feyerabend) is by far the longest article in the series, and leads to the conclusion that the tenet is both incomplete and contains undesirable assumptions. The former limitation is removable if the relation between man and the universe is accepted as a cosmological hypothesis. Finally, "The Ethical Dimension of Scientific Research" (Rescher) brings us face to face with those moral problems which the success of the scientific method has forced on us. It amounts to a deliberate assertion that we are responsible for our talents, and for whatever may follow from their cultivation and deployment.

F. I. G. RAWLINS

ASPECTS OF LEARNING

How and Why do we Learn?

Edited by W. R. Niblett. Pp. 196. (London: Faber and Faber, Ltd., 1965.) 21s. net.

THIS series of essays springs from a series of public lectures given at the University of London Institute of Education. Publication in book form is perhaps a good way of making some of the lectures by this very distinguished, and very mixed, group of contributors available

a wider public. But the form, which seems to be coming more popular, is itself a questionable one. Many the lecturers/authors are clearly handicapped by the limitations of time and space and one cannot help regretting that Prof. Wiseman and Prof. Morris were not able to develop their subjects more fully. Moreover, this particular collection is an extreme example of the mixture of styles and levels from which this form almost inevitably suffers. The lectures/essays fall into three marked classes: Prof. Wiseman on "Learning versus Teaching" and Prof. Morris "How does a Group Learn to Work Together" review with great learning, but necessarily in very condensed form, the latest research findings in these fields and support their views with copious references; Dr. Wall, Dr. Taylor and Dr. Kay are more concerned to put forward personal interpretations of their subjects "Learning to Think", "Learning to Live with Neighbours", and "Programmed Learning"; Miss Lee's essay on "Perception, Intuition and Insight" falls half-way between the two groups. Finally we have in Part 2 three personal essays by writers who are not professional 'educationists': Stephen Potter, Lord Caradon and Richard Hoggart.

The student who is looking for a handy summary and authoritative list of sources will probably find the first group most useful. The general reader interested in education will get little from these but much from the second group, while the third group are only marginally related to the title of the collection.

There are important points excellently made in many of the essays; for example, Dr. Wall's warning that an judicious use of visual aids may tend to fixate children at a pre-conceptual stage and make it harder for them to develop verbal symbolization; or Dr. Kay's suggestion that the next and really important development in programmed learning should come with the instruction of groups rather than individuals. If there is a theme which emerges more than others it seems to be a growing recognition of the importance to learning of relationships within the group and a diminished interest in the paradigm case of an individual in a 'learning situation'. In this respect Dr. Taylor's is one of the most interesting and certainly among the best written of the essays.

The three tail-pieces are all worth reading, though whether it was worth reprinting them in this series seems to me more doubtful. Richard Hoggart deals firmly but justly with the claim of the advertisers that they are really exceptionally honest public benefactors. Lord Caradon's analysis of the genuine concept of trusteeship is sad in its over-optimistic predictions about the future of Cyprus. But the most fascinating is Stephen Potter's revelation of how one man has learnt to enjoy so much of life. How many of us, I wonder, realized that before he turned to gamesmanship he had edited the *Nonesuch Chaucer*?

A. D. C. PETERSON

COSMIC RAY PHYSICS

Cosmic Ray Physics

By A. E. Sandström. Pp. x + 421. (Amsterdam: North-Holland Publishing Co., 1965.) 80s.

DURING the past twenty-five years a world-wide network of stations has been built up to record the intensity variations of the secondary cosmic rays reaching the surface of the Earth. The detectors comprise neutron monitors which respond to the nucleonic component of the secondary radiation and counter telescopes and ionization chambers which mainly record the mu-meson component. Information on the energy and directional dependence of the intensity variations of the primary cosmic rays far out in interplanetary space is obtained by correcting for atmospheric effects and for the deflexion of the charged particles in the geomagnetic field.

Cosmic Ray Physics, by Sandström, provides a comprehensive account of the methods used in these cosmic ray

studies and of the results found. Secondary production and cascade processes in the atmosphere are outlined, and the radiation detectors now in use are described. A detailed account is given of the statistics of particle counting with especial emphasis on the errors involved in the harmonic analysis of the daily variation in intensity. Some difficulty exists in locating the direction of anisotropies in the primary radiation due to the large opening-angles of cosmic ray telescopes and to the scattering of secondary particles in the atmosphere. Further, there is the large dispersion in the magnetic deflexion of different energy primary particles in the Earth's field. Sandström describes what is known about these optical aspects of cosmic ray telescopes.

The well-established intensity variations of the primary cosmic rays are the eleven-year cycle, inversely correlated with solar activity; the Forbush decreases in which a short-term fall of intensity by up to 15 per cent takes place and which may last for a week or more, and the diurnal variation of approximately 0.2 per cent in amplitude. There are also variations correlated with the twenty-seven-day period of solar rotation. Sandström describes the variations, in particular giving a detailed account of the different types of onset and decay of Forbush events. Various theories to explain these primary particle modulations are briefly outlined. Sandström favours the solar beam model in which plasma from the Sun streams out, carrying with it a rather regular, frozen-in magnetic field. Owing to the plasma motion a terrestrial observer also sees an electric field. Forbush decreases are interpreted in terms of particle energy-loss in the electric field together with a screening effect of the magnetic field in the beam. Some early space-probe observations are used to support this interpretation. Experimental results on the daily variation indicate a small excess of particles coming from the evening side of the Earth. Various theoretical interpretations are advanced, including the possibility that this simply represents cosmic rays co-rotating with a large-scale magnetic field, attached to the Sun, but extending far out into interplanetary space. The propagation of solar flare particles to the Earth is discussed in terms of the solar beam theory. Rather short chapters are devoted to extensive air showers, the problem of the origin of cosmic rays and the radiation trapped in the magnetosphere.

The aim of much cosmic ray work is to understand the interaction between the energetic particles, fields and plasma in interplanetary space. Direct measurements are now made of all these physical quantities using vehicles such as the *Interplanetary Monitoring Platform (IMP)* series of eccentric orbit satellites. An important early result from *IMP* is the confirmation of the idea that the interplanetary field can be roughly represented by a spiral lying in the ecliptic plane. However, the sea-level recording on which Sandström mainly concentrates yields important complementary results to the satellite work, especially with regard to the accurate measurement of small variations occurring over long periods.

The book may be recommended to undergraduates taking a special course in cosmic rays and to graduates and research workers in the field. J. J. QUENBY

SEDIMENTARY STRUCTURES

Atlas and Glossary of Primary Sedimentary Structures By F. J. Pettijohn and P. E. Potter. Pp. xv + 370 (including plates). (Berlin, Göttingen, Heidelberg and New York: Springer-Verlag, 1964.) 59 D.M.

THE first concern of all geologists is to describe logically and accurately the effects wrought by past events in the history of the Earth. Any tool that assists the professional geologist or student in training in this task is therefore to be welcomed. With their book *Atlas and*

Glossary of Primary Sedimentary Structures, Prof. F. J. Pettijohn and Prof. P. E. Potter make a notable addition to the resources of undergraduates reading earth science and of teachers in the field of soft-rock geology. The *Atlas* will also be valued by those professionals whose work lies largely outside the stratigraphical and sedimentological fields.

Most of the book is taken up with 198 photographic plates illustrating many different kinds of sedimentary structure, the majority of which are dependent on current action or on deformation of the sediment prior to lithification. Most of the plates represent natural or artificial rock-exposures, but twenty-nine come from the field of present-day sedimentary environments. The plates are preceded by a brief statement in which primary sedimentary structures are defined and classified. After the plates comes a glossary of names and terms commonly applied to sedimentary structures. The text and descriptions of the plates are also given in German, French and Spanish.

The scope of the book is a very fair measure of the advances in descriptive knowledge of sedimentary structures made since Sorby, in Britain, and Hall, in the United States, began work in this field more than a century ago. The authors' aim is descriptive and the adopted classification of structures logical within the self-imposed limitations of a field guide. Notwithstanding, a classification based on genesis of the higher ranks of structure will be regarded by many teachers as equally valid within these same limitations, on the grounds that the description and analysis of a body of rock should be so arranged as to provide the most direct path to an interpretation. Thus, for example, load casts were perhaps better illustrated with ball-and-pillow, ripple marks with cross-stratification, and current crescents with flute marks, than with the structures chosen as immediate neighbours for them in the *Atlas*.

Although the book is wide in scope there are important omissions. Aeolian dunes and related phenomena are treated very sketchily, although a wealth of photographic data exists for this important group of structures in both North America and Europe. A much fuller treatment of trace fossils could have been given without the authors having risked over-specialization. Many teachers will regret that there is no discussion of the limitations of rock-exposures as evidence of the complete form of sedimentary structures. In the case of cross-stratification, for example, surely some of the many plates used to illustrate this structure could have come from outcrops that provided more than one plane of section. Such evidence is all too rare, but because three-dimensional views are provided is to be valued the more highly.

The *Atlas* is well produced and the publishers are to be congratulated. The plates are excellent technically, if over-abundant in the case of certain structures, and the number of printer's errors is relatively small. However, it is a pity that a text is not provided in Russian, in view of the large amount of sedimentological work published by Soviet scientists in recent years. None the less, Profs. Pettijohn and Potter have performed a notable service, and their book is to be warmly recommended to teachers and students alike.

J. R. L. ALLEN

A JOURNEY THROUGH MODERN BIOCHEMISTRY

Essays in Biochemistry

Vol. 1. Edited by P. N. Campbell and G. D. Greville. Pp. xi+170. (Published for The Biochemical Society by Academic Press, London and New York, 1965.) 18s. 6d.

THE major purpose of *Essays in Biochemistry* is to provide the advanced student with an annual group of essays, less detailed than the usual specialist reviews, at a price that is within the scope of students' budgets.

In addition, the editors hope the essays will be helpful teachers and research workers as a vehicle to transport them to sectors of the biochemical front where they are not personally engaged. With the ever-increasing diversification and pace of biochemical research, the second objective seems at least as important as the first.

Volume 1 opens with an account by H. G. Wood and M. F. Utter of the role of carbon-dioxide fixation metabolism. The three main mechanisms of carbon dioxide fixation, which involve formation of carbon-carbon bonds, are described and the metabolic significance of such reactions in gluconeogenesis, fatty acid synthesis and metabolism of propionate is critically assessed. The second essay, by R. E. Davies, describes the gradual elucidation of the mechanism of muscular contraction. Although the author begins with the ancient Greeks, he rapidly progresses through the important pre-war work of R. Hill, K. Lohmann, W. A. Engelhardt and others to the massive effort which has been undertaken in the past two decades and finally to his own detailed theory. One is left with the impression that the time is ripe for a determined effort to investigate the mechanism of muscular contraction at molecular level. The important problem of determining nucleotide sequences in nucleic acids has been surveyed by K. Burton. After describing the analytical methods used for the separation and determination of fragments of nucleic acids, the author discusses enzymatic and chemical methods of degrading nucleic acids and determining the sequence of oligonucleotides. The prediction that the complete sequence of transfer ribonucleic acids will soon be known has already been vindicated by Holley *et al.* (*Science*, 147, 1462; 1965). The remaining articles on oxidative phosphorylation (D. E. Griffiths) and the photosynthetic electron transport chain in plants (R. Hill) are closely related. These are difficult subjects, both for the student and the research worker, and the authors have produced excellent accounts of them by leaving out a lot of detail and work which is of nebulous significance at the present state of our knowledge. The final article in particular is written in a refreshing style; it requires considerable courage to summarize a lifetime's work in a few pages and devote a significant proportion of the available space to indicate the problems that remain to be solved. R. Hill's account of the knowledge that accrued from his early work on haematin and haemoglobin should be an object lesson to young research workers to familiarize themselves with work that is proceeding in fields contiguous to their own.

For me, it has been a rewarding experience to journey through these carefully distilled accounts of present-day important topics to within sight of our present horizons.

D. T. ELMORE

ENGLER REVISED

Syllabus der Pflanzenfamilien

Von A. Engler. II Band: Angiospermen-Ubersicht über die Florenggebiete der Erde. Zwölfte völlig neugestaltete Auflage. Herausgegeben von Prof. Hans Melchior. Pp. 666. (Berlin-Nikolassee: Gebrüder Borntraeger, 1964.) 110 D.M.

ENGLER'S *Syllabus* has been a valued work of reference for systematic botanists since its first publication in 1892. With the appearance of the present volume covering the angiosperms the twelfth edition is completed, the volume dealing with the wide range of organisms from bacteria to gymnosperms having been published eleven years ago.

The volume has been completely rewritten by eleven contributors, the whole being edited by Prof. Hans Melchior. The numerous illustrations are all new, the old blocks having been destroyed during the Second World War. Many of the well-known, one may almost say

miliar, features of the original work are retained: the "ormation about economic and, particularly, medicinal ants, the extensive use of *Unterreihen*, and so on. There s, however, been a considerable rearrangement of the -ajor taxa, the most conspicuous feature of which is the -ansfer of the Monocotyledoneae to the end. Perhaps of -eater significance is the fact that the Pandanales, with hich the original Engler sequence of orders started, are ow placed near the end of the monocotyledons, presm -bly an acceptance of the view that the apparent sim- ilarity of their flowers is the result of reduction, not an dication of primitiveness.

In curious contrast to this is the retention of the petalous dicotyledons in the traditional order at the -eginning of that group, in spite of their syncarpous -varies and the widely held view that anemophily is a -econdary development in flowering plants. There is thus a -apparently unexplained inconsistency between the -inciples adopted for classifying the monocotyledons and the dicotyledons. This is perhaps not very important. What does seem to be of much greater importance arises -om the old, but often overlooked, question: "What is the -urpose of this classification?". The original *Syllabus* is he basis for the arrangement of many large herbaria and -umerous Floras, and so forms a guide to them; it is also o familiar to most taxonomists that when they want to ook something up they rarely need to refer to the index. The new edition has, at present, neither of these merits -nd, because of the labour involved, no large herbarium will be rearranged according to it, so it will still be neces- -ary for a working taxonomist to be familiar with the old Engler system and the Bentham and Hooker system, and -perhaps some others.

The urge to 'improve' systems of classification by -making them more 'natural' is one to which many, perhaps -most, taxonomists succumb sooner or later. It is an -understandable and perhaps laudable urge, but it seems to me unfortunate when it is given rein in a standard work of reference. In such works, for practical purposes an unchanging classification, even though rather artificial, is the most convenient. It is interesting to see that the differences between the Engler and the Bentham and Hooker systems, formerly so great, are now diminished.

In spite of this drawback, the twelfth edition of the *Syllabus* is a most valuable reference work, with its admirably clear descriptions of orders, sub-orders, families, sub-families and tribes, and the brief, but often illuminating, mention of important genera and species. It is particularly useful to have notes on pollen morphol- ogy, embryological characters and anatomical peculiar- ities included in the family descriptions. The bibliography is extensive and up to date, though perhaps rather deficient in its coverage of Russian literature. There are, inevitably, a few misprints, for example, *Astrodiscus* for *Astrodaucus* and *Bifora radicans* for *B. radians*; other- wise the book is well printed and the illustrations are clear and informative. It is certainly a work to keep near at hand.

T. G. TUTIN

PERISHABLE RICHES

Fish as Food

Edited by Georg Borgstrom. Vol. 3: Processing, Part 1. Pp. xiv+489. 125s. Vol. 4: Processing, Part 2. Pp. xiv+518. 132s. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1965.)

I N the preface to Volume 4 of this book the editor says that fish as food is part of the epic of mankind. But, he points out in Volume 3 that: "Only through wise utilization and efficient processing is it possible to save these extremely perishable riches of the oceans and fresh waters and place them within reach of the consuming public independent of seasons and of fishing fortunes . . .".

After death, fish deteriorates extremely quickly: even when packed with ice, biochemical changes commence immediately as indicated by the progressive accumulation of dimethylamine in the flesh. For reasons not yet under- stood calamitous breakdown of fish catches may some- times occur to produce, for example 'jellied' plaice or 'milky' hake. When environmental temperatures rise above 0° C spoilage can convert a "bin of sound fish to a soup or porridge. . . ." in 24 h.

The value of fish as food for man and its perishable nature have led from ancient times to the use of methods of preservation. Until comparatively recently, salting, drying and smoking were used. In addition to these techniques increasing use has been made since the last century of cooling and canning, and more recently of chemical additives.

No method of fish preservation is free from possible hazards. More than 4,000 years ago a Chinese wrote about the red discoloration of sea-water when it was allowed to stand and evaporate. This discoloration is due to the 'red-halophil' bacteria which can grow in high concentra- tion of salt and are the cause of the repulsive smell which sometimes develops in salted cod. Whereas most foods keep as well at temperatures up to 5° C as at 0° C, this is not the case with fish, the bacterial contaminants of which begin to flourish as soon as temperatures rise above freez- ing-point. Preservation of fish by cooling therefore re- quires special means of refrigeration.

By and large, man has been successful in preserving fish so as to allow its wide utilization as food. Fish sauces, fish pastes, marinades, anchovies and other products have become established either as essential or luxurious items in the diets of different countries. The biological mechan- isms of many traditional processing methods, and even the composition of some fish products, are not known. The techniques have evolved, not by the application of scien- tific knowledge, but from trial and error or by force of circumstance. The high cost of salt about 200 years ago, for example, probably led the people of northern Sweden and Norway to use less of it than was needed to prevent growth of bacteria. As a result fermentation occurred and, by good chance, gave a product, fermented herring, which is harmless to eat and has become prized as a delicacy.

The practice of food technology is increasing rapidly, and as it does so much new knowledge and many scientific and technical problems become apparent. This is particu- larly so in the case of fish processing; the discussions contained in the two volumes under review reveal the com- plexity of the problems and also that much is being done to try to resolve them. It is not enough to know that our forefathers preserved fish by smoking them in the house- hold chimney. On the scale that smoking of fish is now required it is necessary to know about the chemical and physical nature of smoke itself, the costs of producing it, and how temperatures in smoke kilns can be controlled. The fish sauce of Vietnam is known in France as an exotic addition to the diet. But, in a world facing catas- trophic shortage of food, the product may have much greater significance for as revealed by modern analytical techniques, it contains appreciable amounts of essential amino-acids. Until about thirty years ago the watery by- product obtained in the process of making fish meal was discarded. But the release of this waste not only caused serious pollution, but was a loss of valuable material. Fish solubles are now used as additions to animal feeds; they promote growth not only because of their vitamin content, but also because of the presence of a 'fish factor' the nature of which is unknown.

These are but one or two examples of the many topics discussed by the different authors of the two volumes, which bring to completion the treatise, *Fish as Food*. Dr. Borgstrom is to be congratulated on editing, and sub- stantially contributing to, a work which will surely remain for many years the standard exposition of the subject.

G. R. WADSWORTH

The Basic Laws of Arithmetic

Exposition of the System. By G. Frege. Translated by M. Furth. Pp. lxiii+143. (Berkeley and Los Angeles: University of California Press; London: Cambridge University Press, 1964.) 40s.

IT is scarcely surprising that Frege's two great works, the *Grundgesetze der Arithmetik* (1884) and the two-volumed *Grundgesetze der Arithmetik* (1893, 1903), made little contemporary impression. Frege's view, that mathematical propositions are truths of pure logic, ran contrary to accepted philosophical opinions of the day; his style, partly because of the severely abstract topic, was crabbed, and his symbolism idiosyncratic. Russell's almost accidental discovery of Frege's work, and his celebrated antinomy which struck away the basis of much of Frege's elaborate construction, gave due recognition to Frege as a great pioneer in the field of mathematical logic. Mr. Furth provides a new translation of the introductory parts of the *Grundgesetze*, with the appendix in which Frege somewhat pathetically announces the receipt of the Russell paradox just as his own work was completed and sets about trying to repair the damage. An introduction of some 50 pages discusses Frege's work and explains his themes, his language and symbolism, and adds a very necessary account of the way in which certain technical terms have been translated. Frege hoped to start with a small number of logical truths and a small number of rules of inference, and thence derive all the superstructure of arithmetic and analysis. Perhaps to-day there is less interest in building the superstructure than in examining the logical foundations, and Frege's semantics and his whole philosophy of language seem more relevant to modern enquiry than his inferred consequences.

A mathematical classic has been defined as "a book which must be written but need not be read"; it may be asking too much of modern students that they should read the originals, but Mr. Furth gives them a good opportunity of understanding why books such as Frege's had to be written. T. A. A. BROADBENT

The Application of Continued Fractions and Their

Generalizations to Problems in Approximation Theory
By A. N. Khovanskii. Translated by Peter Wynn. (Library of Applied Analysis and Computational Mathematics.) Pp. xii+212. (Groningen: P. Noordhoff, Ltd., 1963.) n.p.

THIS book is written in a pleasant and easy style. It is a welcome addition to the sparse and inaccessible literature on the application of continued fractions to approximation theory. The advent of digital computers has renewed interest in this subject as in many situations it provides a more efficient form of approximation than power series or other forms. The book provides a suitable introduction to the larger and more theoretical treatise on the subject by H. S. Wall.

The first chapter (75 pages) is introductory; it describes various notations and basic concepts: convergents and the related recurrence relations, equivalence between continued fractions and power series and their transformations, convergence and its domain, and convergence tests for various forms of continued fractions.

The second chapter (75 pages) derives a continued fraction expansion for the solutions of various differential equations of the Riccati type. This leads to the expansion in continued fraction form of a comprehensive list of basic mathematical functions: binomial, trigonometric, exponential, logarithmic, hypergeometric, Bessel's, gamma functions and others.

The last two chapters describe various further techniques such as the derivation of continued fractions expansions using Cesaro summation methods and the use of matrices to describe generalized recurrence relations and their convergence. I. M. KHAZABA

Determination of pH

Theory and Practice. By Dr. Roger G. Bates. Pp. xv+435. (New York and London: John Wiley and Sons, 1964.) 100s.

THIS book is a revised and enlarged version of *Electrometric pH Determinations* by the same author. The scope of the book has been extended to include no instrumental determinations of pH and these are fully surveyed and contrasted with instrumental measurement.

The early chapters of the book are devoted to the various definitions of pH scales from theoretical and practical points of view; these are extended to par aqueous and non-aqueous systems. The treatment of the theoretical aspects is clearly stated with concise definitions of terms, symbols and conventions in accordance with the International Union of Pure and Applied Chemistry recommendations. A discussion of the choice and use of secondary standards is included in a chapter on pH standards, while the primary standards are fully defined and tabulated. The performance of primary standard solutions is usefully summarized.

Various forms of electrodes used with pH instrument are fully discussed. These include hydrogen, quinhydrone, antimony, silver/silver chloride, calomel and glass electrodes. Understandably, the various commercial electrodes and types of pH meters reviewed are mainly of American origin.

The book is concluded with a short section on the principles of automatic control using pH sensing systems. Regrettably, a number of useful tables are lost in the general text of the book; one would have expected such tables as those denoting pH values of standard buffer solutions to have been included in the appendixes. In these days of high cost books this is remarkably good value for money; it is clearly presented and readable. R. SAWYER

L'Homme Face à Son Destin

Par Charles Léopold Mayer. Pp. 251. (Paris: Éditions Marcel Rivière et Cie., 1964.) n.p.

IN this little volume the reader will find the results of a philosopher's thoughts extending over a quarter of a century. It is distinguished by its lucid French logic, combined with a certain classical inevitability of argument. There are thirty-four short chapters, and their general trend can be seen as an attempt to expose the main facets of human experience, giving due weight to both sides of a question. For example, no absolute truth can be claimed, but only relative truth, and one law to govern the universe (necessity), or failing that, the law of chance. An interesting quasi-mechanical principle is introduced, namely, that of "least irritability" which controls the behaviour of the living world much as Maupertuis's law of least action functions in dynamics. For the author, Nature is amoral, but axiological judgments belong to human beings. It may well be, too, that natural law is a useful and necessary fiction, though no less a person than Leonardo da Vinci venerated it, and in terms of it made both artistic and technical progress.

If an estimate is to be made of the value of a collection of meditations such as this, it is perhaps prudent to recommend it as an academic study, since its conclusions are significant, but not immediately applicable to the daily round and common task. Much of it is in the style of soliloquy, very attractively expressed in the French idiom. F. I. G. RAWLINS

The Thymus in Immunobiology

Structure, Function, and Role in Disease. Edited by Robert A. Good and Ann E. Gabrielsen. Pp. xxii+778. (New York and London: Hoeber Medical Division, Harper and Row, 1964.) 24.50 dollars.

THIS volume is based on the proceedings of the first International Symposium on the Thymus, and, with its contributions by 72 authors, collects together

ost of the groundwork on which our present knowledge is based. Of the 36 contributions, most are concerned with the relation of the thymus and bursa of Fabricius with lymphocytopoiesis and the development of immune responsiveness. Much of this material has been published elsewhere, and some has been reviewed also. Thus, it is pity that this book was not published more promptly after the symposium (held in 1962) when it might have had greater value.

Many of the old problems still remain: there is still no convincing evidence that the thymus, either directly or via the lymphocyte, has any trophic function on other tissues; and there is still no convincing way of determining whether thymic lymphocytes have an epidermal or mesodermal origin. However, the distinct functions of the thymus and bursa in the chicken become clearer now that thymectomy has been achieved while leaving the bursa intact.

There are lighter moments: as when one author presents the same unconvincing experiment which he first described in 1953. Another author, starting with an *idée fixe* that the thymus is concerned with growth, is continually frustrated by finding that each of his 'thymic' growth factors is a general tissue constituent. The clinical papers, though often long-winded, are perhaps the most valuable, since much of this material is not always readily accessible. Here is a fascinating collection of facts which will correct any tendency to over-simplify from the results of pure research.

There are many illustrations, which are mostly good, although the finer points of lymphoid tissue structure are not well conveyed by the usual black-and-white print. While the book will be of value in some libraries, there may be few individuals to whom it is worth the high cost.

R. B. TAYLOR

Advances in Enzyme Regulation

Vol. 2. Edited by George Weber. (Proceedings of the Second Symposium on Regulation of Enzyme Activity and Syntheses in Normal and Neoplastic Liver, held at Indiana University School of Medicine, Indianapolis, Indiana, September 30 and October 1, 1963.) Pp. xii + 405. (London and New York: Pergamon Press, 1964.) 100s. net.

THE symposia on "Regulation of Enzyme Activity and Syntheses" seem now to be established annual events, and the proceedings of the second meeting show that enzyme regulation in mammalian systems is nowadays the subject of as much research as is that of micro-organisms.

The volume, like the symposium, is arranged in six sections, each under a distinguished chairman. The first, on the regulation of gluconeogenesis, contains valuable discussions on the roles of the enzymes pyruvate carboxylase, phospho-enol-pyruvate carboxylase and fructose diphosphatase in gluconeogenesis and suggests an action of insulin as a repressor of some essential enzymes of this pathway. The second and third sections remain centred on carbohydrate metabolism and present data on acetyl-coenzyme A metabolism, the role of some steroids in gluconeogenesis, human hypoglycemia and the regulation of glucokinase. The synthesis of this last enzyme is inducible, being stimulated by glucose and by insulin.

Section four, on feed-back regulation in hepatic systems, contains papers on the enzymes lactic dehydrogenase, aspartic transcarbamylase and phosphofructokinase, on glucose regulation of enzyme synthesis and on regulation of cholesterol biosynthesis. Section five includes discussion of the substrate and coenzyme-stimulated induction of tyrosine transaminase; the final section, on enzyme regulation in hepatomata, presents studies of the variation of enzyme activities in tumours of different characteristics. Some correlation between enzyme activities, metabolic patterns and growth rates is analysed.

The volume is attractively presented, and represents a valuable progress report on a very active field of research.

C. J. R. THORNE

Seed-borne Fungi

Description of 77 Fungus Species. By J. P. Malone and A. E. Muscott. (Proceedings of the International Seed Testing Association, Vol. 29, No. 2.) Pp. 179-384. (Wageningen: Association Internationale d'Essais de Semences, 1964.) n.p.

THE authors of *Seed-borne Fungi* are well known for their experience in seed testing, and theirs is a compact and strictly practical production. After a brief historical introduction, the book contains a description of 77 species of seed-borne fungi, each illustrated by clear photographs of growth habit, fructification and/or spores, as may be appropriate to the species. The approved method of testing for its presence is indicated under each. Most of the fungi found on germinating seeds are harmless saprophytes but it is important that the tester shall be able to recognize these for what they are, and they are accordingly figured as carefully as the known parasites. The latter include 5 smuts (one, however, a common contaminant from cereal weeds) and 14 ascomycetes, or conidial states presumed to belong to ascomycetes. The 57 species in 36 genera of saprophytes described include 4 genera of ascomycetes which normally produce perithecia under test conditions, including 11 species of *Chaetomium*, 2 of *Melanospora* and 2 of *Sordaria*, all clearly figured, as well as 4 genera of phycomycetes. The fungi are arranged alphabetically, irrespective of their systematic position, often under the name of the ascus state even though only the conidial state is to be expected to appear under the test conditions. This has the awkward effect of dispersing the species of *Fusarium* described under *Fusarium*, *Gibberella* and *Griphosphaeria*, while those of *Helminthosporium* have to be sought under that name and under *Cochliobolus* and *Pyrenophora*. The treatment is not consistent, for *Batrachium cinerea* appears under that name, not as *Sclerotinia fuckeliana*, nor does *Cladosporium herbarum* masquerade under *Mycosphaerella tulasnei*, though to find *Stemphylium botryosum* we must look under *Pleospora herbarum*. Such difficulties as may arise from this treatment are resolved by an adequate index, but if one has to find a species through the index after all, the advantage of an alphabetical arrangement over a taxonomic one is lost. One feels that a taxonomic arrangement based on conidial states where they exist would be more helpful to an inexperienced worker seeking to name an unfamiliar species.

R. W. G. DENNIS

Methods for the Examination of Root Systems and Roots

By Dr. J. J. Schuurman and Dr. M. A. J. Goedewaagen. Pp. 86. (Wageningen: Centre for Agricultural Publications and Documentation, 1964.) 10.50 D.fl.; 21s.; 3 dollars.

THE underground parts of plants have been consistently neglected by botanists, most of whom have little idea how to set about investigating the morphology of a root system. This little book is therefore to be welcomed, for it describes concisely the various methods of investigation which have been used, those favoured by the authors being supported by numerous practical hints. The methods covered include the transferring of a soil monolith to a pinboard, the mapping of a profile face, the sampling of root systems by means of auger borings, and the growing of experimental plants in special containers. The scope of the book is confined to methods, and the sceptical are left to wonder whether the arduous task of disinterring roots yields a commensurate return of information. Production of the book is good; but the reader will encounter a few eccentricities of vocabulary, such as pendulum for crank handle.

W. D. CLAYTON

THE SMITHSONIAN INSTITUTION, WASHINGTON, D.C.

A COLOURFUL academic procession opened the three-day (September 16-18) celebration, marking the two-hundredth anniversary of the birth of James Smithson, founder of the Smithsonian Institution, Washington, D.C. Prof. S. Dillon Ripley, the present chief executive and secretary of the Institution, said that the bicentennial paid tribute to the Institution's past, affirmed its intent to fulfil a vital role in society, and examined the nature of knowledge and creative discovery as conceived by James Smithson and as understood to-day.

On September 16 the procession, which began with a clarion call from a tower of the world-famous red Smithsonian 'castle', included some 500 world scholars and scientists. Colonel John H. Magruder, of the U.S. Marine Corps, acted as the grand marshal, leading the distinguished gathering from the original Smithsonian Institution building across the Washington Mall, to a site near the Museum of Natural History.

Here, the President of the United States addressed the delegates in an outdoor convocation. Dr. Leonard C. Carmichael, former secretary of the Smithsonian, also gave an address, the substance of which follows.

Smithson, an English scholar, scientist, and prominent member of the Royal Society of London, left his entire estate to the United States, a country he had never seen, "to found at Washington an establishment for the increase and diffusion of knowledge among men". He died in Genoa, Italy, in 1829.

To-day, his 'legacy' comprises twelve far-reaching units:

(1) The Museum of Natural History, which has separate offices of anthropology, ecology and systematics, and departments of vertebrate and invertebrate zoology,

entomology, botany, palaeobiology, and mineral science and an oceanography programme.

(2) The Museum of History and Technology, which has departments of science and technology, arts and manufactures, civil history, and armed forces history.

(3) The National Air Museum, containing the world largest collection of historical aviation and aerospace material.

(4) The Freer Gallery of Art, specializing in near and far eastern art.

(5) The National Collection of Fine Arts.

(6) The new National Portrait Gallery, which will feature portraits and statues of men and women who have made significant contributions to the history, development and culture of the United States. This gallery will not be fully developed until 1967.

(7) The National Zoological Park.

(8) The International Exchange Service, which exchanges scientific, cultural, literary, and governmental publications with other countries.

(9) The Science Information Exchange, which receives organizes and disseminates information about scientific research in progress in the United States.

(10) The Astrophysical Observatory, located at Cambridge, Massachusetts.

(11) The Canal Zone Biological Area, a biological preserve located on Barro Colorado Island in Gatun Lake, Canal Zone.

(12) The National Gallery of Art, which has a separate Board of Trustees.

The John F. Kennedy Center for the Performing Arts, to be built soon in Washington, is also a bureau of the Smithsonian Institution, administered separately by its own Board of Trustees.

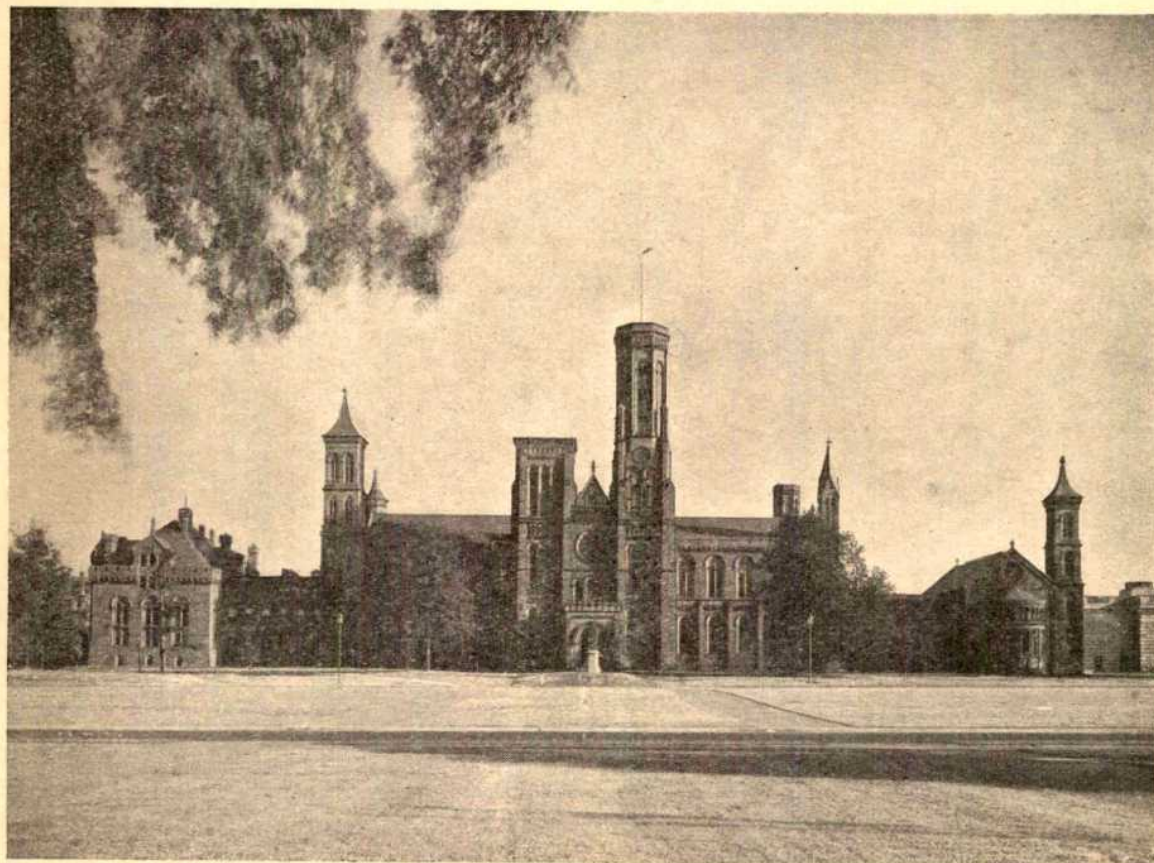


Fig. 1. The Smithsonian Institution

JAMES SMITHSON: PATHFINDER IN SCIENCE AND PHILANTHROPY

By DR. LEONARD CARMICHAEL

Vice-President for Research and Exploration, U.S. National Geographic Society,
and Former Secretary of the Smithsonian Institution

"Why did he do that?" Her Majesty Queen Elizabeth, the Queen Mother, asked me. I was walking with her as I showed her the Museum displays of the Smithsonian Institution. I had just said that James Smithson, the Institution's founder, was an English philanthropist who had never been on the American continent, but when he died he left his whole large fortune to found the Smithsonian in Washington.

"Why did he do that?" I had to reply that no one knew. But, as a partial answer, I would like to say something of the life of this idealistic lonely English scientist whose bequest has done so much for America and for the world and whose birth 200 years ago is now being honoured.

The basic facts of the life of this benefactor are sad and hard. H. R. Tedder, his English biographer, says he was "a man of gentle character whose life was devoted to study uncheered by domestic affection". He was the natural son of Sir Hugh Percy, first Duke of Northumberland of the third creation. This nobleman, in spite of a reputation for arrogance, was one of the great figures of his day. A contemporary called him the handsomest man in England. He had a large personal fortune and he held many posts of honour. He served as a Privy Councillor and Lord-Lieutenant of Ireland. His own family name was Smithson before it was changed to that of Percy by Parliament.

James Smithson's mother was a widowed heiress, Mrs. Elizabeth Hungerford Keate Macie, who was herself descended from King Henry VII through the line of the Dukes of Somerset. Much of his mother's considerable wealth came from her own family, the Hungerfords, and

from the estate of her deceased husband, John Macie, a country gentleman of Bath, Somerset. Her son, James, was born in France in 1765, and for this reason it was later necessary for him to be naturalized as an English subject, even though both his parents were English. Strangely, when this petition was granted by the Crown, it contained unusual provisos. It granted James the advantage of being a British subject except for certain stipulations such as that he was never to be allowed to be a member of the Privy Council or of Parliament or to be a civil or military officer. Neither could he receive any hereditaments from the Crown. Thus, unlike many scientists of his day and ours, he was never knighted.

J. C. Long, who has recently written a new life of Smithson based on extensive research in England and to whose scholarship I owe much in preparing this address, points out that these restrictions in his grant of naturalization were most extraordinary. He feels that they must have been inserted by someone with power in court circles who wished to guard against a possible political challenge later in life by this personable, wealthy, and well-born young man. Since the Duke had been a Privy Councillor, the specific mention of this office as barred to his natural son may not be without significance in hinting at the author of the surprising prohibitions. Many facts about Smithson's life are in doubt and may always remain so, because his personal papers, which were sent to America when his Institution was founded, were lost in the disastrous Smithsonian fire of 1865. Two hundred of Smithson's unpublished manuscripts and thousands of items of his personal memorabilia and his mineral collection of some ten thousand specimens perished at that time.



Fig. 2. Museum of History and Technology, Smithsonian Institution

In 1782, at the age of seventeen, James matriculated at Pembroke College, Oxford. Here, because of his family, he wore the special gown and had the privileges of a 'gentleman commoner'. On matriculation he signed the thirty-nine articles of the Church of England, and throughout his life he remained loyal and constant in his membership in this church. This was true even during the extensive periods of his life which were spent on the Continent of Europe.

James mastered well the regular classical studies of his period at Oxford. Almost from the first, however, he became interested in the then extra-curricular activity of the study of experimental science. Soon he was recognized as one of the leading authorities at Oxford in the infant science of chemistry. He also became an intensive student of geology and mineralogy. While still an undergraduate he was a scientific member of an important and well-organized geological expedition under the direction of a distinguished French mineralogist.

At twenty-one years of age, in 1786, he graduated with the degree of master of arts. His already achieved distinction as a chemist is attested by the fact that eleven months after he received his degree he was elected a Fellow of the Royal Society. He seems to be the youngest man ever to have received this honour. The Royal Society, then as now, was recognized as having no peer among the learned academies of the world. It is indeed amazing to think of a scientist of twenty-two being able to write after his name the great letters, F.R.S.

He soon established himself as an almost-official helper and close colleague of Henry Cavendish, the discoverer of hydrogen and one of the most distinguished scientists of the eighteenth century. This scholarly man, son of Lord Charles Cavendish and grandson of the Duke of Devonshire, was the possessor of a large personal fortune, but he elected to live almost a hermit's life and to develop the excellent private laboratory in which Smithson worked with so much satisfaction.

James Macie, as Smithson was known at that time, also established close friendships with other scientists in London and especially with Sir Joseph Banks, president of the Royal Society. Other friends were Sir Humphry Davy and the American-born Benjamin Thompson, Count Rumford.

Before his mother's death she expressed the wish that her son adopt the name Smithson, his father's family name. As I have noted, his father had taken the name Percy when he was created Duke of Northumberland. It is interesting that Smithson's mother and also Sir Hugh's wife were related by blood to the Percy family, but the Duke himself was not. In 1801 the petition for his change of name was granted, and thereafter the founder of the Institution is officially James Smithson.

As a young scientist Smithson was assiduous in attending meetings of the Royal Society and was active in his own programme of experimental research. He soon began the publication of important scientific papers. To-day we hear of the so-called 'publish or perish' policy of some large universities. Smithson would have passed this test with honour. In all he published some twenty-seven papers in the most respected scientific research journals of his day.

Smithson never married, and in spite of the gay world into which he was born and his ample fortune he devoted his life to study and research. Not only was he active in scientific associations but he also visited and worked with leading research men of his day in Berlin, Paris, Rome, Florence, Genoa, and Geneva. He knew France well under the monarchy, but when anti-Royalist sentiments became common in Paris he found himself sympathetic with them. Indeed, all his life he was philosophically favourable to a republican form of government rather than to a monarchistic one, but he deeply disapproved of the excesses of the French Revolution. He did, however, understand its basis in the errors of the old régime. He wrote in this

connexion: "Stupidity and guilt have had a long reign and it begins indeed to be the time for justice and common sense to have their term".

No one knows how close Smithson was to his autocratic, handsome, and politically powerful father, the Duke of Northumberland, or to the Duke's other children who were his half-brothers and -sisters. It is interesting that one of his half-brothers, Earl Percy, who later succeeded his father as Duke of Northumberland, was Lieutenant-General of the British forces during the American Revolution. He led British troops back from the battle of Lexington and Concord. He secured his recall from America because he had long disapproved of the policy of George III towards the colonies. General Percy, indeed like James Smithson and some other members of his family, was a very moderate Tory and much attracted to republican theories. He was known as an admirable landlord and as a most generous officer by those under his command. How much these attitudes and especially his view of America influenced his younger half-brother, James, we probably will never know.

James Smithson was disturbed by the success of Napoleon in Europe, but he continued to visit the Continent for study and research. In 1807, he was seized as a prisoner of war by the Napoleonic authorities as he was about to sail back to England from Denmark. His treatment as a prisoner was harsh, and he never fully recovered from the chest illness which he acquired at this time. Almost certainly as a result of the intervention of his friend Sir Joseph Banks of the Royal Society, the King of Denmark interceded on behalf of Smithson. Even with this help, although his lot was brightened, he did not succeed in returning to England until Napoleon's power waned after the disastrous Russian campaign of 1812.

In 1800 and 1801, before these difficult prison years, Smithson had been one of the group which established in London the Royal Institution. This new organization had and has a remarkable mission which in many ways suggests the purposes of Smithson's own later institution in Washington. The purpose of the Royal Institution as set forth in its charter is:

"For diffusing the knowledge and facilitating the general introduction of useful mechanical inventions . . . and for teaching by courses of philosophical lectures and experiments the application of science to the common purposes of life."

As I have already stated, most of Smithson's own scientific work dealt with chemistry or mineralogy and with what would now be called applied science. One of his most notable contributions was his development by the ingenious use of the still-primitive chemical methods available of the basic distinction between the native carbonates and silicates of zinc. As a result of this work the beautiful and economically important zinc carbonate ore is now called 'smithsonite'. One of Smithson's real memorials at his Institution in Washington is a specially constructed case in which various forms of brilliantly coloured smithsonite slowly revolve on a turntable so that each year hundreds of thousands of visitors to the Institution's Hall of Minerals and Gems can see the full beauty of the founder's great mineral.

Smithson also was one of the pioneers in what is to-day called microchemistry. He was able to analyse quite minute quantities. On one occasion he effectively identified the salts in solution of half a drop secretion from a human lacrimal gland. He also developed a method for the detection of very minute quantities of arsenic and mercury.

In spite of his concern with experimentation he was also interested in the theory and philosophy of science. One of his papers deals in an effective way with the errors of a contemporary scientist who tried to see unjustifiable biblical significance in fossil bones collected in a Yorkshire cave.

Smithson was well aware of the insufficiency of scientific knowledge of his time. He said: "What we know in

mistery bears so small a proportion to what we are ignorant of; our knowledge in every department of it is so incomplete, consisting so entirely of isolated points, thinly scattered, like lurid specks on a vast field of darkness, that researches can be undertaken without producing some results leading to consequences which extend beyond the boundaries of their immediate object".

He was also far ahead of his time in concerning himself with the fundamentals of scientific research and scientific method. At one point he wrote in adumbration of much later knowledge: "The particle and the planet are subject to the same laws, and what is learned of one will be known of the other".

The eighteenth century was not a period noted for great personal morality on the part of many members of its wealthy and influential families; but no breath of scandal, even by surmise, has attached itself to James Smithson. It may be noted, however, that he did have one form of recreation that may call for the raising of at least some proper modern eyebrows. He liked to gamble. He was well known in the casinos of European capitals. But Smithson was a good mathematician, and he early convinced himself that the only consistent winner in a gambling hall was the proprietor. He even calculated with care what such a man's winnings were apt to be. Because of this knowledge he always limited his expenditures at the table of chance to a predetermined sum which was well within his means for an evening's entertainment.

Three years before his death Smithson wrote his famous will. It began as follows: "I James Smithson Son to Hugh, 1st Duke of Northumberland, and Elizabeth, Heiress of the Hungerfords of Studley, and Niece to Charles the 4th Duke of Somerset, . . . make this my last Will and testament. . .". The Will provided that the income of his estate should go to his nephew, and if the nephew later had children the principal should go to them. But if his nephew should die without heirs he bequeathed the whole of his property, "to the United States of America to found in Washington under the name of the Smithsonian Institution an establishment for the increase and diffusion of knowledge among men".

On June 26, 1829, Smithson died in Genoa, Italy. In this city he had close scientific colleagues. He had been a devout attendant at the Church of England services in that city, and it was after services in this church that he was buried in the Protestant cemetery of that city. To-day there is in the English Church of the Holy Ghost at Genoa a fine marble relief monument to Smithson erected by the Regents of the Smithsonian Institution.

His nephew died without heir on June 5, 1835, and even weeks later President Andrew Jackson in an address to Congress made formal recognition of the gift to the American nation.

After prolonged discussion and largely as a result of the effective work of John Quincy Adams, who had returned to the House of Representatives after his period as President of the United States, an act authorizing the Smithsonian Institution was passed by the Congress and signed by President Polk on August 10, 1846. The English gold sovereigns in which the bequest was paid were brought to America and recoined at the Philadelphia Mint. In new American money the bequest was 508,418.46 dollars.

The bequest may not seem very great to-day, but at that time it was correctly recognized as a most significant sum. No comparable amount of money then existed in America in any university or other establishment to be used as an endowment to support research or the diffusion of knowledge.

Under the administration of the first Smithsonian secretary, the distinguished scientist Joseph Henry, and with the support of an able Board of Regents, the Institution was soon actively engaged in its work of fostering new scientific research. Through the years Smithson's phrase, "the diffusion of knowledge", has been interpreted at his

Institution to mean the issuing of books and monographs and the development of truly instructive museum displays. At least 12,000 separate publications have been issued by the Smithsonian Institution.

At about the turn of the century word was received that land changes were threatening the area of the cemetery in which Smithson was buried in Genoa. The Regents of the Smithsonian delegated their very distinguished member, the great telephone inventor, Alexander Graham Bell, to go to Genoa and investigate the condition of the founder's grave. As a result it was decided that Smithson's remains should be brought to Washington. His marble sarcophagus was transported with honour to the United States, and borne from the Washington Navy Yard to the Institution, accompanied by a troop of cavalry, a detachment of Marines, and the Marine Band. To-day, under English and American flags, the sarcophagus rests in a beautiful chapel in the Institution's original building on the Mall here in Washington.

So, as we think of Smithson's quiet and useful years and of his amazing gift to the United States, we can again consider the Queen Mother's query: "Why did he do that?" Still it must be said no one can answer this question with assurance, but I hope some people may feel with me that an understanding of Smithson's life makes it apparent that he was a prudent but true son of the 'Enlightenment'. In youthful and active America he saw much that was hopeful for the human race everywhere. His own Institution has certainly been true to his ringing charge, and in each year since its founding it has transformed some good aspect of his dream into solid serviceable reality.

When the Smithsonian was established, Washington was a swampy and fever-ridden community of some 5,000 people, and the country of which it was the capital had scarcely begun the kind of scientific research which even then was providing much humanly important, pure and applied knowledge in the older European countries. The careful handling of the income from Smithson's bequest and of funds provided by other gifts, and above all by appropriations of the Congress of the United States, has allowed the Smithsonian Institution through the years to serve as a pioneer in the establishment of the great scientific tradition of the United States. To-day and indeed through all its years, the success of the Smithsonian has been due in large part to the devotion of its always-eminent and always diligent Board of Regents.

The utterly unselfish generosity and the broad and wise terms of Smithson's bequest aroused great interest in the United States and indeed in the world when it became known. In many ways this unprecedented gift still helps set a pattern for charitable and educational benefactions. Histories of the development of the great philanthropic foundations of the United States which are doing and have done so much for the world rightly begin with a reference to James Smithson's beneficence.

As a man Smithson was a loyal son of England. But as a scientist his horizon always was global. He once wrote: "The man of science is of no country, the world is his country, all mankind his countrymen". Surely those of us who have been privileged to know Smithson's Institution intimately cannot help feeling that by his gift he established an Institution which certainly in the future, as in the past, will continue the great work of the increase and diffusion of knowledge in a way that will benefit all mankind everywhere.

It is well that the following words of Smithson himself are cut in the stone of the Institution's great new Museum of History and Technology:

"Every man is a valuable member of society who, by his observations, researches, and experiments, procures knowledge for men . . . it is in his knowledge that man has found his greatness and his happiness, the high superiority which he holds over the other animals who inhabit the earth with him, and consequently no ignorance is probably without loss to him, no error without evil. . . ."

Such a "valuable member of society" indeed was James Smithson, the sagacious, far-sighted and truly philanthropic founder of the now honoured and superlatively useful Smithsonian Institution.

SCHOLARLY SESSIONS AT THE SMITHSONIAN BICENTENNIAL CELEBRATIONS

The following sessions were held during September 17-18. The chairman is named (in brackets) followed by the speakers.

(1) *Man's Origin and Progress* (Dr. Caryl P. Haskins, president of the Carnegie Institution and regent of the Smithsonian Institution): Prof. F. L. Whipple, director of the Smithsonian Astrophysical Observatory; Prof. Claude Levi-Strauss, director of the Institute of Ethnology, University of Paris.

(2) *Environmental Influences on Man* (Dr. Crawford H. Greenewalt, chairman of the Board, E. I. du Pont de Nemours and Co., and regent of the Smithsonian Institution): Dr. Ian McTaggart Cowan, dean of the faculty of graduate studies, University of British Columbia; Prof. J. Robert Oppenheimer, professor of physics and director of the Institute of Advanced Study, Princeton, formerly director of the Los Alamos Scientific Laboratory.

(3) *Man and Technology* (The Hon. A. M. Burden, former American Ambassador to Belgium and regent of the Smithsonian Institution): Prof. Herbert Butterfield, master of Peterhouse and professor of modern history in the University of Cambridge; Dr. Lewis Mumford, president of the American Academy of Arts and Letters.

(4) *Inheritance, Values and Creativity* (The Hon. John Nicholas Brown, former assistant secretary of the U.S. Navy for Air and regent of the Smithsonian Institution): Prof. G. Evelyn Hutchinson, Sterling professor of zoology, Yale University; Sir Kenneth Clark, former director of the National Gallery, London; Mr. Arthur Koestler, author.

(5) *Man's Developing Intellect and Attitudes*: George H. Mahon, United States Representative (Te and regent of the Smithsonian Institution); Prof. Jer-S. Bruner, professor of psychology and director of Center for Cognitive Studies, Harvard University; Prof. Stephen E. Toulmin, professor of the history ideas and philosophy, Brandeis University, former director of the Nuffield Foundation Unit for the History of Ideas in London.

JAMES SMITHSON MEDAL: ROYAL SOCIETY OF LONDON

The James Smithson Medal, instituted in memory of British founder of the Smithsonian Institution, is award for distinguished contributions to "the increase and diffusion of knowledge among men", the role of Institution as set forth by James Smithson in making bequest of his fortune to the United States.

The Medal is the creation of Paul Vincze of London following closely a medallion made in 1817 by the well-known sculptor and engraver at the Paris Mint, Nicolas Pierre Tiolier, which was marked on the back in Smithson's own hand as "my likeness". Vincze has created on the obverse a portrait of James Smithson, surrounded by his name and dates of his birth and death, 1765-1829. The reverse bears a rendering of the familiar outline of the first Smithsonian building, for which the cornerstone was laid on the Mall on May 1, 1847.

The Medal was presented for the first time on September 18, 1965, to the Royal Society of London, during the bicentennial celebration. In the future it will be presented for exceptional achievement in the various disciplines of science and the humanities embraced by the Smithsonian Institution.

The first Smithson Medal for outstanding contribution in the fields of science, technology, history, and art was presented by Robert V. Fleming, chairman of the Board of Regents of the Smithsonian Institution.

EVOLUTION IN THE BIOSPHERE

By PROF. HAROLD F. BLUM*

National Cancer Institute, National Institutes of Health, Bethesda, and Department of Biology, Princeton University

EVOLUTION in the biosphere—whether biological or cultural—is based on the persistence of patterns which are on occasion subject to alteration, the altered pattern persisting in turn until another alteration occurs. The genotype pattern of a species persists through replication in the germ cells from generation to generation, subject to rare modification which constitutes mutation. Cultural patterns are also perpetuated; we do not know much about the mechanism in this case; but it is clear that they may persist for long periods although readily modifiable when circumstances arise¹.

Perpetuation makes possible the selection of some patterns—according to the dictates of environment or other factors—with the consequent rejection of others, for example, the natural selection of a mutant form from a number of possible alternatives. By restricting our view to the selected patterns and disregarding the rejections we see evolution as an increase in order, although the order is related inversely to the number of rejections. We may picture the increase in order in the evolving system as accompanying the addition of facets of pattern to the pattern as a whole; somewhat analogous to increase in order with growth of a crystal. Since change in pattern is relative to existing pattern, the number of facets may be expected to grow exponentially; thus

increase in order is accompanied by limitation of subsequent change to a general type of pattern, but at the same time the possibilities of change within the scope of that pattern may increase.

Analogy may be made to a computer, where the number of possible answers is decreased by selection of one answer instead of another, each question being postulated on the previous one². The increase in order of the evolving system should, then, be expressible in a form similar to that applied to computer operation:

$$-\Delta s = q \ln \frac{f_2}{f_1} \quad (1)$$

where $-\Delta s$ is the negentropy change, f is the number of facets of biological or cultural pattern in the evolving system, q is a constant, and \ln indicates the natural logarithm. Successful mutations and adopted cultural innovations represent additions of facets to respective existing patterns.

Perpetuation of both genetic and cultural patterns depends on perpetuation of the kinds of organisms which carry them. This involves maintenance as well as reproduction, paid for in terms of free energy amounting to about 2×10^{18} kg cal/yr captured from sunlight by photochemical reduction of carbon dioxide. The fraction of the total free energy expenditure which supports the

* Present address P.O. Box 704, Princeton, New Jersey, U.S.A.

rection of changes in pattern directly concerned in the increase of negentropy must be extremely small, so no violation of the second law of thermodynamics need be feared in the increase of order which accompanies evolution.

Although the supply of free energy limits the rate of accumulation of negentropy, it does not control the direction in which evolution may take, any more than the amount of electrical energy supplied to a computer influences the answers it gives to questions. Thus, although the biosphere is ultimately dependent on the Sun for its supply of energy, the course of evolution may be treated as virtually independent of events in, and properties of, the universe beyond Earth's limits. Some influence from outside our planet must, of course, be admitted—the rate of occurrence of mutations may be influenced by ionizing radiation from external sources, and investigation of the heavens has played a profound part in Man's cultural evolution—but we may neglect these as minor factors in our view of the process as a whole.

Evolution in the biosphere does not constitute movement toward greater order *per se*; but is guided by changes in specific properties, as contrasted with changes in number of arrangements of properties in terms of which change in order is measured, and these two things need not run closely parallel. (May we not think of free energy depending on specific arrangements of microscopic properties, as contrasted to the number of arrangements of microscopic properties in terms of which entropy is measured?) For example, natural selection of a mutant over a parent form depends on specific properties of the two phenotypes and on specific properties of the environment with which they impinge—the resulting change in the genotype pattern entails, but does not depend on, a change in order.

Whether dealing with computer operation or with evolution, the assumption is usually tacit that increase in order is accompanied by increase in meaning², which must presumably be defined in terms of specific properties. Even though we might conceive of measuring meaning in terms of all the specific properties of the system, we would still not know how to fit our measurements into the same dimensional framework with the number of facets of orderly pattern. In practice we assess meaning in terms of subjective judgments (although sometimes this is concealed), thus substituting attributed meaning for the true meaning we cannot hope to measure; this would seem a potential source of error in thinking about the evolution of living systems.

It may be useful to formalize this situation by assuming quantitative relationship between attributed meaning, true meaning, and orderly arrangement of facets, representing this by:

$$x' \ln \frac{m'_2}{m'_1} = x \ln \frac{m_2}{m_1} = q \ln \frac{f_2}{f_1} \quad (2)$$

where m is a quantitative measure of true meaning, m' a measure of attributed meaning, and x and x' are respective constants which we imagine can be related to each other and to q within a common dimensional framework, although knowing that this cannot really be done.

Exponential growth of each of the variables, m' , m and f , expressed in equation (2), may be rationalized on the basis that change in pattern is always relative to existing pattern, whether involving change in number of facets or specific change in properties, and that our subjective estimates presumably follow the latter in a general way. Thus, in whatever terms measured, the addition of each facet increases the possibility of expansion of the pattern. We may, for example, expect culture to evolve exponentially because usually each cultural innovation increases the chance of further innovations³; even when there may be loss of facets, this should be relative to the existing pattern.

Evolution of the biosphere may be thought of as composed of many interplaying parts, and equation (2) may be applied to the whole or to a single one. In practice, our attention is usually focused on a particular part, say the species of a given genus or a single aspect of culture, which it is convenient to treat as a separate system independently of the whole. In so doing, however, we should not lose sight of the possible effects of other parts on the one we study, but we cannot expect to fit x' and x for different parts into the same dimensional framework.

The changes in specific properties which may be regarded as steps in evolution—successful mutations, and cultural innovations—may be thought of as chance events distributed successively in time. But since any given step depends on an existing pattern, the probability of the step occurring must be relative to the existing pattern, and hence not to be treated as a strictly random happening. Thus, the rate of evolution cannot be regarded as completely independent of the pattern of the evolving system, but for purposes of description we may write:

$$x' \ln \frac{m'_2}{m'_1} = \int_{t_1}^{t_2} f(t) dt \quad (3)$$

where $f(t)$ is a function which may take a variety of shapes; the equation is written for attributed meaning since it is this we perforce investigate.

In the simplest form equation (3) might take, $f(t)$ would be a constant, α :

$$x' \ln \frac{m'_2}{m'_1} = \alpha t \quad (4)$$

Equation (4) should be thought to hold in only a statistical sense, since it is unlikely that all evolutionary steps can be given equal weight or that they occur at regular intervals of time, as the equation might imply. Such simple exponential growth seems fairly closely followed, nevertheless, in some instances, by various aspects of cultural evolution for short periods of time³, and by number of species of molluscs⁴. But it would seem impossible that a curve of this form could be followed indefinitely, evolution being eventually restricted for various reasons; for example, increasing adaptation to an ecological niche would seem to impose restrictions on evolution of the given species, development of one aspect of culture may place restrictions on another, and the size of the system must itself impose limits.

If we assume that the probability of selecting new facets of pattern falls off exponentially with time due to such restriction, we may write as a general expression:

$$x' \ln \frac{m'_2}{m'_1} = \beta(1 - e^{-\gamma t}) \quad (5)$$

where β and γ are constants, and e indicates the base of natural logarithms.

Curves described by equation (4) may represent early parts of curves described by equation (5), since for small values of t the function $(1 - e^{-\gamma t})$ is very nearly equal to γt . On the other hand, for large values of t the function approaches unity, thus describing approach to a static condition, as may be exemplified in the brachiopods and in *Sphenodon*, where there has been little evolution for very long periods of time. In a preliminary search I have not found many data on biological evolution which seem appropriate for such analysis. On the other hand, aspects of cultural evolution often indicate relative growth; some curves approach a limit after the manner of equation (5), others follow equation (4) more or less closely, but sometimes show fluctuations which suggest acceleration and deceleration of relative growth. It is proposed to discuss elsewhere a variety of curves describing aspects of cultural evolution, among which there may seem little similarity at first glance; but which may be reasonably well fitted by equation (3) when simple, logical forms are

assigned to $f(t)$. All this suggests that evolution follows variations on an exponential theme, and may be thought of as a relative growth process.

Let us imagine, for purposes of contrast, an environment with a fixed number of ecological niches each one of which can best be filled by a species having a specific inherited pattern. Then, given adequate time, all species with inherited patterns that do not fit best will be eliminated, leaving every niche perfectly filled. In this kind of panglossian world, where everything happens for the best if one waits long enough, evolution might be expected to follow a more or less linear course, and to have only one ultimate outcome. One wonders at times whether some such a world is not tacitly assumed in a good deal of present thinking⁵. Recent achievements in biochemistry indicating nice evolutionary series of molecular structure may have encouraged this thinking⁶: but such series are, of course, to be expected if evolution follows a course of relative growth.

If one adopts the idea that each chance step in evolution can only be taken in terms of an existing pattern—which seems imposed by modern concepts of genetics—one admits the possibility that evolution in another biosphere than ours might, although starting from nearly identical initial conditions, arrive at an array of species and cultures widely different from those we know, or even none what-

soever. Only by tacitly disregarding the possible alternate pathways open each time a facet of pattern was selected does one arrive at the notion of inevitableness in evolution; yet selection from among alternate pathways is essence of Darwinian theory.

I thank Prof. Roger S. Pinkham for his advice.

Note added in proof. After writing this article, one Sir Karl Popper has appeared (*Nature*, 207, 233; 1965) which has been taken by at least one reader to suggest that evolution in the biosphere runs counter to the second law of thermodynamics. (My book, *Time's Arrow & Evolution*, Princeton Univ. Press, 1951, revised printing Harpers, 1962, is wrongly cited there, which may be confusing to a reader wishing to examine the original statements referred to.) In both this and another recent paper (*Nature*, 206, 131; 1965) it is pointed out that a thermodynamic balance sheet cannot be drawn up for evolution in the biosphere, because the dimensions of the system cannot be properly evaluated; but there seems no reason to assume that the second law is disobeyed.

¹ Blum, H. F., *Amer. Scientist*, 51, 32 (1963).

² Blum, H. F., *Nature*, 206, 132 (1965).

³ Lehman, H. C., *Social Forces*, 25, 281 (1947).

⁴ Cailleux, A., *C. R. Soc. Geol. France*, 222 (1950).

⁵ Fremlin, J. H., *Nature*, 207, 668 (1965).

⁶ Ehrensavard, G., *Life: Origin and Development* (Chicago Univ. Press, 1962)

NEWS and VIEWS

Pacific Science Center Foundation Arches of Science Award : Dr. Warren Weaver

DR. WARREN WEAVER has been elected to receive the Arches of Science Award of the Pacific Science Center Foundation. This comprises a cash prize of 25,000 dollars and a gold medal. It will be presented to Dr. Weaver at the Pacific Science Center on October 25. The Arches of Science Award is presented to an American who has made "the outstanding contribution to the public understanding of the meaning of science to contemporary man". The first recipient of the Award, Dr. Weaver is well known as a 'communicator of science', and is recognized as one of the outstanding public interpreters of the scientific revolution of the past half-century. Associated with the Rockefeller and Sloan Foundations for more than thirty years, he is also an internationally known mathematician, administrator of some of the leading scientific research institutions in the United States, an author, editor and lecturer. In announcing the Award, Dr. Edward E. Carlson, president of the Pacific Science Center Foundation, said: "Dr. Weaver's selection for this new Arches of Science Award adds prestige comparable to the highest prizes for science achievement. The scientific community, because of their regard for Warren Weaver, is characterizing the Award as an American Nobel Prize—and it is significant that more than fifteen Nobel Laureates in science have received Dr. Weaver's early recognition and support".

During his response to the announcement of the Award, Dr. Weaver said: "Most of the previous significant rewards for scientists have been given exclusively for what the individual has done inside of science, strictly for notable contributions to scientific research. Individuals who can make such contributions clearly deserve society's highest honours. But the relations of science to society, to our total culture, and incidentally to our government, are now quite unlike what they were even twenty-five years ago. It is essential that we to-day have individuals who are capable of understanding science, and who are willing to live their lives partly within science but also partly within the world of affairs. These persons, working at the interface of science and society, are more than

useful—they have become essential". Sponsor of the Award is the Pacific Science Center Foundation of Seattle, Washington. The Foundation is a non-profit agency composed of business, scientific and educational leaders: the Pacific north-west who have been joined by leaders of science throughout the nation to create an active and permanent centre dedicated to increasing the public understanding of science. The Foundation operates the Pacific Science Center which was the popular U.S. Science Exhibit at the Seattle World's Fair of 1962. The Arches of Science Award was named for the five instantly recognizable arches soaring into the sky above the Pacific Science Center. The arches have become symbols of the technological strides and superior climate for science and science understanding emerging from the Pacific north west.

New Zealand Department of Scientific and Industrial Research : Dr. K. A. Wodzicki

DR. K. A. WODZICKI, director of the Animal Ecology Division of the New Zealand Department of Scientific and Industrial Research, retired recently after holding the position for sixteen years. Dr. Wodzicki was born in Poland and educated at the University of Cracow where he completed his M.Sc. and Ph.D. degrees. After serving as a lecturer at this University he was later appointed professor of animal physiology at the University of Warsaw, where his research work was concerned mainly with the genetical and ecological aspects of birds and animals. At the outbreak of the Second World War he escaped to France where he joined the Polish Government in Exile, and in 1941 was appointed as its Consul-General in New Zealand. In 1946 he was employed by the Department of Scientific and Industrial Research to survey the wild-life problems in New Zealand, and following the preparation of his report—which was later issued as a Departmental *Bulletin*—it was decided to set up an Animal Ecology Section of the Department with Dr. Wodzicki as officer in charge. Under Dr. Wodzicki's direction the Section has undertaken basic research on the ecology and physiology of introduced small animals, such as rabbit, hare and the mustelid family, and of birds such as

s. Dr. Wodzicki made a special study of the gannets in their nest at Cape Kidnappers, Hawke's Bay. This study is recognized by ornithologists as one of the most rewarding of its type carried out anywhere, and has produced a great deal of new information about this bird.

Dr. J. A. Gibb

DR. J. A. GIBB, who will succeed Dr. Wodzicki as director of the Animal Ecology Division of the Department of Scientific and Industrial Research, was born in Dorset, England, and educated at Sherborne School and at the University of Oxford, where he gained his M.A. and Phil. in zoology for research work on bird and insect populations carried out at the Edward Grey Institute. During the Second World War he served with the Royal Artillery in the Mediterranean Sector. Dr. Gibb joined the New Zealand Department of Scientific and Industrial Research in October 1957 as an animal ecologist, and was in charge of the Division's major research project in habitat ecology concerned with a study of population dynamics and feeding behaviour. In 1963 Dr. Gibb visited the United Kingdom and the United States to attend a meeting of the British Ecological Society and to visit various research institutions; in 1964 he visited Australia to attend a conference on "Vermin Control".

Mr. E. W. Hullett

MR. E. W. HULLETT has retired as director of the Wheat Research Institute of the New Zealand Department of Scientific and Industrial Research. Mr. Hullett went to the University of Canterbury (New Zealand), where he gained his M.Sc. degree with honours in chemistry. He was later awarded a National Research fellowship for study overseas, and on return to New Zealand became lecturer at Lincoln College before joining the staff of the Wheat Research Institute in 1934; in 1936 he was appointed chief cereal chemist, and in 1950 succeeded Dr. O. Frankel as director of the Institute. Mr. Hullett is specialized in cereal chemistry and its application to milling and baking techniques, and under his directorship the milling and baking industries have benefited greatly from the research undertaken and the testing and information services provided by the Institute.

Mr. R. W. Cawley

MR. R. W. CAWLEY has been appointed director of the Wheat Research Institute in succession to Mr. E. W. Hullett. Mr. Cawley graduated M.Sc. with honours in chemistry from Auckland University College in 1948. After lecturing for two years at Massey University, Palmerston North, he was appointed to the staff of the Wheat Research Institute as assistant chemist in 1950. His research work has been concerned with cereal biochemistry, particularly with the process of dough fermentation. In 1963 he was granted a research fellowship at the Wheat Research Unit of the Commonwealth Scientific and Industrial Research Organization, Australia, where he made a special study of pentosans, and their role in bread-making.

Mr. N. Modriniak

MR. N. MODRINIAK has retired from the position of superintendent of the Geophysical Survey, Geophysics Division, New Zealand Department of Scientific and Industrial Research. Mr. Modriniak, who was educated at Leoben, Austria, arrived in New Zealand in 1927 and was engaged on geophysical prospecting with private companies for the following six years. In 1933 he joined the New Zealand Department of Scientific and Industrial Research, and until the outbreak of the Second World War he was concerned mainly with prospecting alluvial deposits for gold. In this period he pioneered the use in New Zealand of seismic methods for the investigation of shallow structures. Mr. Modriniak was among the first

to become interested in the possibility of deriving power from geothermal steam in New Zealand, and the exploration of geothermal resources led to a major expansion of the Geophysical Survey in the late 1940s. During the early 1950s, when interest in and optimism about New Zealand's oil resources were at a low ebb, Mr. Modriniak persevered with seismic reflexion tests in the major sedimentary basins with the view of stimulating the interest of the major oil companies. While geothermal power, engineering and oil have been the main concerns of Mr. Modriniak as a Government scientist, he has always been actively interested in the fundamental geophysical structure of New Zealand and the south-west Pacific region generally.

Dr. T. Hatherton, O.B.E.

DR. T. HATHERTON has been appointed to succeed Mr. N. Modriniak as superintendent of the Geophysical Survey, Geophysics Division, New Zealand Department of Scientific and Industrial Research. Dr. Hatherton was born in Yorkshire and received his education at Normanton Grammar School and the Universities of Birmingham and London. In 1950 he became the first United Kingdom recipient of a New Zealand National Research Scholarship and went to New Zealand to carry out research on the magnetic properties of New Zealand volcanic rocks, for which he was awarded a Ph.D. by the University of London. Dr. Hatherton spent several seasons in Antarctica during 1955-64, and wintered over at Scott Base in 1957 as leader of the International Geophysical Year party. For this work he was awarded an O.B.E. and a Polar Medal for 1958. In 1959-60 Dr. Hatherton spent a year at the California Institute of Technology on a Commonwealth Civil Service (Harkness) fellowship. He is editor of a treatise on *Antarctica*, recently published in the United Kingdom, New Zealand, and the United States.

Promotions at Woods Hole Oceanographic Institution

IN recognition of scientific achievements of major importance over the past several years, the Woods Hole Oceanographic Institution has promoted seven members of its scientific staff to higher positions. Foremost among these was the appointment of Dr. Howard L. Sanders (zoologist) to the position of senior scientist, the highest rank of scientific appointment which is made by the Institution. Other appointments were to the position of associate scientist, and these included Dr. Carl O. Bowin (geologist), Dr. John E. Gordon (chemist), Dr. Robert R. Hessler (palaeozoologist), Dr. Richard M. Pratt (geologist), Dr. John M. Teal (biologist), and T. Ferris Webster (geophysicist). Dr. Sanders has been a member of the staff of the Woods Hole Institution since 1955 when he received his doctoral degree from Yale University. His special scientific interest lies in the area of marine benthic ecology, but he has also made extensive investigations of crustacean phylogeny. Of the other members of staff recently promoted, Dr. Bowin has made intensive studies of the density distribution of the Earth's crust and upper mantle. He has also done much to develop new techniques for the use of a digital computer on board a research vessel at sea. Dr. Gordon, on the other hand, has investigated electrolyte and non-electrolyte interactions in sea water, while Dr. Hessler has been concerned with studies on the evolution and morphology of Arthropoda and deep-sea animal communities. Dr. Pratt's special interest is in deep-sea topography. Dr. Teal has concerned himself with physiological ecology, and Dr. Webster with ocean current measurements.

Recruitment to the Civil Service

THE sixth report of the Estimates Committee for the session 1964-65, dealing with recruitment to the Civil Service, is discussed on p. 311, but the report is noteworthy for the memoranda submitted in evidence. Among

these, those submitted by the Civil Service Commission on the function and organization of the Civil Service Commission and that of the Treasury on recruitment to the Civil Service may be mentioned. Recruitment to the Civil Service is dealt with in memoranda by Prof. W. J. M. Mackenzie, by Mr. B. J. Holloway, by the staff side of the Civil Service National Whitley Council, and by the Acton Society Trust. The last-mentioned is supported by a further memorandum on the attitudes of civic university students towards an administrative career in the Civil Service. This is based on a survey of third-year undergraduate students at the University of Hull, undertaken following a suggestion made in the statement of evidence made to the Committee by the Acton Society Trust. A further memorandum on recruitment to the Civil Service is by the Warden of Nuffield College, Oxford, Mr. D. N. Chester. Another on the same subject by the Business Economists Group is also appended; the Institution of Professional Civil Servants has a memorandum on recruitment to the professional and technical classes; finally, one on the recruitment of girls to the Civil Service is submitted by Miss Margaret Miles.

Anglo-Romanian Agreement on Co-operation

A FORMAL agreement on co-operation has been concluded between the Royal Society and the Academy of the Socialist Republic of Romania with the signature in Bucharest by Academician D. Dumitrescu, First Secretary of the Romanian Academy, and in London by Sir Patrick Linstead, Foreign Secretary of the Royal Society. The agreement makes provision for; (1) the annual exchange of three scientists from each side for lectures and visits to specialized scientific institutions in the other country for periods of fourteen days; (2) the annual exchange of up to two scientific research workers from each side, for a nine-month period in each case, to study fundamental problems in some aspects of the physical and biological sciences, including their applied aspects. The agreement also calls for the exchange of periodical publications of the two sides and for the encouragement of further such exchanges of publications in specialized branches of the physical and biological sciences between the respective corresponding institutions in the United Kingdom and Romania.

Eleventh Pacific Science Congress

THE eleventh Pacific science congress of the Pacific Science Association will be held at the University of Tokyo, Japan, during August 22–September 10, 1966, under the auspices of the Science Council of Japan. The inaugural meeting will be held on August 22 and the general meeting on September 3. The first week of the congress will be devoted mainly to symposia, the second to divisional meetings and the third to scientific tours. The organizers are not limiting the number of scientists attending the congress. The branches of science covered by the Pacific Science Association include: meteorology; oceanography; geophysics; geology and soil science; biology; agriculture; forestry; animal science and conservation; fisheries; marine and freshwater sciences; nutrition; public health and medical science; social sciences; anthropology; geography; scientific information and museums. Symposia and divisional meetings will be held on specific topics in these fields. There will also be two congress symposia on (a) population problems in the Pacific, and (b) air and water pollution in the Pacific area, as well as four special symposia with exhibitions and demonstrations on vessels and instruments for oceanic and freshwater research, marine park, primate biology of the Pacific area and tsutsugamushi disease. It is hoped that various meetings of other international scientific organizations will be held in Japan concurrently with the congress.

The Royal Society, as the United Kingdom representative institution in the Pacific Science Association,

will be sending an official delegation of ten members to the congress. Members attending other meetings to be held concurrently with the Pacific science congress are invited to become members of the congress, and in addition any qualified scientist may attend the congress and participate in the two weeks of meetings without presenting a paper. Representative institutions have been requested to accredit all members of the congress: the Organizing Committee, and any United Kingdom scientist planning to attend is asked to inform the Executive Secretary, the Royal Society, Burlington House, London, W.1.

High-pressure Science and Technology

THE recent international conference on "High Pressures", held at Le Creusot, France, emphasized important common interests, particularly in engineering and experimental techniques, which link all high-pressure activities. At a special meeting, British delegates felt that better interchange of information between specialist activities would greatly accelerate progress in the United Kingdom. A committee was elected to: (1) organize meetings promoting contacts between specialists in different fields at which informal discussion would be encouraged; (2) provide a source of information on high-pressure activities in Britain, and liaison with American, Continental and other groups; (3) set up an association in which all interested in high pressures could join; (4) act as an advisory body to the Government and other authorities. The committee was chosen to represent a variety of interests and will be enlarged as necessary. Members are: K. E. Bett (Imperial College of Science and Technology); C. C. Bradley (National Physical Laboratory); C. H. L. Goodman (Standard Telecommunications Laboratories, Ltd, Harlow, *Acting Chairman*); W. R. Manning (St. Albans, Herts). All groups in Britain working with high pressures are urged to write to the committee giving a brief outline of their interests. This will enable a provisional directory of high-pressure research to be compiled which will be circulated to all who write in. It will also form the basis for the association, and for deciding the topics for the first research meetings. Letters should be sent preferably to the acting chairman, but any committee member would be pleased to answer queries.

Scientific Periodicals from the U.S.S.R.

THE 1966 catalogues of Russian newspapers and magazines procurable on a subscription basis through national agencies for Soviet books list several hundred scientific periodicals and more than 250 sections of the *Journal of Abstracts* and the *Engineering Index*, all of which are readily obtainable by Western readers if order are placed six weeks in advance of publication. An unwelcome feature of these 1966 lists is the rising cost of Russian journals of an academic or technical character, the price of which in most instances has advanced by 18–19 per cent since 1963. Annual charges for volume of the *Journal of Abstracts* (*Referativnyi Zhurnal*) series have gone up by some 30–40 per cent in the same period. In numerous instances these Russian scientific periodicals, characterized a decade ago by their relative cheapness, are now appreciably more expensive than their Western counterparts. It is significant that no comparable advances are scheduled in the price of newspapers and popular magazines and propaganda literature.

Soils of the Mendip District of Somerset

A RECENT memoir of the Soil Survey of Great Britain, by D. C. Findlay, gives an account of the soils and land-use of the region between Weston-super-Mare and Bridgwater and extending inland to Shepton Mallet (*The Soils of the Mendip District of Somerset*. Sheets 279 and 280. Pp. viii + 204 + 9 plates. Harpenden: Rothamsted Experimental Station, 1965. 35s.). The area surveyed extends to 279 square miles and includes the Mendip

and of limestone plateau at about 800 ft., surmounted four Old Red Sandstone hills, and the flat belt of alluvium, constituting the Levels, along the eastern side of the Severn Estuary. Exposed rocks range from the Devonian to the Jurassic systems, while the superficial deposits accumulated since the Pleistocene period include beds of estuarine and riverine alluvium interbedded with peat. Distance from the coast and increases in altitude account for wide variations in local climate and length of growing season. For example, the average rainfall is about 32 in. on the coast and 50 in. on the highest ground; sunshine and temperature display similar tendencies, while moisture-deficient periods may last during six months on the coast but only during June and July on the Mendips. There is a short discussion of the vegetation changes brought about by clearances of the oak-ash woodlands of the uplands, by agricultural improvements in the vales and by peat cutting on the moors. Six chapters are devoted to an explanation of soil formation and classification, and to details of the mapping units, including profile descriptions and analytical data. The soil conditions are then discussed in relation to land-use and types of farming, and there is a useful appendix dealing with the land-use limitations of the soil series. There is a list of references, a subject index, and two maps.

Tropical Timbers

FROM Belgium comes a very detailed account of the members of 67 tree species of the Congo (*Publications de l'Institut National pour l'Etude Agronomique du Congo. Mois du Mayumbe*. Par Joseph Fouarge et Georges Gerard. Pp. 579+57 planches. Bruxelles: Institut National pour l'Etude Agronomique du Congo, 1964). These are the principal timber species of Mayumbe, but not a few are to be found in the West African high forest and others are very closely related species. Each species is treated separately. The results of physical and mechanical tests are listed and notes are provided on the relative susceptibility of the timber to insect and fungal attacks. For many of the species a set of four microphotographs illustrates anatomical characteristics, and these are accompanied by detailed descriptions. A final section to the volume is intended for timber merchants and others interested in the uses of timbers, but a word of warning is added directing attention to variations which may occur in the same species. Statistics of the

physical and mechanical properties and the relative durability are tabulated and sections are given recommending timbers for particular purposes such as cabinet work, furniture, mouldings, carpentry, turnery, shingles, pulleys, sleepers, paving-blocks, piles, sluice-gates, marine constructional work, small boats, barrel-making, matches, pulping and other uses. This is a most useful reference work and the authors deserve praise for what they have accomplished.

Announcements

PROF. J. C. WHEATLEY, University of Illinois, has been awarded the fourth Simon Memorial Prize in recognition of his outstanding work on the properties of liquid helium-3 at very low temperatures. The Prize of £250 was established as a memorial to Sir Francis Simon and is awarded by the Committee of the Low Temperature Group every two or three years for outstanding contributions in the field of low-temperature physics.

THE second conference on "The Countryside in 1970" will be held in London during November 10-12. Further information can be obtained from the Royal Society of Arts, John Adam Street, London, W.C.2.

A SYMPOSIUM on "The Chemistry and Technology of Polyvinyl Chloride" will be held at the Bradford Institute of Technology during November 5-6. Further information can be obtained from the Registrar, Bradford Institute of Technology, Bradford 7.

A COLLOQUIUM on "Diet and Diabetes", arranged by the Scottish Group of the Nutrition Society, will be held at the Royal Infirmary, Edinburgh, on October 30. Further information can be obtained from Dr. C. F. Mills, Rowett Research Institute, Bucksburn, Aberdeen.

A SEMINAR on "Formulating International Science Policy", arranged by the Science of Science Foundation, will be held at the Foundation on November 15. Further information can be obtained from the Science of Science Foundation, c/o the Ciba Foundation, 41 Portland Place, London, W.1.

A MEETING on "Nuclear and Engineering Ceramics", arranged by the Basic Science Section of the British Ceramic Society, will be held at Harwell during October 25-27. Further information can be obtained from Dr. J. P. Roberts, Houldsworth School of Applied Science, the University, Leeds 2.

THE NIGHT SKY IN NOVEMBER.

All times are in Universal Time

MOON		CONJUNCTIONS WITH THE MOON	
New Moon	23d 04h	Venus	27d 06h, 0° 7' N.
Full Moon	9d 04h	Mars	26d 05h, 2° N.
		Jupiter	12d 08h, 3° S.
		Saturn	3d 22h, 3° N.

PLANETS

Times of Rising (R) and Setting (S) during the month

Name	R/S	Beginning	Middle	End	Mag.	Dg (10 ⁴ miles)	Zodiacal position
Mercury	—	Unfavourable for observation				91	—
Venus	S	18h 25m	18h 35m	18h 55m	-4.1	63	—
Mars	S	18h 20m	18h 10m	18h 05m	+1.4	190	Sagittarius
Jupiter	R	19h 05m	18h 00m	16h 55m	-2.2	399	Gemini→Taurus
Saturn	S	1h 20m	0h 25m	23h 30m	+1.1	865	Aquarius

Dg is the distance of planet from the Earth on the 15th of the month

OCCULTATIONS OF STARS BRIGHTER THAN MAGNITUDE +6 AT GREENWICH

Star	R/D	Time	Mag.
69 Aqr	D	3d 18h 08 4m	+5.8
89 Psc	D	7d 02h 58 8m	+5.3
121 Tau	R	11d 20h 15 3m	+5.3
37 Gem	R	13d 04h 11 0m	+5.8
α Gem	R	13d 21h 44 4m	+3.7
ν Vir	D	18d 03h 25 6m	+4.2
ν Vir	R	18d 04h 29 2m	+4.2

(D, disappearance, R, reappearance)

METEORS

Name	Active period	Date of maximum	Radiant	Remarks
Taurids	Oct. 26d-Nov 16d	1d-10d	54° R.A. + 14° Dec.	Early stages favourable
Leonids	14d-17d	16d	152° R.A. + 22° Dec.	Unfavourable because of moonlight

SECOND INTERNATIONAL CONFERENCE ON PROTOZOOLOGY

THE first International Conference on Protozoology was held in Prague in 1961, and the second at the Imperial College of Science and Technology in London during July 29–August 5, 1965. Approximately 570 delegates attended the Conference from 26 countries; 370 papers or films were read or shown.

In place of full proceedings, a shortened version of the papers was printed and given to delegates on arrival at the Conference, in a volume entitled *Progress in Protozoology*¹. This service was much appreciated and certainly is preferable to reports of congresses, which are not produced sometimes until as long as seven years after the meeting has ended, while the abolition of formal proceedings allows speakers a greater freedom to speculate and report on very recent work.

The patron of the Conference was H.R.H. the Duke of Edinburgh; the honorary president was Prof. E. Fauré-Fremiet, and the Conference was organized by a national committee under the chairmanship of Prof. P. C. C. Garnham.

The annual meeting of the Society of Protozoologists was held under the presidency of John Corliss (Chicago) during the course of the Conference.

The Conference was divided into two parts: the mornings were devoted to plenary sessions based on single topics; the afternoons to special symposia and contributed papers in four simultaneous sessions. At the end of the afternoons, ciné films were shown. The plenary sessions were selected to give non-specialized workers the present views on the following subjects: morphogenesis and life-cycles, types and preservation of strains, genetics, biochemistry and physiology, cytology and ultrastructure, and locomotion.

The afternoon sessions were devoted to specialized subjects, including: marine protozoa, piroplasms, protozoa of invertebrates, metabolism and drug action, cultivation of Protozoa, ecology of free-living Protozoa, ecology and host-parasite relationships, and little-known parasitic Protozoa. The remainder of the contributed papers were arranged around their individual subjects, such as *Toxoplasma*, malaria parasites, coccidia, and ciliates. The day after the close of the Conference itself, a symposium was held on the classification of the Protozoa, when various schemes, originating from the Society of Protozoologists, from the U.S.S.R. and from Poland, were discussed. No finality was reached, but arrangements have been made for the continuation of this discussion by the International Commission on Protozoology. This body was formally instituted at the Conference, following a proposal by Prof. Pierre de Puytorac (Clermont-Ferrand). It has the responsibility for organizing future International Conferences, to work for the establishment of relations with other international biological organizations, such as the International Union of Biological Sciences and Unesco, and particularly to appoint various committees to consider such subjects as the preservation of types, the maintenance of culture collections, the revision of systems of classification of the Protozoa, and the participation of protozoology in the International Biological Programme. The Conference itself passed a resolution requesting that the World Health Organization should be invited to convene a meeting for discussing how international co-operation could assist in the preservation of strains and types of Protozoa. The Conference also resolved that an abstracting service for protozoology is highly desirable, and the feasibility of the provision of this service is to be investigated. The final resolution of the Conference was that the third International Conference on Protozoology should be held in Leningrad in 1969.

The importance of the Protozoa in the whole field of genetics was emphasized by Sonneborn (Indiana), who pointed out that while work on viruses, bacteria, yeasts etc., appeared to have overshadowed that on Protozoa, it was nevertheless the protozoologist who, in the past, had pioneered a number of important routes for use in gene genetics. The Protozoa are likely to become increasingly important in this field, by virtue of their special suitability for investigating certain types of genetical activities including the special role they have in cytoplasmic genetics. This aspect was further developed by Preer (Philadelphia) in a discussion on *Kappa* and its relatives; showed that the mate-killing *mu* particle is maintained by the gene *M*, by producing "'metagons'"—particles which prevent destruction of *mu*. Metagons in extracts prevent loss of *mu* in metagon-depleted paramecia. The latter are produced by treating mate-killers with ribonuclease, replacing *M* genes by *m* genes using suitable crosses at autogamy. Metagons are present in the RNA extracts from ribosomes of paramecia bearing the *M* gene. Metagons hybridize well with DNA from paramecia containing *M* genes and poorly with DNA from paramecia containing *m* genes. Consequently metagons are thought to be messenger RNA. Metagons from paramecia, when introduced into certain ciliates such as *Didinium*, become self-reproducing, like RNA viruses². Recent Russian work, for example, by Judin (Leningrad), involving nuclear transplantation and amoebae, appears to minimize the control of genetic effect by the cytoplasm; he concludes that the inheritance of all the characters studied is conditioned exclusively by the nucleus. Grell (Tübingen) explained the new ideas on sexuality in foraminiferans, in particular autogamy or the mating of gamonts which could be differentiated into two morphologically similar types, reacting differently in the course of their lives.

There were numerous contributions from the French School (headed by Fauré-Fremiet, de Puytorac, Hovasse-Savoie, Mme Noirot-Timotheé and others) on the ultrastructure of the ciliates, and the structures were not merely described, but also considered from the point of view of their nature, morphogenesis, and mode of action.

The paper by Satir (Chicago) presented evidence for a sliding filament mechanism in the peripheral fibres of flagella and cilia. The 'doublets' consist of a longer and shorter filament; the shorter filaments are apparently different in the two cases and they are always distal to the cell of origin compared to the longer filaments. This agrees with expectations for sliding-filament models of filament function where the filaments at the bottom of the cross-section would move out past those at the top to accommodate curvature.

Progress has also been made in other aspects in the study of flagella and cilia. The great versatility of these organelles, especially in relation to the presence and arrangement of mastigonemes, was stressed by Jahn (Los Angeles). The mathematical analysis of the hydrodynamic principles involved in both flagella and cilia has advanced considerably, as illustrated by the papers of Rikmenspoel (New York), Holwill (Dorking) and Machin (Cambridge). Further, the co-ordination of ciliary action was shown by a number of speakers to be mediated mechanically and not, as has often been assumed, by the transmission of neuro-motor impulses by infra-ciliary fibres. The Polish workers in particular (Dryl, Grebecki and others) reported extensive experimental work correlating various forms of ciliary activity with surface potential, thereby providing a physiological interpretation of ciliate movement and reaction (for example, the induction of anodal galvanotaxis).

as in *Paramecium caudatum* by membrane calcium). Nelson and Warmouth (Boston) made an attempt to demonstrate learning in *Paramecium caudatum*, in which paired mechanical stimuli, paired with light or absence of light, were presented to individual animals in a designated region of a capillary test chamber. The animals avoided the area in which the mechanical stimuli were applied only under conditions of low-intensity illumination. Although avoidance persisted for a period of several minutes following cessation of mechanical stimuli, this response cannot be interpreted as learning since it was not dependent on pairing of any other stimulus with the mechanical stimulus. Further, during stimulation of one animal, a second animal also present in the chamber, but not receiving the stimuli, developed the avoidance response to the area in which the first animal was stimulated. Since it is unlikely that mechanical stimulation produces changes directly in the medium, it is suggested that a material is elaborated by paramecia in response to noxious stimulation. A simpler aspect of locomotion was beautifully demonstrated by Freyvogel (Basle) in a speeded up 16-film to show the movements of malarial ookinetes. In recent years their inherent motility had been denied. The film clearly demonstrated that two types of movement are to be distinguished: in the first the ookinete is attached to the substrate with one pole as a sucker; in the second the free part of the body it carries out circling movements, but it remains on the spot. By the second a more or less even locomotion is made possible; this is brought about by circular contraction waves which originate at the anterior pole and which migrate on the surface of the body, all over its central portion. With reference to the substrate these contraction waves remain on the spot and the ectoplasm with its inclusions is driven forward through them. In this way, on one occasion, an ookinete was shown to cover about 100 μ within 40 min.

Few papers dealt with amoeboid movement, doubtless because this has been the subject of a recent symposium somewhere, but the demonstration by Seravin (Leningrad) that amoeboid locomotion is produced not by a single but by many physiological mechanisms strikes a new note.

While the importance of the organelles, as revealed by electron microscopy, is fully recognized, Cheissin (Leningrad) pointed out that their presence or absence should not be regarded as an indication of the taxonomic position of the organism concerned; such organelles have a common function throughout the animal and plant kingdoms, and their existence is an indication of function rather than of taxonomy. On the other hand, as Jahn (Los Angeles) states, locomotion itself has always been regarded as the most important and generally accepted single taxonomic criterion of non-fossil Protozoa from subphyla to species, and this function of the organism is dependent on such organelles. Polyansky (Leningrad) extended these ideas in an interesting discussion on evolution in the Metazoa and Protozoa; in the former, there is a tendency to a decrease in the number of organs, in the latter an increase (polymerization). This is accomplished either by increasing their actual number (for example, the nuclei of opalinids), their duplication as in *Giardia* or their differentiation into two types as in the macro- and micro-nuclei of ciliates. A further extension of the process is by polyploidization with the emergence of new evolutionary forms.

The foregoing topics largely concerned the free-living Protozoa, although many of the conclusions can also be applied to the parasitic forms. The latter represent specialized subjects, progress on which has advanced since the preceding conference on predictable lines.

The subject of piroplasms was selected as representing an area where important gaps in knowledge exist, in the hope of stimulating fresh work, but unfortunately little major progress in relation to the life-history of these organisms or even on their taxonomic position has yet been effected.

It was obvious that a great deal of work is being done on the biochemistry of the Trypanosomatidae, and this is commencing to throw light on their phylogeny and classification. Guttman (New York) explained the different nutritional requirements in special media of *Leishmania*, *Leptomonas* and *Crithidia*.

Artificial immunization of animals against the malaria parasite (Margaret Weiss, Michigan) and the trypanosomes (Soltys, Cambridge) has at last been accomplished. In the former case, mice were actively immunized by the injection of a strain of *Plasmodium berghei* which had become non-invasive for mice. This strain had lost its infectivity for mice, though not for rats, following a number of sojourns in tissue culture in a medium containing hamster serum. An initial immunizing dose containing few parasites was followed 2 weeks later by a booster injection containing 1-2 million parasites of the same strain. The mice were afterwards challenged by the parent strain which kills control mice within 3 weeks. Immunity was slow to develop, and during an initial period extending for about 4 weeks after the booster injection the mice were particularly susceptible, and succumbed even faster than the controls to challenge infection with the virulent strain. But after this sensitive period good immunity developed which was solid in many cases; in others single parasites were seen, and in a few cases peak parasitaemias of 1-2 per cent red blood cells parasitized were observed. This degree of immunity persisted for about 4 months. Soltys showed that *Trypanosoma brucei*, inactivated by treatment with β -propiolactone, could be used as a living vaccine to protect mice against the homologous, virulent strain.

The recent discovery by Mme Landau (Paris) of a new species of rodent malaria parasite is likely greatly to facilitate malaria research, because *Plasmodium chabaudi* represents a model where an infection in a small laboratory mammal can be used with much greater ease and success than the well-known *P. berghei* in mice or *P. cynomolgi* or *P. knowlesi* in monkeys. The latter systems present many technical difficulties; on the contrary, the new species is easily transmitted by mosquitoes and enormous numbers of exoerythrocytic forms are produced. This work stems from the highly successful recent experiments of Yoeli² (New York) on the transmission of *P. berghei* and the discovery of its exoerythrocytic cycle in the liver of mice and hamsters.

Further knowledge of the ultrastructure of the Sporozoa was advanced by Scholtyssek's (Bonn) work on sporozoites of *Eimeria perforans*, Cheissin's (Leningrad) on *E. bovis*, Sheffield and Hammond's (Utah) also on *E. bovis*, Vivier's (Lille) on gregarines and coccidia, and Bardele's (Tübingen) on *Eucoccidium dinophili*.

A special topic of the first Conference was *Toxoplasma*; little new information has emerged in the past four years relating to the life-history of this organism until very recently when Hutchinson (Glasgow)³ described the apparent transmission of *Toxoplasma* through the egg of *Toxocara cati*. He did not give a paper on this subject, but discussed the implications in the session dealing with the organism.

A newly discovered disease of man, due to the free-living amoeba, *Hartmannella castellani*, was described by Culbertson (Indiana), who gave details of his experimental work with this organism in rabbits and mice, and showed slides of the organism as found in the tissues from 7 fatal human cases.

The role of *Pneumocystis carinii* as a pathogen of man becomes more formally established following papers by Kučera (Prague), Frerking (Kansas) and Yaeger (New Orleans).

P. C. C. GARNHAM

¹ Intern. Congr. Ser., No. 91. Excerpta Medica Foundation, Herengracht 119-123, Amsterdam, C).

² Yoeli, M., Trans. Roy. Soc. Trop. Med. Hyg., 59, 255 (1965).

³ Hutchinson, W. M., Nature, 208, 961 (1965)

TYPOGRAPHY OF BACTERIA

AT an informal meeting of editors of journals in which bacteriological papers appear, and some other interested persons, it was agreed that a greater uniformity in typography was desirable; the following proposals, which arose from the meeting and from subsequent discussion, may be of wider interest.

It must be made clear that these proposals are concerned solely with typography and not with the more controversial subjects of classification or nomenclature.

(1) Nomenclature. The International Code should be followed. (*International Code of Nomenclature of Bacteria and Viruses: Bacteriological Code*: Ames, Iowa, Iowa State University Press, 1959.)

(2) Major ranks, such as the names of orders, tribes or families, are written in roman with an initial capital; for example, Enterobacteriaceae

(3) Binomials consisting of genus and species together are italicized, only the genus having a capital letter; for example, *Staphylococcus aureus*. [Note: A binomial which is considered invalid may be enclosed in quotes; for example, 'Bacillus typhosus'.]

(4) Sub-species (varieties) are italicized, without a capital, and preceded by 'subsp.' (or 'var.') in roman; for example, *Corynebacterium diphtheriae* subsp. *mitis*.

(5) Strains are printed in roman and are capitalized only when derived from proper names; they are preceded by 'strain' in roman or may be regarded as part of a trivial name (see 9); for example, *Staphylococcus aureus* strain Oxford, the Oxford staphylococcus.

(6) Groups and types are printed in roman and designated by capital letters and arabic figures respectively; for example, *Streptococcus pyogenes* group A type 1.

(7) Generic names are italicized either when used alone to mean the whole genus as a genus or followed by 'sp.' or 'spp.' to indicate a culture which has been identified only as far as the genus. Otherwise when used either as nouns or adjectives they are regarded as trivials and printed in roman; for example, the genus *Brucella* . . ., culture yielded *Bacillus* sp., some escherichias may have salmonella antigens.

(8) Specific epithets are occasionally used alone, and are usually regarded as trivials and printed in roman; for example, the strains were of the abortus type.

(9) Trivial names should be in roman type and capitalized only when derived from proper names. (Not when names of diseases are used.) Generic names may be used trivially; for example, meningococcus, Koch-Weeks bacillus, haemophilus, spirochaete, typhoid bacillus.

(10) Anglicized plurals may be used for trivial names to avoid classically derived forms which may appear pedantic or clumsy; for example, salmonellas, mycoplasmas, actinomycetes, pseudomonads, leptospores.

(11) Hyphens should be used sparingly and never in italicized binomial (or trinomial) forms. A single word must be used to avoid confusion; for example, *Salmonella choleraesuis*, *Bacillus pseudotuberculosis* rodentium; but note: *Salmonella paratyphi* B.

(12) Abbreviations should be used with caution. Generic names may be abbreviated to a single capital letter except in titles; in summaries; when first mentioned in the text and when any possibility of confusion could exist; for example, *S. aureus* (see Cowan, S. T., *Science*, 120, 1103; 1954); specific epithets should never be abbreviated, except possibly in tables.

(Note: Special *ad hoc* abbreviations may sometimes be acceptable in text-books or abstract journals, so long as they are clearly explained in the text.)

(13) Salmonellas present a special problem as their nomenclature is still in dispute. It is not the purpose of this communication to discuss the merits of the various proposals but to suggest appropriate typography. The

following examples illustrate some of the ways in which same culture may be described. (a) A separate species, so written as a binomial in italics with the specific epithet in latinized form and in lower case; for example, *Salmonella londonensis* (this example is after Haupt, cited in *Bergey's Manual*, sixth ed. The latinized form of the word 'London' should, in fact, be 'londiniensis'). (b) As above but with the specific epithet unlatinized; for example, *Salmonella london*. (c) A variety of a large inclusive species and so written with the epithet in lower case italics; for example, *Salmonella kauffmanni* var. *londonensis*. (d) Serotype of a large inclusive species and written in roman with an initial capital if derived from a proper name; for example, *Salmonella kauffmanni* [serotype] London. (e) A trivially named serotype written in roman throughout with an initial capital if indicated; for example, *Salmonella* London.

(14) Notes: (i) Specific epithets should be a single word in lower case, but serotypes, being trivials, may have more than one word and capitals where required. See pars. 10 and 11; for example, *Salmonella saintpaul*, c.f. *Salmonella* Saint Paul. (ii) A hybrid or bastard composed of an italicized generic name and a roman serotype cannot be defended and should not be used; for example, *Salmonella* London.

(15) The nomenclature of salmonellas is discussed more fully by Cowan, S. T. (*Bull. Hyg.*, 32, 101; 1957). Here we would merely suggest that wherever possible a single usage should be adopted for use in a single communication.

A Note on the Typography of Other Micro-organisms

(16) Fungi and protozoa. Nomenclature is governed by the international botanical and zoological codes. In general, our suggestions for bacterial typography could apply.

(17) Viruses. The nomenclature of viruses is at present being considered by the Provisional Virus Nomenclature Committee of the International Association of Microbiological Societies. Until their findings have been accepted and approved we consider that names of viruses should be regarded as trivials. The following tentative suggestions for their typography may be helpful: (a) Viruses named after a disease should be written as separate words in lower case roman, except where the name of the disease is derived from a proper name; for example, measles virus, poliomyelitis virus, varicella zoster virus, herpes simplex virus, Newcastle disease virus. (b) Viruses named after a place or person should be written as separate words with an initial capital; for example, Cocksackie virus. (c) Classical or euphonious synthetic prefixes should be combined with the suffix 'virus' and written as a single word in lower case; for example, adenovirus, cytomegalovirus, echovirus, picornavirus, poliovirus. (d) Unpronounceable synthetic prefixes should be written as a separate 'word' entirely in capitals; for example, LCM virus.

Among those who make these recommendations are Sir Christopher Andrewes, formerly deputy director, National Institute for Medical Research; Dr. H. J. O'D. Burke-Gaffney, editor, *Bulletin of Hygiene*; Dr. S. T. Cowan, director, Central Public Health Laboratory formerly curator, National Collection of Type Cultures; Dr. R. M. Fry, editor, *Journal of Hygiene*; Dr. J. C. Kelsey, editor, *Monthly Bulletin of the Ministry of Health and Public Health Laboratory Service: Section II*; Prof. C. L. Oakley, editor, *Journal of Pathology and Bacteriology*; Mr. A. F. B. Standfast, editor, *Journal of General Microbiology*; Mr. G. Sykes, editor, *Journal of Applied Bacteriology*.

ANGLO-AFRICAN JOINT RESEARCH SCHEMES AND RECRUITMENT

A CONFERENCE was held under the auspices of the Centre of African Studies, University of Edinburgh, in association with the Ministry of Overseas Development, at the "Joint Research Schemes and Recruitment", at the University of Edinburgh during September 13-17.

Ninety delegates from universities, training colleges and colleges of advanced technology in Scotland, the North of England and Northern Ireland and Africa attended the conference. Representatives came from thirteen British institutions of higher education and fourteen African, together with representatives of the British Ministry of Overseas Development, the Commonwealth Association of Architects, the Architectural Association, the British Council and the Inter-University Council for Higher Education Overseas.

Although this conference was called originally to discuss joint research schemes between institutions of higher education in Scotland, the North of England and Northern Ireland and African universities and also recruitment in his northern region of Great Britain for African universities, it soon extended its terms of reference to include almost the whole field of relations between British and African higher educational institutions. This tendency undoubtedly grew from the fact that both the British and African universities find themselves in an exciting period of parallel development in a rapidly changing world.

In the past it has been common to consider that the relations between British and African universities were heavily weighted on the side of British initiatives. It soon emerged from the conference, particularly as a result of the contributions of the African delegates, that this was a two-way traffic and that the British universities would also benefit from an extension of their relations at all levels with African universities.

The conference, in its conclusions, stressed the necessity of improving, in all their ramifications, the channels of communication between British and African universities. Not only was it necessary to improve the existing channels of communication between the Ministry of Overseas Development, the Inter-University Council for Higher Education Overseas, and British and African higher educational institutions and to improve the information services on African educational possibilities in Great Britain of British professional organizations, but it was also important to set up regional committees of British universities to improve their own communications with African universities. In particular, there should be a committee of the Scottish, North of England and Northern Ireland universities, training colleges and colleges of advanced technology for this purpose.

A major decision of the conference—one in which it struck out in a new direction in British-African higher educational relationships—was that throughout the British Isles a number of committees based on the major academic disciplines and organized on trans-university lines should be set up to initiate new joint research schemes and activities between British and African academic institutions and to review existing joint activities periodically.

The conference urged that a directory of research in progress in African universities should be produced as soon as possible. Furthermore, it urged that a directory of specific post-graduate opportunities in British universities (their individual research interests and achievements; the facilities which they offered for research; the size of their post-graduate departments; and their reputations in their respective fields) aimed at the particular needs of African post-graduate students should be compiled as soon as possible. This, it was suggested, should be revised frequently, in order to present the

potential African postgraduate student with as up-to-date a picture as possible of postgraduate prospects in a rapidly changing scene.

The conference stressed strongly the importance of both official and unofficial funds being made available, not only in improving the conditions of secondment of British academic workers in African universities but also for improving the position of the permanent staff, many of whom were from Great Britain, in African institutions of higher education. The financial implications of secondment from British to African universities should give greater attention to ensuring that seconded personnel do not lose financially, as is so often the case, during their period of secondment to an African university. Careful safeguards to this effect should be written into their agreement with an African university. Greater efforts should be made at the British end to supply African universities with technicians and 'middle level' administrators and secretaries.

Secondment of senior personnel (such as deans of faculties and heads of departments) from British to African universities, it was considered, should be for periods sufficiently long to ensure that programmes which such individuals initiated in African universities were properly launched.

The conference directed special attention to the problems of African post-graduate research students and stressed that greater attention should be given to placing them in those British universities in which their interests and talents could be most effectively developed. Departments in British universities should be discouraged from assuming responsibility for African postgraduate students for whom they could not provide viable research topics, adequate supervision and the necessary materials for research.

In its detailed consideration of joint research schemes between British and African higher educational institutions—many of which were developed in detail in a series of inter-disciplinary discussion groups—the conference stressed that British universities should endeavour to participate in existing joint research schemes with African universities and in schemes which have been initiated in African universities themselves. While recognizing, however, that such approaches are less wasteful in time, money and manpower than joint research schemes launched independently in Great Britain, it was considered important not to discourage independent schemes in those fields where British institutions of higher education were in a position to give a lead to African universities.

The conference urged that experiments should be made by the universities of Scotland, the North of England and Northern Ireland in setting up international halls of residence and British-African halls of residence in order to go some way towards ameliorating the growing crisis in student accommodation in British universities.

The extent of the conference's detailed deliberations will be reported in the *Proceedings* which it is hoped to publish shortly. In anticipation of these, it should be indicated that those deliberations ranged into unexpectedly detailed fields. For example, there was a lively discussion on the necessity of continuing the external examiner system in African universities. Again, it was suggested by a considerable number of individuals at the conference that there were certain fields such as African history in which external examiners could be used from African universities in British universities.

Finally, the conference expressed a strong wish that the many recommendations for improving relations between British and African universities which had been made in its various sessions should be translated into action as

soon as possible at all levels, both institutionally and individually. The conference saw in the suggestion indicated here that academic disciplinary committees should be established for this purpose, one of the most hopeful signs

for the future. It was considered that in their meetings the future such academic disciplinary committees should consider the possibility of holding further conferences the kind held in Edinburgh.

MODERN TECHNIQUE IN RESTORATION OF ROMAN MOSAICS

THERE is an inborn fascination to many of us, besides archaeologists and historians, in the discovery and unearthing of relics of life as it existed centuries ago. None of these is perhaps more generally exciting and popular than the Roman mosaic pavements, those skilfully constructed floorings in regular cubes of stone of many colours, beautifully contrived in patterns and pictures, frequently found in western Europe, far less common in Britain. Proof of life as it existed in Yorkshire during A.D. 100–400, as evidenced by the discovery of two excellent examples of Roman mosaic handiwork, is furnished by the Rudstone pavement, a few miles inland from Bridlington (1933), and by one at Brantingham, near the north bank of the Humber, a few miles west of Hull, unearthed in 1962. Some faithfully illustrated records of these two mosaics and particularly the manner of their preservation are contained in a recent publication entitled *Araldite in the Restoration of Roman Mosaics**.

At Rudstone three mosaics were originally uncovered and housed on site for many years. However, they began to deteriorate and were ultimately removed to Hull Museums for permanent preservation, this work being undertaken by the Museum staff and successfully completed in 1962. "One mosaic, 12½ ft. × 10½ ft., has a figure of Venus as its centrepiece; another, 11 ft. by 11 ft., shows a geometric pattern, while the third, depicting marine life, measures 10 ft. × 5 ft. These panels are now beautifully displayed in the museum." Their removal from site was, however, a matter of great concern and cost. The geometric panel, for instance, with its steel reinforcement, weighed 4½ tons; its display on a wall entailed the use of temporary scaffolding for its erection, a costly and laborious job. The lessons had been learnt with these Rudstone mosaics the hard way; when it came to the question of lifting the newly discovered Brantingham pavement, different methods of preservation were resorted to, involving the use of an 'Araldite' (CIBA) epoxy resin formulation and a novel technique.

The Brantingham pavement apparently formed the floor of the largest room in what is thought to have been a distinguished Romano-British villa. It originally measured 36 ft. × 26 ft. It is the largest example of Roman mosaic work so far discovered in Yorkshire. Unfortunately part of this mosaic had suffered damage by

collapse into flues of an underlying hypocaust, but number of large panels were well preserved, remove and are now in course of preparation for exhibition in Hull Museums. Some smaller sections of the mosaic were taken away flat, but other larger portions were transported by what is known as the 'rolling-up' technique. Briefly, the procedure is as follows: to ensure absence of moisture a metal scaffold frame is erected over the pavement and on this a plastic sheet is stretched; butane gas heaters are then directed on to the pavement for some three days or so; at the end of this drying, two coats of a polyvinyl chloride solution are spread over the mosaic, a bandage material laid thereon, and a top sealing coat of polyvinyl chloride. The next operation—a difficult one—is the actual rolling up of the pavement; according to this account: "This was done on a cardboard roller, 2 ft. in diameter, strengthened internally and filled with a suitable axle. The tesserae (the individual stone cubes forming the pavement) were cut away from the mortar with long iron blades and several workers helped to turn the roller and ensure that the pavement was evenly wound. As they went on to the roller, the layers were interleaved with two sheets of corrugated paper and a strong dust sheet which was pulled tightly over the tesserae to keep them in place". Thus the sections of the pavement are transported to the museum.

The unrolling stage is the next operation and it is here that the 'Araldite' resin plays its part. The major sections of the pavement are unrolled face down on a sloping surface; the edges are boxed in with timber to make a container for the backing; any loose mortar is cleaned off; then an 'Araldite' coat is applied together with chopped glass fibre. Finally, this is backed up with 2 in. of vermiculite and iron reinforcing bars bonded with an 'Araldite' resin formulation. The whole process results in a light but rigid structure. Compared with the Rudstone mosaic panels, it is estimated that this technique results in the weight of the Brantingham panels being less than a quarter per unit area.

The great advantages of these cured 'Araldite' epoxy resins for this sort of work are their stability, resistance to chemical attack and moisture. Furthermore, they possess strong adherent properties to a host of different materials, such as stone, glass fibre and vermiculite. It is indeed no boastful claim that "... these beautiful pavements are now permanently preserved for the benefit of posterity ..."

* Ciba (A.R.L.), Ltd., *Technical Notes* (July, 1965): *Araldite in the Restoration of Roman Mosaics*. Pp. 12. (Duxford: Ciba (A.R.L.), Ltd., 1965.)

A PROPOSAL ON NATURE'S TIME-SCALE

By PROF. NEIL G. McCORMICK

Department of Microbiology, School of Medicine, University of Virginia, Charlottesville

IT has become increasingly obvious through the various cosmological and relativity theories that we live in a non-linear universe. As has been aptly pointed out¹, there is a tendency to make simplifying assumptions about all observable phenomena in order to explain them in a linear manner. In a way this has lulled us into the belief that most processes may be considered to exist in some theoretically idealized state. Hence, many observations of non-linear processes are compromised by considering "... equations to be linear for a sufficiently small displacement

..." or, "... in a certain range the curve can be approximated by straight lines as shown". However, these idealized assumptions (that is, homogeneity, frictionlessness, weightlessness, temperature of absolute zero, perfect gas, perfect vacuum, etc.) are never observed in reality, and in all likelihood never occur in Nature. Even though such assumptions may aid greatly in understanding the mechanisms involved, they do not lead to a complete description of the process. As stated by D. W. Thompson² in a discussion concerning the regeneration of tadpole

s: "... it is obvious that the value which we have used for the latter portion of the curve (however closely it conformed to) is only an empirical value; it has only a temporary usefulness, and must in time give place to a formula which shall represent the entire phenomenon, from start to finish".

This communication proposes a non-linear function of time which appears to describe the time-course relationships of a variety of processes.

Several proposals have been made recently on re-evaluating some of the basic units of measurement. Aldwin and Tonks⁶ propose the adoption of a logarithmic pressure scale which would encompass both extreme vacuums and high pressures with equal facility. The authors emphasize the advantages of converting to a logarithmic scale and refer to the established precedence for such a scale in the pH system. This scale would permit the recording of vacuums approaching that of an absolute vacuum, a limit (it should be emphasized) which has never been attained experimentally. Likewise, a proposal for the re-orientation of the temperature scale has been advanced by Georgian⁴ in which he maintains that new units are necessary to describe the proper dimensions for temperature, that is, specific energy. When the proper conversion is made, the gas constant, R , becomes unity and the Boltzmann constant, k , is recognized as the reciprocal of the vagadro number. In addition to the resulting simplification it may be appropriate to consider the temperature scale as logarithmic also. As in the case of an absolute vacuum, a temperature of absolute zero is a limit which may be closely approached but never attained. The adoption of logarithmic scales would provide a continuous connecting link between the infinitesimally small and the infinitely large.

Proposals which consider the nature of macrocosmic time as exponential or logarithmic functions have been advocated by Milne and by others⁵⁻⁷. A logarithmic nature to psychological perceptions of time has also been noted^{8,9}. If time is truly non-linear then this non-linear function should be equally applicable to processes describing microcosmic events. In whatever process the time parameter is involved there should exist some descriptive function which incorporates a non-linear function of time.

An investigation of the kinetics of germination of bacterial spores has led to the derivation of an empirical equation which accurately describes the sigmoidal time-course curves obtained¹⁰⁻¹². The equation, $Y = \exp(-kt^{-c})$, may be converted into a linear function by plotting $\log \log 1/Y$ versus $\log t$, where Y is the fraction of the process completed in time t , k is a function of the intercept at $\log t = 0$, and $-c$ is the slope.

The equation also successfully describes the sigmoidal time-course curves obtained from a variety of different processes. Further applications suggest that time-course curves which appear to be hyperbolic rather than sigmoidal are also described by the equation. Representative types of time-course curves from a variety of processes are shown in Fig. 1. The time-scale is expressed in arbitrary units depending on the nature of the specific process being measured. Curve A is representative of processes which appear to reach completion very rapidly, such as fast chemical reactions and, if inverted, even survival curves. Curves B and D represent processes describing rectangular hyperbolas passing through the origin, such as observed with many enzymatic and chemical reactions. Curves C and E represent processes which describe sigmoidal time-course curves such as growth, certain chemical reactions, spore germination, enzyme induction, and a whole host of other naturally occurring phenomena. Curve F represents the type of process which appears to be linear with time, such as one which completes only a very small fraction of the total process during the period of observation. The curves shown in Fig. 1 were all generated by use of the equation by assigning to the constants, c and k , the values given in Table 1.

Table 1 THE VALUES OF THE CONSTANTS USED IN THE EQUATION $Y = \exp(-kt^{-c})$ TO GENERATE THE CURVES IN FIG. 1

Process	c	k
A	3.725	0.000286
B	0.6502	0.3558
C	1.536	3.224
D	0.4307	1.709
E	1.096	6.909
F	0.3010	4.606

Because of the variety of processes which have been examined and found to conform to the equation, the following premise is suggested: the time-course curves of all natural processes are sigmoidal whether or not an induction period or inflexion point can be detected during a particular observation. Examination in the classical sense of curves A , B and D in Fig. 1 suggests that a tangent to the curve at the origin might be constructed and that the slope of such a line would give the maximum rate of the process. These suppositions have been extensively utilized in the formulation and development of classical kinetic theory. The same argument holds for curve F in which it appears that a straight line passing through the origin may be drawn through the points. If the foregoing premise is true, however, then all the curves actually must possess an inflexion point at a time other than zero although it may approach zero to any degree desired. Since each of the curves depicted was generated by the equation $Y = \exp(-kt^{-c})$, the inflexion points may be estimated mathematically¹³. Inspection of curves A , B and C suggests that they tend toward a limit of 1.0. However, this limiting value is not as apparent with the remaining curves. Curve F may appear either to increase indefinitely (if considered a straight line) or to approach a limit significantly less than a value of 1.0 (if curvature is acknowledged); however, the equation used to generate each curve is based on a limiting value of 1.0 as time approaches infinity. Curves D and E also appear to approach limits somewhat less than the maximum of 1.0 even when the usual methods of extrapolation to infinity are employed. It can be estimated by means of the foregoing equation that process F would require 2.82×10^6 units of time to reach 90 per cent of completion, process D would require 750 units of time, and process E , 64 units of time. Thus, the equilibrium value of a process determined by application of the foregoing equation may be appreciably greater than the value estimated by extrapolation procedures at present available.

Investigation of the possible physical significance and properties of the equation has led to the formulation of the

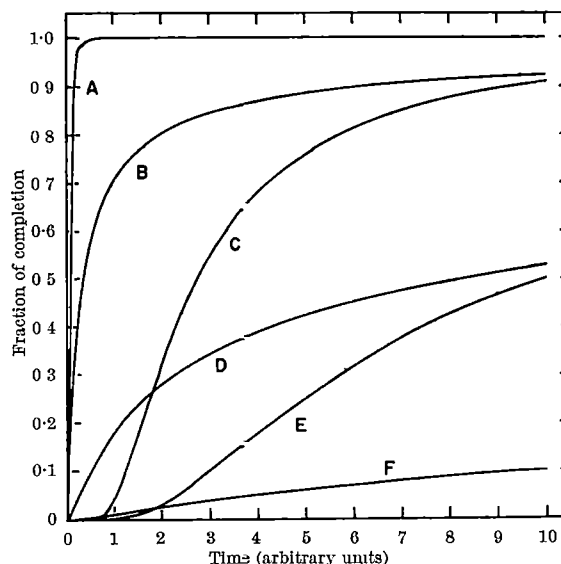


Fig. 1. Time-course curves typical of various types of processes generated by the equation $Y = \exp(-kt^{-c})$. The corresponding values for the constants are given in Table 1.

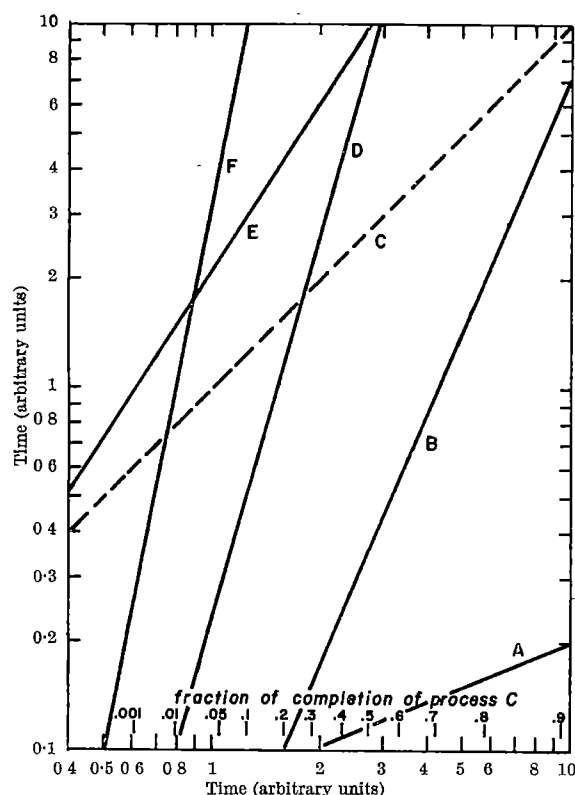


Fig. 2. Exponential relationships of the times required for processes A, B, D, E, and F to attain various fractions of completion versus the time required for the reference process (C) to achieve the same fraction of completion

following hypothesis: all naturally occurring phenomena proceed according to exponential functions of time and each process in Nature conforms to its own unique time scale. The refutability of the hypothesis may be examined by testing a corollary to the hypothesis; namely, that a process need not necessarily be timed by a clock, but may

be 'clocked' by reference to any other reaction in Nature. That is, the time required for any specific process in Nature to attain a certain degree of completion (or fraction of a limiting value) is exponentially proportional to the time required for any other natural process to attain the same fraction of completion. This is illustrated in Fig. 2. The times required for the process represented by curve (the reference 'clock' in this case) to attain various fractions of completion (superimposed scale) are plotted along the abscissa while the times required for each of the other processes to reach the respective fractions of completion are plotted along the ordinate. If a complete and accurate record of the reference process is known, and if the extent of completion of the second process is known at two different times then the course of that process may be predicted for any time. The dashed line represents a guideline of the reference curve C plotted on both the ordinate and abscissa.

The final proof of the hypothesis lies in testing the foregoing corollary with accurate data from a wide variety of different processes. In order to calculate the fraction of completion at any time and to apply the experimental data, it is imperative that the exact zero time be known as well as the true limiting (or equilibrium) value of the process as time approaches infinity.

This work was supported in part by U.S. Public Health Service research grant AI-06037-01 from the NIAID, and by Research and Development Funds, University of Virginia.

- ¹ Kovach, L. D., *Amer. Scientist*, **48**, 218 (1960).
- ² Thompson, D. W., *On Growth and Form*, 138 (Camb. Univ. Press, 1917).
- ³ Baldwin, G. C., and Tonks, L., *Nature*, **203**, 633 (1964).
- ⁴ Georgian, J. C., *Nature*, **201**, 695 (1964).
- ⁵ Milne, E. A., *Kinematic Relativity* (Clarendon Press, Oxford, 1948).
- ⁶ Johnson, M., *Time, Knowledge and the Nebulae* (Dover Publications, New York, 1947).
- ⁷ Dirac, P. A. M., *Nature*, **139**, 323 (1937).
- ⁸ Fraisse, P., *The Psychology of Time* (Harper and Row, New York, 1963).
- ⁹ von Schelling, H., *Bioastronautics*, 361 (The Macmillan Co., New York, 1964).
- ¹⁰ McCormick, N. G., *Biochem. Biophys. Res. Comm.*, **14**, 443 (1964).
- ¹¹ Vary, J. C., and McCormick, N. G., *Spores*, **3**, 188 (Amer. Soc. Microbiol., 1965).
- ¹² McCormick, N. G., *J. Bact.*, **89**, 1180 (1965).

SUBCUTANEOUS SARCOMATA IN HAMSTERS INDUCED BY ROUS SARCOMA VIRUS (BRYAN)

By PROF. M. L. EIDINOFF, B. BATES, M. STEINGLASS and PROF. J. R. HADDAD

Divisions of Biophysics and Microbiology, Sloan-Kettering Institute for Cancer Research, New York

WHEN the investigation reported here was begun (June, 1964) the Bryan strain of the Rous sarcoma virus (RSV) had been found to be oncogenic for fowl only, while several strains of RSV, including the Carr-Zilber and Schmidt-Ruppin, produced tumours in a variety of

mammalian species¹⁻³. The experiments of Stewart and Landon (reported by S. E. Stewart at the Stern symposium, "Perspectives in Virology", February 1964) suggested that RSV (Bryan) could be 'activated' to be oncogenic for mammals by suitable treatment with a hamster embryo

Table 1. INCIDENCE OF TUMOURS FOLLOWING INOCULATION OF ROUS SARCOMA VIRUS IN NEW-BORN HAMSTERS

Group	Inoculum	Pock-forming units	Animals	Incidence of tumours (days)						Died	+	-	% +
				0-14	15-20	21-30	31-60	61-90	91-200†				
A	RSV (Bryan)*	2×10^4	21	0	0	1	0	0	1	1	2	18	10
B	RSV (Bryan)† reconstituted	1×10^5	122	0	1	12	4	2	7	25	26	71	27
C	RSV (Bryan) plus hamster embryo extract†	1×10^5	37	0	8	2	1	0	6	2	17	18	49
D	RSV (Schmidt-Ruppin)§	2.5×10^2	24	0	2	2	0	0	4	2	8	14	36
E	RSV (Schmidt-Ruppin)§	1×10^4	71	0	1	3	12	8	18	10	42	19	69
F	Hamster embryo extract		40							11	0	29	0
G	Eagle's basal medium		71							1	0	70	0
H	No injection		37							0	0	37	0

* Combined data from several experiments using CT 951, CT 955 and CT 957. Original sample (in 0.05 M sodium citrate) diluted with Eagle's basal medium plus 5 per cent calf serum.

† Bryan stocks, pelleted and resuspended in Eagle's basal medium plus 5 per cent calf serum.

‡ See text for preparation of sample.

§ Schmidt-Ruppin strain, Lots 11 and 29, University Laboratory, Highland Park, New Jersey. Samples diluted with Eagle's basal medium plus 5 per cent calf serum.

¶ The experiments were terminated at 200 days.

tract⁴. These findings led to an investigation in our laboratory in which RSV (Bryan) was inoculated into v-born hamsters under several experimental conditions including treatment with hamster embryo extract. We have found that RSV (Bryan) can yield sarcomata in hamsters following inoculation (10^4 – 10^5 pock-forming units in 0.1 ml.) in new-born hamsters in the absence of any extract from rapidly growing tissue. Since these experiments were started, Rabotti *et al.* have reported the formation of gliomas and choroid plexus papillomata induced by intracerebral inoculation of RSV (Bryan) in w-born hamsters⁵.

RSV (Bryan, lots CT 951, CT 955 and CT 957) was provided by Dr. W. Ray Bryan, National Cancer Institute, a partially purified, pelletized preparation stored in 0.5 M sodium citrate. RSV (Schmidt-Ruppin, lots 11 and 29) was supplied by University Laboratories, Highland Park, New Jersey. Viral suspensions and control materials (0.1 ml.) were inoculated subcutaneously (at the nape of the neck) into golden Syrian hamsters (Manor Farms, New York) 24–48 h after birth. Virus preparations were assayed in pock-forming units by enumeration of pocks on the chorioallantoic membrane of chick embryos using standard procedures⁶.

In order to prepare RSV (Bryan), 'reconstituted' (Table 1, Group B), a pellet was prepared by centrifugation at 40,000g for 1 h followed by resuspension in Eagle's basal medium plus 5 per cent calf serum to the original volume. To prepare hamster embryo extract containing RSV (Bryan), embryos taken one day before parturition were minced and an equivalent volume of virus suspension was added. This mixture with an equal volume of 0.153 M potassium citrate (with 1.5 mg per cent hyaluronidase) was homogenized ('Virtis 45') at 4° for five 1-min runs at a setting of 10 with a 20-sec rest between runs. Eight volumes of 0.153 M potassium citrate were then added. The mixture was allowed to stand at room temperature for 30 min and afterwards kept at 4° for 24 h. The mixture was sedimented (International centrifuge) for 20 min at 4° and 1,500 r.p.m. and the supernatant was removed and spun again as above. The supernatant was cleared at 8,000g for 1 min (Servall centrifuge). The virus was then pelletized at 40,000g for 1 h (Spinco L) at 4°. The pellet was resuspended in a volume of Eagle's basal medium plus 5 per cent calf serum equal to the original volume of virus suspension. The preparation of hamster embryo extract (without addition of virus) was similar to that already described here and used as a control inoculum (Table 1, Group F).

The combined results of several experiments using RSV (Bryan), 10^5 pock-forming units, are shown in Table 1, Group B. Since the presence of 0.05 M sodium citrate in the RSV (Bryan) was observed to be toxic to the new-born hamsters, the virus suspension was 'reconstituted' at full titre in Eagle's basal medium plus 5 per cent calf serum. Progressively growing sarcomata had been observed in 26 of the 122 animals when the experiments were terminated after 200 days. Approximately half the animals receiving 1×10^5 pock-forming units of RSV (Bryan) plus hamster embryo extract developed tumours (Group C). Using a dose of only 2×10^4 pock-forming units of RSV (Bryan), about 10 per cent of the animals developed tumours (Group A).

Of the 148 control animals either inoculated with hamster embryo extract or Eagle's basal medium or not inoculated at all, no tumours were found after 200 days (Groups F, G and H).

Several experiments using the Schmidt-Ruppin strain are also summarized (Groups D, E). Based on the pock-forming units of RSV administered, the Schmidt-Ruppin strain was more oncogenic for the new-born hamsters used in this investigation than was the Bryan strain.

The results for Group B and C (Table 1) suggest a slightly enhanced oncogenic activity for the RSV (Bryan) exposed to the treatment with hamster embryo extract

described here. After we had observed substantial oncogenic activity in hamsters using the RSV (Bryan) samples available in our laboratory, we abandoned any further search in our system for the identity of the 'activating factor' suggested by the work of Stewart and Landon⁴.

The tumours were histologically sarcomatous (Fig. 1A), poorly differentiated and similar to those described by

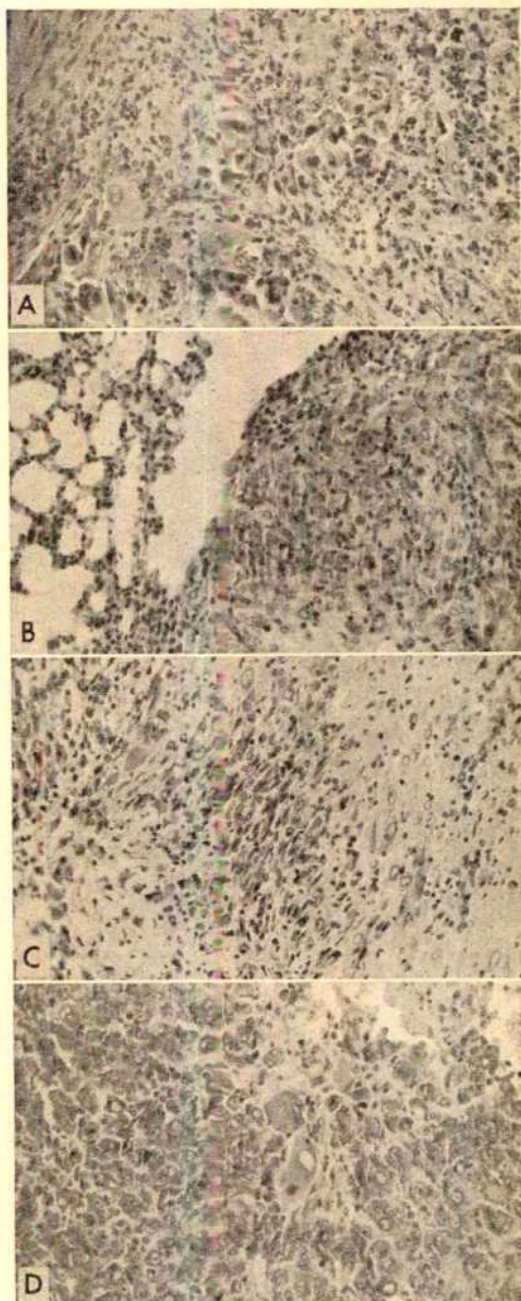


Fig. 1. Sections of tumours in hamsters inoculated at birth with Bryan strain of Rous sarcoma virus (haematoxylin-eosin stain, $\times 128$). A, Poorly differentiated sarcoma. Pleomorphism; multiple nuclei; mitotic figure (lower left). Bryan CT 957; 2×10^4 pock-forming units diluted in Eagle's basal medium plus 5 per cent calf serum. Tumour onset: 24 days; excised after 10 days; 3.3 g. B, Metastasis to lung. Bryan CT 955, pelletized and resuspended in Eagle's basal medium plus 5 per cent calf serum. 1×10^5 pock-forming units; tumour onset 25 days; excised after 13 days; 19.9 g. C, Fibrosarcoma. Bryan CT 951; pelletized and resuspended in Eagle's basal medium plus 5 per cent calf serum; 1×10^5 pock-forming units; tumour onset 17 days; excised 37 days after onset; 3.8 g. D, Strap cells (in centre) suggesting rhabdomyosarcoma in the vicinity of skeletal muscle (not shown in the figure). Bryan CT 951, pelletized and resuspended in Eagle's basal medium plus 5 per cent calf serum. 1×10^5 pock-forming units; tumour onset 18 days; excised after 10 days; 3.2 g.

Ahlstrom and Forsby² using the Schmidt-Ruppin strain of RSV. The latter virus strain was also used in our laboratory as a control and produced similar tumours (Groups D and E, Table 1). In a few instances, metastasis to the lungs was observed (Fig. 1B). In some tumours the cells were sufficiently differentiated to suggest a fibrosarcoma (Fig. 1C). In several cases, when the tumour was invading skeletal muscle fibres, the latter appear to have been transformed to rhabdomyosarcomatous cells (Fig. 1D).

The formation of rhabdomyosarcomatous cells when the tumour produced by either the Bryan or Schmidt-Ruppin strain invades skeletal muscle suggests that the virus can induce several tumour types depending on the tissue infected. This suggestion is further supported by the production of gliomata and choroid plexus papillomata following intracerebral inoculation of the Bryan virus

strain into new-born hamsters⁵ and the production of pleomorphic gliomata following inoculation of the Schmidt-Ruppin strain⁷.

We thank Mr. Stephen Goldberg for his assistance and Drs. W. Ray Bryan, S. E. Stewart and J. C. Landon for their advice. This study was aided by grants from the National Cancer Institute (C-3811) and the Atomic Energy Commission AT (30-1)—910.

¹ Zilber, L. A., *Intern. Conf. Avian Tumour Viruses: J. Nat. Cancer Inst. Mono.*, **17**, 261 (1964).

² Ahlstrom, C. G., and Forsby, N., *J. Exp. Med.*, **115**, 839 (1962).

³ Munroe, J. S., and Southam, C. M., *Fed. Proc.*, **21**, 453 (1962).

⁴ Stewart, S. E., and Landon, J. C., *Intern. Conf. Avian Tumour Viruses: J. Nat. Cancer Inst. Mono.*, **17**, 237 (1964).

⁵ Rabotti, G. F., Raine, W. A., and Sellers, R. L., *Science*, **147**, 504 (1965).

⁶ Prince, A. M., *Virology*, **5**, 435 (1958).

⁷ Rabotti, G. F., and Raine, W. A., *Nature*, **204**, 898 (1964).

IMPLICATIONS OF THE IMMUNOLOGICAL THEORY OF CANCER TO MULTIPLE CAUSES

By DR. MARY R. ANDERSON and PROF. H. N. GREEN

Department of Experimental Pathology and Cancer Research, University School of Medicine, Leeds 2

EXPERIMENTAL neoplasia has been induced in animals by radiation¹, by viruses^{2,3}, and by intrinsic and extrinsic chemical carcinogens. In man, too, these factors have been associated with cancer.

The evidence is still uncertain that the virus is more than a passenger in the cancer cell in man, but the lymphomatous tumour described by Burkitt⁴ among children in Uganda suggests that viruses can cause some types of human cancer. Brookes and Lawley^{5,6} maintain that carcinogens act by altering the nucleic acids binding to the adenine or guanine components of DNA. Gause *et al.*⁷ recently described lesions in the DNA molecule, caused either by ultra-violet light or 5-fluorouracil, which they think are repaired by deoxyguanic and deoxycytidilic acids. X-rays also can cause breaks in the DNA and RNA molecule⁸. Nowell and Hungerford⁹ found loss of a normal chromosome in human myeloid leukaemia; Court Brown *et al.*¹⁰ described the Philadelphia chromosomal abnormality in the bone marrow in myeloid leukaemia, and Upton¹¹ found this Philadelphia chromosome in irradiated bone marrow. So far, no consistent genotype abnormality has been found in malignant cells from fixed tissues¹², although karyotype abnormalities have been found in most cases of chronic myeloid leukaemia¹³, in acute leukaemia¹⁴ and in the bone marrow cells of patients with malignant lymphomas¹⁵. Witts¹⁶ has described an association between the experience of foetal irradiation and the subsequent development of leukaemia, and Holland¹⁷ has pointed out that patients with Down's syndrome (small acrocentric additional chromosome and mongolism) experience eighteen times the national average of leukaemia.

A low concordance for the incidence of cancer in monozygotic twins has been noted, however, which argues against chromosomal abnormality being a direct cause of carcinogenesis¹⁸. Recent work by Rich *et al.*¹⁹ on virus-induced murine leukaemia suggests that aneuploidy results from, rather than initiates, the disease. The observations of Sulzberger, Sherwin and Hermann²⁰ suggest that a chemical could be a carcinogen in one species, and cause contact dermatitis in another. If protein binding were the sole mechanism involved in the aetiology of chemical carcinogenesis by skin painting, one would expect tumour formation in a species such as a rat or mouse where carcinogen-protein binding has been described²¹. In species which do not show protein binding such as the guinea-pig²², the skin would be expected to be inert. Sulzberger has shown, however, that guinea-

pig skin responds by developing contact dermatitis to chemicals which, in other species, cause tumour formation.

The precise site of attack of carcinogens in the chemical induction of cancer is not yet known. Green²³ suggested that carcinogen-protein complexes were formed at lipoprotein sites on the cell surface, and the resultant complexes altered the antigenic nature of the cell, which called forth an immune response from the host. The immune attack deleted, or occluded, the tissue specific antigens on the cell surface, causing a loss of cell identity. He first proposed this immunological theory of cancer in 1954 (ref. 24), when he suggested that loss of identity markers in, or on, the cell (tissue specific antigens) caused a displaced cell to be antigenetically anonymous, and that this anonymity was the fundamental cause of cancer. The maintained loss of tissue specific antigens (TSA) through subsequent cell generations may imply an alteration in the nucleic acids in all neoplasia, such as has been suggested for virus- and radiation-induced cancers. However, this is not necessarily so, as heterologous tumours have been grown for several generations in normal adult animals after having been induced under acquired tolerance²⁵. These tumours re-grew in their species of origin only when induced under acquired tolerance again.

Boyden²⁶ has described a cytophilic antibody which is only elicited to an antigen plus Freund's complete adjuvant, and which adheres to selective cells that can then absorb the antigen. He suggests that this antibody plays a part in the reaction of delayed hypersensitivity, and is normally masked in the serum by excess of classical antibody, which competes for the antigen with that small fraction of cytophilic antibody not attached to the cells. It is thought that any antibody, or antibodies, taking part in carcinogenesis must be similarly masked by the classical responses. It is believed that, in cancer, this antibody, acting on the cell surface, deleting the tissue specific antigens, and rendering the cell anonymous, possibly also modifies the surface membranes so that the malignant cell has a metabolic advantage over normal cells.

There is evidence that malignant cells have all, or nearly all, the species and iso-antigens²⁷⁻²⁹, so the cell surface may have been incorporating serum protein into the cell. The tumours in foreign hosts appeared to have been 'heterogenized' by binding host proteins, and this antigenic alteration was apparently maintained through several generations. It is believed that TSA-depleted cells replicate in a manner similar to these heterogenized tumours,

and that at somatic cell division the nucleus is unable to reconstitute deleted cell components.

Hamburg and Svet-Moldavsky³⁰ altered the antigenicity of trypsinized tumour cell suspensions by virus infection. This antigenic change prevented the tumour growing in normal mice of the strain of origin and caused enhancement in mice pre-immunized with the specific virus. Habel³¹, Sjogren *et al.*³² and Sachs³³ all reported independently on polyoma-induced tumours which could not grow in the pre-immunized strain of origin unless a very large number of tumour cells were injected. This is evidence of malignant cells incorporating a virus which partly alters the antigenic state of the cells so that an injection of a small number of heterogenized tumour cells regressed in a susceptible, pre-immunized host. An injection of a larger number, however, was able to grow and kill the host.

Loss of tissue-specific antigens in tissues undergoing carcinogenic change has been shown to take place³⁴⁻³⁷. If the TSA is deleted it must, presumably, leave a hitherto unexposed site open to immunological attack, and this could, at any rate theoretically, modify the antigenic nature of the cell. It is possible that a structural alteration, resultant on loss of TSA in or on the cell membranes, may also alter the functional behaviour of these membranes. Evidence has been presented to try to demonstrate this alteration in function, which is believed to be immunologically recognizable³⁸. Rat spleen was sensitized to a piece of autologous 20-methylcholanthrene-induced tumour enclosed in a 'Millipore' chamber and replaced intraperitoneally. Cell suspensions were made of the autologous liver and tumour. Autologous tumour and sensitized spleen suspensions were injected subcutaneously into one flank of a guinea-pig, while the other flank received sensitized spleen and autologous liver cells. It was found that the experimental flank showed a larger flare than the control side, thus the sensitized spleen appeared to be capable of recognizing autologous malignant cells. This again suggests some antigenic alteration.

It is believed that there is a structural alteration in the membranes of a malignant cell that can be caused by factors which are endogenous or exogenous to the cell. This is supported by the work of Oppenheimer *et al.*³⁹, who induced sarcomata in rats to subcutaneous implants of strips of 'Cellophane', whereas Goldhaber⁴⁰ did not get sarcomata to subcutaneous 'Millipore' implants, which are porous. A mechanical alteration in the physical environment of the cell caused by an impermeable strip could possibly induce an alteration in the surfaces of adjacent cells, and initiate an immune response.

If an increased permeability to nutrients of the cytoplasmic membranes of malignant cells is postulated, the cell may be working under a metabolic advantage compared with normal cells, which, with the advance of the disease, may possibly cause gradual protein depletion of

the host. This could be one of the causes of cachexia, and of the specific impairment of antibody formation, as compared with other patients, recently described in malignant disease⁴¹.

It is possible that a variety of agents, which act on different cell components including spontaneous gene mutations⁴², find their ultimate expression at the same cell sites and, by inducing an antigenic alteration on the cell surface, initiate an immune response which can culminate in malignancy.

¹ Glucksmann, A., Lamerton, J. F., and Mayneord, W. V., in *Cancer*, edit. by Raven, R. W., 1, 497 (Butterworth, 1957).

² Dmochowski, L., in *Cancer*, edit. by Raven, R. W., 1, 214 (1957).

³ Gross, L., *Proc. Soc. Exp. Biol.*, **88**, 362 (1955).

⁴ Burkitt, D., *Brit. J. Cancer*, **16**, 379 (1962), *Brit. Med. J.*, **ii**, 1019 (1962).

⁵ Brookes, P., and Lawley, P. D., *Biochem. J.*, **80**, 496 (1961).

⁶ Lawley, P. D., and Brookes, P., *Biochem. J.*, **89**, 127 (1963).

⁷ Gause, G. G., Loshkareva, T. P., Zbarsky, I. B., and Gause, G. F., *Nature*, **203**, 598 (1964).

⁸ Hauschka, T. S., *Exp. Cell Res.*, **Supp.**, **9**, 569 (1963).

⁹ Nowell, P. C., and Hungerford, D. A., *Science*, **132**, 1497 (1960).

¹⁰ Court Brown, W. M., Harnden, D. G., Jacobs, P. A., Maclean, N., and Mantle, D. J., *Spec. Report Ser.*, Med. Res. Council, London, No. 305 (1964).

¹¹ Upton, A. C., in *Cellular Basis and Aetiology of Late Somatic Effects of Ionizing Radiation*, edit. by Harris, R. J. C., 67 (Academic Press, London).

¹² Ishihara, T., Kikuchi, Y., and Sandberg, A. A., *J. Nat. Cancer Inst.*, **30**, 1303 (1963).

¹³ Fitzgerald, P. H., Adams, —, and Gunz, F. W., *Blood*, **21**, 183 (1963).

¹⁴ Sandberg, A. A., Ishihara, T., Kikuchi, Y., and Crosswhite, L. H., *Ann. N.Y. Acad. Sci.*, **113**, 662 (1964).

¹⁵ Sandberg, A. A., Ishihara, T., Kikuchi, Y., and Crosswhite, L. H., *Cancer*, **17**, 738 (1964).

¹⁶ Witts, L. J., *Brit. Med. J.*, **i**, 1197 (1957).

¹⁷ Holland, W. W., Doll, R., and Carter, C. O., *Brit. J. Cancer*, **16**, 177 (1962).

¹⁸ Von Vershuer, and Kober, E., *Excerpta Medica*, Sect. 165, 189 (abstract) (1957).

¹⁹ Rich, M. A., Tsuchida, R., and Siegler, R., *Science*, **146**, 252 (1964).

²⁰ Sulzberger, M. B., Sherwin, R. W., and Hermann, F., *J. Invest. Dermat.*, **39**, 179 (1962).

²¹ Clayton, D. B., *Chemical Carcinogenesis*, 374 (Churchill, London, 1962).

²² Clayton, D. B., *Chemical Carcinogenesis*, 375 (Churchill, London, 1962).

²³ Green, H. N., *Ann. N.Y. Acad. Sci.*, **68**, 268 (1957-58).

²⁴ Green, H. N., *Brit. Med. J.*, **ii**, 1378 (1954).

²⁵ Anderson, M. R., and Greer, H. N., *Nature*, **198**, 859 (1963).

²⁶ Boyden, S. V., *Immunol.*, **2**, 474 (1964).

²⁷ Moller, E., *Transplantation*, **1**, 165 (1963).

²⁸ Moller, E., and Moller, G., *Acta Un. Intern. Contra Canc.*, **19**, 205 (1963).

²⁹ Klein, E., *Transplant Bull.*, **6**, 420 (1959).

³⁰ Hamburg, V. P., and Svet-Moldavsky, G. J., *Nature*, **203**, 772 (1964).

³¹ Habel, K., *Proc. Soc. Exp. Biol. and Med.*, **106**, 722 (1961).

³² Sjogren, H. O., Hellstrom, L., and Klein, G., *Exp. Cell Res.*, **23**, 204 (1961).

³³ Sachs, L., *Exp. Cell Res.*, **24**, 185 (1961).

³⁴ Nairn, R. C., Richmond, E. G., McEntegart, M. G., and Fothergill, J. E., *Brit. Med. J.*, **ii**, 1335 (1960).

³⁵ Nairn, R. C., Fothergill, J. E., McEntegart, M. G., and Richmond, H. G., *Brit. Med. J.*, **ii**, 1791 (1962).

³⁶ Weiler, E., *Brit. J. Cancer*, **10**, 533, 560 (1956).

³⁷ Baldwin, R. W., *Brit. Assoc. Cancer Res.*, Leeds (1963).

³⁸ Anderson, M. R., *Nature*, **211**, 202 (1964).

³⁹ Oppenheimer, R. S., Oppenheimer, E. T., and Stout, A. P., *Proc. Soc. Exp. Biol. and Med.*, **47**, 33 (1948).

⁴⁰ Goldhaber, P., *Science*, **133**, 2065 (1960).

⁴¹ Lytton, B., Hughes, L. E., and Fulthorpe, A. J., *Lancet*, **i**, 69 (1964).

⁴² Burnet, F. M., and Holmes, M. C., *Nature*, **207**, 368 (1965).

SELF-ADAPTIVE CONTROL AND THE RESPIRATORY SYSTEM

By DR. I. P. PRIBAN and DR. W. F. FINCHAM

Autonomics Division, National Physical Laboratory, Teddington, Middlesex

IN order to design an experimental investigation of a complex biological system it is desirable, if not essential, to have a model or hypothesis which will explain the operation of the system. This is a short report of a study resulting in a hypothesis which offers a simple explanation of the normal behaviour of the entire respiratory control system. Wherever possible the hypothesis is based on structures and mechanisms known to exist.

There are many similarities between the mechanism controlling respiration and those used in the control of some engineering plants. Therefore, engineering concepts have been applied to examine the respiratory system. The main principle underlying the operation of this system is that of self-adaptive control. An adaptive control system (also called self-optimizing) operates by continuously maximizing an over-all performance index in the presence

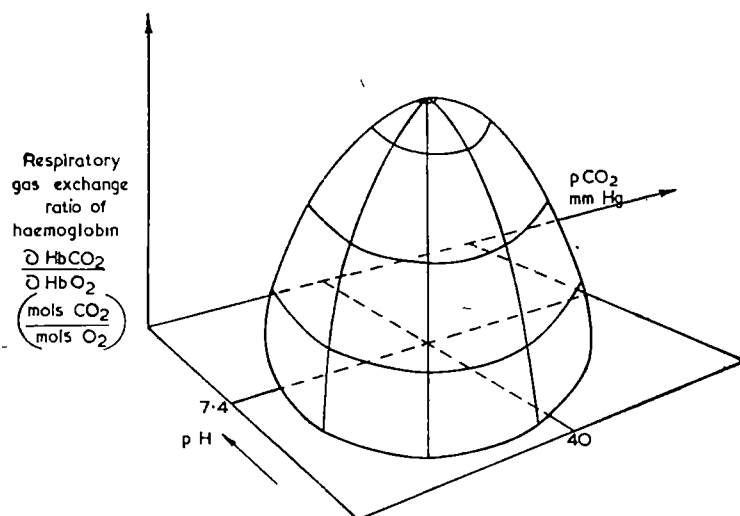


Fig. 1. Relation between the respiratory variables of blood (based on data from ref. 3)

of major external disturbances. This is achieved by adjusting the characteristics of the system¹.

The respiratory control system may be considered to consist of three interacting control loops. The processes associated with these loops are:

(1) *Chemical control.* The process by which for any given metabolic exchange requirement of both oxygen and carbon dioxide the level of ventilation is kept at a minimum.

(2) *Muscle control.* The process by which for any level of ventilation demanded by the blood chemistry, a pattern of activity of respiratory muscles is selected in which the average expenditure of energy is kept at a minimum.

(3) *Airway control.* The process by which for any alveolar gas exchange requirement the energy required to ventilate the dead space is kept at a minimum.

Each of these processes is co-ordinated and controlled by the over-all controller, a network of respiratory neurones in the brain. The controller predicts the activity which will keep the performance of the respiratory system at its optimum. For this prediction the controller uses information fed back during previous breaths.

Chemical Control

An examination of the physico-chemical properties of blood, in particular of haemoglobin, shows that there is an acidifying (Bohr) effect when haemoglobin is oxygenated in the absence of carbon dioxide. This effect is a function of pH and occurs in the range pH 6-9. It is maximal at pH 7.4 (ref. 2). The presence of carbon dioxide changes this effect. Above certain values of the partial pressure of carbon dioxide, $p\text{CO}_2$, and pH, oxygenation tends to make blood more alkaline³. The presence of carbon dioxide also increases the buffer power towards addition of fixed acid or fixed base. The relation between the function of haemoglobin and the respiratory variables may be represented by the shape of a hill (Fig. 1). This shows that the maximum number of molecules of carbon dioxide that can be exchanged per molecule of oxygen occurs at pH 7.4 and a $p\text{CO}_2 = 40$ mm mercury, the values normally found in arterial blood. Results by Naeraa, Strange Petersen and Boye⁴ suggest that the partial pressure of oxygen, $p\text{O}_2$, is also involved in determining the optimum operating range of haemoglobin. It is likely that at a lower $p\text{O}_2$ the base of the hill is smaller (Fig. 2).

Engineering processes having these characteristics are often controlled by so-called hill-climbers. These are designed to drive the operating point of the process toward some optimum position which maximizes a performance index, for example, minimum operating cost. The mechanism involved basically finds the direction to this position by measuring, for example, the slope of the hill, and uses this information to move the operating point in this direction.

A signal which can be used by the body to define the operating point of the blood are the fluctuations in $p\text{CO}_2$ and pH in the arterial blood produced by breathing. At a constant frequency and depth of breathing these fluctuations are minimal at the optimum operating point and increase on moving away from this (Fig. 2). A theoretical analysis showed that the fluctuations in the arterial $p\text{CO}_2$ increased with increasing metabolic (exercise) carbon dioxide production⁵. It was suggested that these fluctuations and slower ones might have been

signals involved in the control of ventilation. The fluctuations in arterial pH due to breathing that have been recorded in resting man show a swing of 0.01 to 0.015 pH units⁶.

A control system which will keep the blood variables at the optimum values with minimum ventilation is the biological hill-climber shown in Fig. 3. Its goal is to keep the pH- $p\text{CO}_2$ fluctuations at a minimum. The magnitude of the signal in the arterial blood is determined by two factors. One is the mean level of ventilation which determines the position of the operating point. The other is the perturbation due to breathing which causes the fluctuations of pH and $p\text{CO}_2$ in the blood. This perturba-

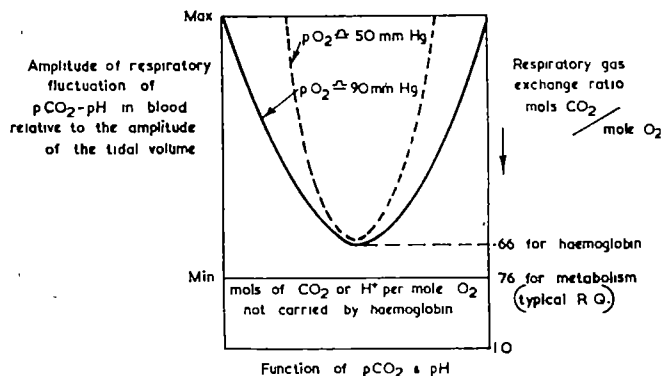


Fig. 2. The control and functional characteristics of blood

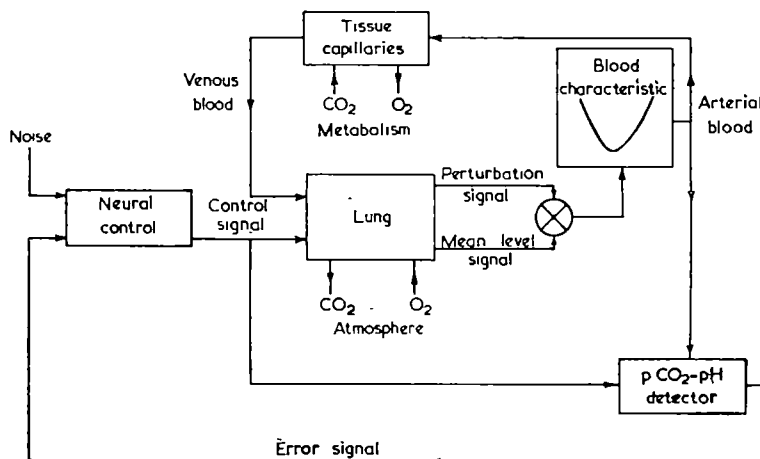


Fig. 3. Control of the respiratory gas exchange. 'Noise' is used to describe signals that are important in keeping the system operational, but which have no relevant control component

on signal $f(p\text{CO}_2, pH)$, and a signal which is a function of the signal controlling breathing are combined in the detector to give an error signal. The control signal input to the detector is necessary to give the sign of the error signal and also to make allowance for changes in the depth and frequency of breathing. The natural choice for the detector is the area postrema. The relevant factors are its structure, its closeness to the medullary respiratory neurones and the relative absence of the blood-brain barrier in the area. The error signal from the detector is fed back to the neural control where the control signal is corrected in the direction that will take the operating point of the blood towards the optimum. This control will keep the ventilatory gas exchange equal to that required by the metabolism of the body.

In order that this control can be effective (in particular that associated with the perturbation signal due to breathing) it is necessary that the noise-level in the mixed venous blood, of which the random fluctuation about the mean level of the $p\text{O}_2$ is a component, is kept at a minimum. This would be accomplished if the proportion of the cardiac output distributed to a particular region were a function of the metabolism in this region. Such control will optimize the respiratory function of the circulation. Receptors of the type found by Hughes⁹, lying on the venous side of any tissues, could be part of the control mechanism which accomplishes this control. The adjustment of the peripheral circulation through the corresponding tissue would be via the autonomic nervous system.

The brain is without an oxygen store and its metabolism is essentially aerobic, so that its functioning is critically dependent on a satisfactory oxygen supply. The arterial chemo-receptors lying in the circulatory path to the brain would seem to be in the most suitable position to detect any potential lack of oxygen supply to the brain and bring about a feed-forward compensatory adjustment to the cerebral circulation and to the cardiac output generally as well as influencing respiration. This emergency type control mechanism is considered to bring about a rapid qualitative (coarse) adjustment which over-rides the optimizing control mechanism discussed.

Muscle Control

The muscles that are used for breathing may also be involved in maintaining posture or in producing movement of the trunk and limbs. Taking up a new posture or movement of limbs may result in a new pattern of breathing, for example, a new frequency of breathing and/or a new mean thoracic volume. The response usually takes a few breaths to settle down and is associated with a different spatial and temporal distribution of muscle activity.

This aspect of respiratory control involves muscle spindles and spinal cord structures. It has received considerable attention¹⁰⁻¹² since Nathan and Sears¹³ showed its importance in normal breathing. The general arrangement of the structures is shown in Fig. 4.

The behaviour of this sub-system may be described in terms of a number of time-varying contours. One set of

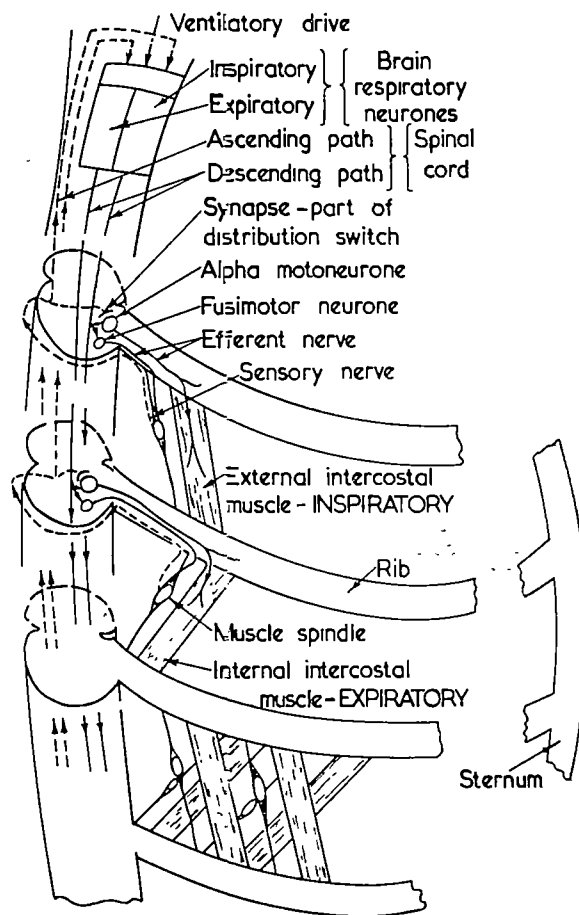


Fig. 5. Neuro-muscular control system

contours represents values of muscle activity on the grid of a muscle map (Fig. 5). The physical properties and the structure of the chest respiratory apparatus are such that progressively more energy is required for operation towards the extremities of the system. This property is represented by contours of performance index values on the muscle map. The performance index is defined in terms of the maximum number of units of ventilation obtainable per unit energy expenditure in the respiratory muscles. The contours are different for different levels of ventilation and different postures. The function of the control process is seen as the matching of the muscle activity contours with the performance index contours of highest value.

The highest level of control is the neural respiratory activity in the brain as represented by brain activity contours. The nervous activity is seen as equivalent to energy, for it is the signal determining the instantaneous energy expenditure during a breath and the duration of a breath. Once a breath has begun this control activity is not modified during natural breathing. The muscles that are activated are selected by a scanning process involving the fusimotor (γ -loop) system (Figs. 4 and 5). The scanning process selects the route of activation by a continuous appraisal of the muscle tension as determined by the muscle spindles. In this way the output from the spindles determines the spatial distribution of muscle activity. The muscles with the maximum performance index correspond to those in which the spindle output is at a minimum. This scanning process probably involves a low-amplitude 10-c/s component of muscle activity.

The process selects the optimum pattern of respiratory muscle activity by making the best

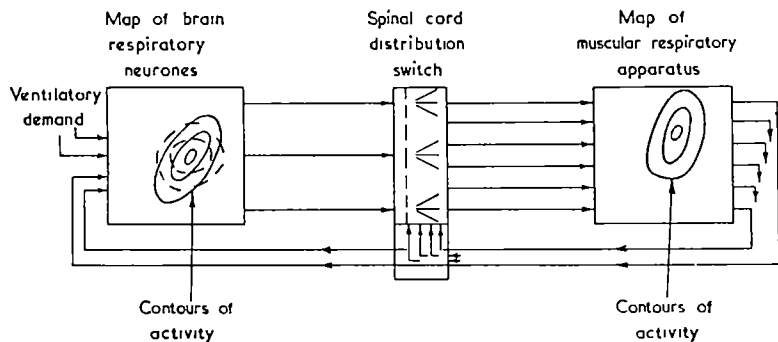


Fig. 4. Idealized structure of the neuro-muscular respiratory system

choice of inspiratory and expiratory muscles to be activated. It does this even when some of the muscles concerned with respiration are also involved in producing movement or maintaining posture.

The spindles also feed back information to the brain¹⁴. If the muscles shorten according to the predicted control signal then the muscle tension corresponds to the electrical activity. In this condition the ratio of the signal output from the spindles to the input to both muscles and spindles is unity and no correction of the brain activity (which determines the pattern of the next breath) is necessary. If, however, the load on the muscles has changed, the information fed back alters correspondingly. This informs the brain about the new state of the muscular system and enables it to make a new prediction of the optimum activity.

Airway Control

Temporary blocking of the vagus nerve causes an increase in the variance of the depth and frequency of breathing¹⁵. This implies that the energy consumption was increased for the same level of ventilation. The vagus nerve carries both the efferent fibres to the smooth muscles in the airway walls and afferent fibres coming from the receptors in the airway walls¹⁶.

From the structure and behaviour of the respiratory system one can predict that the function of the airway control mechanism is to keep the airway diameter adjusted, by means of the smooth muscles, to those dimensions at which the forces acting on the walls of the airway due to the main muscles, are minimal. The brain activity and corresponding activity in the vagus nerve are seen as determining the size of the airway diameter at any instant

relative to the activity of the main respiratory muscle. The purpose of the airway control process is to attain the condition of the airways in which the energy required to both ventilate the dead space and overcome airway resistance is minimal. The process controlling this optimization must, therefore, interact with the chemical control and muscular control mechanism.

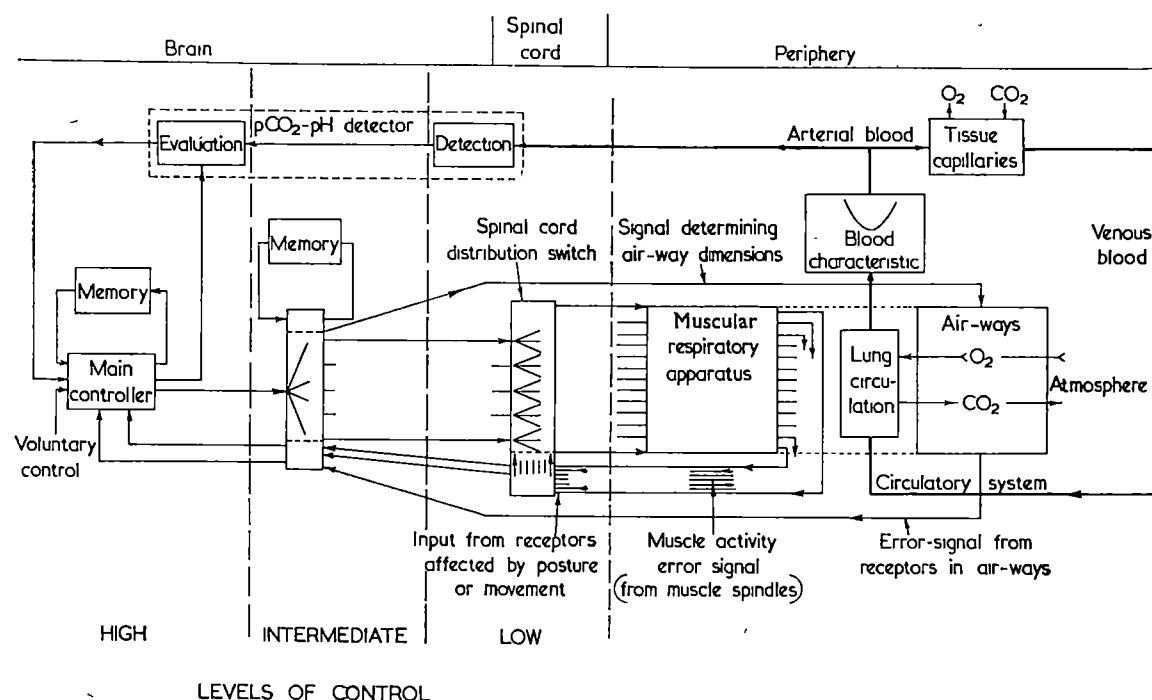
Over-all Control of Respiration

The function of the over-all control process is the combination of the functions of the three sub-systems—that is to regulate respiration so that this is accomplished with the minimum consumption of energy. The control ensures that the ventilatory gas exchange and the metabolic gas exchange are equal.

The control action of the entire system is seen as being divided according to their importance into three levels of control (Fig. 6). A high level defines the total amount of energy, E , to be expended in a breath and the time, τ , over which it is to be dissipated. It does this by evaluating the optimal predicted activity of a breath with the actual activity in that breath. The result is the prediction of the next breath in terms of energy and time.

The optimal prediction by the high-level controller is the activity that should keep the operating point of the blood at the optimum while using a minimum of energy. The prediction is conditioned by the response of the system during previous breaths.

An intermediate-level controller interprets the orders from the higher level and produces a more detailed temporal distribution of the pattern of activity. It also produces some spatial resolution of activity. A low-level



High	Intermediate	Low
Prediction of the total energy, E , to be expended in the next breath in terms of duration, τ , tidal volume and airway diameter	Increase in resolution of E in time τ and in coarse resolution of E in space.	Detailed resolution of E in space
Data reduction and storage.	Assessment in time of muscle activity accomplished	Assessment in space of muscle activity accomplished
Assessment of operating point on blood characteristic		Detection of amplitude of signal $f(pCO_2, pH)$

Fig. 6. Organization of structure and function of the over-all respiratory control system

controller accomplishes the fine spatial resolution of activity. This resolution has also a small temporal component.

Consider a control sequence: a change in the operating point of blood may be the result of a change in: (1) ventilation; (2) metabolism; (3) efficiency of airway control. The chemical detector mechanism effectively determines both the magnitude and the direction of the operating point of blood from the optimum value. It does this by comparing the chemical state of the blood in the last few (probably two) breaths with that in earlier breaths. The purpose of the comparison is to decide whether or not a change of respiratory energy dissipation is required to change ventilation.

In order to resolve ventilation into tidal volume, V_t , and breath duration, τ , the main controller uses the previous prediction, that is, the memory of the activity controlling the present breath and the error signals fed back from the muscular sub-system and airway sub-system. These error signals contain the information about the mismatch in performance with respect to the best input to the sub-system. These data are reduced at the low and intermediate level controllers so that only the significant information about the accuracy of the prediction is fed back to the main controller.

The prediction and relative adjustment of the values of V_t and τ at which the mean value of E is at a minimum (for a given ventilatory demand) involve information passing around the control loops in the three sub-systems. That such a control process exists is suggested from an analysis of small variations in V_t and τ which have been observed during breathing¹⁷. The values of V_t and τ are positively correlated. The average cycle time for these variations is 3.6 breaths, while the cycle time expected from a random process is 3 breaths. The shortest cycle time of a search process of a controlled system of the type discussed in which there are delays and which is subjected to random disturbances would be expected to have a length of 3-4 breaths.

The function of any sub-system or component of the respiratory system can be represented by contours having values of a functional index, as was done for the respiratory muscles. The index contours of many, if not all, structures take the form of a hill. The over-all purpose of the control is to regulate the different functions so as to obtain coincidence of the hills representing the different components. In other words the individual component systems are adjusted so that these operate at their optima. By combining the hills representing different structures the map of functional index contours of the entire system is obtained. In this context the over-all control should be seen as a hill-climbing process involving an over-all functional hill of the respiratory system.

The nervous activity associated with a conscious voluntary effort is seen as over-riding the self-optimizing control process for a short period. Similar nervous activity is thought to produce the sharp rise in ventilation at the beginning of exercise¹⁸. The immediate response would

seem to be a very qualitative increase in tidal volume (or E). This may be seen as a feed-forward signal anticipating an increase in ventilatory requirement. The adjustment of the total level of ventilation V_t and τ to the optimum values can then occur in the usual time. The selection of the optimal pattern of respiration for a new posture or during movement also involves the spinal mechanisms and muscle receptors (Fig. 6). This aspect is more complex than has been implied here. More details about the structures and mechanisms involved have been discussed by C. von Euler¹¹.

Only the essential points have been presented here to describe a new way of thinking about the organization and mode of operation of the respiratory control system. The underlying principle of operation, the maximization of an average functional index, may be applied to the study of most complex systems, even to the entire living organism. Certain of the aspects of respiratory control are discussed elsewhere in more detail and in relation to new experimental findings¹⁹. The relevance of this type of investigation to understanding such aspects of respiratory impairment as those producing the sensation of breathlessness are also discussed elsewhere²⁰.

We thank Dr. A. M. Uttley, superintendent of the Autonomics Division, National Physical Laboratory, for providing facilities at the laboratory, and Mr. P. H. Hammond for initiating and guiding this interdisciplinary investigation.

This work was supported by a grant from the Medical Research Council.

- ¹ Westcott, J. H., *An Exposition of Adaptive Control* (Pergamon Press, Oxford, 1962).
- ² Roughton, F. J. W., in *Handbook of Physiology*, Sect. 3, *Respiration*, 1, 767, edit. by Fenn, W. O., and Rahn, H. (Amer. Physiol. Soc., Washington, 1964). Margaria, R., *Chim. Chim.*, 9, 745 (1963).
- ³ Rossi, J., and Roughton, F. J. W., *J. Physiol.*, 167, 15P (1963). Roughton, F. J. W., in *Oxygen in the Animal Organism*, 5, edit. by Dickens, F., and Neil, E. (Pergamon Press, Oxford, 1964).
- ⁴ Naerum, N., Strange Petersen, E., and Boye, E., *Scand. J. Clin. Lab. Invest.*, 15, 141 (1963).
- ⁵ Yamamoto, W. S., *J. Appl. Physiol.*, 15, 215 (1960).
- ⁶ Semple, S. J. G., in *Breathlessness*, edit. by Howell, J. B. L., and Campbell, E. J. M. (Blackwell, Oxford, in the press).
- ⁷ Brizze, K. R., and Neal, L. M., *J. Comp. Neurol.*, 100, 41 (1954).
- ⁸ Dawson, H., *Physiology of the Ocular and Cerebrospinal Fluids* (Churchill, London, 1956).
- ⁹ Hughes, T., *Nature*, 205, 249 (1965).
- ¹⁰ Campbell, E. J. M., and Howell, J. B. L., in *Pulmonary Structure and Function*, edit. by de Reuck, A. V. S., and O'Connor, M., 29 (Churchill, London, 1962).
- ¹¹ Euler, C. von, in *Breathlessness* (see ref. 6).
- ¹² Sears, T. A., in *Breathlessness* (see ref. 6).
- ¹³ Nathan, P. W., and Sears, T. A., *J. Neurol. Neurosurg., Psychiat.*, 23, 10 (1960).
- ¹⁴ Yamamoto, S., Miyajima, M., and Urabe, M., *Jap. J. Physiol.*, 10, 509 (1960).
- ¹⁵ Guz, A., in *Breathlessness* (see ref. 6).
- ¹⁶ Widdicombe, J. G., in *Handbook of Physiology*, Sect. 3, *Respiration*, 1, 585 (see ref. 2).
- ¹⁷ Pribram, I. P., *J. Physiol.*, 136, 425 (1963).
- ¹⁸ Dejours, P., in *Handbook of Physiology*, Sect. 3, *Respiration*, 1, 631 (see ref. 2).
- ¹⁹ Fincham, W. F., and Pribram, I. P., in *Proc. Second I.F.A.C. Symp., The Theory of Self-Adaptive Control Systems* (to be published by Inst. Soc. Amer.). Pribram, I. P., and Fincham, W. F., *Autonomic Control of Respiration*, National Physical Laboratory, *Special Rep. (AUTO)* (1965).
- ²⁰ Pribram, I. P., in *Breathlessness* (see ref. 6).

REACTION OF HUMAN LYMPHOCYTES IN CULTURE TO COMPONENTS OF THE MEDIUM

By G. J. JOHNSON* and PROF. P. S. RUSSELL

Department of Surgery, Harvard Medical School, and General Surgical Services, Massachusetts General Hospital, Boston, Massachusetts

THE *in vitro* lymphocyte transformation reaction has already found many applications^{1,2}. In the hands of Bain, Vas and Lowenstein³, and Bach and Hirschhorn⁴ it has been shown to be potentially of use for histocompatibility matching in human beings. However, in order to

be able to discriminate between members of a family when choosing a suitable donor for tissue transplantation, fine degrees of difference in stimulation of lymphocytes must be capable of detection.

Early in the development of this system for human histocompatibility testing, using a modification of Hirschhorn's technique⁵, it was found that an unexpectedly

* Present address: Woolwich Memorial Hospital, Shooters Hill, London, S E.18.

Table 1. REACTIONS OF LYMPHOCYTES TO MEDIUM

Additions to Eagle's medium	% R.H.	% Large cells C.C.	% Large cells plus mitoses at 7 days E.O.	% Large cells plus mitoses at 7 days E.R.	% Large cells plus mitoses at 7 days R.W.	% Large cells plus mitoses at 7 days D.C.
Foetal calf serum + pen. and strep.	29.6	24.8	18.9	19.0	27.8	22.6
Foetal calf serum alone	—	20.5	16.2	—	31.1	15.7
$\alpha\gamma$ -Calf serum + pen. and strep.	16.6	—	—	6.9	16.9	6.8
Autologous serum + pen. and strep.	6.8	4.6	5.0	5.5	6.6	2.3
Autologous serum alone	—	2.0	5.3	3.4	3.7	1.9

large number of blastoid cells and mitoses occurred in the control culture tubes, that is, those tubes in which the lymphocytes of one individual were cultured alone in the medium for 7 days. The present experiments were designed to investigate the hypothesis that these 'high control counts' might represent a primary immune response to various constituents of the medium.

The semi-purified lymphocytes used in these experiments were prepared from 50 ml. of venous blood drawn into a syringe containing 3 ml. of heparin (1,000 units/ml.). Gelatine ('Plasmagel') was added in the proportion of 1 : 2.5 of blood, and mixed in 50-ml. centrifuge tubes, inclined at 45°. After 40 min the red cells were sedimented. The supernatant, containing white cells and gelatine in addition to plasma, was transferred to 100-ml. bottles, and a knife-point of iron powder⁷ added. These bottles were incubated at 37° C, with agitation, for 1 h. The plasma was then spun in 15-ml. tubes in an International Clinical Centrifuge at 400 r.p.m. for 3 min. At this speed almost all those granulocytes which had engulfed iron-particles were sedimented. The remaining cells were then obtained from the supernatant by centrifugation in new tubes at 1,850 r.p.m. for 4 min, washed with Eagle's medium, and counted. The majority of these cells were typical small lymphocytes but there was an average granulocyte contamination of 38 per cent.

The cells were grown in 110 mm × 16 mm culture tubes, tightly closed, in a medium consisting of Eagle's minimal essential medium with 20 per cent foetal calf serum, 1 per cent L-glutamine (200 mM) and 125 units penicillin and 125 γ -streptomycin/ml. For each subsequent variation in the medium there were 2 parallel tubes and also phytohaemagglutinin ('Difco M') controls.

The cells were developed after 7 days by treatment with 1 per cent sodium citrate solution (to separate the clumps of cells) and fixation in 25 per cent glacial acetic acid in absolute ethyl alcohol. A minimum of 1,000 cells, fixed on slides and stained by Jenner-Giemsa, were counted microscopically by a standardized technique. One person counted the whole of one experiment without knowing the identification of the slides. The percentage of large cells plus the percentage of mitoses were added to give the 'percentage of change'. The final result was the average of the counts of 2 parallel tubes.

A few of the large cells possessing large, pale nuclei with two or three nucleoli had a grey cytoplasm which differed from the densely basophilic cytoplasm of the typical lymphoblasts. These cells were considered to be macrophages. Some other cells could not be put into either category. Therefore, all these cells with large nuclei which were clearly of a different size and type from those of the small lymphocytes were counted together as 'large cells'. The great majority of them, however, were typical lymphoblasts.

In the first 30 normal individuals it was found that the percentage of change in control tubes containing the total medium ranged from 5.0 to 48.0 per cent, with an average of 17.2 per cent.

This variable but high count in the controls was at first ascribed to the granulocyte contamination. However, the percentage of enlargement bore no relation to the percentage of granulocytes in each lymphocyte preparation. Even a 99 per cent pure lymphocyte preparation from peripheral blood gave an 11 per cent change when grown alone in the medium.

Therefore, the possibility that the 'high control counts' represented a primary immune response to various constituents of the medium was tested by deleting these constituents in a step-wise fashion. The foetal calf serum was replaced by new-born calf serum from which the γ globulins had been removed⁸ or autologous human serum (prepared from fresh clotted blood), and the penicillin and streptomycin mixture was also removed. The resultant step-wise reduction in counts in six different individuals is shown in Table 1.

Except in two instances where duplicates were not completed, each compartment in Table 1 represents the mean of two assays. The two assays obtained for these 23 pairs were tested to determine whether chance circumstances could account for discrepancies which existed or whether unknown factors were responsible. Separate χ^2 values on the original cell counts computed by a four-fold contingency table from 13 pairs totalled 16 (degrees of freedom = 13; $P > 0.1$). The remaining 10 pairs each had large χ^2 values, suggesting that the assays were not true duplicates.

Therefore, in subsequent comparisons to determine the significance of the effect produced by foetal calf serum or penicillin and streptomycin, the only cell counts used were those derived from pairs where good agreement existed.

In considering the effect of the presence of foetal calf serum in the medium instead of autologous serum, 4 comparisons between 8 compartments revealed a highly significant difference ($\chi^2 = 1.100$; $d.f. = 4$; $P < 0.001$). Similarly, 4 comparisons of the values in the presence and absence of penicillin and streptomycin were also significant ($\chi^2 = 52$; $d.f. = 4$; $P < 0.001$).

The values obtained when $\alpha\gamma$ -globulin calf serum was used were among those in which less satisfactory agreement between the duplicate assays was found. Therefore, in comparing these results with those for foetal calf serum on one hand, or autologous serum on the other, the assay selected to represent the $\alpha\gamma$ -globulin serum was the one of the pair which was closest to the value with which it was being compared. This selection provided the least favourable value for demonstrating differences between the $\alpha\gamma$ -globulin serum and adjacent compartments. Despite this conservative method of analysing the data, the differences for 4 comparisons in each direction are significant (for each $\chi^2 > 120$; $d.f. = 4$; $P < 0.001$).

At first glance the criticism might be raised that these results could be accounted for on the basis of nutrition: that foetal calf serum is a better nutrient than $\alpha\gamma$ -calf serum or autologous human serum. There are three reasons for thinking that this is not the case.

(1) The percentage of cells which are transformed by three days under the influence of phytohaemagglutinin is consistently greater when the medium contains autologous serum than when it contains foetal calf serum.

(2) In other experiments in which cells sensitive to tuberculin or Candida antigen are stimulated by these antigens, the net count (the count in the stimulated culture minus the count in the controls) is greater when the medium contains autologous serum than when it contains foetal calf serum.

Both these results suggest that the autologous serum is in fact a better nutrient than is foetal calf serum.

(3) The addition of penicillin and streptomycin to the medium significantly increased the percentage of large cells and mitoses. It would seem to be more likely that these antibiotics are acting as antigens or as stimulants, than that they improve the nutritive properties of the media.

In the experiments in Table 1, a semi-purified lymphocyte preparation was used; however, if the same type of experiment was attempted using the total white cells present in the supernatant after sedimentation of the red cells with gelatine, the results were more erratic; the step-

wise reduction could not always be produced; and the spontaneous enlargement in the absence of foetal calf serum and antibiotics could not be reduced to such a low level.

It is possible that, in addition to the components of the medium, white blood cells other than small lymphocytes and granulocytes play a part in producing the 'high control count'. McFarland and Heilman⁹ have recently reported their observations on living cultures which suggest that the macrophages play an important part in the transformation of lymphocytes to lymphoblasts. The preparation of the semi-purified lymphocytes used in the experiments reported here involved a stage of incubation and mild agitation with iron powder. This was followed by centrifugation at low speed. Examination of both the sediment and supernatant from this centrifugation reveals that the supernatant contains the nuclei of disrupted monocytes. Almost all the monocytes are removed from the lymphocyte preparation, not by engulfing iron and being sedimented, but by destruction. Monocytes, which are present in greater numbers in the total white blood cell preparations, may cause, or permit, a spontaneous enlargement in the cultures which is not so clearly controlled by alteration of the constituents of the medium.

A similar 'high control count' has been observed with the total white blood cells of the rat in culture. Unlike the human situation, these counts could not be reduced by subtraction of foetal calf serum or penicillin and streptomycin. These experiments have not yet been repeated with purified lymphocytes.

The time curve of the reaction of human lymphocytes to foetal calf serum shows that there is an upswing at 7-8 days. By contrast, the reactions of lymphocytes from individuals sensitive to PPD or Candida antigen, when cultured with these antigens, show their main response at five to six days. It may be that this difference in the time of development of large cells reflects the difference between a primary and secondary immunological response

of the lymphocytes. If that were the case, the reaction to foetal calf serum would be a primary response.

Another observation emerges from these experiments. This is the apparent 'summation' effect produced by the addition of antigens. The impression is gained that the addition of antigens of different specificity causes additional lymphocytes to become involved in the reaction. A similar observation was made by Chapman and Dutton¹⁰ in rabbits. The responses of lymphoid cells from immunized rabbits to the simultaneous presence of the immunizing antigen (heterologous serum protein) and allogeneic lymphoid cells was greater than the response to either alone.

These results are encouraging from the point of view of the application of this system to histocompatibility testing. It is reasonable to think, from what has already been shown, that allogeneic lymphocytes which differ from each other by two antigens of the same strength would stimulate one another to a greater extent than if they were to differ at only one of the two sites.

It is hoped that the avoidance of substances in the medium which, by themselves, will stimulate the lymphocytes to enlarge and divide will make it easier to detect minor differences in antigenicity between potential allograft donors.

This work was supported by U.S. Public Health Service grants AM 07055 and AI 06320. We thank Dr. B. A. Barnes for his assistance with the analysis of the data.

¹ Nowell, P. C., *Cancer Res.*, **20**, 462 (1960).

² Pearmain, G., Lycette, R. R., and Fitzgerald, P. H., *Lancet*, **i**, 637 (1963).

³ Bain, B., Vas, M. R., and Lowerstein, L., *Blood*, **23**, 108 (1964).

⁴ Bach, F., and Hirschhorn, K., *Science*, **143**, 813 (1964).

⁵ Hirschhorn, K., in *Histocompatibility Testing*, edit. by Russell, P. S., Winn, H., and Amos, D. B., 77 (National Research Council, Washington, D.C., 1965).

⁶ Laboratoire Roger Bellon, Neuilly (Seine), France.

⁷ Carbonyl Iron Powder, General Aniline and Film Corp., Linden, New Jersey.

⁸ Hyland Laboratories, Los Angeles, California.

⁹ McFarland, W., and Heilman, D. H., *Nature*, **205**, 887 (1965).

¹⁰ Chapman, N. D., and Dutton, R. W., *J. Exp. Med.*, **121**, 85 (1965).

AFLATOXIN: EFFECT ON CULTURED HETEROPLOID HUMAN EMBRYONIC LUNG CELLS

By DR. MARVIN S. LEGATOR, SAVERIO M. ZUFFANTE and ANTHONY R. HARP

Bureau of Scientific Research, Division of Pharmacology, Tissue Culture Section Food and Drug Administration, U.S. Department of Health, Education and Welfare, Washington, D.C.

AFLATOXIN, a mixture of metabolites produced by *A. flavus*, has been characterized chemically^{1,15}, and sensitive methods for the detection of the major aflatoxin components have been reported^{4,7}. In certain sensitive animal species this group of mycotoxins is acutely toxic at low concentrations, usually showing hepatotoxicity. In addition to its acute toxicity, aflatoxin B₁ is an exceedingly potent carcinogen in rats⁸ and trout². In biological studies the major effort has been directed towards the development of bioassays to augment and confirm the chemical procedures of analysis. There have been few reports on the biological action of this group of toxins and only three reports on the action of aflatoxin in *in vitro* systems. Juhasz and Greezi found that low concentrations of various groundnut samples destroyed cultured calf kidney cells⁹. Smith found that the toxicant inhibited the incorporation of ¹⁴C-leucine into protein in various liver preparations¹³; Legator and Withrow reported that the crude and crystallized aflatoxin preparations suppressed mitosis in cultured human diploid and heteroploid embryonic lung cells¹⁰. The experiments reported here were designed to expand the information concerning the biological activity of aflatoxins in cell

culture. The effect of aflatoxin on cell growth, cell morphology, and the synthesis of DNA was investigated.

A heteroploid human embryonic lung cell line, L-132, was used. The cells were cultured in monolayer, using basal medium (Eagle) with Earle's 'BSS' and 10 per cent calf serum. Aflatoxin was added to the growth medium by one of the following procedures:

(1) The chemical was dissolved in propylene glycol and added directly to the culture medium. The amount of solvent in the culture medium never exceeded 0.1 per cent; this concentration in preliminary experiments was ten times below any detectable toxic concentration.

(2) The aflatoxin preparation was dissolved in chloroform and added to the culture vessel. The chloroform was removed under nitrogen and sterile medium was added.

Usually, to achieve maximum solubility, the uninoculated medium containing the toxicant was sonicated in a Branson 'S-75' unit for 30 sec. After sonication the medium was inoculated with approximately 100,000 cells per c.c. of medium. The cells were incubated at 37° C in an atmosphere of 3 per cent carbon dioxide.

Cell growth in the presence of aflatoxin. To determine the effect of aflatoxin on cell growth, cells were counted by a Coulter counter, and protein and DNA were determined. An aflatoxin preparation (495) containing 15 per cent aflatoxin B_1 , 9 per cent G_1 and less than 1 per cent B_2 and G_2 was used. The final concentration added to the culture vessel was based on the known concentration of B_1 and G_1 in this preparation. Up to the 48th hour after the addition of the toxicant, cells exposed to 0.05, 0.1, 0.5 and 1.0 p.p.m. increased in number; the 0.05 p.p.m. concentration differed very little from the control. There was little increase in cell numbers between 48 and 93 h at 0.05, 0.1 and 0.5 p.p.m. At 1.0 p.p.m. there was a slight decrease in cell numbers. During this same period of incubation the cells in the control divided rapidly. At the highest concentration used, 5.0 p.p.m., there was an entirely different response, since only a negligible amount of growth was found at any of the time-intervals investigated. Fig. 1 summarizes the results of this experiment.

DNA was determined according to the method of Webb and Levy¹⁶; protein was assayed according to the method of Lowry *et al.*¹¹. The DNA and protein determinations essentially confirmed the results obtained with the cell count determination. Fig. 2 indicates the effect of 0.5 p.p.m. on protein content when the cultures were examined over an incubation period of 4–93 h.

To determine the viability of the cells after various time exposures to aflatoxin, trypan-blue was used as a vital stain. No difference was found in viability between the control and the cells exposed to the toxicant except at the concentration of 5.0 p.p.m.

Morphology and giant cell formation. The L-132 cells exhibited many abnormal morphological patterns including

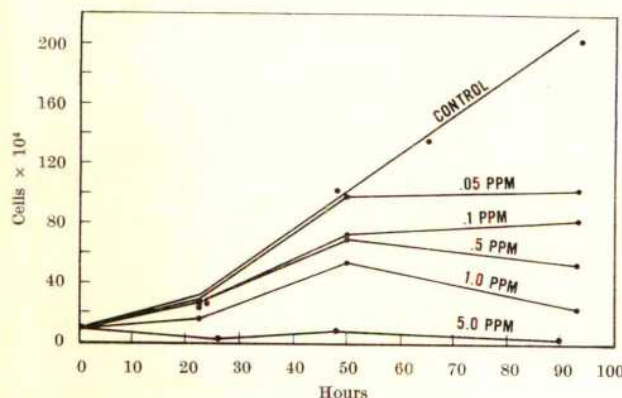


Fig. 1. Effect of aflatoxin on cell growth: cell count by Coulter counter

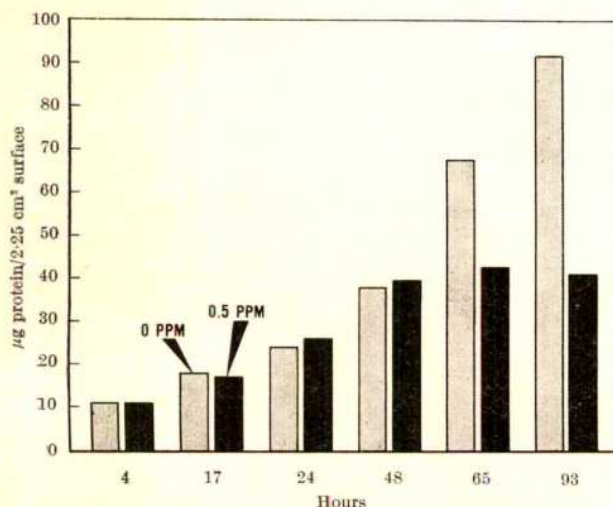


Fig. 2. Effect of aflatoxin on cell growth: total protein determination

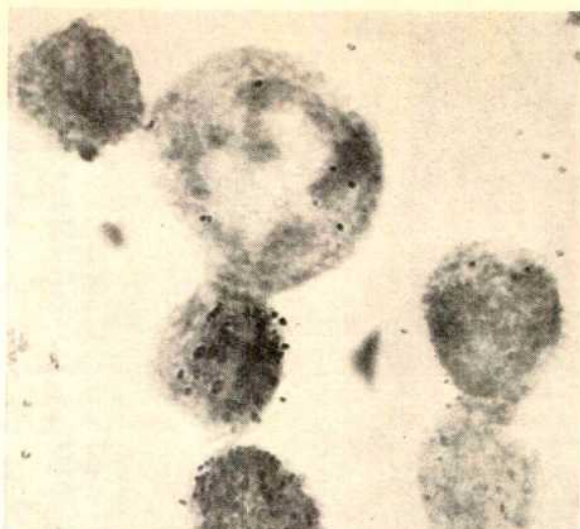


Fig. 3. Giant cells, showing vacuoles after exposure to aflatoxin, 1 p.p.m.

vacuolization and an accumulation of cellular debris. Cell vacuolization was similar to the response reported by Smith in monolayer growth of monkey kidney cells¹². In addition there was an abnormally high number of giant cells. Fig. 3 illustrates a typical giant cell showing cell vacuolization after an exposure to 0.1 p.p.m. of aflatoxin. To determine the number of giant cells, cells were trypsinized and fixed in acetic acid-ethanol (1:3) and stained with 1 per cent toluidine blue. Approximately 4,000 cells were counted to determine the number of giant cells in the population. The normal population contained approximately 0.3 per cent giant cells. After an exposure of 8–12 h to 1 p.p.m. crystallized aflatoxin B_1 , a 92 per cent increase in giant cells was found over the control. To determine the persistence of giant cells after exposure to the toxicant, L-132 cells were exposed to 1 p.p.m. of aflatoxin (495) for 24 h. After this exposure period the medium containing the toxicant was removed and fresh medium minus the toxicant was added. The number of giant cells was then determined at various time-intervals after the removal of aflatoxin. After a 24-h exposure to aflatoxin, followed by removal of the chemical, an 81–100 per cent increase in giant cells was found for a period up to 10 h. From 12 to 24 h after the removal of the toxicant, proportionately fewer giant cells were found. Table 1 shows these results.

Autoradiographic studies. To determine the effect of aflatoxin on DNA synthesis, tritiated thymidine was employed. The labelling procedure was essentially that of Moorhead and Defendi¹³. The cells were incubated in the presence of aflatoxin for 16 h before 'pulsing'. The cells were then exposed for 20 min to tritium-labelled thymidine at a concentration of 0.5 µc. per ml. The medium was removed and replaced with 50 per cent fresh medium and 50 per cent of the medium that had been removed before pulsing. After a further 12 h the cells were removed, fixed and dipped in NTB2 liquid emulsion (Eastman Kodak Co., Rochester, N.Y.), and the slides were incubated for 10 days before being developed.

Table 1. GIANT CELL FORMATION AFTER EXPOSURE TO AFLATOXIN

Hours after removal of aflatoxin	Increase over control (%)
0	81
2	81
6	92
8	88
10	100
12	70
14	68
18	70
20	60
22	51
24	34

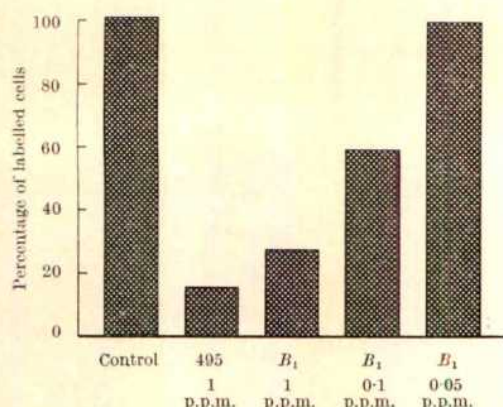


Fig. 4. Inhibition of DNA synthesis after 16 h exposure to aflatoxin

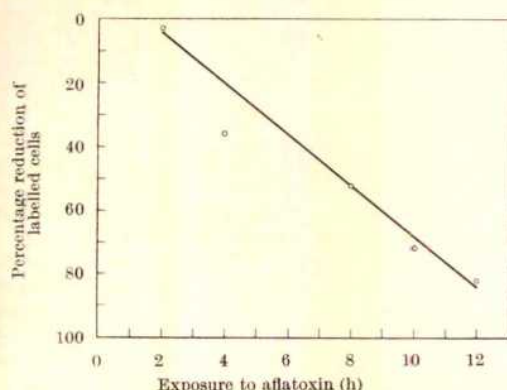


Fig. 5. Inhibition of incorporation of tritiated thymidine into DNA after exposure to aflatoxin, 1 p.p.m., for various time intervals

The number of labelled cells was determined, a minimum of 1,000 cells being counted for each determination. In this study the aflatoxin (495) and crystallized aflatoxin B₁ were used. A 40 per cent inhibition of DNA synthesis was produced by 0.1 p.p.m. of B₁ and more than 80 per cent inhibition of DNA synthesis by 1 p.p.m. of aflatoxin (495). The crude aflatoxin (495) at 1 p.p.m. was more effective than 1 p.p.m. aflatoxin B₁. The results of this experiment are summarized in Fig. 4.

Fig. 5 shows the inhibition of DNA synthesis after exposure of cells to 1 p.p.m. aflatoxin (495) for various time-intervals from 4 to 12 h. During this period, DNA synthesis was reduced by 4–84 per cent.

Discussion. A probable sequence of events for the effect of aflatoxin on this biological system can be constructed, based on the present investigations and the previous work

on mitotic suppression⁹. The earliest effect of this toxicant is to suppress DNA synthesis and mitosis; this effect is detectable within the first few hours after exposure of the cells. The inhibition effect on division and DNA synthesis leads to the survival of the cell in a non-dividing stage which can be quantitated after 48 h of exposure to aflatoxin. In the static population there is no equilibrium between the lethal effects of the chemical and the number of dividing cells. This has been shown by the viability counts. The abnormal production of giant cells could well be accounted for by the enlargement of non-dividing cells. The giant cell formation seems to be of the non-syncytial type seen in tissue culture cells infected with certain viruses, and is associated with cells in which mitosis has been arrested in metaphase³.

The suppression of mitosis, inhibition of DNA synthesis, and formation of giant cells in this system, as well as the previously reported carcinogenic action of aflatoxin, may well indicate that aflatoxin affects biological systems much as do known alkylating agents. Alkylating agents that are chemically reactive compounds are known to interact with nucleophilic (electron-rich) centres of receptor molecules of the cell⁸. Dickens and Jones have shown that aflatoxin, as well as other chemicals that contain an α - β unsaturated lactone ring, are carcinogenic⁵. It is possible that this functional group may be responsible for the overall biological effect of aflatoxin.

Alkylating agents are known to be mutagenic, carcinogenic and anti-neoplastic⁶. The carcinogenic effect may well be a manifestation of the mutagenic action^{6,14}. If aflatoxin is a unique alkylating agent, the demonstration of mutagenicity and evaluation as a candidate anti-neoplastic agent may be the next steps in investigating its biological effects.

¹ Asao, T., Buchi, G., Abdel-Eader, M. M., Chang, S. B., Wick, E. L., and Wogan, G. N., *J. Amer. Chem. Soc.*, **85**, 1706 (1963).

² Ashley, L. M., Haver, J. E., and Wogan, G. N., *Fed. Proc.*, **23**, 105 (1964).

³ Barski, G., and Robineaux, R., *Proc. Soc. Exp. Biol. and Med.*, **101**, 639 (1959).

⁴ Broadbent, J. H., Cornelius, J. A., and Shone, G., *Analyst*, **88**, 214 (1963).

⁵ Dickens, F., and Jones, H. E. H., *Brit. J. Cancer*, **17**, 691 (1963).

⁶ Haddow, A., *Brit. Med. Bull.*, **14**, 79 (1958).

⁷ Hartley, R. D., Nesbitt, B. E., and O'Kelly, J., *Nature*, **198**, 1056 (1963).

⁸ Johnson, J. M., and Berge, F., *Metabolic Inhibition*, **2**, 161 (Academic Press, New York, 1963).

⁹ Juhasz, S., and Greczi, E., *Nature*, **203**, 861 (1964).

¹⁰ Legator, M., and Withrow, A., *J. Assoc. Off. Agr. Chemists*, **47**, 1007 (1964).

¹¹ Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. Biol. Chem.*, **193**, 265 (1951).

¹² Moorhead, P. S., and Defendi, V., *J. Cell Biol.*, **16**, 202 (1963).

¹³ Smith, R. H., *Biochem. J.*, **88**, 50 (1963).

¹⁴ Strong, L. C., *Proc. Eighth Intern. Congr. Genetics*, July, 1948, Stockholm, 486 (1949).

¹⁵ Van Dorp, D. A., Van Der Zijden, A. S. N., Beerthuis, R. K., Sparreboom, S., Ord, W. O., De Jong, K., and Keuning, R., *Rec. Trav. Chim.*, **82**, 587 (1963).

¹⁶ Webb, J. M., and Levy, H. E., *J. Biol. Chem.*, **213**, 107 (1955).

NICOTINAMIDE MONONUCLEOTIDE PYROPHOSPHORYLASE ACTIVITY IN ASCITES CELL EXTRACTS

By PROF. L. S. DIETRICH, L. FULLER, I. LEONOR YERO and LAURA MARTINEZ

Department of Biochemistry, University of Miami School of Medicine, Florida

NICOTINAMIDE MONONUCLEOTIDE (NMN) pyrophosphorylase activity has been detected previously in acetone powder extracts of erythrocytes¹ and ascites cells². In both these cases, the *K_m* for the nicotinamide was extremely high, being 0.1 M and approximately 0.1 M for the erythrocyte¹ and ascites acetone powder extracts², respectively. The very low affinity for nicotinamide would seem to indicate that the physiological role of this enzyme in the biosynthesis of nicotinamide adenine dinucleotide from nicotinamide is doubtful. Work in our laboratory has shown that the

supernatant fluid obtained from ascites cells homogenized in a tris-chloride buffer contains NMN-pyrophosphorylase. The enzyme, in this crude form, has a *K_m* for nicotinamide of 1.6×10^{-5} M and requires adenosine triphosphate (ATP) as well as 5-phosphoribosyl-1-pyrophosphate (PRPP).

Ehrlich-Lette ascites cells were grown in Swiss albino (Webster) mice, 2–3 months of age. Cells were gathered 7–10 days after incubation and collected in heparinized vessels. Pooled cell suspensions were centrifuged at 600g for 5 min at room temperature and the supernatant

fluid discarded. The cells were resuspended in 6 volumes of isotonic NaCl solution and the washing procedure repeated four times to remove contamination erythrocytes. The cells were then suspended in 9 volumes of 0.05 tris-chloride buffer, pH 7.3, and homogenized for 1-2 min employing a homogenizer of the Potter-Elvehjem type. The supernatant fluid obtained by centrifuging this homogenate at 21,500g for 30 min served as the enzyme source.

Assay of NMN-pyrophosphorylase activity. Reaction mixture contains: 0.3 ml. of ascites supernatant, 0.7 μ moles PRPP, 2.0 μ moles ATP, 10 μ moles $MgCl_2$, 0.1 μ moles nicotinamide- ^{14}C (8.0 $\mu c./\mu$ mole) and 50 μ moles of tris, pH 7.3, in a total volume of 1 ml. Incubation was carried out at 37° C for 1 h, at which time the reaction was stopped by heating in a boiling water bath for 1½ min. The samples were then centrifuged and the supernatant material was chromatographed in paper employing the solvent C of Preiss and Handler³. The areas corresponding to NMN were cut out and counted in a liquid scintillation spectrometer as previously described⁴.

The requirements for the NMN-pyrophosphorylase activity of ascites cells is shown in Table 1. Mg^{++} , PRPP and ATP are required for the complete reaction. Stimulation produced by ATP appears to be specific, not being replaced by guanosine triphosphate (GTP), uridine triphosphate (UTP), and cytidine triphosphate (CTP) or the di- and mono-adenine derivatives.

Identification of enzymatic product as NMN. The reaction product obtained has been characterized employing four different paper chromatographic systems: the solvent B and C of Preiss and Handler³, isobutyrate-ammonia⁵, and N-butanol saturated with water in an NH_4HCO_3 atmosphere⁶ and paper electrophoresis. Under these conditions the unknown behaved in an identical manner to NMN. Mild alkaline hydrolysis with NH_4OH resulted in the release of a radioactive compound that behaved in an identical manner to nicotinamide in the aforementioned chromatographic and electrophoretic procedures.

A Lineweaver-Burk plot of the NMN-pyrophosphorylase activity in ascites cell supernatant employing nicotinamide as substrate is presented in Fig. 1. The K_m for nicotinamide calculated from these data is 1.62×10^{-5} M.

Optimum-pH experiments for the enzyme under these conditions demonstrate that the enzyme has a broad peak exhibiting maximum activity from pH 6.5 to pH 9. No attempt was made to determine whether the decrease in activity at either side of the broad activity peak is revers-

NMN Pyrophosphorylase in Ascites Cells

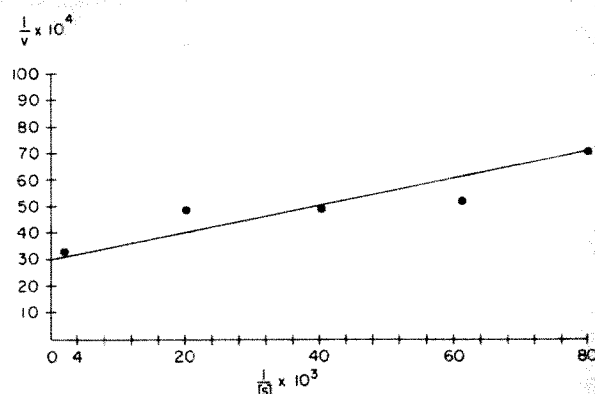


Fig. 1. Lineweaver-Burk plot of pyrophosphorylase activity employing nicotinamide as substrate

ible. However, exposure of the enzyme to a pH of 4-8 for short period of time does irreversibly inhibit the enzyme.

Under the condition of the assay the enzymatic activity is proportional to enzyme concentration and time of incubation. The effect of various inhibitors and treatment on the activity of NMN-pyrophosphorylase activity of ascites cells is presented in Table 2. The enzymatic activity is destroyed by boiling, freezing or exposure to cold acetone. It is inhibited by phosphate and pyrophosphate. The enzymatic activity is stable to storage at 4° C overnight at 55° C for 1 min. No activity was lost during protamine sulphate treatment of the supernatant. The activity of whole homogenate is considerably less.

The presence of an enzyme capable of converting nicotinamide to NMN in the presence of PRPP and ATP with a K_m for nicotinamide of 1.6×10^{-5} M is significant and demonstrates that these cell types possess the enzymatic capacity to convert nicotinamide directly to NMN without necessitating deamidation of nicotinamide to nicotinic acid⁷ and the subsequent utilization of the Preiss-Handler pathway³ to NAD. The ascites cells studied contain endogenous nicotinamide levels of approximately 4 μ moles/g wet weight (unpublished data). Thus, it is feasible that this reaction could, in the absence of inhibitors, proceed at a significant rate in intact cells.

The marked stimulation of NMN-pyrophosphorylase activity by ATP is similar to that observed for nicotinic acid mononucleotide pyrophosphorylase activity⁸. Whether the function of ATP is to control the direction of the reaction, as has been recently demonstrated by Nakamura *et al.*⁹ for nicotinic acid mononucleotide pyrophosphorylase, remains to be determined.

Evidence that NMN-pyrophosphorylase is not the same enzyme that catalyses the conversion of nicotinic acid to nicotinic acid mononucleotide is demonstrated by the fact that NMN-pyrophosphorylase is irreversibly inactivated at pH 4.8 while the nicotinic acid mononucleotide pyrophosphorylase activity of the extract remains unaltered.

This work was supported by grants AM 03049-05 and CA 04868-05 and a career development award K3-GM-15,186 from the United States Public Health Service.

¹ Preiss, J., and Handler, P., *J. Biol. Chem.*, **225**, 759 (1957).

² Dold, U., Mielsch, M., and Holzer, H., *Biochem. Z.*, **338**, 349 (1963).

³ Preiss, J., and Handler, P., *J. Biol. Chem.*, **233**, 488 (1958).

⁴ Dietrich, L. S., and Fuller, L., *J. Biol. Chem.*, **239**, 1653 (1964).

⁵ Magasanik, B., Vischer, E., Doniger, R., Elson, D., and Chargaff, E., *J. Biol. Chem.*, **186**, 37 (1950).

⁶ Tsuboi, K. K., and Price, T. D., *Arch. Biochem. Biophys.*, **81**, 223 (1959).

⁷ Petrack, B., Greengard, P., Craston, A., and Kalinsky, H. J., *Biochem. Biophys. Res. Comm.*, **13**, 472 (1963).

⁸ Imsande, J., and Handler, P., *J. Biol. Chem.*, **236**, 525 (1961).

⁹ Nakamura, S., Nishizuka, Y., and Hayaishi, O., *J. Biol. Chem.*, **239**, PC 2717 (1964).

Table 1. REQUIREMENTS FOR THE NMN-PYROPHOSPHORYLASE ACTIVITY IN ASCITES CELLS

Flask addition	NMN formed/ml. reaction mixture/h (c.p.m.)
Complete medium	96,900
-ATP	5,800
-PRPP	4,400
- Mg^{++}	3,700
-ATP + ADP	18,540
-ATP + AMP	5,840
-ATP + GTP	16,040
-ATP + UTP	18,580
-ATP + CTP	8,100

Reaction mixture contained 0.7 μ moles PRPP, 2.0 μ moles nucleotide, 10 μ moles $MgCl_2$, 0.1 μ moles of nicotinamide-7- ^{14}C and 50 μ moles of tris, pH 7.3.

Table 2. FACTORS AFFECTING NMN-PYROPHOSPHORYLASE ACTIVITY IN ASCITES CELL EXTRACTS

	Activity (c.p.m./ml. reaction mixture/h)
Control supernatant	113,600
Boiling H_2O bath 2 min	280
55° C for 1 min	82,246
Crude homogenate	40,620
Acetone powder extract*	1,852
Supernatant from protamine sulphate† precipitation	109,920
Stored overnight 4° C	115,250
Stored overnight -20° C	7,895
Phosphate, pH 7.3, 0.04 M	40,800
Pyrophosphate, pH 7.3, 0.03 M	2,272

* Acetone powder obtained from aliquot of ascites supernatant used in these studies. Acetone powder extracted in 9 volumes of 0.05 M tris, pH 7.3. † 0.2 volumes of a 1 per cent protamine sulphate solution added.

MATURATION IN VITRO OF MOUSE, SHEEP, COW, PIG, RHESUS MONKEY AND HUMAN OVARIAN OOCYTES

By DR. R. G. EDWARDS

Physiological Laboratory, University of Cambridge

THE investigation of early development in many mammalian species is restricted by the difficulty of obtaining sufficient numbers of oocytes and embryos at particular stages of development. Post-dietyate ovarian oocytes are usually obtained after stimulation by luteinizing hormone (LH) either during the female cycle or after the injection of exogenous hormone, and ovulated eggs can be obtained in relatively large numbers after super-ovulation treatments. While these methods are highly satisfactory for many types of investigation, they are obviously unsuitable in the examination of species such as primates or man where the effect of exogenous hormones is undesirable or inadequately understood and in experiments demanding very large numbers of oocytes. An alternative technique has been reported in rabbits¹ and rodents², namely, to liberate the oocyte from its follicle into a culture medium whereon the prolonged dietyate stage is abruptly terminated, the germinal vesicle breaks down, and meiosis is resumed at diplotene/diakinesis within 2-3 h. But, with dog, primate and human oocytes, the germinal vesicles persisted after 20 h of culture³. The results of the present work indicate, however, that in many if not all mammalian species the culture of liberated oocytes for periods longer than 20 h will induce a resumption of meiosis at a rate exactly comparable to that occurring *in vitro* after an injection of LH into the mother. With this technique it should eventually be possible to study pre-ovulatory development, and perhaps fertilization and pre-implantation development in various species including man.

Maturation of Oocytes *in vitro*

Oocytes from mice, pigs, cows, sheep, monkey and man were liberated from their follicles into a culture medium containing heparin to prevent clotting of the follicular fluid, and then cultured in various media: Waymouth's Medium MB 752/1 (ref. 3), Medium 199 (ref. 4), or Hanks's saline supplemented with human and/or calf serum, antibiotics, various other additives, all media being buffered with bicarbonate (pH 7.2) against 5 per cent carbon dioxide in air. Samples of the oocytes were examined as whole mounts stained with aceto-orcein at various intervals after liberation in order to record the presence of a germinal vesicle or the stage of meiosis. The culture techniques were designed to encourage the migration of the corona and cumulus cells from the oocytes on to the 'Falcon' plastic used for culture, for these cells make microscopy difficult and are difficult to remove enzymatically. In almost all species examined a low proportion of the oocytes possessed a polar body and were at metaphase of the second meiotic division (metaphase-II) or were pycnotic when recovered; these oocytes were usually devoid of granulosa cells and were presumably atretic⁴.

Mouse. With Difco Medium 199 supplemented with 15 per cent serum, 80 per cent of the oocytes resumed meiosis (Table 1), which is in good agreement with earlier estimates⁵. The hormonal state of the mother had no detectable effect on the proportion of oocytes resuming their maturation. A high pH in the medium, prolonged exposure to heparin, or high concentration of antibiotics (1,000 i.u./ml.) reduced the proportions maturing, often to nil. Almost all maturing oocytes reached early anaphase of the first meiotic division (anaphase-I), their rate of maturation to this stage being similar to the rate found

in vivo after injecting LH into the mother⁶. Some oocytes became blocked in their maturation at early anaphase-I (Fig. 1), while the remainder progressed normally to metaphase-II and extrusion of the first polar body (Table 1). The block at early anaphase-I will be discussed below.

Table 1. MATURATION OF MOUSE OOCYTES IN DIFCO MEDIUM 199 WITH 15 PER CENT BOVINE SERUM AND 100 I.U. PENICILLIN/ml.

Duration of culture (h)	Germinal vesicle (dietyate)	Developmental stage of oocyte			
		Meta-phase-I	Early ana-phase-I	Late ana-phase-I or telophase-I	Metaphase-II and first polar body
0	76	1	—	—	3*
8.5	8	7	11	—	1
10	9	5	15	6	1
12.5-13	8	—	3	—	27
15	16	—	16	—	31
18	4	—	3	—	9

* These oocytes were presumably atretic.

After priming mice with follicle-stimulating hormone preparations followed by LH-preparations, oocytes are in anaphase between 8 and 11 h after the injection of LH, polar body extrusion begins at 10 h and is virtually complete in all oocytes by 12 h after the LH injection (ref. 6).

Cow. Ovaries were obtained from two cows in the luteal and one in the follicular stage of the cycle. Nine of the oocytes were examined immediately, and each possessed a germinal vesicle. The remaining 14 oocytes were cultured and all had a polar body and were in metaphase-II when examined 31 h later. Ovulation occurs 24-36 h after injecting LH into cows⁷.

Sheep. Ovaries were obtained from two sheep in their follicular phase. Ten oocytes examined immediately possessed a germinal vesicle. Six oocytes examined after 22.5 h in culture were in metaphase-I, and out of seven examined after 46 h four had a polar body and were in metaphase-II. Polar body extrusion *in vivo* requires less than 40 h after an injection of LH into the mother⁸.

Pig. I am indebted to Dr. C. Polge for permission to quote these results of our joint work. In this species, a delay of 20-24 h occurs between the injection of LH into the mother and the breakdown of the germinal vesicle⁹. A similar delay *in vitro* after liberation of the oocytes would help to explain our earlier results where germinal vesicles persisted for 20 h in cultured human and primate oocytes. Pigs were killed on day 12 of their cycle, that is, when corpora lutea were still active and the numbers of Graafian follicles maximal, or on day 18, when the corpora lutea

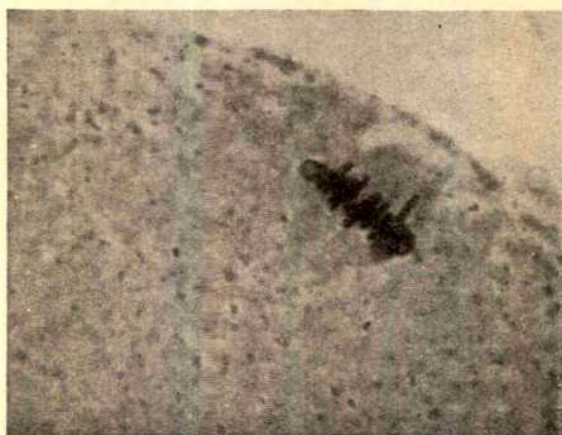


Fig. 1. Mouse oocyte blocked in its maturation at early anaphase-I. The spindle is formed and the chromosomes are extended along it. Whole mount stained in aceto-orcein. ($\times 1,500$)

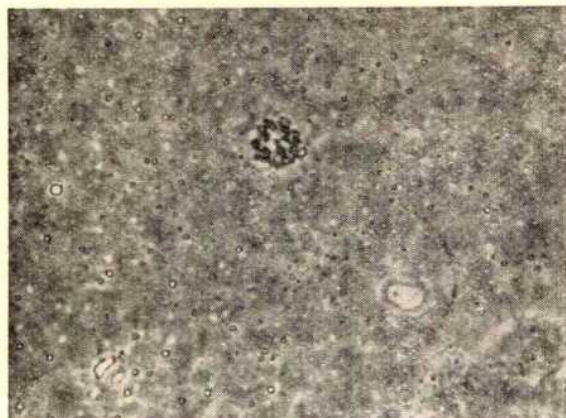


Fig. 2. Metaphase-II and first polar body (slightly out of focus in lower part of figure) in a cultured pig oocyte. Whole mount stained in aceto-orcein. ($\times 675$)



Fig. 3. Group of metaphase-II chromosomes in a human egg. Slight pressure applied after staining has brought many of the chromosomes into focus but has destroyed the outlines of the spindle. Whole mount stained in aceto-orcein. ($\times 600$)

had regressed and the wave of atresia had passed. In culture there was a delay of 20–24 h before breakdown of the germinal vesicle, and meiosis was then resumed (Table 2). Most oocytes had polar bodies, and were in metaphase-II after 43 h in culture (Fig. 2), although some were blocked at early anaphase-I. The rate of maturation was similar in oocytes from pigs killed on the 12th or 18th day of the cycle, and was closely comparable with that occurring *in vivo* during the natural cycle or after injecting LH into the mother⁹.

Rhesus monkey. Ovaries from 4 monkeys were available. Polar body extrusion required approximately 30 (Table 2). Several oocytes displayed blocking at anaphase I, especially if grown in Burroughs Wellcome Medium 199. Ovulation occurs 24–48 h after the injection of LH in female monkeys¹⁰.

Man. Only small pieces of ovary from patients undergoing surgery for various disorders were available, and the number of oocytes from any one piece were seldom sufficient to provide samples for examination after different periods of culture. All oocytes from one Steir Levant patient failed to mature *in vitro* and have been excluded from Table 2. This might have been an example of the endocrinological state of the mother influencing the maturation of oocytes *in vitro*. Several oocytes examined after 24 h had prometaphase chromosomes. Polar body extrusion evidently required a maximum of 40–48 h (Table 2, Fig. 3). Ovulation occurs 24–48 h after the injection of LH into women¹¹.

Block in Development at Early Anaphase-I

The block at anaphase-I might have physiological significance, for a natural arrest of development occurs in most mammalian species at late metaphase-II just before fertilization. With Difco medium 199 supplied ready for use, between 10 and 45 per cent of mouse oocytes were blocked in replicate cultures (Table 1), whereas with Burroughs Wellcome 10 times concentrated 199 approximately 80 per cent were blocked, the great majority at early anaphase-I and a few at telophase (Table 3). In many blocked oocytes a distinct bulge from the vitellus could be seen next to the spindle after fixation. Maturation was blocked rather than delayed, for prolonging the culture period did not alter the incidence of blocked oocytes (Tables 1 and 3). Examination of the listed contents of the two media 199 revealed no differences in composition to explain why one should cause more blocking than the other, the major difference being the inclusion of Earle's saline in Burroughs Wellcome 199 as opposed to Hanks's saline in Difco 199.

Nevertheless, blocking was evidently due to deficiencies in the culture medium as shown by the following experiments. First, a sample of mouse oocytes was divided into three groups and cultured in Difco 199, Burroughs Wellcome 199, or a mixture of equal parts of the two. The incidence of blocking was profoundly influenced by

Table 3. MATURATION OF MOUSE OOCYTES IN BURROUGHS WELLCOME 199 WITH 15 PER CENT BOVINE SERUM AND 100 I.U. PENICILLIN/ml.

Duration of culture (h)	Germinal vesicle (dictyate)	Stage of development of the oocytes			
		Meta-phase-I	Early anaphase-I	Late anaphase-I or telophase-I	Metaphase-II and first polar body
11–5–12	4	0	6	1	0
13–14	8	3	16	2	4
16–17	15	2	16	2	4
18–19	34	8	58	6	11
25	9	—	5	—	6

Table 2. MATURATION OF PIG, RHESUS MONKEY AND HUMAN OVARIAN OOCYTES *in vitro*

Species	No. of animals	Duration of culture (h)	Stage of development of the oocytes				
			Germinal vesicle (dictyate)	Prophase-I	Metaphase-I	Early anaphase-I	Late anaphase-I or telophase-I
Pig	8	0	20	—	—	—	—
		9–9.5	14	—	—	—	—
		17.5–20	14	3	5	—	—
		24–25	6	11	—	—	—
		29–30	10	—	5	2	—
Rhesus†	4	43–46	17	—	—	2	—
		0	19	—	—	—	3
		23	10	5	5	—	—
		29	8	—	1	2	1
Man	†	41	8	—	1	4	—
		0	11	—	—	—	—
		24	7	7	—	—	—
		40	2	—	1	—	—
		48	6	—	—	—	—
		63	6	—	2?	—	—

* Several degenerating.

† Pieces of ovary from several donors.

‡ Oocytes from two monkeys grown in Burroughs Wellcome 199 were blocked at early anaphase-I at 41 h.

Table 4. INFLUENCE OF THE MEDIUM ON THE MATURATION OF MOUSE OOCYTES AFTER 16 h IN CULTURE

Medium*	Any extra treatment	Stage of development of the oocytes	
		Meta-phase-I or early anaphase-I	Meta-phase-II first polar body
100 199+15 per cent bovine serum	—	3	32
100 199+15 per cent bovine serum	—	6	39
100 199+15 per cent bovine serum	—	19	7
100 199+15 per cent bovine serum	Mice super-ovulated, oocytes recovered 2 h after HCG†	7	12
100 199+15 per cent bovine serum	Pig follicular fluid (33 per cent v/v)	3	5
100 199+15 per cent bovine serum	—	14	5

* 100 i.u. penicillin added to all media.

† HCG, human chorionic gonadotrophin.

the culture medium (Table 4). Secondly, mice were super-ovulated with gonadotrophins⁶, and 2 h after the injection of HCG their oocytes were liberated. Polar body extrusion from these oocytes would have occurred *in vivo* within 9 h. After 15 h in Difco 199 and 15 per cent calf serum, 7 of the 19 oocytes were blocked. Thirdly, even the use of neat or diluted pig follicular fluid failed to alleviate the block in mouse oocytes (Table 4).

Various attempts, so far unsuccessful, have been made to alleviate the block at anaphase-I by modifying the culture medium. Blocking could not be attributed to epiparin, the source of serum or distilled water, the pH of the medium (although higher pHs reduced the proportion slightly), or to the antibiotics in the medium. Nor was the incidence of blocked oocytes influenced by the provision of feeder cells (*L*-strain or granulosa monolayers), addition of extra ATP, addition of extra oxidized and/or reduced glutathione in case extra disulphide or sulphhydryl groups were required by the oocytes for anaphase separation¹², addition of folic acid and ascorbic acids to facilitate the shedding of RNA attached to chromosomes at metaphase¹³, or changing the gas phase to 5 per cent carbon dioxide in oxygen. Fertilized 1-celled mouse eggs are difficult to grow *in vitro* and the block at early anaphase might be due to similar deficiencies in the culture medium.

Experimental Studies with Oocytes maturing *in vitro*

Oocytes maturing *in vitro* are evidently not atretic. This is shown by: (1) the similarity in all species examined between the duration of maturation *in vitro* and *in vivo*; (2) the profound influence of the culture medium on the incidence of blocking an early anaphase-I; and (3) observations that matured rabbit¹ and pig¹⁴ oocytes can be fertilized *in vivo* and that the fertilization of matured human oocytes can proceed *in vitro* at least as far as penetration of spermatozoa into the perivitelline space¹⁵. The technique thus promises to provide excellent material for investigating the dynamics of early mammalian development. For example, the role of the centrioles in

meiosis and fertilization could be investigated using the techniques applied to the sea-urchin egg¹⁶; the spindle of the second maturation division is formed within 30 min of the previous telophase which implies a rapid re-organization of the centrioles. Distinct asters with astral fibres attached to the nearest point on the egg membrane could be seen in several mouse oocytes at the first and second meiotic division, and this eccentric attachment of the fibres to the membrane could account for spindle rotation at telophase and the smallness of the polar bodies. Chromosome mechanics at meiosis could also be examined, especially with reference to meiotic anomalies leading to trisomy in oocytes from older women².

The technique also offers the opportunity of investigating early development in species difficult to study otherwise, for example, primates, and of obtaining many oocytes and increased numbers of progeny from valuable livestock by egg transfer. Large numbers of oocytes can be handled in culture: we have handled up to 700 pig oocytes on several collections without undue difficulty¹⁴. With these numbers it should be possible to apply biochemical analyses to mammalian eggs similar to those applied to invertebrate and amphibian eggs. Ribosomal RNA is synthesized during the pre-ovulatory and pre-neurula stages in amphibians, sRNA and mRNA synthesis increasing considerably during late cleavage¹⁷. A similar situation might hold in mammals, where increasing amounts of RNA are synthesized after the second cleavage¹⁸. Several phenomena occurring in the mammalian morula might be related to the initiation of mRNA synthesis at this stage, for example, the initiation of heterochromatin in the sex chromosomes and sex chromatin in the nucleus¹⁹.

I thank Miss C. Jackson for her assistance.

This work was supported by the Ford Foundation.

¹ Pincus, G., and Enzmann, E. W., *J. Exp. Med.*, **62**, 665 (1935); *J. Morphol.*, **61**, 351 (1935). Chang, M. C., *J. Exp. Zool.*, **123**, 379 (1955).

² Edwards, R. G., *Nature*, **196**, 446 (1962).

³ Waymouth, C., *J. Nat. Cancer Inst.*, **22**, 1003 (1959).

⁴ Morton, H. J., Morgan, J. F., and Parker, R. C., *Proc. Soc. Exp. Biol. and Med.*, **73**, 1 (1950).

⁵ Ingram, D. L., Chapter 4 in *The Ovary*, edit. by Zuckerman, S., Mandl, A. M., and Beckstein, P. (Academic Press, 1962).

⁶ Edwards, R. G., and Gates, A. H., *J. Endocrinol.*, **13**, 292 (1959).

⁷ Dzuik, P. J., Donker, J. D., Nichols, J. R., and Petersen, W. E., *Univ. Minnesota Agric. Exp. Sta. Tech. Bull.*, **222**, (1958). Hafez, E. S. E., Sugie, T., and Gordon, I., *J. Reprod. Fertil.*, **5**, 359 (1963).

⁸ Berry, R. O., and Savery, H. P., in *Third Symp. Reproduction and Fertility*, edit. by Gassner, F. X., 75 (Pergamon Press, 1958).

⁹ Spalding, J. F., Berry, R. O., and Moffit, J. C., *J. Anim. Sci.*, **14**, 609 (1955). Polge, C. (unpublished results).

¹⁰ Simpson, M. E., and van Wagenen, G., *Fertil. and Steril.*, **13**, 140 (1962).

¹¹ Gemzell, C. A., *Fertil. and Steril.*, **13**, 153 (1962).

¹² Mazia, D., Chapter 2 in *The Cell*, edit. by Brachet, J., and Mirsky, A. E. (Academic Press, 1961).

¹³ Jacobson, W., in *The Treatment of Cancer*, edit. by Mitchell, J. S., 30 (1965).

¹⁴ Edwards, R. G., and Polge, C. (unpublished results).

¹⁵ Edwards, R. G. (unpublished results).

¹⁶ Mazia, D., Harris, P. J., and Bibring, T., *J. Biophys. Biochem. Cytol.*, **7**, 1 (1960).

¹⁷ Brown, D. D., *J. Exp. Zool.*, **157**, 101 (1964). Gurdon, J. B., and Brown, D. D., *J. Mol. Biol.*, **12**, 27 (1965).

¹⁸ Austin, C. R., *The Mammalian Egg* (Blackwell Scientific Publications 1961). Mintz, B., *J. Exp. Zool.*, **157**, 85 (1964).

¹⁹ Melander, Y., *Hereditas*, **48**, 345 (1962).

PROCESSES OF EXCITATION AND INHIBITION IN SINGLE MECHANORECEPTORS (PACINIAN CORPUSCLES)

By DR. O. B. ILYINSKY

I. P. Pavlov Institute of Physiology, U.S.S.R. Academy of Sciences, Leningrad

INVESTIGATING various properties of Pacinian corpuscles we found that in a number of cases their electrical response contains positive deviations of potential, the origin of which is impossible to understand within the limits of present concepts of their activity¹⁻⁴. Some peculiarities of this phenomenon are discussed in the

present article. A detailed description of the experimental technique has been presented elsewhere⁵⁻⁸.

Fig. 1 demonstrates gradual reaction of the receptor elicited by mechanical shocks of triangular form. It is seen that with increase in duration of the stimulus the response declined in amplitude, decreased in rate of rise

and increased in duration. In some records²⁻⁵ a little hyperpolarization of gradual character appears. It may be noticed also that the appearance of depolarization is associated only with the effect of the posterior front of the stimulus. Its anterior front either produces no changes or gives rise to positive deviations of the potential. The diminution in the amplitude and in the rate of rise of the gradual response of the receptor with the decrease of the velocity of displacement has already been described⁹.

It is well known¹⁰ that the capsule of the receptor, while strongly attenuating the static component of the stimulus, conducts its dynamic component well. The prolongation of the triangular stimulus is actually equal to the increase in duration of the dynamic component of such stimulation. Therefore the observed increase in the duration of the gradual response means that the receiving elements of the mechano-sensitive membrane of the Pacinian corpuscles adapt themselves to the long-lasting stimulus much more slowly than was previously thought (see also refs. 11 and 12). The fact that the reactions of the receptor to the ascending and descending parts of the mechanical shock differ is more difficult to understand, for it is well known^{5,8,9} that responses of the intact corpuscles to the on- and off-stimuli are more or less identical. Furthermore, it is now clear⁸ that mechanical shock produces oscillations in the capsule of the receptor which, provided the stimulation is sufficiently long, induce off-reaction, that is, the mechanism of generation of off-responses is actually the same as that of on-responses. If the amplitude of mechanical stimulus is sufficiently large these oscillations evoke multiple responses of the receptor (see also Fig. 2, 11).

The systematic study of a great number of receptors showed that, in practice, depolarization of the nerve ending is always accompanied by hyperpolarization phenomena and not infrequently the reactions to anterior and posterior fronts of the stimulation may be essentially different especially when long-lasting triangular (sinusoidal) mechani-

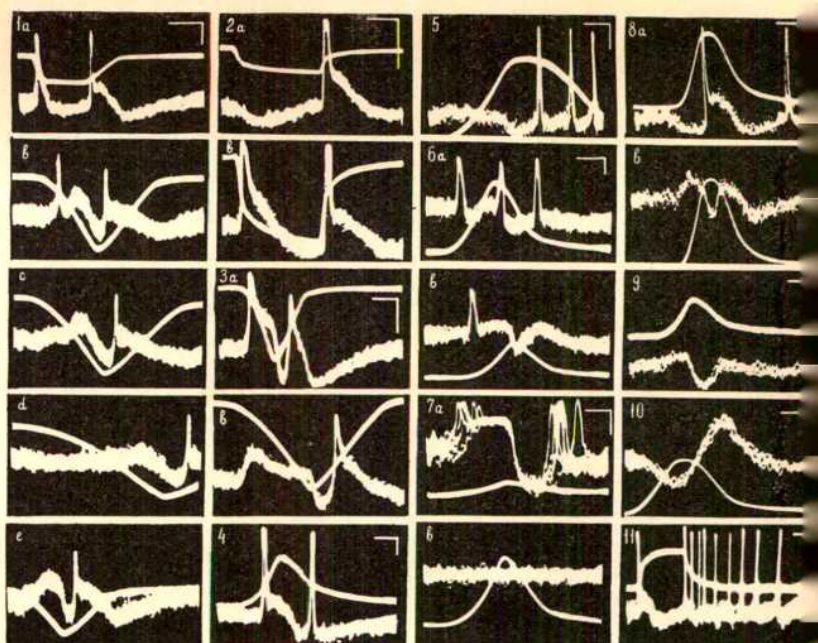


Fig. 2. Response of the receptor under different conditions of stimulation (11 different Pacinian corpuscles). In 6b, amplification in the system of recording the mechanical shock (lower beam) is diminished in relation to 6a and the direction of the shock is changed. In 7b, the receptor is killed and the absence of mechanical artefact in the system of recording under stimulation greater than in 7a is demonstrated. In 8b the direction of shock is changed as compared to 8a. Peak potentials in 8b, 9, 10 are blocked by procaine. The appearance of depolarization responses in almost all records in switching off the stimulation is evidently connected with the origin of oscillation process in the capsule of the receptor (see record 11); time mark: 1, 3, 5-0 msec; 2, 5, 8, 10, 11, 4-0 msec; 4, 3-0 msec; 6, 7, 9, 2-0 msec; calibration: 1, 3, 4, 5, 6, 7, 8, 11, 20-0 μ V; 2, 30-0 μ V; 9, 10, 15-0 μ V.

cal shocks are applied. Records presented in Fig. 2 illustrate the phenomena in question. It can be seen that the amplitude, rate of rise, duration and shape of the positive response of the receptor vary both from experiment to experiment and in the same experiment. The appearance of hyperpolarization is in no way connected with the moment of the spike origination and is observed even in its absence. However, positive deviations of the potential are most clearly observed on the background of the preceding depolarization. The character of the reaction is to a great extent determined by the amplitude and duration of the mechanical shock as well as by the site of its application, etc.

It is known¹³ that the nerve ending in the Pacinian corpuscle has the shape of an elliptic cylinder. Hubbard¹⁴ suggested that the ratio between the great (*a*) and small (*b*) transverse axes of the nerve ending is increased by stimulation. Consequently the surface of the receptor membrane increases, which results in excitation.

However, the increase in the ratio *a* : *b* occurs only if the mechanical stimulus is applied along the axis 'b'. The stimulus directed along the axis 'a' decreases the ratio *a* : *b* and consequently decreases the surface of the receptor membrane. Suppose that depolarization of the nerve ending membrane is caused by an increase (distension) and hyperpolarization by a decrease (constriction) in the surface of the latter. Then stimulation applied along the axis 'b' would produce depolarization, while that falling in the direction of the axis 'a' would evoke hyperpolarization. In experimental verification of this assumption possible appearance of oscillation processes in the capsule of the receptor had to be taken into consideration⁶. In this connexion short-lasting shocks with considerable amplitude and rate of rise were not to be used, as the phenomena of hyperpolarization would have been masked by arising depolarizing responses.

In Fig. 3 are shown oscillograms which demonstrate a good agreement between expected and experimental data. It is obvious that with rotation of the corpuscle around its longitudinal axis the anterior front of the long-

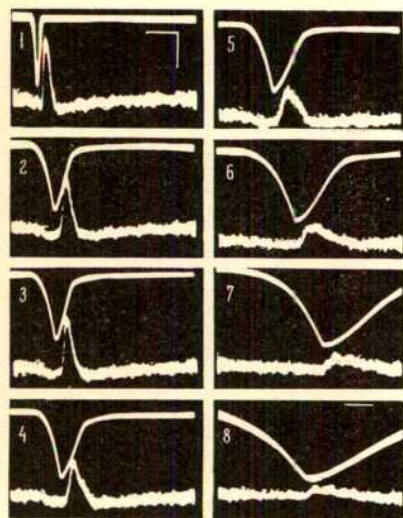


Fig. 3. Changes in the response of mechanoreceptor (lower beam) caused by alterations in duration of mechanical stimulation (upper beam). Peak potentials are blocked by procaine. Calibration 50-0 μ V; time mark (msec): 1-7, 5-0; 8, 10-0.

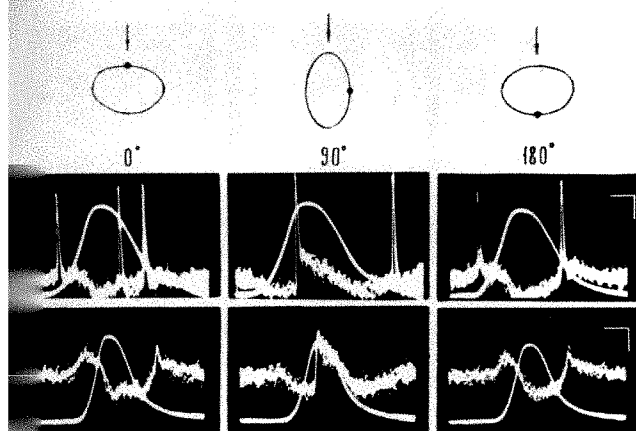


Fig. 3. Functional asymmetry of the receptor. Top: scheme of supposed correlation between the direction of stimulus and position of the nerve ending (its small transverse axis). Bottom: corresponding schemes of responses of Pacinian corpuscles in two different experiments. In 2 spikes blocked by procaine; time mark (msec): 1 and 2, 4.0; calibration (μ V): 1, 10.0; 2, 5.0

sting stimulus which is responsible for depolarizing action produces hyperpolarization response and vice versa. It should be noted that reverse-directed deformation due to elastic properties of the capsule at the moment of cessation of stimulation arises⁸ and therefore the

posterior front of the stimulus acts contrary to the anterior one.

On the basis of the data obtained, the responses of Pacinian corpuscles presented in Figs. 1 and 2 may be readily explained (a detailed consideration of the hyperpolarization phenomenon will be presented elsewhere). Positive deviations of potentials are usually accompanied by some decrease in the receptor excitability (Fig. 2, cf. 2a and 2b); therefore, on the analogy with inhibitory synaptic potentials, they may be referred to as inhibitory receptor potentials (IRP), while the negative changes in the potential may be called excitatory receptor potentials (ERP).

In view of the data obtained the participation of mediators in the processes of excitation of Pacinian corpuscles¹⁴ seems scarcely feasible.

¹ Gray, J. A. B., *Prog. Biophys. Biophys. Chem.*, **9**, 285 (1959).

² Loewenstein, W. R., *Ann. N.Y. Acad. Sci.*, **81**, 367 (1959).

³ Loewenstein, W. R., *Ann. N.Y. Acad. Sci.*, **94**, 510 (1961).

⁴ Alvarez-Buylla, R., and Remolina, J., *Acta Physiol. Latinoamericana*, **9**, 178 (1959).

⁵ Ilyinsky, O. B., *J. Physiol. U.S.S.R.*, **49**, 201 (1963).

⁶ Ilyinsky, O. B., *Bull. Exp. Biol. Med.*, **58**, 8, 123 (1964).

⁷ Ilyinsky, O. B., *Bull. Exp. Biol. Med.* (in the press).

⁸ Ilyinsky, O. B., *J. Physiol. U.S.S.R.*, **51** (in the press).

⁹ Gray, J. A. B., and Sato, M., *J. Physiol.*, **122**, 610 (1953).

¹⁰ Hubbard, S. J., *J. Physiol.*, **111**, 198 (1953).

¹¹ Mendelson, M., and Loewenstein, W. R., *Science*, **144**, 554 (1964).

¹² Ilyinsky, O. B., *Bull. Exp. Biol. Med.* (in the press).

¹³ Pease, D. C., and Quilliam, T. A., *J. Biophys. Biochem. Cytol.*, **3**, 331 (1957).

¹⁴ Koelle, C. B., *Nature*, **190**, 208 (1961).

A NEW FLUORESCENT ANTIBODY METHOD: MIXED ANTIGLOBULIN IMMUNOFLUORESCENCE OR LABELLED ANTIGEN INDIRECT IMMUNOFLUORESCENCE STAINING

By PROF. E. H. BEUTNER*, DR. E. J. HOLBOROW and G. D. JOHNSON

Medical Research Council Rheumatism Research Unit, Canadian Red Cross Memorial Hospital, Taplow, Maidenhead, Berkshire

INVESTIGATIONS by Coombs *et al.* on mixed agglutination¹ and mixed antiglobulin tests² have revealed that an antibody combined with its specific antigen on a cell may have a free antibody combining site capable of reacting with another molecule of its specific antigen. In the mixed agglutination tests these free combining sites are detected with antigens (immunoglobulins for the mixed antiglobulin tests) attached to red cell indicators. We have investigated the use of antigens (immunoglobulins) labelled with fluorescein^{3,4} in a similar system using frozen tissue sections⁵ as primary antigens. This method may be referred to as mixed antiglobulin immunofluorescence (IF) or mixed immunofluorescence (MIF). Unfortunately the latter term may be confused with the 'mixture of colours'⁶ obtained by labelling with different fluorochromes, so that the cumbersome but more accurate term 'labelled antigen indirect IF staining' may be preferable to describe the method. For the sake of clarity the established IIF (indirect immunofluorescent) staining procedure⁶ may now be referred to as the 'labelled antiglobulin indirect IF staining' in contradistinction to the 'labelled antigen indirect IF' or MIF method.

Fig. 1 provides a diagrammatic comparison of the principles involved in labelled antiglobulin and labelled antigen indirect IF staining. Table 1 summarizes the specific techniques employed in the labelled antigen or MIF staining method.

Serial dilutions of human sera containing tissue antibodies were tested on frozen sections of tissue cut in a cryostat⁷. Labelled immunoglobulins were prepared by

conjugating either commercial human serum fraction FII or IgG prepared by DEAE chromatography. For conjugation, 0.08 mg FITC/mg protein was found to yield satisfactory reagents (FITC, fluorescein isothiocyanate, was obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio). Both the antiglobulin (step 3) and the normal rabbit serum used as the diluent (after 1:15 dilution in buffered saline) of the labelled antigen (step 5) were heat-inactivated before dilution because some slight enhancement of MIF staining (sometimes as much as a whole dilution in the staining end-point of the human serum) could be effected thereby.

The precipitating activity of the antiglobulins and of the labelled antigens provided estimates of their respective antibody and antigen activities. A concentration of 1 unit/ml. of antiglobulin was defined as the highest dilution which would give a visible line of precipitation in a standard agar-gel diffusion precipitation test (6 wells 8 mm from a centre well, all 2.5 mm in diameter) using serial 2-fold dilutions of the antiglobulin in the peripheral wells and 1 mg/ml. of IgG or FII in the centre well. Conversely, 1 unit/ml. of labelled immunoglobulin was

Table 1. PROCEDURE FOR MIXED ANTIGLOBULIN OR LABELLED ANTIGEN INDIRECT IF

Step	Reagents	Conditions	Time (min)
1	Human sera	Serial dilutions to end-point	20
2	Wash	PO ₄ buffered saline	60
3	Anti-human immunoglobulin	Heated 56° C 30 min, diluted to 1-4 units	20
4	Wash	PO ₄ buffered saline	60
5	Labelled human immunoglobulin	Diluted to 1-2 units in heated normal rabbit serum (1/15 dilution)	20
6	Wash	PO ₄ buffered saline	120
7	Glycerin mount	1/10 buffer: 9/10 glycerin	

* Permanent address: State University of New York at Buffalo, Schools of Medicine and Dentistry, Buffalo, New York.

defined as the concentration present in the highest dilution yielding a visible line of precipitation with 2 units of an antiglobulin in the centre well. Most labelled preparations of *IgG* or *FII* which we have used have contained about 0.05 mg of protein/ml. in 1 unit. A more detailed report on the relation between the unitages of antiglobulins and labelled immunoglobulins and their respective reactivity in MIF staining will be made at a later date¹⁴.

Phosphate-buffered saline⁸ served as a diluent for all reagents as well as a washing solution. Prolonged washing (60 min for steps 2 and 4 and 120 min for step 6 in Table 1) was used to minimize non-specific staining. While some exploratory studies indicated that washing times of 20, 20 and 120 min (for steps 2, 4 and 6, respectively) might yield equally satisfactory results, the prolonged washing times listed in Table 1 were adhered to throughout most of these investigations to provide an added margin of safety. Fluorescence microscopy was performed as described previously⁷.

Studies of MIF or labelled antigen IF staining revealed that under the conditions used non-specific staining ranged from an undetectable to a low acceptable level. The latter appeared as a dull-green background staining which could readily be distinguished from specific IF staining. In most of these investigations it was difficult to distinguish between some traces of green auto-fluorescence of tissue and traces of non-specific staining.

The appearance of specific MIF staining patterns given by tissue antibodies, such as antinuclear factor* (ANF), could not be distinguished qualitatively (in readings of coded preparations) from those obtained in parallel studies by the established labelled antiglobulin indirect IF staining procedure. At the quantitative level, comparisons were made by titrating human sera (usually in doubling dilutions) to IF staining end-points. An end-point was defined as the highest dilution of the patient's serum which yielded an IF staining reaction clearly distinguishable from that of negative control sera. Preparations were randomized and read as unknowns by two or more observers. Under these conditions, IF staining end-points varied by as much as plus or minus one doubling dilution.

Table 2. TITRATIONS OF ANTINUCLEAR AND ANTINUCLEOLAR ANTIBODY BY THE LABELLED ANTIGEN (MIF)* AND LABELLED ANTIGLOBULIN (IIF) INDIRECT IF* STAINING METHODS

Case	Sera tested Type of antibody	Dil. of sera	IF staining reactions			
			Rabbit anti-human		Goat anti-human	
			MIF	IIF	MIF	IIF
B	None	1:25	-	-	-	-
		1:100	+	+	+	+
		1:200	+	+	+	+
		1:400	+	+	+	+
		1:800	W*	W	W	W
		1:1,600	W	-	-	W
W	Nucleoli	1:25	+	+	-	+
		1:50	W	W	-	W

* IF, immunofluorescence. MIF, mixed (antiglobulin) IF or labelled antigen indirect IF. IIF, indirect IF or labelled antiglobulin IIF.

Rabbit and goat anti-human antisera to human *IgG*. Other antibodies could be demonstrated in these by immunoelectrophoresis but did not appear to be relevant to the MIF or the IIF staining reactions as judged by comparison with monospecific antisera to *IgG*.

ANF, antinuclear factor.

+, moderate to strong positive.

W, weak positive.

Table 2 summarizes some typical titrations performed with human sera containing two types of antibodies. Titrations were performed by the labelled antigen and labelled antiglobulin methods using both goat and rabbit antisera to human immunoglobulins. For both the MIF and IIF staining 4 units of the goat antiserum were used while the rabbit antiserum was used at dilutions containing 2 units and 1/5 units for the MIF and IIF staining respectively. Both antiglobulins contained not only antibodies to human *IgG* (as indicated by their unitage) but also antibodies to other serum proteins as revealed by immunoelectrophoresis. For MIF staining 2 units of labelled human immunoglobulin (*FII*, Koch Light Laboratories, Ltd., Colnbrook, Buckinghamshire) with an estimated molar fluorescein to protein ratio of 8.1/1 was used as antigen for the top layer.

The serum of patient *D*, as shown in Table 2, yielded nuclear staining titre of 1:800 or 1:1,600 by both the MIF and the IIF staining methods under the conditions used. Essentially comparable results were obtained with the goat and rabbit antiglobulins. Similarly, the serum of patient *W*, which contained antibodies to nucleoli

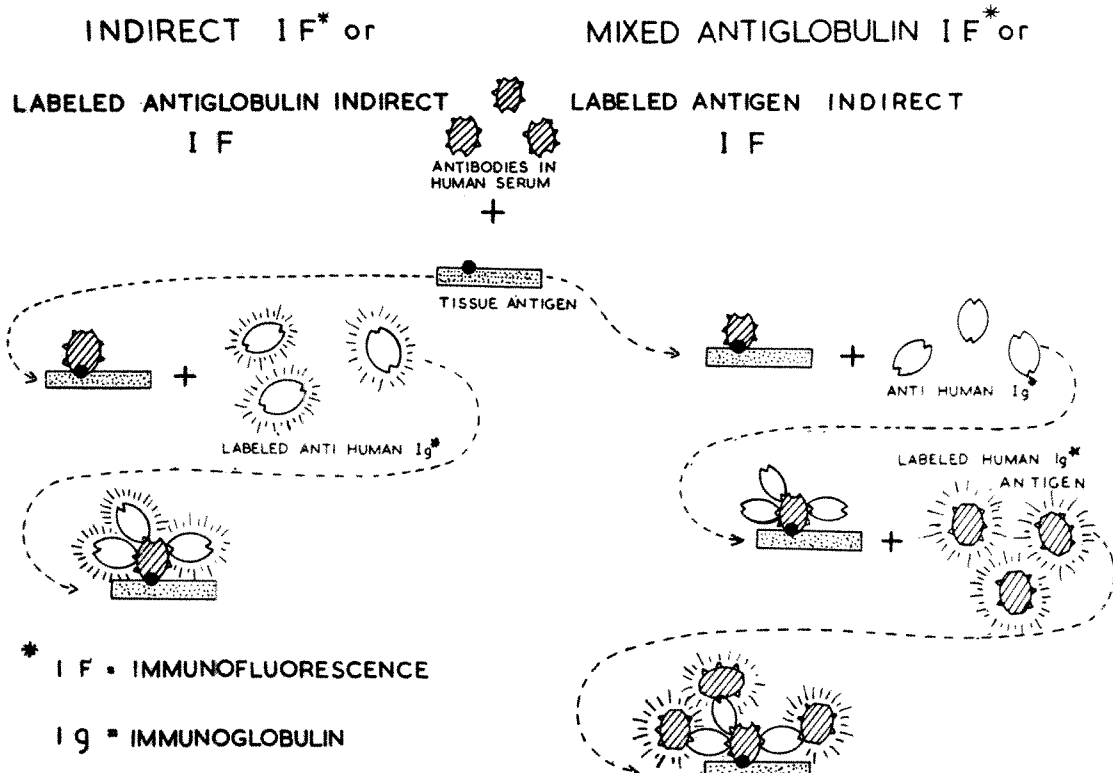


Fig. 1

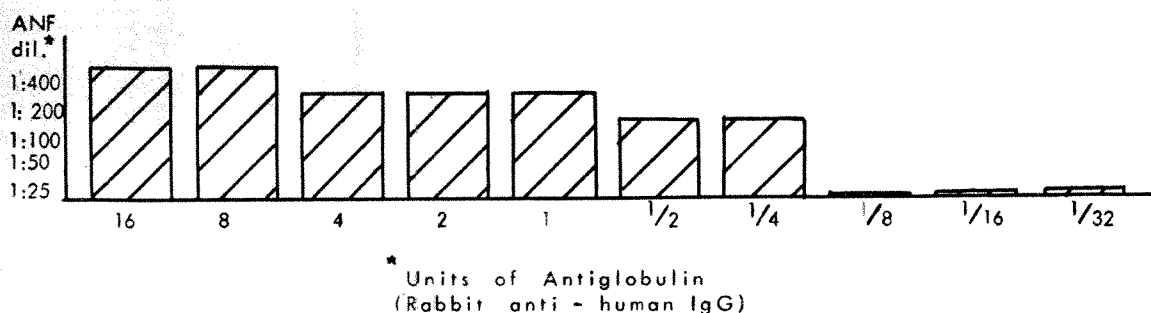


Fig. 2

ielded comparable reactions by the MIF and IIF staining methods with the rabbit antiglobulin. However, the cat antiglobulin only yielded a detectable reaction by IF, not by the MIF staining method. This is the most iscrepant result in this series of experiments; it is doubtful if much significance can be attached to it because the difference is close to the limits of error of the method ± 1 dilution).

Several types of tissue antibodies (probably all autoantibodies) were tested by both the MIF and IIF staining methods. The results of these studies are summarized in Table 3.

Table 3. EXAMPLES OF TITRES OBTAINED WITH SOME HUMAN ANTIBODIES TO TISSUE ANTIGENS BY THE LABELLED ANTIGEN (MIF)* AND LABELLED ANTIGLOBULIN (IIF)* INDIRECT IF* STAINING METHODS

Systems tested	Literature ref.	Tissues	Examples of titres	
Type of antibody			By MIF	By IIF
Antinuclear factor	8, 9, 17			
Smooth		Calf thyroid	1:200	1:200
Speckled		Calf thyroid	1:200	1:400
Nucleolar		Calf thyroid	1:50	1:50
Thyroid microsomal	10	Human thyroid	1:100	1:100
Muscle striations	11, 12	Human heart	1:100	1:200
Skin antibodies:				
Intercellular	13	Guinea-pig lip	1:30	1:90
Basement zone	13	Guinea-pig lip	1:270†	1:270†
Cytoplasmic	14	Guinea-pig lip	1:30	1:30

* Abbreviations as for Table 2.

† No end-points were obtained in these particular titrations.

Antinuclear factors^{8,9,17} of various sorts could be demonstrated by both MIF and IIF staining as shown in Table 3. Identical staining patterns were seen in preparations stained by the two methods. IF staining of thyroid cells¹⁰ yielded strongly positive reactions by the labelled antigen method. Preliminary studies of muscle autoantibodies found in sera of patients with myasthenia gravis^{11,12} yielded comparable reactions by the two IF staining methods. Three types of skin antibodies^{13,14} could be demonstrated by MIF staining as well as IIF staining.

The prime objective of these investigations on labelled antigen indirect IF staining was to seek a better understanding of the quantitative relationships that obtain in the conventional labelled antiglobulin method. The approach used in these investigations was that of chess-board titrations. Fig. 2 illustrates the results obtained in one experiment of this type. An antiglobulin monospecific for IgG was tested at varying dilution against doubling dilutions of an ANF. The precipitation end-point of this antiglobulin (1 unit per ml.) was 1:32. The labelled IgG antigen used for the top layer has an estimated $F:P$ ratio of 3.6/1 and was used at a concentration of 1 unit/ml. (0.05 mg protein/ml.).

The results summarized in Fig. 2 reveal that the ANF titre maintains a plateau (in the range of 1:200–1:400) down to an antiglobulin dilution of 1:32 (1 unit/ml.). At higher dilutions of the antiglobulin the ANF titre declines, falling off to a negative reaction at $\frac{1}{8}$ of a unit per ml.

These observations provide a basis for making the following assumptions in quantitative studies of indirect IF staining (both IIF and MIF). (1) Titration of a standard human serum yields a constant IF end-point (± 1

dilution) over a range of antiglobulin dilutions, forming a plateau in a chess-board titration as in Fig. 2. The highest antiglobulin dilution in this plateau yielding the maximal titre (± 1 dilution) of IF staining for a given human serum is the antiglobulin plateau end-point. (2) The antiglobulin plateau end-point is roughly proportional to the precipitation end-point of the antiglobulin. (3) Non-specific staining is proportional to the fluorescein concentration in the conjugate dilution used for IF staining. (4) The titre of IF staining obtained with a given human serum is proportional to the $F:P$ ratio of the labelled antiglobulin (in IIF) or labelled antigen (MIF). It must be added that over-conjugation, that is, too high a fluorescein to protein ($F:P$) ratio, so alters the protein that it gives rise to unacceptable non-specific staining¹⁵. (5) In labelled antigen indirect IF staining the maximal titre obtained with a given human serum is maintained over a range of dilutions of the labelled antigen of the top layer, again in a plateau fashion. The highest antigen dilution in this plateau which yields maximal titres is the labelled antigen plateau end-point. This labelled antigen end-point is also roughly proportional to its precipitation end-point.

More recent studies^{12,16} have revealed that essentially these assumptions hold true for either MIF staining or IIF staining.

From the foregoing it appears that the control of the following is required for quantifying indirect immunofluorescence: (1) Characterization of antiglobulins by immunoelectrophoresis (2) Titration of antiglobulins by precipitation tests (for MIF staining the labelled antigen must also be titrated). (3) Estimation of the fluorescein concentrations and fluorescein to protein ratios in conjugates. These parameters are minimal requirements for quantitative indirect immunofluorescence.

This work was supported by the Nuffield Foundation (E. H. B.) and U.S. Public Health Service grant DE-01801-03. We thank Miss Natasha Bell for assistance.

¹ Coombs, R. R. A., Bedford, E., and Rouillard, L. M., *Lancet*, i, 461 (1956).

² Coombs, R. R. A., Mark, H., and Bedford, D., *Brit. J. Haemat.*, 2, 84 (1956).

³ Coons, A. H., and Kaplan, M. H., *J. Exp. Med.*, 91, 1 (1950).

⁴ Riggs, J. L., Seiwald, R. D., Burkhalter, J. H., Downs, C. M., and Metcalf, T. G., *Amer. J. Path.*, 34, 3084 (1958).

⁵ Colberg, J. E., and Dray, S., *Immunology*, 7, 273 (1964).

⁶ Willer, T. H., and Coons, A. H., *Proc. Soc. Exp. Biol. and Med.*, 85, 789 (1954).

⁷ Holborow, John, and Johnson, Gerald D., *Arthr. and Rheum.*, 7, 119 (1964).

⁸ Holborow, E. J., Weir, D. M., and Johnson, G. D., *Brit. Med. J.*, ii, 732 (1957).

⁹ Friou, G. J., Finch, S. C., and Detre, K. D., *Fed. Proc.*, 16, 413 (1957).

¹⁰ Holborow, E. J., Brown, P. C., Reiff, D. M., and Doniach, D., *Brit. J. Exp. Path.*, 40, 583 (1959).

¹¹ Strauss, A. J. L., Seegal, B. C., Hsu, K. C., Burkholder, P. M., Nastuk, W. L., and Osserman, K. E., *Proc. Soc. Exp. Biol. and Med.*, 105, 184 (1960).

¹² Beutner, E. H., Witebsky, E., Ricken, D., and Adler, R. H., *J. Amer. Med. Assoc.*, 182, 46 (1962).

¹³ Beutner, E. H., Lever, W. F., Witebsky, E., Jordan, R., and Chertock, B., *J. Amer. Med. Assoc.*, 192, 682 (1965).

¹⁴ Beutner, E. H., Holborow, E. J., and Johnson, G. D. (in preparation).

¹⁵ Beutner, E. H., *Bact. Rev.*, 25, 49 (1961).

¹⁶ Holborow, E. J., Johnson, G. D., and Beutner, E. H. (in preparation).

¹⁷ Beck, J. S., *Lancet*, i, 1203 (1961).

EFFECT OF A LIPID FRACTION FROM RAT THYMUS ON DELAYED HYPERSENSITIVITY REACTIONS OF NEONATALLY THYMECTOMIZED RATS

By PROF. BRANISLAV D. JANKOVIĆ, DR. KATARINA ISAKOVIĆ and JOZEF HORVAT

Microbiological Institute, Faculty of Pharmacy, and Immunology Unit, Institute of Biology, University of Belgrade, Yugoslavia

IT is now clear that the thymus has an important role in the development of immunological capacity in mammals¹. Neonatal thymectomy is followed by a striking depletion of lymphocyte population in various lymphoid tissues². The immunological role of the thymus has been considered by many investigators to be in the main indirect, this organ providing the body with cells which colonize different lymphoid tissues where they undergo further immunological maturation. Such a cellular function of the thymus does not, *a priori*, exclude the possibility that the thymus may have endocrine activity³ and that the development of the immune system may be influenced by active principles elaborated by the thymus and discharged into the circulation⁴. Interest in this possibility has been revived recently by Szent-Györgyi *et al.*⁵, who isolated two substances from thymus, among other organs, capable of affecting tumour growth. The observations presented here form part of a large experiment in which various fractions of rat thymus have been used in attempts to restore immunological potential to rats thymectomized at birth.

Thymuses from 18–28-day-old outbred albino rats were used as source for the preparation of lipid fraction: 12 g tissue was homogenized with 150 ml. of saline, followed by lyophilization of homogenate. Dried tissue was extracted with chloroform-methanol (2:1 by volume) at boiling-point for 12 h. The lipid fraction from whole rat central nervous tissue was prepared in a similar way. Lipid fractions were emulsified separately in saline before injection. Inbred Lewis strain rats (Microbiological Associates, Washington) were used in the experiment. Thymectomy was performed at birth, and operated animals were divided into three groups. The first group was treated with the thymus lipid fraction; the second group with the central nervous tissue lipid, and the third group was not treated. Two control groups of normal rats were also included. One control group (sham-operated) was treated with the central nervous tissue lipid, whereas another group (unoperated rats) was untreated. Each treated thymectomized or sham-operated rat received a total of 11 injections, beginning at 21 days and ending at 58 days of age. The individual inoculations contained 2.6 mg of thymus or nervous tissue lipid extract. Thymectomized animals showing the wasting syndrome were excluded from the experiment.

At 8 weeks all rats were immunized with crystallized bovine serum albumin dissolved in saline and mixed with an equal volume of complete Freund's adjuvant. 0.1 ml. of a stable water-in-oil emulsion (0.8 mg of bovine serum albumin) was injected into the left hind foot-pad. The first skin test was done 10 days, and the second 18 days, after immunization. 40 µg of bovine serum albumin in 0.1 ml. saline were injected into the skin of either left or

right depilated flank. Differential leucocyte counts were performed on tail blood taken 2 days after the first skin test. At 11 weeks all animals were killed and the mediastinum was inspected grossly and histologically for the presence of thymic remnants. Axillary lymph nodes and spleen were taken for histological appraisal.

Immunological responses of thymectomized and control rats, untreated or treated with the cell-free fraction of allogeneic thymus, are given in Table 1. Delayed reaction to bovine serum albumin were sharply reduced in untreated thymectomized rats. In contrast are the delayed hypersensitive responses of thymectomized animals treated with thymic lipid. At the time of the second skin test delayed reactivity in those rats was comparable to that of non-thymectomized controls. The responses of thymectomized rats treated with central nervous tissue lipid (CNS lipid) fell between those of untreated thymectomized rats and those of thymectomized rats repeatedly injected with thymic lipid.

Untreated thymectomized rats showed a moderate retardation in weight gain, a low incidence of wasting disease and a marked reduction in circulating lymphocytes (Table 2). The body-weight of thymectomized rats injected with thymic lipid, on the other hand, was comparable to that of sham-operated and intact controls. No wasting disease was seen in this group, and there was a partial restoration of total number of lymphocytes in the peripheral blood. Histological examination of spleen and lymph nodes from thymectomized rats, untreated or treated with central nervous tissue lipid, revealed a profound depletion of small lymphocytes, whereas the plasma cell population was found to be normal². The absence of small lymphocytes was less striking in the spleen of thymectomized animals treated with thymic lipid, and in the lymph nodes masses of these cells were larger than in other thymus-free rats which were not injected with thymic lipid. There were many foci of cellular proliferation in the cortical areas of those lymph nodes. These histological findings fit fairly well with the immunological responses of thymectomized animals treated with thymus lipid fraction.

None of several thymic materials used by other authors⁶ has been effective in preventing wasting disease or in

Table 2. BODY-WEIGHT AND TOTAL LYMPHOCYTE COUNT IN TREATED AND UNTREATED THYMECTOMIZED AND CONTROL RATS

Group	No. of rats	Treatment	Average body weight at 11 weeks (g)	Average total lymphocyte count at 11 weeks
Thymectomized	10	—	170.0	6,425
	9	CNS lipid	164.1	6,591
	12	Thymus lipid	189.1	10,183
Non-thymectomized	10	—	181.5	11,301
Intact	10	—	188.2	12,892
Sham-operated	10	CNS lipid	188.2	12,892

Table 1. DELAYED HYPERSENSITIVITY TO BOVINE SERUM ALBUMIN IN TREATED AND UNTREATED THYMECTOMIZED AND CONTROL RATS

Group	No. of rats	Treatment	Skin test at 10 days No. of rats with reaction:				Skin test at 18 days No. of rats with reaction:			
			0	+	++	+++	0	+	++	+++
Thymectomized	10	—	9	1	0	0	9	1	0	0
	9	CNS lipid	5	3	1	0	3	2	2	2
	12	Thymus lipid	3	7	2	0	1	4	4	6
Non-thymectomized	10	—	0	2	3	5	0	2	2	6
Intact	10	—	0	1	2	7	0	3	3	4
Sham-operated	10	CNS lipid	0	1	2	7	0	3	3	4

toring immunological competence to thymectomized ce. It has been claimed, however, that injection of thymus extracts into animals produces a temporary rease in lymphocyte number⁷. The results presented re suggest that the thymus is capable of elaborating ive principles which influence immunological reactivity thymectomized rats in terms of restoring their immune tential. One could assume that the humoral activity the thymus, as described in this experiment, might be iliar to the activity exerted by the tumour growth-omoting substance isolated by Szent-Györgyi *et al.*⁵. should be mentioned, however, that Dalmasso *et al.*⁶ led to restore immunological potential to neonatally ymectomized animals by repeated injections of 'pro-ne'. The reported humoral activity of the thymus ems to be related to thymic lipid, lipoprotein or lipoluble components. Several injections of highly polymered deoxyribonucleic acid isolated from young rat ymus increased, to some extent, the delayed hypernsitivity response in rats thymectomized at birth (unpublished results). Thus the immunological reactivity tained was quantitatively similar to that of rats treated ith central nervous tissue lipid, but it never reached the vel of delayed reactions observed in thymectomized rats jected with thymus lipid fraction.

Thymus lipid(s) exerted restorative effects on lymphocytes in the circulation and lymphoid tissues. This ovides the histological substrate to the corrected munological inadequacy, but does not itself explain e re-population of lymphoid cells, that is, whether umoral thymic factor(s) act solely by stimulating

mesenchymal cells of lymphoid tissues, or through the mechanism of immune recognition. Although the present results do not offer evidence as to the thymus cells to which humoral function could be attributed, it might be assumed that this activity is related to the central cellular architecture of this organ. The implied humoral role of the thymus, however, is not inconsistent with the possibility that the thymus acts as the source of cells involved in delayed hypersensitivity responses.

This work was supported by grants from the Yugoslav Foundation for Scientific Research (Belgrade), and in part by the National Institutes of Health (Bethesda). Part of the work was carried out while one of us (B. D. J.) was a member of the Department of Bacteriology and Immunology, Harvard Medical School, and Allergy Unit, Massachusetts General Hospital, Boston.

¹ Miller, J. F. A. P., *Lancet*, ii, 748 (1961). Arnason, B. G., Janković, B. D., and Waksman, B. H., *Nature*, **194**, 99 (1962). Martinez, C., Kersey, J., Papermaster, B. W., and Good, R. A., *Proc. Soc. Exp. Biol. and Med.*, **109**, 193 (1962).

² Waksman, B. H., Arnason, B. G., and Janković, B. D., *J. Exp. Med.*, **116**, 187 (1962).

³ Eecke, A. ver., *Bull. Acad. Roy. Méd. Belg.*, **13**, 67 (1899). Hammar, J. A., *Anat. Anz.*, **27**, 23 (1905). Bell, E. T., *Amer. J. Anat.*, **5**, 29 (1906). Hoskins, E. R., *Endocrinology*, **2**, 241 (1918). Cosma, J., *Acta Endocrinol.*, **30**, 621 (1959).

⁴ Metcalf, D., *Brit. J. Cancer*, **10**, 442 (1956). Osoba, D., and Miller, J. F. A. P., *Nature*, **199**, 553 (1963). Levey, R. H., Trainin, N., and Law, L. W., *J. Nat. Cancer Inst.*, **31**, 199 (1963).

⁵ Szent-Györgyi, A., Hegyeli, A., and McLaughlin, J. A., *Proc. U.S. Nat. Acad. Sci.*, **48**, 1439 (1962). Hegyeli, A., McLaughlin, J. A., and Szent-Györgyi, A., *ibid.*, **48**, 230 (1963).

⁶ Miller, J. F. A. P., *Ann. N.Y. Acad. Sci.*, **99**, 340 (1962). Dalmasso, A. P., Martinez, C., Sjödin, K., and Good, R. A., *J. Exp. Med.*, **118**, 1089 (1963).

⁷ Bomskov, C., and Sladovic, L., *Deutsch. med. Wchnschr.*, **22**, 589 (1940). Cosma, J., *Acta Endocrinol.*, **26**, 361 (1957).

PRECIPITIN ACTIVITY IN THE SERA OF PATIENTS WITH DEFICIENCY OF β -LIPOPROTEINS (ACANTHOCYTOSIS)

By DR. R. F. MURRAY, jun.

The National Institutes of Health, Bethesda, Maryland

AND

PROF. B. S. BLUMBERG*

Institute for Cancer Research, Philadelphia, Pennsylvania

WHILE searching for isoprecipitins in the sera of multiply-transfused patients¹, the sera from two patients with a deficiency of β -lipoproteins were tested by double diffusion in agar² against a panel of normal human sera. Weak but definite precipitin lines formed. The lines were stained by the specific lipid-stain, sudan black B (ref. 3). The studies reported here were undertaken to confirm this observation and to characterize the reaction further.

Sera which had been stored at -25° for approximately one year were available from the two original patients (M. S. and A. C.), and fresh specimens were obtained from two additional patients (L. Z. and N. S.) with the same disease. The precipitin reaction was detected in all of these, with the exception of a second specimen from M. S. Serum from N. S. showed the strongest reaction of the four specimens tested. The best results were obtained when reactant proportions of two (N. S. serum) to one (normal serum) were used. Precipitin lines that developed within 16 h and stained with sudan black B were observed between N. S. and 29 of 30 sera from American white individuals. Under similar conditions sera from L. Z. and A. C. gave weaker and less-well-defined reactions. The low-density lipoproteins (specific gravity < 1.063) from 36 individuals were isolated by flotation in a salt medium of specific gravity 1.063 by the method of Havel *et al.*⁴. Thirty-five of these 36 isolated lipoprotein specimens

reacted with serum from L. Z., A. C. and N. S. (Fig. 1). The isolated lipoproteins failed to react with any of ten-fold concentrated sera from ten normal, white individuals. The > 1.063 fraction of the fractionated sera did not produce a precipitin reaction even when concentrated by ultrafiltration to a degree comparable to that of the low-density lipoprotein fraction (ten-fold concentrated).

In order to characterize the precipitin in the sera of the acanthocytosis patients, the immunoelectrophoretic method of Grabar and Williams⁵ was modified as follows. Sera from A. C. and L. Z. were concentrated three-fold by ultrafiltration, placed in the centre well, and subjected to electrophoresis in 1 per cent agar in veronal buffer, pH 8.6, ionic strength 0.05, for 1 h at 6 V/cm. Isolated low-density β -lipoprotein was placed in one trough and whole serum in the other. A single arc developed when the whole serum was used, and two arcs formed opposite the isolated low-density β -lipoprotein. The arc common to both troughs was broad, with indistinct borders. It had a cathodal mobility intermediate to that of β - and γ -globulins. The second arc had an anodal migration which corresponded to that of albumin or an α_1 -globulin, and was about one-half as intense as the slower arc. Both arcs tended to form very near the trough, and neither alteration of the concentration of reactants nor moving the trough farther from the well influenced their position significantly (Fig. 1). The clearest arcs were noted when isolated lipoprotein diluted 1:3 with saline was used.

* Present address: Division of Medical Genetics, Department of Medicine, University of Washington School of Medicine, Seattle.

Specific goat anti-human immunoglobulin antisera obtained from Dr. W. Terry, National Institutes of Health, were used in an attempt to relate the precipitin activity in the acanthocytosis serum to that of three of the known immunoglobulins (γA , γM and γG)⁶. The position of the major component was similar to but not identical with that of the γA -immunoglobulin (Fig. 2). Serum from patient A. C. was absorbed in the test-tube and in agar gel with these three antisera, but the precipitin activity was not removed. Five ml. serum from patient A. C. was subjected to column chromatography on DEAE in phosphate buffer at pH 7.0. The serum was placed on a column 1 cm \times 25 cm and the protein fractions were eluted in a stepwise fashion using 0.005, 0.025 and 0.5 M phosphate buffers. The precipitin activity could not be demonstrated in any of the four fractions obtained despite five- to ten-fold concentration of each of the fractions.

Serum from patient A. C. was tested, using the Ouchterlony technique, against the isolated β -lipoprotein specimens from 24 normal individuals. This same panel was tested with the human anti- β -lipoprotein antiserum C. de B.⁷ Nineteen of the 24 specimens reacted with C. de B. while all 24 of the specimens reacted with serum from patient A. C. The precipitin reaction with A. C. occurred after 4 h, while that with C. de B. occurred after 16 h. Precipitin arcs with C. de B. formed approximately midway between the centre (antiserum) and peripheral (panel sera) wells, while that with patient A. C. formed immediately adjacent to the peripheral well. These observations suggest that the precipitins in the acanthocytosis sera are different from those developed in multiply-transfused patients. Other experiments were performed which showed that the precipitin band stains only weakly, if at all, with the protein stains, azo carmine and amido black 10B, but strongly with lipid stains. Repeated testing of a single normal serum specimen under the same experimental conditions usually, but not always, produced a precipitin line. The failure of precipitin band formation was not related to variations in pH, temperature, or buffer composition. The reaction did not fix complement using the technique of Sever⁸. The complex formed between the C. de B. antiserum and β -lipoprotein does not fix complement, but the reaction does conform to most other criteria for an antigen-antibody reaction⁹.

These findings and several recent reports in the literature suggest that the reaction observed is probably a non-specific precipitation reaction.

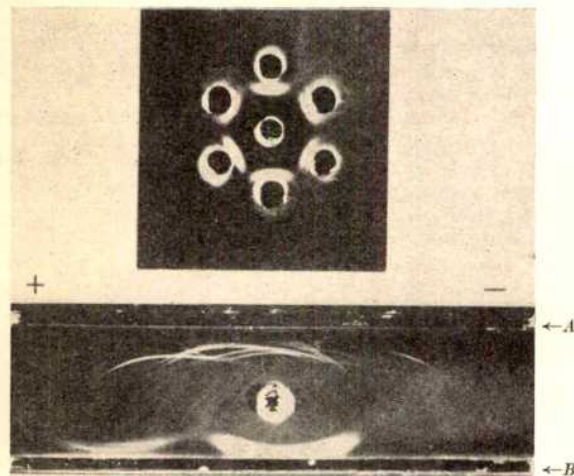


Fig. 1. The upper portion shows a representative Ouchterlony pattern. The centre well contains serum from a patient with deficiency of β -lipoproteins. Peripheral wells contain isolated low-density β -lipoproteins. The lower portion is an immunoelectrophoretic pattern of the serum from an acanthocytotic patient showing precipitin activity in the β_2 - γ -globulin and α_1 -globulin regions. Trough A contains horse anti-human antiserum. Trough B contains isolated low-density β -lipoproteins.

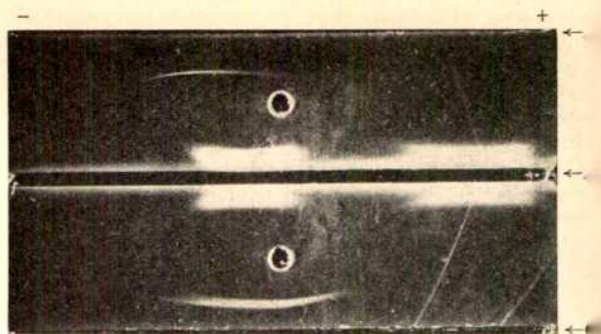


Fig. 2. Immunoelectrophoretic pattern of the serum of a patient with acanthocytosis, comparing the position of the precipitin region with that of known immunoglobulins. Wells contain the serum of patient A. C. Trough A contains goat anti- γM antiserum. Trough B contains isolated low-density β -lipoproteins. Trough C contains goat anti- γA antiserum.

Tomasi¹⁰ noted that certain extracts of human organ gave a non-specific precipitation reaction with normal human sera using a modified Ouchterlony technique². He presented evidence that albumin was the reactive component in the serum and that the tissue extract component migrated slightly toward the cathode or immunoelectrophoresis. Teichman and Vogt¹¹ also noted non-specific precipitation reactions occurring between the sera of patients with tuberculosis and old tuberculi obtained from an *H37Rv* strain of *M. tuberculosis*. They identified the serum component as an α_1 -lipoprotein by its immunoelectrophoretic mobility. The same serum component was also found in sera of normal individuals as well as in sera of patients with carcinoma of the lung. They further suggest that Tomasi's serum component as well as that of Peetom *et al.*¹² (who reported a precipitation reaction between normal human and certain animal sera and haemolysates of normal human red cells) are also α_1 -lipoprotein in nature. Other authors^{13,14} have reported non-specific precipitin reactions between tissue extracts and normal sera, but such a reaction has not been previously reported between two human sera or with the low-density β -lipoprotein of human sera. In addition, precipitin activity is found in two fractions of the acanthocytotic serum. It is possible, however, that the α_1 -globulin fraction contains antibody activity, since G. Strejan and I. Flechner were able to produce antibody with α_1 -globulin mobility in rabbits¹⁵.

In summary, the following findings support the hypothesis that the precipitin activity in the acanthocytosis patients is probably non-specific.

- (1) The precipitin arcs on immunoelectrophoresis have an atypical appearance compared with those seen using human, anti-human lipoprotein antisera such as C. de B.
- (2) Precipitin activity is noted in the albumin or α_1 -globulin region. Non-specific precipitin reactions, which have been previously described, also occurred in these regions.
- (3) The precipitin activity was not removed by absorption with three common anti-immunoglobulin antisera.
- (4) Precipitin activity has been demonstrated with nearly all the normal sera against which it has been tested.
- (5) Precipitin bands stain well with lipid stains but weakly, if at all, with protein stains.
- (6) It is unusual for antibody activity against the same antigen to develop in two classes of immunoglobulins in the same individual.
- (7) The complex formed does not fix complement.

Neither demonstration of a classical antigen-antibody precipitation curve nor localization of the precipitin activity in the β - or γ -globulin region on immunoelectrophoresis is sufficient evidence to classify the reactant in the acanthocytosis patient's serum as an antibody¹⁴. Niece and Barrett¹³ noted an atypical precipitin band when chymotrypsin was tested in agar against an anti-

arboxypeptidase antiserum produced in rabbits. The and was unlike that produced with carboxypeptidase A and stained with sudan black but not with bromphenol blue. The antiserum would neither agglutinate latex particles coated with chymotrypsin nor provoke passive cutaneous anaphylactic reactions in guinea-pigs with chymotrypsin as antigen. Both phenomena occurred when carboxypeptidase A was used as antigen. They concluded that the precipitin was spurious, probably resulting from precipitation in the agar of lipid released by enzymatic degradation of the lipoproteins in the antiserum. The precipitin bands noted in our experiments are similar to theirs and may also result from the action of an enzyme or enzymes in the serum of these patients with deficiency of β -lipoproteins.

A reaction between the low-density β -lipoproteins and two components in the serum of patients with deficiency of β -lipoproteins is clearly demonstrated for the first time. Further work is required to determine whether these components are peculiar to the sera of acanthocytotic patients and whether they are a result of, or play a part in production of, the deficiency of β -lipoprotein.

We thank Dr. Leonard Laster of the National Institute of Arthritis and Metabolic Diseases for giving us serum from patients L. Z. and A. C., and Dr. P. Ways of the University of Washington in Seattle for serum from patients M. S. and N. S.

- ¹ Blumberg, B. S., Alter, H. J., Riddell, N. M., and Erlandson, M., *Vox Sang*, **9**, 128 (1964).
- ² Ouchterlony, O., *Acta Path. Microbiol. Scand.*, **32**, 231 (1953).
- ³ Blumberg, B. S., *Clin. Res.*, **11**, 202 (1963).
- ⁴ Havel, R. J., Eder, H. A., and Bragdon, J. H., *J. Clin. Invest.*, **34**, 1345 (1955).
- ⁵ Grabar, P., and Williams, C. A., *Biochim. Biophys. Acta*, **17**, 67 (1955).
- ⁶ Committee on Nomenclature for Human Immunoglobins, *Bulletin W. H. O.*, **30**, 447 (1964).
- ⁷ Blumberg, B. S., Dray, S., and Robinson, J. C., *Nature*, **194**, 656 (1962).
- ⁸ Sever, J. L., *J. Immunol.*, **38**, 320 (1962).
- ⁹ Allison, A. C., and Blumberg, B. S., *Lancet*, **i**, 634 (1961).
- ¹⁰ Tomasi, T. B., *J. Immunol.*, **86**, 427 (1961).
- ¹¹ Teichman, B., and Vogt, R., *Naturwiss.*, **51**, 141 (1964).
- ¹² Peetom, F., Rose, N., Sudčy, S., Micheli, A., and Grabar, P., *Ann. Inst. Pasteur*, **96**, 252 (1960).
- ¹³ Niece, J. L., and Barrett, J. T., *Nature*, **197**, 1021 (1963).
- ¹⁴ Deckers, C., and Maisin, J., *Nature*, **197**, 397 (1963).
- ¹⁵ Strejan, G., and Flechner, I., *Proc. Soc. Exp. Biol. and Med.*, **115**, 352 (1964).

DOUBLE CHROMOSOME REDUCTION IN A TETRAPLOID SOLANUM

By G. E. MARKS, DR. R. K. MCKEE and DR. J. B. HARBORNE

John Innes Institute, Bayfordbury, Hertford

REPEATED attempts to cross *Solanum demissum* ($2n=6x=72$) with *S. stoloniferum* ($2n=4x=48$) gave one seeded berry containing one seed. This yielded a plant (*M.271*) which had $2n=4x=48$, instead of the expected $2n=5x=60$. The cytological, morphological and biochemical studies of *M.271* reported here provide good evidence that it is a true hybrid and therefore the product of double chromosome reduction.

The plants used in this investigation were as follows: *Parents*. *Solanum demissum* (C.P.C. 2249) (*dms* 2249). This clone, collected by Dr. J. G. Hawkes in 1949 in Hidalgo State, Real del Monte, Mexico, is morphologically typical of the species. It is both male and female fertile, producing 94.6 (± 1.64) per cent stainable pollen of a regular diameter and a mean of 96 seeds per berry on self-pollination. *Solanum stoloniferum* (C.P.C. 2282.2) (*sto* 2282.2). This clone was grown from seed collected by Dr. J. G. Hawkes in 1949 in Puebla State, Mexico. It is a form of the species characterized by dark green leaves, many interjected leaflets, a high leaflet index and blue flowers. It sets abundantly seeded berries by self-pollination.

Hybrid M.271. This one seed obtained in the cross *dms* 2249 (ϕ) \times *sto* 2282.2 (σ) germinated and grew very slowly into a small plant with extremely short internodes and a tufted, almost cushiony habit; it produced only a single flower. By contrast, when grafted on tomato, its

growth was upright and vigorous, it produced long internodes and flowering was profuse and continuous. Comparison of the leaf and flower characters of *M.271* with those of its parents (Table 1) shows that in most ways it resembles the *demissum* parent more closely than it does *sto* 2282.2. A predominance of *demissum* would also be expected in a pentaploid hybrid ($2n=60-3\text{ dms}+2\text{ sto}$ genomes). Morphologically, *M.271* resembles polyploid *S. demissum* ($2n=3x=36$)¹⁻³ and could be taken for one were it not for its chromosome number. An analysis of meiosis gave the results shown in Table 2.

M.271 has no stainable pollen and attempts to back-cross it by both parents as males gave one berry when *sto* 2282.2 was used. This contained two seeds, but neither germinated.

Possible Origins

There is no definite morphological evidence to suggest, let alone prove, that *M.271* is a true hybrid of *S. demissum* \times *S. stoloniferum*. Before giving the biochemical evidence on this point, we may consider three other possible origins, as follows:

(1) *Contamination*. It is possible that *M.271* is a hybrid between *dms* 2249 and a diploid *Solanum*, since three such species, *S. chomatophyllum*, *S. santolallae* and *S. verrucosum*, were growing in the same glasshouse as *S. demissum* and were being used in the same crossing programme. However, all attempts to cross *demissum* with these diploids failed, and, in addition, *M.271* shows no evidence of the characteristic morphology or phenolic pattern (see later) of any of these species. Hybrids between *demissum* and *verrucosum* have been recorded^{4,5}; they differ both morphologically and cytologically from *M.271*.

Table 1. LEAF CHARACTERS OF PARENTS AND HYBRID

	<i>dms</i> 2249	<i>M.271</i>	<i>sto</i> 2282.2
	Mean* (range)	Mean* (range)	Mean* (range)
Pairs of lateral leaflets	2.90 (2-3)	2.00 (0)	3.40 (3-4)
Number of interjected leaflets	2.55 (1-4)	0.90 (0-1)	6.40 (3-10)
Terminal leaflet index (Breadth/Length %)	58 (52-68)	61 (50-72)	66 (60-73)

* Based on ten measurements.

Table 2. MEIOTIC AND FERTILITY DATA

Plant	Meiosis in pollen mother cells							Per cent \pm S.E. stainable pollen
	No. of cells	Mean \pm S.E. per cell					Term coeff.	
		IV	III	II	I	Xta		
<i>sto</i> 2282.2	25	0	0	24.00	0	32.64 \pm 0.51	0.56	60.4 \pm 4.02
<i>M.</i> 271	25	0.08	1.48 \pm 0.25	12.64 \pm 0.36	17.96 \pm 0.40	22.4 \pm 0.73	0.72	0

(2) *Female parthenogenesis*. Polyhaploid plants of *S. demissum* are occasionally produced as a result of female parthenogenesis in selfed progenies of *S. demissum*³ or in progenies obtained from crosses between *demissum* and diploid species^{2,6,7}. All had $2n = 3x = 36$ except for two plants: one which Dodds² found to have $2n = 3x + 3 = 39$ and another which Beamish⁷ found to have $2n = 2x + 2 = 26$.

A tetraploid plant could arise parthenogenetically from a hexaploid only by an extremely abnormal maternal meiosis. Meiosis and embryo-sac development were therefore studied in ovules of *dms* 2249, using sections stained with Heidenhain's haematoxylin. Meiosis was regular and embryo-sac development was of the type normal for *S. demissum*³. Thus the occurrence of a parthenogenetic tetraploid seedling of *demissum* is extremely unlikely.

(3) *Male parthenogenesis*. *M.271* could have conceivably arisen by male parthenogenesis, in which case it would be genetically equivalent to a segregate in a progeny raised by selfing *sto* 2282.2. Such a progeny was grown and all the individuals were found to resemble closely the parent plant; furthermore, all were fertile and set berries by self-pollination. Thus, this parental clone is highly homozygous, and it is therefore inconceivable that a male parthenogenetic seedling should resemble *demissum* rather than its *stoloniferum* parent.

Biochemical Evidence

Except for the anthocyanin pigments of flowers which exhibit dominant-recessive relationships in their inheritance, the genetic control of most chemical constituents is more or less quantitative and hybrids contain most or all of the substances present in the parents⁹. Thus chemical constituents characteristic of presumed parents can give good evidence of the origin of a putative hybrid. On this assumption, the flavonol glycosides of the flowers and the leaf alkaloids of *dms*, *sto* and *M.271* were examined, since these substances were known to show significant variation among the wild tuber-bearing *Solanum* species.

Phenolic patterns in flowers. Ten flavonol glycosides are known to occur (and vary) in potato flowers¹⁰. Their distributions in *M.271*, in its putative parents and in three related diploid species were therefore examined (Table 3). The results eliminate the possibility that *M.271* could have arisen by a chance hybridization between *demissum* and a related diploid species. The following points about the phenolic patterns of the flowers may be noted.

(1) The pattern of *M.271* is identical with that of *demissum*, agreeing with the morphological evidence (see earlier) showing that *demissum* is the predominant parent.

(2) The pattern of *M.271* does not disagree with the idea that *stoloniferum* is the other parent. One constituent of *stoloniferum*, it is true, is not present in the hybrid. This substance, luteolin 7-glucoside, though characteristic of the species, is not always present: it was lacking in four out of 38 clones examined¹⁰. Its absence from *M.271* is therefore not critical.

Table 3. PHENOLIC CONSTITUENTS OF FLOWERS

	<i>dms</i> 2249	<i>M.271</i>	Occurrence in <i>sto</i> 2282.2	<i>chm</i> * 2708	<i>san</i> 2519	<i>ver</i> 2644
Flavonol glycosides						
Kaempferol 3-glucoside	—	—	—	—	+	—
Kaempferol 3-rutinoside	—	—	—	—	—	+
Kaempferol 3-sophoroside	—	—	—	+	—	—
Kaempferol 3-(2 ⁶ -glucosylrutinoside)	—	—	—	+	—	+
Quercetin 3-glucoside	—	—	—	—	+	—
Quercetin 3-rutinoside	+	+	+	—	—	+
Quercetin 3-sophoroside	—	—	—	—	+	—
Quercetin 3-(2 ⁶ -glucosylrutinoside)	—	—	—	+	—	+
Myricetin 3-rutinoside	+	+	—	+	—	—
Luteolin 7-glucoside	—	—	+	—	—	—
Other phenolics						
Caffeoyl-glucose-quinic complex	+	+	—	—	—	+
Unknown colourless → mauve	—	—	—	+	—	—

* Also contains two other unidentified flavonol glycosides.

Key: *dms*—*demissum*; *sto*—*stoloniferum*; *chm*—*chomatophyllum*; *san*—*santallinae*; *ver*—*verrucosum*.

Table 4. ALKALOIDS IN THE LEAVES

Plant	Alkaloid	<i>R</i> * α -S	<i>R</i> †	Products of acid hydrolysis Aglycone(s)	Sugars
<i>dms</i> 2249	Demissine	0.94	0.29	Demissidine	Xylose, glucose and galactose
<i>sto</i> 2282.2	Solanine	1.0	0.28	Solanidine	Rhamnose, glucose and galactose
	α -chaconine	1.35	0.46		
<i>M.271</i>	Demissine (?)	0.88	0.30	Demissidine + traces of solanidine§	Rhamnose, xylose, glucose and galactose
	New demissidine glycoside α -chaconine (?)	† 1.37	0.48		

* *R** relative to α -solanine: chromatograms run on citrate-buffered Whatman No. 1 paper in butanol-citric acid-water.

† Chromatograms run on silica-gel plates in acetic acid-ethanol (1:3).

‡ Identified as a mixture of two components, since this spot gave a strong Dragendorff reaction for alkaloid but only a weak reaction with Clarke reagent (used for detecting solanidine glycosides in the presence of demissidine glycosides, which do not react).

§ Solanidine was detected, in the presence of demissidine, by the use of Sarett's reagent (compare ref. 13). The crude mixture of alkaloid aglycones failed to respond to the nitroso test, indicating that tomatidine was absent.

(3) The pattern of *M.271* is quite different from those of the three diploid species (Table 2), which makes it very unlikely that any of these species is involved in its parentage. Thus, all three not only contain kaempferol as well as quercetin glycosides but, in addition, the glycosidic patterns (sophoroside, glucoside and (2⁶-glucosylrutinoside)) are quite different from *M.271* or its parents (which have only the 3-rutinoside). Production of (2⁶-glucosylrutinoside) in flowers of *S. stoloniferum* and *S. chacoense* is known¹⁰ to be controlled by a dominant gene *G* (3-rutinoside being the recessive character), and it is unlikely that this glycosidic form would not appear in hybrids involving *chomatophyllum* or *verrucosum*.

Alkaloids of the leaves. Steroidal alkaloids occur, combined with sugars (branched tri- or tetra-saccharides), quite characteristically in the genus *Solanum*, and their distribution has been studied intensively in recent years¹¹. While glycosides of solanidine are widely distributed in the tuberous solanums, those based on demissidine are found only in *S. demissum*. Thus it should be possible to characterize hybrids of *demissum* with other species on the basis of their alkaloid content and this has, in fact been done with *M.271*.

The alkaloids of both parents were examined, with the results shown in Table 4. The clone of *S. demissum* contained the expected demissine¹² and *S. stoloniferum* contained solanine and α -chaconine, the two commonest potato alkaloids. *M.271* contained three glycosides of which two corresponded in *R** value with glycosides from the different parents, that is with demissine and α -chaconine; the third appeared to be a new demissidine glycoside. On acid hydrolysis, these glycosides together yielded demissidine, with traces of solanidine, and four sugars: glucose, galactose, xylose and rhamnose. Thus, *M.271* has the aglycones of both parents and also the constituent sugars of both, since the bound xylose is derived from *S. demissum* and the bound rhamnose can only have come from *S. stoloniferum*.

Since major intraspecific variation in alkaloid content is unknown in the potatoes, this result is effective proof that *M.271* is a true hybrid between *S. demissum* and *S. stoloniferum*.

Origin of *M.271*

The morphology and biochemistry of *M.271* indicate that it is far nearer its female parent, *demissum*, than it is to *stoloniferum*, suggesting that double chromosome reduction occurred in the latter, the male parent.

Meiosis was normal in the sample of pollen mother cells examined from *sto* 2282.2 (Table 1). However, pollen grain measurements from ten different anthers showed a range of size within each anther. Using within-anther variance as a measure of this variation, Bartlett's test showed a highly significant heterogeneity between anthers ($\chi^2_{[9]} 487.4$). Furthermore, the mean variance within anthers of *sto* 2282.2 was above that for other *stoloniferum* clones. Pollen stainability was also extremely variable in *sto* 2282.2, ranging from 12 to 75 per cent per anther. Of a

ample of 1,000 'good' pollen grains 5.6 per cent had diameters equal to or below the modal diameter of pollen grains measured in a diploid species. Since, within species, the size of a pollen grain is often correlated with its chromosome number, it is quite likely that some of the small pollen grains found in *sto* 2282.2 were double-reduced, with 12 chromosomes.

Matsubayashi¹⁴ found that certain clones of *stolonium* produced occasional tripolar spindles at MI of meiosis which resulted in hexads containing six nuclei of almost equal size. Such a mechanism could well be the source of double-reduced pollen grains. Unfortunately, re-investigation of meiosis in *sto* 2282.2 is impossible since the clone was lost through virus infection. There is therefore no information about the cytological mechanism of the presumed double reduction.

Discussion

Double reduction has been invoked on a number of occasions to account for the appearance of unusual hybrids. Thus Steere¹⁵ obtained diploid progeny which resembled both parents, from the cross *Petunia axillaris* $2n=2x=14$ \times *P. hybrida* ($2n=4x=28$). Nishiyama¹⁶ made similar observations on *Avena* hybrids, Karprechenko¹⁷ on *Brassica* \times *Raphanus* crosses and Ewart and Walker¹⁸ on *Poinsettia*. In the cherry, three diploid cultivars are reputed to have arisen from crosses between diploid *Prunus avium* and a tetraploid cultivar, 'May Duke'¹⁹; Darlington²⁰ thought that the chromosome number of the parents disproved the supposed origin of these cultivars but, in retrospect, it now seems that the original interpretation is worth reconsideration.

In none of these examples is there definite proof of double chromosome reduction: for the potato hybrid,

although direct cytological evidence is lacking, the biochemical data appear to be decisive. The fact that Matsubayashi¹⁴ independently obtained a tetraploid plant from the same cross that produced *M.271* suggests that double chromosome reduction may be less uncommon than we think. But it seems unlikely that it is an important general mechanism in Nature for decreasing chromosome numbers.

From a biochemical point of view, *M.271* is particularly interesting since it contains a new hybrid substance, a glycoside of demissidine not present in either parent. The identification of the sugar linkages in this glycoside will clearly be of interest, as it should throw some light on the control of alkaloid glycoside synthesis, about which very little is at present known.

¹ Howard, H. W., and Swaminathan, M. S., *Genetica*, **26**, 381 (1953).

² Dodds, K. S., *Nature*, **166**, 755 (1950).

³ Marks, G. E., *J. Genet.*, **53**, 262 (1955).

⁴ Propach, H., *Z. Indukt. Abstamm. Vererb.*, **74**, 376 (1937).

⁵ Kawakami, K., and Matsubayashi, M., *Sci. Rep. Myogo Univ. Agric.*, **3**, 17 (1957).

⁶ Bains, G. S., and Howard, H. W., *Nature*, **166**, 795 (1950).

⁷ Beamish, K., *Amer. J. Bot.*, **43**, 297 (1955).

⁸ Walker, R. I., *Bull. Torrey Bot. Club*, **82**, 87 (1955).

⁹ Alston, R. E., and Turner, B. L., *Biochemical Systematics*, 295 (Prentice-Hall, 1963).

¹⁰ Harborne, J. B., *Biochem. J.*, **84**, 100 (1962).

¹¹ Schreiber, K., *Die Kulturpflanzen*, **11**, 422 (1963).

¹² Schreiber, K., and Aurich, O., *Z. Naturforsch.*, **18b**, 471 (1963).

¹³ Schreiber, K., Aurich, O., and Osske, G., *J. Chromatog.*, **12**, 63 (1963).

¹⁴ Matsubayashi, M., *Chromosome Information Service*, No. 3, 7 (1962) and personal communication.

¹⁵ Steere, W. C., *Amer. J. Bot.*, **20**, 340 (1932).

¹⁶ Nishiyama, I., *Cytologia*, **5**, 146 (1933).

¹⁷ Karprechenko, G. D., in *Recent Advances in Cytology*, edit. by Darlington, C. D., 193 (Churchill, 1937).

¹⁸ Ewart, L. C., and Walker, D. E., *J. Heredity*, **50**, 203 (1960).

¹⁹ Knight, T. A., quoted by Darlington, C. D., *J. Genet.*, **22**, 19 (1923).

²⁰ Darlington, C. D., *J. Genet.*, **23**, 327 (1934).

RETICULOCYTE RIBOSOME FRACTION WITH AN EXCEPTIONAL CAPACITY FOR POLYPHENYLALANINE SYNTHESIS

By DR. JOHN O. BISHOP

Institute of Animal Genetics, Edinburgh

RETICULOCYTE ribosomes, isolated from the cell lysate by differential centrifugation, form a heterogeneous population with respect to size¹⁻⁴. The accepted unit is the ribosomal monomer, with a sedimentation coefficient in the region of 80 S (ref. 5). Polyribosomes⁴ (or ergosomes⁶) consist of aggregates of two or more monomers, held together probably by a strand of informational RNA. Two types of ribosomal sub-units are also found, with sedimentation coefficients of about 60 S and 40 S (ref. 3).

It is widely recognized that the monomer is the active unit in protein synthesis. However, the monomers found free in the cell lysate do not synthesize protein²⁻⁴; those ribosomal monomers which make up the polyribosome do so, each monomer synthesizing a single polypeptide chain^{7,8}. In this communication the term 'monosome'⁹ is used to refer to any ribosomal monomer which is not incorporated in a polyribosome. It is convenient to be able to refer in molar terms to the monomers which constitute the polyribosomes, and to the monomers which would be formed by the aggregation of a given quantity of sub-units. The term 'monomer equivalent'⁷ is used to refer to the amount of any given homogeneous or heterogeneous ribosome preparation equal in mass to a ribosomal monomer. The term 'native', used to describe a particular ribosome species, indicates that it was found in the cell lysate. This does not imply that it is present as such within the cell. A 'derived monosome'¹⁰ is one released

from a polyribosome during protein synthesis in the cell-free system. 'Derived sub-units' are sub-units prepared by dissociation of monosomes⁸.

The macromolecular components required for the synthesis of polypeptides under the direction of synthetic polynucleotides are activating and transfer enzymes, transfer RNA, and ribosomes. In the preparations used, the predominant ribosomal species has usually been the monosome. The newly synthesized polypeptide chains are sometimes found associated with artificial polyribosomes which are thought to consist of two or more ribosomal monomers attached to a single polynucleotide molecule¹¹⁻¹⁴. These sediment in a centrifugal field more rapidly than the monosomes¹¹⁻¹⁶. In none of the cases cited has more than a small proportion of the available ribosomal material been seen to become involved in the formation of complexes with polynucleotide. This suggests that only a minor ribosome fraction is capable of reacting with the synthetic polynucleotide.

This conclusion has recently been confirmed by measuring the chain-length of the polyphenylalanine which is synthesized in the reticulocyte cell-free system under the direction of polyuridylic acid (poly U)¹⁷. Total phenylalanine incorporation in this system rarely exceeds 5 μ moles per mg of ribosomes, or, taking the molecular weight of the monomer to be 4×10^6 (ref. 21), 20 phenylalanine residues per monomer equivalent. The average chain-length was found to be of the order of 200 phenylalanine

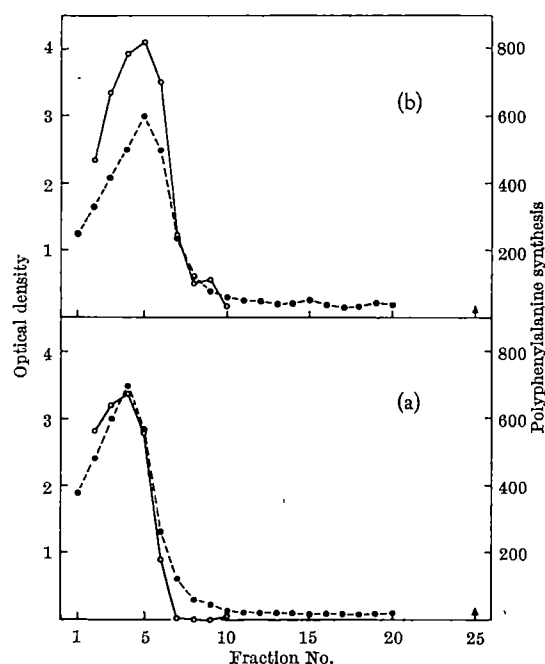


Fig. 1. Polyphenylalanine synthesis by native and derived monosomes following zone sedimentation. (a) Native monosomes. (b) Derived monosomes. The broken lines show optical density at 260 $m\mu$; solid lines show polyphenylalanine synthesis, measured as c.p.m. incorporated per 0.2 ml. under the standard assay conditions described below. The direction of sedimentation is from right to left. The preparation of monosomes is described in the text. One ml. of the monosome fraction, in standard buffer containing 5 per cent sucrose, was layered on a 29 ml., 10–23 per cent sucrose gradient, prepared in standard buffer, and centrifuged for 10 h at 25,000 r.p.m. in the Spinco 'No. 25' rotor at 0° C. Suitable dilutions of fractions 1 to 10 were assayed spectrophotometrically. Fractions 2 to 10 were assayed for polyphenylalanine synthesis in the cell-free system by mixing 0.2 ml. with an equal volume of a solution containing, in μ moles: ATP, 0.25; GTP, 0.08; creatine phosphate, 2.8; $MgCl_2$, 2; *tris*-HCl, pH 7.2, 16; glutathione, 6; 17 L-amino acids, 0.01 each; and uniformly labelled ^{14}C -phenylalanine, specific activity 10 c./mole (approximately 5,000 c.p.m. per μ mole), 0.02, together with 5 μ g creatine kinase, 50 μ g yeast sRNA¹⁵, 25 μ g poly-U and 1.5 mg of an enzyme fraction prepared by precipitating the ribosomal supernatant between 40 and 70 per cent ammonium sulphate saturation¹⁷. The optimum amount of KCl (20 μ moles) was introduced with the gradient fraction. The mixtures were incubated for 40 min at 37° C and chilled. 0.1 ml. of 6 N NaOH was added and after 2 min at 37° C the mixtures were again chilled and 2 ml. of 10 per cent TCA was added. After 30 min at room temperature the precipitates were collected on 2-cm diameter 'Oxoid' membrane filters, washed 8 times with 5 per cent TCA, twice with ethanol/ether (1/1) and once with ether. Radioactivity was measured using a 'Tricarb' liquid scintillation counter, by placing the filter in a 20 ml. vial containing 15 ml. of counting fluid (0.5 per cent w/v PPO, 0.03 per cent w/v dimethyl POPOP in toluene).

residues¹⁷. If one monomer equivalent is involved in the synthesis of each chain, then only 10 per cent of the ribosomal material is active in polyphenylalanine synthesis.

This paper shows that two minor ribosomal species, the native ribosomal sub-units, are very active in polyphenylalanine synthesis. Both types of sub-units are required, together with poly-U. The specific activity of sub-units is 3–4 times greater than that of monosomes. Up to 80 phenylalanine residues are incorporated in the cell-free system per sub-unit pair.

The ribosomes used in these experiments were prepared from rabbit reticulocytes¹⁸. The cells were washed 3 times by centrifugation and lysed by stirring for 2 min at 0° C with 3 volumes of buffer (10 mM *tris*-HCl, pH 8.1 at 0°, 30 mM KCl, 2 mM $MgCl_2$). Lysis was terminated by adding 0.45 times the original cell volume of ice-cold 2 M sucrose. Cell debris was removed by centrifuging for 15 min at 13,000g. Ribosome fraction P1 was the pellet obtained by centrifuging the clarified lysate for 1 h at 30,000 r.p.m. in the Spinco 'No. 30' rotor (78,000g). Measurements made with the analytical ultracentrifuge with Schlieren optics showed P1 to contain polyribosomes and monosomes in the approximate proportion of 2:3, together with a trace (about 5 per cent) of ribosomal sub-units. Fraction P2H was the pellet obtained by centrifuging the lysate for 2 h. The composition of

Table 1 ENDOGENOUS AND POLY-U-STIMULATED PHENYLALANINE INCORPORATION BY RIBOSOME FRACTIONS

Conditions	Fraction P2H	Fraction P2-4
Complete system	0.644	0.219
Plus 40 μ g poly-U	2.895	2.040

One mg of the ribosome fraction was incubated at 37° C for 40 min in total volume of 0.4 ml. containing other reagents in the amounts given in the legend to Fig. 1. The samples were precipitated, collected, washed and counted as described there. Phenylalanine incorporation is presented as n moles of phenylalanine incorporated per mg of ribosomes.

P2H was similar to that of P1, but it contained high proportions of monosomes and sub-units. Fraction P2- was the pellet obtained by centrifuging the 2-h supernatant for 4 h. This fraction consisted largely of monosomes (about 70 per cent) and sub-units (20 per cent), and contained no polyribosomes except for a small proportion of dimers (about 10 per cent). Purified native monosomes were prepared by zone centrifugation: 1 ml. (15–20 mg) of fraction P1 in standard buffer (0.1 M KCl, 1 mM *tris* HCl, pH 8.1 at 0° C, 1.5 mM $MgCl_2$) containing 5 per cent sucrose was layered on a 29-ml., 10–23 per cent sucrose gradient prepared in standard buffer, and centrifuged for 8 h at 25,000 r.p.m. (Spinco 'No. 25' rotor). The bottom 5 ml. of the gradient was diluted with 15 ml. of standard buffer and centrifuged for 3 h at 78,000g to obtain a pellet of purified native monosomes. The pellet from the 8-h gradient contained polyribosomes virtually free of monosomes. These were gently resuspended and 'derived' monosomes were prepared from them by incubation in the complete cell-free system (see legend to Fig. 1). The mixture was diluted with 7 volumes of standard buffer, and a pellet, which contained both derived monosomes and residual polyribosomes, was obtained by centrifuging for 3 h at 78,000g. The derived monosomes were separated from polyribosomes during the subsequent analysis (Fig. 1). 40-S native ribosomal sub-units were purified by centrifuging 1 ml. of fraction P2-4 through a 29-ml. 10–23 per cent sucrose gradient (in standard buffer) for 10 h at 25,000 r.p.m. in the Spinco 'No. 25' rotor. 40-S sub-units were well resolved, but 60-S sub-units appeared

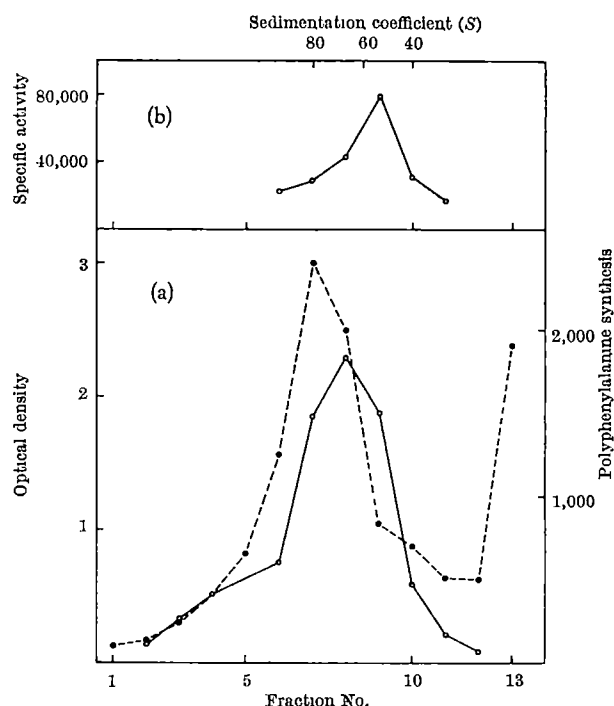


Fig. 2. Polyphenylalanine synthesis by ribosomal fractions following zone sedimentation. (a) Optical density at 260 $m\mu$ (broken line) and polyphenylalanine synthesis (solid line) measured as c.p.m. incorporated under the standard assay conditions described in the legend to Fig. 1. (b) Specific activity, as c.p.m. incorporated per mg of ribosomal material. Direction of sedimentation from right to left. Fraction P2-4 (1 ml.) was applied to a 29-ml sucrose gradient (10–23 per cent) in standard buffer and centrifuged at 25,000 r.p.m. for 5 h. Thirteen 2-ml. fractions were collected and assayed as described in the legend to Fig. 1.

a shoulder on the monosome peak (see Fig. 3). 3 ml. of the 60-S shoulder was applied to a 53-ml. 20-33 per cent sucrose gradient and centrifuged for 10 h at 25,000 r.m. in the Spinco 'No. 25.2' rotor. The 60-S sub-units were resolved from contaminating monosomes and 40-S sub-units as a clear peak near the middle of this gradient (Fig. 4).

Poly-U was synthesized as previously described¹⁷. The preparation used in the experiments had $S_{20,w} = 9.3$, and an average chain-length of about 300 residues, determined by measuring the inorganic phosphate released by bacterial alkaline phosphatase.

The very low polyribosome content of fraction P2-4 is reflected in a low level of endogenous protein synthesis in the cell-free system (Table 1) compared with fraction 2H. In contrast, poly-U-stimulated phenylalanine incorporation was rather higher in fraction P2-4, relative to 2H. Amino-acid incorporation by both fractions, whether endogenous or poly-U-stimulated, was completely dependent on the addition of ATP.

Polyphenylalanine synthesis by native and derived monosomes. Purified native monosomes were centrifuged through a 29-ml. sucrose gradient for 10 h (Fig. 1a). A single peak of optical density was found towards the bottom of the gradient. When the fractions were assayed for their capacity to synthesize polyphenylalanine in a cell-free system with added poly-U the amount of synthesis followed the optical density profile closely. In a parallel experiment derived monosomes were analysed in the same way with similar results (Fig. 1b). However, the specific activity of the derived monosomes was about 10 per cent greater than that of the native monosomes (Fig. 1 and Table 2).

In a previous report¹⁷ it was suggested that polyphenylalanine synthesis by ribosome fractions containing a high proportion of polyribosomes was partly due to monosomes released from polyribosomes during the synthesis of haemoglobin in the cell-free system. Here the activity of derived monosomes is clearly demonstrated. The activity of both types of monosome in this experiment represents

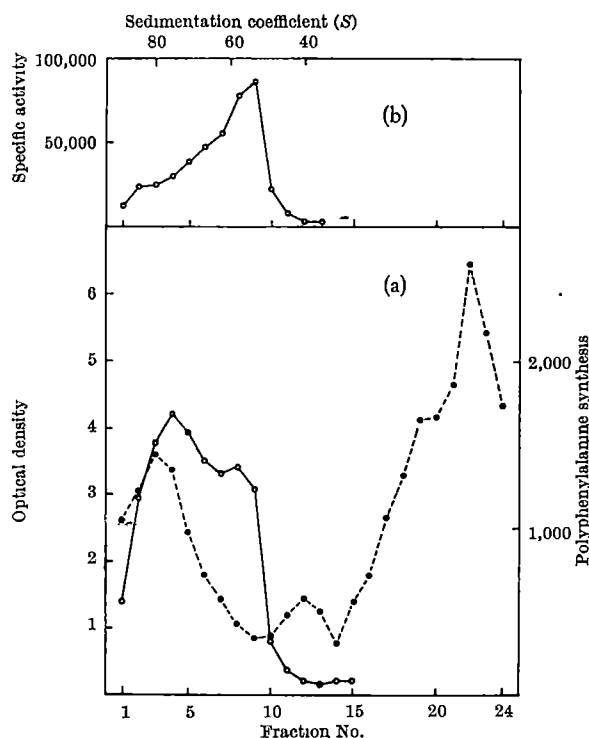


Fig. 3. Polyphenylalanine synthesis by ribosomal fractions following zone sedimentation. (a) Optical density at 260 mμ (broken line) and polyphenylalanine synthesis (solid line). (b) Specific activity. Direction of sedimentation from right to left. Zone centrifugation and assay procedure as for Fig. 2 except that sedimentation was for 10 h and 24 fractions of 1.25 ml. were collected.

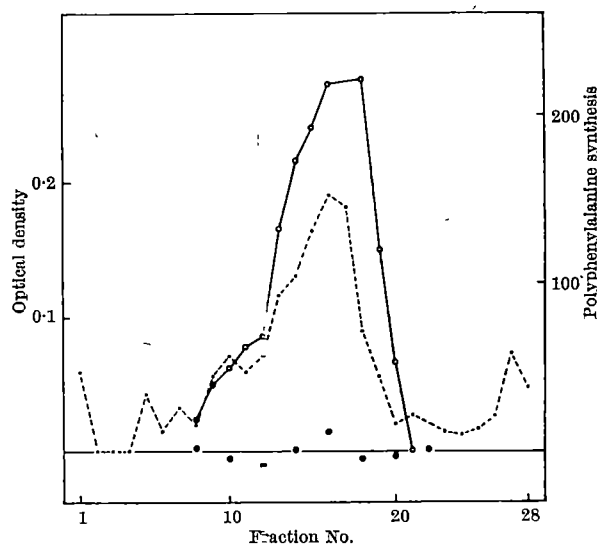


Fig. 4. Purification of native 60-S sub-units. Optical density (broken line) and polyphenylalanine synthesis as c.p.m. incorporated per 0.13 ml. in the absence (closed circles) and in the presence (open circles) of 40-S sub-units (5.6 μg). Direction of sedimentation from right to left. The preparation of 60-S sub-units is described in the text. Twenty-eight fractions of about 2 ml. were collected. Assay conditions were as for Fig. 1.

the incorporation of less than 20 phenylalanine residues per monosome. If the average chain-length is about 200 (ref. 17), less than 10 per cent of these monosomes synthesize polyphenylalanine. The lesser activity of the native monosomes most probably indicates that a lower proportion is active; since the derivation of the two types was rather different, it is quite likely that one was more extensively inactivated than the other. However, the alternative interpretation, that the two types of monosome synthesize different lengths of polyphenylalanine, remains as a possibility.

Polyphenylalanine synthesis by native ribosomal sub-units. To determine which components of fraction P2-4 were active in polyphenylalanine synthesis, 1 ml. of the fraction was centrifuged for 5 h through a sucrose density gradient (Fig. 2), bringing the preponderant ribosome species, the monosomes, to the middle of the gradient. Setting the peak optical density at 80 S, the remainder of the gradient was calibrated by the approximation of Martin and Ames²⁰. The 40-S ribosomal units were partially resolved, forming a shoulder at the trailing edge of the monosome peak. Endogenous protein synthesis was measured by incubating aliquots of the gradient fractions with ¹⁴C-phenylalanine in the cell-free system in the absence of poly-U. No significant phenylalanine incorporation was observed. When poly-U was added to the cell-free system extensive phenylalanine incorporation occurred (Fig. 2a). A single peak of activity was found,

Table 2. POLYPHENYLALANINE SYNTHESIS BY SUCROSE GRADIENT RIBOSOME FRACTIONS

Exp.	Fraction	Approximate sedimentation coefficient	Volume analysed (ml)	Phenylalanine incorporation (c.p.m.)	Specific activity (phenylalanine residues per monomer equivalent)
Fig. 1a	4	80 S	0.2	673	9
	2-5 (av.)	80 S	0.2	818	10
Fig. 1b	5	80 S	0.2	818	13
	2-5 (av.)	80 S	0.2	1,473	14
Fig. 2	9	80 S	0.2	1,499	24
	7	50 S	0.2	1,510	62
Fig. 3	8	80 S	0.2	1,510	20
	8	60 S	0.2	1,364	62
	9	55 S	0.2	1,228	69
	7	60 S	0.1	658	42
	12	40 S	0.1	41	3
	7+12	60 S+40 S	0.1 each	2,358	80
Fig. 4	18	60 S	0.13	11	4
	40-S sub-units		0.07	20	3
	60 S+40 S		0.13+0.07	239	24

The fractions were assayed as described in the legend to Fig. 1. Specific activity is calculated from the optical density and phenylalanine incorporation of the fractions taking the optical density of a 1 mg per ml. solution of ribosomes to be 12 and the molecular weight of the monosome as 4×10^6 (ref. 21).

overlapping the optical density peak, but displaced towards the top of the gradient. The fraction with maximum activity lay between 60 and 70 *S*. A distinct peak of specific activity was also found (Fig. 2b), and the fraction with the highest specific activity lay still nearer to the top of the gradient, between 50 and 60 *S*. In the monosome region (80 *S*) the specific activity was three times lower. The peak fraction, fraction 9, incorporated approximately 60 phenylalanine residues per monomer equivalent (Table 2). Similar profiles, though showing lower levels of incorporation, were obtained when the fractions were incubated with smaller (8 μ g) or greater (80 μ g) amounts of poly-U.

In order to separate the various types of ribosome more completely, a similar sample of fraction P2-4 was centrifuged through the same gradient for 10 h (Fig. 3). Monosomes were found at the bottom of the gradient, clearly separated from the 40-*S* sub-units. The 60-*S* sub-units formed a shoulder on the trailing edge of the monosome peak (fractions 6-8, Fig. 3a). Haemoglobin was partly resolved from heavier contaminating proteins at the upper end of the gradient (fractions 15-24). Aliquots of the gradient fractions were incubated with poly-U and 14 C-phenylalanine in the cell-free system. Two peaks of activity were observed. The first tended to follow the monosome optical density profile, but merged into the second, which was maximum at about 60 *S*. The optical density and incorporation profiles resolved into a single peak of specific activity (Fig. 3b) with its maximum at about 55 *S*. Once again, the specific activity in the monosome region was about three times less than the maximum specific activity (Table 2).

The results show that the ribosomal sub-units are very active in polyphenylalanine synthesis. If both types of sub-unit were required, this would explain the fact that the highest specific activity is found between 40 and 60 *S*, where the two types of sub-unit probably overlap on the gradient. A direct demonstration of the requirement for both sub-units was obtained by mixing fractions from the 10-h gradient. When fractions 7 and 9 were incubated together, polyphenylalanine synthesis was 3.5 times greater than the sum of the two when incubated separately (Table 2). 40-*S* sub-units (fraction 9) showed little activity when incubated alone but the 60-*S* fraction, as isolated from the 10-h gradient, had considerable activity. Since the 60-*S* sub-units were poorly resolved, their activity might be due to contamination either by monosomes or by 40-*S* sub-units. To test this possibility the 60-*S* fraction was applied to a second gradient and centrifuged for a further 10 h. This separated the 60-*S* sub-units from contaminants which appeared as minor peaks on each side of the 60-*S* peak (Fig. 4). At this point the 60-*S* sub-units no longer showed any capacity for polyphenylalanine synthesis when incubated alone, but considerable activity was found when 40-*S* and 60-*S* sub-units were incubated together (Table 2). The profile of polyphenylalanine synthesis in the presence of 40-*S* sub-units followed the optical density profile of the 60-*S* sub-units quite closely (Fig. 4). The greatest specific activity observed in this experiment corresponded to the incorporation of 24 phenylalanine residues per monomer equivalent, which is much lower than the values found in the previous experiments (Table 2). This may be partly because in the assays of the peak tubes the ratio of 40-*S* to 60-*S* sub-units was about 5:1 on a molar basis. In fact, about 60 phenylalanine residues were incorporated per 60-*S* sub-unit present. A second factor may be the inactivation of purified sub-units. Variable inactivation (10-60 per cent) occurred when the sub-unit fractions were held at 0° C for the period of the second gradient run.

These experiments show that native ribosomal sub-units participate with poly-U in the synthesis of polyphenylalanine. Alone, each type of sub-unit appears to be inactive. The obvious inference is that the two types co-operate in the synthesis of each polyphenylalanine

molecule. In these experiments sub-units incorporate up to 80 phenylalanine residues per monomer equivalent. Taking 200 as the chain-length of polyphenylalanine synthesized in the reticulocyte system¹⁷, 40 per cent the sub-units would be participating in polyphenylalanine synthesis, if each chain is synthesized by a single pair of sub-units. If more sub-units were required, the proportion of active sub-units would be higher, and the figure of 80 residues sets at 2.5 the upper limit to the number of pairs which might be involved in the synthesis of a single polyphenylalanine molecule. These arguments are based on the assumption that a given sub-unit can participate in the synthesis of only one polyphenylalanine molecule.

Discussion. The native ribosomal sub-units described here have sedimentation coefficients of about 40 and 60 (ref. 7), as have the derived sub-units prepared by the disaggregation of monosomes⁵. Under conditions which have been reported to effect the reaggregation of derived sub-units⁵, native sub-units are completely stable. Furthermore, native sub-units are isolated from the cell lysate under conditions in which monosomes are stable. These facts suggest that there is an inherent structural difference between native and derived sub-units, although it is clear that before a definitive statement can be made the differently derived sub-units must be compared side by side.

It has not yet been determined whether the native sub-units are in sub-unit form during polyphenylalanine synthesis, or whether they are aggregated together to form monosomes or higher order structures.

The specific incorporation figures for native monosomes and sub-units (Table 2) allow an estimate to be made of the relative contributions these fractions would make to total polyphenylalanine synthesis in cell-free systems. Native sub-units make up about 15 and monosomes about 60 per cent of the total ribosomal material in the clarified lysate (unpublished experiments). In the lysate, about 60 per cent of polyphenylalanine synthesis would be due to both native and derived monosomes, and about 40 per cent to sub-units. The same would be true of purifier systems using ribosome preparations which had been sedimented long enough to precipitate all sub-units, that is for 3-4 h at 78,000*g*. Where shorter sedimentation times are used the proportional contribution of the monosomes will be greater. The same will be true of pre-incubated systems, regardless of the method of preparation, since pre-incubation under conditions favouring protein synthesis leads to the disappearance of sub-units⁷.

E. coli ribosomes have rather different properties than reticulocyte ribosomes, and it is not at all clear what homologies, if any, exist between ribosomes and sub-units from the two organisms. Green and Hall²² isolated sub-units from an *E. coli* lysate which failed to aggregate under conditions which led to the aggregation of derived *E. coli* sub-units. It is possible that these correspond in some way to the native reticulocyte sub-units, but it is not known whether they are active in polyphenylalanine synthesis. Gilbert¹³, and Okamoto and Takanami²³, prepared two types of ribosomal sub-unit from *E. coli* and showed that when incubated together, but not when incubated alone, they would synthesize polyphenylalanine. In each case the sub-units were prepared after the complete dissociation of all monosomes. It is therefore not clear whether the activity observed was due to the derived or the native sub-units.

The question arises of whether native sub-units or monosomes or both are responsible for the initiation of endogenous polypeptide chains, the chains of the globin molecule in the present case. Recent evidence⁷, much of it indirect, indicates that sub-units are required. In certain circumstances a simple one-to-one relationship was demonstrated between the number of sub-unit pairs available and the number of chains initiated. Strong indications were found that monosomes do not initiate endogenous polypeptide chains.

On the other hand, Hardesty *et al.*¹⁰ and Lamfrom andopf²⁴ have suggested that monosomes do initiate globin ins. Under the conditions used by these authors for preparation of monosomes, sub-units would have been aggregated. It is likely, however, that the monosomes are not resolved from the disaggregated sub-units. The monosomes used by Goodman and Rich²⁵ in reaching the conclusion from experiments with an ascites cell stem were more thoroughly purified. However, it is not known whether or not ascites cell native sub-units could be aggregated under the conditions of isolation. The results presented here support the idea that sub-units are active in polypeptide initiation; but at first, they tend to contradict the conclusion that monosomes are inactive. Monosomes are clearly shown to be active in polyphenylalanine synthesis, although less than sub-units. However, it is by no means certain that the properties of poly-U and the endogenous informational RNA are the same. Secondly, in the experiments reported here the ratio of poly-U molecules to ribosomes was of the order of 100:1. In the endogenous situation it is likely to be less than 1:1. The reactivity of the ribosome fractions with poly-U may not be a certain index of their behaviour either within the cell or in the cell-free system without added polynucleotide.

I thank Mrs. Norma Robertson for her assistance, and Dr. B. B. Cohen for his advice. This work was supported by grants from the Medical Research Council.

¹ Arnstein, H. R. V., *Biochem. J.*, **81**, 24P (1961).

² Gierer, A., *J. Mol. Biol.*, **6**, 14E (1963).

³ Marks, P. A., Burka, E. R., and Schlessinger, D., *Proc. U.S. Nat. Acad. Sci.*, **48**, 2163 (1962).

⁴ Warner, J. R., Knopf, P. M., and Rich, A., *Proc. U.S. Nat. Acad. Sci.*, **49**, 122 (1963).

⁵ Ts'o, P. O. P., and Vinograd, L., *Biochim. Biophys. Acta*, **49**, 113 (1961).

⁶ Wettstein, F. O., Staehelin, T., and Noll, H., *Nature*, **197**, 430 (1963).

⁷ Bishop, J. O., *Biochim. Biophys. Acta* (in the press).

⁸ Warner, J. R., and Rich, A., *J. Mol. Biol.*, **10**, 202 (1964).

⁹ Mathias, A. P., Williamson, R. Huxley, H. E., and Page, S., *J. Mol. Biol.*, **9**, 154 (1964).

¹⁰ Hardesty, B., Hutton, J. J., Erlinghaus, R., and Schweet, R., *Proc. U.S. Nat. Acad. Sci.*, **50**, 1078 (1963).

¹¹ Spyrides, G. J., and Lipman, F., *Proc. U.S. Nat. Acad. Sci.*, **48**, 1977 (1962).

¹² Barondes, S. H., and Nirenberg, M. W., *Science*, **138**, 813 (1962).

¹³ Gilbert, W., *J. Mol. Biol.*, **6**, 574 (1963).

¹⁴ Hardesty, B., Erlinghaus, R., Schaeffer, J., and Schweet, R. S., *Cold Spring Harbor Symp. Quant. Biol.*, **28**, 215 (1963).

¹⁵ Haschemeyer, A. E. V., and Rich, A., *Biochim. Biophys. Acta*, **55**, 994 (1962).

¹⁶ Bishop, J. O., *Nature*, **202**, 86 (1964).

¹⁷ Bishop, J. O., *Nature*, **203**, 40 (1964).

¹⁸ Allen, E. H., and Schweet, R. S., *J. Biol. Chem.*, **237**, 760 (1962).

¹⁹ Monier, R., Stephenson, M. L., and Zamecnik, P. C., *Biochim. Biophys. Acta*, **43**, 1 (1960).

²⁰ Martin, R. G., and Ames, B. N., *J. Biol. Chem.*, **236**, 1372 (1961).

²¹ Dintzis, H. M., Borsook, H., and Vinograd, J., in *Microsomal Particles and Protein Synthesis*, 95 (Pergamon, N.Y., 1958).

²² Green, M. H., and Hall, B. D., *Biophys. J.*, **1**, 517 (1961).

²³ Okamoto, T., and Takanami, M., *Biochim. Biophys. Acta*, **68**, 325 (1963).

²⁴ Lamfrom, H., and Knopf, P. M., *J. Mol. Biol.*, **9**, 558 (1964).

²⁵ Goodman, H., and Rich, A., *Nature*, **199**, 318 (1963).

INTERMEDIATE STATES IN THE PRECIPITATION OF HYDROXYAPATITE

By PROF. E. D. EANES, DR. I. H. GILLESSEN and PROF. A. S. POSNER

Hospital for Special Surgery, Cornell University Medical College, New York

TO distinguish between the physico-chemical and physiological factors underlying the formation of biological hydroxyapatites, investigations have been undertaken in this laboratory on the ageing of aqueous preparation of synthetic calcium phosphates. In these studies, chemical and X-ray diffraction analyses are being employed to elucidate certain aspects of the nucleation and growth dynamics of the emerging solid phase under a wide variety of experimental conditions. The present article describes the precipitation behaviour of these calcium phosphates under conditions of high pH and high initial concentrations of reactants, and gives a description of some of the alterations which occur in the physical and chemical properties of the solid phase while in contact with the preparative solution.

To minimize the formation of more soluble acid phosphates, the following hydroxyapatite preparation, a modification of one proposed by Hayek and Stadlmann¹, was employed: 0.250 molar solutions of $(\text{NH}_4)_2\text{HPO}_4$ were rapidly added while stirring to 0.750 molar solutions of either $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ or $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ to achieve a final phosphate concentration of 0.15 molar in the Ca/PO_4 molar ratio 1.71. All solutions were brought to a pH value of 10.5 with concentrated NH_4OH before mixing. The reactions were carried out at 25° C in closed systems to minimize CO_2 absorption. Constant stirring kept the slurry in intimate contact with the reaction solution for periods up to 2 weeks. Samples of the reaction slurry were withdrawn at fixed intervals after initial mixing for chemical, pH and X-ray diffraction analyses. To avoid changes in the solid during the drying of the slurry, all samples were lyophilized. The samples chosen for chemical studies were washed four times with ammoniated wash water (pH 10.5) before freeze-drying and then analysed for Ca by standard EDTA titration techniques and for PO_4 by the differential spectrophotometric technique of Gee and Deitz². The supernatant and washings were also analysed for Ca and PO_4 .

X-ray diffraction patterns of the samples were recorded on an electronic diffractometer equipped with a scintillation counter and a pulse-height analyser. Nickel-filtered copper $K\alpha$ radiation was employed. The scattering region 24–36°2 θ was principally surveyed; this portion of the diffraction pattern includes the main hydroxyapatite diffraction profile. Because of extensive overlaps, only the 002 peak at 25.8°2 θ was sufficiently resolved for line-breadth measurements. The width at one-half the maximum height of this peak, $\beta_{1/2}$, was measured in degrees 2 θ and corrected for instrumental aberrations according to the method of Warren³. The quantity, $1/\beta_{1/2}$, which is proportional to the mean crystallite size and/or degree of perfection in the direction along the c-axis of the crystals was taken as the measure of the crystallinity index. Since it was observed qualitatively that variations in the degree of resolution of the remainder of the diffraction pattern parallel the changes in the 002 reflexion, this crystallinity index can be taken as representative of the crystal as a whole.

The initial solid phase, which separated immediately on mixing of the reactants, was 'non-crystalline' inasmuch as the diffraction patterns showed no discrete diffraction peaks (see Fig. 1A). The molar Ca/PO_4 ratio of this amorphous phase after washing was well below the 1.71 ratio of the overall reaction system and was found to be dependent on the Ca salt used in the preparation. The molar Ca/PO_4 ratio was remarkably consistent for any given salt; a ratio of 1.52 was found when the nitrate solution was used, a value of 1.46 was observed when the chloride solution was used. At most only 90 per cent of the total Ca available in solution was incorporated into the amorphous phase while the unincorporated Ca either remained in solution or was washed out of the precipitate. No detectable amount of PO_4 was found in either the reaction solution or the wash waters after solid formation.

X-ray diffraction patterns taken on the unwashed, freeze-dried product, as well as patterns taken directly of

the slurry, centrifuged to remove excess water, established that the 'non-crystalline' character of the precipitate persisted in contact with the precipitating solution for several hours before completely transforming into a poorly crystalline hydroxyapatite (Fig. 1B). The observable conversion of the pre-apatitic phase into hydroxyapatite did not occur abruptly, but extended over a period of about 2 h. Measurements made on the 002 diffraction peak of samples collected during this period revealed that even though there was a continuous five-fold increase in relative peak area, indicative of a five-fold increase in converted apatite, the diffraction breadth remained unchanged at $1/\beta_{1/2} = 1.44/^\circ 2\theta$ ($\sigma = \pm 0.04$), a value smaller than normally found in poorly crystallized bone apatite⁴. When the pre-apatitic phase has fully converted, as evidenced by the constancy of the 002 diffraction peak area, a gradual sharpening of the 002 reflexion was observed ($1/\beta_{1/2} = 1.75/^\circ 2\theta$ at 25 h, $1/\beta_{1/2} = 2.39/^\circ 2\theta$ at two weeks).

Increase in the Ca/PO_4 molar ratio of the solid and corresponding decreases in the concentration of solution Ca occurred concomitantly with the crystallographic conversion. By the end of two weeks the Ca/PO_4 molar ratio of the solid had increased to 1.67 ± 0.01 , equalling that for pure hydroxyapatite and the Ca in solution had been considerably reduced from 10 per cent to about 2 per cent of the total Ca available in the solution at the start of the reaction. As before, no phosphate was detected in the reaction solution. An overall Ca/PO_4 molar ratio in slight excess of 1.67 was employed in these experiments to ensure that the final ratio of the solid was independent of the availability of solution Ca. The pH of the reaction solution, on the other hand, dropped from 10.5 to a value of 9.8 immediately on mixing and remained at this value during the entire period of crystallization. The crystal-chemical events described here were greatly accelerated when the reaction was conducted at 30.5°C instead of 25°C .

The ageing of freshly precipitated calcium phosphate as prepared under conditions described in this article can be divided into three stages (Fig. 2). The first stage, which commences immediately on mixing and continues for about 5 h, is characterized by an amorphous-like diffraction pattern which does not undergo any overt changes. Stage two is marked by the progression from a barely discernible apatitic profile superimposed on the amorphous pattern to a fully developed apatite pattern with the complete disappearance of any underlying amorphous features. The third stage covers the period following total conversion of the amorphous phase to a crystalline apatite and is characterized by the constancy of the 002

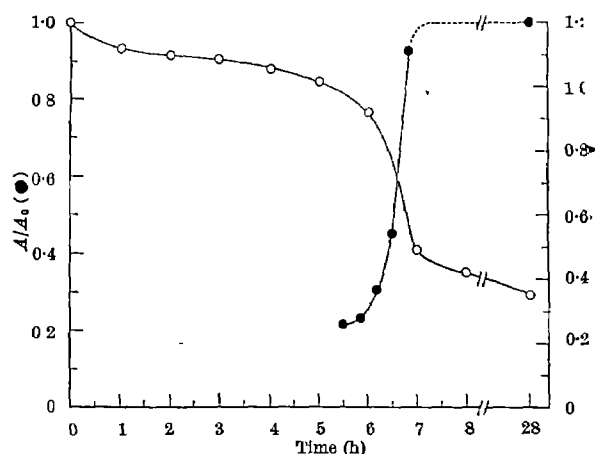


Fig. 2. The curve connecting the closed circles shows the per cent conversion of non-crystalline to crystalline calcium phosphate (A/A_0) as a function of time. The plot connecting the open circles indicates the drop in solution Ca concentration (mg Ca/ml.) with time.

peak area. This final stage commences about 7 h after the reaction is initiated, and continues indefinitely.

Despite the apparent constancy in the diffraction pattern, the first stage is not chemically static. Slight but significant decreases in solution Ca occur during this period (from 1.20 mg/ml. to 1.02 mg/ml.; Fig. 2). Moreover the demarcation between stages one and two is arbitrarily based on the initial appearance of a discrete apatite diffraction pattern. Since the 002 peak area at the beginning of the second stage is already about 1/5 the value achieved at maximum conversion, it is clear that about 20 per cent of the amorphous material has been converted to the crystalline phase by the time stage two can be detected. Thus, crystallographic as well as chemical changes are taking place in this initial period. Nearly coincident with the emergence of a discernible diffraction profile, however, is a more rapid decrease in solution Ca levels (from 1.02 mg/ml. at the beginning of stage two to 0.42 mg/ml. at the end; Fig. 2). In addition, the 002 diffraction peak, once discernible, proceeds to develop at a rate commensurate with the accelerated rate of fall in solution Ca levels, so that the remaining 80 per cent of pre-apatite converts over a much shorter interval than the initial 20 per cent. One can conclude, then, that while no fundamental distinction may exist between stages one and two, a distinction between rates of conversion can be made with validity, with the crystal phase formation taking place at a much accelerated pace in the second stage than in the first. The constancy of the 002 crystallinity values up to the end of the second stage suggests that the rate of conversion of the amorphous phase in stage two depends on the nucleation of new crystals rather than the growth or perfection of pre-existing crystals.

The most characteristic feature of the third stage is the gradual increase in crystallinity. The improvement in hydroxyapatite crystallinity during this period is typical of the gradual enlargement of mean particle size of many low-solubility precipitates as a result of re-precipitation in solution. This growth of the precipitate, known as Ostwald ripening, is generally attributed to the dissolving of the smaller, more soluble crystals and re-deposition of the dissolved material on the growing faces of the larger, less soluble crystals⁵. No doubt this re-crystallization is in progress from the earliest stages of the conversion, but the time interval in stage two is too short for this gradual increase in crystal dimensions to have a measurable effect on the crystallinity values measured during this period. The gradual decrease in solution Ca from 0.42 to 0.20 mg/ml. during this final stage indicates that a perfection of the crystalline phase parallels the crystal growth.

To describe the initial phase as 'non-crystalline' is to imply only that there has been insufficient development of

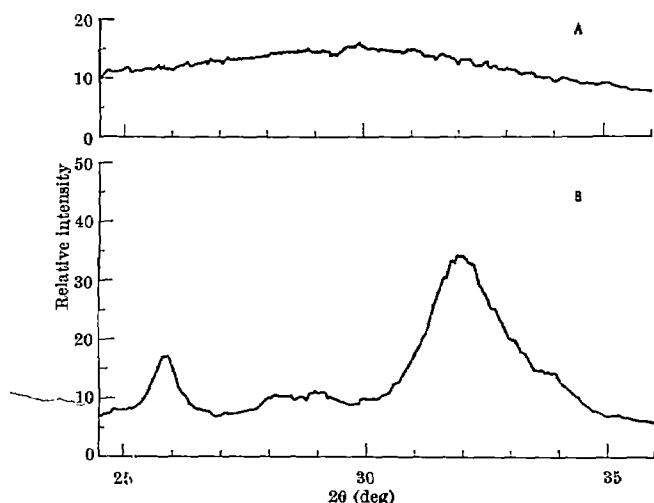


Fig. 1. X-ray diffraction patterns of (A) non-crystalline and (B) crystalline (apatitic) calcium phosphates (copper $K\alpha$ radiation).

angular, repetitive, spatial array of ions to define a crystal structure. This does not preclude the possibility of alized order and structure. Indeed, the fact that the lar Ca/PO_4 ratio does not vary under a given set of tial conditions suggests a chemically well-defined local uctural unit.

The formation of 'non-crystalline' calcium phosphate is netic phenomenon. The rapid mixing of the reaction utions creates strong interactions between Ca^{++} and PO_4^{--} ions leading to irregular co-ordination complexes ge enough in size to separate from solution. The bsequent temperature-dependent transformation into atite indicates that this initial phase is isothermally etastable with respect to the more ordered apatite onfiguration. In addition, the sigmoid-shaped relationship the transformation with time strongly suggests that

the conversion mechanism is autocatalytic. It is possible that the interfacial surfaces of the emerging crystalline phase act as sites for heterogeneous nucleation, thereby accelerating the rate of conversion. In this regard, the 'non-crystalline' calcium phosphate remains stable indefinitely if kept dry, pointing out the necessity for water for the process of nucleation to take place in this system.

This work was supported in part by U.S. Public Health Service grant *DE-01945* from the National Institute of Dental Research.

¹ Hayek, E., and Stadlmann, W., *Angew. Chem.*, **67**, 327 (1955).

² Gee, A., and Deitz, V. R., *Anal. Chem.*, **25**, 1320 (1953).

³ Warren, B. E., *J. Appl. Phys.*, **12**, 375 (1941).

⁴ Posner, A. S., Kanes, E. D., Hesper, R. A., and Zipkin, I., *Arch. Oral. Biol.*, **8**, 549 (1963).

⁵ Wagner, C., *Z. Elektrochem.*, **4E**, 581 (1961).

EFFECTS OF SEEDING ON THE CONDENSATION OF ATMOSPHERIC MOISTURE IN NOZZLES

By DR. E. R. BUCKLE* and DR. A. A. POURING†

Yale University, New Haven, Connecticut

A PRELIMINARY investigation has been made of the effects of inorganic smokes on the condensation of atmospheric moisture in the wind tunnel. The operation of the tunnel, and earlier results obtained with nadulterated air, have been described elsewhere^{1,2}. Theoretical calculations had indicated^{3,4} that chance impurities such as dust particles could not interfere detectably with homogeneous nucleation of the moisture unless present in concentrations of 10^6 particles per c.c. or greater. It was thought desirable to test this experimentally.

In the experiments, smokes of AgCl , AgI , CdI_2 , CuBr , KI , and PbI_2 were generated in the atmospheric inlet of the wind tunnel by evaporation of the compounds on 30-ohm heater coils of nichrome wire. Evaporation rates at various supply voltages were measured by the periodical weighing of test sections of the coils loaded with salt. From 90 to 150 V, the useful range for smoke generation, evaporation was steady and reproducible with a rate of the order of $5 \mu\text{g}/\text{sec}/\text{cm}$ of coil.

Smoke particles were collected for examination by supporting microscope slides and electron microscope grids in the air flow about 10 cm downstream of the smoke generator. The deposit which built up on the support of the pressure probe in the test section was also sampled. Methods of examination included optical and electron microscopy and X-ray diffractometry. Microscopy disclosed a variety of particle sizes. At high heater voltages particles of up to 1μ diam. were present, but at low voltages only sub-micron particles occurred. A useful idea of the relative abundance of small and large particles could be gained with the optical microscope, but the electron microscope was more informative about the structure. In AgI smoke generated at 140 volts there were considerable numbers of 1μ coagula containing up to 20 particles of 0.01μ diam. or less. At 120 V there were fewer large aggregates, sizes below 0.1μ being more frequent. Coagula were rare in smoke produced at 100 V and most of them consisted of only two particles. Nearly all the remaining particles were less than 0.01μ in diam., and even the aggregates were smaller than 0.1μ . It is evident that the total concentration of particles in the smokes increased rapidly as the voltage was lowered because the mass evaporation rate was much the same at

all voltage settings. In cloud chamber experiments it has also been found that AgI aerosols tend to contain coagula when the generating temperature is high⁵.

The course of condensation was followed in two nozzles (Nos. 4 and 6) by measuring the static pressure with an axial probe¹. The air temperature at the inlet was $24^\circ \pm 2^\circ \text{C}$. Mass flow rates of dry air in the nozzles were 972 and 1,110 g per sec respectively. The maximum speed of dry air in the fast nozzle (No. 4) occurred at a Mach number of 1.8. The temperature gradient in this nozzle was approximately 14°C per cm. In the slow nozzle (No. 6) maximum speed was attained at a Mach number of 1.6, the temperature gradient being about 2°C per cm. Other experimental details are given in Table 1.

Typical results are shown in Figs. 1 and 2, where the ratio of the local static pressure, p , to the supply total pressure, p_{01} , is plotted against the position of the probe along the nozzle axis. Positive deviations from the isentropic curves, that is, 'humps', are the result of heat evolved during the condensation of moisture. A level of heat addition exists above which there occurs a normal shock, and this gives rise to a marked peaking in the pressure curves. This critical heat addition depends on the Mach number of the flow in the vicinity of the condensation zone^{1,2}. The positions of shocks in supercritical runs are indicated in the figures.

Seed addition caused the hump to be flattened and displaced downstream. The effect was produced by all compounds and was most drastic in the slow nozzle (curves 644, 645 in Fig. 1 and curves 592, 594 in Fig. 2). In both nozzles the extent of the flattening and displacement decreased as the humidity was lowered. Increase in the voltage of smoke generation also caused a decline in the effect (curves 593, 594 in Fig. 2), presumably through the reduction in the total concentration of seed particles.

Table 1. EXPERIMENTAL DETAILS

Exp.	Nozzle	Relative humidity* (%)	Seed	Heater voltage
601	4	27.0	—	—
602	4	27.0	—	100
644	4	48.0	—	—
645	4	48.0	AgI	100
603	4	74.0	—	—
604	4	74.0	AgI	110
674	4	80.5	KI	110
582	6	19.0	—	—
581	6	19.0	AgI	120
578	6	34.0	—	—
608	6	34.0	CuBr	110
592	6	45.5	—	—
593	6	45.0	AgI	140
594	6	45.5	AgI	90

* At atmospheric inlet.

* On leave (1964) from the Department of Chemical Engineering and Chemical Technology, Imperial College of Science and Technology, London, S.W.7.

† Present address: Department of Engineering, U.S. Naval Academy, Annapolis, Md., U.S.A.

Conditions for the onset of the seeding effect could not be determined exactly because of the lack of precise control over humidity and smoke concentration. It began at an inlet moisture-level between 27 and 48 per cent relative humidity in nozzle 4 and between 19 and 45 per cent relative humidity in nozzle 6 (Figs. 1 and 2). The exact level was probably highest in the fast nozzle (No. 4). Irregular pressure readings obtained at 34 per cent relative humidity in nozzle 6 (curve 698) were possibly connected with the threshold of the seeding effect, but this needs clarification. From the mass flow rate of air and the evaporation rate of the smoke compound it is calculated that about $10^3/D^3$ smoke particles per c.c. of air were present in the intake, where D is the particle diam. in μ . For the given sizes this means that the seeding effect was observable at concentrations of 10^8 particles per c.c. and above.

The shape of the residual condensation hump in a seeded run, and the location and visual structure (schlieren pattern) of the shock wave (if any) in the hump, were closely similar to those in an un-seeded run at a lower humidity (curves 663, 674 in Fig. 1 and curves 578, 594 in Fig. 2). This leads to the following conclusions. In a seeded run moisture condenses on the smoke particles upstream of the hump, the quantity so condensed depending on the smoke concentration and the flow conditions imposed by the nozzle. The hump is caused by spontaneous condensation, and this process is substantially independent of the preceding heterogeneous process.

It is possible to compare in a rough way the amounts of catalytically and spontaneously condensed moisture by matching humps for seeded and un-seeded runs. Two suitable examples occurred fortuitously in the present

experiments. Curves 663 and 674 (Table 1) show that about one-fifth of the water condensed out on the KI nuclei in nozzle 4 at an inlet humidity of 80.5 per cent relative humidity. The moist air took 120 μ sec to travel down the nozzle from the point at which saturation was reached to the point where the hump began, cooling through 57° C on the way. Curves 578 and 594 show that in nozzle 6 at an initial humidity of 45.5 per cent relative humidity some two-fifths of the moisture condensed out on the AgI. In this case the air cooled through 28° in 400 μ sec while flowing between the saturation point and the hump. Bearing in mind the fall in pressure p/p_0 , decreasing from 0.9 to 0.5 in the first example and from 0.5 to 0.4 in the second, it is seen that termination of the heterogeneous process by the homogeneous occurs sooner the more rapid the expansion.

The fact that the isentropic curve is indistinguishable from the moist air curve in the region where condensation occurs only on the seed shows that this condensation is far more gradual process than the homogeneous one. In the first process, a fixed number only of artificially established nuclei are available, while in the second much larger numbers of natural centres are possible. Apart from the lowering of available moisture the presence of 10^8 seed particles per c.c. has little or no effect on spontaneous condensation, as appears likely from the present results, numbers of self-nuclei exceeding 10^8 per c.c. must build up very rapidly in the beginning of the hump. In fact, since growth of seeded condensate particles is supplanted in this region, multiplication of spontaneous centres rather than their growth is indicated. While nucleation continues in the hump the homogeneous condensate probably consists of particles smaller than the seed (that is, less than 0.01 μ diam.).

The discrepancy between the maxima in curves 663 and 674 suggests that at high-supply humidities the presence of seeded condensate may interfere with homogeneous nucleation. It should be borne in mind, however, that the possibility of disturbance in the flow by the condensation shocks present in these runs throws some doubt on the accuracy of the pressure readings downstream of the shocks. The evaluation of any interference between the condensation processes from pressure data alone would therefore be difficult for super-critical runs.

A reversal of the trend towards sharper humps with higher humidities was observed in un-seeded nozzle expansions of very humid air. This was gas-dynamical in origin², but it is still possible that the inversion shown by curves 664 and 674 was contributed to by differences in AgI and KI as seeding materials. Apart from this, there were no obvious differences in any of the compounds studied.

The high activity of AgI as a catalyst for ice nucleation in water and water vapour has been attributed⁶ to the close matching of the crystal structures. This proposal was recently questioned⁷ on the basis of the limited adsorptive power of AgI for water vapour and the role of surface topography⁸ in heterogeneous ice nucleation. Experiments on the crystallization of ice from water under pressure have since confirmed that for a given substrate material the polymorphic form most closely resembled in structure by the substrate is preferentially nucleated, even when it is metastable⁹.

The chemical influences on the nucleating properties of different compounds are still not clear, and it may be asked whether the results recorded here lead to any new conclusions. The central problem would seem to be whether an ice or a water nucleus first forms on the seed. The X-ray evidence showed that AgI smokes containing large particles were crystalline with the hexagonal structure. Distinction between the hexagonal and cubic forms was obscured for small-particle smokes by the poor resolution of peaks in the diffractometer recordings. Manson¹⁰, whose mean particle size corresponded to the largest single particles in the present smokes (0.1 μ),

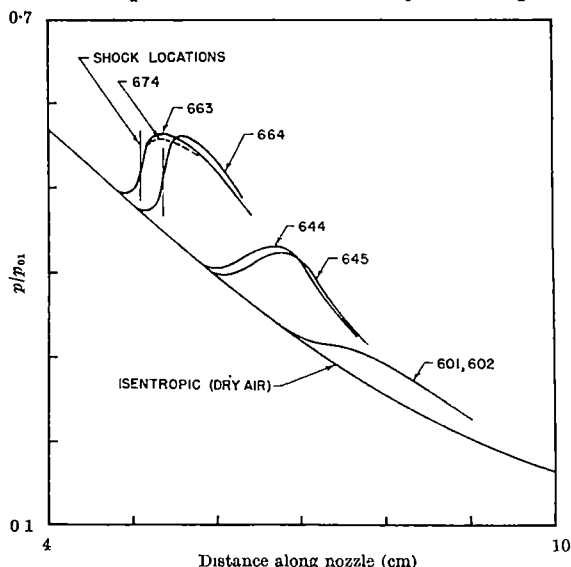


Fig. 1. Static pressure ratios for fast nozzle (No. 4) sonic point at 4.5 cm). Curve numbers refer to experiments in Table 1

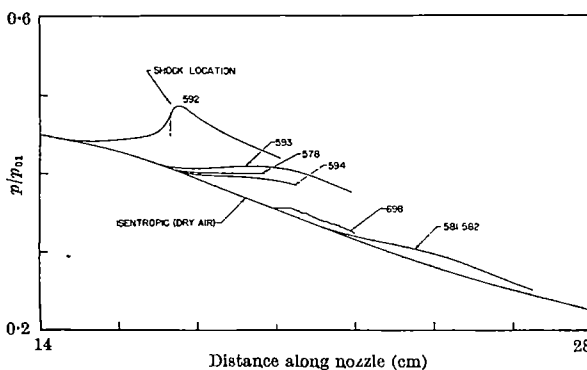


Fig. 2. Static pressure ratios for slow nozzle (No. 6) sonic point at 8.0 cm). Curve numbers refer to experiments in Table 1

erved that the hexagonal AgI structure gradually loses the cubic as the volatilization temperature is raised. Morphological changes accompanied by an increase in particle size have since been observed in a variety of condensation aerosols on raising the temperature generation^{5,11}. It may be concluded that the hexagonal form of AgI was more abundant in the high-temperature zones. If the nozzle condensate begins as two-dimensional ice nuclei, the greater seeding power of AgI smoke generated at low temperatures would imply that the effect of a decrease in the proportion of hexagonal material is outweighed by the increase in the particle concentration. Alternatively, the topographical requirements for adsorption are likely to be a good deal less stringent generally than those for ice nucleation. The apparent similarity in heterogeneous activity of all the compounds, with the implied unimportance of equilibrium crystal structure, might be more easily explained if, under the conditions of a nozzle expansion, the initial step was the formation of a water layer on the seed.

To recall our main conclusions, it is confirmed that sufficient heterogeneous nuclei are present in normal air to interfere with the homogeneous condensation of water vapour in the wind tunnel. Seeding with foreign particles in concentrations of 10^6 per c.c. or above has a marked effect when the humidity in the intake is raised above

a certain level dependent on the nozzle flow. Under moist climatic conditions, reduction of humidity by seeding makes it possible to study homogeneous condensation in shock-free flow. The experiments provide some idea of the conditions under which spontaneous and catalysed condensation of water vapour occurs competitively. To put this on a quantitative basis to test, among other things, theories of nucleation and growth in aerosols requires further work to establish exact relations between seed concentration, inlet conditions, and flow variables.

This work was partially sponsored by the Power Branch, U.S. Office of Naval Research, Washington, D.C., under contract Nonr-609(47). We thank G. D. Stein for his assistance.

¹ Wegener, P. P., and Pouring, A. A., *Phys. Fluids*, **7**, 352 (1964).

² Pouring, A. A., *Phys. Fluids* (in the press).

³ Oswatitsch, K., *Jahrb. Deutsch. Luftfahrtforsch.*, **1**, 703 (1941).

⁴ Wegener, P. P., and Mack, L. M., *Adv. App. Mechanics*, **5** (Academic Press, Inc., New York, 1953).

⁵ Buckle, E. R., and Hooker, C. N., *Trans. Faraday Soc.*, **58**, 1939 (1962).

⁶ Vonnegut, B., *J. App. Physics*, **18**, 593 (1947).

⁷ Zetlemoyer, A. C., Teheurekjian, Noubar, and Chessick, J. J., *Nature*, **192**, 653 (1961).

⁸ Mason, B. J., *Faraday Soc. Disc.*, **30**, 20 (1960).

⁹ Evans, L. F., *Nature*, **206**, 82E (1965).

¹⁰ Manson, J. E., *J. App. Phys.*, **36**, 423 (1955).

¹¹ Buckle, E. R., in *Condensation and Evaporation of Solids*, edit. by Rutner et al. (Gordon and Breach, New York and London, 1964).

METAL CONCENTRATION PROFILES IN OXIDE SCALES ON NICKEL-10.9 PER CENT COBALT ALLOY

By Dr. G. C. WOOD and J. M. FERGUSON

Corrosion Science Division, Department of Chemical Engineering, Faculty of Technology, University of Manchester

FOR many years it has been widely accepted that when nickel is oxidized in oxygen at high temperature a simple, single-phase, single-layer scale of NiO grows on its surface according to a parabolic relationship. The NiO is supposed to be a metal-deficient, *p*-type semiconductor growing by the outward movement of electrons and Ni²⁺ ions to form fresh oxide at the scale/oxygen interface¹. Cobalt behaves similarly above 900° C, but the CoO scale grows much more rapidly², an observation in agreement with measured self-diffusion coefficients of the metal ions in their respective oxides³⁻⁵. Recently, however, the situation has been shown to be more complex because double-layer scales, each consisting of the single oxide, have been found on both metals^{6-13,15}, with the consequence that ideas of oxygen ion or gas movement through parts of the scale have been invoked. The present state of knowledge has recently been reviewed elsewhere¹⁴.

Now, nickel-cobalt alloys, which are homogeneous and single-phase, also produce either single or double layers of a single oxide solid solution (Ni,Co)O when oxidized above 900° C—cobalt being only slightly preferentially oxidized^{14,15}. This almost unique alloy oxidation situation provides an excellent opportunity for following the concentration profiles in alloy and scale directly by electron-probe microanalysis at various stages of growth. The method has problems of its own, but it has some advantages over marker experiments and certain tracer techniques. Such an investigation is reported here because it provides new precise evidence for oxidation of the alloy, with possible interpolation of the results to the case of the pure metals.

Annealed specimens of Ni-10.9 per cent Co, of purity in excess of 99.95 per cent, $2.5 \times 0.6 \times 0.066$ cm, were electropolished at 0.7 amp/cm² (2.5 V) in 60 per cent sulphuric acid (s.g. 1.6) at 30° C for 1 min. After drying, they were immediately oxidized in pure oxygen in a silica-spring thermobalance for 15, 30 min and 1, 2, 3, 6, 10, 24 and 48 h respectively at 1,000° C, followed by cooling over

a 15-min period to minimize scale cracking. Other short-term specimens were simply exposed in flowing oxygen in the hot zone of a furnace for 10 and 15 min respectively without slow cooling. The latter specimens were examined directly in plan in the microanalyser whereas the former were prepared metallographically in cross-section before analysis.

All the specimens gave reasonably reproducible growth kinetics with a close, if not quite exact, agreement with the parabolic relationship (Fig. 1). The growth rate was the same on specimens given a 5-sec etch in 50/50 (v.) glacial acetic acid/nitric acid after electropolishing and was only slightly less on specimens mechanically polished on 600 g silicon carbide paper. The mean parabolic rate constant for the electropolished specimens was 4.9×10^{-10} g²cm⁻⁴sec⁻¹.

Each specimen displayed in section a uniform, single-layer single-phase oxide, generally adherent to the alloy

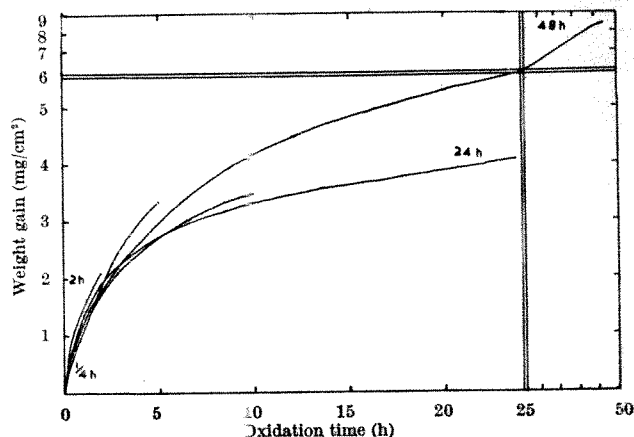


Fig. 1. Weight gain/time curves for Ni-10.9 per cent Co oxidizing in oxygen at 1,000° C

and with no observable colour change through its depth (Fig. 2). Internal oxide particles were present, but were few in number. The outer surfaces of all specimens examined in plan were blue-grey with characteristic topographical features which will be discussed elsewhere. It is likely that oxide colour in this system is characterized more by grain size than by chemical composition.

Typical microanalyses for the thin scales examined in plan are given in Fig. 3. Since for both films the total wt. per cent metal curves would lie within a few per cent of 78 per cent, the X-ray source was clearly entirely enclosed within the scale over the kilovoltage range used, namely 15–35 kV. As the kilovoltage was reduced, the source became located to a greater extent in the outer region of the scale (the source sizes giving 95 per cent of the radiation were about 0.8, 1.1, 1.5, 1.8 and 2.2 μ at 15, 20, 25, 30 and 35 kV respectively). The results prove that when the surface scale was formed on this alloy it was immediately enriched in cobalt. They also appear to show that this enrichment at the outer surface was greater after 15 min than after 10 min. This could be genuine, but an alternative explanation is that at all

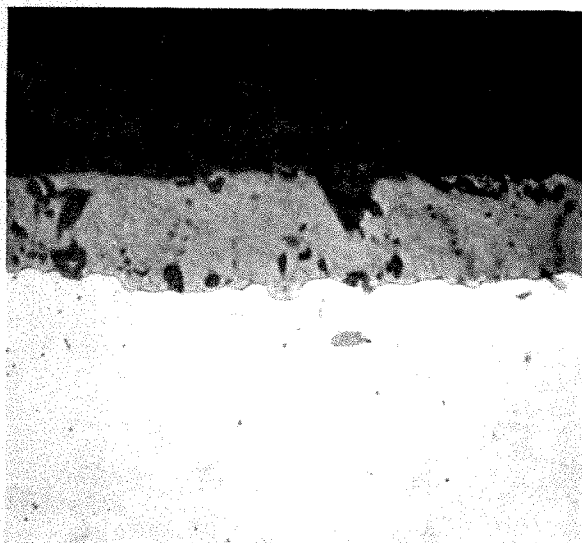


Fig. 2. Typical cross-section through scale on Ni-10.9 per cent Co oxidized in oxygen for 3 h at 1,000°C ($\times 1,000$)

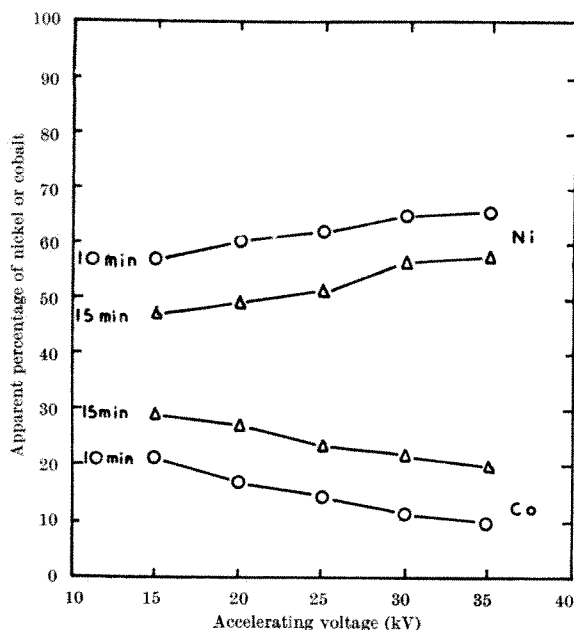


Fig. 3. Examination of thin scales on Ni-10.9 per cent Co in plan by electron-probe microanalysis

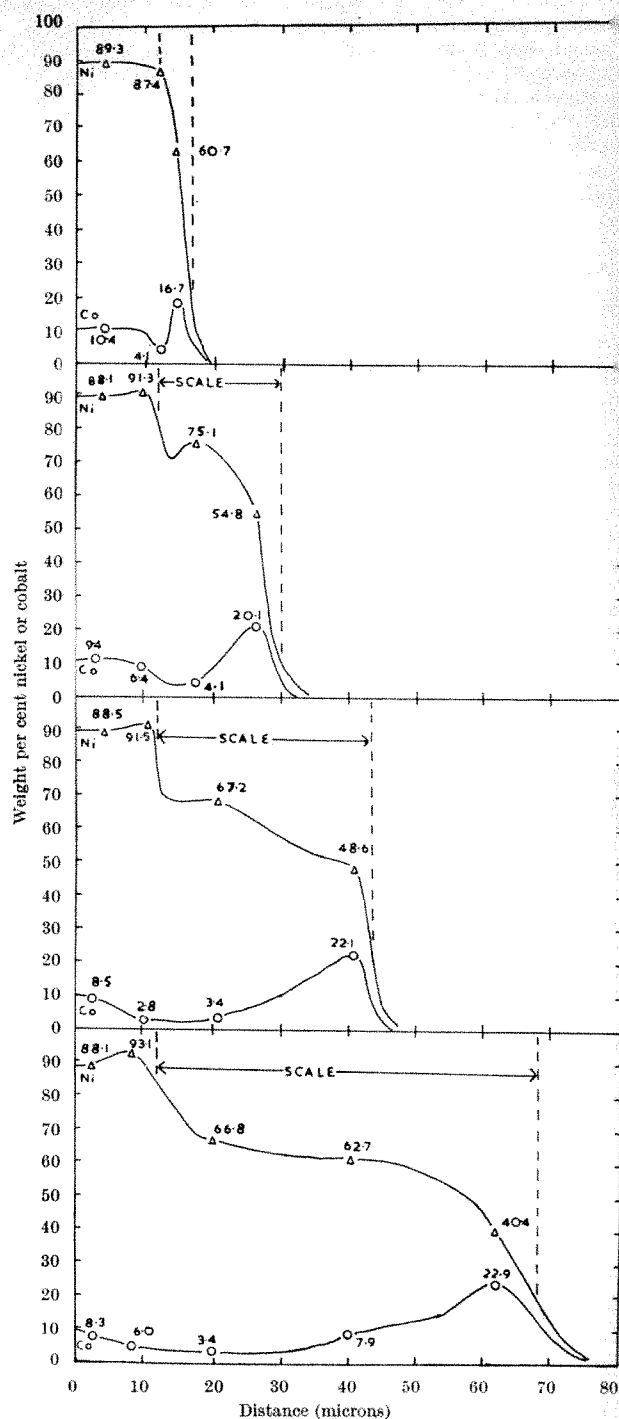


Fig. 4. Distribution of nickel and cobalt through alloy and scale for Ni-10.9 per cent Co oxidized in oxygen at 1,000°C. Top to bottom: 15 min, 2 h, 24 h, 48 h

kilovoltages more of the nickel-rich inner region was incorporated in the source for the 10-min specimen because the outer region was quite thin. Plan measurements of this kind permit a more accurate analysis of the outer micron or so of scale than do measurements in section because of their greater resolution.

This general situation is confirmed in Fig. 4, where concentration profiles in cross-section at various stages of oxidation are presented. The profiles are smooth with a pronounced cobalt peak, apparently containing from 16.7 to 22.9 per cent cobalt, near the outer surface. This value is probably rather low, due to relief polishing problems near the outer interface and to the lower resolution of the microanalyser under these conditions

measurements were made at 25 kV). An obvious observation is the similarity in shape of the cobalt and nickel profiles in the films of different thickness, almost as though the cobalt peak were merely growing a longer tail extending to the alloy/oxide interface. Closer scrutiny, however, shows that the outer cobalt-rich peak is in fact growing in thickness and possibly in cobalt content, although the latter effect was small and obscured by the reasons given above. Measurement of the areas under the curves shows that the percentage cobalt in the scales lay between 20.4 and 11.1 per cent, but this varied in a slightly irregular manner with time, probably due to minor measuring errors, and is being studied further. The alloy was depleted in cobalt and enriched in nickel over a small distance.

It is possible to draw several other conclusions from this investigation, some definite but others more tentative and requiring further thought and experiments which are in progress:

(1) The reasonable agreement with a parabolic growth relationship may not be particularly meaningful because of the large compositional variations through the scales. Presumably the cobalt-rich regions offer less resistance to the movement of the diffusing species than the nickel-rich regions because they probably contain a larger percentage of cation vacancies. This is further proof of the need to be careful when carrying out kinetic studies of alloy oxidation. On the other hand, the present situation is really an extreme case of one previously reported for the oxidation of pure cobalt⁵, where it was inferred from marker experiments that the parabolic relationship held even for a non-linear vacancy distribution through CoO. Since the compositions of scale in contact with the alloy and atmosphere respectively did not change much with time in the present case, at least after the first hour and probably from a much earlier stage, it is perhaps reasonable to conclude that the cation vacancy concentration difference across the scale remained constant, which was the only requirement of this type specified in Wagner's original derivation of the parabolic relationship.

(2) As mentioned earlier, at first sight it appears that after the initial cobalt-rich peak was rapidly established, it was pushed further and further from the alloy, fresh oxide being formed near the alloy/oxide interface. This conclusion would support early ideas^{16,17} and more recent revolutionary ones¹⁸ for oxygen ion movement in the scale. However, the gradual growth in thickness and possibly cobalt content of the outer cobalt-rich peak could

only be achieved by the outward diffusion of Co²⁺ ions. There is therefore strong evidence for scale growth by cation movement in the present case, supporting most of the previous evidence with the pure metals. The cobalt peak was constantly reformed, the cobalt and nickel profiles being determined partly by the relative diffusion rates of the cations and partly by thermodynamic factors. It also appears that there was a general outward diffusion of ions and that newly ionized metal did not pass right through the film. Although Co²⁺ ions diffuse about 100 times as fast in CoO as do Ni²⁺ ions in NiO, the closeness of the ionic radii suggests that there should not be any vast difference in their rate in any given composition of (Ni,Co)O. The present direct measurement of concentration profiles in growing scales has certain obvious advantages over marker experiments and tracer techniques, at least in the present system.

(3) Scales on the pure metals almost certainly grow in the same way, by cation movement¹.

(4) Since concentration profiles through single-layer and double-layer scales are very similar¹⁵, this possibly suggests, but does not prove, that they grow by the same mechanism. This would involve cation movement outwards in the inner layer rather than oxygen gas diffusion inwards.

We thank Prof. T. E. Ross for providing facilities and for his advice, Mr. B. W. Lambert for his collaboration with the electron-probe microanalysis and the Science Research Council for the provision of a maintenance grant to one of us (J. M. F.).

¹ Wagner, C., and Gruenewald, K., *Z. Phys. Chem.*, B, **40**, 455 (1938).

² Moore, W. J., and Lee, J. K., *J. Chem. Phys.*, **19**, 255 (1951).

³ Shim, M. T., and Moore, W. J., *J. Chem. Phys.*, **26**, 802 (1957).

⁴ Lindner, R., and Åkerström, Å., *Faraday Soc. Disc.*, **23**, 133 (1957).

⁵ Carter, R. E., and Richardson, F. D., *J. Metals*, **6**, 1244 (1954); **7**, 336 (1955).

⁶ Berry, L., and Paidassi, J., *C. R. Acad. Sci., Paris*, **258**, 2810 (1964).

⁷ Pettit, F. S., and Felten, E. J., *J. Electrochem. Soc.*, **111**, 135 (1964).

⁸ Sartell, J. H., and Li, C. H., *J. Inst. Metals*, **90**, 92 (1961-2).

⁹ Preece, A., and Lucas, G., *C. Inst. Metals*, **61**, 219 (1952).

¹⁰ Czerski, L., and Franik, F., *Arch. Gornictwa i Hutnictwa*, **3**, 43 (1955).

¹¹ Vasyutinitskiy, B. M., and Kartmazov, G. N., *Fiz. Metal. i Metalloved.*, **15**, No. 1, 132 (1963).

¹² Mrowec, S., and Werber, T., *Acta Met.*, **8**, 819 (1960).

¹³ Birks, N., and Rickert, H., *J. Inst. Metals*, **91**, 308 (1963).

¹⁴ Wood, G. C., Wright, I. G., and Ferguson, J. M., *Corrosion Science* (in the press).

¹⁵ Wood, G. C., and Wright, I. G., *Corrosion Science* (in the press).

¹⁶ Valensi, G., *J. Metallurgie Italiana*, **42**, 77, 104 (1950).

¹⁷ Valensi, G., *Métaux et Corrosion*, **25**, 283 (1950).

¹⁸ Arkharov, V. L., and Blankova, Y. E., *Phys. Met. Metal.*, **10**, 62 (1960).

STRUCTURE OF THE QUASI-STELLAR RADIO SOURCE 3C 273 B

By M. J. REES and Dr. D. W. SCIAMA

Department of Applied Mathematics and Theoretical Physics, University of Cambridge

IN this article we show that: (1) the radio variations¹ of 3C 273 B are consistent with its red shift being cosmological; (2) the variable component of the optical continuum² of 3C 273 B may be partly due to inverse Compton collisions between relativistic electrons and their own synchrotron radiation. In this case the optical variations would be correlated with the radio variations, though with a phase delay.

The problem involved in (1) is to reconcile (a) the upper limit on the size of the source derived from the period of the variations³; (b) the lower limit on the angular diameter of the source derived from the absence of self-absorption down to some particular frequency^{4,5} (assuming the synchrotron mechanism and that the electrons are radiating incoherently); (c) the distance derived from the red shift of the optical counterpart of the radio source. This problem first arose with the quasi-stellar source CTA 102, which Sholomitsky⁶ reported to vary with a period of about 100 days at 940 Mc/s. In this case the distance

derived from the red shift of 1.037 (ref. 7) is ~1,500 times greater than the upper limit permitted by (a) and (b). We recently proposed⁸ a model of CTA 102 which would resolve this discrepancy, but recognized that it required a rather improbable geometry and so was very implausible. Since then Maltby and Moffet⁹ have reported that CTA 102 did not vary appreciably at 970 Mc/s over a 3-year period ending about 2 years before Sholomitsky's observations began. While it is possible that CTA 102 varies sporadically, the more likely inference is that Sholomitsky's observations are in error.

The same problem arises with the quasi-stellar source 3C 273 B, the flux density of which at 8,000 Mc/s was found by Dent¹ to have increased by about 40 per cent in 3 years. This source shows no self-absorption in its spectrum down to at least 200 Mc/s (ref. 10) (in fact the spectrum is flat down to this frequency). For a magnetic field strength of 10⁻² gauss this implies a lower limit on the angular diameter of 0.03 sec (while the observed¹¹

angular diameter is 0.5 sec). If the period of the radio variations is comparable with that of the optical variations, that is ~ 12 years, the size of the radio source cannot exceed 3 pc and its distance cannot exceed 20 Mpc. On the other hand, the red shift of 0.158 (ref. 12) implies a distance of 470 Mpc.

The discrepancy would be resolved if the varying part of the source had an angular diameter $\sim 10^{-3}$ sec. We therefore propose¹³:

Model I. This model consists of a source of angular diameter 0.5 sec and (roughly constant) flux density ~ 25 flux units down to at least 200 Mc/s, in the centre of which is a varying source of angular diameter 1.3×10^{-3} sec (corresponding to a linear diameter of 3 pc), and at minimum a flux density ~ 2.5 flux units down to the frequency ν_a at which self-absorption sets in. Such self-absorption would not show up in the observed spectrum of this composite source. For a magnetic field H gauss in the central source, $\nu_a \sim 4,000 H^{1/2}$ Mc/s. For simplicity we assume that the central source varies because of an increase in its relativistic electron flux, while its angular diameter and magnetic field are unchanged. If the total flux density of the source is doubled at high frequencies at maximum, the flux from the central source must increase by a factor ~ 10 . Thus the frequency ν_b at which self-absorption sets in at maximum is given by $(\nu_b/\nu_a)^{2.5} \sim 10$, so $\nu_b \sim 10,000 H^{1/2}$ Mc/s.

We can now predict the time variations that would be observed at various frequencies. For simplicity we take the central source to vary sinusoidally at high frequencies. For $\nu > \nu_b$ no self-absorption occurs, so the observed variation would be sinusoidal. In the intermediate range $\nu_b > \nu > \nu_a$ the flux density would at first increase sinusoidally, but when it reaches the level at which it would be self-absorbed at that frequency the increase would be cut off, and the flux density would remain constant until it decreases again in the second half of the cycle. For $\nu < \nu_a$ the variations would be negligible.

A lower limit on H (the value of which has not yet been specified) can be obtained if we require that the energy density of relativistic particles shall not exceed the magnetic energy density. If only ~ 1 per cent of the total energy is in the electrons, we find that the field strength cannot be much less than 1 gauss. If $H = 1$ gauss, ν_a and ν_b have the values $\sim 4,000$ and $\sim 10,000$ Mc/s respectively, and the expected variations at 8,000 Mc/s are large enough to account for the observations. Our estimates for ν_a and ν_b are only rough, but they indicate what might be expected, and the measurement of their values and of the variations at intermediate frequencies would be a useful test of the model and would provide considerable information about the structure of the source.

It is not known how electrons attain relativistic energies in radio sources. The generation process may occur throughout the volume of the source, or the electrons may be ejected from a massive object at its centre. It is easily seen that the electrons must be produced by a mechanism of the former kind if the source in fact resembles Model I. The reason is that the lifetime of an electron which radiates at, say, 10^4 Mc/s in a field of 1 gauss is only $\sim 5 \times 10^6$ sec, which is much less than the time taken to cross the source, even for an electron moving at the speed of light. If such an electron were ejected from the centre it would not reach the edge, unless it were emitting radiation at a frequency at which the source was opaque. Thus for frequencies exceeding ν_a our discussion of the variations will be incorrect. To allow for this we now introduce:

Model II. In this model we assume that the electrons are all generated in a very small region (with diameter < 0.1 pc), which presumably contains a massive object. As in Model I, nearly all the flux at frequencies $< \sim 4,000$ Mc/s is assumed to come from a halo, and we only concern ourselves with an intense spherically symmetric core with radius ~ 1 pc, in which the variable higher frequency

radio flux is supposed to originate. We assume the $H \sim 1$ gauss throughout this region, and that its radiation emission has a flux density $S(\nu)$ which may be variable. For each ν we can determine the minimum radius $R(\nu)$ of a sphere from which $S(\nu)$ could come, if it in fact comes from a region which is opaque at all frequencies up to ν . We find that $R(\nu) \sim 0.3 (S(\nu))^{1/2} (\nu/10^4)^{-1.25}$ parsecs, where S is measured in flux units and ν in Mc/s. This radius can be compared with the distance $r(\nu)$ which an electron radiating at frequencies $\sim \nu$ would travel in its lifetime assuming that it moves with speed $\sim c$. This is $\sim 5 \times 10^{-2} (\nu/10^4)^{-1/2}$ pc. If $R(\nu) > r(\nu)$, electrons moving outward from the centre cannot radiate freely without producing a radiation field the brightness temperature of which a frequency ν is higher than their kinetic temperature. Therefore they will conserve most of their energy until they reach a distance $\sim R(\nu)$ from the centre, where they can radiate freely.

The available data do not enable us to specify the flux density of the core precisely, but it is consistent with the observations to take $S(\nu)$ to be 10–30 flux units in the frequency range from 5,000 up to $\sim 10^5$ Mc/s. $R(\nu)$ is then several times greater than $r(\nu)$ throughout this range, and so we conclude that the radiation at frequency ν comes from the surface of a sphere of radius $R(\nu)$.

If electrons are ejected from the centre at a steady rate, $S(\nu)$ and $R(\nu)$ will adjust themselves to values which depend on the energy spectrum of the input electrons, and the flux of the source will be constant. If, however, the rate of input of electrons alters, $R(\nu)$ (and consequently $S(\nu)$) will change after the lapse of an interval of the order of the time for light signals to travel a distance $R(\nu)$. Thus changes in the behaviour of the massive object will cause changes in the radio flux. The maximum rate of increase of flux will be attained if $R(\nu)$ expands with speed $\sim c$. An increase of $S(8 \times 10^3)$ from 2.5 to 25 flux units, which corresponds to an increase in $R(8 \times 10^3)$ from ~ 0.6 to ~ 1.9 pc, could therefore certainly occur rapidly enough to explain Dent's observations. We can also predict, on the basis of this model, that more rapid variations could occur at higher frequencies. Furthermore, if a sudden change in the rate of injection of particles produces changes in $S(\nu)$ at different frequencies, we would expect to observe the variations at higher frequencies before those at lower (even in the absence of intergalactic dispersion¹⁴).

We have seen that, if the core emits a significant amount of radiation at, say, 10^5 Mc/s, this radiation must come mainly from a sphere which is opaque up to that frequency. The total energy density of all the radio frequency radiation up to 10^5 Mc/s within it will be $\sim 6 \times 10^{-3}$ ergs/c.c. (compared with $\sim 10^{-6}$ ergs/c.c. within Model I), and this very high value suggests that inverse Compton scattering might be important. The Compton lifetime of an electron of energy $\gamma m_e c^2$ in this radiation field is $8 \times 10^9/\gamma$ sec. If $S(10^5) \sim 25$ flux units, $R(10^5) \sim 0.1$ pc and the time which an electron takes to drift out of this sphere is $\sim 2 \times 10^7$ sec if its outward velocity $\sim c/2$. But the electrons radiating at 10^5 Mc/s have $\gamma \sim 300$, and so their Compton lifetime is of the same order. The most energetic photons would result from the scattering of 10^5 Mc/s photons by electrons with $\gamma \sim 300$, and their frequency would be $\sim 10^{10}$ Mc/s, which is in the ultra-violet range. Photons of lower energy (including the whole visible range) would be produced, and the spectrum of the scattered radiation would have a low-energy cut-off at a frequency depending on the smallest value of γ represented in the electron energy spectrum. The energy radiated by this process in the visible range may well be greater than that of the synchrotron radio emission. The exact relative importance of the Compton and synchrotron losses is very sensitive to the high-frequency cut-off in the radio spectrum (which we have taken as 10^5 Mc/s) to the value of H , to the precise position where the electrons are accelerated, and to their mean rate of outward drift. It would there-

re clearly not be worth while to base an exact calculation on this crude model.

The foregoing rough arguments do, however, suggest that at least a part of the visible light emitted by 3C 273 B may have been produced by Compton scattering. Moreover, such a hypothesis would provide a natural explanation for the observed optical fluctuations of the source, since, if electrons are accelerated in the massive object in regular bursts, there will be variations in the intensity of the scattered light. Nearly all the scattering occurs in region of radius ~ 0.1 parsecs (since not only the radiation energy density, but also the particle density, is much higher near the centre). Therefore, fluctuations with time-scales of the order of months, or even less may occur, and these are in fact observed¹⁶. The long period variations³ (~ 12 years) in the optical luminosity may also arise from periodic variations in the rate of injection of electrons, which, as we have already seen, can produce the observed variation at radio frequencies. This model therefore suggests that the radio and the long-period optical variations may be connected.

The increase in the radio flux observed by Dent coincided with a decrease in the optical luminosity, which had the most recent of its 12-yearly maxima in 1962. But according to this model one would expect periodic radio variations to lag (perhaps by several years) behind the variations at the centre which cause them, so this fact also accords with our model, and suggests that the radio variations, when they have been observed for longer, will also turn out to have a period ~ 12 years.

The visible light from 3C 273 B is unpolarized¹⁷, whereas the radio flux (at $\sim 3,000$ Mc/s) is ~ 3 per cent polarized¹⁸. It will not be possible to find out whether the central part of the source is polarized at radio frequencies until the extent to which the degree of polarization changes when the flux varies has been examined. However, even if the radio flux from the core were polarized (implying large-scale uniformity in the direction of H), it would not follow that the scattered visible light must appear polarized, since it is mainly produced in an extremely opaque region, where the radio-frequency radiation will not be polarized even if the field is uniform.

It has been assumed in the foregoing that H does not change significantly when the density of the relativistic particles alters. This will be a good approximation if the magnetic energy is greater than the particle energy (a strength of 1 gauss is sufficient). The field is presumably 'anchored' to the massive object.

It should be emphasized that the Compton losses are significant in this model mainly because of the occurrence of synchrotron self-absorption, which prevents the electrons from radiating away their energy as fast as they otherwise would. Any electrons which are accelerated to high energies ($\gamma > 300$, say), and which therefore emit synchrotron radiation at frequencies which can escape freely from the source, will lose their energy in a time much shorter than the Compton lifetime, so they will contribute to the radiation at high radio frequencies ($> 10^5$ Mc/s), rather than in the far ultra-violet ($> 10^{10}$ Mc/s).

Model III. The first two models have taken no account of the fact that lines are present in the optical spectrum of 3C 273 B. According to Greenstein and Schmidt¹⁹, these lines could be produced by gas of density $\sim 10^7$ particles/c.c., temperature $\sim 2 \times 10^4$ K, and mass $\sim 10^6 M_\odot$. Though this gas need not radiate all the optical continuum, it must be incorporated in a complete model of the source. The model which we shall now describe indicates one way in which this can be done.

If the gas were in the form of a uniform spherical cloud, its diameter would be ~ 0.5 pc, and it would be opaque to light of all frequencies because of scattering by free electrons. It would therefore be difficult to account for the observed fluctuations in optical luminosity with a time-scale of a few months, since if the variable flux were

emitted by a small region within the cloud its variations would be smeared out and would not be observed. This difficulty would be eased if the density within the cloud were non-uniform, or if it had a filamentary structure²⁰, but there would remain the problem of feeding energy into the gas to balance its losses.

In an alternative configuration, suggested by Shklovsky²¹, the gas is distributed in a thin spherical shell. We shall adopt this suggestion, and show that a model can be constructed on the basis of which the radio variations, and both the long and short period optical variations, can be explained.

If the radius of the shell is taken²¹ as $4 \cdot 10^{18}$ cm, and its density as $\sim 10^7$ particles/c.c., its thickness will be $\sim 10^{17}$ cm if its total mass is $\sim 10^6 M_\odot$. The observed broadening of the spectral lines places an upper limit of $\sim 1,500$ km/sec on the velocity of the shell if it is expanding. If there is a mass of $\sim 10^6 M_\odot$ at its centre, a field of ~ 1 gauss would be strong enough to prevent the shell collapsing. Scattering by free electrons would be unimportant at all frequencies. The shell will be transparent to most photons of optical frequency, but will be opaque to radio waves at all frequencies below $\sim 10^5$ Mc/s because of free-free absorption. The observed radio flux must therefore originate outside the shell.

The gas in the shell would produce the observed line emission, and would radiate at a rate $\sim 10^{46}$ ergs/sec, mainly at visible wave-lengths. Its thermal energy content is insufficient to maintain this rate of energy loss for more than a few weeks, and so it must be absorbing energy at an equal rate from the interior. Since the shell absorbs radio waves, intense but unobserved radio radiation may exist in the spherical region within it, and this suggests the possibility that the shell may be absorbing enough energy in the form of radio waves to compensate for its losses in the visible range. This would require the production of $\sim 10^{46}$ ergs/sec at frequencies below $\sim 10^5$ Mc/s, which is ~ 30 times as great as the observed power radiated in this frequency range. However, such a high rate of energy production is not unreasonable since a sphere within which synchrotron self-absorption were taking place at all frequencies up to 10^5 Mc/s would need to have a radius of only ~ 0.8 pc (little more than half the radius of the shell) to emit $\sim 10^{46}$ ergs/sec. Alternatively the required amount could be produced if the whole interior were opaque up to $\sim 7 \times 10^4$ Mc/s. The exact situation within the shell will depend on the energy spectrum of the electrons injected into it, but a sufficiently high rate of energy generation will be achieved if relativistic electrons with $\gamma < 300$ are produced at a rate $\sim 10^{46}$ ergs/sec. We must assume that there is a cut-off in the electron energy spectrum for $\gamma > 300$, since otherwise there would be intense radiation at frequencies above 10^5 Mc/s which would escape through the shell and produce observable radiation with a higher flux-density than is observed.

The observed radiation at frequencies below 10^5 Mc/s (apart from the component which comes from the halo) is, according to this model, emitted outside the shell by electrons which have escaped from the interior. An electron ejected from the massive object at the centre will be absorbing and emitting radiation at the same rate until it approaches the shell. Since the synchrotron lifetime of the electrons (in the absence of self-absorption) is of the order of the time which they take to pass through the shell, most electrons will lose their energy before reaching the exterior. Thus there radiation will not be observed directly, but will simply heat the gas. However, since the power of the observed radio radiation is much less than the power required to heat the gas, the observed radio flux would be produced even if only a few per cent of the electrons succeeded in escaping. Despite the high density of the gas in the shell, an electron passing through it has a 90 per cent chance of reaching the exterior without losing a significant amount of energy in ionization and

collisional losses. If the field ~ 1 gauss extends outside the shell, these electrons will radiate away their energy before they have travelled a further distance ~ 0.1 pc unless self-absorption occurs outside the shell as well. Such self-absorption will occur at sufficiently low frequencies, though the situation is somewhat complicated by the fact that the electrons can radiate energy inwards into the shell.

The production of the visible light is more complicated. Much of it, both in spectral lines and in the continuum, comes from the gas in the shell. However, Compton scattering may also, as in Model II, produce radiation at optical frequencies. The radio radiation energy density in the volume enclosed by the shell is $\sim 2 \times 10^{-3}$ erg/c.c. if synchrotron self-absorption occurs at frequencies up to $\sim 7 \times 10^4$ Mc/s, and the corresponding Compton lifetime of an electron is $2 \times 10^{10}/\gamma$ sec. The highest energy electrons the synchrotron losses of which are balanced by self-absorption have $\gamma \sim 250$, and their Compton lifetimes are of the same order as the time they would take to travel outwards from the centre to the shell. The Compton losses, therefore, are significant, though their exact magnitude is highly sensitive to the precise model adopted. The frequencies of the scattered photons will, as in Model II, be mainly in the visible and near-ultra-violet, and most photons will escape through the shell, though those the frequencies of which correspond to intense spectral lines may be absorbed. Compton scattering of radio frequency radiation outside the shell will be insignificant.

We now consider how the observed variations in flux can occur in this model. We discuss first the long period variations. If there were fluctuations with a time-scale ~ 12 years in the rate of production of relativistic particles at the centre, there would be changes in the radiation density in the sphere enclosed by the shell, and a consequent change in the temperature of the gas. The rate of radiation of visible light by the shell would therefore alter. There would also be changes in the rate at which electrons escape through the shell, and consequently in the observed radio flux. Variations with periods less than, say, 5 years would be partly smeared out and so could not have a large amplitude.

The observed short period fluctuations in the optical luminosity have a time-scale of a few months, and obviously cannot be produced in the shell, which has a radius of ~ 4 light years. In Model II they were attributed to the inverse Compton effect. Since we have shown that light will be produced by Compton scattering in this model, a similar explanation might also be possible here. This would require the scattering to occur in a region smaller than a few light months in radius. However, the scattering will not be concentrated in such a small region as in Model II, where the radiation energy density was high enough for scattering to be significant only within a sphere of radius ~ 0.1 pc. Nevertheless, if all the electrons are ejected from a massive object, their number density will be higher near the centre, and the amount of scattering will be greater there even if the radiation density is no higher. Consequently, the variations in the component of the optical continuum produced by the Compton effect may be sufficiently rapid to account for the observed variations with time-scales of a few months.

We note that whereas the line strengths will alter with the slow period ~ 12 years, it would be inconsistent with this model for them to be involved in the rapid fluctuations in optical luminosity. We understand that attempts are now being made to see whether the lines do in fact vary in intensity. This feature of our model will thus soon be tested.

If we could construct a complete model of this kind, we would be able to calculate the expected phase lag between the variations in luminosity at optical frequencies and the related radio variations. However, we lack sufficient information to enable us to do this. To illustrate the complexity of the problem we enumerate some of the

factors which would determine the magnitude of the lag.

(1) The variations in particle density and radiative energy density propagate outwards with different velocities.

(2) The phase delay of the radio variations will depend on whether self-absorption is taking place outside the shell at the frequency of observation. If it is taking place the observed flux would come from the surface of a sphere with radius greater than 1.5 pc, rather than from just outside the shell, thus increasing the lag of the radio variations. At $8,000$ Mc/s, the frequency of Dent observations, self-absorption would occur if more than ~ 14 flux units of the observed flux density were coming from the compact source (rather than from the halo). Since the observed variations amount to 10 flux units (and this flux cannot come from the halo), it is likely though not certain, that self-absorption is in fact occurring.

(3) The sense in which the emission rate of the gas in the shell varies as the additional radio energy falls on it must be known.

(4) The relative intensity of the light produced by the inverse Compton effect and the light emitted by the shell must be known. These two components will have the same period, but may be out of phase with one another. The times at which maxima in the resultant optical luminosity occur will thus depend on the phase lag between the two components, and on the ratio of the amplitude of their variations.

This discussion shows that existing observations do not suffice to determine a well-defined working model of 3C 273 B. However, we can draw the important conclusion that neither the optical nor the radio variations require the source to be closer to us than the 470 Mpc implied by a cosmological red shift of 0.158 . This conclusion considerably weakens the case for the local model^{3,22} of quasi-stellar radio sources which places them a few megaparsecs away and in which the observed red shift implies a large velocity relative to their surroundings. More detailed observations of optical variations, and of radio variations as a function of frequency, should enable us to decide whether models of the type described here are appropriate for 3C 273 B, and for other quasi-stellar sources as well.

Note added in proof. H. Gent and H. P. Palmer reported at the Dublin meeting of the Royal Astronomical Society (September 7, 1965) that they have succeeded in resolving 3C 273 B at 21 cm with a long base-line interferometer. Their proposed interpretation of their observations is that the source consists of two components separated by ~ 0.4 sec of arc, the angular diameter of each component being less than ~ 0.1 sec. This limit on the angular size of the components is still much greater than the angular diameter of the variable core in our model ($\sim 10^{-3}$ sec), and of the lower limit of 3×10^{-2} sec for the halo.

¹ Dent, W. A., *Science*, **148**, 1458 (1965).

² Smith, H. J., *Quasi-stellar Sources and Gravitational Collapse*, 221 (Univ. Chicago Press, 1965).

³ Terrell, J., *Science*, **145**, 918 (1964).

⁴ Sligh, V. I., *Nature*, **199**, 682 (1963).

⁵ Williams, P. J. S., *Nature*, **200**, 56 (1963).

⁶ Sholomitsky, G. B., *I.A.U. Inf. Bull. Variable Stars*, No. 83 (Feb. 27, 1965).

⁷ Schmidt, M., *Astrophys. J.*, **141**, 1295 (1965).

⁸ Rees, M. J., and Sciama, D. W., *Nature*, **207**, 738 (1965).

⁹ Maltby, P., and Moffet, A. T., *Astrophys. J.*, **142**, 409 (1965).

¹⁰ Dent, W. A., and Haddock, F. T., *Quasi-stellar Sources and Gravitational Collapse*, 381 (Univ. Chicago Press, 1965).

¹¹ Hazard, C., Mackey, M. B., and Shimmins, A. J., *Nature*, **197**, 1037 (1963).

¹² Schmidt, M., *Nature*, **197**, 1040 (1963).

¹³ Sciama, D. W., *Proc. Intern. School of Physics 'Enrico Fermi', Course 35*, July 12-24, 1965 (to be published).

¹⁴ Haddock, F. T., and Sciama, D. W., *Phys. Rev. Letters*, **14**, 1007 (1965).

¹⁵ Feenberg, E., and Primakoff, H., *Phys. Rev.*, **73**, 449 (1948).

¹⁶ Sandage, A., *Astrophys. J.*, **139**, 416 (1964).

¹⁷ Moroz, V. I., and Yesipov, V. R., *I.A.U. Inf. Bull. Variable Stars*, No. 31 (1963).

¹⁸ Morris, D., and Berge, G. L., *Astro. J.*, **69**, 641 (1964).

¹⁹ Greenstein, J. L., and Schmidt, M., *Astrophys. J.*, **140**, 1 (1964).

²⁰ Schmidt, M., *Proc. Second Texas Conf. Relativistic Astrophysics* (to be published).

²¹ Shklovsky, I. S., *Soviet Astronomy*, **8**, 638 (1965).

²² Hoyle, F., and Burbidge, G. R. (to be published).

LETTERS TO THE EDITOR

ASTROPHYSICS

Rotation Period of the Planet Mercury

THE recent radar measurements of Mercury indicate at the period of rotation of the planet is 59 ± 5 days¹. This result is in complete disagreement with the previously noted value of 88 days based on the visual observations of the markings on Mercury²⁻⁶. In this communication we show that the same visual observations can not only be reconciled with the radar-determined rotation period of Mercury but, in addition, can be used to derive an improved value for the period of rotation of the planet, namely, 58.4 ± 0.4 days.

We have examined nearly 50 drawings of Mercury published by Lowell², Antoniadi³, Lyot⁴, Dollfus⁵, and Baum⁶. Of these, six pairs of drawings show near duplication of markings and phase.

	Time-interval <i>T</i> (days)
1) Antoniadi (August 11, 1924) and Antoniadi (June 21, 1927)	1,105
2) Antoniadi (August 23, 1927) and Antoniadi (August 6, 1928)	349
3) Antoniadi (October 4, 1927) and Antoniadi (August 23, 1929)	689
4) Antoniadi (August 6, 1928) and Antoniadi (July 20, 1929)	348
5) Lyot (July 22, 1942) and Dollfus (October 12, 1950)	3,004
6) Baum (March 15, 1952) and Baum (March 1, 1953)	351

Duplication of markings and phase for any single pair of drawings of Mercury does not necessarily indicate that synchronous rotation of the planet is the only possible

solution. A number of other periods of rotation are possible as given by the following equation:

$$P \approx \frac{T}{n + \frac{\theta}{360}}$$

where P is the period of rotation of Mercury in days, T the time-interval between the two observations, n the number of complete rotations of the planet, and θ is the average angular displacement of the earth and Mercury in their orbits, with respect to the stars, in time T . Fig. 1 shows all possible values of P between 50 and 70 days as calculated for each pair of drawings. It is noted that in addition to an 88-day period (not shown in Fig. 1) there are at least three more values of P , namely, 50.1, 58.4 and 70.2 days which will be consistent with all the six pairs of drawings. However, only one of these values is within the allowed limits of radar results (59 ± 5 days). Therefore the rotation period of 58.4 ± 0.4 days is consistent with both the visual and the radar observations of Mercury.

Peale and Gold⁷ have pointed out that because of the large eccentricity of the orbit of Mercury, the tidal torque will be greatest at the perihelion, and the planet will acquire a rotation period lying between 56.6 and 88 days. Our value of 58.4 ± 0.4 days is very close to the lower limit, indicating a significant amplitude dependence of the tidal dissipation on Mercury.

We thank Dr. A. G. W. Cameron for discussions.

W. E. MCGOVERN

Department of Meteorology
and Oceanography
New York University,
New York.

Airborne Instruments Laboratory,
Melville, New York.

S. H. GROSS

S. I. RASOOL

Institute for Space Studies,
National Aeronautics and
Space Administration,
Goddard Space Flight Center,
New York.

¹ Pettengill, G. H., and Dyce, E. B., *Nature*, **206**, 1240 (1965).

² Lowell, P., *Mem. Amer. Acad. Arts and Sci.*, **12**, 431 (1902).

³ Antoniadi, E. M., *La Planète Mercure* (Paris: Gauthier Villars, 1934).

⁴ Lyot, E., *Astronomie*, **67**, 3 (1943).

⁵ Dollfus, A., in *Planets and Satellites*, Kuiper, G. P., and Middlehurst, B. M., eds. (Chicago Univ. Press 1961).

⁶ Baum, R. M., in *The Planet Mercury*, by Sandner, W. (Macmillan Co., New York, 1963); and plate II in *A Guide to the Planets*, by Moore, P. (Norton and Co., Inc., New York, 1960).

⁷ Peale, S. J., and Gold, T., *Nature*, **206**, 1240 (1965).

Tidal De-spin of Planets and Satellites

RECENTLY, Peale and Gold¹ have shown that the non-synchronous rotation of Mercury is likely to be a consequence of tidal friction. They point out that in an eccentric orbit the spin of an axially symmetric planet will not relax to the orbital mean motion, but instead will approach a final value which is somewhat larger. The final spin rate will be somewhere between the mean orbital angular velocity and the orbital angular velocity at perihelion. The precise value for the final spin is determined by the condition that the net tidal torque on the planet around each orbit be equal to zero. The spin rate at which this condition is satisfied is determined by the frequency and amplitude dependence of the planet's 'Q' ($1/Q$ is the specific dissipation function²). According to Peale and Gold: "The condition discussed here is based on the supposition

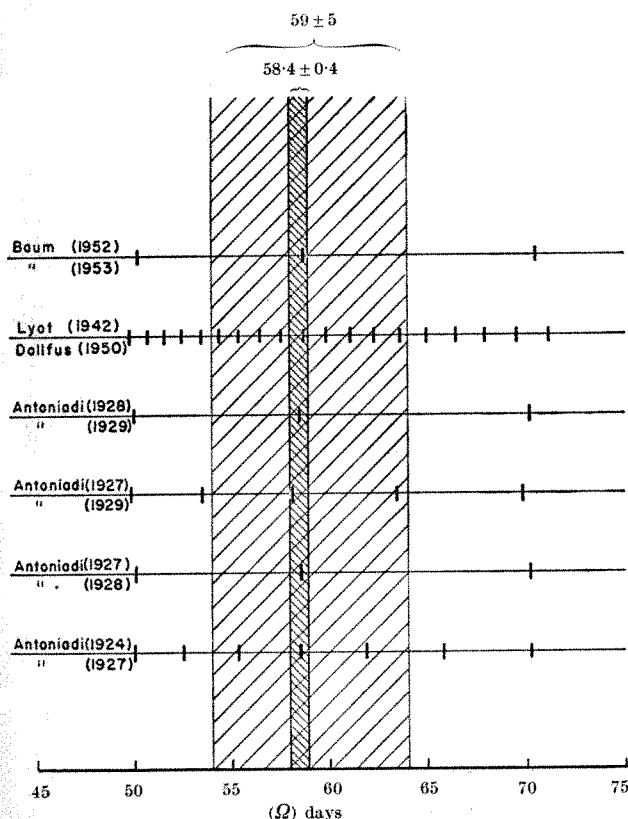


Fig. 1. Rotation periods of the planet Mercury, in days, as derived from six pairs of drawings. The single-hatched area shows the limits in the rotation period allowed by the radar observations of Mercury. Visual observations indicate a value of 58.4 ± 0.4 (double-hatched area).

that the solar torque exerted on the tidal bulge exceeds that exerted on any permanent deformation from axial symmetry. In the converse case a period of 88 days for the rotation would indeed result." This last statement, if true, would imply that Mercury's principal moments of inertia, in the plane perpendicular to its spin axis, differ by less than a few parts in 10^7 . This value is very small when compared with the values known for the Moon—another solid, slowly rotating body.

I have re-examined the conditions which determine which of the two final states of rotation tidal friction will bring about. While the conclusions of this investigation cannot be expressed in exact analytical form, good approximations do exist in the limiting case of low orbital eccentricity. These conclusions do not agree with those expressed by Peale and Gold in the statement already quoted here, except for the case of an axially symmetric planet.

As mentioned previously, the vanishing of the net torque on an axially symmetrical planet (the rotation and symmetry axes are assumed to be coincident with the normal to the planet's orbit plane) when averaged over an orbit leads to a final value for the spin, s , which is somewhat larger than the mean motion n . If the figure of the planet has a sufficiently small permanent deformation from axial symmetry, the only new feature which arises is an oscillation of s about its value for the axially symmetric case. Otherwise, if this deformation is large enough, s will eventually begin to oscillate about n and tidal friction will cause the amplitude of this oscillation to decay. Quantitatively, the division between a sufficiently small and a sufficiently large permanent deformation occurs at:

$$\frac{B-A}{C} \approx \frac{2}{3} \left(\frac{s-n}{n} \right)^2 \quad (1)$$

where s is the final value for the spin in the case of axial symmetry. In equation (1), C denotes the moment of inertia about the spin axes while A and B are the smaller and larger of the principal moments about axes perpendicular to the spin axis. For completeness, it should be mentioned that if the initial spin rate of a planet is smaller than its orbital mean motion (this includes retrograde spins) tidal friction may produce synchronous rotation unless the planet's tidal deformation is of the order of its permanent deformation from axial symmetry.

The Moon is unique in the solar system in that its spin has obviously been affected by tidal friction and the value of $(B-A)/C$ for its figure is known. Therefore, for the Moon we may ask the following question: If in its present orbit the Moon were given a much more rapid spin, would tidal friction once again bring the Moon into synchronous rotation? The answer to this question appears to be: No. To arrive at this result we must first calculate $(s-n)/n$ for the Moon. In the special circumstance that Q is independent of both frequency and amplitude, it is easily shown that $\frac{s-n}{n} \approx 9.5e^2$. Taking $\frac{B-A}{C} = 2.05 \times 10^{-4}$ (ref. 3), we find

that the right-hand side of equation (1) is 2.75 times as large as the left-hand side. Thus the Moon would end up spinning with a mean period about 2.86 per cent shorter than its present orbital period and would not return to synchronous rotation. This brings up the question of how the Moon's rotation became synchronous in the first place. To answer this question we would need to know both how the Moon's orbital eccentricity and its principal moments varied in the past. Of course, little can be deduced about the Moon's figure and we shall assume that it has not changed. On the other hand, it is possible to calculate the change in the Moon's orbital eccentricity due to tides. This calculation shows that tides raised by the Moon on the Earth act to increase the eccentricity while tides raised on the Moon tend to decrease it^{4,5}. Without knowledge of the Moon's Q no definite conclusions can be drawn about the sign of de/dt . However, it seems likely that de/dt is positive and e was smaller in the past. This might explain

why the Moon has a synchronous rotation since equation (1) would be satisfied for $e = 0.043$, 22 per cent below present value. Of course, once the Moon attained synchronous rotation this condition would be maintained even though the orbital eccentricity increased. Similar conclusions hold if the Moon's Q is dependent on frequency and amplitude so long as this dependence is not unusually severe.

Equation (1) allows us to derive an upper limit $\frac{B-A}{C}$ for Mercury. The value we obtain (again assuming to be independent of amplitude and frequency) is 0.1 which is undoubtedly much larger than the actual value.

PETER GOLDBREICH

Department of Astronomy and
Institute of Geophysics and Planetary Physics,
University of California, Los Angeles.

¹ Peale, S. J., and Gold, T., *Nature*, **206**, 1240 (1965).

² MacDonald, G. J. F., *Rev. Geophys.*, **2**, No. 3 (1964).

³ Jeffereys, H., *Mon. Not. Roy. Astro. Soc.*, **122**, No. 5 (1961).

⁴ Goldreich, P., *Mon. Not. Roy. Astro. Soc.*, **126**, No. 3 (1963).

⁵ Kaula, W. M., *Rev. Geophys.*, **2**, No. 4 (1964).

PHYSICS

Laboratory Observations of Plasma Instabilities in a Dipole Magnetic Field

THERE have been a number of laboratory experimental investigations concerning the nature of plasma motion in a dipole magnetic field. Recently results have been reported concerning an investigation of a stable trapped plasma belt in the field of a small, permanent, uniform magnetized sphere¹.

Further investigations using a much larger sphere indicate the existence of plasma instabilities. The experimental arrangement is quite simple. A permanent magnetized sphere, 6 in. in diameter, is placed in a suitable large vacuum chamber and made a negative electrode while the wall of the chamber is made the positive electrode. A d.c. voltage of sufficient magnitude to break down the gas is then applied to the configuration.

When the gas breaks down, a number of plasma configurations occur. The first to be described is that of a stable, visible, plasma belt in the equatorial plane as shown in Fig. 1. As time progresses, an intense arc discharge occurs along a field line as shown in Fig. 2. When the arc occurs the visible belt disappears. The reason that the belt and two arcs appear in the photograph in Fig. 2 is because the picture is a time exposure. After a short time the arc is quenched and the stable belt reappears. The duration of the stable configuration

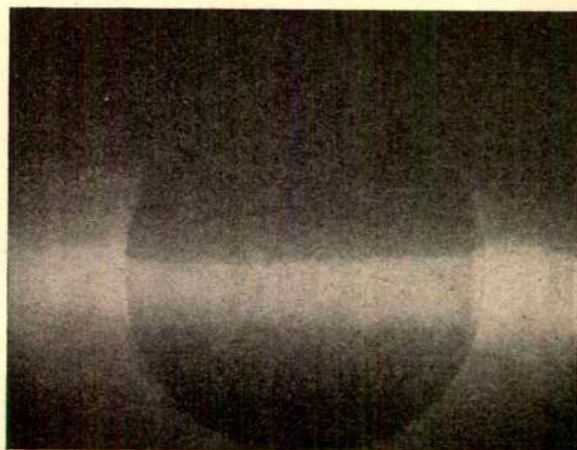


Fig. 1. Photograph showing stable equatorial plasma belt

increases with decreasing pressure and applied voltage. As the ambient pressure or the applied voltage is increased, both the stable belt and the arcs occur in a continuous manner. Such a condition is shown in Fig. 3.

The points of precipitation of the arcs also vary with applied potential. For large values of applied voltage the points all occur in equatorial regions as shown in Fig. 4.

Buneman² has discussed the various conditions under which instabilities in a streaming plasma can occur. Essentially the theory describes how the directed energy of an electron stream is dissipated in a plasma by collective collisions with ions. The theory treats both random fluctuations and fluctuations caused by the application of a constant field.

Swift³ has applied the theory to a particular model in which the stream is the equatorial ring current and a local electric field transverse to the magnetic field causes a perturbation. The model is applied to geophysical conditions, and the subsequent analysis suggests that, with a large enough ring current, an accelerating potential can be established along a field line provided the conductivity in that direction is not infinite. This potential then serves as an accelerating mechanism for auroral electrons.

Present experimental results indicate that the laboratory model is a particular case of instability arising from the action of a constant transverse electric field. The visible observations in the photographs certainly indicate that a stable configuration first becomes unstable, then returns to its former configuration. Additional evidence

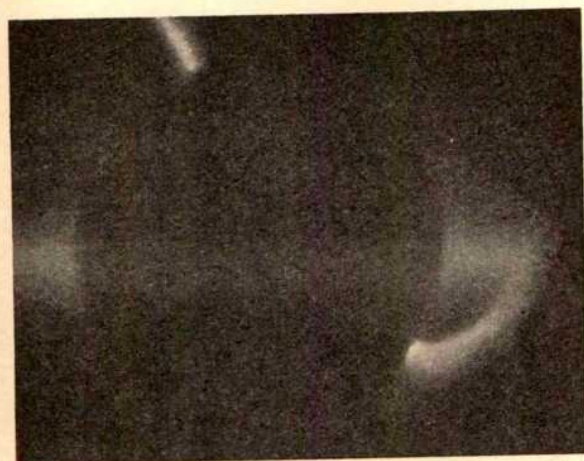


Fig. 2. Photograph showing arc instability. Two appear as a result of 5-sec exposure

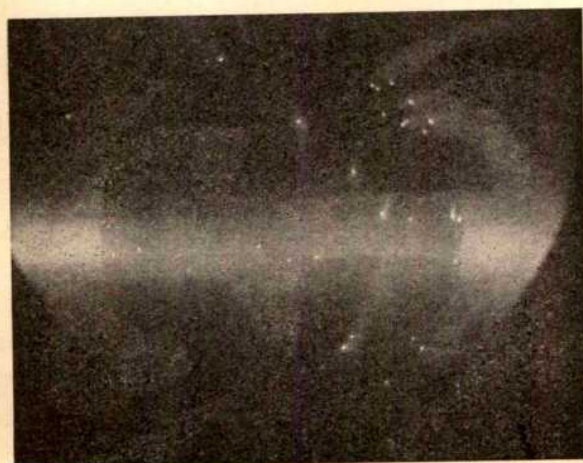


Fig. 3. Photograph showing more rapid occurrence of instabilities at higher pressure and voltage. (Darkened area is outline of viewport)



Fig. 4. Photograph showing constriction of precipitation points to low latitudes at high voltage. (Darkened area is outline of viewport)

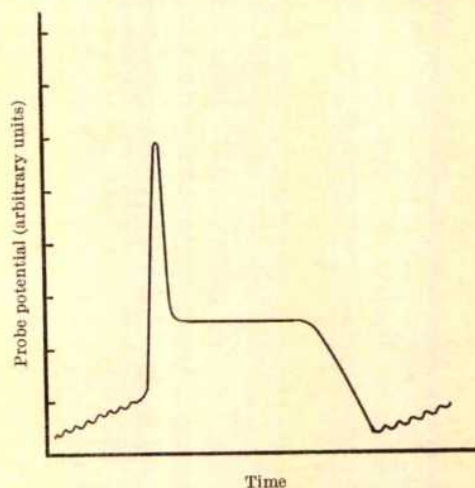


Fig. 5. Sketch of probe potential versus time. The sharp increase and decrease time-interval is of the order of microseconds. The constant voltage time-interval is approximately 100 msec, while the decrease to initial conditions lasts approximately 50 msec. The wavy portion which indicates the slow rise to instability is of the order of seconds

is also found in the measurement of the temporal dependence of the instability by another method. A Langmuir probe was placed in the stable belt in the equatorial plane. The potential of the probe with respect to the sphere was measured as a function of time. A sketch of a typical oscilloscope trace of the event which is remarkably reproducible in almost all its details is shown in Fig. 5. Starting with the stable configuration, the voltage is seen to increase slowly with time until a critical value is reached. At this potential there is a sharp rise by at least a factor of 5 and a sharp decrease to some value above the stable case. This sharp rise and fall occur in the order of microseconds. There follows a constant voltage regime which lasts for approximately 100 msec during which the arc is sustained. Then the voltage drops to the stable value in approximately 50 msec. The whole process is then repeated. It appears that with proper variation of pressure and voltage the stability conditions can be varied over a wide range.

The present experimental evidence, while incomplete and only moderately quantitative, suggests that much can be learned from further, more extensive, investigations which will include a complete mapping of the temporal and spatial dependence of the instabilities by measurement of electric and magnetic field variations as well as transient current systems. Such investigations are at present in progress.

While the model experiments do not accurately reproduce geophysical conditions it is hoped that a careful experimental study will lead to the formulation of a theory which can then be extended to explain various aspects of auroral phenomena.

ROBERT G. QUINN

Department of Space Science and
Applied Physics,
Catholic University of America,
Washington, D.C.

¹ Quinn, R. G., and Chang, C. C., *Trans. Amer. Geophys. Union*, April (1965).

² Buneman, O., *Phys. Rev.*, **115**, 503 (1959).

³ Swift, D., *J. Geophys. Res.*, **70**, 3061 (1965).

Sub-millimetre Wave Spectra of Polar Liquids

IN an earlier communication¹, it was reported that liquid phase absorption of the monohalogenated benzenes at a frequency $\approx 10^{12}$ c/s (1 teracycle) was higher than would be expected from the assumption that a simple Debye process was operative. The measurements supporting this conclusion were made using a CN maser source which gives only the single frequency 29.715 cm^{-1} (337 μ wave-length). We have now supplemented these observations by wide-band spectral investigations using Fourier transform interferometric techniques.

The lower part of Fig. 1 shows results for chlorobenzene in the spectral range 10–400 cm^{-1} . The sharp absorption features at 297 cm^{-1} and 195 cm^{-1} are known molecular vibration bands ν_{24} (B_1) and ν_{30} (B_2), respectively, and are the lowest frequency modes of the molecule². However, the main interest here is the region below 100 cm^{-1} , and in the upper part of Fig. 1 this region is plotted on a logarithmic frequency scale. The microwave observations are those of Poley³ and that of Garg and Smyth⁴, and the interferometric measurements confirm the earlier maser observation. It can be seen that there is a broad but strong feature reaching a maximum at a frequency of 45 cm^{-1} and that the peak is substantially greater than the asymptotic value to be expected at high frequencies from Debye's expression. These observations are believed to be the first reported of a complete band of this type though the high-frequency end of it is to be seen in the observations of Wyss *et al.*⁵. That such bands should exist is not surprising since they may be considered to arise from the

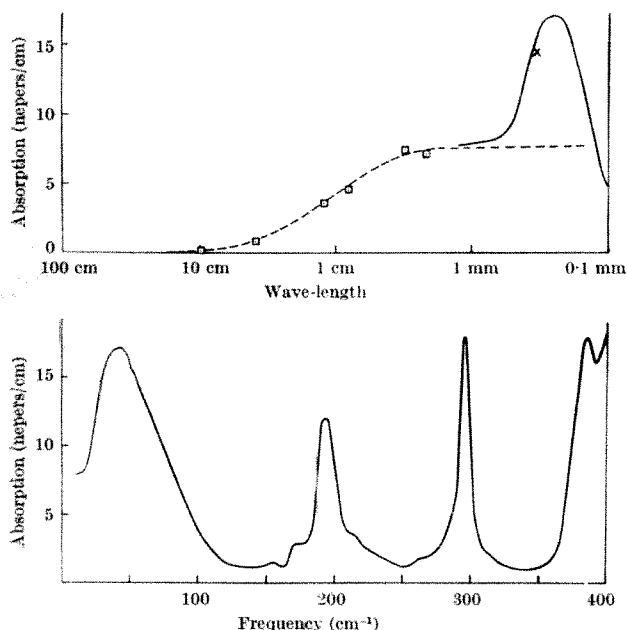


Fig. 1. Far infra-red spectra of liquid chlorobenzene. \square , Microwave observations; \times , teratron observation; ---, interferometer observations; ----, Debye behaviour

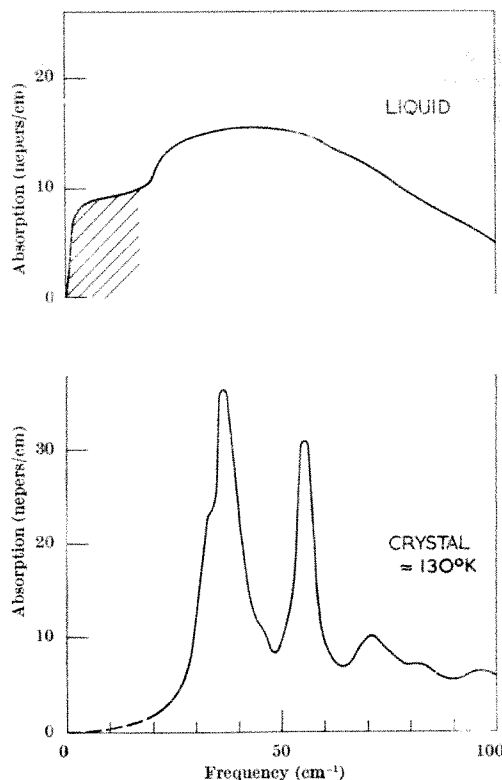


Fig. 2. Sub-millimetre absorption of chlorobenzene

'pseudo-lattice' modes of the liquid, and for this there is support from the observations shown in Fig. 2. This shows the absorption spectrum of solid chlorobenzene at a temperature of $\approx 130^\circ$ K with again the liquid spectrum (at room temperature) for comparison. The solid spectrum shows the relatively sharp features characteristic of a lattice spectrum, and it will be seen that these occur in the same frequency region as the diffuse band observed for the liquid. It should also be noted that the absorption in the region below 20 cm^{-1} for the liquid (shown cross-hatched) where Debye processes are dominant is absent in the solid, as is expected since it arises from the overall rotation of dipoles.

The exact nature of the pseudo-lattice in such liquids will be clarified by the results of further work, but the general picture of a polar molecule undergoing resonant oscillations in the force field of its immediate neighbours is acceptable. In such a model, the width of any absorption line would be determined by the lifetime of the pseudo-lattice state or, in other words, the reciprocal of the time over which the surrounding cage does not alter appreciably. This latter is the relaxation time, which is known from viscosity measurements or more directly from the frequency at which the Debye asymptote is nearly achieved. This is about 10 cm^{-1} for chlorobenzene, and it is not hard to believe that the observed band is made up of a number of lines having widths of this order. The well-defined peak of the band is then explained by the reasonable assumption that the orientation of the oscillator in the 'cage' is not random, but has a most probable value which could be similar to that found in the crystal.

G. W. CHANTRY
H. A. GEBBIE

National Physical Laboratory,
Teddington, Middlesex.

¹ Gebbie, H. A., Stone, N. W. B., Findlay, F. D., and Pyatt, E. C., *Nature*, **205**, 377 (1965).

² Whiffen, D. H., *J. Chem. Soc.*, 1350 (1956).

³ Poley, J. Ph., *App. Sci. Res.*, B, **4**, 337 (1954).

⁴ Garg, S. K., and Smyth, C. P., *J. Chem. Phys.*, **42**, 1397 (1965).

⁵ Wyss, H. R., Werder, R. D., and Günthard, Hs. H., *Spectrochim. Acta*, **20**, 573 (1964).

METALLURGY

Effect of Temperature on the Discontinuity in the S/N Fatigue Curve of a Stainless Steel

RECENT investigations of the fatigue characteristics of a wide range of alloys have revealed a discontinuity in the S/N fatigue curves at high-stress levels. Finney¹ reports the discontinuities in extruded high-strength aluminium alloys of Australian manufacture to specifications D 683/3 and 2024-T4 involve a shift to shorter fatigue lives at stresses above the discontinuities. These tests were carried out in rotating cantilever machines with notched test pieces ($K_t = 1.5$), and Finney also showed that the size of the discontinuity increases with increasing rate of cyclic loading in tests within the range 100–1,200 c/min. A similar tendency was observed by Williams and Mitchell² in rotating cantilever tests of smooth test pieces prepared in weldable aluminium alloy—'Alean 3032WP'. The discontinuities reported for tests on steels³ show an opposite tendency to increased fatigue lives immediately above the discontinuities, and the trend is similar for both ferritic steel to BS970EN3B and an austenitic stainless steel S130, when smooth test pieces are fatigued in a R.R. Royce fatigue machine.

The strain ageing phenomenon in steels induces special characteristics in their fatigue behaviour, for example, the appearance of a 'knee' in their S/N curves⁴. It has been suggested that the discontinuity is associated with 'cyclic slip' involving a general yielding of the metal due to exhaustion of strain hardening. The plastic deformation involved would induce hysteretic heating in steels and thus cause an increase in both dislocation density and diffusion rates to enhance strain ageing effects. To assess the role of strain ageing during the fatigue cycling of an S130 stainless steel, comparative fatigue tests were undertaken at room temperature and at -80°C . Calculations showed that the diffusion rate would be very slow at -80°C and strain ageing thus suppressed.

The tests were performed in a pull-pull fatigue machine utilizing flat specimens 0.061 in. thick and containing a semi-elliptical hole ($K_t = 2.5$). Figs. 1 and 2 show the results

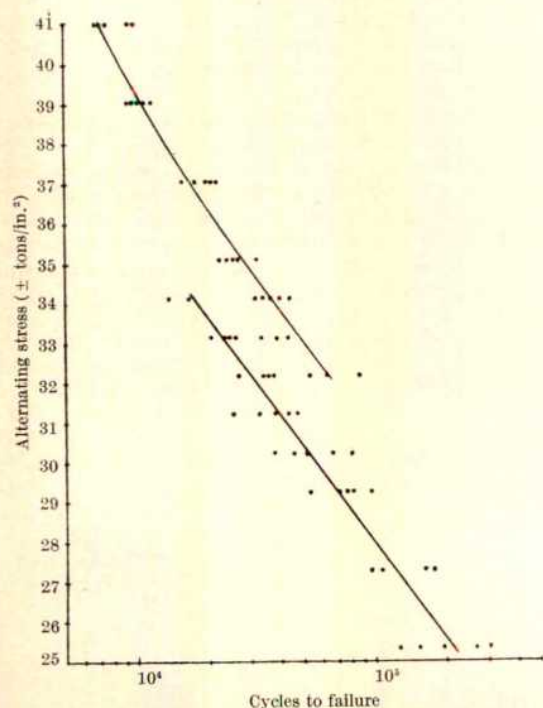


Fig. 1. Conditions: temperature, $+20^\circ\text{C}$; loading, sinusoidal; frequency, 120 c/s.; stress concentration factor, 2.5

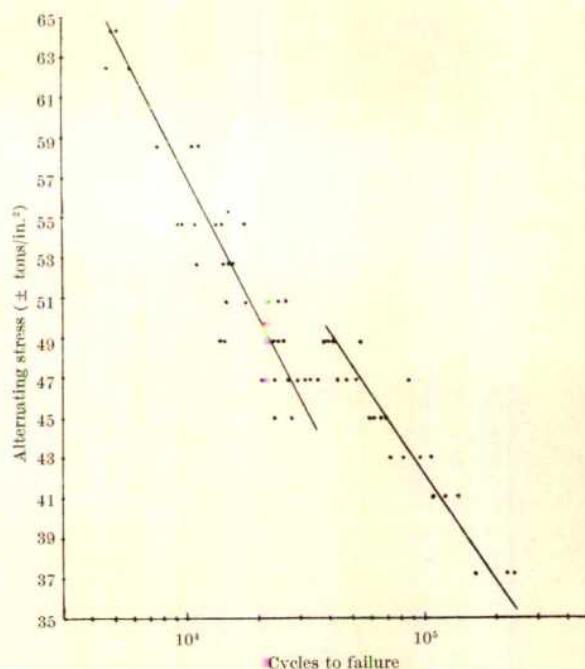


Fig. 2. Conditions: temperature, -80°C ; loading, sinusoidal; frequency, 120 c/s.; stress concentration factor, 2.5

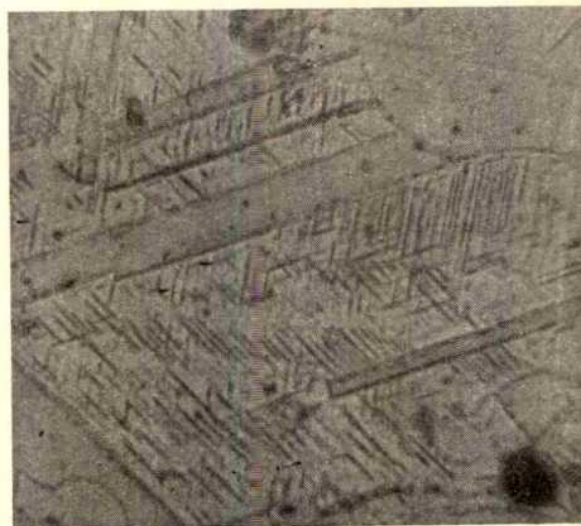


Fig. 3. ($\times c. 666$)

obtained. Testing at -80°C has raised the stress-level of the discontinuity by about 15.9 tons per in.² and the shift of the curve above the discontinuity is to shorter fatigue lives. Metallographic examination of the fatigued stainless steel revealed extensive martensitic transformation at the slip bands (Fig. 3). It appears, therefore, that suppression of the strain ageing phenomenon is associated with a reduced resistance of the stainless steel to fatigue damage at stresses above the discontinuity.

T. R. G. WILLIAMS
C. R. SHURMER

Department of Mechanical Engineering,
University of Southampton.

¹ Finney, J. M., *J. Inst. Metals*, 480 (July 1964).

² Williams, T. R. G., and Mitchell, P. J., *Inst. Sound and Vibration Mem.*, No. 118 (Southampton Univ.).

³ Williams, T. R. G., and Abdulla, J. A., *The Engineer*, 325 (August 28, 1964).

⁴ Levy, J. C., and Kanitkar, S. L., *J. Iron Steel Inst.*, 296 (April 1961).

⁵ Benham, P. P., and Ford, H., *J. Mech. Eng. Sci.*, 3, 119 (1961).

CHEMISTRY

Correlation of the Third Excited State of Polycyclic Aromatic Hydrocarbons with the Ionization Potential

In two earlier communications^{1,2} it has been shown that the means of the first excited singlet state 1L_b and first excited triplet state 3L_a of some polycyclic aromatic hydrocarbons obey the equation:

$$I = 4.39 + 1.15 \frac{^3L_a + ^1L_b}{2} \quad (\text{eV}) \quad (1)$$

where I denotes the ionization potential of the molecule. The designation of the states is due to Platt³.

This equation is similar to the Hückel⁴ equation:

$$I = \alpha + bE$$

where E is the energy of the first state and ' α ' corresponds to the ionization potential of an infinite planar carbon molecule which can be assumed to be graphite, which has an ionization potential of 4.39 eV (ref. 5).

Hückel's theory does not consider electron spin and does not therefore differentiate between states of different spin multiplicity. It does seem that averaging out over the states of different multiplicity brings the experimental data into close agreement with the theoretical predictions.

The third excited states of the polycyclic aromatic hydrocarbons have been examined to see whether they show any correlation. The energies of higher excited triplet states are obtained by the techniques of flash-photolysis^{6,7}. Values of T_3 are listed in Table 1. A plot of I against T_3 is shown in Fig. 1. A very rough correlation only is observed.

The energy of the third excited singlet state can be obtained from absorption spectra. The singlet state corresponding to the T_3 triplet state would appear to be the state designated 1B_b by Platt. This in general corresponds to the level designed β by Clar⁷. In obtaining the values of 1B_b shown in Table 1, the experimental values of Clar have been used throughout. In the case of phenanthrene

and chrysene, theoretical studies by Ham and Rueden⁸ suggest that the levels designated β^1 by Clar correspond to 1B_b and these have been used. The β^1 state has rarely been designated 1B_b for 1,2,5,6-dibenzanthracene it having been found earlier¹ that these were a crossover of the first excited state. In all other cases the state of Clar has been designated 1B_b .

A plot of I against 1B_b is shown in Fig. 1, but no rough correlation can be seen.

Fig. 1 also shows a plot of I against $\frac{^1B_b + T_3}{2}$. A fair good correlation is seen to exist. The equation of the straight line passing through the data is:

$$I = 3.67 + 0.82 \frac{^1B_b + T_3}{2} \quad (\text{eV})$$

using the method of least squares. The sum of the standard deviations is 0.204 where $\frac{^1B_b + T_3}{2}$ is treated as dependent variable.

The best straight line extrapolating to 4.39 is given

$$I = 4.39 + 0.667 \frac{^1B_b + T_3}{2} \quad (\text{eV})$$

The sum of the standard deviations is 0.315.

Hückel's theory predicts a correlation between I_2 , second ionization potential, and the third excited state, not between I and this state. However, the capacitive energy model of Smith⁹, which gives good predictive states that there is a direct linear relationship between I and I_2 which will then explain the correlation shown in equation 2. The capacitive energy model predicts that

$$I \approx I_2 - 4.39 - 3 \times 1.5 \frac{^3L_a + ^1L_b}{2}$$

comparing equation 4 of ref. 9 with equation 1 of this report. This would therefore suggest that equation 3 is more correct one to take, for, as the energy levels go to zero with increasing molecular dimensions, I should go to 4.39, the work function of graphite⁵, the infinite carbon planar molecule. A good test of equations 2 and 3 is the prediction of the third triplet state of benzene. The T_3 state of benzene is 6.94 eV (refs. 7, 8). Equation 2 predicts that T_3 is 6.56 eV whereas equation 3 predicts that T_3 is 7.43 eV. It is hoped to build apparatus¹⁰ which will enable the $T_1 - T_3$ transition of benzene to be measured.

For molecules having ionization potentials in the region of 7–8 eV, which includes nearly all twenty aromatic hydrocarbons listed in ref. 2, either equation can be applied. It is thus possible to predict the T_3 energies of those molecules for which it has not yet been observed. Alternatively, knowing T_3 , the correct assignment of the 1B_b singlet excited state can be made.

In Table 2 are shown the predicted energy-levels of some polycyclic aromatic hydrocarbons assuming 1B_b equal to β and β^1 . In all cases it is more probable that β^1 is 1B_b , but values of β^1 are not available in all cases.

An interesting case is perylene. It has an ionization potential of 7.03 eV (ref. 2) and a T_3 energy of 4.10 eV (ref. 6). Equation 2 predicts a 1B_b energy of 4.09 eV. The spectrum of perylene is greatly different from that of the

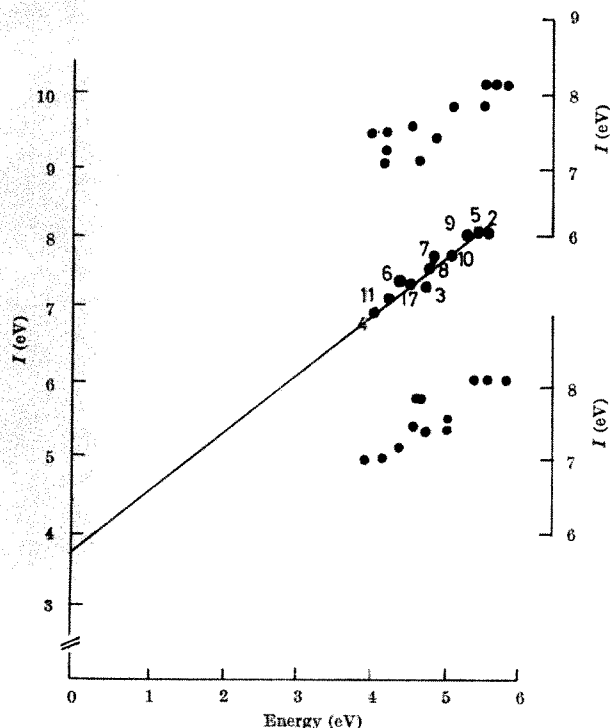


Fig. 1. Upper diagram: right-hand ordinate, plot of I vs. 1B_b . Middle diagram: left-hand ordinate, plot of I vs. $\frac{^1B_b + T_3}{2}$. Lower diagram: right-hand ordinate, plot of I vs. T_3 .

Table 1. POLYCYCLIC AROMATIC HYDROCARBONS (ENERGIES IN eV)

	I (refs. 1, 2)	1B_b (ref. 7)	T_3 (ref. 6)	$\frac{^1B_b + T_3}{2}$
(2)* Naphthalene	8.10	5.60	5.62	5.61
(3) Anthracene	7.37	4.92	4.74	4.83
(4) Tetracene	7.00	4.22	3.96	4.09
(5) Phenanthrene	8.09	5.65	5.26	5.46
(6) 1,2-Benzanthracene	7.45	4.34	4.60	4.47
(7) Chrysene	7.80	5.12	4.62	4.87
(8) Pyrene	7.55	4.55	5.05	4.80
(9) Triphenylene	8.09	4.81	5.81	5.31
(10) 1,2,5,6-Dibenzanthracene	7.80	5.55	4.59	5.07
(11) 3,4-Benzpyrene	7.19	4.18	4.39	4.29
(18) Coronene	7.44	4.05	5.05	4.55

* Numeration as in refs. 1 and 2.

Table 2

	I (ref. 2)	β (ref. 7)	β^1 (ref. 7)	T_2 (β)	T_2 (β^1)
1,8-Dibenzanthracene	7.68	4.07		5.72	
1,4-Dibenzanthracene	7.61	4.33	5.68	5.31	4.12
ne	7.80	4.32		5.73	
1,5-Dibenzpyrene	7.27	4.04		4.74	
anthrene	7.00	3.99	4.82	4.13	3.30
-Benzpyrene	7.35	4.08		4.90	
Benzpyrene	7.56	4.28	5.23	5.20	4.25

er aromatic hydrocarbons⁶. It has a new series of sorption bands denoted α^1 lying between the α - and β -levels. The energy of the α^1 -level is 4.25 eV, which agrees reasonably well with the predicted value of 1B_u . As this level is the third highest energy transition observed in naphthalene, the assignment to 1B_u seems very sensible.

Equations 2 and 3 were computed by Miss E. Watson.

M. A. SLIFKIN*

Department of Physics,
Newcastle College of Technology,
Newcastle upon Tyne.

* Present address: Department of Pure and Applied Physics, Royal College of Advanced Technology, Salford 5.

Birks, J. B., and Slifkin, M. A., *Nature*, **191**, 761 (1961).

Slifkin, M. A., *Nature*, **200**, 877 (1963).

Platt, J. R., *J. Chem. Phys.*, **17**, 484 (1949).

Hückel, E., *Z. Phys.*, **69**, 423 (1930); **70**, 204 (1931).

Brann, A., and Busch, G., *Helv. Phys. Acta*, **20**, 33 (1947).

Porter, G., and Windsor, M. W., *J. Chem. Phys.*, **20**, 2008 (1953); *Proc. Roy. Soc. A*, **245**, 238 (1958).

Clar, E., *Polycyclic Hydrocarbons*, Vols. I and II (Academic Press, London, 1964).

Ham, N. S., and Ruedenberg, K., *J. Chem. Phys.*, **25**, 13 (1956).

Smith, F. T., *J. Chem. Phys.*, **34**, 793 (1961).

Dyson, D. J., and Slifkin, M. A., *J. Sci. Inst.*, **40**, 599 (1963).

Labelling of Beta-lipoprotein with Tritium

LABELLING of organic compounds and bio-molecules with tritium is of great importance in biochemistry. In general, the method of choice may be radiochemical synthesis¹, the gas exposure labelling of Wiltzsch²⁻⁴, or biochemical synthesis or different types of exchange reactions⁵. However, for the labelling of certain bio-molecules in radiochemically pure form, without any destruction of the natural constitution, only biochemical synthesis could be expected to be successful.

In our laboratory β -lipoprotein was labelled with tritium by a simple exchange reaction in radiochemically pure form, without any change in its natural constitution. The method developed is a modification of that reported by Yavorsky and Gorin⁶, and seems to be widely applicable for the labelling of different types of organic compounds and certain bio-molecules as well.

The tritiating reagent is $\text{CH}_3\text{COOHT.BF}_3 + \text{CH}_3\text{COO}^-$. This complex can easily be prepared by refluxing a small excess of acetic anhydride with tritiated water and saturating the formed trihydride acetic acid with BF_3 gas. The complex was isolated by distillation (b.p. 139°–142° C).

Labelling of β -lipoprotein was effected by simply contacting and shaking it for 24 h with an acetic acid solution of the reagent in heterogeneous phase at room temperature. When the equilibrium was reached, the tritiated complex was decomposed by adding 3–5 ml. of water with subsequent lyophilization. The labile tritium was washed out

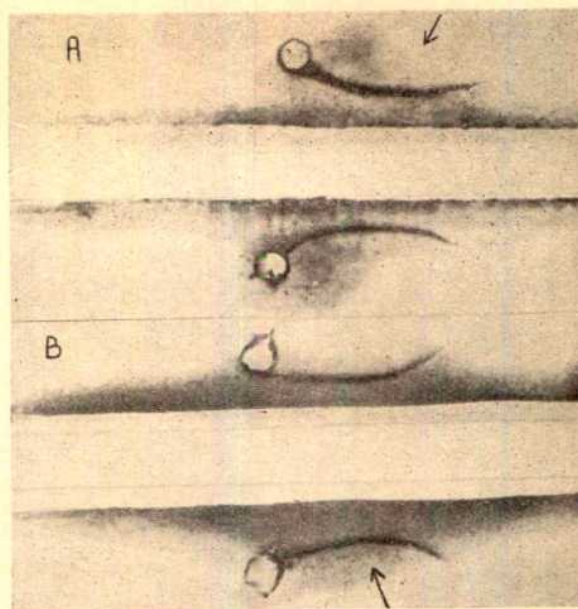


Fig. 1. Immunoelectrophoretic pattern of β -lipoprotein of rabbit. A, Above, after isolation by Gofman's method (arrow); below, the control β -lipoprotein used in our laboratory for identification; antisera, anti-rabbit β -lipoprotein produced in cockers. B, Above, isolated β -lipoprotein before labelling; below, radio-immunoelectrophoretogram of isolated tritium labelled β -lipoprotein; antisera, the same as A.

with distilled water by repeated lyophilization. The labelled β -lipoprotein was used without any further purification.

Similarly, some other compounds have also been labelled in order to get more detailed information about the scope and mechanism of this labelling method. Results are summarized in Table 1.

Natural β -lipoprotein was isolated by the method of Gofman^{6,7}. The purity of the isolated sample was checked by the immunoelectrophoretic method of Scheidegger⁸ (Fig. 1A). The same control proved (Fig. 1B) that during labelling the β -lipoprotein remained unchanged. The radiochemical purity of the labelled sample was checked by autoradiography using Kodak 'AR-10' stripping film (Fig. 1B, lower picture). The labelled sample could be recovered from aorta homogenate in the same way⁹⁻¹¹ as unlabelled samples after having been administered intravenously into rabbits. The labelled β -lipoprotein has been used for some mechanistic investigation of plaque formation on cholesterol-fed rabbits. More details of this investigation will be published elsewhere¹².

Preliminary experiments made on different types of organic compounds showed that hydrogen atoms bonded to carbon can be replaced with tritium by this reagent (Table 1). The rate of these exchange reactions was found to be markedly dependent on temperature. However, the scope and particularly the mechanism of this exchange reaction require more detailed investigation.

The main advantages of this labelling method are: radiochemically pure labelled compounds can be prepared without any post-labelling purification that may be

Table 1. EQUILIBRATION OF DIFFERENT TYPES OF COMPOUNDS WITH TRITIATED ACETIC ACID-BORON-TRIFLUORIDE COMPLEX

Compounds	Weight (mg)	Reagents spec. act. ($\mu\text{C./mg}$)	Reaction				Products spec. act.	
			Weight (mg)	Time (h)	Temp. (°C)	Yields (%)	($\mu\text{C./mg}$)	($\mu\text{C./mmole}$)
β -lipoprotein	400	35.20	3,700	20	24	—	0.722*	
Phenylalanine	500	133.75	1,000	20	24	62	1.060	175.10
L-serine	500	133.75	1,000	20	24	90	0.877	92.16
L-thyrosine	500	133.75	1,000	20	24	46	0.914	165.61
L-tryptophan	500	133.75	1,000	20	24	30	2.523	515.25
L-methionine	500	133.75	1,000	20	24	63	—	
Cholesterol	500	131.28	1,000	20	24	45	3.023	1,168.81
Acetic acid†		133.75	1,000	2,000	24	—	114.08	6,845

* $\mu\text{C./ml. solution}$.

† Measured as the self-exchange of the complex.

required after other labelling methods, for example, after Wiltzsch-labelling⁴. The procedure is rather rapid, requiring no special equipment, and may take place under mild conditions permitting the successful labelling even of sensitive molecules with suitably high specific activity. Activity yield is about of the same order as in the case of Wiltzsch-labelling. Radioactivity was measured by the liquid scintillation technique using a Packard liquid scintillation spectrometer (model 3003).

We thank Dr. S. Virág for the isolation and purity control of the β -lipoprotein sample.

T. GOSZTONYI
J. MÁRTON
A. KOVÁCS

National Atomic Energy Commission,
Institute of Isotopes, Budapest,
Hungary.

¹ Murray, A., and Williams, D. L., *Organic Synthesis with Isotopes* (Interscience Pub. Inc., New York, 1958).

² Wiltzsch, K. E., *J. Amer. Chem. Soc.*, **79**, 1013 (1957).

³ Riess, P., and Wiltzsch, K. E., *J. Phys. Chem.*, **62**, 6 (1958).

⁴ Wenzel, M., and Schulze, P. E., *Tritium Markierung* (Walter de Gruyter Co., Berlin, 1962).

⁵ Yavorsky, B. M., and Gorin, E., *J. Amer. Chem. Soc.*, **84**, 1071 (1962).

⁶ Gofman, J. W., Lindgren, F. T., and Elliot, H. A., *J. Biol. Chem.*, **179**, 973 (1949).

⁷ Gofman, J. W., et al., *Science*, **111**, 166 (1950).

⁸ Scheidegger, J. J., *Intern. Arch. Allergy*, **7**, 103 (1955).

⁹ Ott, H., Lohss, F., and Gergely, J., *Klin. Wschr.*, **33**, 383 (1958).

¹⁰ Kayden, H. J., Seegal, B. C., and Hsu, J., *J. Clin. Invest.*, **38**, 1016 (1959).

¹¹ Gerö, S., et al., *J. Atherosclerosis Res.*, **1**, 88 (1961).

¹² Virág, S., et al. (to be published).

Pure Quadrupole Resonance Frequency Shift and Internal Stress in Gamma-ray Irradiated Sodium Chlorate

THE effects of irradiation and crystalline defect on pure quadrupole resonance (PQR) have been extensively investigated¹. Randall *et al.*² observed an increase of the line width and second moment after X-ray irradiation, and concluded that strain broadening is the predominant mechanism. The changes in the electric field gradient induced by an applied stress were also measured by Collins and Bloembergen³. Colour centres occur in sodium chlorate crystals after γ -ray irradiation. These are probably due to V centres and O_2^- centres^{4,5}. Such impurities are likely to set up stresses and bring about changes in the electric field gradient. For this reason, it would be expected that a shift of the pure quadrupole resonance would occur.

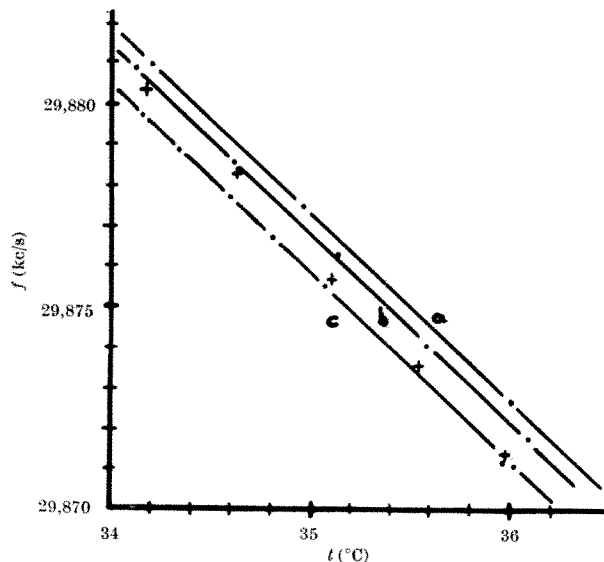


Fig. 1. Temperature dependence of PQR frequency of ^{35}Cl isotope: a, before; b and c, after γ -ray irradiations of 0.9×10^6 and 2.09×10^6 r, respectively

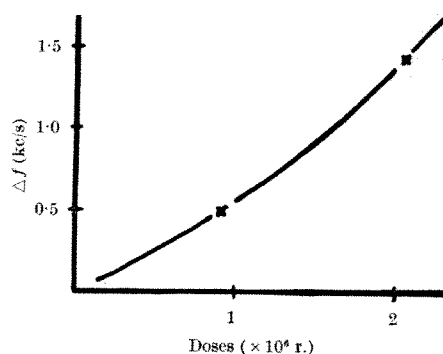


Fig. 2. PQR frequency shift of ^{35}Cl due to γ -ray irradiations

The aim of the work reported here was to measure the shift and to interpret the internal stress. The results of the preliminary measurements are shown in Fig. 1. It was necessary to eliminate the temperature effect. For this reason we made the measurements in a thermostat at about 35°C (ref. 6). In Fig. 1, a is for the non-irradiated sample is for the sample irradiated with γ -rays from reactor TH (Çekmece Nükleer Research Center, Istanbul) with doses of 0.9×10^6 r, and c gives the results after cumulative doses of 2.09×10^6 r. During the measurements the temperature fluctuation was less than 0.01°C . Measurements were made with a conventional Deane-type spectrometer. Frequency was measured with a Hewlett-Packard 524 period counter. For each point on Fig. 1 the mean square root error of frequency was about 30 c/s. In Fig. 2 PQR frequency shift is shown as a function of the irradiation dose. With these results and using theoretical analysis it is possible to interpret the stress produced in sodium chlorate crystals by γ -rays. Since the piezo-electric effect are negligible⁸, and since the changes in the electric field gradient may not explain the line broadening², the main effect should be due to strains. $TE_{33,33}^{E}$ is very much greater than $TE_{33,11}^{E}$. The stress produced by rays of doses 10^6 r in sodium chlorate crystals in the Na-Cl direction is:

$$\sigma_{33} \approx 23 \text{ kg/cm}^2 \text{ Mr.}$$

I thank Dr. S. Akpınar, director of ÇNAEM, and Dr. A. Çilesiz for providing the irradiation facilities.

HAYATI BUDAK

Experimental Physics Institute,
University of Istanbul.

¹ Depierreux, J., and Duchesne, J., *Nature*, **181**, 759 (1958), and refs. 1-10 on ref. 2.

² Randall, J. L., Moulton, W. G., and Ard, W. B., jun., *J. Chem. Phys.*, **31**, 730 (1959).

³ Collins, F. A., and Bloembergen, N., *J. Chem. Phys.*, **40**, 3479 (1964).

⁴ Sastry, S. B. S., and Ramasastry, C., *J. Phys. Soc. Japan*, **18**, 1220 (1963).

⁵ Hasty, T. E., Ph.D. thesis, Univ. Alabama (1959).

⁶ Budak, H., *Rev. Fac. Sci. Univ. Istanbul*, **C28**, 142 (1963).

⁷ Deane, C., *Phys. Rev.*, **96**, 1053 (1954).

Acid Phosphatases

WE have been concerned with the isolation of acid phosphatases (orthophosphoric monoester phosphohydrolases, 3.1.3.2.) in quantities sufficient for chemical studies of these enzymes. The sources chosen were: the potato, the human prostate gland and human seminal plasma. We have found that the potato is a poor source and, although the enzyme has been obtained in a form which is homogeneous to starch-gel electrophoresis and to ultracentrifugation, the method is tedious and, because of the very large purification factor involved (about 25,000), leads to a low final yield (about 10 mg from 50 kg of potatoes). The best preparation previously reported was apparently associated with a purification factor of about 1,000 (ref. 1).

Several reports of the complete purification of prostatic acid phosphatase have been made^{2,3}, but, seemingly, the

ounts obtained were too small to allow the usual tests of homogeneity to be applied. It has also been reported that the pure enzyme is unstable² particularly so when in contact with glass surfaces. Recently it has been demonstrated by immunoelectrophoresis⁴ that there are, in fact, two prostatic acid phosphatases. We now report that, by relatively simple and reproducible procedures, two distinct phosphatases can be isolated from a homogenate of prostatic tissue; a typical preparation from thirteen glands giving 25 mg of P_2 and 10 mg of P_1 . Both enzymes are homogeneous in the ultracentrifuge and on starch-gel electrophoresis and are unchanged on further attempts at purification. Both are stable and resist the following procedures: prolonged dialysis, ultra-filtration, gel-filtration, chromatography on cellulose ion-exchange resins, precipitation by salts, filtration through glass wool, prolonged periods (6 months) in solution at 4° interspersed with days at room temperature, low pH (about 2.0) freezing and thawing and contact with certain proteolytic enzymes. Both are unstable above 40° and on precipitation with acetone at 0°.

The assay method used for the enzymes is based on the fact that the ultra-violet absorption spectrum of a monoaryl phosphate differs from that of the parent phenol. For phenyl phosphate (pH 5.0, 0.1 M acetate) the difference in extinction coefficient at 276 mμ is 1,000. Assays are, therefore, conveniently carried out using a solution of phenyl phosphate (5×10^{-3} M) maintained at 35° in a spectrophotometer cell. The specific activity is defined as the rate of change of $O.D._{276}/\text{min}$ produced when 1 μl. of enzyme solution is added to 2 ml. of substrate solution divided by the absorbancy of the enzyme solution at 280 mμ. This assay method is much more convenient and introduces fewer artefacts than aliquot methods based on the formation of molybdenum blue. Greater sensitivity can be achieved by using pyridoxal-5'-phosphate as substrate, but control of pH is then more important since the difference spectrum is very sensitive to pH changes.

Purification starts from an aqueous homogenate of minced tissue. The supernatant is fractionated by addition of ammonium sulphate; the enzymes precipitate between 50 and 75 per cent saturation. The fraction is freed from ammonium sulphate and brought to pH 5.2, 0.02 M acetate buffer. It is then absorbed into a column of DEAE-cellulose which has been equilibrated with the same buffer. After washing with dilute buffer, the column is washed with 0.2 M acetate buffer, pH 5.2. The active fraction is then concentrated by ultrafiltration and passed through 'Sephadex G-100' until its specific activity reaches 0.3. It is then re-chromatographed on DEAE-cellulose under the same conditions as described here except that gradient elution is used. The enzyme emerges as two separate peaks, P_1 coming before P_2 . The specific activities are constant across the peaks and both have the value about 1.2 (this compares with the best value so far obtained with the potato enzyme of 0.4). The enzymes run at slightly different speeds on starch-gel electrophoresis, P_1 running slightly ahead. The Svedberg constants (20°, about 0.5 per cent protein concentration) are 5.23S and 5.36S for P_1 (3 determinations) and P_2 (6 determinations), respectively. Preliminary measurements of the diffusion constants indicate, assuming partial specific volumes of 0.745, molecular weights in the region 120,000–150,000. The rate of passage through 'Sephadex G-100', however, indicates rather lower molecular weights, that is, 110,000–130,000. Amino-acid analyses show some significant differences notably in the relative amounts of valine and alanine. With phenyl phosphate as substrate the two enzymes show similar kinetic behaviour. Both are inhibited by (+) tartrate. An important problem is whether both enzymes occur in each individual gland. In one experiment a single gland was found to contain both enzymes, but it is not yet known whether this is general.

Human seminal plasma might seem, at first sight, to be a convenient source of the prostatic enzymes. Accordingly, a sample of plasma, after dialysis, was chromatographed on DEAE-cellulose as described here. Starch-gel electrophoresis showed that the enzyme obtained, which had a higher specific activity than the prostatic enzymes (about 1.6), was free of contaminating protein. However, activity was found to be split into two bands and, consistently, in the ultracentrifuge the enzyme sample separated into two components, S_1 and S_2 , with Svedberg constant (20°) of 2.65S and 4.43S, respectively. Afterwards a slight change in procedure separated the two enzymes on DEAE-cellulose. These enzymes clearly have much lower molecular weights than the enzymes isolated directly from the prostate and they may not be prostatic in origin. This problem is now being investigated.

The prostatic enzymes and the potato enzyme are inhibited by phosphate ions and by molybdate ions. In all cases the inhibition is kinetically strictly of the 'competitive' type. However, the inhibition by molybdate ions can be reversed by the addition of EDTA, whereas inhibition by phosphate ions is unaffected. The simplest explanation would seem to be that molybdate ions, unlike phosphate ions, combine with the enzyme in a way not directly involving the active site and produce a protein conformation unfavourable for combination with substrates. Consistently, the potato enzyme is considerably activated by EDTA and the effect can be shown not to be due to removal of trace metal inhibitors. These presumed conformational changes should, of course, be detectable by ORD measurements and the appropriate experiments are now in progress.

We acknowledge grants from the Department of Scientific and Industrial Research (to Jennifer M. H. and P. E. S.), the Medical Research Council (to J. M. H.) and the National Research Council of Canada (to J. G.).

C. A. VERNON
J. GAULDIE
JENNIFER M. HANSON
J. M. HUMPHREYS
P. E. SMITH

Department of Chemistry,

Department of Pharmacology,

Department of Physiology,
University College, London, W.C.1.

A. J. LAWRENCE

BARBARA E. C. BANKS

- ¹ Andreu, M., Fernandez Alvarez, E., and Lora-Lamayo, M., *Anales real soc. espan. fis y quim.* (Madrid) **56B**, 67 (1960).
² Hudson, P. B., and London, M., *Arch. Biochem. Biophys.*, **46**, 141 (1953).
³ Boman, H. G., *Arkiv Kemi*, **12**, 453 (1958).
⁴ Shularo, S., Mamrod, L., Goncer, M. J., and Soanes, W. A., *J. Immunol.*, **93**, 474 (1964).

Structure of Phosphobenzene

WE have already shown that of the polyphosphines of formula $(C_6H_5P)_n$ and melting points (A) 149–150°, (B) 190°, (C) 252–256°, (D) 260–285°, compound A is a five-membered ring compound in the solid state¹.

Compound B, which has been prepared according to the directions given in the literature², crystallizes in at least four different forms; two of these, the monoclinic (from tetrahydrofuran) and rhombohedral (from benzene) modifications, have already been described¹. The other two forms are triclinic and trigonal and have also been obtained by recrystallization from tetrahydrofuran. The triclinic form melted at 185°–189° in an open tube and 193°–198° in an evacuated tube, while the trigonal form melted at 190°–195° in an open tube and 236°–240° in an evacuated tube: all four forms decompose on melting. The only time the trigonal form was obtained the triclinic form was also present. It was not possible to define the conditions which would give any one of the three forms obtainable from tetrahydrofuran. The unit cell and space group data for the trigonal and triclinic forms are: Trigonal: $a =$

13.026, $c = 11.547$ Å, $D_m = 1.265$, $D_c = 1.269$ g/c.c., $Z = 2$, space group $P\bar{3}c1$. Triclinic: $a = 10.551$, $b = 14.717$, $c = 13.000$ Å, $\alpha = 73.7^\circ$, $\beta = 63.2^\circ$, $\gamma = 91.6^\circ$, $D_m = 1.265$, $D_c = 1.266$ g/c.c., $Z = 2$, space group $P1$ or $P\bar{1}$.

We have determined the structure of the trigonal modification by single crystal X-ray diffraction methods. 879 of the 1,397 intensities measured with the aid of a Hilger and Watts linear diffractometer³ were assigned a non-zero value and used in the structure determination. The position of the independent phosphorus atom, obtained from the three-dimensional Patterson function, showed that the six phosphorus atoms in the molecule were arranged in a puckered ring of the chair form. The positions of the light atoms were determined from the $[c]$ -axis projection and a model. The structure was refined by the least squares method with individual anisotropic temperature factors. The final value of R for 879 planes was 0.072. Full details of the structure will be published elsewhere.

The analysis shows that, in the crystalline state, the trigonal form of phosphobenzene *B* is a hexamer, $(C_6H_5P)_6$, and contains a six-membered ring of phosphorus atoms in the chair form with phenyl groups occupying the equatorial positions (see Fig. 1). The molecule has the symmetry $\bar{3}$ (C_{3i}).

Although many attempts were made to prepare suitable single crystals of compound *C*, none was successful. In the only solvent found (diphenyl ether), the suspension had to be heated to refluxing point (258°) to get a clear yellow solution. On cooling only the pentamer, $(C_6H_5P)_5$, could be isolated. This conversion was most probably effected by heat alone, since it is known that, on melting compound *C*, compound *A* is obtained. It has now also been found that compound *A* is converted to *C* by heating in piperidine. Phosphobenzene *C*, m.p. 260° – 265° , has been described as polymeric, but the value of n cannot be very large since an X-ray powder photograph shows it to be highly crystalline. Attempts to index this powder photograph have failed.

It is interesting to note that the related cyclohexyl derivative, $(cyclo-C_6H_{11}P)_n$, is apparently tetrameric as suggested by previous workers^{2,4}. It crystallizes in the space group $P4_3/n$ with $a = 15.33$ and $c = 5.63$ Å. The observed density is 1.161 g/c.c. which, with the cell volume, gives 924.2 for the molecular weight of the cell contents. This implies that there are two molecules of $(cyclo-C_6H_{11}P)_4$ (mol. wt., 456.5) in the unit cell with the molecular symmetry 4 (S_4). The sample used was supplied by Prof. Issleib of Halle.

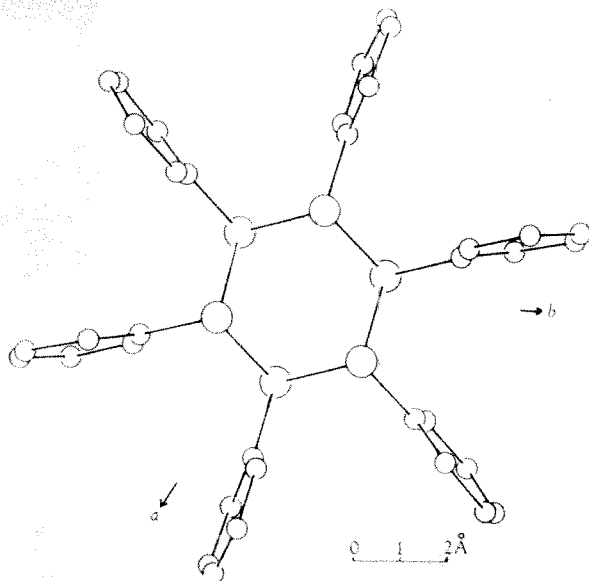
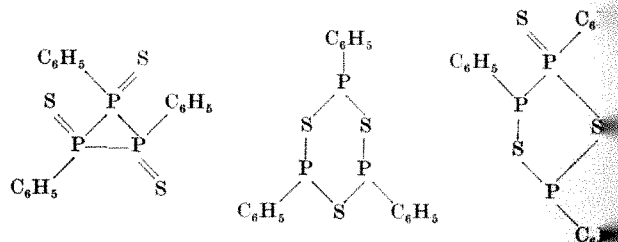


Fig. 1

Crystals of the sulphur derivative of $(C_6H_5P)_6$, $(C_6H_5P)_6S$ which were obtained from the reaction of phenylphosphine with sulphur (ref. 5) and were identical in every respect with those obtained from *A* and sulphur (ref. 6) (m.p. 148° , mix m.p. 148° , superimposable infra-red spectra), crystallize in the monoclinic system. The space group is either $P2_1$, $P2_1/m$, and a survey of the $h0l$ and $h1l$ intensities suggest that the non-centric $P2_1$ is more probable. The c dimensions are $a = 8.90$, $b = 13.71$, $c = 8.35$ Å, $\beta = 107^\circ$. The observed density is 1.438 g/c.c. which, with the known volume, gives six units (actually 6.005) of (C_6H_5PS) in the unit cell. Since in both possible space groups there may be a minimum of two molecules in the cell, it may be assumed that the molecule is a trimer. Some of the possible structures are:



It is not possible to evaluate a recent paper⁷ describing the synthesis of co-ordination compounds obtained from transition metal carbonyls and what is apparently phosphobenzene *A* until full experimental details are published. Nevertheless the synthesis of $(C_6H_5P)_4Ni(CO)_2$ and $(C_6H_5P)_5Mo(CO)_3$ could be equally well explained by postulating a contraction of $(C_6H_5P)_6$ to give the nickel compound instead of an expansion of $(C_6H_5P)_4$ to give the molybdenum compound. We have made $(C_6H_5P)_6$ in two different ways¹ and have recrystallized it from three different solvents (benzene-alcohol, acetonitrile and tetrahydrofuran) and have always obtained crystals of the pentamer $(C_6H_5P)_5$. Thus it does not appear likely that acetonitrile has any special effect on phosphobenzene as has been suggested by other workers⁷.

J. J. DALY
LUDWIG MAIER

Monsanto Research S.A.,
8045 Zürich, Binzstr. 39,
Switzerland.

¹ Daly, J. J., and Maier, L., *Nature*, **203**, 1167 (1964). Daly, J. J., *J. Chem. Soc.*, 6147 (1964).

² Henderson, W. A., Epstein, M., and Seichter, F. S., *J. Amer. Chem. Soc.*, **85**, 2462 (1963).

³ Arndt, U. W., and Phillips, D. C., *Acta Cryst.*, **14**, 807 (1961).

⁴ Issleib, K., and Seidel, W., *Z. Anorg. Allgem. Chem.*, **303**, 155 (1960).

⁵ Maier, L., *Helv. Chim. Acta*, **46**, 1812 (1963).

⁶ Kuchen, W., and Buchwald, H., *Chem. Ber.*, **91**, 2296 (1958).

⁷ Ang, H. G., Shannon, J. S., and West, B. O., *Chem. Commun.*, **1**, 10 (1965).

BIOPHYSICS

Intercellular Matrix of the Intervertebral Disk in Ageing and in Prolapse

At the present time an increasing amount of attention is being directed to the non-collagenous proteins in connective tissues. Like collagen, these proteins are associated with polysaccharides in the form of mixtures or complexes, though the exact nature of the linkages between the moieties is a controversial subject. These compounds are essential components of the intercellular matrix of intervertebral disks, and a knowledge of them is needed for an understanding of the mechanisms of intervertebral disk prolapse.

Prolapse of intervertebral disk tissue in the lumbar region is a frequent cause of back pain and sciatica. Displacement of tissue follows after the loss of normal mechanical properties. It is known that in connective

es the collagenous fraction of the intercellular matrix associated with tensile strength and the non-fibrillar ion with resilience. The latter fraction requires further investigation.

he term 'herniation of the nucleus pulposus', though occasionally applicable, is an unfortunate misnomer. prolapse is more correct, because the prolapsed tissue consists of varying proportions of nucleus pulposus and annulus fibrosus. Frequently large fragments of cartilage plate are also to be found in sequestered tissue, and misleading to compare the results of analyses on prolapsed tissue with similar investigations on the material removed from the centre of a normal control disk. The significant annular contribution to a disk prolapse is noted in the high collagen content of prolapsed tissue reported by Davidson and Woodhall¹, and Mitchell, Dry and Billewicz².

In a recent investigation on intervertebral disk tissue the main techniques were used, namely X-ray fibre diffraction and electron microscopy³. Subsidiary methods included *in vitro* culture, histology, and biochemical analyses. A wide age range of fresh autopsy specimens (each week of foetal life to 93 years) and tissue removed at the course of 56 lumbar laminectomies for prolapse were examined.

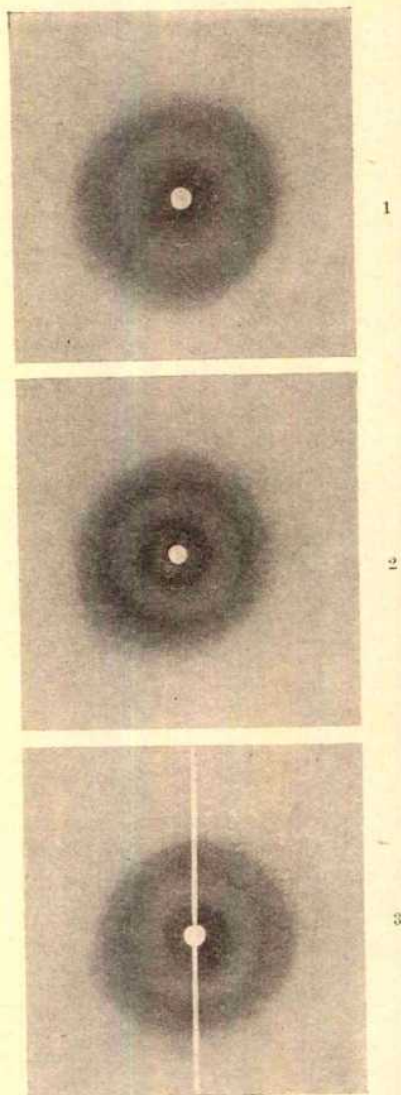
Little direct attention was paid to the polysaccharides because these are poorly crystalline and not suitable for X-ray study by the methods used. However, whenever non-collagenous proteins are discussed the associated polysaccharides are implicated. In human intervertebral disk tissue, at least three non-collagenous proteins have been distinguished from each other by their X-ray fibre diffraction patterns and their appearances in the electron microscope. The differences are slight but quite distinct. In young children the chondrocyte-like cell produces large amounts of a non-collagenous protein. This component has a 'fluffy' appearance when viewed in the electron microscope, indicating that there was a highly swollen gel structure before drying, and the 4.6 Å backbone spacing is a broadened reflexion (Fig. 1). The absence of definite side-chain spacing is notable. By the middle of the second decade the cells produce what has been termed 'mature' β -protein. This is found in quantity in the nucleus pulposus and the transitional zones of the disk, and is present in comparable amounts at all spinal levels; it tends to become more crystalline with advancing age.

After the age of fifty years, and sometimes before, a third non-collagenous protein becomes a major component of disk tissue. In contrast to the proteins mentioned already it gives a better resolved backbone spacing and a well-defined side-chain spacing in the 10 Å region (Fig. 2). When observed in the electron microscope, it was seen to be relatively electron-dense. Since with advancing years this protein becomes a major tissue component, the physical properties of the tissue partly depend on it. Similar fibre diffraction patterns were not obtained in a study of normal articular and nasal septum cartilage, tendon or knee joint menisci³. The central fibro-cartilaginous area of the symphysis pubis from elderly subjects gives a comparable diffraction pattern, and in the electron microscope a similar dense protein has been noted in the media of the aorta. These observations suggest that the presence of this type of protein in certain connective tissues is connected with the demands of physical forces the menisci are non-weight-bearing).

In sequestered disk tissue there is a greater quantity of the mature type of non-collagenous protein as compared with tissue from the interior of the same disk; and in prolapsed disk tissue there is an increase in the non-collagenous protein content as compared with control tissue from the same age range. The tissue gives fibre diffraction patterns in which there is a prominent β -protein backbone spacing but the side-chain spacing is not present, indicating irregularity in the mode of packing of the polypeptide chains (Fig. 3). The chondrocyte-like cells

were observed to be surrounded by large amounts of matrix, apparently of recent origin, suggesting that the increase of β -protein is a change associated with effect rather than cause. Moreover, the overall appearance of the tissue was consistent with a considerable reduction in quantity of the original matrix. The electron-dense protein was infrequently observed in disks where prolapse was present. A number of the specimens were from subjects in the age range where this component begins to be present in appreciable quantities.

Prolapse occurs most frequently in the age range 20-40 years and afterwards becomes decidedly less common⁴. This age distribution alone is sufficient to discount the hypothesis that prolapse is the result of pathologically accelerated ageing of the structure. Such a hypothesis



Figs. 1-3. X-ray fibre diffraction patterns copper $K\alpha$ radiation of wavelength 1.54 Å. Specimen to film distance \approx 3 cm

Fig. 1. X-ray fibre diffraction pattern from the gelatinous nucleus pulposus of a child aged eight years. There is a broadened reflexion in the 4.6 Å region, but no definite side-chain spacing is resolved. The central scatter is from amorphous material. A weak reflexion at 2.86 Å is present from collagen

Fig. 2. X-ray fibre diffraction patterns from nuclear material of a subject aged 87 years. A well-defined β -protein diffraction pattern is present with clear resolution of the side-chain spacing. There is no evidence of orientation

Fig. 3. Comparison X-ray fibre diffraction patterns of prolapsed lumbar disk tissue (left) and nucleus pulposus from an elderly subject (right). The difference in the sharpness of the backbone spacings is evident. The non-collagenous protein in the prolapsed tissue has not given a side-chain spacing. (The region outside the collagen side-chain spacing is 'clean')

was tentatively supported by Blakely *et al.*⁵, who also observed an increase in the β -protein content of prolapsed disk tissue, although they assumed that this was due to the same protein as is prominent in the disks of elderly subjects. Lyons *et al.*⁶ reported the results of biochemical investigations during which they isolated an 'insoluble complex' from the disks of subjects over the age of 60 years taken as normal controls, and a 'similar type of complex' from eight 'herniated' specimens. Both complexes contained chondroitin sulphate *B*. These authors also supported the theory of pathologically accelerated ageing. The hexosamine/nitrogen ratios they reported for the insoluble complexes showed wide variations which could well be indicative either of a mixture or inconstant composition.

The changes in the intercellular matrix are, in the main, a reflexion of cell activity. In prolapse the polysaccharide content is reduced^{1,2} and the non-collagenous protein content increased, an alteration in the quality as well as the quantity of the matrix. This indicates disordered cell function, which may well stem from a disturbed nutritional state similar to that which has been proposed as a cause of osteoarthritis. No differences have been detected between the tissue from patients with or without a history of a significant spinal injury. In ageing there is also a change in the type of matrix, with the emergence of the dense β -protein as a major matrix component. Once this is present in appreciable quantities it seems reasonable to suggest that it affords a measure of protection against prolapse.

T. K. F. TAYLOR
K. LITTLE

Nuffield Department of Orthopaedic
Surgery,
Nuffield Orthopaedic Centre,
Oxford.

¹ Davidson, E. A., and Woodhall, B., *J. Biol. Chem.*, **234**, 2951 (1959).

² Mitchell, P. E. G., Hendry, N. G. C., and Billiewicz, W. Z., *J. Bone and Joint Surg.*, **43**, B, 141 (1961).

³ Taylor, T. K. F., D.Phil. thesis, Univ. Oxford, 1964.

⁴ O'Connell, J. E. A., *J. Bone and Joint Surg.*, **33**, B (1951).

⁵ Blakely, P. R., Happey, F., Naylor, A., and Turner, R. L., *Nature*, **195**, 73 (1962).

⁶ Lyons, H., Jones, E., Quinn, F. E., and Sprunt, D. H., *Proc. Soc. Exp. Biol. and Med.*, **115**, 610 (1964).

BIOCHEMISTRY

Enzymatic Synthesis of the Skin-lightening Agent, Melatonin, in Amphibians

MELATONIN (*N*-acetyl-5-methoxytryptamine) has been shown to be the most effective skin-lightening agent in amphibians^{1,2}. This compound produces changes in pigmentation by causing the aggregation of melanin granules within the amphibian melanocyte. Although melatonin has been found to occur in mammals, no evidence for its formation has been obtained in amphibians where it exerts its most potent effects. High concentrations of radioactivity have been observed in the pineal area of the amphibian *Xenopus laevis* after the administration of the melatonin precursors ¹⁴C-5-hydroxytryptamine and ¹⁴C-methylmethionine³.

The formation of melatonin is catalysed by an enzyme, hydroxyindole-*O*-methyl transferase, which requires *N*-acetylserotonin as the substrate and *S*-adenosylmethionine as the methyl donor⁴. So far, this enzyme has been found only in the pineal gland of mammals⁵ and birds⁶. In mammals melatonin has been shown to inhibit the oestrous phase of the rat oestrous cycle and to produce decreases in ovary weight⁷. This communication describes the presence of hydroxyindole-*O*-methyl transferase in two amphibian species, *Xenopus laevis* larvae and adult frogs (*Rana pipiens*).

Table 1

Species	Hydroxyindole- <i>O</i> -methyl transferase activities
<i>Xenopus laevis</i> larvae	1.3
<i>Rana pipiens</i> adults:	
Brain	11.6
Pineal area	36.6
Optic tectum	34.5
Hypothalamus	36.0

Results expressed as μ moles/¹⁴C-melatonin formed per g tissue per hour when incubated with *N*-acetylserotonin and ¹⁴C-methyl-*S*-adenosylmethionine.

Xenopus larvae, stages 48–50 (ref. 8), were homogenized in 0.5 ml. ice-cold water using an all-glass homogenizer. A 0.2-ml. aliquot was taken to measure the hydroxyindole-*O*-methyl transferase activity⁹. A control incubation was run concurrently without the addition of substrate correct for a small amount of endogenous material present in the *Xenopus* larvae which forms a methylated metabolite on incubation of *Xenopus* larvae with *N*-acetylserotonin and ¹⁴C-methyl-*S*-adenosylmethionine, a radioactive product was formed which was extractable into chloroform. This material had the same *R_F* value on three thin-layer chromatographic systems as authentic melatonin. The amount of melatonin formed enzymatically (Table 1) was more than enough to cause blanching in the *Xenopus*².

Hydroxyindole-*O*-methyl transferase was also examined in the adult frog brain using the procedure already described. Considerable enzyme activity was found in the frog brain (Table 1). Since the brain would also include the pineal, hydroxyindole-*O*-methyl transferase was measured in several brain areas. All areas examined contained enzyme activity. The melatonin-forming enzyme was found to be absent in the adult frog skin, liver, intestine and heart. These results demonstrate that the enzyme that forms the amphibian skin-lightening agent, melatonin, is present in the brain and pineal area of the frog, while it is present only in the pineal gland of other mammalian and bird species so far reported. Further discoveries and more detailed analyses of hydroxyindole-*O*-methyl transferase activities in pineals, brain regions and eyes in various vertebrate species will be published elsewhere.

JULIUS AXELROD

National Institute of Mental Health,
Bethesda, Maryland.

WILBUR B. QUAY
PETER C. BAKER

Department of Zoology,
University of California,
Berkeley.

¹ Lerner, A. B., Case, J. D., Mori, W., and Wright, M. R., *Nature*, **183**, 182 (1957).

² Quay, W. B., and Bagnara, J. T., *Arch. Intern. Pharmacodyn.*, **150**, 13 (1964).

³ Charlton, H. M., *Nature*, **204**, 1093 (1964).

⁴ Axelrod, J., and Weissbach, H., *J. Biol. Chem.*, **236**, 211 (1961).

⁵ Axelrod, J., MacLean, P. D., Albers, R. W., and Weissbach, H., in *Regional Neurochemistry*, edit. by Kety, S. S., and Elkes, J., 307 (Pergamon Press Oxford, England, 1961).

⁶ Axelrod, J., Wurtman, R. J., and Winget, C. M., *Nature*, **201**, 1134 (1964).

⁷ Wurtman, R. J., Axelrod, J., and Chu, E. W., *Science*, **141**, 277 (1963).

⁸ Nieuwkoop, P. D., and Faber, J., *Normal Table of Xenopus laevis* (Daudin) (North Holland Pub. Co., Amsterdam, 1956).

⁹ Axelrod, J., Wurtman, R. J., and Snyder, S. H., *J. Biol. Chem.*, **240**, 949 (1965).

Agar-gel Electrophoresis of Soluble Lens Proteins in Galactose-fed Rats

A NUMBER of reports have recently been published dealing with the electrophoretic properties of water-soluble lens proteins of albino rats and the presence of a 'fast' anodic fraction in electrophoretic patterns of lens proteins from galactose- and lactose-fed rats^{1,2}. It is well established that dense cataractous opacities develop in rat lenses within two to three weeks of galactose feeding³. Electrophoretic analysis of such lens extracts shows that the fraction with the highest anodic mobility is con-

erably increased when compared to normal rat lens⁴. There is, furthermore, an overall decrease in fractions migrating toward the cathode.

Occasionally, however, a rat will not develop a severe opacity of the lens despite prolonged feeding with large amounts of galactose. In some instances the typical cataractous opacity is present in one eye only, while the other lens remains clear.

In an attempt to correlate the appearance of the 'fast' anodic fraction with the stage of cataract development, the following experiments were carried out. From a colony of approximately 100 male Wistar rats, which had been placed on a 40 per cent galactose diet, the following two animals were chosen at random from their respective groups: one rat from the group with mature bilateral cataract after 21 days of galactose feeding, and one rat from the group that had developed only a unilateral cataract. The rat from this second group had been on the lactose diet for 14 days during which time a nuclear opacity developed in one lens while the fellow eye remained clear. One rat of the same strain and comparable age, which had been fed normal laboratory rat chow, was selected as control animal.

After killing the rats under light ether anaesthesia, the lenses were immediately extracted from the eyes under a dissecting microscope and washed free of vitreous humour in iced physiological saline. Along with the dissection a schematic drawing was made of each lens to indicate the degree of cataract development. The lenses were then homogenized individually in 50 μ l. iced saline in a small porcelain weighing-pan, using a glass rod as pestle. The homogenate was transferred quantitatively into a micro-haematocrit capillary tube, using an additional 50 μ l. of saline to rinse the spoon. The haematocrit tube was then sealed and centrifuged in a micro-haematocrit centrifuge for 4 min at 4° C. Following centrifugation, the supernatant was used for electrophoresis.

Agar-gel electrophoresis was carried out following Grabar's method⁵, in a medium of 2 per cent agar in 0.025 M barbital buffer, pH 8.9, and 4 m.amp per cm for 5 min at room temperature. Immediately upon completion of electrophoresis, the agar slides were transferred into a staining bath of 0.05 M thiazine red in 5 per cent acetic acid. After staining for 1 h, excess dye was removed by washing in 5 per cent acetic acid with the final rinse containing 2 per cent glycerol in 5 per cent acetic acid.

Fig. 1 shows the results of the electrophoresis. The two patterns represent lens protein extracts from the normal control rat, right and left lens being indicated at the cathodic side of the slide by R and L, respectively. The drawings next to the electrophoretic patterns indicate the appearance of the lenses under the microscope. As expected, the normal lenses were absolutely clear.

The next two patterns are those obtained from the lens extracts of the rat with mature bilateral cataract. These patterns correspond exactly to those which we obtained repeatedly when using pooled lens extracts from galactose-ataractous rats. The drawings show the presence of a dense opacity in the centre of the lens with small vacuoles in the periphery. Electrophoresis revealed a decrease in the cathodic fractions as well as a considerable increase in the fraction nearest the anode.

The third set of patterns stems from the lens extracts of the rat which had developed unilateral cataract after 14 days on galactose. The dense opacity in the left eye is smaller than that of the other rat due to the shorter feeding time. However, though the left lens still contains a normal amount of the cathodic fractions, the change in anodic fractions is already present. The right lens of this animal was perfectly clear except for a few isolated vacuoles along the periphery. The electrophoretic pattern of this lens is normal in appearance.

A sample of normal rat serum was electrophoresed along with the lens extracts to provide a reference marker. The serum protein pattern, indicated by the letter S, appears

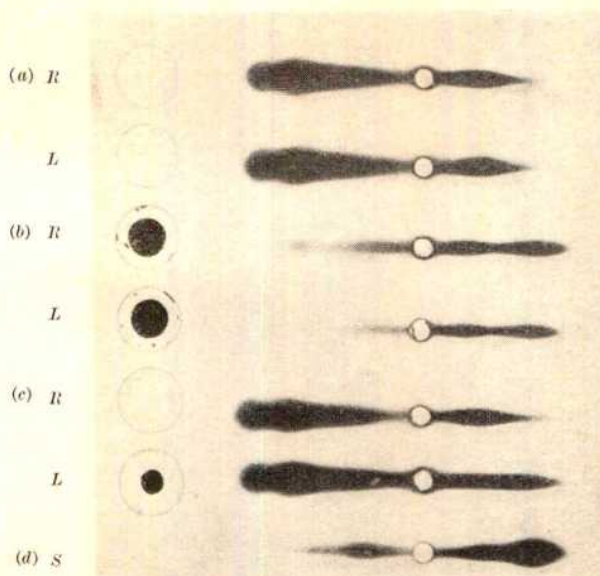


Fig. 1. Agar-gel electrophoresis of individual rat lens proteins. Right and left lens of the same animal are indicated by R and L, respectively. Drawings next to lens protein patterns at cathodic side show stage of cataract development as appearing under dissecting microscope at time of lens extraction. Anode at right. From top to bottom: (a) Normal rat; (b) rat with mature cataract after 21 days on galactose; (c) rat with unilateral cataract (left lens) after 14 days on galactose; (d) normal rat serum.

at the bottom of Fig. 1, the albumin showing at the right-hand side.

Throughout our experiments, 38 out of 635 galactose-fed male Wistar rats, or roughly 6 per cent, did not develop bilateral cataract, even when feeding with galactose was continued for 4 weeks. The development of dense nuclear opacities in those rats was inhibited either in both eyes or in one eye only. There was, furthermore, no consistency as to which eye was affected when unilateral cataract was present. When the extracted clear lenses were examined under the dissecting microscope, the apparently unaffected lens showed the formation of vacuoles similar to those presented in Fig. 1.

In this work, the development of a unilateral cataract made it possible to use the clear fellow lens as a control, thus eliminating differences between animals. It raises the question whether a protective mechanism could be involved, lessening the effect of galactose on the metabolism of the rat lens. Why this should occur in one lens and not in the other is even more puzzling.

This work was supported by research grant NB-03443 from the National Institute of Neurological Diseases and Blindness, National Institutes of Health, U.S. Public Health Service.

EVA WITTGENSTEIN
KENNETH W. ROWE, JUN.

Children's Hospital Research Foundation
and Department of Ophthalmology,
University of Cincinnati College of Medicine,
Cincinnati, Ohio.

¹ Kore, I., and Calcagno, M., *Nature*, **203**, 691 (1964).

² Wittgenstein, E., and Rowe, K. W., jun., *Fed. Proc.*, **22**, 478 (1963).

³ Mitchell, H. S., *Proc. Soc. Exp. Biol. and Med.*, **32**, 971 (1935).

⁴ Wittgenstein, E., and Rowe, K. W., jun., *Investig. Ophthalm.*, **2**, 285 (1963).

⁵ Grabar, P., and Williams, C. A., *Biochim. Biophys. Acta*, **10**, 193 (1953).

Freeing of Amino-acids from Proteins

The large amount of free amino-acid which surrounds haemoglobin freshly released from red blood cells cannot be dialysed away from the protein even after vigorous washing (48 h, running water). The amino-acids still released after such washing may come from (a) metabolism of bacterial contaminants, (b) cellular enzymes,

Table 1

Time from completion of autoclaving (min)		Amino-acid released into dialysate from:		Time from completion of autoclaving (h)		Amino-acid released into dialysate from:	
		Bag A	Bag B			Bag C	Bag D
0	10	Cooling		0	1/6	Cooling	
10	15	3	4	These bags dialysed against running water from the conclusion of cooling until:			
15	20	2	6				
20	25	2	3				
25	30	4	3				
30	60	4	4	18	18.5	4	4
60	90	4	6	18.5	19	2	4
90	120	6	9	19	19.5	3	2
120	150	3	5	19.5	20	2	3
150	180	3	25*	20	20.5	3	23*
180	210	5	33*	20.5	21	4	17*
210	240	3	29*	21	21.5	6	10*
240	270	3	24*	21.5	22	8	25*
13.5 h overnight		9	42*				

Amino-acids expressed as μg of nitrogen in 100 ml. of dialysate.

* These dialysed against normal saline.

(c) amino-acids secondarily bonded to the protein, and (d) the cleavage of peptide bonds. It is impossible to rule out (a) and (b) in the case of haemoglobin.

Commercial casein, however, makes solutions with water which will withstand autoclaving (15 lb. per sq. in. for 30 min) without coming out of solution. A solution of casein subjected to this treatment does not have a large pool of amino-acids around it, but it does progressively release amino-acids on dialysis. (20 g of soluble casein (B.D.H.) were slowly added to 400 ml. of water at 30° C. 25 ml. of this solution contained 150 mg of nitrogen and was put into 'Visking' tubing double knotted at each end. These bags were immersed in water and autoclaved. They were dialysed against 100 ml. of liquid. Each dialysate was evaporated to dryness (1 g sodium chloride was added to the aqueous dialysates first, in order to standardize extraction). After extraction into acetone-hydrochloric acid mixture, which was removed at 120° C, the amino-acids were estimated by the method of Moore and Stein¹.) The autoclaving must exclude those having been produced by causes (a) or (b)².

The amount of amino-acid produced is always small, but it increases significantly if the dialysis is against normal saline instead of water. This is shown in Table 1, as is also the continual production of free amino-acids. The quantity released is in some way related to the concentration of amino-acid in the surrounding solution since the amount freed after 15 h dialysis against 100 ml. of unchanged water is no greater than the sum of the amounts given off during four successive half-hour dialyses.

The freeing of more amino-acids by salt was first noticed with solutions of haemoglobin. While in the present report on casein the cause (c) cannot be excluded, the evidence is in favour of the rupture of primary bonds.

L. J. HASTEWELL
R. N. PRIESTLAND

Department of Physiology,
University of Manchester.

¹ Moore, S., and Stein, W. H., *J. Biol. Chem.*, **178**, 367 (1948).

² Warner, R. C., and Pollis, E., *J. Amer. Chem. Soc.*, **67**, 529 (1945).

Red Pigment in Leaf Galls of *Salix fragilis* L.

THE galls of *Salix fragilis* L. leaves are produced by *Pontania proxima* Lep. and contain a red pigment which is not present in mature leaves or leaves freed from galls, but has been found in young leaves¹. Pigment production has been stimulated in young willow leaves by slight mechanical injury, but the injured leaves generally died². Pontanin was isolated as orange-yellow needles from the galls of *S. fragilis* dried at 100° C and on hydrolysis gave 1 mol. of purpurogallin and 2 mol. of glucose, the probable formula being $\text{C}_{23}\text{H}_{28}\text{O}_{15}$ (ref. 3). Related glycosides of purpurogallin were isolated from other dried galls³, but it was uncertain whether these compounds were natural products or artefacts, and from unpublished

work⁴ it was evident that fresh galls from one of the species lacked purpurogallin glycosides. Hence the present work was done with a fresh approach to clarify the identity of the pigment as part of a larger investigation.

The pigment was extracted from the fresh galls of *fragilis* leaves by maceration with methanol containing 1 per cent hydrochloric acid, the extract concentrated a few ml. under reduced pressure, and purified by the method of Harborne⁵ on washed sheets of Whatman N 3MM paper by band chromatography, using *n*-butanol-2 N hydrochloric acid (1:1 v/v, upper phase). The pigment band was then eluted with methanol-acetic acid (95:5 v/v), concentrated to a few ml. under reduced pressure and further purified by band chromatography using, first, *n*-butanol-acetic acid-water (4:1:5 v/v, upper phase), and, secondly, water-acetic acid (85:15 v/v). The pigment was eluted as before.

The purified pigment was evaporated to dryness *vacuo*, re-dissolved in 2 N hydrochloric acid, heated in boiling water bath for 20 min, then cooled and extracted with iso-amyl alcohol. Purification was achieved by band chromatography on washed sheets of Whatman N 3MM paper, using water-acetic acid-12 N hydrochloric acid (10:30:3 v/v). The acid-treated pigment was eluted as already described. Mineral acid was removed from the pigment-free aqueous solution by washing the solution with chloroform containing 10 per cent di-*n*-octylmethylamine.

The R_F values of the purified pigment were obtained using Whatman No. 1 paper with solvent systems described for anthocyanins⁶, namely, (1) *n*-butanol-2 N hydrochloric acid (1:1 v/v); (2) *n*-butanol-acetic acid-water (4:1:5 v/v); (3) water-12 N hydrochloric acid (97:3 v/v); (4) water-acetic acid-12 N hydrochloric acid (85:15:3 v/v). In addition R_F values of the pigment were determined using the original acidified methanolic extract of fresh galls. With all 4 solvent systems the R_F values of the pigment obtained from the original extract and the purified pigment were similar, showing that there had been no decomposition during the various purification stages. The R_F values of the purified pigment obtained after heating with 2 N hydrochloric acid were determined with reference to a sample of cyanidin chloride using the solvent systems recommended for anthocyanins^{6,7}. These were (1) and (2) as here, (5) formic acid-12 N hydrochloric acid-water (5:2:3 v/v) and (6) water-acetic acid-12 N hydrochloric acid (10:30:3 v/v). Mixtures of the acid-treated pigment and cyanidin chloride were chromatographed in all the given solvent systems and did not form separate spots.

Spectral measurements of the pigment before and after acid treatment were made in the visible region as described by Harborne⁵ using a Unicam S.P. 500. The λ_{max} for the original pigment in methanolic hydrochloric acid was 527 m μ (quoted value for cyanidin-3-monoglucoside 525 m μ ⁸), for the pigment after acid-treatment 535 m μ and for cyanidin chloride 535 m μ . On the addition of 3 drops of a solution of anhydrous aluminium chloride in ethanol (5 per cent, w/v) to the cell solution a shift in the λ_{max} was obtained of 19 m μ for the acid-treated pigment and 18 m μ for cyanidin chloride. This shift shows the compound to have *o*-dihydroxyl groups. The λ_{max} was also determined using as solvent ethanolic hydrochloric acid. The λ_{max} for the unknown and for cyanidin chloride was 545 m μ .

From chromatographic and spectral characters it was concluded that the pigment obtained after acid treatment is cyanidin, this being confirmed by the distribution and oxidation tests, and the tests with the cyanidin and delphinidin reagents, sodium acetate and ferric chloride (Robinson's method⁹).

From the chromatographic data obtained it was concluded that the red pigment of the galls is either cyanidin-3-monoglucoside or cyanidin-3-monogalactoside. The free sugar obtained after acid hydrolysis of the antho-

Table 1. R_F VALUES OF THE PIGMENT FROM *S. fragilis* LEAF GALLS BEFORE AND AFTER ACID TREATMENT

	(1) BuOH/ 2 N HCl (1:1)	(2) BuOH/ AcOH/H ₂ O (4:1:5)	(3) H ₂ O/ 12 N HCl (97:3)	(4) H ₂ O/AcOH 12 N HCl (85:15:3)	(5) Formic acid/ 12 N HCl/H ₂ O (5:2:3)	(6) H ₂ O/AcOH/ 12 N HCl (10:30:3)
Unknown pigment*	0.24	0.37	0.06	0.25	—	—
Unknown pigment after acid treatment	0.70	0.68	—	—	0.21	0.50
Cyanidin chloride	0.69	0.68	—	—	0.22	0.50

R_F values quoted (ref. 6) for cyanidin-3-monoglucoside are: (1) 0.25, (2) 0.38, (3) 0.07, (4) 0.26; and for cyanidin-3-monogalactoside: (1) 0.24, (2) 0.37, (3) 0.07, and (4) 0.26.

nin was investigated by chromatography on Whatman 1 paper using ethyl acetate-water-pyridine (2:2:1), (upper phase), a development time of 26 h, and aniline hydrochloride (Merck) as the locating reagent. The unknown pigment had the same R_F as glucose, and separated from fructose but not from glucose. The same results were obtained when the sugar was chromatographed on thin layers of kieselgel G (Merck) using the solvent system of ethyl acetate-methyl ethyl ketone-methanol-acetic acid (1:1:1 v/v). The spray reagent used was anisaldehyde-sulphuric acid-95 per cent ethanol (5:5:90 v/v), followed by heating at 100° C for 10 min. Glucose and fructose gave a blue colour with the spray reagent, whereas galactose gave a greenish grey, and it was concluded that the sugar obtained from the glycoside was glucose. The latter was confirmed by preparing the osazone which was identical with glucosazone.

The foregoing results, therefore, show that the red pigment in freshly collected galls of *S. fragilis* is cyanidin-3-monoglucoside. The pigment isolated by Nierenstein and Swanton, having been obtained from dried galls, could have been formed during drying and was not the natural pigment.

GERALD BLUNDEN
STEPHEN B. CHALLEN

School of Pharmacy,
Portsmouth College of Technology,
Portsmouth, Hants.

- Challen, S. B., *J. Pharm. Pharmacol.*, **11**, 223T (1959).
Magnus, W., from Carleton, M., *J. Linn. Soc. (Zoo.)*, **40**, 575 (1939).
Nierenstein, M., and Swanton, A., *Biochem. J.*, **38**, 373 (1944).
Erdtman, H., *Modern Methods of Plant Analysis*, **3**, 352 (Springer-Verlag, Berlin, 1955).
Harborne, J. B., *Biochem. J.*, **70**, 22 (1958).
Harborne, J. B., *J. Chromatog.*, **1**, 473 (1958).
Bate-Smith, E. C., *Biochem. J.*, **70**, 473 (1958).
Robinson, G. M., and Robinson, R., *Biochem. J.*, **25**, 1687 (1931).
Pastuska, G., *J. Chromatog.*, **15**, D20 (1964).

Isolation of a Lymphoid-cell Protein with Relation to Delayed Hypersensitivity

THE reports on passive transfer of delayed hypersensitivity not only by intact cells but also with cellular extracts¹⁻⁶ have initiated studies regarding the nature of the substance responsible for this effect^{2,4,5,9}. Using free electrophoresis according to Tiselius, it has been shown⁵ that peritoneal-cell extracts from guinea-pigs with delayed hypersensitivity contain a protein fraction moving as the alpha-1 globulins. This protein was absent in extracts from the control animals. With this fraction it was possible to transfer passively the delayed hypersensitivity.

The aim of this investigation was to analyse the protein pattern of lymphoid-cell extracts from guinea-pigs sensitized to 2,4-dinitrochlorobenzene (DNCB) and from normal animals. The main interest was focused on the comparison between the lymph nodes regional to the sensitization and normal lymph nodes, but other lymph nodes from the sensitized animals were also studied.

In the first step of the investigation electrophoresis in starch gel⁷ in a discontinuous buffer system⁸ was used. Ten DNCB-sensitized animals and six control animals provided the material. The experimental set-up was essentially the same as that described below for the disc electrophoresis. By electrophoresis in starch gel it was not possible to discover any differences in the extracts from the different lymph nodes. This is in accordance

with the negative results with human leucocyte extracts separated on cellulose acetate strips⁹.

In the second step disc electrophoresis¹⁰ was employed. Thirty-six white guinea-pigs weighing 300–450 g were used. Sixteen of these served as normal controls, twenty were sensitized epicutaneously with DNCB (20 vol. per cent in acetone, two applications with a 24-h interval). After a test dose of 9 µg DNCB in acetone per square cm all the animals showed positive reactions (erythema and sometimes oedema). The tests were performed on the eleventh day after the first application of DNCB and the animals were killed on the thirteenth day. The lymph nodes were homogenized in a cooled Potter-Elvehjem homogenizer. After freezing-thawing six times the samples were centrifuged in a Beckman Spinco 'Microfuge' for 4 min. The supernatants were mixed with crystals of sucrose to a final concentration of 5 per cent, and could then be used for electrophoresis. This was carried out according to a modification of the disc electrophoresis technique developed in our laboratory. Instead of using a large-pore gel of 2.5 per cent polyacrylamide on top of the column, an anticonvection medium consisting of 'Sephadex G-200' sedimented in 20 per cent sucrose was used and mixed with the sample. The electrophoretic procedure is shown schematically in Fig. 1. Electrophoresis was first carried out in gels of 7.5 per cent concentration for 30 min at 3.3 m.a.p. Sections were cut out of the gels and transferred to the top of a column 6.8 mm wide containing 7.5 per cent gel and carefully homogenized with a glass rod. The end of the column was covered with a 'Visking 18/32' cellulose membrane. Thus there was a little space of about 300 µl. between the lower end of the gel and the membrane, which, after electrophoresis for 2 h at about 3 m.a.p. contained the non-dialysable proteins from the gel sections originally placed on the column. This specimen could be additionally concentrated about three-fold by stirring dry 'Sephadex G-25' into the solution until a thick suspension was obtained. The gel grains were removed by centrifugation. The supernatant was mixed with sucrose and layered above a 6.8 × 40 mm column containing 15 per cent polyacrylamide gel. Even this column was charged with an anticonvection medium of 'Sephadex G-200'. Electrophoresis was carried out using a current of 3.0 m.a.p. for 100 min. Staining was performed in 0.1 g per cent amido black in 7 per cent acetic acid.

In the first electrophoretic run (7.5 per cent gel) fifteen bands were separated in extracts from lymph nodes of whatever sort. However, the band corresponding to albumin in extracts from the nodes regional to the sensitization appeared to be more concentrated than that of the control nodes. This band was further resolved in the run in the 15 per cent gel; in samples from the hypersensitive animals four distinct bands appeared compared

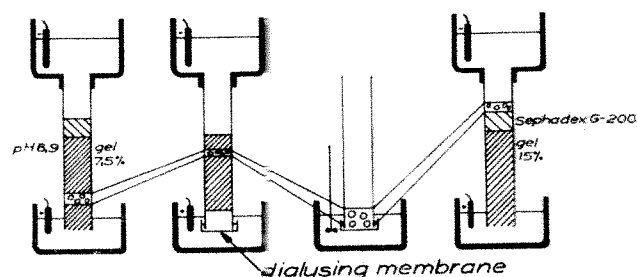


Fig. 1

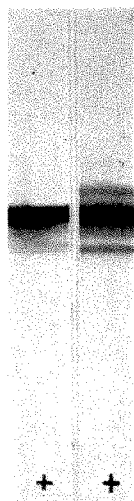


Fig. 2. Pherogram of lymph node extract from a normal animal (left) and a DNCB-sensitized animal (right). Two fast-moving components are visible ahead of the albumin (strong band) in both extracts from normal and DNCB-sensitized animals; the latter show a specific fraction with an electrophoretic mobility lower than that of albumin.

with three bands from the control animals (Fig. 2). It was also possible to visualize the fourth band by electrophoresis in 15 per cent gel at once without the preceding runs in 7.5 per cent gel. However, the separation was not very good in this case. The pre-albumins, also separated, have been shown to vary a little between the animals, possibly due to genetic differences.

In this investigation extracts from lymph node cells regional to the place of sensitization were used. A very weak fourth band was also found in extracts from the lymph nodes regional to the test place, while in extracts from the neck lymph nodes of the same animal this band was barely discernible. In other experiments we recently have found that the fourth band seems to be present at the highest concentration about a fortnight after sensitization. This coincides with the maximum lymphocyte infiltration in the epidermis of the test area¹¹. To exclude a possible effect of the small quantity of serum left in the nodes, the sera from the animals were also separated using both the electrophoretic methods. No differences between normal and sensitized animals were seen.

We thank Prof. Ove Groth, who kindly supplied some of the sensitized animals.

Note added in proof. During the course of publication the protein fraction described here has been further analysed and found to be an IgA (= beta-2A) globulin.

The investigation was supported by the Swedish Medical Research Council.

ULF ROTHMAN
STURE LIDÉN

Departments of Histology and Dermatology,
University of Uppsala,
Sweden.

¹ Jeter, W. S., Tremaine, M. M., and Seebohm, P. M., *Proc. Soc. Exp. Biol. and Med.*, **86**, 251 (1954).

² Cole, L. R., and Favour, C. B., *J. Exp. Med.*, **101**, 391 (1955).

³ Cummings, M. M., Patnode, R. A., and Hudgins, P. C., *Amer. Rev. Tuberc.*, **73**, 246 (1956).

⁴ Lawrence, H. S., in *Cellular and Humoral Aspects of the Hypersensitive States*, edit. by Lawrence, 281 (1959).

⁵ Jeter, W. S., Laurence, K. A., and Seebohm, P. M., *J. Bact.*, **74**, 680 (1957).

⁶ Rapaport, F. F., Lawrence, H. S., Millar, J. W., Pappagianis, D., and Smith, C. E., *J. Immunol.*, **84**, 358 (1960).

⁷ Smithies, O., *Biochem. J.*, **61**, 629 (1955).

⁸ Poulik, M. D., *Nature*, **180**, 1477 (1957).

⁹ Baram, P., and Mosko, M., *J. Allergy*, **33**, 489 (1962).

¹⁰ Ornstein, L., and Davis, B. J., *Disc Electrophoresis*, preprinted by Distillation Products Industries, Rochester, N.Y.

¹¹ Groth, O., *Acta dermato-venereol.*, **44**, 1 (1964).

Formation of Complexes between Basic Proteins of Leucocytes and Plasma Globulin

RECENT studies have shown that cationic proteins from leucocytes produce tissue injury and responses characteristic of immunological reactions¹⁻⁴. In an earlier publication we put forward the hypothesis that homograft rejection may be caused by such cationic proteins⁵. The specificity of the homograft reaction could only occur if the basic protein were transported to the homograft site via specific antibody (globulin) or in cells which have specificity through some chemotactic response of the cells to antigen.

This report presents a new technique in disc electrophoresis for the purpose of demonstrating the formation of a complex between basic proteins and purified globulin of serum. The method is a general one which may be applied to other complexes, but is described below from the standpoint of complexes involving plasma globulin.

The technique combines the standard system of disc electrophoresis⁶ which resolves anionic proteins with a reversed system⁷ that resolves cationic proteins. Resolution in the first system frees the complex (anionic) from any uncomplexed cationic component. The total protein migrating in this system is then transferred, in its gel, the sample layer of the reversed system. Depending on the pH of the second or reversed system and the stability of the complex at that pH, resolution will show the cationic component of the complex. Any such protein appearing at this final stage must have been brought into the lower gel of the original sample tube on a 'carrier', complexed with a globular protein.

The pH of the sample gel as well as the 'running pH' of the first system must be those at which the complex is stable, whereas the same pH values in the second system would be those at which the complex is split into its components. These considerations led to a modification of the standard disc electrophoresis system regarding the pH of the various gels employed. This system normally resolves anionic substances having free electrophoretic mobilities in the range -0.6 to -7.5. The new system is specifically designed to give the greatest resolving power in the mobility range -0.75 to -1.0 which includes the slow α -, slow β -, and γ -globulins. The pH of the sample gel has been raised from a slightly acid pH of 6.9 to a pH of 7.5 which closely approximates to that of whole blood. The pH at which electrophoresis actually occurs is reduced from 9.5 to 8.6, thus maintaining a mildly alkaline medium for the sample at all times.

The changes made are only in the amounts of *tris* employed in the upper gel, lower gel and buffer. The new values for 100 ml. of solutions A (pH 7.2), and B (pH 7.3) are 10.65 g and 6.0 g, respectively, and the buffer quantity for 1 l. is changed from 6.0 g to 10.0 g (pH 8.5). All other solutions as well as techniques for gel formation and processing are as adopted at present. Because of the selectivity of this system for molecules of high molecular weight, it is recommended that gel concentration of 15 per cent and higher be used to effect the greatest possible resolutions.

In practice, the technique is carried out as follows. The two components of the complex to be tested are allowed to conjugate and they are then polymerized into the sample gel of the first system. No indicator dye is used because of the possible combination of the dye with one of the constituents of the complex. The ionic boundary is identified during the run by noting the difference in the refractive index between the upper and lower solutions. When the eye is placed on the level of this front it can be plainly seen. The ionic front is allowed to migrate into the lower gel only a short distance. The lower gel containing the protein is removed from the glass tube and the upper portion, containing the sample, is cut off with a sharp knife. This gel is then transferred, with the same orientation (not inverted), to the sample layer of a tube made up in the reverse system. It is covered with

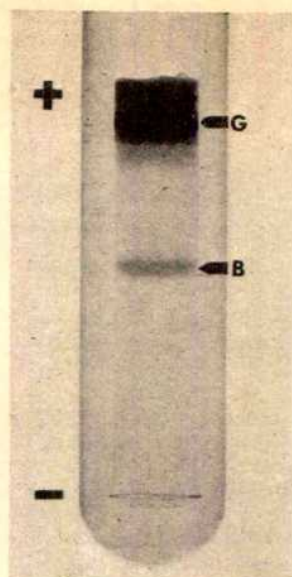


Fig. 1. Acrylamide-gel disc electrophoretic separation of basic (cationic) protein (B) from the carrier, α -globulin (G). The orientation of migration is shown on the left side of the gel.

upper gel solution, all trapped air bubbles removed and then polymerized into the tube. When the ionic boundary has entered the lower gel, the hard sample gel may be removed using a bent syringe needle or hook. The boundary is allowed to migrate a previously measured distance, the gel removed from the tube, stained and de-stained in the usual manner.

Fig. 1 shows the separation of basic protein (B) carried as a complex with α -globulin fraction (G) by the technique described above. The basic protein was isolated from polymorphonuclear leucocytes of rabbits using the method of Cohn and Hirsch⁸. The α -globulin was obtained commercially from International Chemical and Nuclear Corporation, City of Industry, California. The purity of this material was checked by disc electrophoresis prior to use.

Similar experiments were conducted in which protamine was substituted for the basic protein of the leucocyte. No protamine complexed with the α -globulin in the technique previously described.

The results of these experiments clearly demonstrate the transporting of basic (cationic) proteins from polymorphonuclear leucocytes on globulin molecules. These observations become extremely provocative in the light of other work which has demonstrated that basic protein from a similar source has bactericidal activity² and also provokes inflammatory-like reactions¹. Such complexes would facilitate an accumulation of toxic substance at the site of antigen-antibody interaction.

A question immediately arises whether the complex of the cationic protein and globulin remains after the globulin reacts with antigen of foreign tissue. Experiments are under way to determine the toxicity of the complex versus the free basic protein.

A possible mechanism by which one might get dissociation of such complexes would be through discharge of electrostatic attraction at the time of globulin-antigen combination. An alternative method for the dissociation could be proposed if one assumes that the low molecular weight basic protein exists as a helix. Helical configurations have been shown in various peptide chains⁹⁻¹¹. Cohn and Hirsch have shown that the basic proteins exist as granules in polymorphonuclear cells and are released whenever the cell pH drops to approximately five¹². Granule release at such a pH might occur if the protein configuration changed from a helix to a random coil.

The formation of a random coil might present such spatial differences that the globulin-cationic protein com-

plex would dissociate. Since no complex with protamine could be demonstrated, structural differences could be assumed to have importance and not simply the basicity of the molecule.

We have already shown that removal of basic proteins from the peritoneal cavity with a Millipore 'trap' increases homograft survival⁵.

This research was supported in part by U.S. Public Health Service grants AM 06020 and CA 05813, National Institute of Arthritis and Metabolic Diseases.

Note added in proof. Since submitting this article for publication, additional experimental details covering the electrophoresis of ionic complexes have appeared¹³.

WELDON B. JOLLEY
HENRY W. ALLEN

Department of Surgery,
Loma Linda University School of Medicine,
Los Angeles 33, California.

- ¹ Janoff, A., and Zweifach, B. W., *Science*, **144**, 1456 (1964).
- ² Zeya, H. I., and Spitznagel, J. K., *Science*, **142**, 1085 (1963).
- ³ Golub, E. S., and Spitznagel, J. K., *Fed. Proc.*, **23**, 509 (1964).
- ⁴ Hirsch, J. G., *J. Exp. Med.*, **103**, 589 (1956).
- ⁵ Jolley, W. B., and Hinshaw, D. B., *Amer. J. Surg.*, **108**, 127 (1964).
- ⁶ Ornstein, L., and Davis, B. J., *Disc Electrophoresis* (Distillation Products Industries, 1962).
- ⁷ Reisfeld, R. A., Lewis, U. J., and Williams, D. E., *Nature*, **195**, 281 (1962).
- ⁸ Cohn, Z. A., and Hirsch, J. G., *J. Exp. Med.*, **112**, 983 (1961).
- ⁹ Crick, F. H. C., and Rich, A., *Nature*, **176**, 780 (1955).
- ¹⁰ Elliott, A., *Proc. Roy. Soc., A*, **226**, 508 (1954).
- ¹¹ Yang, J. T., and Doty, P., *J. Amer. Chem. Soc.*, **79**, 761 (1957).
- ¹² Cohn, Z. A., and Hirsch, J. G., *J. Exp. Med.*, **112**, 983 (1960).
- ¹³ Ornstein, L., *Ann. New York Acad. Sci.*, **121**, 321 (1964).

PHYSIOLOGY

Effect of Altered Perfusion Rates on the Retention of Noradrenaline by the Spleen

In the majority of investigations concerning the mechanism of retention of circulating noradrenaline by tissues, the amine has been administered by intravenous injection. Noradrenaline itself, as well as a wide variety of physiological and pathological conditions, are known to exert profound effects on the flow of blood through tissues. It is important, therefore, to establish the effect of changes in flow rate on the uptake of circulating noradrenaline or that liberated by sympathetic nerve stimulation. The evidence in the literature on this point is conflicting. It has been shown that the greater the flow of blood through the organ, the greater is the amount of noradrenaline in the venous effluent following stimulation of the post-ganglionic sympathetic nerves to spleen¹⁻³, intestine⁴ and skeletal muscle⁵. On the other hand, spontaneous changes in flow rate have been reported to be without effect on the retention of noradrenaline by the perfused heart⁶ and the perfused spleen⁷. Consequently, we have examined the effect of different, controlled flow rates on the uptake of tritiated noradrenaline by the perfused spleen.

Spleens of cats were perfused by means of a technique similar to that recently described by Fischer *et al.*⁸. However, the procedure differed in the following respects: the intestines were left intact while both the left gastric and hepatic arteries were ligated and the coeliac artery was cannulated. The spleen was perfused, at a constant flow rate, with oxygenated Krebs solution at 37° C, by means of a 'Sigmamotor' pump (model TM11). Perfusion pressure distal to the pump was monitored continuously using a Statham P-23A pressure transducer, the output of which was displayed on a Grass model 5 polygraph. The spleen was kept warm with cotton pads soaked in mineral oil at 37° C. The splenic nerves were cut immediately prior to the infusion of tritiated noradrenaline. Chromatographically pure tritiated *dl*-noradrenaline as a solution of the hydrochloride (New England Nuclear Corp.), with a specific activity of 7.6 c./mmole, was infused into the tubing leading to the perfusion pump using a Harvard infusion pump (model 500-910). The rate of infusion of

the amine was such as to ensure that the concentration of tritiated noradrenaline was 4.8 mg/ml. regardless of the flow rate. At each flow rate used, a total of 50 ng tritiated noradrenaline base was infused. Each spleen was perfused at least three times with the labelled amine, the first and last perfusion always being at the same flow rate. The venous effluent was collected for 2 min before, during and for 15 min after the infusion of tritiated noradrenaline, each sample being collected for 1 min. To each sample, one-tenth of the volume of 4 M perchloric acid was added. Fifteen minutes later the samples were centrifuged in a clinical centrifuge. In each case, the supernatant fluid was decanted, its volume measured, and a 0.1-ml. aliquot mixed with 10 ml. Bray's phosphor. The total radioactivity of the aliquot (^3H -total) was then determined in a Packard 'Tri-carb' liquid scintillation spectrometer having an efficiency for tritium of 25 per cent. Quenching was determined by the internal standard method using tritiated water. Results are corrected for quenching, which ranged from 20 to 25 per cent. Retention of tritiated noradrenaline was taken as the difference between the total amount of tritiated noradrenaline infused (assessed by direct measurement of tritium) and the total amount of tritium recovered in the venous effluent during and for 15 min after the infusion. At least 75 per cent of the tritium recovered was found in the venous effluent within 6 min of terminating the infusion.

The results of these experiments are shown in Table 1. The amounts of tritiated noradrenaline retained in the spleen during the first and last perfusions at the same flow rate were very similar. With increasing flow rates significantly decreased amounts of total- ^3H were retained by the spleen.

At the concentration used in this investigation the retention of noradrenaline would be dependent mainly on a saturable membrane transport mechanism⁹⁻¹². When the noradrenaline concentration is constant, such a process would be expected to operate at a constant rate⁹⁻¹². It follows, therefore, that when the noradrenaline was infused, its passage across the membrane should proceed at the same rate regardless of flow through the organ. In our investigation, the only experimental variable was the flow rate, alterations of which necessitated increasing or decreasing the total time during which the noradrenaline was added to the perfusion fluid. The durations of noradrenaline infusion at the different flow rates are given in Table 1. Table 1 shows that there was an inverse relationship between total retention of tritiated noradrenaline and flow rate. This finding shows that retention of noradrenaline by tissues is a function of the duration of the time during which the amine is in contact with the uptake sites. After the completion of our investigations the results of a single experiment with similar results was reported¹³.

If the transport and/or diffusion mechanism operates at a constant rate, then the retention of amine per unit time should be the same regardless of blood flow provided that the concentration is fixed. When the data of Table 1 were used to calculate the fraction of the total noradrenaline retention that took place per minute (that is, the rate of retention), the relationship shown in Table 2 resulted. This reveals an apparent increase in the rate of retention with increasing flow rate. If retention, in fact, takes place at a constant rate, it may be that the higher flow rates caused the apparent increase in the rate of retention (Table 2) by virtue of their perfusing more of

Table 2. PERCENTAGE OF TRITIATED NORADRENALINE RETAINED IN THE SPLEEN PER MIN AT DIFFERENT FLOW RATES

Flow rate (ml./min)	Mean tritiated noradrenaline retained (percentage/min)
3.5	13.7
7.0	18.7
14.0	28.7

the splenic tissue, thus bringing the tritiated noradrenaline into contact with more sites at which its retention can take place. Perfusion pressure was only slightly elevated by increasing the flow rate from 3.5 to 14 ml./min.

This investigation directs attention to the importance of fluid flow rate as a determinant of noradrenaline retention by tissues. Altered flow rates change the time available for retention of noradrenaline by binding sites as well as the extent of functional tissue perfusion. A practical implication of this work is that a single, rapid injection of noradrenaline, by altering the blood (or perfusion fluid) flow through a tissue, may thus influence the magnitude of retention. Slow infusion of the amine would circumvent this hazard. It has also been shown that the fraction of the cardiac output delivered to an organ is another determinant of noradrenaline retention.

We thank Miss Jean Bach for her assistance. This work was supported by U.S. Public Health Service grant H-7249 from the National Heart Institute, and by grant G-65-46 from the Life Insurance Medical Research Fund. One of us (D. M. P.) was the recipient of a Wellcome research travel grant.

D. M. PATON*
C. N. GILLIS†

Department of Pharmacology,
Yale University School of Medicine,
New Haven, Connecticut.

* James Hudson Brown Memorial Postdoctoral Fellow.

† Established Investigator of the American Heart Association.

¹ Brown, G. L., and Gillespie, J. S., *J. Physiol. (Lond.)*, **138**, 81 (1957).

² Blakeley, A. G. H., Brown, G. L., and Ferry, C. B., *J. Physiol. (Lond.)*, **167**, 505 (1963).

³ Hertting, G., and Schiefthaler, T., *Arch. Exp. Path. Pharmacol.*, **246**, 1 (1963).

⁴ Brown, G. L., Davies, B. N., and Gillespie, J. S., *J. Physiol. (Lond.)*, **141**, 41 (1958).

⁵ Carlsson, A., Folkow, B., and Häggendal, J., *Life Sciences*, **3**, 1335 (1964).

⁶ Lindmar, R., and Muscholl, E., *Arch. Exp. Path. Pharmacol.*, **247**, 40 (1964).

⁷ Gillespie, J. S., and Kirpekar, S. M., *J. Physiol. (Lond.)*, **176**, 205 (1965).

⁸ Fischer, J. E., Horst, W. D., and Kopin, I. J., *Brit. J. Pharmacol.*, **24**, 47 (1965).

⁹ Iverson, L. L., *Brit. J. Pharmacol.*, **21**, 523 (1963).

¹⁰ Iverson, L. L., *J. Physiol. (Lond.)*, **175**, 71P (1964).

¹¹ Iverson, L. L., quoted by Zaimis, E., *J. Physiol. (Lond.)*, **177**, 35P (1965).

¹² Potter, L. T., Cooper, T., Willman, V. L., and Wolfe, D. E., *Circ. Res.*, **16**, 468 (1965).

¹³ Hertting, G., *Proc. Sec. Intern. Pharm. Meeting*, **3**, 277 (1965).

¹⁴ Wurtman, R. J., Kopin, I. J., Horst, D., and Fischer, J. E., *Amer. J. Physiol.*, **207**, 1247 (1964).

¹⁵ Johnson, P. O., *Statistical Methods in Research*, 62 (Prentice Hall, New York, 1949).

Role of Portal Blood Supply in Liver Regeneration

It has been known for many years that in laboratory animals, such as the rat, partial hepatectomy is followed by liver regeneration. In recent years it has been generally accepted that this response is initiated by some sort of humoral mechanism¹. This view was, however, originally based on parabiotic and serum injection experiments the validity of which has been contested. We have therefore been led to re-examine the alternative and much older 'blood-flow' theory². This postulates that after partial hepatectomy the remaining liver fragment acquires a much more generous blood supply, since it has to accommodate portal vein blood previously destined for the excised lobes. It is this over-generous blood supply which is said to act as the stimulus to hyperplasia. The strongest evidence in favour of this theory was derived from experiments on domestic fowls, by taking advantage of the anastomosis between the post-caval and

Table 1. PERCENTAGE OF INJECTED TRITIATED NORADRENALINE RETAINED IN THE SPLEEN AT DIFFERENT FLOW RATES

Flow rate (ml./min)	Duration of tritiated noradrenaline infusion (sec)	Tritiated noradrenaline retained (mean percentage \pm S.E.)
3.5	180	41.2 \pm 1.2 (a) (n = 12)
7.0	90	28.1 \pm 1.6 (b) (n = 6)
14.0	45	21.5 \pm 2.3 (c) (n = 4)

Weighted \pm test¹⁵: (a) versus (b), $P < 0.001$; (a) versus (c), $P < 0.01$.

portal veins through the coccygeo-mesenteric vein. It is therefore possible to increase portal blood flow by simple ligation of the postcaval vein between the kidneys and the liver. According to the 'blood-flow' theory this should itself cause hyperplasia of the liver. Furthermore, the 'blood-flow' theory would predict that, in the fowl, partial hepatectomy would not be followed by liver regeneration, since the blood which would normally be accommodated by the missing lobes could instead return to the heart via the anastomosis. It was claimed that both these theoretical predictions were fulfilled experimentally¹, since the number of fowls used in these original experiments was small, and since the criteria of liver growth were very crude, it seemed worthwhile to repeat them in an improved form using mitotic frequency as an index of liver growth. For this purpose we have used 26-week-old pullets of the "Sussex Legbar" strain weighing about 200 g. For both operations the fowls were anaesthetized lightly with intravenous 'Nembutal', and anaesthesia completed with ether. In the partial hepatectomy the entire left lobe of the liver was removed through an incision on the left side parallel to the edge of the sternum and 1 cm above it. Ligation of the postcaval vein was performed through an incision in the right flank⁴.

Table 1 shows the effect of partial hepatectomy. Before the operation the mitotic frequency is very low—less than 20 per 100,000 nuclei. After the operation it shows a rather irregular increase for the first 3 or 4 days and then appears to return to a figure not very different from the pre-operative level. This suggests that, contrary to the blood flow theory, there has been a regenerative response of a sort; admittedly less dramatic than that seen in the rat, but it must be remembered that only 40 per cent of the fowl liver was removed compared with 60–70 per cent removed in most rat experiments. Table 2 shows the effect of ligation of the postcaval vein, as compared with that of a sham operation. Both procedures give mitotic frequencies rather higher than the pre-operative figures shown in Table 1; but there is no indication that the ligation has any more effect than the sham operation. This result also is in flat contradiction to the 'blood flow' theory.

Taken together, the results shown in Tables 1 and 2 seem to deprive the 'blood flow' theory of its one apparently secure experimental support. It seemed, however, desirable to carry out an analogous series of experiments in some species which, unlike the fowl, is known to show the classical regenerative response to partial hepatectomy. We have therefore compared the effects in the dog of partial hepatectomy⁵ and of the establishment of a reverse Eck fistula⁶. The latter procedure, by increasing the blood flow through the liver, would be expected, according to the 'blood flow' theory, to cause liver growth similar to that which follows partial hepatectomy. The results are shown in Table 3. Partial hepatectomy, as expected, produced a very large increase in mitotic activity demon-

Table 3. EFFECT OF REVERSE ECK FISTULA AND PARTIAL HEPATECTOMY ON MITOSIS IN THE LIVER

Animal No.	Operation	Mitotic figures per 100,000 nuclei			
		Before operation	3 days later	6 days later	9 days later
1	Reverse Eck fistula	5	6	—	—
2	Reverse Eck fistula	55	13	—	—
3	Reverse Eck fistula	3	7	—	—
4	Reverse Eck fistula	18	0	—	—
5	Reverse Eck fistula	7	—	6 + 529*	—
6	Reverse Eck fistula	47	—	34	—
7	Reverse Eck fistula	3	—	10	—
8	Reverse Eck fistula	3	—	—	15
9	Reverse Eck fistula	5	—	—	5
10	Reverse Eck fistula	7	—	—	8
11	Partial hepatectomy	3	382	198	381

* Biopsy from left lobe (see text).

strable 3, 6 and 9 days after the operation. By contrast, establishment of the shunt in general produced no significant increase over the pre-shunt level. There was, however, one exception. In general, when the animals were under anaesthesia before being killed, the livers were a dark bluish colour, they appeared turgid, and the normally sharp edges of their lobes had become rounded and the surface often presented a fine nutmeg appearance. At death, the liver of animal number 6, though showing the usual rounding of the sharp edges, did not have the usual homogeneous dark colour. The left central and left lateral lobes were a bright red in contrast to the bluish colour of the rest of the liver. This colour difference disappeared after death when the liver was excised. The mitotic index in the red lobes was extremely high and of the order of that seen following partial hepatectomy (Table 3). In the rest of the liver it remained at the pre-shunt level. This anomalous result can most easily be explained on the assumption that in some unknown fashion the reverse Eck fistula had disturbed the haemodynamics of the liver in such a way that blood being delivered to the left lobes was better oxygenated than that being delivered to the right. The high mitotic frequency observed in the left lobes might well be attributable to a relative increase in the oxygen saturation of its blood supply. However that may be, it seems quite clear from Table 3 that increased blood flow does not by itself bring about growth of liver tissue.

The blood flow theory of liver regeneration would therefore seem to be untenable, at least in the form in which it was originally stated.

R. Y. THOMSON

Department of Biochemistry,

A. M. CLARKE

Department of Surgery,
University of Glasgow.

¹ Bucher, N. L. R., *Intern. Rev. Cytol.*, **15**, 245 (1963).

² Leong, G. F., Grisham, J. W., Hole, B. V., and Albright, M. L., *Cancer Res.*, **24**, 1496 (1964).

³ Mann, F. C., *J. Mt. Sinai Hosp.*, **11**, 65 (1944).

⁴ Higgins, G. M., Mann, F. C. and Priestley, J. T., *Arch. Path. (Lab. Med.)*, **14**, 491 (1932).

⁵ Sigel, B., *Arch. Surg. (Chicago)*, **87**, 788 (1963).

⁶ Markowitz, J., *Experimental Surgery* (Baillière, Tindall and Cox, London, 1954).

Chemotopic Organization in the Bulbar Gustatory Relay of the Rat

In the relay nuclei of the mammalian auditory system, one representation of sound frequency is the location of maximum neural activity within a nucleus. This spatial representation of stimulus quality is called tonotopic organization¹. In the gustatory system, change in the response magnitudes to chemicals as a function of change in tongue region stimulated has already been demonstrated in the nucleus of the fasciculus solitarius (NFS)². The preliminary data to be presented here indicate that discrete sub-areas which are highly responsive to some chemicals are located in the anterior tongue zone of the bulbar gustatory relay of the rat. The recording and stimulating techniques have been described previously^{2,3}.

Table 1. FREQUENCY OF MITOSIS IN THE LIVERS OF FOWLS BEFORE AND AFTER PARTIAL HEPATECTOMY

Time between hepatectomy and death (days)	Mitoses per 100,000 nuclei	
	At operation	At death
1	6	10
2	—	167
3	5	465
	5	125
4	14	106
5	9	14
	5	38
6	8	15
7	14	10
	4	53

Table 2. FREQUENCY OF MITOSIS IN THE LIVERS OF FOWLS AFTER LIGATION OF THE POSTCAVAL VEIN OR SHAM OPERATION

Time between operation and death (days)	Mitoses per 100,000 nuclei at death	
	Sham operation	Postcaval vein ligation
2	5, 6, 10, 6	12, 20, 34, 144
3	8, 26, 16, 37	31, 30, 14, 54, 18
5	10, 11, 17, 26, 57	78, 29, 16, 42, 24
7	23, 10, 10	63, 9, 12, 7

The active electrode was a nickel-chrome wire, 25-4 μ in diameter, insulated except at the tip. The multi-unit neural activity was led through an electronic summator⁴ into a recording milliammeter. The stimuli were liquids which flowed over the anterior tongue through a tongue chamber.

In the first experiments, neural responses were recorded at two depths along a single vertical electrode track in the bulbar gustatory relay. The order of response magnitudes at the more dorsal recording loci was: 0.1 M NaCl > 0.005 M HCl > 0.1 M KCl > 0.01 M NaCl > 0.01 M quinine hydrochloride (QHCl) = 0.1 M sucrose. In contrast, at the more ventral loci, the order was: 0.1 M NaCl > 0.005 M HCl > 0.1 M sucrose > 0.1 M KCl > 0.01 M NaCl > 0.01 M QHCl (summed ranks from seven animals). Note that sucrose moved from least effective at the more dorsal loci to two from the top at the more ventral loci. These different orders of stimulus effectiveness were each, for the seven animals used, statistically consistent: dorsal, $W = 0.35$, $P < 0.05$; ventral, $W = 0.61$, $P < 0.01$ (Kendall's W , ref. 5). However, the differences between the magnitudes of the more dorsal and the more ventral responses (taken relative to 0.1 M NaCl at each depth) were significant only for sucrose and for QHCl (Fig. 1) ($P < 0.05$, Wilcoxin signed rank (WSR), ref. 5). The more ventral sucrose response was larger in all but one animal; the QHCl response was smaller in all but one. The bulbar surface co-ordinates for the electrode tracks in these animals correspond to the location of the NFS.

The two-depth responses were next studied, using a range of sucrose concentrations. Fig. 2 shows that the absolute magnitudes of the more ventral sucrose responses were larger than those of the more dorsal responses ($P = 0.025$, WSR, ref. 5). Therefore, the previously

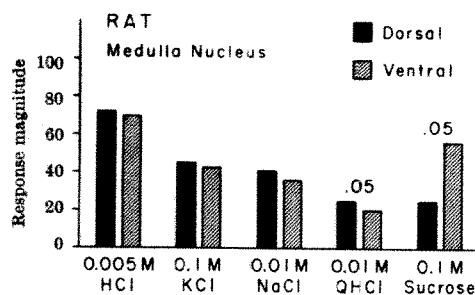


Fig. 1. Median summated relative response magnitude (response to 0.1 M NaCl at each depth, 100) at two electrode depths along a single vertical puncture in the bulbar gustatory relay. Median separation: 50 μ based on seven rats. Anterior tongue stimulated by each of the five chemicals shown, and by the 0.1 M NaCl reference standard

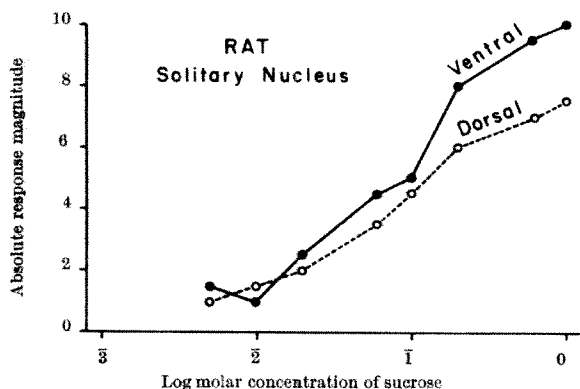


Fig. 2. Absolute response magnitudes recorded at two depths along a single electrode track to sucrose solution stimulation of the anterior tongue. Done in three animals (not those included in Fig. 1), median values shown. Separation between recording depths: 50 μ (median). The brain of one rat studied histologically. The electrode track marking the responsive area ended in the nucleus of the fasciculus solitarius

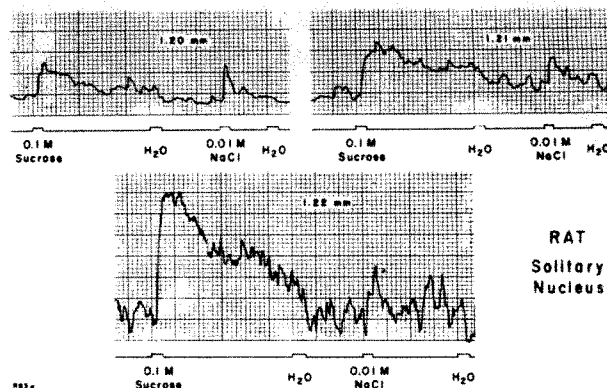


Fig. 3. Photograph of summated multi-unit responses recorded in the medulla oblongata of one rat. Records taken at 1.20 mm, 1.21 mm, and 1.22 mm ventral to the dorsal surface of the medulla. Heavy vertical time lines are 20 sec apart. Deflexion in signal marker below each record indicates approximate onset and duration of flow of each liquid. Tongue chamber empty of liquids except during stimulus flow. Records read from left to right. Critically damped, rectilinear recording milliammeter. An electrolytic lesion which was made in the responsive area was found in the nucleus of the fasciculus solitarius of the myelin stained brain. The response to sucrose is seen to double relative to NaCl; to triple, in absolute magnitude

observed increase in sucrose responsiveness with depth was not actually a decrease in responsiveness to the other stimuli. Further, the relative response magnitudes (re: 0.1 M NaCl at each depth) for the wide range of sucrose concentrations were significantly larger at the more ventral loci ($P < 0.01$, WSR, ref. 5), thus demonstrating that the increase is not just a rise in the response magnitudes to all chemicals. Thus, responsiveness to sucrose increased at deeper recording sites in the bulbar gustatory relay, while responsiveness to QHCl decreased at the deeper sites.

When the recording electrode was driven through NFS in small steps, it was observed that, with an increase in electrode depth of only 20 μ , the response magnitude to 0.1 M sucrose often enlarged considerably, both relative to 0.1 M NaCl and in absolute response magnitude (Fig. 3). In order to examine the highly responsive regions more closely, additional experiments were made. The electrode was driven through NFS in steps of either 20 or 25 μ , and several stimuli were applied to the tongue at each step. Two patterns of response versus depth were observed (Fig. 4): a superficial, narrow area within which QHCl gave large responses, while no large responses to sucrose were recorded anywhere along the electrode track (Type A); and a deep, narrow area in which sucrose gave large responses, while nowhere along the track did QHCl give large responses (Type B). In two other animals, the response magnitudes to sucrose and QHCl were both large, separated by ≤ 21 per cent points. The highly responsive areas were narrow. In one of these animals, the maxima occurred at the same depth, half-way through the responsive area (50 per cent of total depth). In the other animal, the QHCl maximum was quite superficial; the sucrose maximum, deeper. An analysis of variance was applied to the maximum response magnitudes of the A- and B-pattern animals, as well as to those animals whose maxima differed by ≤ 21 per cent points. The analysis indicated that the responses differed significantly for both sucrose ($P < 0.008$) and for QHCl ($P = 0.05$; Kruskal-Wallis⁵).

It is unlikely that the sub-areas observed are highly responsive only to sucrose or only to QHCl. However, glucose and maltose did not yield large responses in 'sucrose' sub-areas, while quinine sulphate, sucrose octa acetate, and caffeine (all bitter)⁶ produced only small responses in a 'QHCl' sub-area. Thus, although indications of a chemotopic organization have been observed, the defining chemical characteristics, and many other factors, remain to be determined.

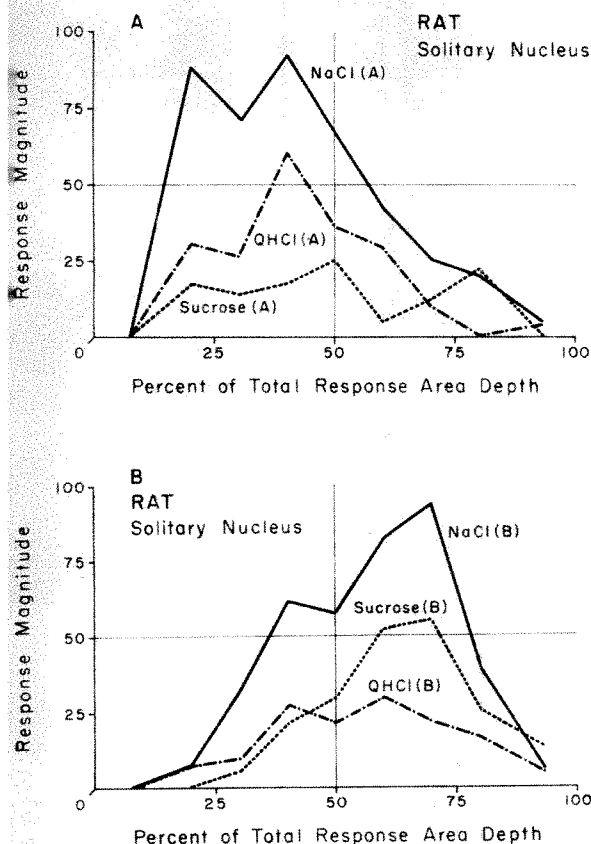


Fig. 4. Median summated response magnitudes to 0.05 M NaCl, 0.02 M quinine hydrochloride (QHCl) and 0.1 M sucrose, recorded in the nucleus of the fasciculus solitarius. Response magnitudes are expressed as percentages of the maximal response of each rat to 0.05 M NaCl. The distance from the beginning (dorsal limit) to the end (ventral limit) of the responsive area was converted into percentages (median size was 380 μ). An electrolytic marker lesion was made at the recording site in each animal, and located in the anterior tongue zone of the nucleus of the fasciculus solitarius. A. Animals (N, 3) which had (1) a maximum response to QHCl ≥ 60 per cent; (2) a maximum response to sucrose ≤ 30 per cent; (3) a separation of at least 38 per cent points between QHCl and sucrose responses. B. Animals (N, 2) which had (1) a maximum sucrose response ≥ 50 per cent; (2) a maximum QHCl response ≤ 39 per cent, and (3) a sucrose-QHCl response difference of at least 25 per cent points.

This work was supported by U.S. National Institutes of Health grant NB 04121.

BRUCE P. HALPERN

Department of Physiology,
Upstate Medical Center,
State University of New York,
Syracuse.

- Ades, H. W., in *Handbook of Physiology, Section 1: Neurophysiology*, 1, edit. by Field, J., 585 (Amer. Physiol. Soc., Washington, 1959).
Halpern, B. P., and Nelson, L. M., *Amer. J. Physiol.*, **209**, 105 (1965).
Pffman, C., Erickson, R. P., Frommer, G. P., and Halpern, B. P., in *Sensory Communication*, edit. by Rosenblith, W. A., 455 (Wiley, New York, 1961).
Halpern, B. P., in *Biological Prototypes and Synthetic Systems*, edit. by Bernard, E. E., and Kare, M. R., 22 (Plenum Press, New York, 1962).
Siegel, S., *Nonparametric Statistics* (McGraw-Hill, New York, 1956).
Pffman, C., in *Handbook of Physiology, Section 1: Neurophysiology*, 1, edit. by Field, J., 507 (Amer. Physiol. Soc., Washington, 1959).

Evidence for a Poisson Distribution of Miniature End-plate Potentials and some Implications

MINIATURE end-plate potentials (m.e.p.p.s), first discovered at the frog neuromuscular junction, have now been detected at mammalian, avian, piscine, crustacean, and insect neuromuscular junctions, at frog and chick ganglionic synapses and at frog and cat spinal cord synapses¹.

Invariably the spontaneous release of transmitter has been shown to occur at random intervals indicating no interaction between consecutive potentials. It is surprising, therefore, that the next step in such an analysis—the determination of the statistical distribution best suited to the description of the m.e.p.p. frequency—appears not to have been undertaken, particularly in view of the fact that it is generally believed that an end-plate potential represents a momentary acceleration of m.e.p.p.s, and the number of quanta in each e.p.p. evoked by nerve stimulation is distributed according to a Poisson distribution². It seemed worthwhile, therefore, to determine whether the spontaneous release of quanta recorded as m.e.p.p.s also fits such a distribution. This was done in rat hemidiaphragm-phrenic nerve preparations *in vitro*. The method of dissection, the mounting of the preparation, and the bathing solution have been previously described³. M.e.p.p.s were recorded with microelectrodes filled with 3 M potassium chloride and frequencies were counted from photographed oscilloscope sweeps. Eight series of m.e.p.p.s were analysed (Table 1). In each, the mean frequency (m) per unit time interval (usually corresponding to one oscilloscope sweep) was calculated. The probability of finding sweeps containing $k=0, 1, 2 \dots n$ m.e.p.p.s was derived from the Poisson formula $p(k) = e^{-m} \frac{m^k}{k!}$. The closeness of fit between the theoretical ($N_p(k, m)$) and observed frequencies (N_k) was determined by use of the χ^2 test with $n-2$ degrees of freedom (where n = the number of comparisons of k). The expected number of intervals for any k was usually kept greater than 5 by grouping k 's. The column 'total N ' shows the total number of m.e.p.p.s observed in each series. The last column in Table 1 (χ^2 level per cent) indicates the approximate percentage of ideal cases in which chance fluctuations would give a worse agreement between observation and theory.

In the first three experiments shown in Table 1, m.e.p.p.s were recorded from neuromuscular junctions in control solution. It is clear that the distribution of m.e.p.p.s in all three trials closely fitted a Poisson. At first sight, the type of distribution may appear to be of little importance, but the demonstration of a Poisson distribution has some important implications. First, it is consistent with the hypothesis that the e.p.p. is the result of synchronization of m.e.p.p.s; that is, quanta which would normally be released in periods of 50 or more sec are released in less than a millisecond under the influence of the presynaptic spike. Secondly, if it is assumed that quanta are reformed in presynaptic terminals (vesicle hypothesis), and that the probability of release is determined by the probability of collision of these quanta with the presynaptic membrane⁴, then some interesting conclusions can be drawn from the fact that the spontaneous release of quanta conforms to a Poisson distribution. The release of each m.e.p.p. must be independent of past or future events, that is, the release of quanta cannot have influenced the probability of release of quanta remaining in the terminal. This implies that the population of releasable quanta remained constant, despite losses. This could occur in many ways; the lost quanta may be very rapidly replaced, or the population of releasable quanta may be so large that those lost cause little alteration of the release probability and are slowly replaced.

When the release rate of quanta was increased to 50/sec by raising the potassium concentration to 10 mM (exp. 7, Table 1), the distribution of m.e.p.p.s was still highly consistent with the Poisson hypothesis (χ^2 level 85 per cent). It has been suggested that the deviation of e.p.p. amplitude from a Poisson distribution when the quantal content of e.p.p.s is high (50–200) may be caused to some extent by an increase in the probability of release of remaining quanta⁵. It was afterwards shown that a better fit to a Poisson distribution could be obtained by

Table 1

Experiment number	Solution		Intervals with k m.e.p.p.s						Total N	χ^2 level (%)				
			k	0	1	2	3	≥ 4						
1	Control	k							109	75				
		Observed N_k $N_p(k; 1.43)$	27 27	38 37.6	25 22.2	9 12.3	10 9.9							
2	Control	k	0	1	2	3	≥ 4	144	80					
		Observed N_k $N_p(k; 0.78)$	64 64.8	56 51	18 20.2	5 5.2	1 2.8							
3	Control	k	0	1	2		≥ 3	117	60					
		Observed N_k $N_p(k; 0.71)$	54 58	46 40.8	14 14.2		3 4							
4	Ca-free 30 min	k	0	1	2		≥ 3	100	60					
		Observed N_k $N_p(k; 0.7)$	51 49.7	34 34.8	10 12.2		5 3.3							
5	9 mM Ca 50 min	k	0	1	2	3	4	≥ 5	79	45				
		Observed N_k $N_p(k; 2.2)$	12 8.8	13 19.3	23 21.2	14 15.6	9 8.5	6 5.6						
6	16 mM Ca 50 min	k	≤ 1	2	3	4	5	6	≥ 7	77	60			
		Observed N_k $N_p(k; 4.02)$	6 7.1	10 11.3	15 15	20 15	8 12	9 8	9 8.6					
7	10 mM K > 1 h	k	≤ 4	5	6	7	8	9	10	11	12	≥ 13	98	85
		Observed N_k $N_p(k; 8.6)$	8 6.7	5 7.01	10 10.1	12 12.4	11 13.4	18 12.8	12 11	7 8.6	7 6.15	8 9.84		
8	HC3 45 min	k	0	1	2		≥ 3	111	75					
		Observed N_k $N_p(k; 0.47)$	67 67.2	36 33.6		7 8.4				1 1.4				

using a compensating factor for the non-linear summation of quanta⁶. The present results show that quantal release rates of 50/sec cause no change from a Poisson distribution of quantal release and indicate, in terms of the hypothesis of preformed quanta, that adequate refilling of the store must occur to maintain constant parameters for the Poisson.

In exp. 8, Table 1, the drug hemicholinium No. 3 (α - α -dimethylethanolamine 4,4'-biacetophenone, HC3) was used at a concentration of 4×10^{-6} M to inhibit ACh synthesis^{7,8}. An attempt was then made to deplete the transmitter store in magnesium-blocked preparations by stimulating the phrenic nerve at a rate of 12/sec. Under these conditions, both e.p.p.s and m.e.p.p.s could be observed, and the quantal content of the e.p.p.s remained at approximately 10 throughout the experiment. After 45 min, when e.p.p. and m.e.p.p. amplitudes had decreased 60 per cent, which presumably corresponded to a similar reduction in the store of transmitter⁸, the series of m.e.p.p.s shown in experiment 8 was analysed. It is evident that there is a remarkably good fit to a Poisson distribution in spite of the depletion of ACh. This result suggests that the population of releasable quanta remained approximately constant so that each quantum might be expected to contain less ACh, and in fact it was observed that m.e.p.p.s had become smaller in amplitude.

An alternative hypothesis⁵, which fits these observations and does not depend on the postulate of preformed quanta, cannot be ignored. The probability of transmitter release may depend on the random activation of a small fraction of a large population of potential release sites in the presynaptic membrane. On the assumption that the probability of activation of potential release sites is a function of the extracellular calcium concentration⁹, several series of e.p.p.s in solutions containing varying calcium concentrations were examined. In expts. 4, 5 and 6, Table 1, the calcium concentrations of the bathing solutions were 0, 9 and 16 mM respectively, but all three series were again in excellent agreement with the Poisson predictions. In several experiments with 16 mM calcium chloride, however, the χ^2 level appeared to be less than with the normal concentration of calcium chloride (2 mM). At present it is difficult to think of decisive experiments

to differentiate between the two hypotheses. It has been shown that quantal size does not fall during depletion of the transmitter store during a tetanus, yet the reduced e.p.p. amplitudes still conform to a Poisson distribution¹⁰. In terms of the vesicle hypothesis, the reduction of quantal content implies that the store of quanta was reduced and consequently one might have expected some deviation from a Poisson distribution. The second of the two hypotheses described is more consistent with these observations.

The experiments presented demonstrate that the conformity of spontaneous transmitter release to a Poisson distribution is very significant under a variety of experimental conditions which might have been expected to affect the probability of release and thus cause deviations from this distribution. The very persistence of this conformity to a Poisson distribution must provide a powerful test of the various hypotheses of transmitter release.

We thank Dr. W. Ewens of the Statistics Department of this University for his advice.

P. W. GAGE
J. I. HUBBARD

Department of Physiology,
John Curtin School of Medical Research,
Australian National University,
Canberra.

¹ Fatt, P., and Katz, B., *J. Physiol.*, **117**, 1094 (1952). Boyd, I. A., and Martin, A. R., *ibid.*, **144**, 176 (1956). Lilley, A. W., *ibid.*, **132**, 650 (1956). Elmquist, D., Johns, T. R., and Thesleff, S., *ibid.*, **154**, 602 (1960). Ginsborg, B. L., *ibid.*, **150**, 707 (1960). Takeuchi, A., *J. Cell. Comp. Physiol.*, **54**, 211 (1959). Dudel, J., and Kuffler, S. W., *J. Physiol.*, **155**, 514 (1961). Usherwood, P. N. R., *ibid.*, **169**, 149 (1963). Blackman, J. G., Ginsborg, B. L., and Ray, C., *ibid.*, **167**, 389 (1963). Martin, A. R., and Pilar, G., *ibid.*, **175**, 1 (1964). Katz, B., and Miledi, R., *ibid.*, **168**, 389 (1963). Kuno, M., *ibid.*, **175**, 81 (1964).

² Katz, B., *Proc. Roy. Soc., B*, **155**, 455 (1962).

³ Hubbard, J. I., *J. Physiol.*, **159**, 507 (1961).

⁴ del Castillo, J., and Katz, B., *Prog. Biophys.*, **6**, 121 (1956).

⁵ del Castillo, J., and Katz, B., *J. Physiol.*, **124**, 560 (1954).

⁶ Martin, A. R., *J. Physiol.*, **130**, 114 (1955).

⁷ Schueler, F. W., *Intern. Rev. Neurobiol.*, **2**, 77 (1960).

⁸ Elmquist, D., and Quastel, D. M. J., *J. Physiol.*, **177**, 463 (1965).

⁹ Gage, P. W., and Quastel, D. M. J. (submitted to *J. Physiol.*).

¹⁰ Elmquist, D., and Quastel, D. M. J., *J. Physiol.*, **178**, 505 (1965).

PHARMACOLOGY

Anticoccidial Activity of Nicotinamide Antagonists

THE life-cycle of poultry coccidia can be inhibited by certain *p*-aminobenzoic acid¹⁻³, folic acid³⁻⁶ and thiamine⁷ antagonists. This knowledge led to our testing three known antagonists of another vitamin, nicotinamide, for anticoccidial activity. These substances were 3-acetylpyridine⁸, pyridine-3-sulphonamide⁹ and 6-aminonicotinamide¹⁰.

To evaluate the activity of the compounds, they were mixed in chick starter mash of known composition and fed to one-week-old cockerels, starting one day before they were inoculated orally with approximately 200,000 sporulated oocysts of *Eimeria tenella* or *E. necatrix*, or 1,000 sporulated oocysts of *E. acervulina*. In each test one infected and one uninfected control group of birds were fed unmedicated ration. The criteria to assess activity were a comparison of mortality and oocyst reduction¹¹. The toxicity of the compounds was assessed in both infected and uninfected groups by a comparison of death rates and weight gains of the birds individually weighed at intervals.

3-Acetylpyridine at 0.1 per cent w/w in the food was inactive against both *E. tenella* and *E. acervulina*.

Pyridine-3-sulphonamide at 0.025 per cent w/w was inactive against *E. tenella* and *E. necatrix*, and showed no signs of toxicity when fed for eight days. It was active against *E. acervulina*, but less effective than sulphaquinoxaline, the reference compound (Table 1). The results of exp. 3 indicate the possibility of different strain responses. The activity was neutralized by simultaneous feeding of an equal concentration of nicotinamide (exps. 4 and 5, Table 1). In another single experiment the activity of pyridine-3-sulphonamide was not antagonized by equal concentrations of *p*-, *o*- or *m*-aminobenzoic acid.

6-Aminonicotinamide showed a different spectrum of activity to pyridine-3-sulphonamide. It was active against *E. tenella* at low concentrations comparable with those of the control drug amprolium (1-(4-amino-2-n-

Table 2. 6-AMINONICOTINAMIDE ACTIVITY AGAINST *E. tenella* AND TOXICITY IN CHICKS

Exp. No.	Drug	Per cent in food	Deaths* by seventh day after infection	
			Acute coccidiosis	Toxicity
1	Nil (control)	—	13/15	—
	6-aminonicotinamide	0.001	0/15	13/15
		0.0005	0/15	0/15
		0.00025	11/15	0/15
		0.002	0/15	0/15
2	Amprolium	0.001	3/15	0/15
		0.0005	7/15	0/15
		—	8/10	—
		0.0008	0/10	0/10
	6-aminonicotinamide	0.0004	3/10	0/10
		0.0002	5/10	0/10

* Numerator, No. of chicks dead. Denominator, No. of chicks per group.

Table 3. ANTAGONISM OF THE ACTIVITY AGAINST *E. tenella* AND THE TOXICITY IN THE CHICK OF 6-AMINONICOTINAMIDE (6-ANC) BY NICOTINAMIDE (NC)

Per cent drug in food	Deaths* due to acute coccidiosis in infected groups after one week	Uninfected groups	
		Deaths* due to toxicity after 1 week	Av. wt. (g) gain after 2 weeks
6-ANC	NC	1 week	2 weeks
—	—	0/28	39.3
0.002	—	0/15	103.3
0.001	—	15/28	5.8
0.0005	—	1/20	20.2
—	0.004	6/20	37.1
—	0.008	0/10	21.3
—	0.02	0/10	86.4
—	0.1	0/10	40.8
0.002	0.0005	0/8	99.8
0.002	0.001	0/8	46.0
0.002	0.002	0/10	32.0
0.002	0.004	0/10	95.4
0.002	0.008	0/10	102.4
0.002	0.02	0/5	0.6
0.002	0.1	0/5	17.4
0.002	0.002	4/18	46.5
0.002	0.004	7/18	14.2
0.002	0.008	13/18	15.8
0.002	0.02	9/18	33.3
0.002	0.1	0/8	81.6
0.002	0.005	0/10	32.4
0.002	0.01	0/10	92.1

Results of three experiments added together. Groups balanced for weight at the start of each test.

* As Tables 1 and 2.

propyl-5-pyrimidinylmethyl)-2-picolinium chloride hydrochloride) (Table 2), but there was little margin between the active and toxic concentrations. This nicotinamide analogue was active against *E. necatrix* and inactive against *E. acervulina* at the maximum tolerated concentration. Lower concentrations were not examined against *E. necatrix*.

The results in Table 3 show that one-half to twice the concentration of nicotinamide added to 6-aminonicotinamide has some effect in offsetting its toxicity as judged by the deaths and depression of gain in weight; but four times or more the amount of the vitamin was required to neutralize the anticoccidial activity and toxicity of this analogue.

These observations suggest that nicotinamide is a growth factor for *E. tenella*, *E. acervulina* and presumably for *E. necatrix*.

It is interesting to note that pyridine-3-sulphonamide showed activity only against *E. acervulina*, which is also generally more sensitive than *E. tenella* and *E. necatrix* to sulphanilamide derivatives. This may indicate that for *E. acervulina* transport of drugs to the site of action is enhanced by a weakly acidic group such as $-\text{SO}_2\text{NHR}$.

S. J. BALL

E. W. WARREN

May and Baker, Ltd.,
Veterinary Research Station,
Ongar, Essex.

E. W. PARNELL

Research Laboratories,
Dagenham, Essex.

¹ Horton-Smith, C., and Boyland, E., *Brit. J. Pharmacol.*, **1**, 139 (1946).

² Waletzky, E., and Hughes, C. O., *Amer. J. Vet. Res.*, **7**, 365 (1946).

³ Joyner, L. P., and Kendall, S. B., *Brit. J. Pharmacol.*, **11**, 454 (1956).

⁴ Lux, R. E., *Antibiot. Chemother.*, **4**, 971 (1954).

⁵ Joyner, L. P., and Kendall, S. B., *Vet. Rec.*, **68**, 468 (1956).

⁶ Kendall, S. B., and Joyner, L. P., *Vet. Rec.*, **70**, 632 (1958).

⁷ Rogers, E. P., Clark, R. L., Pessolano, A. A., Becker, H. J., Leanza, W. J., Sarett, L. H., Cuckler, A. C., McManus, E., Garzillo, M., Malanga, C., Ott, W. H., Dickinson, A. M., and Van Iderstine, A., *J. Amer. Chem. Soc.*, **82**, 2974 (1960).

⁸ Woolley, D. W., *J. Biol. Chem.*, **157**, 455 (1945).

⁹ McIlwain, H., *Brit. J. Exp. Path.*, **21**, 136 (1940).

¹⁰ Johnson, W. J., and McColE, J. D., *Science*, **122**, 834 (1955).

¹¹ Long, P. L., and Rowell, J. F., *Lab. Pract.*, **7**, 515 (1958).

Table 1. ACTIVITY OF PYRIDINE-3-SULPHONAMIDE AGAINST *E. acervulina* AND THE EFFECT OF ADDING NICOTINAMIDE

Exp. No.	Per cent drug in food			Strain of <i>E. acervulina</i>	Millions of oocysts passed per chick*
	P3S	NC	SQ		
1	—	—	—	Ongar†	222.65
	0.0125	—	—		0.15
	0.006	—	—		10.47
2	—	—	0.0125	Ongar†	0
	0.0125	—	—		88.26
	0.006	—	—		0
3	—	—	0.006	Ongar†	1.18
	0.0125	—	—		0
	—	—	0.006		113.02
4	—	—	—	Houghton‡	2.14
	0.0125	—	—		0
	0.006	—	—		189.18
5	—	—	0.006	Andover§	101.66
	0.0125	—	—		210.75
	0.006	—	—		0.02
6	—	—	—	Ongar†	111.26
	0.025	—	—		0.02
	0.0125	—	—		37.60
7	—	—	—	Ongar†	79.60
	0.006	—	—		85.79
	0.003	—	—		0.72
8	—	—	0.025	Ongar†	116.18
	—	—	0.0125		261.49
	—	—	0.006		0
9	—	—	—	Ongar†	0.76
	0.0125	—	—		14.30
	0.006	—	—		78.90
10	—	—	—	Ongar†	179.68
	0.0125	—	—		113.55
	—	—	—		32.23
11	—	—	—	Ongar†	0
	0.05	—	—		1.15
	0.025	—	—		25.03
12	—	—	—	Ongar†	14.07
	0.05	—	—		22.28
	0.025	—	—		—

P3S, pyridine-3-sulphonamide; NC, nicotinamide; SQ, sulphaquinoxaline.

* From fourth to thirteenth days post-infection.

† Isolated 1958.

‡ Isolated 1956.

§ Isolated 1960.

PATHOLOGY

Induction of Malignant Lymphomas by Urethane in Adult Mice bearing Syngeneic Thymus Grafts

In recent years it has repeatedly been proved that neonatal or very young animals are very susceptible to carcinogenesis induced by different agents. One possible explanation is that tissues of new-born animals contain a large number of incompletely differentiated cells, particularly responsive to carcinogenic stimuli. It is relevant to this speculation that a close correlation has been noted between susceptibility of mice to develop malignant lymphomas and the presence in the thymus of a great concentration of immature cells, whether by reason of early age¹, genetic constitution², whole-body X-irradiation³, or regenerative changes in implanted thymus⁴. In thymectomized, virus-inoculated⁵ or X-irradiated⁶ mice, implantation of new-born thymus proved much more effective than adult thymus in restoring leukaemogenesis.

Previous work showed that urethane (ethyl carbamate) in adult mice was practically devoid of leukaemogenic activity^{6,7}, although effective in inducing thymic changes⁸. On the other hand, administration of this chemical to new-born animals was found to provoke a significant number of malignant lymphomas⁹. Urethane-induced lymphomas originated in nearly all cases in the thymus, only later acquiring the character of a generalized disease.

The experiments reported here were undertaken to investigate whether, by making use of single or multiple grafts of new-born thymus as a more suitable target tissue, adult mice would be rendered susceptible to the leukaemogenic action of urethane.

C57BL male mice aged 2-3 months were grafted subcutaneously into the right flank with one or five thymuses obtained from 1- to 5-day-old syngeneic donors. Starting 5 days after implantation, they received urethane in drinking water at the 0.3 per cent level for 30 days, continuously. Adult C57BL mice of both sexes treated only with urethane served as controls. The total quantity of the chemical administered to animals of the different groups amounted to an average of 220 mg per mouse. A certain number of mice died within 30 days from the end of treatment, presumably owing to the toxic effect of urethane. They were not included in the investigation.

Table 1. MALIGNANT LYMPHOMAS INDUCED BY URETHANE IN C57BL MICE IMPLANTED WITH SYNGENEIC SINGLE OR MULTIPLE THYMUSES

Thymus graft	No. of mice in group	Mice with lymphomas No.	%
None	44	1	2
1 thymus	15	2	13
5 thymuses	22	9	41

The results, which refer to an 8-month period of observation from the end of urethane administration, are summarized in Table 1. It is evident that the highest incidence of malignant lymphomas occurred in the group grafted with 5 thymuses (41 per cent), while in animals receiving only a single thymus the incidence was markedly lower (13 per cent). However, only in the case of multiple grafts was the incidence statistically significant in comparison with the control group ($\chi^2 = 14.15$; $0.001 < P < 0.01$). Neoplastic disease in all thymus-implanted animals was initiated after a mean latent period of four months as a localized growth at the graft site, and reached a maximum diameter of 40-50 mm in about 40 days (Fig. 1). Generalized leukaemia appeared later, except in three animals implanted with 5 thymuses, which at death had only local tumours. *In situ* thymus of leukaemic mice, although appearing grossly of almost normal size, showed histologically, in all cases in which the organ could be recognized with certainty, initial or limited neoplastic changes. Lymphomatous tissue at the site

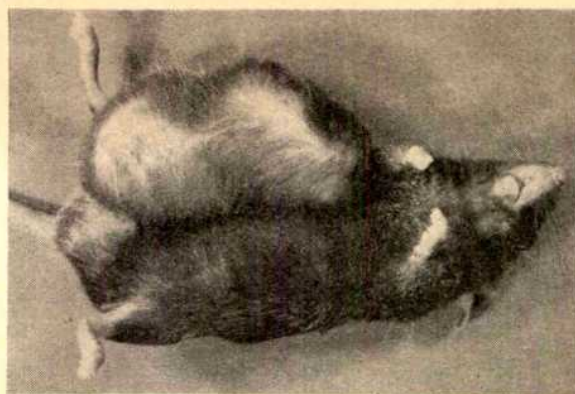


Fig. 1. C57BL mouse implanted with multiple syngeneic new-born thymuses and treated with urethane in drinking water. Large neoplastic mass at the graft site, 130 days after the end of urethane administration.

of implantation, as well as in the various organs involved by the generalized disease, was composed of cells of lymphoid type.

Present data suggest that grafts of neonatal thymus make susceptible to the leukaemogenic action of urethane the otherwise resistant mice. Since in our experiments lymphomas constantly arose from the implanted thymus(es), it is possible that the positive results obtained in both the experimental groups are correlated with the availability in the graft of cells particularly susceptible to urethane-induced damage leading to neoplasia. On the other hand, the finding of the highest susceptibility in the group implanted with multiple thymuses seems to indicate that a quantitative factor is involved in the process of neoplastic transformation of the graft. Thus it could be assumed that in a single thymus graft the number of cells sensitive to the weak leukaemogenic activity of urethane scarcely reaches the critical level necessary to initiate successfully a lymphomatous growth under the present experimental conditions. However, the mechanism through which a greater mass of thymic tissue influences leukaemogenesis, that is, by contribution of more target cells and/or production of larger amounts of humoral substance, remains to be elucidated.

The work was supported by grant DRG-808 from the Damon Runyon Memorial Fund for Cancer Research and by a grant from Consiglio Nazionale delle Ricerche, Roma. One of us (G. T.) is also holder of a fellowship from the Lady Tata Memorial Fund.

L. FIORE-DONATI
L. CHIECO-BIANCHI
G. TRIDENTE

Istituto di Anatomia Patologica,
Divisione di Cancerologia Sperimentale,
Università di Padova, Italy.

L. MAZZARELLA

Istituto di Anatomia Patologica,
Divisione di Cancerologia Sperimentale,
Università di Bari, Italy.

¹ Axelrod, A. A., and van der Gaag, H. C., *J. Nat. Cancer Inst.*, **28**, 1065 (1962).

² Nakamura, K., and Metcalf, D., *Brit. J. Cancer*, **15**, 306 (1961).

³ Kaplan, H. S., *Acta Un. Intern. Cancer*, **17**, 143 (1961).

⁴ Carnes, W. H., Kaplan, H. S., Brown, M. B., and Hirsch, B. B., *Cancer Res.*, **16**, 429 (1956).

⁵ Miller, J. F. A. P., in *Tumour Viruses of Murine Origin* (Ciba Symposium), edit. by Wolstenholme, G. E. W., and O'Connor, M., 262 (Churchill Ltd., London, 1962).

⁶ Fiore-Donati, L., De Benedictis, G., Chieco-Bianchi, L., and Maiorano, G., *Acta Un. Intern. Cancer*, **18**, 134 (1962).

⁷ Chieco-Bianchi, L., Fiore-Donati, L., De Benedictis, G., and Tridente, G., *Nature*, **199**, 292 (1963).

⁸ Fiore-Donati, L., and Kaye, A. M., *J. Nat. Cancer Inst.*, **33**, 907 (1964).

⁹ Fiore-Donati, L., Chieco-Bianchi, L., De Benedictis, G., and Maiorano, G., *Nature*, **190**, 278 (1961).

RADIOBIOLOGY

Bone Marrow Allografts in Thymectomized X-irradiated Dogs

ALLOGENEIC bone marrow transplantations in 1,000-r. irradiated dogs are therapeutically ineffective. Our earlier results¹ made it possible for us to suggest that transplanted haemopoietic cells became immunologically active against the recipient's lymphocyte antigens. All the dogs invariably indicated within 6-7 days of transplantation profound leucopenia followed by a sharp increase in white blood cell count at the cost of peculiar mononuclear hyperbasophilic cells resembling immune B precursors or transformed lymphocytes in phytohemagglutinin leucocyte cultures. On the next day only lymphocytes were observed in the blood; their count progressively decreased, and the animals died.

Taking into consideration previous results²⁻⁷ which showed that adult thymectomized mice failed to restore immunogenesis after radiation and bone marrow transplantation, and that immunological competence of radiation chimeras apparently are restored by transplanted bone marrow cells which act as lymphoprecursors in thymus^{8,9}, we supposed that transformation of transplanted haemopoietic cells into lymphocytes in dogs also requires a thymic factor.

To prove this assumption we performed experiments in thymectomized adult dogs. Thymectomy was performed under intravenous pentobarbital anaesthesia and artificial respiration through sternal cutting. One to two months after the operation the dogs were X-irradiated with 1,000-r. (2 days running with 500-r.). The day following the last radiation the dogs were injected intravenously with $7.4-9.2 \times 10^8$ viable nucleated bone marrow cells in 250-300 ml. Hanks's solution. Bone marrow cells were pressed out from the vertebrae and epiphysis femur and humerus. Simultaneously the dogs were transplanted with donor's skin (the pinch graft method¹⁰ was used). The dogs received daily intramuscular injection of 200,000 units of penicillin and 100 mg of streptomycin.

Thymectomy as such did not influence the haematological picture, in particular lymphopenia. The degree of radiation disease in these dogs was very heavy. Four dogs died 6-7 days after radiation before any re-population of the bone marrow graft appeared. At autopsy they showed large haemorrhages. Two dogs died 21 and 12 days after radiation. In one of these dogs (Fig. 1) there was sharp and quickly developing leucopenia, especially lymphopenia. However, 5-6 days after bone marrow transplantation the leucocyte count rapidly increased due to proliferation of donor cells. From the 7th day until death, donor leucocytes were identified in the blood of the dog (sex chromatin). As can be seen from Fig. 1, almost all leucocytes of this dog were polymorphonuclear. No hyperbasophilic cells appeared except during the 7th-8th days, when a small quantity of these cells was seen; scarcely any lymphocytes were present. White blood cells continued to increase—5,350 cells/mm³ on the 13th day and 13,150 cells/mm³ on the 21st day. In myelograms an insignificant quantity of hyperbasophilic cells was observed during the 7th-13th days after radiation. It should be noted that since the operation was performed on the sternum, bone marrow for investigation was taken from

the iliac crest which was badly re-populated in this experiment. In spite of a good leucopoietic restoration, anaemia progressed; the haemoglobin value had fallen by the 17th day from 15.2 g per cent to 8.6 g per cent and the red blood cell count from 5.4×10^6 to 3.0×10^6 cells/mm³. Death of the dog was caused by renal haemorrhage. At autopsy fatty bone marrow was found in the hind legs, while in the sternum, ribs and vertebrae bone marrow was active, rich in haemopoietic red and white cell lines. Lymphoid tissues of the spleen and lymphoid nodes were atrophied. The skin graft up to the death of the dog was in good condition, and was vascularized; there were no signs of any rejection.

Similar results were indicated by the second dog. Re-population of the bone marrow graft was active. Three days later, the bone marrow was injected and there appeared, in myelogram: stem cells, immature red and white cell lines which were practically absent before the bone marrow graft. Five days after transplantation the number of white blood cells increased; their amount rose from 100 cells/mm³ to 1,400 cells/mm³ in 2 days. Donor leucocytes (female in male) were identifiable up to the dog's death. The white blood cell count was fully conditioned by the increase of polymorphonuclear cells. Hyperbasophilic cells were absent in peripheral blood; lymphocyte content was not above 2 per cent. In bone marrow few hyperbasophilic cells were observed until just before death (12th day). At autopsy hypoplasia of haemopoietic and aplasia of lymphoid tissues were seen. The haemorrhagic syndrome was insignificant, and the cause of death was not established. The skin graft was in good condition.

Thus in neither dog did we observe any significant transformation of haemopoietic cells into lymphocytes. The decisive role of the thymus in such transformations is obvious from the fact that, without any exception, in all irradiated dogs (more than 40), similarly treated but without thymectomy, clear transformation of haemopoietic

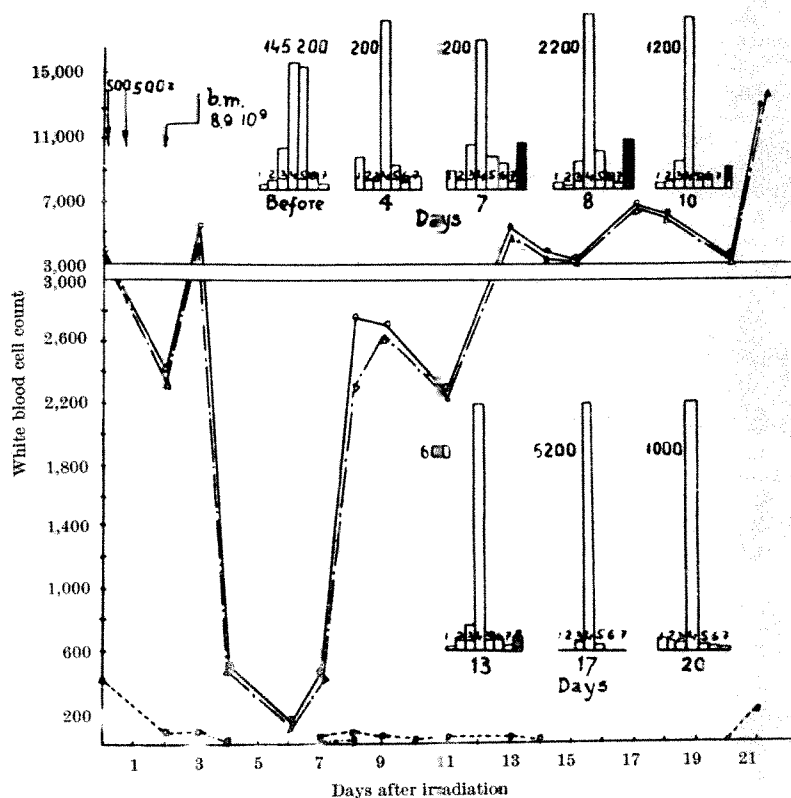


Fig. 1. White blood cell count and myelograms in a thymectomized dog irradiated with 1,000 r. On the day after radiation the dog received allogeneic bone marrow. (1) Reticular cells; (2) stem cells; (3) immature myeloid cell lines; (4) mature myeloid cell lines; (5) erythrocytic cell lines; (6) lymphocytes; (7) plasmacytes; (8) hyperbasophilic cells. Above myelograms—bone marrow cell count in 1 mm³. ○, Leucocytes; △, neutrophils; □, lymphocytes; ■, hyperbasophilic cells.

cells into lymphocytes developed. These results suggest that for large animals, including man (just as for mice), the thymus is necessary for functional differentiation of polypotent stem cells into lymphoid precursor. These preliminary results indicate that even a strongly reduced thymus in adult dogs is effective. By analogy with the results already cited²⁻⁹ one is led to believe that the thymus of adult large animals and man plays a part in the restoration of immunogenesis after radiation, 'teaching' remaining stem cells. In connexion with this assumption it seems reasonable to attempt to delay the restoration of immunogenesis (depressed by massive whole-body irradiation and cytostatics) by preliminary thymectomy prior to transplantation of tissues and organs in man.

I. L. CHERTKOV
A. S. MAXIMENKO
M. N. NOVIKOVA
M. O. RAUSHENBACH

Central Order of Lenin Institute of Haematology
and Blood Transfusion, Moscow, U.S.S.R.

¹ Chertkov, I. L., Novikova, M. N., Maximenko, A. S., et al., *Med. Radiol. (Rus.)*, **6**, 8 (1964); **6**, 51 (1963); *Nature*, **200**, 1170 (1963).

² Cross, A. M., Davies, A. J., Doe, B., and Leuchars, E., *Nature*, **201**, 1045 (1964); **203**, 1239 (1964).

³ Cross, A. M., Leuchars, E., and Miller, J. F., *Intern. J. Rad. Biol.*, **6**, 485 (1963); *J. Exp. Med.*, **119**, 837 (1964).

⁴ Lengerova, A., and Vojtisková, M., *Folia Biol. (C.S.S.R.)*, **10**, 245 (1964).

⁵ Miller, J. F., Doak, S. M., and Cross, A. M., *Proc. Soc. Exp. Biol. and Med.*, **112**, 785 (1963).

⁶ Tyan, M. L., Cole, J. L., and Davis, W. E., *Science*, **142**, 584 (1963).

⁷ Zaalberg, O. B., *Intern. J. Rad. Biol.*, **6**, 484 (1963).

⁸ Ford, C. E., and Micklem, H. S., *Lancet*, **i**, 359 (1963).

⁹ Harris, J. E., and Ford, C. E., *Nature*, **201**, 884 (1964). Barnes, D. W. H., and Evans, E. P., *Nature*, **201**, 886 (1964).

¹⁰ *Transplantation of Tissues and Cells*, edit. by Billingham, R. E. (Silvers, W. K., Philadelphia, 1961).

An Unusually Radioactive Fossil Fish from Thurso, Scotland

A PLATE of *Homosteus* from Clardon Haven about two miles east of Thurso was found to be radioactive to the extent of 1.2×10^4 γ /min/g. In order to characterize the radioactive isotopes present, the energies of the emitted γ -rays were measured using a γ -scintillation spectrometer.

The spectrometer was a 3 in. \times 3 in. sodium iodide phosphor used in conjunction with a photomultiplier, a high-gain amplifier and a 512-channel pulse-height analyser.

The energy range 80 keV–3,500 keV was examined and from the absorbed energies it was evident that the radioactivity was due to the decay chain of ^{238}U with

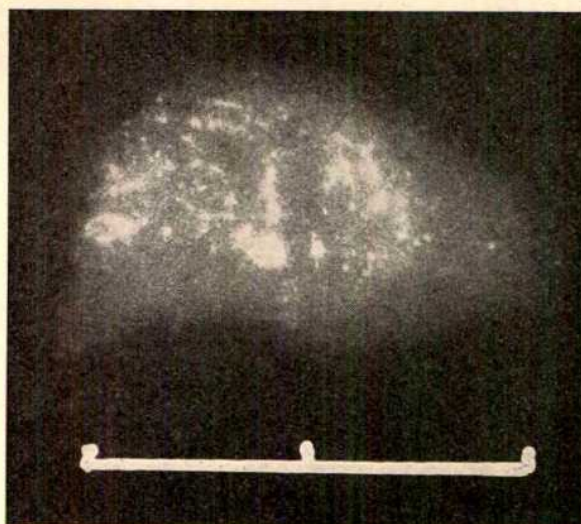


Fig. 1. Autoradiograph of a polished section of the bone of *Homosteus*. Scale, cm. The extremely bright spots are due to α -particles

some contribution from the ^{232}Th chain. The component of these chains and the appropriate gamma energies as shown in Table 1.

To assess the distribution of activity across the specimen a section was polished and an autoradiograph was obtained using industrial X-ray film (Fig. 1). The distribution is not uniform but is concentrated into a few small areas.

A quantitative examination using the spark excite emission spectrum examined by a Hilger medium spectrum graph indicated the chemical constituents as being major, calcium; minor, strontium, phosphorus, magnesium; heavy trace, barium.

The fact that the major activity was due to the decay chain of ^{238}U differs from previous results reported by Bowie and Atkin¹, who, working on an unidentified *Homosteus* plate (GSM 89090), found the major activity to be due to the decay chain of ^{232}Th .

Similar examinations were made on a small specimen of *Dipterus valenciennesi* from Scrabster Brae about 1.5 miles west of Thurso and on a specimen of *Thursius pholidotus* from the shore of Thurso Bay about one mile west of Thurso. These specimens, of similar age from stratigraphical evidence, showed no unusual activity. It would therefore seem that we only find excessive radioactivity in those fossil fishes having heavy dermal armour, for example, the arthrodira.

The penetrating γ -photons from the $4n+2$ series can be readily observed using a sensitive portable γ -detector which could be a useful aid in locating specimens in the field.

W. R. DIGGLE
J. SAXON

U.K. Atomic Energy Authority,
Dounreay Experimental Reactor
Establishment,
Thurso, Caithness.

¹ Bowie, S. H. U., and Atkin, D., *Nature*, **177**, 487 (1956).

Continuous Irradiation of HeLa Cells at –196° C

THE incorporation of a suitable quantity of tritiated water in the growth medium permits the continuous irradiation of cells cultured *in vitro*. A uniform source of β -radiation is thus provided which can be used in a variety of cell culture techniques. Some observations on the effects of continuous irradiation of HeLa cells grown as a monolayer at 37° C have already been published^{1,2}. This communication reports some results of the continuous irradiation of HeLa cells at –196° C.

Isotope	Half-life	Gamma energy (keV)
^{238}U chain		
^{238}U	4.5×10^9 y	48
^{234}Th	24.1 d	29, 63, 91
^{234}Pa	1.18 min	750, 1,000
^{234}U	2.5×10^5 y	51
^{230}Th	8.0×10^4 y	67
^{226}Ra	1,630 y	188
^{222}Rn	3.815 d	—
^{218}Po	3.05 min	—
^{214}Pb	26.8 min	243, 295, 317
^{214}Bi	19.9 min	610, 1,120, 1,760 and others up to 2,430
^{214}Po	1.6×10^{-4} s	Several very weak
^{214}Pb	1.3 min	
^{210}Pb	21 y	47
^{210}Bi	5.0 d	—
^{210}Po	138.4 d	800 (very weak)
^{206}Pb	Stable	—
^{232}Th chain		
^{232}Th	1.41×10^{10} y	59
^{228}Ra	6.7 y	—
^{228}Ac	6.13 h	57 to 1,640 many gammas
^{228}Th	1.91 y	84
^{228}Ra	3.64 d	240
^{228}Ac	51.5 s	—
^{212}Pb	0.158 s	—
^{212}Bi	10.6 h	120, 240, 300
^{212}Po	0.3 μ s	40
^{208}Tl	3.1 m	280, 510,
^{208}Pb	Stable	580, 860, 2,620

HeLa cells in logarithmic growth were suspended at 10^6 cells/ml. of Eagle's medium plus 20 per cent pooled man serum plus 10 per cent dimethyl sulphoxide and added into 1 ml. aliquots. Suitable amounts of tritiated water were incorporated in the medium of the aliquots to be continuously irradiated. The aliquots were sealed in glass ampoules and cooled by the technique of Nagington and Greaves³ down to -196°C in a liquid nitrogen refrigerator (by the courtesy of Dr. T. S. L. Beswick and Miss Ann Mostratos, Department of Virology, University of Manchester). In each experiment all the ampoules, control and treated, were maintained at this temperature for the same length of time, and the treated cells would be continuously irradiated at two different dose-rates to totals of 1,000 or 2,000 rads, respectively, in either 20 or 40 days.

At the end of each experiment the ampoules were removed from the liquid nitrogen and plunged directly into a water bath at 37°C . Each 1 ml. aliquot of cells was then diluted to 10 ml. in fresh growth medium (Eagle's medium plus 10 per cent pooled human serum) and the cell number was again determined to confirm that all the cells had survived at this stage of the experiment. Replicate single cell clonal cultures were then inoculated by the technique of Puck and Marcus⁴ using the same inoculum size for all cultures, irradiated and control. After incubation for 10 days at 37°C the colonies were scored; comparison with the controls gave the fraction of cells surviving each régime of continuous radiation at -196°C , determined from the average of six plates.

The surviving fractions are shown (Table 1) for each experiment and combined as an average percentage for each dosage régime. Although these average percentages are all lower than the controls, the only fractions which are significantly different are those after the 2,000 rads total dose given over 20 days at 100 rads/day. The variation between individual experiments is attributable to the lower plating efficiencies which follow the freezing technique. Nevertheless, one definite conclusion can be drawn from all these experiments: namely, that the large total doses of continuous irradiation had a very small effect on HeLa cells at -196°C .

In considering the interpretation of these results, the following facts are relevant. Continuous irradiation at 37°C leads to very little depression in the number of HeLa cells at dose-rates of 50 rads/day or less¹ since the radiation damage is spread over many cell cycles, and growth of the surviving cells may conceal the small proportion of cells sterilized per cell cycle. On the other hand, with acute doses (for example, 100 rads/min) the radiation is delivered during a small fraction of one cell cycle, and growth during that time is negligible. The proportion of surviving cells after an acute dose of 2,000 rads is reduced to about 10^{-6} (leaving a negligible number of colonies to be counted in a constant inoculum experiment).

One might expect that a dose of 2,000 rads delivered at 100 rads/day at -196°C would produce the same effect as an acute dose delivered at 100 rads/min at 37°C . Cellular metabolism will be reduced to a minimum at -196°C and one would expect the accumulated 2,000

rads to produce as much damage over at least as small a fraction of one cell cycle as an acute dose at 37°C .

It is known, however, that acute radiation is less effective at lower temperatures. Stapleton and Edington⁵ found the sensitivity of *E. coli* to be reduced at sub-freezing temperatures. Similar observations have been described by Webb *et al.*⁶ for dry bacterial spores, and the phenomenon has been discussed in subsequent papers by Powers and his colleagues⁷.

In mammalian cells (HeLa) Belli and Bonte⁸ found an increase of radio-resistance by a factor of 1.25 by decreasing the temperature from 37°C to 5°C . They point out quite correctly that the phenomenon is unlikely to be due to any degree of anoxia. At 5°C , however, diffusion of radicals is so little affected that it is difficult to think of a suitable radiation chemical explanation of their observation. Dawson *et al.*⁹ found a two-fold increase in radio-resistance of HeLa cells at -79°C when the mobility of free radicals would presumably be reduced.

At the temperature used in our experiments irradiation still produces ions, electrons, hydrogen atoms and free radicals in the solid matrix. The electrons will be captured by positive holes and/or imperfections in the solid matrix. The species capable of movement at a temperature of -196°C are presumably hydrogen atoms; the larger molecular fragments would be expected to be held in position. The hydrogen atoms may react with free radicals and may reduce some of the effects of radiation by reducing the number of radicals. Recombination of free radicals, especially fragments of macromolecules, may occur during the rise of temperature before thawing.

A. H. W. NIAS
L. G. LAJTHA

Paterson Laboratories,
Christie Hospital and Holt Radium Institute,
Manchester.

¹ Nias, A. H. W., and Lajtha, L. G., *Nature*, **202**, 613 (1964).

² Nias, A. H. W., Gilbert, C. W., Lajtha, L. G., and Lange, C. S., *Intern. J. Rad. Biol.*, **9**, 275 (1965).

³ Nagington, J., and Greaves, R. I. N., *Nature*, **194**, 993 (1962).

⁴ Puck, T. T., and Marcus, P. I., *J. Exp. Med.*, **103**, 653 (1956).

⁵ Stapleton, G. E., and Edington, C. W., *Rad. Res.*, **5**, 39 (1956).

⁶ Webb, R. B., Ehret, C. F., and Powers, E. L., *Experientia*, **14**, 324 (1958).

⁷ Powers, E. L., Webb, R. B., and Ehret, C. F., *Rad. Res.*, Suppl. 2, 94 (1960).

⁸ Belli, J. A., and Bonte, F. J., *Rad. Res.*, **18**, 272 (1963).

⁹ Dawson, K. B., Field, E. O., and Madoc-Jones, H., *Nature*, **195**, 1012 (1962).

BIOLOGY

Hatching Time of Turtle Eggs

Jayakar and Spurway¹ have reported possible bimodality not only in egg-laying time by a turtle (*Testudo elegans* Schoepff) but also in hatching time. Evidence from other species of turtles may be helpful in interpreting their observations.

Several species of marine turtles, of tropical or sub-tropical regions, are known to lay more than once per season²⁻⁴, whereas terrestrial and fresh-water forms of temperate zones typically lay all the eggs of the year at one time⁵. Duration of incubation is dependent on environmental conditions, particularly temperature^{2,3,5,6}.

In my own experiments on turtle embryos over the past several years, I have found that hatching time for controls varies by only a few days for a given species even of different clutches, according to the temperature of incubation (Table 1). Within a given clutch of eggs there is an even smaller time-range. Hatchling size (carapace length) is positively correlated with egg size; in the very few instances in which the hatchlings were weighed the larger turtles were heavier than the smaller. There seems to be no pattern as to hatching sequence of large and small specimens.

Turtle hatchlings differ notably in their behaviour (cf. Carr⁷ for adults of *Terrapene carolina carolina* (Linné)). Some are markedly aggressive, whereas others appear

Table 1. SURVIVAL OF THE COLONY-FORMING ABILITY OF HeLa CELLS AFTER CONTINUOUS IRRADIATION AT -196°C

	Experiment No.	% of control survival
20 days' irradiation 1,000 rads (50 rads/day)	1	105
	2	54
	3	89
	4	58
2,000 rads (100 rads/day)	1	52
	2	32
	3	68
	4	49
40 days' irradiation 1,000 rads (25 rads/day)	1	66
	2	77
	1	63
	2	85

Table 1. TEMPERATURE OF INCUBATION AND HATCHING TIME OF TURTLES

Species	Temperature*	No. of hatchlings†	Hatching day Range	Mean
<i>Chelydra serpentina serpentina</i>	Room (1951)	9 (2)	65-69	66.9
<i>Chelydra serpentina serpentina</i>	Room (1952)	23 (8)	62-67	64.7
<i>Chelydra serpentina serpentina</i>	Room (1959)	3 (3)	66-70	67.7
<i>Chrysemys picta marginata</i>	Room (1959)	6 (6)	64-68	66.5
<i>Caretta caretta caretta</i>	30° C	2 (1)	54-55	54.5
<i>Chelydra serpentina serpentina</i>	28° C	9 (3)	55-62	58.0

* Incubation of eggs in any one group was begun at one time, in June or July. Room temperature varied considerably, but averaged close to 28° C. The dishes of eggs at constant temperature were kept in a water bath.

† The number in parentheses after number of hatchlings indicates the number of different clutches of eggs represented in each group.

timid and retiring. In my investigations the eggs are incubated in glass dishes between two layers of moistened absorbent cotton⁷. At the onset of hatching observations are recorded at least every 12 h. Some newly hatched specimens of *Chelydra serpentina serpentina* (Linné) have made their way out of the dishes, off the table, out of the laboratory, and one even down a flight of steps, all within a 12-h period. Others, on the contrary, have hidden themselves under the cotton or within the egg shell and have made no observed attempt to explore even after several days.

Miss Karen Diamond of Rockville, Maryland, told me of watching a Carolina box turtle, *Terrapene carolina carolina* (Linné), make a nest and lay her eggs under a rose bush in nearby Kensington on May 10, 1963. As the summer wore on, the spot was examined frequently for signs of emerging hatchlings. Finally, near the end of September, about 140 days later, the nest, between 4 and 6 in. deep, was dug up and revealed egg shell fragments and three live hatchlings with no sign of the yolk sac on the plastron. All three were 'timid' turtles⁸.

Domantay⁴ reports for *Chelonia mydas*, and Caldwell⁵ for *Caretta caretta caretta* (Linné), that the hatchlings usually emerge from the nest together, a fact which suggests that the more active ones open the way for the others.

In view of the foregoing evidence concerning other species of turtles, I suggest that the late emergers of *Testudo elegans* Schoepff as reported by Jayakar and Spurway¹ were not slow in developing but rather inactive as hatchlings, and that the correlation of low weight with late emergence from the hatching site is due to the yolk having been used up during the prolonged stay in the soil.

This work was supported in part by U.S. Public Health Service research grant AM 01766 from the National Institute of Arthritis and Metabolic Diseases.

MARIE THERESE DIMOND

Department of Biology,
Trinity College,
Washington, D.C.

¹ Jayakar, S. D., and Spurway, H., *Nature*, **204**, 603 (1964).

² Caldwell, D. K., Berry, F. H., Carr, A., and Ragotzkie, R. A., *Bull. Florida State Mus., Biol. Sci.*, **4**, 309 (1959).

³ Carr, A., *Handbook of Turtles, the Turtles of the United States, Canada, and California* (Comstock Publ. Assoc., Cornell Univ. Press, Ithaca, N.Y., 1952).

⁴ Domantay, J. S., *Notes on the Development of Chelonia mydas, a Marine Turtle, and its Breeding Instincts* (Bureau of Fisheries, Philippines, n.d.).

⁵ Caldwell, D. K., *Bull. Florida State Mus., Biol. Sci.*, **4**, 319 (1959).

⁶ Lynn, W. G., and Ullrich, M. C., *Copeia*, 253 (1950).

⁷ Dimond, M. T., *J. Exp. Zool.*, **127**, 93 (1954).

⁸ Diamond, K. (personal communication).

Development of the Ovule in a Species of *Cissus*

In an investigation of the floral morphology and embryology of the Vitaceae the following features were observed in the development of the ovule of *Cissus trilobata* Lamk. collected from Changanacherry, Kerala State, India.

The ovule is anatropous, bitegmatic and crassinucellate. Of the two integuments, the inner which projects beyond the outer is initiated first by periclinal divisions in the protoderm simultaneous with the differentiation of the



Fig. 1. Longitudinal section of the ovule ($\times 130$). oi, Outer integument; ii, Inner integument.

ovular archesporium in the first hypodermal layer. develops faster than the nucellus and not only encloses the latter but also grows very long, 2-3 times the length of the nucellus, into a tubular structure which is thrown into folds and coils (Fig. 1). Although it is known that in other members of the Vitaceae the micropyle is formed by the inner integument¹⁻⁴, coiling and folding of the integument have not so far been mentioned. Moreover, such a long, coiled, and folded micropylar canal is not reported in any of the flowering plants.

N. C. NAIR

Botanical Survey of India,
Dehra Dun.

¹ Adatia, R. D., Mulay, B. N., and Hingorani, G. R., *J. Univ., Bombay Sect. B*, **21**, 51 (1953).

² Kashyap, G., *J. Indian Bot. Soc.*, **37**, 240 (1958).

³ Mulay, B. N., Nair, N. C., and Sastry, M. S. R., *Proc. Rajasthan Acad. Sci.*, **4**, 17 (1953).

⁴ Nair, N. C., and Parasuraman, V., *Phyton*, **18**, 157 (1962).

Marsupial Spermatozoa Pairing in the Epididymis of American Forms

In 1962 Biggers and Creed¹ pointed out that conjugation (henceforth called pairing) of spermatozoa is a normal occurrence in the epididymides of the North American opossum (*Didelphis marsupialis virginiana* Kerr). Evidence has now been obtained which shows that this phenomenon is found in the majority of, if not all, marsupials in the American continents, and that, if it occurs in Australasian marsupials, it is likely to be exceptional. Also, three morphologically distinct types of spermatozoa have been found to exist in the extant American group, which consists of 69 species² grouped into 15 genera and two families. Our observations are summarized in this report.

The species of marsupial we have examined are shown in Tables 1 and 2. In most cases, fixed specimens of the testes and epididymides were examined histologically. However, the living epididymal spermatozoa of *Philander opossum* (four-eyed opossum), *Marmosa mexicana* (murine opossum), and *Caluromys derbianus* (woolly opossum) have also been examined.

Pairing of spermatozoa has been observed in the epididymides of all the species of American marsupials shown in Table 1. In no instance was pairing observed in sections of the testes. The only genera we have not been able to examine are *Glironia*, *Dromiciops*, *Caluromys*, *Lutreolina* and *Notodelphis*. A sufficient number of data are available on the two sub-species of *Didelphis* and *Philander* opossum to indicate the incidence of pairing within species. Out of 92 specimens of *D. m. tabascensis*, 88 (95.7 per cent) were paired, and out of 56 specimens of *P. opossum*, 54 (96.5 per cent) were paired. This incidence is very similar to that found for *D. m. virginiana*¹. Thus the pairing of

* 1. AMERICAN MARSUPIALS IN WHICH EPIDIDYMAL SPERMATOZOA HAVE BEEN EXAMINED FOR PAIRING. ALTHOUGH SEVERAL MORE SPECIMENS WERE EXAMINED, ONLY THOSE UNEQUIVOCALLY IDENTIFIED ARE INCLUDED

Family	Species	Vernacular name	No. of specimens	Source
Didelphidae	<i>Didelphis marsupialis</i>	Common opossum	88	(a)
	<i>Didelphis tabascensis</i>	Four-eyed opossum	54	(a)
	<i>Philander opossum</i>	Water opossum	1	(c)
	<i>Chironectes panamensis</i>	Short-tailed opossum	1	(b)
	<i>Monodelphis brevicaudatus</i>	Brown opossum	1	(b)
	<i>Metachirus nudicaudatus</i>	Murine opossum	1	(a)
	<i>Marmosa mexicana</i>	Murine opossum	2	(b)
Molestidae	<i>Caluromys derbianus</i>	Woolly opossum	3	(a, d)
	<i>Caenolestes</i>	Rat opossum	12	(b)

See footnotes for Table 2.

* 2. AUSTRALASIAN MARSUPIALS IN WHICH TESTICULAR AND EPIDIDYMAL SPERMATOZOA HAVE BEEN EXAMINED FOR PAIRING. ALTHOUGH MANY MORE SPECIMENS WERE EXAMINED, ONLY THOSE UNEQUIVOCALLY IDENTIFIED ARE INCLUDED

Family	Species	Vernacular name	No. of specimens	Source
Sminthopodidae	<i>Sminthopsis crassicaudata</i>	Fat-tailed sminthopsis	2	(b)
	<i>Sminthopsis rufigenis</i>	—	1	(b)
	<i>Neophascogale lorentzi</i>	—	1	(b)
	<i>Antechinus flavipes</i>	Yellow-footed marsupial mouse	1	(b)
Alangeridae	<i>Acrobates pygmaeus</i>	Pigmy glider	2	(b)
	<i>Eudromicia caudata</i>	Pigmy possum	1	(b)
	<i>Trichosurus vulpecula</i>	Brush-tail possum	1	(e)
Ascolomidae	<i>Phascolomys mitchelli</i>	Common wombat	1	(e)
Amelidae	<i>Perameles nasuta</i>	Long-nosed bandicoot	1	(f)
Ceropodidae	<i>Peroryctes longicauda</i>	Bandicoot	3	(b, g)
	<i>Protemnodon bicolor</i>	Swamp wallaby	1	(e)
	<i>Megaleia rufa</i>	Red kangaroo	2	(e)
	<i>Macropus robustus</i>	Wallaroo	3	(e)
	<i>Macropus kanguru</i>	Great grey kangaroo	1	(e)

Dr. L. G. Clark, Leptospirosis Unit, University of Pennsylvania, Philadelphia, Pennsylvania.

American Museum of Natural History, New York. Courtesy of Dr. H. van Deusen.

Dr. W. A. Wimsatt, Department of Zoology, Cornell University.

Dr. J. A. Davis, curator of mammals, New York Zoological Society.

Dr. G. Sharman, Division of Wildlife Research, Commonwealth Scientific and Industrial Research Organization, Australia.

Prof. A. S. Parkes, Department of Physiology, University of Cambridge.

Archbold Collection, American Museum of Natural History, New York. Courtesy of Dr. H. van Deusen, curator.

spermatozoa in the epididymides is a widespread, if not universal, phenomenon in the American stock of marsupials. It is impossible at present to determine the extent of pairing in the epididymis and the degree of variation between individuals. This will only be possible when methods of collecting ejaculates from these species have been devised.

The pairing of spermatozoa in the epididymides has not been observed in any of the species of Australasian marsupial shown in Table 2, and in no case was pairing observed in sections of the testes. Thus it seems unlikely that pairing of spermatozoa occurs in the epididymides of the Australasian stock of marsupials.

During the course of this work three distinct morphological types of spermatozoa have been found in the American marsupials. These will be designated the *Didelphis*, *Caluromys* and *Caenolestes* types respectively, and pairs of them are illustrated in Figs. 1, 2, 3. *Didelphis* is the well-known type, and this has been described elsewhere^{1,3}. *Monodelphis*, *Philander*, *Metachirus*, *Chironectes* and *Marmosa* all have this morphological form of spermatozoa. The spermatozoa of *Caluromys* possess saucer-shaped heads with the mid-piece inserted into the convex side, and the acrosome lying in the concave side. Pairing occurs by apposition of the two concave sides. The spermatozoa of *Caenolestes* have only been observed in fixed material. Unfortunately, this is a very rare species indigenous to localized areas of the Andes, and it is very unlikely that living material will be obtained. The spermatozoa are very rectilinear in shape with a niche on one side from which the mid-piece arises. Pairing occurs by apposition of the edges opposite the insertion of the mid-piece.

The phenomenon we have described as pairing was first observed in *D. marsupialis* by Selenka⁴, who referred

to it as 'copulating' spermatozoa. This name was used by Von Korph⁵, who made the crucial observation that the spermatozoa arise as single cells in the seminiferous tubules, and that the pairing only occurs after the gametes reach the epididymides. Later, Wilson⁶ objected to this label, and introduced the word 'conjugating'. However, this word has acquired a specific meaning in biology with reference to the union of gametes in unicellular organisms. For this reason, we prefer the simple descriptive word 'pairing' to describe the phenomenon. Perhaps, when experimental work has elucidated the phenomena involved in this highly oriented adhesion of cells, a more explicit word may be coined for its description.

The finding of three morphologically distinct types of spermatozoa in the few genera of American marsupials is pertinent from a taxonomic point of view. Osteological and palaeontological evidence was interpreted by Reig⁷ to suggest that *Caluromys*, *Dromiciops* and *Glironia* are related members of the sub-family Microbiotheriinae formerly thought to be extinct. The finding that the spermatozoa of *Caluromys* are morphologically different from that of the sub-family Didelphinae may therefore be systematically significant. An examination of the sperma-

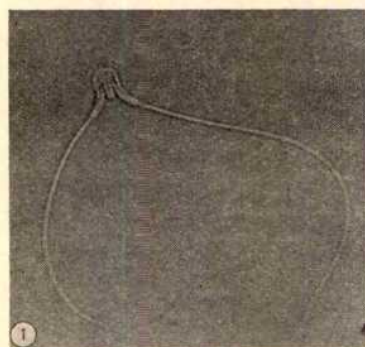


Fig. 1. Paired spermatozoa expressed from the epididymis of the four-eyed opossum (*Philander opossum*). ($\times 805$)

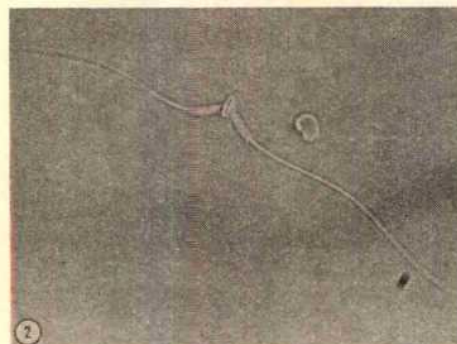


Fig. 2. Paired spermatozoa expressed from the epididymis of the woolly opossum (*Caluromys derbianus*). ($\times 805$)

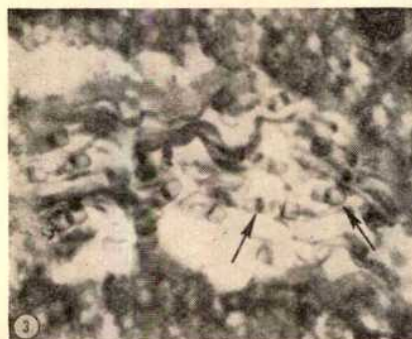


Fig. 3. Section through the epididymis of *Caenolestes*. The arrows indicate the head of paired spermatozoa. ($\times 805$)

tozoa of *Dromiciops* and *Glironia* to see if they are morphologically similar to those of *Caluromys* would elucidate this problem, but unfortunately this has been impossible because of the rarity of these species. The observation that *Caenolestes* has spermatozoa morphologically distinct from other American marsupials is of interest because of the unusual features of this survivor of an ancient marsupial group of the Tertiary epoch⁸. These animals are unique in that they possess affinities with members of the super-family Phalangerioidea found only in Australasia, thus providing a possible connexion between the American and Australasian groups of marsupials⁹. Investigations of the morphology of marsupial spermatozoa, however, indicate no similarity between the American and Australasian forms¹⁰, and therefore, so far as this character is concerned, there is no affinity between the two major marsupial groups.

The fact that pairing of spermatozoa has been observed in all types of American marsupial which have been examined, transcending two natural families and three morphological types, and has not been observed in Australasian marsupials, indicates that both the *Caenolestidae* and *Didelphidae* are derived from a common ancestral stock, the descendants of which evolved only in the Americas. Furthermore, pairing of spermatozoa, whatever its function, must have become established early in the evolutionary history of the group.

This work was supported in part by grant G-17658 from the U.S. National Science Foundation, and general research support grant, U.S. Public Health Service, FR-05464.

J. D. BIGGERS

King Ranch Laboratory of Reproductive Physiology,
School of Veterinary Medicine,
University of Pennsylvania,
Philadelphia.

E. D. DELAMATER

Department of Microbiology,
New York Medical College,
New York.

¹ Biggers, J. D., and Creed, R. F. S., *Nature*, **196**, 1112 (1962).

² Cabrera, A., *Rev. Mus. Argent. Cienc. Nat.* No. 4 (1957).

³ Retzius, G., *Biol. Untersuch.*, **14**, 123 (1909).

⁴ Selenka, E., *Studien über Entwicklungsgeschichte der Thiere*. Viertes Heft, *Das Opossum (Didelphys virginiana)* (C. W. Kreidel's Verlag, Wiesbaden, 1887).

⁵ Korph, K. von, *Arch. mikr. Anat.*, **60**, 232 (1902).

⁶ Wilson, E. B., *The Cell in Development and Heredity* (Macmillan Co., New York, 1928).

⁷ Reig, O. A., *Invest. Zool. Chilensis*, **2**, 121 (1955).

⁸ Osgood, W. H., *Publ. Field Museum Nat. Hist.* No. 207, **14**, 1 (1921).

⁹ Ride, W. D. L., in *The Evolution of Living Organisms*, edit. by Leeper, C. W., 281 (Melbourne University Press, 1962).

¹⁰ Biggers, J. D., *Symp. Zool. Soc., London* (in the press).

Record of the Bivalve Gastropod *Berthelinia limax* (Kawaguti and Baba 1959) from the Indian Ocean

A LIVING bivalve gastropod of the order Sacoglossa, found in Japan by Kawaguti and Baba¹ in 1959, was described as a new genus and species *Tamanovalva limax*. Keen and Smith², Baba³ and Taylor and Soh⁴ synonymized the genus *Tamanovalva* Kawaguti and Baba 1959, with the Eocene Paris Basin fossil genus *Berthelinia* Crosse

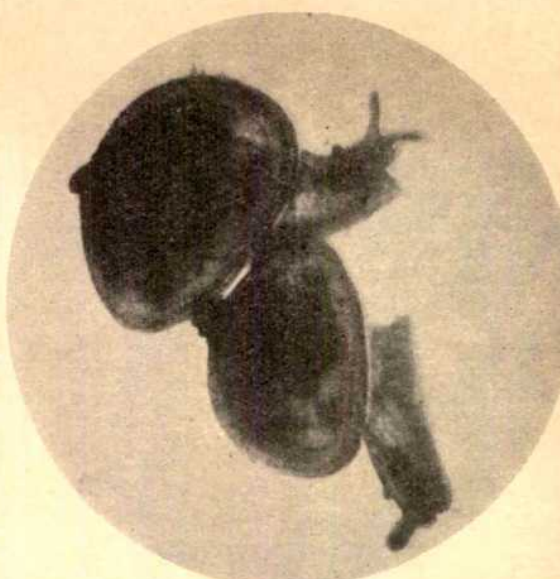


Fig. 1. Two live specimens of *Berthelinia limax* (Kawaguti and Baba 1959) ($\times 25$)

1875 and included it under the sub-family Berthelininae Beets, 1949, of the family Jullidae Dall, 1898. Since the living members of this genus have been reported from number of other Pacific localities in Japan⁵, Australia⁶ and Hawaii⁷, and from Jamaica⁸ in the Atlantic. The purpose of this communication is to record the collection of living specimens of *Berthelinia* for the first time from the Indian Ocean region.

Recently, while examining nudibranch molluscs near the jetty of the Central Marine Fisheries Research Institute at Mandapam Camp facing the Gulf of Mannar, came across four specimens of small bivalve gastropod found attached to the roots of the green alga *Caulerpa racemosa* (Forsskal) J. Agardh, which on close examination resembled *Berthelinia limax* in all essential features (Fig. 1). The measurements of the four specimens are given in Table 1.

Table 1		
Specimen No.	Length (mm)	Height (mm)
1	2.76	1.94
2	2.53	1.76
3	2.41	1.70
4	2.00	1.35

The habitat of *Berthelinia* appears to be restricted to various species of *Caulerpa* on which it feeds. Its known distribution along with the algal habitat is shown in Table 2.

The present specimens resemble the Pacific species and the differences noted are very minor and not sufficient to warrant any specific separation. They are, however, distinct from the West Atlantic species, *Berthelinia caribbea* Edmunds¹⁰, where the shell valves are green or grey-green often tinged brownish, with more or less distinct yellow rays.

The animals are leaf green in colour with auriculate rhinophores ornamented with opaque white spots. The head, neck and sole are light green in colour, while the

Table 2

Spec. No.	Species	Algal habitat	Reference	Locality
1	<i>Berthelinia limax</i>	<i>Caulerpa okamurae</i>	Kawaguti and Baba (ref. 1)	Bisan Seto, Inland Sea, Japan
2	<i>Berthelinia typica</i>	<i>Caulerpa scalpelliformis</i>	Burn (ref. 6)	Torquay, Victoria, Australia
3	<i>Berthelinia australis</i>	<i>Caulerpa scalpelliformis</i>	Burn (ref. 6)	Torquay, Victoria, Australia
		<i>Caulerpa brownii</i>		
4	<i>Berthelinia chloris</i>	<i>Caulerpa simpliciuscula</i>	Keen and Smith (ref. 2)	Puerto Ballandra Bay, Baja, California
		<i>Caulerpa racemosa</i>		
5	<i>Berthelinia</i> sp?	<i>Caulerpa sertularioides</i>	Kay (ref. 8)	Koloa, Kauai, Hawaii
6	<i>Berthelinia caribbea</i>	<i>Caulerpa racemosa</i>	Edmunds (ref. 10)	Port Royal, Jamaica
7	<i>Berthelinia limax</i>	<i>Caulerpa verticillata</i>	Present work	Mandapam Camp (Gulf of Mannar, India)
		<i>Caulerpa racemosa</i>		

tle and the liver are deep green. A few irregularly red dark green spots are present on the liver. The valves of the shell are equal in size, light green in colour, extremely thin and fragile, with a smooth surface which is marked by fine growth lines. The helicoid protoconch is translucent and of one and half whorls, attached to the posterior extremity of the left valve as in *Berthelinia ax.* The protoconch is directed backwards extending horizontally over the right valve. The position of the yellowish white circular attachment of the adductor muscle is as in *B. limax*.

One of the specimens laid an egg string which was kept under observation. The early development agreed in all essential respects with the observations of Kawaguti and Baba¹ and Kawaguti and Yamasu^{11,12} on *B. limax*.

With the record of the genus *Berthelinia* from the Indian coast it could be presumed that the species of the genus are distributed throughout the coastal waters of the warmer seas. A careful search might prove that it is widely distributed in the Indo-Pacific waters.

I thank Dr. Umamaheswara Rao for identifying the species, Dr. P. S. B. R. James for advice, and Mr. K. G. Ambiar for the photograph.

K. PRABHAKARA RAO

Central Marine Fisheries,
Research Institute,
Mandapam Camp,
India.

- Kawaguti, S., and Baba, K., *Biol. J. Okayama Univ.*, **5**, 177 (1959).
Keen, A. M., and Smith, A. G., *Proc. Calif. Acad. Sci.*, **30**, 44 (1961).
Baba, K., *Venus, Kyoto*, **21**, 389 (1961).
Taylor, W. D., and Sohl, N. F., *Malacologia*, **1**, 7 (1962).
Baba, K., *Seto Mar. Biol. Lab.*, **9**, 37 (1961).
Gura, R., *Nature*, **186**, 179 (1960).
Keen, A. M., *Nature*, **185**, 406 (1960).
Kay, E. A., *Nature*, **195**, 96 (1962).
Edmunds, M., *Nature*, **195**, 402 (1962).
Edmunds, M., *J. Linn. Soc. (Zool.)*, **44**, 732 (1963).
Kawaguti, S., and Yamasu, T., *Biol. J. Okayama Univ.*, **6**, 133 (1960).
Kawaguti, S., and Yamasu, T., *Biol. J. Okayama Univ.*, **6**, 150 (1960).

Spore Discharge in *Lepiota konradii*

RECENT investigations¹ based on laboratory experiments have shown that ballistospore discharge is much affected by the humidity and temperature of the ambient air. The observations reported here are based on field studies during which an effort was made to determine the effect of temperature and humidity on ballistospore discharge under natural conditions.

On the morning of January 15, 1965, three fruiting-bodies of *Lepiota konradii* Huijsman ex P.D. Orton² were found growing in the Biological Garden, University of Ife, Ibadan, Nigeria; by noon, their pilei were beginning to expand.

An electric-driven Hirst spore trap was set up near these fruiting-bodies. The trap was placed so that the orifice was at a height of 27 cm above ground-level. The horizontal distance of the orifice from the pileus of the nearest fruiting-body was 56 cm.

Sampling at the rate of 0.6 m³/h was continued until the fruiting-bodies were found to be withering, drying, and tottering as if they were about to fall. The sampling period was January 15–18, 1965.

The ballistospores being sticky themselves, clean slides were used without any adhesive. These were changed daily at 1300 h Nigerian Standard Time. After exposure, slides were mounted for examination in glycerol and scanned under low magnification. Counts were made on cross traverses 34 μ wide and 4 mm apart, representing 2-h intervals. The numbers of spores counted were then converted into an estimated number per cubic metre of air.

Records of temperature and humidity were obtained by means of a Casella thermo-hygrograph situated under the roof of the spore trap.

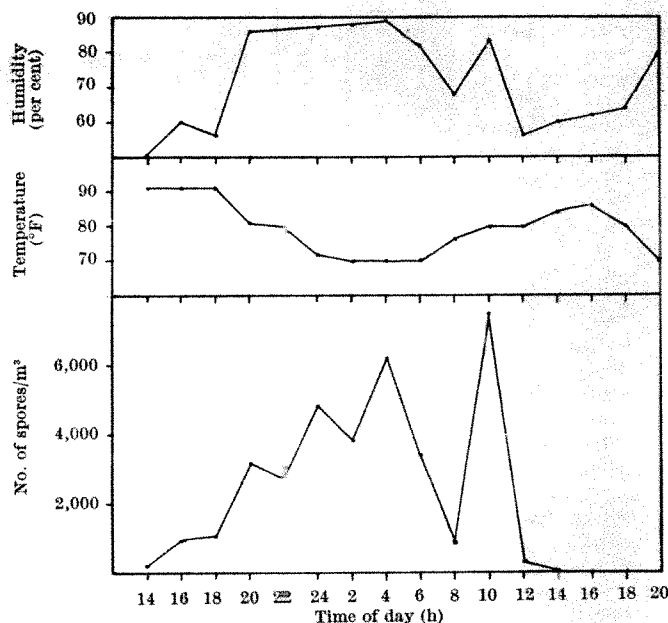


Fig. 1. Hourly mean concentration of ballistospores

The results shown in Fig. 1 clearly indicate a correlation between spore discharge and relative humidity. The increased rate of spore discharge at 1800 h and reaching a maximum (6,200 spores/m³ air) at 0400 h—a period of rapid liberation of ballistospores—corresponds quite closely with that of decreasing temperature and increasing relative humidity of the surrounding atmosphere.

Counts at 2200 h and 0200 h show, however, that the concentration of spores in the 2-h periods preceding these times had been slightly reduced. This needs further investigation, which is being planned. It was probably due to a change in the direction or the velocity of the wind, or possibly to the fact that nearly all mature spores were liberated during the early part of the period of high humidity, so that there were not many spores left for liberation during the later part. On the other hand, the maximum discharge recorded at 0400 h might have been due to a recovery in the number of mature spores.

Shortly after 0400 h the rate of spore discharge decreased gradually and reached a minimum (1,000 spores/m³ air) at 0800 h. The relative humidity then recorded was fairly low (68 per cent).

Again there was a further large increase (to 7,700/m³) in concentration of these ballistospores in the air at 1000 h. Such abundant liberation was correlated with a sudden increase in humidity caused by a slight trace of rain. Thereafter the spore liberation decreased very rapidly, so that by 1200 h the spore concentration in the atmosphere was only 300/m³ and by 1400 h liberation had apparently fallen to zero. For, although subsequent observations indicated numerous dust and smoke particles in the air, no *Lepiota* ballistospores were found impacted on the slides.

These observations also show that the spore discharge period for the fruiting-bodies investigated began at 1400 h in the afternoon and ended about 1200 h the next morning, being accordingly of about 22 h duration.

The observations reported here indicate that temperature and humidity control ballistospore discharge in the field—as in the laboratory. It would be interesting to know to what extent, if any, temperature and humidity control the development of the ballistospores.

M. H. ZOBERI

Department of Botany,
University of Ife,
Ibadan, Nigeria.

¹ Zoberi, M. H., *Trans. Brit. Mycol. Soc.*, **47**, 109 (1964).

² Orton, P. D., *Trans. Brit. Mycol. Soc.*, **43**, 159 (1960).

FORESTRY

Induction of Tension Wood with the Anti-auxin 2,3,5-Tri-iodobenzoic acid

THE xylem formed on the upper side of arborescent angiosperms in reaction to inclination is often of the abnormal tension wood type. A complete discussion of tension wood anatomy is given in a recent review by Wardrop¹. In brief, it is characterized by the presence of a large number of thick-walled, 'gelatinous' fibres of unusually low lignin content, and a reduction in the size and number of vessels over that of normal wood.

The reaction wood observed in leaning coniferous stems occurs on the under side. Since reaction wood in conifers can be induced to form on application of indolyl-3-acetic acid (IAA) to erect stems²⁻⁴, the mechanism of its formation in leaning trees is generally thought to be associated with supra-optimal concentrations of IAA or closely allied growth-promoting substances. However, application of IAA to the upper surface of young, bent seedlings of *Populus monilifera* prevented the formation of tension wood⁴. In this latter study, untreated inclined branches of *Populus alba* were observed to form tension wood on their upper sides in association with low levels of IAA and the presence of growth inhibitors. As a logical extension of this study, we investigated the possibility of inducing the formation of tension wood in an erect stem of a hardwood by applying a synthetic anti-auxin. The use of such anti-metabolites in physiological investigations of tension wood formation has also been suggested by Berlyn⁵.

The anti-auxin 2,3,5-tri-iodobenzoic acid (TIBA) was selected for use. It is known to act as an IAA antagonist either through direct competition with auxin or by blocking its movement about the plant⁶. Larson⁷ has employed this substance with *Pinus resinosa* to induce the formation of tracheids of reduced radial diameter, a response contrary to that evoked by application of IAA. A lateral exogenous application of TIBA in lanolin paste (1:100) was applied to a four-month-old white elm (*Ulmus americana* L.) seedling 5 mm in diameter. After first scraping the outer bark at a point 7 cm above the level of the rooting medium ('Perlite' irrigated with a complete nutrient solution), application was made in a band 5 mm wide completely around the periphery of the stem. At the conclusion of a 35-day period the stem was collected by cutting into 3-cm lengths. These pieces were aspirated in FAA solution and sectioned transversely at three different levels: (1) directly above the point of application of TIBA, (2) 2 cm below, and (3) 5 cm above this point. Sections were stained in safranin and fast green before microscopic examination.

The wood formed by the cambium as a result of application differed markedly from that produced before treatment (Fig. 1). Directly above the point of application, the mature wood formed in response to TIBA consisted of four bands of rectangular conductive cells resembling coniferous tracheids due to their orientation into tangential and radial rows (Fig. 2). These bands of conductive tissue alternated with three bands of fibres, the last of which was almost entirely gelatinous. At the widest point, the number of mature cells in a radial row through these bands totalled about 60, indicating a very rapid rate of cell production. The areas sampled above and below this location had approximately the same features, namely: a zone of response to TIBA only half the radial width of position (1); conductive tissue similar to that described above, except in shorter tangential lines; fibrous zones which were exclusively gelatinous (Fig. 3).

Equal parts of hydrogen peroxide and acetic acid were used to macerate the layer of wood formed in response to TIBA application. Examination of the pulp verified that the conducting elements seen in transverse section were vessel segments, approximately 0.13 mm long, with simple perforations and heavy spiral thickenings. The vessel ele-

ments of the normal wood were likewise simply perforated but were often twice the length of the former, and only weak spiral markings confined to the latewood.

The faster growth rate, reduction in size of vessel elements, and the formation of gelatinous fibres, especially in the two locations more remote from the point of application of TIBA, establish a cambial response leading to tension wood formation. The fact that the total volume of vessels was not reduced must be regarded as an anomaly. Nevertheless, these results lend support to the hypothesis that tension wood forms in response to an auxin deficiency. In naturally leaning hardwood stems and inclined branch the auxin deficiency probably results from the influence of gravity on its distribution, leading to the conclusion

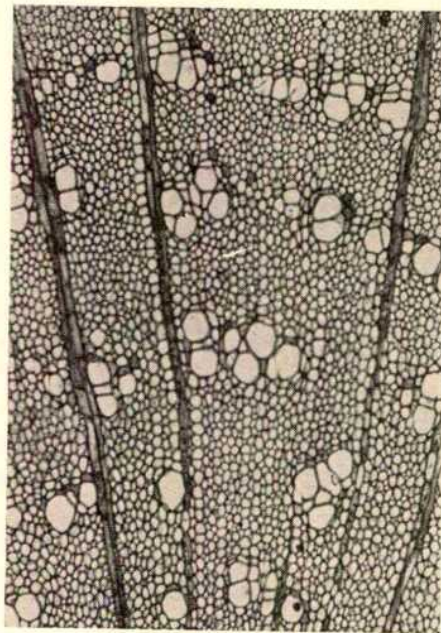


Fig. 1. Appearance of xylem formed in *Ulmus americana* stem before application of TIBA

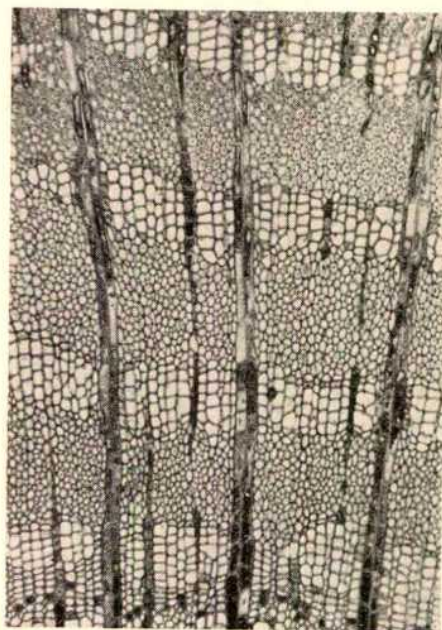


Fig. 2. Appearance of xylem formed in erect *Ulmus americana* stem immediately above the point of application of TIBA. Gelatinous fibres were present only in the third band (top of photograph)

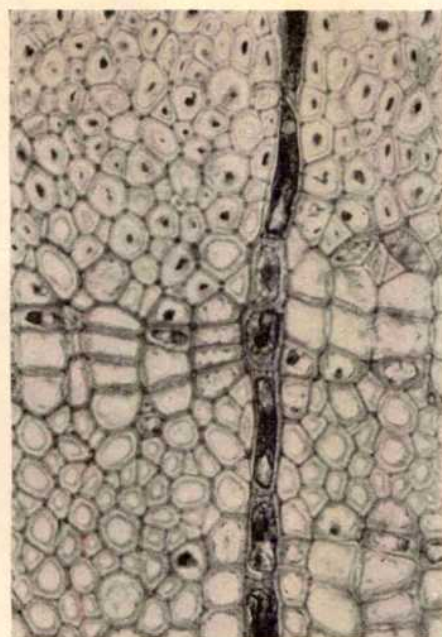


Fig. 3. Comparison of xylem fibres formed in erect *Ulmus americana* stem, 5 cm above the point of application of TIBA. The central tangential band of small vessels and axial parenchyma separates fibres formed before (lower half) and after (upper half of photograph) application of TIBA.

Wardrop¹ that gravity is a major factor governing the formation and distribution of tension wood.

This study is continuing with extensive sampling of 14 angiospermous species on which TIBA has been applied to lateral and excised apical surfaces.

R. W. KENNEDY
J. L. FARRAR

Faculty of Forestry,
University of Toronto,
Toronto 5, Canada.

Wardrop, A. B., *The Formation of Wood in Forest Trees*, 405 (Academic Press, 1964).

Wershing, H. F., and Bailey, I. W., *J. For.*, **30**, 411 (1942).

Fraser, D. A., *Ecology*, **33**, 259 (1952).

Nečesný, V., *Phyton*, **11**, 117 (1958).

Berlyn, G. P., *Iowa State J. Sci.*, **35**, 367 (1961).

Audus, L. J., *Plant Growth Substances*, 80 (Leonard Hill, 1959).

Larson, P. R., *The Formation of Wood in Forest Trees*, 345 (Academic Press, 1964).

Leucoanthocyanins of White Pine in relation to Weevil Attack

LEUCOANTHOCYANINS may serve as growth-promoting substances¹ as well as precursors to tannin formation². Both these functions suggest that leucoanthocyanins may be involved in the complex of factors which influence pest resistance in plants. With this possibility in mind, an investigation of the leucoanthocyanins of eastern white pine (*Pinus strobus* L.) was carried out to determine the relationship between leucoanthocyanin content and susceptibility to white pine weevil (*Pissodes strobi* (Peck)).

In the only previous report dealing with leucoanthocyanins in *P. strobus*³, both leucocyanidin and leucodelphinidin were found in needles and bark, while the wood contained only leucocyanidin. No quantitative data were given in that report.

Parent trees of the control-pollinated progenies used in this investigation were located at the Morris Arboretum of the University of Pennsylvania, Philadelphia. Twelve trees of each progeny were examined in a 1952 test planting established by the U.S. Forest Service in Williamstown, Massachusetts. Significant variation in susceptibility to weevil attack has been reported for these and other pedigreed progenies⁴.

Leaders or leading lateral shoots of the current season were collected during the winter of 1963 and allowed to dry in the laboratory for one month. This procedure eliminated the problems encountered when working with resinous material. The bark (chiefly cortex) was scraped from the wood cylinder and the woody portion of the stem was further separated into wood and pith. All material was ground in a Wiley mill to pass a 20-mesh sieve.

Conversion of leucoanthocyanin to the coloured aglycone was accomplished by hydrolysing small samples of tissue (100 mg bark and wood, 15 mg pith) in 10 ml. of an *n*-butanol-3 N hydrochloric acid (5 : 1 v/v) solution in a sealed test-tube. This mixture was heated at 100° C in a water bath for 40 min. The transformation of leucoanthocyanins to aglycones may not be quantitative but alcoholic solvents are more than twice as efficient as aqueous solvents^{5,6}.

After cooling, the coloured extracts were spotted on Whatman No. 1 paper and chromatographed by the ascending method using Forestal solvent (glacial acetic acid-conc. hydrochloric acid-water, 30 : 3 : 10 v/v/v). Pure cyanidin and delphinidin were used as checks. Cyanidin was the only aglycone that could be identified from both parent and progeny trees. Larger samples of bark (up to 3 g) gave similar results.

In order to check further on the earlier report of the occurrence of both cyanidin- and delphinidin-yielding leucoanthocyanins in *P. strobus* bark, four native trees growing within a small area in New Hampshire were sampled. Of these trees, one yielded delphinidin and cyanidin in approximately equal amounts, while another gave no delphinidin. The two other trees were intermediate. Thus it appears that a detectable content of leucodelphinidin is not invariably present in *P. strobus* bark.

Quantitative determinations of cyanidin content in appropriately diluted extracts were made with a Bausch and Lomb 'Spectronic 20' spectrophotometer. This instrument had been calibrated at a wave-length of 550 mμ with known concentrations of cyanidin prepared from pure leucocyanidin (kindly provided by Dr. K. Weinges, Heidelberg) by hydrolysis in butanol-hydrochloric acid. Results, calculated as percentages of dry weight of tissue, are given in Table 1.

There were no significant or meaningful differences in the cyanidin content of bark or wood between trees that had been weeviled or 'unweeviled' in the past. Although there was significant variation among parents in wood cyanidin, the progeny averages were less than might be expected.

Progeny differences in pith cyanidin were highly significant, but again progeny averages were lower than expected and the difference between weeviled and 'unweeviled' trees was not significant. Cyanidin content of the pith was found to decrease with the time since origin. In the single tree tested, pith of the current season had 2.21 per cent cyanidin, while two-year-old and five-year-old pith contained 1.19 per cent and 0.19 per cent cyanidin, respectively.

Several one-year-old stems showed evidence of localized injury by natural causes. When cross-sections of these stems were stained in methanol-hydrochloric acid, it appeared that leucocyanidin had been translocated from the pith through the rays and had accumulated in the wood at the site of the injury. This movement of leucoanthocyanins in response to injury should be investigated further.

Table 1. CYANIDIN CONTENT OF VARIOUS TISSUES OF WHITE PINE LEADERS

Parent or progeny	Bark (per cent)	Wood (per cent)	Pith (per cent)
G-730	2.54	0.26	3.33
730 x 740	2.53	0.096	1.90
Weeviled	2.54	0.097	1.91
Not weeviled	2.73	0.094	1.86
G-740	2.3	0.08	1.31
730 x 748*	2.6	0.12	1.31
G-748	2.73	0.28	3.71

* All weeviled.

This work was initiated while I was associated with the Northeastern Forest Experiment Station, U.S. Forest Service.

FRANK S. SANTAMOUR, JUN.

Morris Arboretum,
University of Pennsylvania,
Philadelphia.

¹ Steward, F. C., and Shantz, E. M., *Ann. Rev. Plant Physiol.*, **10**, 379 (1959).

² Hergert, H., *For. Prod. J.*, **10**, 610 (1960).

³ Krugman, S. L., *Forest Sci.*, **5**, 169 (1959).

⁴ Santamour, jun., F. S., *Northeast. Forest Tree Improv. Conf. Proc.*, **11**, 49 (1964).

⁵ Bate-Smith, E. C., and Swain, T., *Chem. and Indust.*, 377 (1953).

⁶ Swain, T., and Hillis, W. E., *J. Sci. Food Agric.*, **10**, 63 (1959).

MICROBIOLOGY

Apparent Suppression of Mutation Rates in Bacteria by Spermine

ONE of the chief obstacles to chemotherapy with antibiotics has been the frequent emergence of drug-tolerant bacteria. The availability of means to prevent or suppress the development of resistant cultures could have immense medical import as well as providing a valuable tool for the study of mutagenesis. Sevag and collaborators¹⁻³ reported that when polyamines were included in the culture medium of various micro-organisms, the outgrowth of drug-resistant populations in the presence of low concentrations of antibiotics was significantly delayed. They interpreted these observations as suggesting an antimutagenic role for the polyamines and further proposed that the site for this antimutagenic action is the DNA of the sensitive cells. Because of the theoretical implications of these observations it was of interest to establish in a more rigorous manner whether polyamines are antimutagens or whether the effects described by Sevag and co-workers could be explained in some other manner. This communication presents evidence that the inclusion of spermine in bacterial culture media resulted in a significant decrease in the random mutation rate of *Escherichia coli* and *Staphylococcus aureus*. The mutational events which were investigated were the one-step development of resistance to streptomycin and the back mutation to autotrophic growth by a tryptophan-requiring mutant. Spermine caused even more striking antimutagenic effects when mutations were induced by the inclusion of caffeine in the culture medium⁴ or by ultra-violet light. Future papers will seek to demonstrate that bacterial DNA could be the site of action of this compound⁵. A preliminary report on some of these experiments has already been given⁶.

E. coli (UC 879) and a tryptophan-requiring mutant (UC 707) were cultured in a minimal salts glucose medium⁷ supplemented with 20 µg/ml. L-tryptophan where required. *S. aureus* (UC 76) was cultured on a synthetic medium containing amino-acids, vitamins and salts⁸. To estimate the mutation rates a random fluctuation test, similar to the one described by Luria⁹, was used. This test assumes

that mutation rates are independent of absolute time and are dependent on the number of bacteria present and the generation time of the cultures⁹. The recent work of Kubitschek and Bendigkeit¹⁰ suggests that this assumption is likely to be warranted in this case. For each treatment 50 replicate 2-ml. cultures were inoculated with 100 cells each and at the end of 18 h of growth at 37°C the cultures were plated with nutrient agar containing 8 µg/ml. streptomycin sulphate. When 'back mutation' from tryptophan-requirement was studied, the cultures were twice washed in saline to remove residual tryptophan and plated in glucose salts agar. The number of plates in which no growth occurred was scored and the total number of cells per culture plate was established by plating appropriate dilutions on nutrient agar and counting the number of colonies present. The mutation rate was then calculated from the formula:

$$m = -(2.303 \log_{10} P_0)/N$$

where N is the total number of bacteria in a culture and P_0 the proportion of the total cultures which showed growth.

Table 1 is a summary of a number of experiments in which the random mutation rates to streptomycin resistance by *E. coli* and *S. aureus* were estimated, as well as two experiments in which the 'back mutation' from tryptophan requirement was studied. In all cases the mutation rates of cells which were grown in the presence of 150 µg/ml. spermine·HCl were clearly lower than those of controls. In most experiments the difference was statistically significant ($P = 0.01-0.007$ as determined from a χ^2 distribution). When spermine was added to control cultures at the time of plating, the mutation rate was the same as that of the controls (line 3, Table 1). Similarly, there was no change in the antimutagenic effect of spermine when the compound was removed along with the tryptophan in the washing procedure and not replaced when the cultures were reconstituted for plating (lines 6 and 7, Table 1). Thus to exert its antimutagenic effect, spermine must be present during the growth of the cells and need not be present during the plating. A reduction of the spermine concentration from 150 µg/ml. to 30 µg/ml. resulted in loss of the antimutagenic effect. However, replacement of the glucose salt medium with nutrient broth in no way influenced the antimutagenic action of spermine (lines 4 and 5, Table 1). Finally, in agreement with the observations of Sevag and Drabble¹, the colonies which grew in the presence of streptomycin were shown on sub-culture to be streptomycin-resistant and those which grew in the absence of tryptophan were shown to have lost their requirement for this amino-acid. Thus, growth in the fluctuation test truly reflected mutational events.

While the suppression of random mutation rate by spermine was real, there was a much more pronounced effect on mutations which were induced by the incorporation of 150 µg/ml. caffeine into the culture medium⁴ or by irradiation of the cultures with ultra-violet light. Table presents the results of these experiments. Each exper-

Table 1. ANTIMUTAGENIC ACTION OF SPERMINE IN RANDOM FLUCTUATION TESTS

Organism	Mutational end-point	Treatment	$N \times 10^6$	$M \times 10^{-10}$	$\frac{m \text{ control}}{m \text{ treated}}$	P
<i>E. coli</i> UC 879	Streptomycin resistance	Control	4.3	1.28	3.7	0.007*
		+ Spermine	4.0	0.35		
<i>E. coli</i> UC 879	Streptomycin resistance	Control; spermine added at plating time	4.3	1.18	1.1	> 0.25†
<i>E. coli</i> UC 879	Streptomycin resistance	Control—broth grown	5.4	1.07	1.95	0.002
		Spermine—broth grown	3.5	0.55		
<i>E. coli</i> UC 707	Trypt—Trypt†	Control	0.76	6.4	2.9	0.026‡
		Spermine	0.83	2.2		
<i>S. aureus</i> UC 76	Streptomycin resistance	Control	6.0	1.09	2.5	0.012‡
		Spermine	5.7	0.43		

The mutation rate, m , was computed from the equation $m = -(2.303 \log_{10} P_0)/N$, where N is the total number of bacteria per culture and P_0 the proportion of cultures which were free of mutants. Fifty replicate cultures were used for each treatment. P values are based on χ^2 distribution.

* This experiment is one of 7 similar ones which were performed and is the ranked median of these with respect to magnitude of P . The range in P values was 0.017–0.002 and the overall probability of null hypotheses was ≤ 0.001 .

† Versus control value, line 1.

‡ One of duplicate experiments with identical results.

Table 2. ANTIMUTAGENIC ACTION OF SPERMINE ON MUTATIONS TO STREPTOMYCIN-RESISTANCE INDUCED IN *E. coli* UC 879 BY CAFFEINE AND ULTRA-VIOLET LIGHT

Treatment	(See Table 1 for details)			$\frac{m}{m}$ control treated	P
	Initial $\times 10^{10}$	Surviving $\times 10^8$	$m \times 10^{-10}$		
Caffeine	0.8	—	1.5	11.5	≤ 0.001
Caffeine + spermine	1.7	—	0.13		
Control	1.5	—	0.48		
Ultra-violet, 25 sec	1.4	0.53	220	8.5	0.024
Caffeine + ultra-violet	3.2	0.66	26		
Control	1.4	—	0.46		

ent was repeated once with essentially identical results. The ultra-violet irradiation was accomplished with a testinghouse 36GT5 germicidal lamp at a dose of approximately 150 ergs/mm² to effect a killing rate of approximately 99 per cent¹¹. Individual aliquots of a culture which had been grown for 9 h on a shaker (with or without added spermine during growth) were irradiated in a darkened room. All further handling was carried out in the dark to prevent photoreactivation. In all cases cells grown in the presence of spermine had lower mutation rates than the control cells ($P = 0.02$).

The mutational event in all these experiments occurred in the absence of the indicator by which the event was scored. Furthermore, there was no effect on the growth rate of streptomycin-sensitive or -resistant organisms when spermine was included in the culture medium. Thus there is no chance that any form of selection could account for the observed changes in the incidence of mutations¹². Preliminary results suggest that other polyamines may also suppress the random mutation rate much as they were reported by Sevag and co-workers¹⁻³ to delay the outgrowth of resistant populations in the presence of antibiotics. It is concluded from these results that the antimutagenic action of polyamines is a general property of these compounds, that it is independent of the mutational end-point being measured, and that it is even more pronounced when mutations are induced than when randomly occurring mutations are studied.

We thank Mr. Jack I. Northan for assistance with the statistical analysis of the results.

H. G. JOHNSON
M. K. BACH

Department of Biochemistry,
The Upjohn Co.,
Kalamazoo, Michigan.

¹ Sevag, M. G., and Drabble, W. T., *Biochem. Biophys. Res. Commun.*, **8**, 446 (1962).

² Sevag, M. G., *Arch. Biochem. Biophys.*, **108**, 85 (1964).

³ Sevag, M. G., and Ashton, B., *Nature*, **203**, 1323 (1964).

⁴ Novick, A., *Brookhaven Symp. Biol.*, **8**, 201 (1956).

⁵ Johnson, H. G., and Bach, M. K., *Bact. Proc.*, **G23** (1965).

⁶ Johnson, H. G., and Bach, M. K., *Bact. Proc.*, **G144** (1964).

⁷ Drabble, W. T., and Hinshelwood, C., *Proc. Roy. Soc., B*, **154**, 449 (1961).

⁸ Steers, E., and Sevag, M. G., *Arch. Biochem.*, **24**, 129 (1949).

⁹ Luria, S. E., *Cold Spring Harbor Symp. Quant. Biol.*, **18**, 130 (1946).

¹⁰ Kubitschek, H. E., and Bendigkeit, H. E., *Mutation Res.*, **1**, 113 (1964).

¹¹ Doudney, C. O., White, B. F., and Bruce, B. J., *Biochem. Biophys. Res. Commun.*, **15**, 70 (1964).

¹² Demerec, M., *Proc. U.S. Nat. Acad. Sci.*, **31**, 16 (1945).

VIROLOGY

Derivatives of 2-Aminoethyl-guanidine and of a Eugenol as Virus Inhibitors

It is a well-known fact that certain structurally different chemicals inhibit the synthesis of viral RNA¹⁻³. In this communication a few substances which inhibit virus multiplication are described. These are the following: 2-(β -aminoethyl) pyridine (AEP), its substituted derivatives; the 2-pyridyl-ethyl-guanidine sulphate (Pyg)⁴, the 2-(5-allyl-3-methoxy-2-hydroxy-benzyl)- β -(α -pyridyl) ethyl amine (H-99)⁵ and 'Ismelin' 2-octahydro-1-azocynil ethyl guanidine sulphate⁶. Guanidine hydrochloride was used as control.

Experiments were carried out on primary monkey kidney cell monolayer cultured in Petri dishes by the plaque method⁹. The virus inhibitory effect of chemicals was tested against 10-100 plaque-forming units of Sabin's type 1 (*LSc 2ab*) virus strain. The plaque assay has already been published in detail⁷. Usually 1 h after virus infection, plates were overlaid with nutrient agar containing 20, 50 or 100 μ g/ml. of substances respectively. Sometimes substances were added to the cell sheets, applying a second overlay 4, 24, 32 h after virus infection respectively. The results are presented in Table 1.

Table 1. EFFECT OF CHEMICALS ON THE PLAQUE-FORMING CAPACITY OF TYPE 1 (*LSc 2ab*) VIRUS STRAIN (SABIN)

Exp. No.	Treatment after virus infection (h)	Concentration of substances (μ g/ml.)	Controlled untreated plates	Denomination of substances				
				'Ismelin' Average	Pyg	H-99	AEP	Guanidine
1	1	100	130	Zero	Zero	Zero	N.D.	N.D.
	1	100	98	Zero	Zero	Zero	70	N.D.
	24	100	39	Zero	Zero	N.D.	N.D.	N.D.
3	1	100	137	Zero	Zero	Zero	N.D.	N.D.
	1	20	50	4	Zero	N.D.	N.D.	N.D.
	24	100	100	(Very small) N.D.	17	Zero	N.D.	Zero
4	1	100	49	Zero	N.D.	N.D.	N.D.	N.D.
	1	50	Zero	Zero	N.D.	40	N.D.	3
	1	20	Zero	Zero	Zero	Zero	N.D.	N.D.

*N.D., not done.

As Table 1 indicates, the Pyg was the most effective but its component the AEP had only a slight inhibitory effect. Substance H-99 showed a strong inhibition in a concentration of 100 μ g/ml., but at 20 μ g/ml. its effect was negligible. The inhibitory effect of 'Ismelin' was expressed at any given concentration (100, 50, 20 μ g/ml.). When 'Ismelin' was applied 4 h after infection it could inhibit the formation of visible plaques completely, and applying 24 h after infection the plaque number was strongly but not completely inhibited. In the case of Pyg, 4 and 24 h after virus infection the inhibitory effect was complete; moreover, 32 h after infection it was still at about 80 per cent. Consequently these support the hypothesis that 'Ismelin' and Pyg can inhibit the late phase of virus synthesis.

It is remarkable that the highly active chemicals are known as anti-hypertensive substances⁸, and one of them, 'Ismelin', is already being used as a therapeutic drug.

SÁNDOR PÁCSA

Institute of Microbiology,
Pécs.

OLGA H. HANKOVSKY
KÁLMÁN HIDEG

Institute of Pharmacology,
Pécs, Hungary.

¹ Eggers, H. J., and Tamm, L., *Virology*, **18**, 426 (1962).

² Levintow, L., Thoren, M. M., Darnell, J. E., and Hooper, J. L., *Virology*, **16**, 220 (1962).

³ Crowther, D., and Melnick, J. L., *Virology*, **15**, 65 (1961).

⁴ Mull, R. T., Egbert, M. E., and Dapero, M. R., *J. Org. Chem.*, **25**, 1953 (1960).

⁵ Hankovszky, O., and Hideg, K. (unpublished results).

⁶ Dulbecco, R., and Vogt, M., *J. Exp. Med.*, **99**, 167 (1954).

⁷ Pácsa, S., *Acta Microbiol. Acad. Sci. Hung.*, **9**, 337 (1963).

⁸ Maxwell, R. A., Plummer, A. J., Schneider, F., Povalski, H., and Daniels, A. J., *J. Pharmacol. Exp. Therap.*, **128**, 22 (1960).

GENETICS

Puffing of Salivary Gland Chromosomes after Treatment with Carbon Dioxide

AFTER larvae of the Oregon R strain of *Drosophila melanogaster* had been placed in an atmosphere of CO₂ for 1 h late in the third instar, the salivary-gland chromosomes were examined and compared with those of a similar group of the same age. The chromosomal regions

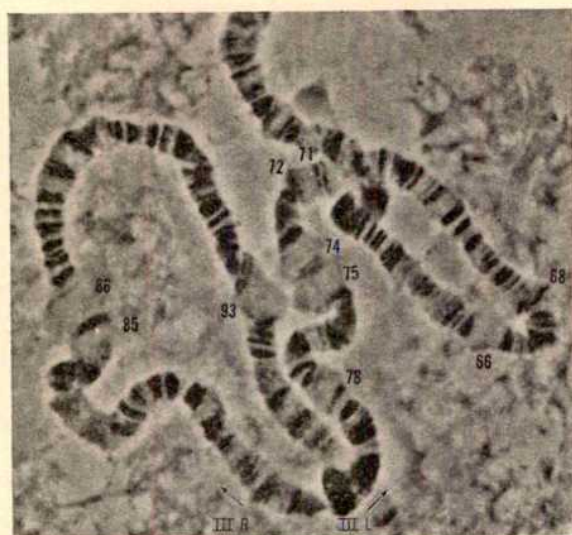


Fig. 1

studied were eight regions known to puff: 85-86, 93, and 95 on the right arm of the third chromosome; 66, 71-72, 74-75, and 78 on the left arm of the same chromosome; and 2B on the X chromosome. The size of the puffs in the 84 glands examined were arbitrarily divided into four classes from smallest to largest and the data tabulated accordingly (Table 1). Although not all puffs were larger in the groups treated, the mean size of puffs III R 85-86, 93, and 95, and III L 66 was larger after exposure to CO_2 . The remainder showed no tendency to enlarge after such treatment. Larvae late in the third instar not used for chromosomal studies survived and developed into adults following the exposure to CO_2 . Many younger larvae died. These observations are reported because of current interest in the puffing pattern as presumed indication of localized production of RNA in response to hormonal stimulation. Recently we have found that ecdysone will produce puffing at this stage of larval development in some regions but not in others. Two not affected by CO_2 (I 2B and III L 74-75) showed an increase in mean size with ecdysone, and one (III L 71-72) failed to respond to either treatment.

Table 1. EFFECT OF CO_2 ON SIZE OF PUFFS EXAMINED IN CHROMOSOMES I AND III

Chromosome	Size of puff	Control Number	Control Percentage	Experimental Number	Experimental Percentage
III R-85, 86*	1	213	91	26	16
	2			14	9
	3	9	4	24	15
	4	15	6	96	60
III R-93*	1	168	72	27	17
	2	51	22	28	17
	3	12	5	55	34
	4	1	1	50	31
III R-95*	1	213	92	62	39
	2			95	59
	3	19	8	3	2
	4				
III L-66*	1	218	94	48	31
	2	11	5	70	44
	3	2	1	27	17
	4			12	8
III L-71, 72	1	88	37	86	54
	2	148	62	74	46
	3	1	1		
	4				
III L-74, 75	1	41	18	35	22
	2	137	59	81	50
	3	34	14	25	16
	4	20	9	19	12
III L-78	1	199	86	156	97
	2	28	12	3	2
	3	6	2	1	1
	4				
I-2B	1	48	21	50	31
	2	161	74	107	67
	3	22	10	1	1
	4	1	1	2	1

* Mean size increased by CO_2 .

This work was supported by grants from the U. S. Public Health Service.

WALTER J. BURDETT
RUTH ANDERSON

Laboratory of Clinical Biology,
Department of Surgery,
University of Utah College of Medicine,
Salt Lake City, Utah.

Evidence for a Second 'Structural' Locus determining Human Phosphoglucumutase

At least seven isoenzyme components¹ of human phosphoglucumutase (PGM) can be distinguished electrophoretically (a-g, Fig. 1). Individual differences occur in the pattern of components present, and three common occurring phenotypes have been recognized (PGM 1, PGM 2-1 and PGM 2). They differ in the distribution of components a, b, c and d. Components a and c are present in PGM 1 and PGM 2-1, while components b and d are present in PGM 2-1 and PGM 2. The genetic evidence indicates that these phenotypic differences are due to pair of autosomal alleles (PGM^1 and PGM^2). Evidently a structural locus is involved, and it seems likely that one of these alleles determines the formation of a polypeptide common to a and c, while the other allele determines the formation of an alternative polypeptide common to b and d. The three phenotypes do not differ from one another in the occurrence of components e, f and g. This suggests that at least one other 'structural' locus is involved in the determination of these latter components.

Evidence for the existence of such a locus has now been obtained from the investigation of a family in which two unusual PGM phenotypes were found to be segregating. These new phenotypes are illustrated diagrammatically in Fig. 1. They will be referred to for the present as PGM (Atkinson) and PGM 2-1 (Atkinson), after the name of the family in which they occurred. The peculiar feature of the 'Atkinson' phenotypes involve the rapidly migrating (efg) set of components. Usually components e and f are very much more intense than component g. In the 'Atkinson' phenotypes components e and f, though present

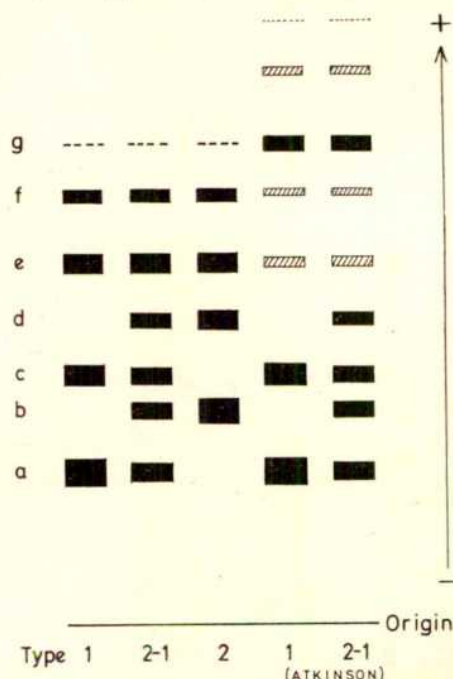


Fig. 1. Diagram of starch gel showing PGM components observed in haemolysates from individuals of the common phenotypes 1, 2-1 and 2, and of the rare phenotypes 1 (Atkinson) and 2-1 (Atkinson). The electrophoretic and staining techniques are described in ref. 1.

relatively weak, but there is an intense component with a mobility similar to *g*, and a further intense component with a mobility greater than *g*. An even faster moving but extremely weak component is also probably characteristic of these phenotypes. This pattern of components was observed in three members of the family (Fig. 2). In one of them (I₁) it was associated with *a* and components typical of PGM 1. In the others (II₁ and I₂) it occurred together with components *a*, *b*, *c* and *d* which are characteristic of PGM 2-1.

Since the unusual pattern of fast moving components as observed in individuals from three successive generations of the same family, it seems likely that it is determined by an uncommon gene for which these individuals are heterozygous. This gene evidently segregates independently of the genes previously designated *PGM*¹ and *PGM*² since it appears to have been transmitted by I₁ to one but not the other of his non-identical twin daughters, both of whom had the *a*, *b*, *c* and *d* components typical of PGM 2-1, and must have received the *PGM*² gene from their mother.

The electrophoretic patterns characteristic of the 'Atkinson' phenotypes suggest that the unusual gene which is present causes a specific structural alteration in components *e*, *f* and probably also in *g*. Individuals I₁, I₂ and II₁ are presumably heterozygous for this gene and its common allele, and the observed patterns can be most simply explained if they are considered as arising from a mixture of components *e*, *f* and *g* determined by the normal allele present, and the altered counterparts of these components determined by the unusual allele. If the structural alteration causes a net decrease in positive charge, such that the altered component *e* now has a mobility similar to that of the usual component *g*, and the alteration to components *f* and *g* results in a similar change in net charge, then the observed pattern can be accounted for.

The pedigree and electrophoretic findings suggest that genes at two distinct and not closely linked loci both of which affect the structure of phosphoglucosmutase components are segregating in this family. We may designate the locus previously recognized as specifically affecting components *a*, *b*, *c* and *d* as *PGM*₁, and the locus which

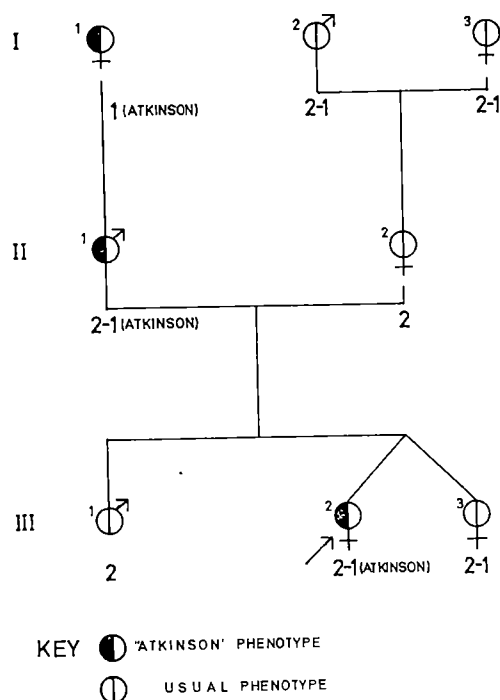


Fig. 2 Pedigree of family (MRC 226) showing segregation of PGM phenotypes

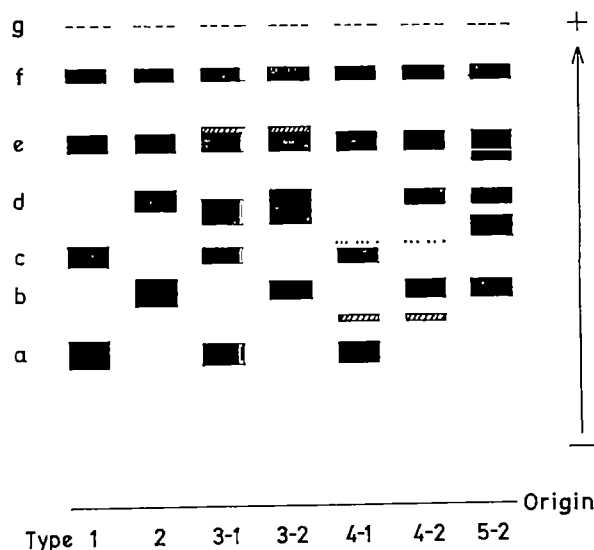


Fig. 3. Diagram of starch gel showing PGM components observed in haemolysates from individuals of the common phenotypes 1 and 2, and the rare phenotypes 3-1, 3-2, 4-1, 4-2 and 5-2

specifically affects components *e*, *f* and *g* as *PGM*₂. The common allele at *PGM*₂ for which most individuals are homozygous may then be designated *PGM*₂¹, and the uncommon allele present in individuals with the 'Atkinson' phenotypes *PGM*₂². In these terms the genotype postulated for the phenotype PGM 1 (Atkinson) is *PGM*₁¹/*PGM*₁¹/*PGM*₂²/*PGM*₂², and the genotype corresponding to PGM 2-1 (Atkinson) is *PGM*₁¹/*PGM*₁¹/*PGM*₂¹/*PGM*₂¹.

In the course of family and population investigations in which PGM in more than 2,000 blood samples from different individuals has been examined, several further uncommon phenotypes have been identified. Five of these are illustrated diagrammatically in Fig. 3. In one family eight individuals with phenotype PGM 3-1 and two individuals with PGM 3-2 were observed. The pedigree suggested that these phenotypes represent heterozygotes for an uncommon allele *PGM*₁³ with *PGM*₁¹ and *PGM*₁², respectively. The allele *PGM*₁³ probably determines the formation of two new components one of which has a mobility slightly slower than *d*, and the other slightly faster than *e*. PGM 4-1 and PGM 4-2 were also encountered among a group of related individuals. There were in all six individuals with phenotype PGM 4-1 and two with PGM 4-2, and the segregation pattern suggested that they were heterozygotes for a further allele *PGM*₁⁴ with the common alleles *PGM*₁¹ and *PGM*₁², respectively. *PGM*₁⁴ probably determines the formation of two components, one of which has a mobility intermediate between that of *a* and *b*, and the other a mobility slightly greater than *c*. Both these new components and particularly the faster one were very weak by comparison with the *a* and *c* or the *b* and *d* components with which they occurred. It seems possible, then, that the rare homozygotes *PGM*₁⁴/*PGM*₁⁴ when identified will exhibit a marked reduction in level of PGM activity. The phenotype PGM 5-2 has so far been identified in only one individual and family data are not available. The PGM of this individual showed two new components, one of which had a mobility intermediate between *c* and *d*, and the other a mobility intermediate between *d* and *e*. Components *b* and *d* were also present, and by analogy it was considered likely that the two new components were determined by an uncommon allele *PGM*₂³ which in this individual occurred in heterozygous combination with *PGM*₂¹. In each of the new phenotypes PGM 3-1, PGM 3-2, PGM 4-1, PGM 4-2 and PGM 5-2, the *e*, *f* and *g* components were apparently not affected and one may suppose that the individuals concerned were all homozygous for *PGM*₂¹. Full details of these family investigations will be published elsewhere.

We are grateful to Dr. J. H. Edwards for sending us samples from the family in Fig. 2.

D. A. HOPKINSON
HARRY HARRIS

Medical Research Council Human Biochemical
Genetics Research Unit,
The Galton Laboratory,
University College, London.

Spencer, N., Hopkinson, D. A., and Harris, H., *Nature*, 204, 742 (1964).

ANTHROPOLOGY

Hair Colour of the Population of Tristan da Cunha

As a result of the volcanic eruption on Tristan da Cunha in 1962 some 268 islanders were evacuated to the United Kingdom. During their stay in this country many of them agreed to co-operate in a number of investigations concerning their physical condition and characteristics organized by the Medical Research Council. Their pigmentation was examined as part of this programme; I was concerned with hair colour.

People the world over show a certain reluctance to part with quantities of their head hair sufficient for detailed laboratory investigation of pigmentation. In this respect the Tristanites were no exception, and only some 131 individuals provided useful quantities of hair.

A number of investigations of hair pigmentation including those of Reed¹, Sunderland² and Barnicot³ have utilized spectrophotometric data including, in particular, percentage reflectance values, as means of denoting brightness and hue. These procedures involving precise quantified information introduced a measure of objectivity into such studies which had been less evident in previous work. Thus Reed¹, for example, denoted redness by the *R* statistic with $R = 100(y_{530} - 0.243y_{400})/Y_{650}$ while Sunderland² used size (*Q*), shape (*P*) and both combined (*L*) statistics in order to characterize the percentage reflectance curves for the hair samples which he studied.

It was hoped that sufficient hair would have been available from the Tristanites to allow the measurement of percentage reflectance values and subsequent statistical analysis of the data, as was possible in the enumerated studies. However, the quantities of hair available disallowed the use of spectrophotometric techniques. The only, fairly satisfactory, alternative means of characterizing this pigmentary variable appeared to be the use of the *Haarfarbentafel nach Fischer-Saller*. This consists of thirty tufts of human hair ranging in colour from very blond (Weiss blond), designated by the letter *A* at one extreme, to the darkest shades of hair (*Braun schwarz*), designated by the letters *U-Y* at the other, together with the intermediate categories *Hell blond* (*B-E*), *Blond* (*F-L*), *Dunkel blond* (*M-O*) and *Braun* (*P-T*) in order of increasing darkness. The Roman numerals I-IV cover a range of red hair shades; V and VI the red-blond range. Hair specimens are matched with these standards and the colours characterized accordingly. In this investigation, the matching was carried out independently by two people, working in good daylight. Their agreed results are summarized in Table 1.

None of the lighter shades of hair colour (*A-L*) is represented in this population, and only three individuals have hair as light as the *Dunkel blond* category. In fact, the great majority of the islanders have dark brown-black hair, the only dramatic exception being the unique vividly red-haired individual. Traces of red hair, usually single red hairs among predominantly brown-black hairs, are occasionally visible in this population.

Table 2 shows the relationship between hair colour and sex.

There do not appear to be any significant sex differences. Therefore, taking both sexes together, Table 3 shows the relationship between hair colour and age.

Table 1. FISCHER-SALLER HAIR COLOUR DESIGNATIONS FOR THE TRISTAN DA CUNHA SAMPLE

Fischer-Saller designation	Number of specimens	Fischer-Saller category
M	1	Dunkel blond
N	1	
O	1	
P	0	
Q	1	Braun
R	0	
S	4	
T	4	
U	8	Braun schwarz
V	9	
W	13	
X	46	
Y	42	Rot
Z	1	
Total	131	100.00%

Table 2. HAIR COLOUR AND SEX

Fischer-Saller category	(No.)	Males (% of total)	Females (No.)	(% of total)
Dunkel blond	2	3.12	1	1.49
Braun	3	4.69	6	8.96
Braun schwarz	59	92.19	59	88.06
Rot	0	0.00	1	1.49
Total	64	100.00	67	100.00

Table 3. HAIR COLOUR AND AGE

Age group (years)	Fischer-Saller categories								Total
	<i>Dunkel blond</i>		<i>Braun</i>		<i>Braun schwarz</i>		<i>Rot</i>		
	No.	% Total	No.	% Total	No.	% Total	No.	% Total	
0-15	2	4.3	7	15.2	38	78.3	1	2.2	48
16-30			1	3.3	29	96.7			30
31-45					26	100.0			26
46-60					15	100.0			15
61-81					8	100.0			8
	2	1.6	8	6.4	114	91.2	1	0.8	125

It is well known from a number of investigations including those of Cowie and Penrose⁴ and Sunderland² that hair colour darkens with age. In the present results it is interesting to note that the fairest people, in the *Dunkel blond* category, are in the youngest age bracket (0-15 years) and that the *Braun* category likewise is found only among individuals aged 30 years and less. Again, red hair fades with increasing age. This was clearly demonstrated by Reed¹ and by Sunderland². Among red-haired individuals, the *R* statistic increases in value with the years; that is, their red hair becomes less so. Here, the only red-haired individual is in the 0-15 age category, and it may well be that at a more advanced age this individual's hair may become much less red or even predominantly brown. All individuals aged 31 years and over have *Braun schwarz* hair.

Quite evidently, this population is predominantly darkly pigmented. Some of the early settlers on Tristan were north-west Europeans, including Glass, Swain and Green, while Rogers and Hagan were American. However, others were from predominantly darker populations, including Repetto and Lavarello from Italy, five women from St. Helena who arrived on the island in 1827 and who are reported to have been of mixed European and 'coloured' parentage and also William Glass's wife from the Cape Coloured population of South Africa. These origins summarized by Harris *et al.*⁵ and the subsequent hybridization on Tristan adequately account for the hair pigmentation characteristics of the islanders.

Any estimate of the quantitative contributions of the Europeans, Americans, Italians, St. Helena people and Cape Coloureds (and possibly other groups also) to this population is fraught with difficulty. So far, even the initial step of comparing the hair colour of the Tristanites with the populations listed is impossible since hair colour data, particularly using the *Haarfarbentafel nach Fischer-Saller*, is not available for those groups.

E. SUNDERLAND

Science Laboratories,
University of Durham.

¹ Reed, T. E., *Ann. Eugen.*, 17, 115 (1952).

² Sunderland, E., *Ann. Hum. Genet.*, 20, 312 (1956).

³ Barnicot, N. A., *Ann. Hum. Genet.*, 21, 31 (1956).

⁴ Cowie, V., and Penrose, L. S., *Ann. Eugen.*, 15, 297 (1951).

⁵ Harris, H., Hopkinson, D. A., Robson, E. B., and Whittaker, M., *Ann. Hum. Genet.*, 26, 359 (1963).

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, October 25

- *LASTICS INSTITUTE, LONDON SECTION REINFORCED PLASTICS SUB-GROUP the Wellcome Building, Euston Road, London, N.W.1, at 3 p.m.—Symposium on "The Changing Face of Reinforced Plastics".
- *LASTICS INSTITUTE, PLASTICS PROPERTIES DISCUSSION CIRCLE (at the Mandeville Hotel, Mandeville Place, London, W.1), at 3 p.m.—Mr. E. B. Sisson: "Flow Behaviour of Molten Polymers".
- *ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), 5 p.m.—Prof. E. G. Bowen: "The Welsh Colony in Patagonia".
- *BRITISH SOCIETY FOR THE HISTORY OF SCIENCE (in the Council Room of the Science Museum, Exhibition Road, London, S.W.7), at 5.30 p.m.—F. W. Gibbs: "Gunpowder Priestley and Dictionary Johnson".
- *INSTITUTION OF CIVIL ENGINEERS (at Great George Street, London, S.W.1), 5.30 p.m.—Sir John Cockcroft, O.M., F.R.S.: "Competitive Nuclear War".
- INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, JOINT I.E.E.—E.R.E. COMPUTER GROUPS (at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 5.30 p.m.—Prof. W. J. Poppelbaum: "Opto-Electronics".
- INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT ASSOCIATION (at Savoy Place, London, W.C.2), at 6.30 p.m.—Mr. J. T. Bull: "C. Traction Power Supplies" (Chairman's Address).

Tuesday, October 26

- SOCIETY OF CHEMICAL INDUSTRY, AGRICULTURE GROUP (at 14 Belgrave Square, London, S.W.1), at 10 a.m.—Meeting on "Land Restoration".
- UNIVERSITY OF LONDON (at Imperial College of Science and Technology, London, S.W.7), at 1.30 p.m.—Mr. E. F. Schumacher: "Levels of Being: What is Man?".
- INSTITUTION OF CIVIL ENGINEERS (at Great George Street, Westminster, London, S.W.1), at 5.30 p.m.—Mr. J. V. Bartlett, Mr. T. M. Noskiewicz and Mr. J. A. Ramsay: "Soft Ground Tunneling for the Toronto Subway".
- INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Emission from Conductors into Dielectrics" opened by Dr. T. J. Lewis.
- UNIVERSITY OF LONDON (at Queen Elizabeth College, Campden Hill Road, London, W.8), at 5.30 p.m.—Prof. H. J. B. Atkins: "Charles Darwin and his Influence" (Inaugural Lecture).
- UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. A. S. V. Burgen: "The Salivary Glands". (Second of fifteen lectures in "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation).
- INSTITUTION OF MECHANICAL ENGINEERS, MANUFACTURE AND MANAGEMENT GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Make Your Workshop Buzz with a Machine Loading Computer Programme".

Wednesday, October 27

- INSTITUTE OF NAVIGATION (at the Royal Geographical Society, 1 Kensington Gore, London, S.W.7), at 4.30 p.m.—Annual General Meeting. Prof. M. S. Blackett, C.H., F.R.S.: "The Continental Drift" (Duke of Edinburgh's Lecture).
- INSTITUTE OF FUEL (at the Royal Institute of British Architects, 66 Portland Place, London, W.1), at 5.30 p.m.—Prof. G. J. Hills: "The Electrochemistry of Deposits of Inorganic Constituents of Fuels at High Temperatures".
- INSTITUTION OF MECHANICAL ENGINEERS (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Mr. H. Norman G. Allen, C.B.E.: "Ex Opera et Industria" (Presidential Address).
- SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP—NUTRITION PANEL (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Prof. J. Yudkin: "Appetite Control".

Thursday, October 28

- UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Dr. C. Vita-Finzi: "The Mediterranean During the Ice Age".
- UNIVERSITY OF LONDON (at Bedford College, Regent's Park, London, N.W.1), at 5.15 p.m.—Prof. Monica M. Cole: "Biogeography in the Service of Man (with particular reference to the Underdeveloped Lands)" (Inaugural Lecture).
- INSTITUTE OF PETROLEUM, EXPLORATION AND PRODUCTION GROUP (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. B. Benthall: "The History of Exploration and Development of Oil in Burma".
- UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. Eleanor Zaimis: "Immunological Sympathectomy". (Third of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation).
- INSTITUTION OF MECHANICAL ENGINEERS, LUBRICATION AND WEAR GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Temperature Limits of Liquid Lubricants".
- TELEVISION SOCIETY (joint meeting with the Institution of Electrical Engineers and the Institution of Electronic and Radio Engineers, at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 6 p.m.—Dr. W. Bruch: "The P.A.L. Colour Television System".

Friday, October 29

- SOCIETY FOR ANALYTICAL CHEMISTRY, MICROCHEMICAL METHODS GROUP (at the Imperial College of Science and Technology, Prince Consort Road, London, S.W.7), at 4.30 p.m.—Meeting on "Microchemistry—Past, Present

and Future". Speakers: Prof. R. Belcher, Mr. C. Whalley and Prof. C. L. Wilson.

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. H. H. Watson: "Some Recent Developments in Ultra-High-Vacuum Technology".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Baroness Jane van Lawick-Goclad: "Infancy, Childhood and Adolescence in the Wild Chimpanzee".

Saturday, October 30

INNER LONDON EDUCATION AUTHORITY (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Mr. A. Robinson: "Natural History of a Landslide".

Monday, November 1

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 9.30 a.m. and 2.15 p.m.—Colloquium on "HS 303 (Early Bird) and the Post Office Earth Station at Goonhilly".

PLASTICS INSTITUTE, PLASTICS PROPERTIES DISCUSSION CIRCLE (at the Mandeville Hotel, Mandeville Place, London, W.1), at 3 p.m.—Mr. D. R. Reid: "Ageing".

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 4 p.m.—Dr. D. A. Price Evans: "Genetics and Drug Idiosyncrasy".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. J. Ralph Audy (U.S.A.): "Red Mites and Typhus. I. Scrub-itch and the Ecologist".

UNIVERSITY OF LONDON (in the Chemistry Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. D. J. Millen: "Molecular Measurements" (Inaugural Lecture).

INSTITUTION OF MECHANICAL ENGINEERS, MEDICAL ENGINEERING WORKING PARTY (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Informal Discussion on "The Doctor's View of Engineering: The Engineer's View of Medicine".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 6 p.m.—"The Island" (film with introduction by Prof. C. A. Fisher).

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dame Kathleen Lonsdale, D.B.E., F.R.S.: "Crystallography as a Research Tool in Chemistry" (Jubilee Memorial Lecture).

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

INSPECTOR GRADE III (Scientific Officer, not over 35, with a recognized first- or second-class honours university degree in which zoology was taken as a major subject) in THE DEPARTMENT OF LANDS, Dublin, for work in connexion with the development of game resources—The Secretary (S.18), Civil Service Commission, 45 Upper O'Connell Street, Dublin, 1, Republic of Ireland (October 26).

SENIOR SCIENTIFIC OFFICER or SCIENTIFIC OFFICER (with a Ph.D. or good honours degree with equivalent research experience in genetics or plant breeding) for breeding work and study of breeding methods with vegetables—The Secretary, National Vegetable Research Station, Wellesbourne, Warwick (October 29).

CHAIR OF ORGANIC CHEMISTRY—The Registrar, University of Kent at Canterbury, Canterbury, Kent (October 30).

LECTURER or ASSISTANT LECTURER in SOCIOLOGY—The Secretary, The University, Aberdeen (October 30).

LECTURERS IN ENGINEERING MATHEMATICS—The Registrar, The University, Newcastle upon Tyne, 2 (November 1).

TUTORIAL STUDENT in the DEPARTMENT OF ELECTRICAL ENGINEERING—The Registrar, King's College, Strand, London, W.C.2 (November 1).

BIOCHEMIST (with an honours degree in biochemistry and some research experience or a Ph.D.) in the PSYCHOSOMATIC RESEARCH UNIT, DEPARTMENT OF PHYSIOLOGICAL MEDICINE (University of Glasgow), to assist with the investigation of aspects of endocrine function in certain bodily illnesses in human subjects—The Director of the Psychosomatic Research Unit, Southern General Hospital, Glasgow, S.W.1 (November 5).

LECTURER in the DEPARTMENT OF ZOOLOGY, University of Tasmania, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 10).

LECTURER (with an honours degree in electrical engineering) in ELECTRICAL ENGINEERING at the University of Tasmania, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 10).

READER in IMMUNOLOGY at the London School of Hygiene and Tropical Medicine—The Academic Registrar, University of London, Senate House, London, W.C.1 (November 10).

LECTURER/ASSISTANT LECTURER (with at least a good honours degree with suitable teaching and research experience, and preferably specialized in some branch of solid state physics) in the DEPARTMENT OF PHYSICS, University of Malaya—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Kuala Lumpur and London, November 15).

LECTURER or ASSISTANT LECTURER in GEOGRAPHY at the University of Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (November 15).

LECTURER (with a veterinary qualification) in VETERINARY MEDICINE—The Secretary, Trinity College, Dublin, 2, Republic of Ireland (November 15).

LECTURER (preferably with research interests in astrophysics, optical astronomy, radio astronomy or plasma physics) in PHYSICS at the University of Tasmania, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 19).

SENIOR LECTURER (preferably with registrable veterinary qualifications) in VETERINARY PHYSIOLOGY in the DEPARTMENT OF VETERINARY PRECLINICAL SCIENCES, University of Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (November 26).

DIRECTOR OF THE MARINE BIOLOGICAL LABORATORY AT MILLPORT, Scottish Marine Biological Association—Dr. C. M. Yonge, C.B.E., F.R.S., 13 Cumin Place, Edinburgh, 9 (November 30).

SENIOR LECTURER or LECTURER (with a degree in mechanical engineering, preferably at honours level, of a recognized Commonwealth University, together with either experience in industry or research and teaching experience at university level) in **MECHANICAL ENGINEERING** at the University of Canterbury, Christchurch, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, November 30).

LECTURER or SENIOR LECTURER in CHEMICAL PATHOLOGY, University of Otago Medical School, Dunedin, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, December 15).

CHAIR OF PHYSIOLOGY—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (January 4).

AGRICULTURAL CHEMIST (national of the United Kingdom or the Republic of Ireland, with a second-class honours degree in agricultural chemistry or chemistry, plus at least two years' postgraduate training or experience in soil science) in Uganda, for research work on soil fertility problems connected with tropical rotations, manures and fertilizers—The Appointments Officer, Ministry of Overseas Development, Room 301, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 213/183/022.

ASSISTANT CONSERVATOR OF FORESTS (national of the United Kingdom or the Republic of Ireland, under 40, with a degree in forestry) in Kenya, for work in connexion with the administration and technical direction of a forest division—The Appointments Officer, Ministry of Overseas Development, Room 301, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 324/95/01.

BIOLOGIST (national of the United Kingdom or the Republic of Ireland, between 24 and 35 years, with an honours degree in zoology and postgraduate experience in animal ecology) in Tanzania, to investigate the ecology of game animals in the woodland areas—The Appointments Officer, Ministry of Overseas Development, Room 301, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 275/173/01.

BIOLOGIST (preferably with a Ph.D. or medically qualified, and interests or experience in the problem of graft versus host reaction, immunological tolerance and mitotic synchronization) for a research post in a unit working on trophoblastic tumours and leukaemia in the Charing Cross Hospital Group—Dr. K. D. Bagshawe, Edgar Laboratory, Fulham Hospital, London, W.6.

CHEMICAL or BIOCHEMICAL TECHNICIAN to assist in research on muscle disease—The Secretary, Institute of Neurology, The National Hospital, Queen Square, London, W.C.1.

DEMONSTRATORS (2) in the DEPARTMENT of PHYSICS for laboratory demonstrating work and assistance with student tutorials—The Registrar, University College of Wales, Aberystwyth.

LECTURER or ASSISTANT LECTURER in THEORETICAL PHYSICS—The Secretary, Royal Holloway College (University of London), Englefield Green, Surrey.

LECTURER (well qualified academically with postgraduate experience in industry and/or teaching) in **PHYSICAL CHEMISTRY** to teach to B.Sc. and Grad.R.I.C. Part II level—The Principal, Medway College of Technology, Horsted, Maidstone Road, Chatham, Kent.

OIL PALM AGRONOMIST (national of the United Kingdom or the Republic of Ireland, with a degree in agriculture and postgraduate training, preferably with previous experience as an agronomist) in Sarawak, to open up and establish a new experimental station, mainly concerned with oil palm and other crops new to Sarawak, for example, cocoa and hemp—The Appointments Officer, Ministry of Overseas Development, Room 301, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 213/155/08.

RESEARCH ASSISTANT or RESEARCH STUDENT to work in the Department of Physics with a small group investigating the application of existing and novel techniques of geophysical prospecting to archaeology—The Registrar, Ref. 126Y/E, Bradford Institute of Technology, Bradford, 7.

RESEARCH FELLOW (with a higher degree and preferably experience in the field of semiconductor physics, technology or chemistry) in the SCHOOL of PHYSICS, to work on new direct-gap semiconductor compounds for use as semiconductor lamps, lasers and Gunn devices—The Secretary and Registrar, Bristol College of Science and Technology, Ashley Down, Bristol, 7, quoting Ref. CST 65/98.

RESEARCH OFFICER (Virologist) (national of the United Kingdom or the Republic of Ireland, with an honours degree in botany or agricultural botany and experience in plant virology) with the East African Common Services Organization, to study virus diseases affecting agricultural crops and to help with the scientific supervision of the East African Plant Quarantine Station—The Appointments Officer, Ministry of Overseas Development, Room 301, Eland House, Stag Place, London, S.W.1, quoting RC 213/214/011.

SENIOR LECTURER in SOCIAL ANTHROPOLOGY; a SENIOR LECTURER or LECTURER in PHILOSOPHY; a LECTURER in PSYCHOLOGY; and a LECTURER in ENTOMOLOGY at the University of Auckland, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1.

SOIL CONSERVATION ENGINEER (national of the United Kingdom or the Republic of Ireland, with a second-class degree in agricultural engineering, or a degree in soil conservation, or a degree in civil engineering and soil conservation, and preferably experience in soil conservation) in Uganda, to carry out soil conservation measures on projects, to check layouts, to train field staff and to collect data and prepare a handbook—The Appointments Officer, Ministry of Overseas Development, Room 301, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 213/183/020.

SOIL SURVEY OFFICER (national of the United Kingdom or the Republic of Ireland, with an honours degree in either natural science, agriculture or geography, with, if possible, postgraduate training or experience in soil science) in Uganda, to carry out soil surveys as a member of a land planting team working on the development of group farms—The Appointments Officer, Ministry of Overseas Development, Room 301, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 213/183/019.

Surface Active Agents: Their Extraction, Characterisation and Determination. By Maurice Bell. Pp. 24. (Leeds: Glovers (Chemicals), Ltd., 1965.)

Loughton College of Further Education. Prospectus for Day and Even Classes, 1965-1966. Pp. 48. (Loughton: Loughton College of Further Education, 1965.)

Department of Education and Science. Chemicals, Additives and Drugs: a Short Working Guide to the Structure of Government Committees and Panels. Pp. 12. (London: Department of Education and Science, 1965.)

Bulletin of the British Museum (Natural History). Geology, Vol. 11, No. Some Lower Cretaceous Terebratuloidae. By Ellis Frederic Owen. 47-72 + 3 plates. (London: British Museum (Natural History), 1965.)

The Role of Chemical Engineering in Process Development. By Dr. G. Reman. (Third Marchon Lecture delivered 6th November 1964 at University of Newcastle upon Tyne.) Pp. 35. (Newcastle upon Tyne: University, 1965.) 6s. 6d. including postage.

City of Liverpool College of Technology. Prospectus of Part-Time Course Session 1965-1966. Pp. 63. (Liverpool: City of Liverpool College of Technology, 1965.)

The Natural Rubber Producers' Research Association. Twenty-seventh Annual Report. Pp. 43. (London: The Natural Rubber Producers' Research Association, 1965.)

Department of Education and Science. National Lending Library Science and Technology. Current Serials Received by the N.L.L., March 1965. Pp. xii + 322. (London: H.M. Stationery Office, 1965.) 27s. 6d. net [1]

Other Countries

Geological Survey of New South Wales. Report No. 17: Quality Classification of Water. Compiled by R. J. Griffin. Pp. 10. Report No. 20: 'Eva' Head Town Water Supply. By R. J. Griffin. Inka Town Water Supply. By R. J. Griffin. Bowling Town Water Supply. By R. J. Griffin. Tumbarum Town Water Supply. By J. Ringis and P. McEwen. Pp. 28. (Sydney: Department of Mines, 1965.)

Commonwealth of Australia: Department of External Affairs. Australian Antarctic Research Expeditions. ANARE Scientific Report Series B (II) Botany. Publication No. 78: Observations on the Fungi Macquarie Island. By J. S. Bunt. Pp. 22. (Melbourne: Antarctic Division, Department of External Affairs, 1965.)

Tables Générales des Mémoires de l'Académie Royale des Sciences, des Lettres et des Beaux-Arts de Belgique, 1772-1897. Pp. 260. (Bruxelles: l'Académie Royale des Sciences, des Lettres et des Beaux-Arts de Belgique, 1964.)

Rubber Research Institute of Malaya. R.R.I.M. Planting Manual No. 1 Clones of *Hevea brasiliensis* of Commercial Interest in Malaya—a Summary Available Information on the Parentage, Distribution, Yields, and Second Characteristics of Clones Planted Commercially or Showing Promise Trials. By C. C. Paardekooper. Pp. 135. (Kuala Lumpur: Rubber Research Institute of Malaya, 1965.) 3 Malayan dollars.

India: Council of Scientific and Industrial Research. Survey and Planning of Scientific Research Unit. Survey Report No. 5: Research Efforts Industrial Establishments in India. By S. H. M. Husain, A. Ghosal and Rahman. Pp. ii + 42. (New Delhi: Council of Scientific and Industrial Research, 1965.)

Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Tribophysics, 1964-65. Pp. 18. (Melbourne: Commonwealth Scientific and Industrial Research Organization, 1965.)

India: Council of Scientific and Industrial Research. Survey and Planning of Scientific Research Unit. Science in India. Compiled by Mohan Bhatia. Pp. ii + 67. (New Delhi: Council of Scientific and Industrial Research, 1965.)

Annual Report of the Geological Survey, Borneo Region, Malaysia, for 1964. By R. A. M. Wilson. Pp. vii + 222 + 60 plates. (Kuching, Sarawak: Geological Survey Department, Borneo Region, Malaysia, 1965.)

Université Léopoldville. "Zooléo" No. 1: Quel est ce Termite Africain Par A. Bouillon et G. Mathot. Pp. 64 + 25 planches. 120 francs; 17s. 2d. "Zooléo" No. 2: Serpents Danger I Par A. Bouillon. Pp. 76. 80 francs 11s. 6d. (Bruxelles: Termite Africains, 33 rue Berckmans, 1965.)

Australia: Commonwealth Scientific and Industrial Research Organization. Mineralogical Investigations—Annual Report 1964-65. Pp. 14. (Melbourne: Commonwealth Scientific and Industrial Research Organization, 1965.)

Colony of Mauritius. Meteorological Observations and Climatological Summaries, April, 1964. Pp. 37. May, 1964. Pp. 37. June, 1964. Pp. 38. (Port Louis: Government Printer, 1965.)

India: Council of Scientific and Industrial Research. Annual Report of the Central Building Research Institute, 1964-65. Pp. ix + 83. (Roorkee (U.P.): Central Building Research Institute, 1965.)

The Resources Agency of California: Department of Fish and Game. Fish Bulletin No. 127: California "Catchable" Trout Fisheries. By Robert L. Butler and David P. Borgeson. Pp. 47. Fish Bulletin No. 128: An Analysis of California's Albacore Fishery. By Harold B. Clemens and William L. Craig. Pp. 301. (Sacramento, California: Office of Procurement, Documents Section, P.O. Box 1612, 1965.)

Séminaire sur la Protection Contre les Dangers du Tritium, Le Vesinet, 16-18 Avril 1964. (Section Française de la Health Physics Society. Service Central de Protection Contre les Rayonnements Ionisants. Société Européenne de Protection Contre les Rayonnements.) Pp. vi + 136. (Le Vesinet (S. et O.): Service Central de Protection Contre les Rayonnements Ionisants, 1965.)

Editorial and Publishing Offices of "NATURE"

MACMILLAN (JOURNALS) LIMITED

4 LITTLE ESSEX STREET, LONDON, W.C.2

Telephone Number: Temple Bar 6633. Telegrams: Phusis London W.C.2

Annual subscription: Inland £14, Overseas £13 15s., payable in advance, postage paid to any part of the world.

Advertisements only should be addressed to

T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2.

Telephone Number: Holborn 4743

Registered as a newspaper at the General Post Office

Copyright © Macmillan (Journals) Limited, October 23, 1965

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Time Units in the Solar System—Summary. By S. Ryzewski. Pp. 23. (Southampton: S. Ryzewski, 15 Landguard Road, 1965.) [149]
British Medical Bulletin, Vol. 21, No. 3 (September, 1965): Symposium on Recent Research in Molecular Biology. Pp. 183-278. (London: The British Council, 1965.) 30s. [149]

NATURE

No. 5009

SATURDAY, OCTOBER 30, 1965

Vol. 208

Published by
MACMILLAN (JOURNALS) LTD.

CONTENTS

	Page
RETHINKING CIVIL SERVICE POLICY	415
DICTIONARY OF ORGANIC COMPOUNDS. By Dr. F. G. Mann, F.R.S.	417
W. M. W. HAFKINE. By Dr. H. I. Jhala	418
PLAIN MAN'S GUIDE TO RADIO PROPAGATION. By Dr. H. Rishbeth	419
THE MARINE ATMOSPHERE. By Prof. J. Darbyshire	419
LIQUID-PHASE OXIDATION OF HYDROCARBONS. By Dr. H. Steiner	420
STATISTICS FOR NON-MATHEMATICIANS. By Prof. D. V. Lindley	420
INTERNATIONAL NUTRITION. By Dr. A. M. Copping	421
CHARACTER RECOGNITION BY HOLOGRAPHY. By Prof. D. Gabor, F.R.S.	422
POLONIUM-210 IN MAN. By Dr. C. R. Hill	423
PATTERN MEASUREMENT. By Dr. A. Ward and G. W. McMaster	428
OBITUARIES:	
TWO PIONEERS OF PEST CONTROLS: DR. W. E. RIPPER AND DR. E. P. JONES. By Dr. G. S. Hartley	430
PROF. K. R. L. HALL. By Dr. F. H. George	432
NEWS AND VIEWS	432
HORTICULTURAL SCIENCE AT THE UNIVERSITY OF READING. By Dr. G. P. Harris	437
THE WORLD HEALTH ORGANIZATION, 1964	437
A COMPARISON OF EUROPEAN HEALTH SERVICES	438
ANTARCTIC AERONOMY AND ICE SHEETS. By Dr. G. de Q. Robin	439
OBSERVATIONS OF POLARIZED OH EMISSION. By Dr. S. Weinreb, Prof. M. L. Meeks, J. C. Carter, Prof. A. H. Barrett and A. E. E. Rogers	440
OH EMISSION IN THE DIRECTION OF RADIO-SOURCE W49. By B. Zuckerman, Prof. A. E. Lilley and H. Penfield	441
COMPARISON OF EXPERIMENTAL DATA OF THE EFFECTIVE ION MASS IN THE UPPER IONOSPHERE WITH A THEORETICAL STRUCTURE. By P. R. Arendt	443
DEPENDENCE OF THE PROPERTIES OF MOVING STRIATIONS IN THE PLASMA OF THE POSITIVE COLUMN ON SOME EXTERNAL AND INTERNAL PARAMETERS. By G. Lakatos and Dr. J. Bitó	445
KINETIC PARAMETERS FOR OXYGEN IN REACTIONS INVOLVING PURIFIED OXIDASES AND TISSUE SLICES. By Dr. M. McCabe and Dr. D. A. Gilbert	450
URINARY METABOLITES IN THE ω -OXIDATION OF 2,4-DIMETHYLHEPTANOIC ACID. By Dr. S. R. Tannenbaum and Prof. S. A. Miller	452
'PINK SPOT' IN THE URINE OF SCHIZOPHRENICS. By R. E. Bourdillon, Prof. C. A. Clarke, A. Pauline Ridges, Prof. P. M. Sheppard, F.R.S., Dr. P. Harper and Mr. Shirley A. Leslie	453
INTERFERON APPEARANCE STIMULATED BY ENDOTOXIN, BACTERIA, OR VILUSES IN MICE PRE-TREATED WITH <i>Escherichia coli</i> ENDOTOXIN OR INFECTED WITH <i>Mycobacterium tuberculosis</i> . By Prof. Julius S. Youngner and Prof. Warren R. Stinebring	456
ALLOGENEIC INHIBITION OF TUMOUR CELLS BY <i>in vitro</i> CONTACT WITH CELLS CONTAINING FOREIGN H-2 ANTIGENS. By K. E. Hellström, I. Hellström and C. Bergheden	458
SPECIFIC UPTAKE IN CORPORA LUTEA OF A NON-STEROID SUBSTANCE WITH ANTI-GESTAGENIC PROPERTIES. By Åke Hanngren, Niels Einer-Jensen and Sven Ullberg	461
AN INSECT 'BIOMETER'. By Philip Kashin and Harold G. Wakeley	462
SYNTHESIS OF RIBOSOMAL RNA IN SYNCHRONIZED HELA CELLS. By Prof. Matthew D. Scharff and Dr. Elliott Robbins	464
HETEROGENEOUS BINDING OF ACRIDINE ORANGE BY POLYRIBONUCLEOTIDES. By Prof. R. F. Beers, jun., and G. Armilei	466
NUCLEOTIDE PEPTIDES IN YEAST. By Dr. D. J. Millin and M. J. Saltmarsh-Andrew	468
A MOLECULAR MODEL FOR A SODIUM PUMP. By Dr. L. J. Opit and Dr. J. S. Charnock	471
<i>Relictogobius kryzhanovskii</i> AND THE PENETRATION OF MEDITERRANEAN GOBIES INTO THE BLACK SEA. By Dr. P. J. Miller	474

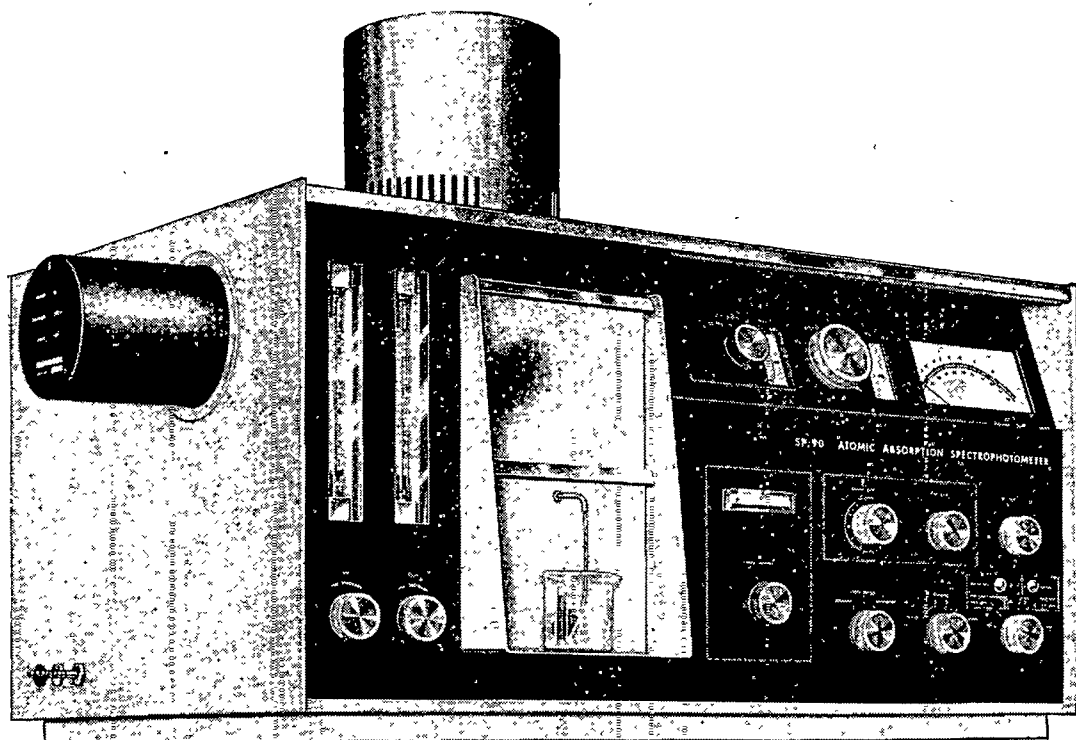
RECENT SCIENTIFIC AND TECHNICAL BOOKS

CCXXV

(Continued overleaf)

NEW UNICAM SP90

ATOMIC ABSORPTION spectrophotometer



ACCURATE • STRAIGHTFORWARD • COMPACT

A precision instrument of modest price bringing analysis by atomic absorption within reach of every laboratory, the Unicam SP90 has the high performance previously associated only with more complex and expensive systems.

for the new user

Self-contained compact instrument
Simple controls clearly marked
Prefocussed lamps in plug-in holders

for the specialist

Quick change burner heads for different fuels
Burner height continuously variable
Adjustable slit for optimum signal to noise ratio

for fast routine analysis

Recorder outlet
Preheating lamp turret accessory
Autosampling accessory for 32 samples

UNICAM

Precision Spectrophotometers

LETTERS TO THE EDITOR

	Page		Page
ASTROPHYSICS			
Atmosphere, and X-ray Images of the Sun.—Dr. C. M. Minnis	476	Response of Melanocytes of Dermis and Epidermis to Lightning Agents.—Prof. Joseph McGuire and Halvor Moller	493
Radiation Balance of Jupiter and Saturn.—Tobias Owen and Thomas E. Walsh	476	Role of the Gut in Synthesizing the Protein Component of Low-density (Beta) Lipoprotein.—P. J. Scott and C. C. Winterbourn	494
GEOPHYSICS			
Daily Variation of Cosmic Rays.—J. B. Mercer and Prof. B. G. Wilson	477	Evidence for Pre-synaptic Inhibition in the Lateral Geniculate Body.—A. Angel, F. Magni and P. Strata	495
Frequency Spectrum of Doppler-shifted Whistler-mode Signals.—R. A. Morris and N. Poletti	479	HAEMATOLOGY	
GEOCHEMISTRY			
Radioactive Elements in the Continental Crust.—Dr. K. S. Heier	479	Chronic Granulocytic Leukaemia: Demonstration of the Philadelphia Chromosome in Cultures of Spleen Cells.—Dr. A. S. D. Spiers and Dr. A. G. Baikie	497
PHYSICS			
Sub-millimetre Dispersion and Rotational Line Strengths of the Hydrogen Halides.—Dr. J. E. Chamberlain and Dr. H. A. Gebbie	480	Stable Messenger RNA in Nucleated Erythrocytes.—Dr. Robert B. Scott and Ronald A. Malt	497
CRYSTALLOGRAPHY			
X-ray Studies on Highly Pure Magnesium Oxide.—Dr. R. A. Brown	481	IMMUNOLOGY	
CHEMISTRY			
Absorption and the Differential Capacitance of the Electrical Double-layer at Platinum/Halide Metal Interfaces.—A. D. Graves and Dr. D. Inman	481	Serological Relationship between Galactans from Normal Bovine Lung and from <i>Mycoplasma mycoides</i> .—Dr. M. Shifrine and R. N. Gourlay	498
Evidence of Thermal and Hot Processes in the 'Hot-zone' produced by Copper-64 Recoil Atoms in Solids.—Kenji Yoshihara and Hiroshi Ebihara	482	RADIOBIOLOGY	
Electron Spin Resonance in X-irradiated Oriented Deoxyribonucleic Acid.—Dr. J. P. Elliott and Dr. S. J. Wyard	483	Influence of Oxygen and Cysteine on the Radical State of X-irradiated Lyophilized Liver.—Mitsuyuki Abe, Hans Mönig and Ruprecht Koch	499
BIOPHYSICS			
Computer Recognition of Cervical Cell Nuclei.—G. W. McMaster	485	'Cell Killing' in Radiobiology.—Dr. D. M. Robinson	500
Distribution of Deoxyribonucleic Acid along Human Chromosomes.—Dr. A. Ward	486	BIOLOGY	
BIOCHEMISTRY			
Secretion of Newly Synthesized Insulin <i>in vitro</i> .—S. L. Howell, D. G. Parry and Dr. K. W. Taylor	487	Ultrastructure of the Antigen-retaining Reticulum of Lymph Node Follicles as shown by High-resolution Autoradiography.—Judith Mitchell and A. Abbot	500
Hormonal Control of Lipid Concentration in Rat Heart and Gastrocnemius.—R. M. Denton and Prof. P. J. Randle	488	Zinc and Other Metallic Ions as Hatching Agents for the Beet Cyst Nematode, <i>Heterodera schachtii</i> Schm.—Dr. A. J. Clarke and Dr. Audrey M. Shepherd	502
Acrylamide-gel Electrophoresis of β -Lactoglobulins stored in Solutions at pH 8.7.—Dr. P. Akroyd	488	Failure of the Zona Reaction in Five Pig Eggs.—Prof. P. J. Dziuk and Z. Diekmann	502
Haemoglobin Polymorphism in the Cod (<i>Gadus morrhua</i>): a Single Peptide Difference.—M. C. Rattazzi and C. Pik	489	Effects of (2-Chloroethyl)trimethylammonium Chloride and Gibberellic Acid on Growth, Fruit Bud Formation and Frost Resistance in One-year-old Pear Trees.—Dr. Irena Modlibowska	503
Factors affecting the Amount and Composition of the Serum Seromucoid Fraction.—Dr. A. J. Anderson	491	Anatomical Changes associated with Juvenile-to-Mature Growth Phase Transition in <i>Hedera</i> .—J. R. Goodin	504
PHYSIOLOGY			
Tubular Site of Urate Secretion in the Rabbit.—Dr. Jesper V. Møller	492	VIROLOGY	
		Milker's Nodule Virus Infections in Dorset and their Similarity to Orf.—Dr. J. Nagington, Dr. G. H. Tee and J. S. Smith	505
		Plant Tumour Virus in Arthropod Host: Microcrystal Formation. — Eishiro Shikata and Dr. Karl Maramorosch	507
		VETERINARY SCIENCE	
		Development of <i>Eimeria tenella</i> in Avian Embryos.—P. L. Long	509
		MISCELLANEOUS	
		Effect of Certain Types of Paper on Sexual Maturation of the Insect <i>Pyrrhocoris apterus</i> .—Prof. C. A. B. Clemetson	510



*microbiological reagents
and media*

THE ONLY COMPLETE LINE OFFERED IN U.K.

<i>Culture Media</i>	<i>Peptones</i>
<i>Microbiological Assay Media</i>	<i>Hydrolysates</i>
<i>Tissue Culture Media</i>	<i>Amino Acids</i>
<i>Serological Reagents</i>	<i>Enzymes</i>
<i>Antisera</i>	<i>Enrichments</i>
<i>Diagnostic Reagents</i>	<i>Dyes</i>
<i>Sensitivity Disks</i>	<i>Indicators</i>
<i>Unidisks</i>	<i>Carbohydrates</i>
	<i>Biochemicals</i>

Requirements of the Bacteriologist, Biochemist, Biologist, Pathologist and Pharmacologist can usually be met promptly from our extensive stocks. We shall always be pleased to obtain other items specially to order.

Please send for the latest technical information.



BAIRD & TATLOCK (LONDON) LIMITED, CHADWELL HEATH, ESSEX, ENGLAND.
Branches in London, Birmingham, Manchester and Glasgow.

Member of the Derbyshire Stone Group.

TAS/DO.11A

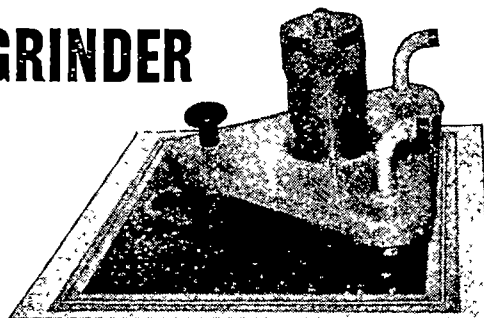
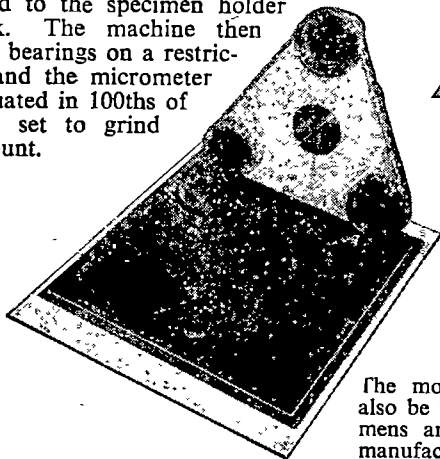
THE CROFT PARALLEL GRINDER

This parallel grinder has been specially designed and constructed for the Palaeontologist

A Brachiopod or similar fossil is mounted in plaster, and attached to the specimen holder with Cerosene wax. The machine then rotates on three ball bearings on a restricted parallel plane, and the micrometer head, which is graduated in 100ths of millimetres, can be set to grind off a requisite amount.



For full information write or telephone



Through various stages of grinding, the fossil is washed, and the structure recorded by means of drawings, photographs or cellulose acetate peels. Thus the complete internal structure of any fossil can be recorded every 1/8th millimetre, or less if required.

The motor driven version of this machine can also be used for controlled grinding of thin specimens and has been successfully applied to the manufacture of thin sections of teeth, bone and gall stones.

CUTROCK ENGINEERING CO., LTD., 35, BALLARDS LANE, LONDON, N.3

TELEPHONE: FINCHLEY 5978/9

RETHINKING CIVIL SERVICE POLICY

TO the scientist and technologist one of the most interesting features of the sixth report from the Estimates Committee for the Session 1964-65*, dealing with recruitment to the Civil Service, is the section which considers the needs of the Civil Service not simply for administrators but for specialists and their role in the service. This is seen not merely through the eyes of the Civil Service itself in the person of Sir Laurence Helsby, head of the Home Civil Service, Sir William Armstrong, Joint Permanent Secretary to the Treasury, and Sir Richard Way, Permanent Secretary to the Ministry of Aviation, for example, but also through those of outside witnesses, including Prof. W. J. M. Mackenzie, Mr. D. N. Chester, Mr. B. J. Holloway and the Institution of Professional Civil Servants. As the Estimates Committee observes, the recruiting methods used depend as much on the assumptions and attitudes about the kind of people required in the Service, the approach to their work which would be required of them, and the division of functions proper within the Service, as on the size and character of the total employment market. It was, therefore, concerned to explore these assumptions and attitudes since they determine the appearance and mode of operation of the Civil Service, both as it is to-day and as it will be during the next generation.

Sir Laurence Helsby believes that there would continue to be a small administrative class, remaining separate from the general service class responsible for the day-to-day run of administration and responsible for somewhat specialized functions which seldom had any precise counterpart in outside organizations—at least below board-level. Staffing at this level called for a particular type of man and for qualities which, he believed, could not be effectively shown in an advertisement, but he did not think that this approach led to a Service which was poised to change. Sir William Armstrong expressed a similar view and, in regard to mobility, thought it was unlikely to reach a point when, at the end of his life, a man could say, "I have spent as much time out of the service as in it". The distinction between the man whose basic career is the Civil Service but who goes out from time to time and the man who is really temporary would remain. He did not deny that there was any need for a reappraisal of the pattern of the Civil Service, and Sir Laurence Helsby suggested that it would be well to wait at least a year before making a broader enquiry into the organization, recruiting and structure of the Civil Service. However, he did agree with the suggestion that such a review would be desirable in spite of the probability that developments in the structure of Government would be rapid for ten years to come.

Sir William Armstrong directed attention to the limitations of the use of techniques such as cost-benefit analysis which were placed by the number of people familiar with these techniques both in the departments concerned and elsewhere in the Government service, local authorities and the nationalized industries. This is accordingly a question of the special trained manpower, and Sir William maintained that the really pressing need was for more specialists, particularly as economic advisers—though in the long term it was

probable that the Service would develop its own brand of expert in what might be called 'Government economics'. Here the Centre for Administrative Studies might help, but other witnesses saw a danger of rigidity in such a development.

Sir Richard Way and Sir Eric Roll, the Permanent Under-Secretary of State at the Department of Economic Affairs, made some interesting observations on the part played by the scientist and other specialists and their relation to administrators. Sir Richard was emphatic that a scientist equipped to do administrative work would not be deterred from entering the scientific grade by the prospects of promotion. However, he thought that for the position of scientific officer the Service required a man competent for a particular piece of research, without special personal qualities, whereas the administrative class Civil Servant in the higher grades should have had wide experience. Scientists destined for more senior posts were helped to widen their experience while remaining specialists. When Sir Richard had encouraged scientists to enter the administrative class he had found that they had usually wished to remain where they were; in his view it was a wholly false picture to imagine that every scientist wanted to become an administrator. The distinction between administrators and scientists was not a hindrance to recruiting. On the contrary, many young scientists who entered the Service would be deterred by the abolition of this distinction. Nor did he think that scientists were deterred by the thought that administrators were amateurs; rather the scientist would regard the administrator as being highly professional in the field he had chosen.

Sir Eric Roll suggested that the relation of economists to administrators was rather different simply because the tasks of Government had a much greater affinity with the field of interest of economists and other social scientists. Nevertheless, though the division between economists and administrators was not so easily defined as that between nuclear physicists and biochemists and administrators, the tasks of the administrator differed in kind from those of the economist. Pointing out that 43 per cent of the administrators in the Department of Economic Affairs had degrees or other qualifications in economics, Sir Eric added that no difficulties from rigid procedures had arisen in obtaining specialized staff. Lack of flexibility had had no real effect on the Department's recruitment possibilities.

These views, however, were not unchallenged in the evidence. The memorandum from the Institution of Professional Civil Servants expressed the unanimous view that if the professional and similar specialists classes were to be given managerial responsibility and if this were to become known, it would have an important and beneficial effect on recruitment. The professional classes, at present, have a strong feeling that they are not given full and proper scope to discharge the responsibilities that they are equipped to carry. Even propaganda in the schools would be more effective if it could be shown that specialists in general were not at a disadvantage in terms of responsibility and advancement to the highest posts. The evidence of the Ministry of Aviation in regard to the transfer of scientists to the administrative class was questioned as inaccurate. It was pointed out that experience in industry shows that transfers to broaden

* Sixth Report from the Estimates Committee together with the Minutes of the Evidence taken before Sub-Committee E and Appendices, Session 1964-65—Recruitment to the Civil Service. Pp. xxxviii+269. (London. H.M.S.O., 1965) 26s. net. (See also *Nature*, 208, 311; 1965)

experience are neither unpopular among young scientists nor a deterrent to recruitment. Mr. D. N. Chester, however, suggests that the evidence indicates that science graduates with first-class degrees prefer to continue with scientific work rather than take administrative or managerial posts. Certainly, he doubts whether this will change when the output of scientists is greatly increased. Nor has it yet been proved that those with a scientific training have a particular aptitude for the higher levels of administration.

The Institution of Professional Civil Servants submits that for the specialist Civil Service the Government's employment policy should rest on three main principles. It should exploit to the full the training and talents of its scientists, technologists and technicians and ensure that there is full employment in terms of the quality of work they are given to do. It should make clear that specialists are full and equal partners with other staff, and that they have an equal opportunity to promotion to the highest posts. It should offer pay and careers which demonstrate that scientists, technologists and technicians are no longer regarded as inferior or second-rate.

In all this evidence there is little that is not to be found in the Barlow Report on scientific staff which was released almost twenty years ago. The Estimates Committee comments that the picture which emerges from this examination is one of considerable development in the patterns and traditions of the Civil Service and of readiness for further development—basically, however, development within an established framework of assumptions. There does not appear to have been any fundamental questioning of the basic structure, and the Estimates Committee finds it hard to accept that the task of Government justifies the unique significance attaching to the administrative class and that only a select few are fitted to undertake this work. It also questions the assumption that all a specialist wants to do on leaving university is to work within his specialized subject. In this connexion, the Committee directs attention to the evidence of Mr. P. F. Nind, of the Shell International Petroleum Co., Ltd., that relatively few among specialist technical staff desire promotion in their own narrow function, whereas many wish to broaden their experience and run small companies elsewhere. This, as Mr. Nind remarked, is entering general management.

While the Estimates Committee welcomes the changes that have already been made or are planned to increase the flexibility of the Civil Service and its ability to undertake new tasks, it considers, nevertheless, that this aspect needs further and much more systematic study. Accordingly, this is one of the considerations which could well be subjected to further enquiry—in particular, the actual tasks undertaken and those that should be undertaken by the administrative class in the near future. The Committee discusses, moreover, recruitment to the Scientific Civil Service in a further section of its report, and here there are some observations that throw further light on the evidence given by Mr. Nind.

The experience of the Civil Service has been that young scientists prefer to become members of a particular team or establishment, rather than to join the Scientific Civil Service as such. This was confirmed by Mr. B. J. Holloway, secretary of the Appointments Board of the University of Manchester, who in expressing the view that the Scientific Civil Service was the section of the Civil Service which had really faced the problems of competitive recruitment—a view which the Estimates Committee endorsed—remarked that to the fresh graduate

it was a more stimulating thought to work, say, at Farborough than to apply to the Civil Service Commission for establishment as a scientific officer. Nevertheless, although Sir Richard Way fully recognized that establishments like Farnborough and Malvern are far more vital and attractive than the Ministry of Aviation as such, his other observations indicate how far some of the senior officers in the Civil Service still are from understanding the scientific life and even the implications of Sir Alexander Barlow's Report.

Rejecting the suggestion that older scientists who have passed their creative years might be transferred to administration, Sir Richard characterized as absurd "the idea that a worn-out scientist can, as an afterthought, come in as an administrator". This is a complete misunderstanding, if not misrepresentation, of how to use to the maximum public advantage the skill and experience of trained manpower. The large industrial firms have long recognized that in research, as in art, creative power are at their peak in youth but tend to decline in middle life; experience, on the other hand, grows and may be used to advantage in many ways. It is here that the Civil Service appears to have fallen behind industrial and commercial practice, for example, and this may well contribute to the unsatisfactory overall situation for scientists, though the general shortage of good graduates and competition are clearly contributory factors. It may be noted that Sir Laurence Helsby in his evidence admitted that mobility in the Service was not so high as he would like it to be, and was clearly not opposed to the use of scientists in administration. The Estimates Committee concludes this section of its report with the remark that, quite apart from the importance of providing good promotion prospects for the scientist, the man with the scientific or mathematical background was more than ever needed in management to-day, in view of the importance of the ability to quantify problems: this is at least as true of public administration.

In discussing recruitment for modern needs, in the final section of its report, the Estimates Committee draws together the evidence which impressed it most with the need for a thorough examination of the problems of recruitment to the Civil Service. All the evidence shows that the Civil Service and the Civil Service Commission are aware that not all the requirements of the Service are being met and that efforts are being made to meet them. Nevertheless, the Estimates Committee believes that the measures taken have not yet been extended to a re-examination of the structure of the Service and other issues underlying recruitment. In three areas in particular detailed examination might be expected to lead to proposals for change.

The first is in the extent of mobility, both within the Service and between the Service and other occupations, including consideration of other means of acquiring wider experience and keeping the Service in touch with the developing community. The second is the need for professional training and the means available for acquiring a knowledge of specialist techniques at the beginning and in the course of a career. The third is the urgent need to examine the supply of qualified manpower on which the Civil Service is to draw. It is a fallacy, as the Estimates Committee remarks, to suppose that, in the long term, the supply of qualified people in any one field is fixed, because the recruiting policies of the Government inevitably affect the total supply. However, in these last two areas, it can be added that enquiry could lead to suggestions for the wise and efficient use of trained manpower,

such as are implicit, though not explicit, in the memorandum from the Institution of Professional Civil Servants.

Research and public discussion on these important developments could contribute to what Prof. W. J. M. Mackenzie termed "the shaping of a public doctrine about the place of the Civil Service in modern Britain". This, he thought, would be a most valuable result of the appointment of a Royal Commission such as he had suggested at the end of his evidence. This Commission should be a relatively small one, of independent standing, empowered to recruit its own research staff, and not placed, as was the Priestley Commission, in a situation which demanded an urgent and specific report. Mr. D. N. Chester also supported the appointment of a Royal Commission, provided a suitable chairman and membership could be found, and the Commission was adequately staffed and empowered to arrange for research. He suggested that it should be established in 1967, thus giving adequate time for preparatory work and discussion.

This date accords with the views expressed in evidence by Sir William Armstrong and Sir Laurence Helsby; the latter, however, suggested that there was great advantage in having a review of the Service undertaken by a group of people, some of whom, in any event, had had direct experience of its working. Moreover, such an internal committee of officials would have access to information which could not easily be made available even to a Royal Commission. A Royal Commission, however, has advantages in authority and in the publicity in which its enquiries are usually conducted. The Estimates Committee was impressed by the desirability of a preliminary internal enquiry and also emphasizes the value of well-directed and independent research. Its recommendations appear to be in accordance with the balance of the evidence. They are: (1) appointment of a committee of officials, aided by members from outside the Civil Service on the lines of the Plowden Committee, to initiate research on, to examine and report on the structure, recruitment and management of the Civil Service; (2) that the Government should review the position immediately on receipt of the report, and report to Parliament the action it proposes to take, appointing forthwith a Royal Commission if further enquiry by such a Commission is then found to be necessary. Meanwhile the Estimates Committee's report should stimulate preliminary discussion and the Parliamentary and Scientific Committee should be able to ensure that the whole question is not side-tracked, whether on pretext of Parliamentary time or any other excuse.

DICTIONARY OF ORGANIC COMPOUNDS

Dictionary of Organic Compounds

The Constitution and Physical, Chemical and other Properties of the Principal Carbon Compounds and Their Derivatives, together with Relevant Literature References. Edited by Dr. J. R. A. Pollock and Dr. R. Stevens. Fourth, completely revised, enlarged and re-set edition. Vol. 1: A-Chlor. Pp. xxx+1-588. Vol. 2: Chlor-Dim. Pp. 589-1230. Vol. 3: Din-Iza. Pp. 1231-1976. Vol. 4: J-Phloi. Pp. 1977-2736. Vol. 5: Phlor-Zym. Pp. 2737-3282. First Supplement 1965. Pp. 207. (London: Eyre and Spottiswoode (Publishers), Ltd; and E. and F. N. Spon, Ltd., 1965.) £100 the set.

THE *Dictionary of Organic Compounds* requires no introduction to organic chemists, to whom it is always known as "Heilbron's Dictionary", for the late Sir Ian

Heilbron was the moving spirit at its initiation and guided it vigorously through its subsequent development until well into the preparation of the present edition.

Workers in neighbouring sciences who may occasionally require information about specific organic compounds may, however, welcome a brief indication of its nature and contents. The first edition appeared during 1934-1937 in three volumes, to be followed during 1943-1953 by the second and third editions, the latter appearing in four volumes. The editors of the first edition accepted the formidable task of selecting from the vast number of recorded organic compounds those to which they considered organic chemists would most frequently want to refer. These were listed in alphabetical order, with their molecular formulae, structural formulae when desirable, and physical properties: this information was followed by a list of the main derivatives of the compound, and a final list of original references. This pattern of the *Dictionary* remains unchanged. The volumes, therefore, provide a rapid and reasonably up-to-date reference to a very large number of compounds. Information about other organic compounds may be sought either in *Beilstein's Handbuch*, which by virtue of its size labours along several years out of date but provides exhaustive treatment, or in the many indexes, annual, five-yearly and decennial, of *Chemical Abstracts*.

The new fourth edition has been completely revised, enlarged to five volumes and very handsomely re-set and bound: the type now adopted gives a very clear and attractive appearance to the pages. The edition includes material obtained by searching *Chemical Abstracts* up to 1962, and by a review of the major chemical journals up to the end of 1963. It is stated to "include all major natural products, pharmaceuticals, pesticides and similar groups of applied chemicals"; natural products, therefore, now appear prominently. It is accompanied by a supplement as a separate volume, which contains new material published in and before 1964. This supplement represents a new feature, for it is the first of a series of annual supplements, of which the fifth will be a cumulative edition containing the material of that year and of the preceding four years: the annual supplements will then continue until the tenth number, which will again be a cumulative volume containing the material of the immediate past five years. (Librarians should note that there is a subscription scheme available for the purchase of these supplements under which the cumulative volumes can be purchased at half-price.)

This fourth edition is about 40 per cent larger than the third, and the considerable increase in the number of entries has largely coincided with various changes of nomenclature: since the names of the compounds form their own index, the editors had to ensure that any compound could be readily found. Many compounds have trivial or common names which are very widely used: in such cases the editors have used their discretion in deciding whether the compound shall be entered in detail under its trivial or its systematic name; since in almost all such cases cross-references (each in its appropriate alphabetical position) are given, no difficulty should arise. Drugs and pesticides in this respect fall into a special category. Since almost all modern drugs, including antibiotics, are known to manufacturers, to the medical profession and to pharmacists and chemists by trade-names (and systematic names would be exceedingly cumbersome), the *Dictionary* uses the names approved by the British Pharmaceutical Commission of the General Medical Council. Similarly, pesticides (although often of more simple structure) are entered under the common names recommended in the *British Standards List B.S. 1931* (1961). Many proteins fortunately now have structure 'names' based on generally accepted conventional abbreviations.

The main difficulty arises from the fact that many systematic names, and also the numbering of (in par-

ticular) many heterocyclic systems, have been changed from time to time by the nomenclature committees of the International Union of Pure and Applied Chemistry; the last set of rules (now fairly widely accepted) were published in 1957. The introduction to Volume 1 summarizes "a few of the outstanding basic rules of nomenclature from the I.U.P.A.C. 1957 rules" in $8\frac{1}{2}$ pages. These rules are, however, often complex and detailed, and cannot be readily understood and applied without ample structural formulae and examples for illustration. The space given in the introduction is insufficient for this purpose, but a reasonably full account would clearly occupy far too much space. The reader could well be referred to the *Handbook for Chemical Authors* (Chemical Society Special Publication No. 14, 1960) by Dr. R. S. Cahn and Dr. L. C. Cross, where the 1957 rules are explained and discussed in detail. It also contains the highly specialized rules agreed in 1952 between the committees of the Chemical Society and the American Chemical Society for organophosphorus compounds, an increasingly common group of compounds to which the *Dictionary* gives little attention.

Two points regarding the presentation may be made. It has already been stated that under the name of the 'parent' compound is a list of its chief derivatives and also a list of references. Now it often happens that a reader is consulting the *Dictionary* to obtain information on one specific derivative: the *Dictionary* usually provides for solid derivatives the melting-point and the solvent for crystallization, and the reader, requiring more information, is given no guidance as to which of the references record this compound, and he has therefore to work steadily and laboriously through them. For example, under "Phenylacetic acid" (p. 2666) there are nearly thirty derivatives listed, followed by eleven references in various journals: the reader who ultimately finds the required information in the eleventh reference will be weary and rather exasperated. This disadvantage could have been overcome, for example, by placing small superscript letters alphabetically above each reference: the names of the derivatives would then each carry the superscript letter of the reference in which they are to be found. This could easily have been done in the early days of the *Dictionary*, but would now involve much tedious work.

The second point, of lesser importance, is that the elements in the molecular formulae "are given in the order C,H,O,N,Cl,Br,F,S,P with any others following in alphabetical order" (Volume 1, p. xi). This is quite clear, but slightly old-fashioned. The modern formulae indexes of *Beilstein*, *Chemical Abstracts*, etc., all adopt the more simple system of giving the elements in the order C,H, with all the remainder following in alphabetical order.

One minor point. One solvent used for crystallization is frequently named 'petroleum ether' or 'pet. ether'. This term is a relic of the old days when a set of solvent bottles bearing permanent titles normally included 'sulphuric ether' and 'petroleum ether'. The first title has long been obsolete and the second should rapidly have followed it: certainly no organic chemist should call a hydrocarbon an ether. The *Dictionary* also uses the rather old-fashioned alternative 'ligroin': the use of 'petroleum', possibly abbreviated to 'petrol', throughout would surely have been preferable?

These points, however, do not seriously detract from the very great value of the immense amount of classified information which this fourth edition contains—information which with the Supplement 1965 is based on more than 40,000 entries. The heavy labours of the editorial staff, moreover, receive deservedly excellent presentation at the hands of the printers. Had Sir Ian Heilbron survived, this edition would certainly have been a source of great pride to him.

F. G. MANN

W. M. W. HAFFKINE

The Brilliant and Tragic Life of W. M. W. Haffkine
Bacteriologist. By Selman A. Waksman. Pp. 87 + 3 plates
(New Brunswick, N.J.: Rutgers University Press, 1964.
3.75 dollars.

THE author of this book visited the Haffkine Institut in the year 1962. While the name of Haffkine resound in the heart of every bacteriologist, his life is an interesting adventure in the early days of microbiology. Now, thirty-five years after his death, Haffkine has attracted the attention of many biographers. Two biographies appeared almost simultaneously—one from the U.S.S.R. and the other from the United States. On the hundredth anniversary of the birth of Haffkine, a special postage stamp was issued by the Government of India. This justifies the description aptly chosen by Waksman to end the biography, that Haffkine was "an immortal man of Universe as a whole".

The author has taken elaborate trouble to trace the early life of Haffkine, which was beset with financial difficulties and political as well as religious upheaval against the Jews in Odessa. Perhaps it was then that the spirit of adventure in Haffkine was kindled, as is apparent from the biography.

The leaving of the University of Odessa and his migration to Switzerland and Paris are described as a course of events, which seemed unpurposeful but compelling. It must have been his scientific curiosity, reading and zeal which brought him to the feet of that acknowledged master, Louis Pasteur.

The author has cited with excerpts from "both sides of the story" many comments on the scientific achievements of Haffkine written by his contemporaries. Reading in between the lines, the opposition was mainly motivated for reasons besides those which are normally associated with scientific controversy. His critics never missed the opportunity to attack him right up until his death. Despite all the set-backs that Haffkine encountered in the Malkowal disaster, so well described by Waksman, reason and justice prevailed.

Such a torment to a scientifically inclined missionary in a foreign land did change Haffkine, who returned to India victorious but without his fiery zeal. Not much is known of his later contributions. It was true in Haffkine's life that, if anything is worse than war lost, it is war gained. His later life is described by Waksman as religious. Perhaps Waksman puts his finger on it when he states that he felt his living with Indian people made him change his philosophy of life.

With all this, the author should have paid a tribute to British justice and British recognition of merit despite intrigues that tortured Haffkine. A tribute is also due to Haffkine himself for the metal he was made of to withstand the opposition, insults, injustice and unfair criticism. These need to be inculcated in all scientists and public servants.

The author has taken excerpts from the *British Medical Journal*, the *Lancet* and other medical journals and has made a very correct appraisal of Haffkine's eminence in science. His eminence, however, does not count because of his persecution at any stage of life, though these made Haffkine what he was. He did not seek recognition and he was humane. Persons with such towering personalities, humility and scientific genius need to be highlighted to posterity: Waksman has done a great service to posterity by preparing this biography. No one will be left in doubt after reading this authentic independent adjudgment of Haffkine that he was one of the pioneers in the field of prophylactic bacteriology, to whom the world owes so much.

The book is briefly, but well, illustrated. There appear to be no printing errors. A bibliography of Haffkine and the references cited by Waksman will help medical his-

arians of the future. Science believes either in one world or that it has a world of its own with no barriers. Biography teaches this lesson and here the message is conveyed through Haffkine's life by Waksman's efforts. Haffkine has become immortal in science and his life will continue to inspire microbiologists for a long time.

H. I. JHALA

PLAIN MAN'S GUIDE TO RADIO PROPAGATION

Ionospheric Radio Propagation

By K. Davies. (National Bureau of Standards Monograph 30.) Pp. xiv+470. (Washington: Superintendent of Documents, U.S. Government Printing Office, 1965.) 2.75 dollars.

IONOSPHERIC Radio Propagation is a successor to the National Bureau of Standards Circular 462, published in 1948 under the same title. It is interesting to compare the contents of the two. The new book has more than twice as many pages, but the difference in volume is not great because of the large page size and closely packed layout of the older book.

Not all the new book was written by Dr. Davies, and some sections have been contributed by his colleagues. However, the book is much more of an entity than Circular 462, in which each chapter has separate authorship. The main difference between the books lies in the subjects treated. Some topics such as magneto-ionic theory and an outline of ionospheric physics find an important place in both. The 1948 book fits its title very closely and could be regarded as a handbook for the communication engineer, with substantial chapters devoted to the practical aspects of radio communication. In the 1965 book these topics represent a much smaller proportion of the total. This is largely because they have since been well treated in other publications, to which Dr. Davies refers; but a contributory reason, one suspects, might be that some topics have not progressed sufficiently to warrant extensive coverage. Their place has been taken in the new book by important subjects developed since 1948. These include very high frequency scatter propagation, and low frequency and very low frequency propagation. Another new chapter deals with ionospheric disturbances and their effect on propagation predictions.

With the change of subject-matter has come a change in emphasis. The new book seems to be written not so much for the communications engineer as for a more academically minded readership, the scientific research worker and perhaps the graduate student. So, being personally unfamiliar with some of the topics included, I tried using the book as a tutorial text. In some places the book seemed to fulfil this purpose; in others the exposition did not seem too clear. The book will perhaps be criticized for being too superficial in some places and too detailed in others. In my opinion, books of this type can scarcely hope to escape such criticism, and this one does not seem especially bad in this respect.

The book is provided with 'subject', 'author' and 'place' indexes. The innocent reader might enjoy the index of places; there are naturally experimental stations and scientific institutions (Comfort Cove rubs shoulders with Central Radio Propagation Laboratory), but the entries also include radio stations (GBR, Decca, Loran C); a conference venue (Lindau); and a publishing house (London). The general and the particular are delightfully mixed, as in the consecutive entries, South America, South Ice, South Pole and, again, Scandinavia, Seattle, 75° geographic meridian.

Seriously, this is a useful book. Its clear type, stiff binding and handy page size are a great improvement on the old Circular 462, and at its very modest price it offers excellent value for money.

H. RISHBETH

THE MARINE ATMOSPHERE

Physics of the Marine Atmosphere

By H. U. Roll. (International Geophysics Series, Vol. 7.) Pp. viii+426. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1965.) 107s. 6d.

PHYSICS of the Marine Atmosphere is written by Dr. H. U. Roll, the distinguished German meteorologist and oceanographer. As he claims in the preface, this book sets out to describe marine meteorology as an exact physical science, and so great emphasis is placed on the physical approach to problems of the interchange of properties between the ocean and the atmosphere. The book is probably the first to be published that deals with the subject as a pure science and not as an applied science. There are six chapters which are further sub-divided. The first chapter is an introduction while the second chapter deals with meteorological observations and measurements at sea and contains a review of instruments and methods. The third chapter deals with the composition and properties of the marine atmosphere and includes a discussion of the properties of atmospheric nuclei above the oceans, the chemistry of the marine atmosphere with a sub-section on the still rather contentious subject of carbon dioxide interchange, and also an account of atmospheric radioactivity and electricity.

The backbone of the book is, however, contained in Chapters 4 and 5. Chapter 4 deals with aerodynamic considerations of the interchange of energy between the atmosphere and the sea and contains sections on the geometry of the sea surface and also on the wind profile over the ocean. Chapter 5 gives the thermodynamic approach. Some concluding remarks are given in Chapter 6.

As an oceanographer, I was most interested in Chapter 4. This starts with an account of waves and ripples and gives a brief résumé of recent theoretical work on the subject. I found this part slightly disappointing, however, as the author confines himself almost entirely to theoretical work and to discussing wavelets and ripples, and gives very little attention to the large waves ordinarily found on the sea surface and which must affect the marine atmosphere. It is true that theory cannot as yet fully explain the formation or predict the magnitude of ordinary sea waves and recourse has had to be had to various empirical methods which have not hitherto been in agreement with each other. The problem should not be ignored, however, simply because it has not been solved, especially as recently a much greater degree of agreement has been obtained by various workers about the empirical approach. It is now generally agreed, for example, that the significant height of fully developed waves is given by:

$$H \text{ (in ft.)} = 0.018W^2 \text{ (in knots)}$$

where W is the surface wind and full development is reached for fetches less than 400 miles for all wind speeds. This relation should be of interest to both the practical and theoretical marine meteorologist.

The section on the determination of wind profiles over the sea surface is very interesting and it leads on to the evaluation of the wind stress constant over the water surface. There are three traditional methods for determining this parameter. The first obtains it from the wind profile over the first few metres over the surface. The second uses the departure of the mean wind speed from sea-level to the level the geostrophic wind speed is reached from the geostrophic wind speed. The third method is purely oceanographic and involves the measurement of the tilt of the water surface in equilibrium with the wind force. These three methods, however, rarely agree although, to judge from the table shown, there is much better agreement with strong winds. The author criticizes the tilt method generally and in particular a determination in which I had a part, because the effect of the set-up due to wave action is ignored. He suggests that wave set-up

might at least partly explain the apparent increase of stress coefficient with fetch which was found. The effect of wave set-up was certainly not appreciated in 1955 when these results were published, but a recent paper by Longuet-Higgins and Stewart has shown that waves only increase the set-up when their height is limited by breaking, as otherwise the effect is reversed and they reduce the set-up. The recording apparatus in this case was laid in about 2-ft. water depth so the mean wave height should be about 3 ft. for continual breaking, but this value was only very rarely attained during the course of the measurements. The effect of wave set-up is very important nevertheless, and the author rightly points out that tilt experiments should be so designed that this effect is a minimum.

These are all minor points of criticism, and the book is an excellent exposition of the subject and will, I hope, inspire a more widespread study of it. It should certainly be in the library of every oceanographer and every meteorologist.

J. DARBYSHIRE

LIQUID-PHASE OXIDATION OF HYDROCARBONS

The Oxidation of Hydrocarbons in the Liquid Phase

Edited by N. M. Emanuel. Translated from the Russian by K. R. Dobson and B. J. Hazzard. Pp. xv+407. (Oxford, London and New York: Pergamon Press, 1965.) 140s.

THE oxidation of hydrocarbons has been the subject of intense investigation for a long time, and particularly so during the past 20–30 years. Oxidation in general is of paramount importance, not only in chemistry, but also in biology and in other related sciences. The oxidation of hydrocarbons is mainly of interest in chemistry, both because of its academic and industrial aspects. In the organic chemical industry oxidation reactions of hydrocarbons play a very important part and great developments are still occurring in this field.

This book deals with certain aspects of the oxidation of hydrocarbons in the liquid phase with gaseous oxygen as oxidant. Even early workers found that the chemistry of these reactions is complex, and it is not surprising, therefore, that progress has been slow. It was established in the early 'thirties that these oxidations proceed by a chain mechanism and show the complex characteristics of reactions of this type. A first real understanding of the mechanism, although confined to the initial stage of the reaction only, was obtained as a result of the work of Criegee and Hock in Germany and of Farmer and Bolland in Britain. This work revealed the central position of hydroperoxides and hydroperoxy radicals in the reaction scheme and led to a mechanism for the first stages of the oxidation of olefines, paraffins, cycloolefines and alkylaromatics which has been generally accepted.

However, if oxidation is carried on to conversions greater than a few per cent, complications arise which greatly influence the course of the reaction. This characteristic feature of oxidation reactions is very important because the industrial processes operate in this range of conversion which extends from about 2 to 30 per cent.

During the past ten years or so the Russian workers, whose collective effort is represented in the book under review, have been concerned with this problem and have made important and far-reaching advances in the understanding of the reaction mechanism at the later stages of oxidation. One must be grateful to N. M. Emanuel for having assembled, as editor, his own work and that of Knorre, Maizus, Denisov, Bashkurov, to name only a few, in this book, which has now been translated from the Russian by K. R. Dobson and B. J. Hazzard, and edited by J. D. Hopton.

Apart from this group of papers dealing mainly with the mechanism of the oxidation reaction, the book also

contains a collection of papers which are concerned with the technical and more phenomenological, as distinct from the detailed mechanistic, aspects of the liquid-phase oxidation of hydrocarbons.

Discussing first the former group of papers, Emanuel and his colleagues have made good use of the theory of gas-phase oxidation reactions developed by Semenov. Particularly, the concept of the degenerate chain branching reaction has proved very useful in explaining many features of the liquid-phase oxidation at the large extent of reaction. In this case degenerate chain branching is due to the decomposition of the hydroperoxides formed as primary products of the reaction. This is the main factor causing the characteristic self-acceleration of the reaction. In addition, other change in the reaction mechanism occur at this stage. For example, Denisov has shown that the original chain carriers are replaced by others derived from the oxygenated products. The new chain carriers may be less reactive than the original ones and thus slow up chain propagation resulting in a retardation of the overall reaction.

These complex conditions have been analysed in a quantitative manner, which leads to an explanation of many features of the oxidation reaction at higher conversions. Other characteristics such as the influence of inhibitors of various types also agree with the reaction scheme. These are important achievements well brought out in the group of papers on mechanism. The only criticism I have is a certain obscurity and even incompleteness in the definition and explanation of the symbols used. As a result it is sometimes unnecessarily difficult to follow the mathematical derivations.

As for the group of technological papers, they are concerned with a variety of industrial aspects of hydrocarbon oxidation such as the oxidation of paraffins to fatty acids, cyclohexane to cyclohexanone and cyclohexanol, the oxidation of fuels under storage and the inhibition of this oxidation.

None of the work reported in the latter group of papers is radically new; but it is of great value to have collected in one volume this information, which is otherwise scattered throughout the literature and patent specifications. It should be of particular value to anyone who wishes to orient himself in an unfamiliar field.

This book clearly is to be welcomed and recommended to anybody interested in the oxidation of hydrocarbons in the liquid phase.

The translation is good and readable, and the publishers are to be congratulated for having made the book available to the many who cannot read it in the original language.

H. STEINER

STATISTICS FOR NON-MATHEMATICIANS

Statistical Inference

By Prof. Jerome C. R. Li. Vol. 1: Pp. xix+658. Vol. 2: Pp. xiv+575. (Ann Arbor: Edwards Brothers, Inc., 1964.) 10 dollars each volume.

THE first volume of this very large work has the sub-title *A Non-Mathematical Exposition of the Theory of Statistics* and is a revised edition of the author's earlier work *Introduction to Statistical Inference*. The second volume has the sub-title *The Multiple Regression and its Ramifications*, and is a new book. A brief listing of most of the chapter headings will give a fair idea of the contents. Volume 1: descriptive statistics, normal distribution, sample mean, test of hypothesis, χ^2 , t - and F -distributions, difference between sample means, confidence interval, one-way classification, randomized blocks, linear regression, factorial experiment, analysis of covariance, binomial and multi-nomial populations, transformations, distribution-free methods. Volume 2: matrix algebra, multiple regression, curvilinear regression,

fitting constants, factorial experiments, multiple-factor experiments.

Modern statistics is a mathematical discipline and yet its results are required by people with the very minimum of mathematics, and, in many cases, with a hostility toward the subject. It is therefore one of the hardest tasks a statistics teacher has, to explain his subject to many users of it. Prof. Li's way out of this difficulty (in Volume 1 at least) is to introduce the student to the ideas by means of sampling experiments. Thus, a simple finite population is repeatedly sampled and the behaviour of the sample means discussed. A little further on in the exposition a normal population is similarly sampled and the t -, F - and χ^2 -distributions obtained. In this way the student gains familiarity with random variation, can understand how the unusual sometimes happens, and even when, in the more complicated situations, he does not perform the sampling experiments, can appreciate the results in simple terms. A serious disadvantage to this method is that the student does not know which of the conditions of his sampling experiment are essential to the result, and which can be removed. But the author is careful over this point and states the results rather clearly in the form of theorems. With the assumptions on which the results are based stated therein, the student should not fall into the trap that most non-mathematical users of statistics succumb to, and apply the results without criticism in entirely inappropriate situations. The author is similarly very careful over his use of mathematical symbols and introduces them with much explanation.

If the exposition is novel in this respect it is no less novel in its adherence to the normal distribution (or population). Until p. 443 of Volume 1 it is the only population discussed, and no other gets a look-in in the second volume. Even when other distributions (for example, binomial) appear, the methods used involve reduction to normal terms. The extreme example of this is the short (for Prof. Li) chapter of 18 pages on distribution-free methods. He says "the selected [methods] are those which need practically no introduction of new principles". It is not surprising therefore that they "turn out to be the very same methods in disguise".

A second unusual feature is the emphasis placed on multiple regression. This comes relatively early in the exposition and, most remarkably, before the usual constants model for analysis of variance situations. The reason for this is that Prof. Li works through this latter situation in terms of 0, 1, or other appropriate variables and performs a multiple regression with them. Both these novelties have the advantage of giving statistics a unity in that everything stems from the minimum of principles, but I felt that the unity was rather forced.

Without the precision of the mathematical language it is difficult not to make errors. There are quite a few in the 1,233 pages. The most serious is the mis-statement of one of the "only two important theorems given in this text [Volume 2]". This refers to the F -distribution of regression over residual mean-squares, and completely omits to state that it only holds under the null-hypothesis (p. 99). In discussing the limiting form of a histogram (p. 14 of Volume 1) the areas are wrong. The necessity for the two samples to be independent is not mentioned when comparing their variances by the F -statistic (p. 123 of Volume 1), though the point does arise later (p. 137) to be forgotten again on p. 146. No attention is paid to the non-additivity of χ^2 (p. 476). The important point of the transformations being monotone is omitted when discussing the invariance of the median (p. 528).

In addition to errors there are some points which seem unfortunate: the emphasis on significance tests instead of confidence intervals, for example, especially when the former are very firmly of the 'accept-or-reject' type. The unusual notation for regression coefficients (partial and otherwise), which is the opposite of the usual practice, is unnecessary.

These books present an interesting approach to the instruction of non-mathematicians in statistics. If the approach fails, it does so not because the approach is not sound but because it is given in a text which has certain deficiencies, mainly arising out of an emphasis on unity, and is perhaps a little out-of-date. D. V. LINDLEY

INTERNATIONAL NUTRITION

World Review of Nutrition and Dietetics

Vol. 5. Edited by G. H. Bourne. Pp. x+381. (London: Pitman Medical Publishing Co., Ltd., 1965.) 115s.

THE fifth volume of reviews of subjects in the international field of nutrition opens with two essays on nutrition education. The first, by Prof. Beeuwkes of the University of Michigan School of Public Health, gives a good account of the history and the methods of education at all levels in the United States. The second, by Mr. McKenzie and Miss Mumford of Queen Elizabeth College, University of London, presents a critical evaluation of some existing education programmes.

The third paper, from Dr. Todhunter of the University of Alabama, considers some aspects of the history of dietetics and attempts to clarify the differences which may be defined between nutrition and dietetics. It is pertinent to quote the last sentence of the review as a reminder to all who work in the field of nutrition or dietetics. "Food and diet are more than a means of existence, or even of a way to health; they are part of a total way of life and culture of a group and both the nutritionist and dietitian must continue to be cognizant of this fact."

An extensive study of present concepts in diet therapy by Dr. Johnson of the Grace-New Haven Hospital follows Dr. Todhunter's historical review and provides much useful information on normal and modified diets. Adjustments of food intake in quantity and in nutrient content have been seriously investigated in the past forty years, and modern dietary treatment is based on solid scientific investigation rather than the empirical and often fallacious concepts that were accepted in the past.

Some aspects of the metabolism of vitamin A and carotene are reviewed by Dr. Owen of the Hannah Dairy Research Institute, Ayr. Particular attention is directed to the origin and function of liver stores of vitamin A in relation to the composition of blood and milk and the effects of hormones. The functions of retinenes in the vision of various animal phyla are considered. Some recent studies of the relation of vitamin A to enzyme systems concerned with metabolism of sulphate and trace elements are reviewed. Dr. Mahadevan, Dr. Malathi and Dr. Ganguly of the Indian Institute of Science, Bangalore, also review vitamin A in relation to the effect of proteins on absorption of the vitamin. The importance of researches in this field is stressed with reference to the observation that protein malnutrition may precipitate vitamin A deficiency in man.

Dr. Holmes, of the University of Adelaide, makes a useful appraisal of the evidence on which recently recommended protein allowances have been based. Recent recommendations from the United Kingdom, the United States, the Food and Agriculture Organization and Australia are compared with critical reference to discrepancies.

An extensive documented review of bone pathology in experimental malnutrition is given by Mr. Stewart of the Human Nutrition Research Unit, National Institute for Medical Research, London. The final paper of the volume is concerned with the fate of selenium in animals. It comes from Dr. Ganther of the University of Wisconsin.

The nine reviews add up to another valuable collection of information of general interest. A. M. COPPING

CHARACTER RECOGNITION BY HOLOGRAPHY

By PROF. D. GABOR, F.R.S.

Department of Electrical Engineering, Imperial College of Science and Technology, London

WAVE-FRONT reconstruction or holography, on which the first report¹ was published in *Nature* seventeen years ago, had a powerful renaissance in the past years. E. N. Leith and J. Upatnieks², G. W. Stroke³ and others have greatly improved the original method, and showed that it was possible to reconstruct complicated two- and three-dimensional objects, with half-tones, in previously unattainable perfection. The revival of holography owed much of its impulse to the invention of the laser, which made it possible to produce holograms with interferences of the order of 10,000, and thus to make full use of the information capacity of fine-grain photographic plates.

I wish to show that it has now become possible to harness holography for the solution of one of the most urgent problems of computers and other data-processing devices; the recognition of characters with many variants.

Wave-front reconstruction contains a principle which has not yet been fully exploited. Expressed in a general form: two coherent waves are made to fall simultaneously on a photographic plate, one coming from an object *A*, the other from an object *B*. The photograph links these together in such a way that if the hologram is illuminated by *A* alone, *B* will appear too, and vice versa. So far this principle has been applied in the form that *A* was the object of interest and *B* a light source, usually a simple one, and in the reconstruction the hologram was illuminated by *B*. I now propose to turn this around. Let *A* be a character, such as a printed or hand-written letter or numeral, which can be read by human beings but not by a machine, and let *B* be a combination of point-sources, forming a code-word which can be read by a machine. Produce the hologram by combining *A* and *B*. When *A*, or a character sufficiently close to it is presented to the hologram, with the original illumination, the code-word *B* will flash out. This means that the hologram can act as a translator, or coding device.

The interest of this principle is in the enormous recognition capacity which can be stored in a single hologram, and which one might not perhaps suspect at first sight. I wish to show that with *N* characters to be discriminated, each with *M* variants, the product *M.N* can be made of the order of a thousand or even more.

Fig. 1 shows the optics for producing the master hologram, and for using it in the read-out. The recording medium is assumed to be a transparency, such as a microfilm; but reflecting media can also be used. The hologram is built up by repeated exposures in what may be called 'layers'. These are not, of course, physically separated in the emulsion. Each layer corresponds to one of the *N* characters to be discriminated, with all its *M* variants, and is marked with one code-word. The layer contains the part-holograms, to be called 'engrams' of the variants side by side, with little overlap. Each engram is produced with one direction of illumination, and as the photographic plate is arranged in the rear focal plane of a lens viewing the character, it is a 'Fourier-hologram'. This has the advantage that the hologram is translation-invariant, that is to say, independent of the position of the character so long as this appears alone in the window. An engram need not occupy much more area in a photographic plate than would be needed for a good record of the corresponding character, but as a cautious example we will assume 120 engrams, each with a diameter of about 5 mm, on a photographic plate of 50 mm × 50 mm. This is sufficient to record without overlap 30 variants, each in four or six 'identical' engrams. Fig. 1 shows how this is achieved.

The light of a laser issues from a point *L*, and a beam splitter consisting of a spherical mirror and a semi-reflecting mirror produces of this two images *L'* and *L''*. The first of these serves the illuminator; the second, in the centre hole of the illuminator plate, serves the code plate. The illuminator plate, backed by a field lens, consists of a plastic plate embossed with, say, 120 lenticules, and is black outside the lenticules. These produce 120 point sources which illuminate the window containing the character through a lens 1, which removes the illuminator points into star space. The point sources correspond one-by-one to the engrams. There is a certain advantage in randomizing them slightly. Four identical engrams are taken at a time of any one variant. These are spaced out, as far as possible, to increase the resolving power of the hologram. They are selected with a mask, and a different mask with four holes is used for every variant.

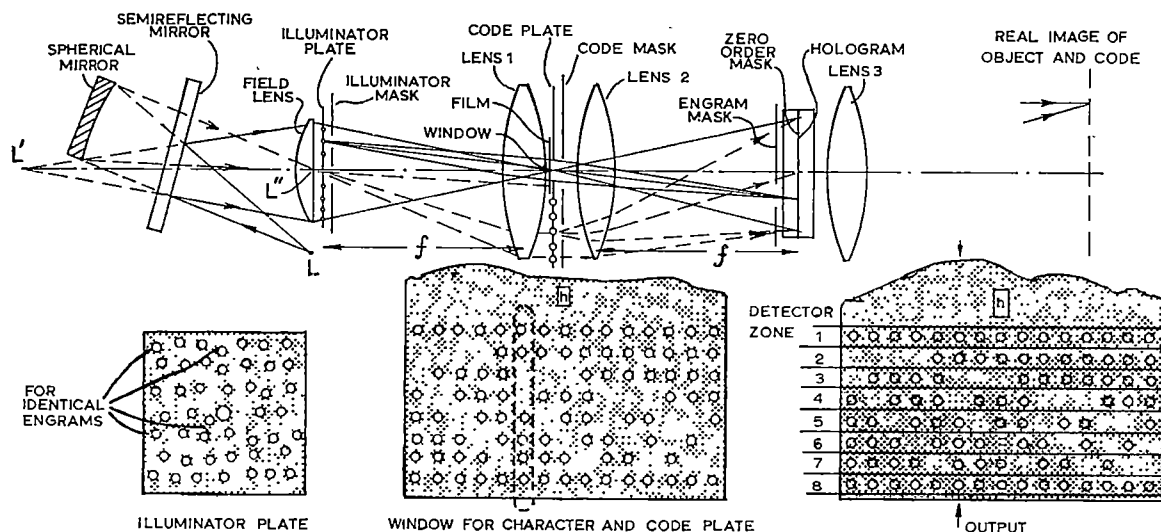


Fig. 1. Apparatus for producing a coding hologram and using it for the read-out

The point source L'' in the centre of the illuminator plate illuminates the code-plate, through the same lens 1, which serves in this area as a field lens. The code-plate, like the illuminator, is an embossed plastic plate, which contains the code-word in the form of groups of luminous points, arranged in one or several arrays. It is advantageous to use self-checking codes, in which every word has the same number of code-points. In the example here are six positions, of which two remain dark and four light up. This code has $6.5/1.2 = 15$ words. Two more positions have been added. These do not contribute to the discrimination of characters, but improve the signal-to-noise ratio, as eight points have to light up for every valid character.

In the making of the master hologram all engrams in one layer are marked, that is to say, exposed simultaneously, with one distinctive code-word, which is selected by a mask. But as each code-word illuminates the whole area of the hologram, a further mask must be used near the plane of the photographic plate, which cuts out the light except in the area of the engrams which are made at any one time. This makes it possible to observe the rule of optimum illumination, which postulates about equal light sums on any engram from the character and from its code-word.

Black-on-white letters are less suitable for discrimination than their negatives, because they have too much in common; all their white area. But this disadvantage can be eliminated by a further mask, in the plane of the hologram, which cuts out all undiffracted light. By Babinet's principle this turns a character into its negative. Such a mask can be easily made by exposing a photographic plate through a clear window simultaneously to all illuminator points.

After $M.N$ successive exposures of the photographic plate, which add up to a convenient medium density, the master hologram is made by processing and printing it, preferably with an overall gamma of 2, and the print is put back in the original position. In the reading all the point-sources of the illuminator are used, while the whole code-plate is covered up. A lens 3 is used for observation, which produces a real image of the code-plate. If now the recording medium is dragged across the window, whenever a character or a variant appears in it, its code-word will flash up. It is advantageous to arrange in the image plane a mask, which is a replica of the code-plate, with very fine holes, so as to exclude all but the signalling light. This mask, too, can be made photographically.

A method of reading the code-words is to sum up all the light which appears in one zone, corresponding to one position in the code, and guide it to a separate photo-electric detector. Each detector is fitted with a level discriminator, so as to reject spurious signals below a certain level. This method is simple; but it has only

moderate discriminating power, because if the characters are not clearly distinct, some light might show up in the same zone in the code-words of other characters. One can reduce this by making the code-words of characters which are not clearly distinct as different as possible. But the maximum of discrimination is achieved by a somewhat more complicated apparatus. In this the image of the code-plate is projected on the screen of an image camera. The code-words flash up at intervals corresponding to the time allotted to each letter, during 10–30 per cent of this period. In the time between flashes all code positions are scanned word by word, and points above a certain level of intensity are transferred to a memory organ, such as a core store. But unless the full number of points appear in a word, the record is erased. If the full number is counted, the code-word is transferred to the computer.

The great discriminating power of the holographic method stems from its high angular resolution. Assume, for example, $N = 35$, $M = 30$, $M.N = 1050$. The group of four engrams corresponding to the character presented to the reader receives $1/30$ of the light, and can diffract about $1/35$ of it, altogether about 10^{-3} of the total. (Not counting, of course, in black-on-white records, the undiffracted light which goes into the zero order.) Of the diffracted light, under the proper conditions, that is to say, when the engrams were taken with about equal light sums from the letter and from the code-word, one-quarter will go into the reconstruction of the code-word. One half appears in the object, another quarter goes into the 'twin' image of the code-word, which, however, is washed out by intermodulation with the character, and is useless for recognition. But the useful quarter is concentrated in extremely small solid angles. For example, if four or six identical engrams are spaced out by about 25 mm, the solid angle in which the major part of the light corresponding to a code-point is concentrated will be of the order 10^{-3} . Let the light of, say, 10^{-4} of the total be distributed among ten code-points, this means that 10^{-5} of the light appears in one code-point, in a solid angle which is perhaps 10^{-3} of the solid angle covered by the whole code; a concentration of the order ten. Moreover, this estimate is somewhat pessimistic, because it takes no account of the confirmation of the character by the engrams of slightly different variants in the same layer.

In conclusion, there is good reason to believe that a single hologram may discriminate between all the numerals and the letters of the alphabet, each with 30 variants.

¹ Gabor, D., *Nature*, **161**, 777 (1948), *Proc. Roy. Soc., A*, **197**, 475 (1949); *Proc. Phys. Soc., B*, **64**, 244 (1951).

² Leith, E. N., and Upatnieks, J., *J. Opt. Soc. Amer.*, **53**, 1377 (1963); **54**, 1295 (1964); **55**, 569 (1965).

³ Stroke, G. W., *Optics of Coherent and Non-coherent Electromagnetic Radiations*, Univ. Michigan (1955), with Falconer, D. G., *Physics Letters*, **13**, 306 (1964); **15**, 283 (1965).

POLONIUM-210 IN MAN

By Dr. C. R. HILL

Physics Department, Institute of Cancer Research, Belmont, Surrey

IT is well known that the atmosphere contains appreciable quantities of radon as a result of emanation from the materials of the Earth's crust. Until recently, however, little interest has been taken in the fate of the long-lived radionuclides produced in the atmosphere by radon decay. Radium *D* (lead-210) has a half-life of twenty-two years, much longer than the twenty–thirty day mean residence time for aerosols in the troposphere, and it is therefore returned to the surface of the Earth as a natural component of fall-out. During residence in the atmosphere, sufficient time elapses for the growth of most of

the equilibrium amount of the five-day β -emitter radium *E* (bismuth-210) and some of the 138-day α -emitter radium *F* (polonium-210), and these therefore also take part in the fall-out process.

In addition to this natural process of production, polonium-210 may be released into the atmosphere by a number of artificial means. Considerable quantities are thought to have been released by the Windscale accident in 1957 (ref. 1). It is being used as a source of thermal power in Earth satellites and there has recently been interest in the possibility that a certain amount may have been

produced by activation of stable lead or bismuth in nuclear weapon explosions. Except, possibly, in the stratosphere the natural source of the nuclide is predominant at the present time, but this may not always remain the case.

Burton and Stewart, in 1956, measured the concentration of radium *D*, *E* and *F* in ground-level air and in rain-water at Harwell³ and their figures show an annual deposition rate for radium *D* of about 1.7 mc./km² (ref. 2), which is nearly half the corresponding mean rate for strontium-90 recorded at the same site during the past five years³. The concentration of radium *D* in ground-level air appears to be dependent on latitude, being highest in temperate latitudes and also being generally higher in the northern than in the southern hemisphere⁴, possibly as a result of the difference in exposed land masses.

Dispersion as an atmospheric aerosol is well known to be an effective means by which many trace materials may gain entry to the biosphere. Lead, bismuth and polonium may all be absorbed by human beings as a result of inhalation and also to an appreciable extent by uptake through the gut⁵: it is therefore of interest to know to what extent human beings may be exposed to these nuclides in the environment and whether, in fact, they are normally present in human tissues.

From knowledge of the energy and linear energy transfer of the radiation emitted by the three nuclides radium *D*, *E* and *F*, it appears that the respective biologically effective doses produced per disintegration will be in the approximate ratios 1:10:1,000 (assuming an RBE of 10 for α -particles). It is radium *F* (polonium-210) that is of primary interest, therefore, in the present context, although it must be borne in mind that its actual presence and pattern of distribution in the human body may be determined very largely by the behaviour of one or more of its radioactive precursors.

Human and animal foodstuffs. Polonium-210 has been observed to occur on exposed foliage, and particularly on grass, in amounts that are directly related to local rainfall⁶. In high rainfall districts in England and Wales concentrations above 10,000 pc./kg of dry grass are not uncommon, with the curious consequence that a load of hay can become legally classifiable as a 'radioactive substance' purely on account of its natural radioactivity. Concentrations of the order of 5,000 pc./kg have also been found to be common in slow-growing plants such as lichens, both in the United Kingdom and also in Lapland and the Canadian Arctic, where they form an important part of the winter diet of reindeer and caribou.

Grazing animals are found to take up polonium-210 in appreciable amounts, and concentrations greater than 1,000 pc./kg have been found in some animal tissues both

in the Arctic and in high rainfall areas of the United Kingdom. Biological concentration of polonium-210 also occurs in some marine organisms and in particular in some varieties of plankton⁷, certain shellfish and crab. Evidently, therefore, there is a considerable variety of ways in which the nuclide may find its way into human and animal foodstuffs, and the amounts that we have measured in a number of these⁸, together with observed values of the lead-210:polonium-210 activity ratio in certain cases, are shown in Table 1.

The data of Table 1 are somewhat fragmentary and are characterized by wide variations in the concentrations of the nuclides, but it may be concluded that the daily intake of both polonium-210 and lead-210 in the food comprising an average mixed western diet will probably be in the range 1–10 pc. This estimate is supported by measurements we have made of the daily faecal excretion of lead-210 by a thirty-year-old male subject consuming an ordinary 'English' diet. For six separate samples collected at intervals over five months the values were between 1.7 and 6.4 with an average of 3.2 pc./day. From similar measurements in the United States, Holtzman has obtained a value of 1.8 pc./day⁹.

Evidently a considerably higher figure might apply, for example, to a connoisseur of sea food, while an Eskimo on a high meat diet based on winter-killed reindeer might ingest of the order of 100 pc./day of each nuclide. It is to be noted that the polonium-210 and lead-210 contents of drinking-waters (other than untreated rain-water) are only of the order 0.01 and 0.05 pc./l. respectively⁹ and so would probably be insignificant in comparison with food as a source of ingestion of the nuclides.

Tobacco and cigarette smoke. It has been pointed out by Radford and Hunt that appreciable concentrations of polonium-210 occur in tobacco and that, as might be expected from the known volatility of polonium, a considerable fraction of the activity in the tobacco may be transferred to the inhaled smoke¹⁰.

I have measured polonium-210 concentrations in a number of cigarette tobaccos of different origin, with the results shown in Table 2. These figures do not indicate any striking differences as between the different major tobacco-producing areas and, in particular, do not support the suggestion recently made by Marsden¹¹, based apparently on total α -activity measurements only, that differences in the polonium-210 content of tobaccos might be related to geographical variations in the incidence of lung cancer.

We have also investigated the fate of the polonium-210 which is present in a cigarette when it is smoked in a standard smoking procedure†. Using a popular brand of cigarette which was found to contain 0.49 ± 0.07 pc. polonium-210 per cigarette, the fate of the original activity in three experiments was as shown in Table 3, where the previous results of Radford and Hunt are summarized for comparison. It will be seen that the main conclusion from the earlier work, that an appreciable fraction of the polonium appears in the main-stream

Table 1. POLONIUM-210 CONTENT OF VARIOUS HUMAN AND ANIMAL FOODS

Materia	No. samples	²¹⁰ Po spec. activ. (pc./kg)	²¹⁰ Pb : ²¹⁰ Po ratio
Grass (dried) U.K.	24	400–16,000	1–5
Dry lichen (<i>Caloplaca elegans</i>) U.K.	2	7,800; 10,000	1
Dry lichen (<i>Cladonia alpestris</i>) Lapland	3	6,600–8,100	1
Dry lichen (<i>Cladonia alpestris</i>) Canada	1	3,500	1
Edible green vegetables (U.K.)	5	0–90	1–3
Carrots and potatoes (U.K.)	2	1	—
Breads and cereals (U.K.)	4	1–7	—
Dried milk powder (U.K.)	3	2–6	—
Beef and lamb mutton (U.K.)	2	3; 3	—
Beef and lamb liver (U.K.)	3	4–100	0.7
Beef and lamb kidney (U.K.)	3	48–270	0.05–1
Lamb kidney (N. Wales)	6	90–1,800	0.2
Reindeer (Lapland) summer killed			
Muscle	6	15–50	—
Liver	5	350–750	—
Kidney	4	110–490	—
Bone	5	520–3,500	—
Reindeer (Canada, N.W. Territory) winter killed			
Muscle	2	200; 210	—
Liver	2	2,400; 5,600	—
Kidney	2	4,200; 2,300	—
Spleen	1	980	—
Camel bone (Jordan)	2	1,100; 1,500	—
Deer bone (Scotland)	1	1,300	—
Cookies (U.K., E. and W. coasts)	3	400–900	0.1–0.2
Crab (U.K., S. coast)	2	1,300; 1,400	—
Plankton (S. Pacific)	1	2,000	—

* Apart from a limited number of α -spectroscopy measurements, the polonium-210 determinations reported in this article have been carried out by the method described by Black (ref. 27), Osborne (ref. 12) and others. Samples are wet ashed using nitric and perchloric acids and polonium is then deposited electrochemically on to silver from a solution in 0.5 N hydrochloric acid, and afterwards α -counted. Yield is checked using duplicate samples spiked with a standard ²¹⁰Pb-²¹⁰Po solution and lead-210 can be estimated by replating polonium-210 from the solution after an interval of several months.

† Smoking was carried out under the following conditions, using a bellows type smoking machine: initial cigarette length 70 mm, weight 1.09 g; butt length 23 mm; puff volume 35 ml., duration 2.0 sec, repeated once a minute to total 10 puffs per cigarette; horizontal smoking position. In experiment No. 3, filter tips were fitted to the cigarette, using adhesive tape. Main-stream smoke (that is, the fraction normally drawn into the mouth) was drawn directly into the collecting device, which was either a Cambridge filter (expts. Nos. 2 and 3) or two wash bottles containing 5 per cent hydrochloric acid in ethanol and immersed in solid carbon dioxide (expt. No. 1). Side-stream smoke was led through a collecting chimney and collected either in two similar wash bottles in series followed by two Cambridge filters in parallel (expt. No. 1) or in the two filters only (expts. Nos. 2 and 3). Following collection, smoke was recovered from the filters by gently flowing hydrochloric acid-ethanol mixture in the reverse direction through the filters, still in their holders. Polonium-210 estimation was carried out as described in the previous footnote.

Table 2. POLONIUM-210 CONTENT OF VARIOUS CIGARETTE TOBACCOS

Origin of sample	No. of samples	²¹⁰ Po activity (pc./kg)		
		Max	Min	Mean
United States	8	650	390	510
Cent. and S. America	6	1,360	290	670
Rhodesia	3	700	600	650
Australia	2	660	610	640
India and Pakistan	2	570	250	410
Indonesia	1	—	—	230
Turkey and Greece	3	280	210	240

Table 3. FATE OF POLONIUM-210 IN SMOKING OF CIGARETTES
(Percentages of total initially present)

Component	Present measurements			Radford and Hunt ¹⁰	
	Expt. 1 (no tips)	Expt. 2 (no tips)	Expt. 3 (filter tips)	(No tips)	(Filter tips)
Main stream	12	10	6.5	23; 25	22; 18
Tips	—	—	3.5	—	—
Side stream	32	28	30	21, 29	27; 24
Butts	35	34	37	30; 25	24; 38
Ash	16	18	19	7; 11	9; 8
Total recovery	95	90	96	81, 90	82; 88

smoke, is confirmed, although the actual fractions found in the present experiments are somewhat lower, possibly as a result of differences in smoking conditions.

In a separate experiment, main-stream smoke from cigarettes from the same batch was weighed after collection on Cambridge filters and found, in two separate measurements, to amount to 0.032 g per cigarette as collected or 0.021 g after drying at 100° C. Taking the polonium-210 content of main-stream smoke as being about 0.05 pc. per cigarette (from the foregoing measurements) it is evident that the specific polonium-210 α -radioactivity in the condensed smoke is of the order of 2 pc./g. The radiation dose rate at the surface of a thick (that is, > 100 μ) slab of such material would be 0.1 rad (or 1 rem) per year and less for thinner slabs. This is of interest in considering the possible occurrence of high local dose rates in the human lung as a result of accumulation of smoke particles, since the foregoing dose rate is in marked contrast with the values of from several hundred to more than 1,000 rems in twenty-five years suggested by Radford and Hunt¹⁰. For such high dose rates to occur it would seem to be necessary to postulate a mechanism for selective clearance from the lung of the bulk constituents of the condensed smoke relative to that of polonium-210. Such a mechanism would not only have to show a high degree of selectivity but also, on the evidence given later for retention time in the lung for the polonium-210, would need to operate rather rapidly.

Normal human tissues. A number of measurements have been reported of the concentrations of polonium-210 and lead-210 in normal human tissues, although, apart from those by Osborne¹² and Stahlhofen¹³, most have been concerned only with bone and teeth^{8,14,15}. In view of the

results presented here, I have made two further sets of measurements on human tissues. In the first place I have examined the polonium-210 levels (equivalent to lead-210, as the samples were several years old at the time of measurement) in bones of ten different Canadian Eskimos. Secondly, I have made comparative measurements of polonium-210 on soft tissues of two groups of individuals known to be non-smokers and regular cigarette smokers respectively, all of whom died sufficiently suddenly that their smoking and other habits would not have been upset seriously for more than a few hours before death. Measurements were made without subdivision of the tissues except in the case of the right lung, where the bronchial tree and samples of peripheral alveolar tissue were dissected out and measured separately. In three cases the secondary bronchial bifurcations were dissected out (to include about 0.5 cm of bronchus above and below the bifurcation) and measured separately. The results of measurements on the Eskimos and on the effect of smoking are presented in Tables 4 and 5 respectively.

The measurements on the Eskimos can be compared with values found by other investigators for bone of 'normal' European and North American subjects (shown in the same Table). From this it will be seen that the Eskimo values range from 'normal' upwards by a factor of twenty or more, and it is interesting that the high values occur in subjects who had been permanently resident in the Arctic while several of the others had been living in southern Canada for some time prior to removal of the bone (either at autopsy or in the course of thoracoplasty). The high values found here seem to be consistent with my observations of high levels in reindeer meat and the known high meat diet of Eskimos.

(Note added in proof. Recent measurements on fresh placental tissue samples derived from nine northern Canadian residents whose diet included reindeer or caribou meat have shown polonium-210 concentrations

Table 4. LEAD-210 LEVELS IN BONES OF CANADIAN ESKIMOS
(Measured as polonium-210 six years after collection)

Case No.	Origin and age of subject (where known)	²¹⁰ Pb activity (pc./kg wet bone)	
1	Igloolik, N.W. Territory	42	710
2	—	—	380
3	Carberry, Man.	67	8
4	Winnipeg, Man.	80	13
5	Edmonton, Alta.	44	27
6	—	—	12
7	Pakatawagan, Man.	21	37
8	Nelson House, Man.	25	42
9	Nelson House, Man.	17	40
10	—	—	100
"Normal" (United Kingdom and United States, refs. 8, 12, 14 and 15)		15-37	

Table 5. POLONIUM-210 TISSUE LEVELS IN SMOKERS AND NON-SMOKERS

Age and sex of subject	²¹⁰ Po Activity (pc./kg tissue)						Activity ratios		
	Liver	Bronch. tree	Alveolae	Mean lung (approx.)	Kidney	Gonad	Lung/liver	Kidney/liver	Bronch. tree/alveolae
Cigarette smokers									
23 M	7.7	6.5	7.2	6.8	19.8	4.0	0.88	1.8	0.9
22 M	22	6.0	10.1	8.0	21	3.6	0.36	0.95	0.6
62 M	27	10.2	15.6	12.9	24.5	—	0.48	0.90	0.65
56 M	23	6.6	6.6	6.6	22.7	4.1	0.29	1.0	1.0
Mean	20	7.3	9.9	8.6	20.5	3.9	0.43	1.02	0.74
Non-smokers									
68 F	7.6	2.0	1.2	1.6	5.7	—	0.21	0.75	1.6
66 F	12.5	1.2	2.0	1.6	—	—	0.13	—	0.6
54 F	19.7	1.9	1.9	1.9	11.4	1.7	0.10	0.58	1.0
63 F	13.2	1.6	1.3	1.4	7.0	—	0.11	0.60	1.2
17 M	12.8	4.0	3.8	3.9	10.5	4.0	0.30	0.82	1.0
57 F	22.6	7.7	10.2	9.0	38.5	—	0.40	1.7	0.75
Mean:	14.8	3.1	3.4	3.2	15	2.8	0.25	1.07	0.75
Measured ²¹⁰Pb/²¹⁰Po ratios									
Max	1.0	—	0.7	—	0.6	—	—	—	—
Min	0.1	—	0.5	—	0.14	—	—	—	—
Mean	0.47	—	0.6	—	0.31	—	—	—	—

twelve times greater, on average, than those found in nine other subjects from the same area.)

From the measurements on smokers and non-smokers (Table 5) the following points emerge:

(1) In both smokers and non-smokers the ratio between gross specific polonium-210 activity in the bronchial tree and that in alveolar tissue is close to unity for any individual.

(2) In liver and kidney the levels of polonium-210 in smokers are little, if at all, greater than those in non-smokers, whereas in lung tissue a difference with a factor of between 2 and 3 seems to occur. This is perhaps best seen in comparison of the activity ratio 'lung/liver' for the two groups. The corresponding ratio 'kidney/liver' may here be regarded as a control parameter indicating variations occurring between two tissues neither of which is directly concerned in the exposure mechanism of interest (inhalation).

(3) In my samples the polonium-210 content of the average smoker's lung exceeds that of the average non-smoker by 5.4 pc. (assuming 1 kg for the total weight of the lungs), which is the approximate content of the nuclide in the main-stream smoke of a hundred cigarettes. Assuming a consumption of twenty cigarettes per day and retention by the lung of half the main-stream smoke, these results suggest a retention time for the polonium-210 in the lung of the order of ten days.

(4) For the three cases of smokers where bronchial bifurcations were dissected out and measured separately, the polonium-210 in each sample (of weight between 5 and 15 g and each consisting of several bifurcations) did not in any case exceed the amount to be expected, from the average value measured for the whole bronchial tree, by more than 0.05 pc.

In view of the possibility suggested by Radford¹⁰ that even such a small quantity of activity might lead to high dose rates if it were concentrated into a small area, I have examined autoradiographically some vacuum-dried samples of epithelium taken from bronchial bifurcations in smokers' lungs. In the specimens examined so far I have not found statistically significant concentrations of α -activity greater than 0.01 pc./cm², and an appreciable part even of this activity is probably attributable to background from the glass plates used in the initial experiments.

My failure to find appreciable excess polonium-210 activity in bronchial bifurcations of cigarette smokers, either when measured as gross tissue concentrations or when examined autoradiographically as surface activity, is in contrast with Radford's observation of hot spot activity¹⁰ and also with his later observations of polonium-210 activities up to 1.25 pc./g in bronchial epithelium¹⁶.

Subjects with high skeletal burdens of radium-226. The decay of radium-226, present in the human skeleton as a result of either natural or abnormal exposure, leads eventually to the formation within the body of a certain amount of lead-210 and its descendants. It will be shown later than in the normal subject this source is of little importance, but in subjects with high radium burdens it can constitute the main source of these nuclides and is of interest therefore as providing an opportunity for an investigation of the effect of differences in the route of their administration to the human body.

I have recently had occasion to examine *post mortem* a subject who had been employed as a radium-dial painter until six weeks before her death, which resulted from acute myeloid leukaemia. Her body-burden of radium-226 proved to be 0.02 μ c.; well below the maximum permissible level, but about one thousand times above normal. The levels of polonium-210 and lead-210 measured in various organs at the time of death are shown in Table 6, both as absolute amounts and as ratios to the corresponding average levels that we have measured in normal individuals. Several interesting points emerge from these figures:

(1) In bone and liver the presence of polonium-210 appears to be due to the quantitative retention of the products of decay of lead-210 in the tissue, since the two nuclides occur in equilibrium. In kidney and ovaries, however, the polonium-210 is significantly in excess of equilibrium and is presumably present largely as a result of active uptake of polonium from the blood. The discrimination between the renal cortex and medulla is in agreement with animal data and with a single previously published case of a human subject¹⁷.

(2) The exposed/normal ratio for polonium-210 in a tissue is much higher for bone than for most soft tissues. This presumably reflects the known fact that an appreciable fraction of the radon produced in bone by decay of radium-226 does not escape but decays there with the eventual deposition of lead-210 and polonium-210. (The high absolute and relative levels of polonium-210 in hair may be due to superficial contamination by radon decay-products in the atmosphere of the luminizing factory.)

(3) The actual distribution of polonium-210 among the soft tissues does not seem to be strongly dependent on the origin of the polonium. It is of particular interest that this observation applies also to the lung: this could be a fortuitous result of the inhalation of high concentrations of radon and daughters, but otherwise it suggests that much of the normal polonium-210 content of the lung originates internally rather than as atmospheric material that has not been cleared physiologically.

(4) In relation to the radium-226 content of the skeleton, the ²¹⁰Pb-²¹⁰Po levels in the soft tissues of the exposed subject are seen to be considerably less, by a factor of about 70, than those in normal individuals. This is in agreement with Holtzman's prediction⁸ that the normal individual acquires only a very small fraction of his polonium-210 burden (at least in soft tissues) as a result of decay of skeletal radium-226.

Measurements have also been made of polonium-210 in the tissues of a number of subjects having radium-226 burdens that had been acquired some twenty-forty years before their death. The results, which are given in Table 7, show a level of polonium-210 in soft tissue, for a given radium-226 burden, that is considerably less than that in the more recently exposed case. This situation is consistent with the older radium deposits being less subject to loss of radon to the blood, but it could also be explained on the basis of high inhalation of radon and daughters by the more recently exposed subject.

If the commonly used value of 0.67 is assumed for the fraction of radon-222 produced in bone that escapes to the blood, the data of Table 7 may be used to calculate the rate of uptake of radon-222 into the blood that would by itself maintain the normal concentration (10 pc./kg) of polonium-210 in the liver. A value of approximately 0.5 pc./min is found in this way.

Table 6. POLONIUM-210 AND LEAD-210 LEVELS IN TISSUES OF A RADIUM-DIAL PAINTER

Tissue	²¹⁰ Po activity (pc/kg)	²¹⁰ Pb : ²¹⁰ Po activity ratio	²¹⁰ Po ratio exposed: normal ¹²
Liver	190	1.0	19
Lung	50	—	15
Kidney cortex	360	0.13	40
Kidney medulla	200	0.10	40
Spleen	34	2.5	11
Ovaries	60	0.25	9
Thyroid	11	—	—
Pancreas	8	—	6
Adrenals	70	—	—
Hair	25,000	1.0	280
Bone	1,500	1.0	88
Bone— ²²⁶ Ra	4,000	—	1,000 (²²⁶ Ra)

Table 7. RADIUM-226 IN BONE AND POLONIUM-210 IN LIVER FOR INDIVIDUALS WITH LONG-ESTABLISHED RADIUM BURDENS

Approx age of ²²⁶ Ra burden (years)	²²⁶ Ra in bone at death (pc/kg wet)	²¹⁰ Po in liver at death (pc/kg wet)	Ratio bone ²²⁶ Ra/liver ²¹⁰ Po
20	9,000,000	11,000	800
40	2,300,000	2,000	1,100
35	2,000,000	4,500	450
30	80,000	70 (normal)	(1,000)
20	40,000	110 (normal)	(400)
40	30,000	80 (normal)	(800)

Isolated proteins. One of the biological properties of polonium that is often overlooked, although it has been known for some forty years¹⁸, is that it is a 'seeker' of soft tissues rather than bone, and it is in fact unique in this way among the α -emitting heavy elements. (The common occurrence of polonium-210 in bone is almost certainly not a result of its own chemical nature but rather of its descent from the bone seeker lead-210.) The reason for this behaviour is not fully understood, although it is known that polonium may be associated with protein, particularly in the blood^{19,20}.

In this connexion it seemed of interest to determine whether the polonium-210 that has been shown to occur in grazing animals may be present in nominally pure crystalline proteins extracted from their tissues. The results of several such measurements on commercially prepared samples are shown in Table 8 together, for one of the insulin samples, with corresponding measurements both on the pancreas from which the sample was derived and on a multiply recrystallized fraction of the commercial grade of protein.

Table 8. POLONIUM-210 CONTENTS OF SOME PURE PROTEINS

Protein	Supplier	²¹⁰ Po activity (pc/kg)
Albumin (crystalline bovine)	B.D.H.	23
Haemoglobin	B.D.H.	98
Gelatine (powder)	B.D.H.	40
Fibrinogen (bovine)	B.D.H.	120
Insulin (crystalline bovine)	B.D.H.	610
Insulin (crystalline bovine)	Boots	1,870
Insulin (crystalline bovine)	Burroughs Wellcome	660
Insulin (crystalline bovine) (multiply recrystallized)	Burroughs Wellcome	370
Raw bovine pancreas (dried)	Burroughs Wellcome	25

The difference in activities between the insulins and the other bovine proteins might simply reflect a difference in origin (the three commercial insulins, although from different manufacturers, were all derived from a common source of supply of pancreas), but it is of interest that, of the various proteins examined, insulin has much the highest content of —SH groups. The reduction in specific activity on multiple recrystallization appears to rule out a very close association between the polonium and the protein, but the possibility is nevertheless suggested that some association, in which —SH groups are involved, may exist. Such a situation would evidently have important implications for the microdosimetry of the polonium isotopes.

Discussion

The selection of data presented in this article has been dictated to a considerable extent by the availability of post-mortem and other specimens and is therefore far from comprehensive. Nevertheless, from these results and others previously reported in the literature, at least the outlines of a consistent picture can be obtained of the part played by the sub-series radium D—E—F in man's radioactive environment.

The correspondence, both for grazing animals and for man, between the levels of polonium-210 in the food of a community and in the tissues of its members clearly indicates that ingestion in food is an important route for acquisition of body-burdens of the nuclide. The conclusion that this is normally the principal source of such body-burdens seems to follow from Holtzman's analysis of other possibilities⁹, including decay of skeletal radium-226 and inhalation of radon-222, radium A, B and C and radium D, E and F, in which he showed that only the last-mentioned of these is of comparable importance with food, and that only if one assumes an atmospheric concentration of the nuclides several times higher than has been measured by most observers²¹.

The variations, by an order of magnitude or more, between polonium-210 body-burdens in 'normal' United Kingdom and United States residents and in some Canadian Eskimos, are probably due to the latter having a high dietary content of meat derived from animals

grazing on poor, slowly growing pasture. Apart from in the Arctic, such conditions might also be expected among the herdsman populations of arid regions such as those in Arabia and East Africa, and it is of interest here that we have found high levels of polonium-210 in camels.

Cigarette smoking does not seem to lead to abnormal levels of polonium-210 in tissues other than lung, and the levels here, examined either as gross tissue concentrations or, so far as I have been able to discover, as localized concentrations, are not such as would lead to abnormally high radiation dose rates. However, the possibility of being able to observe localized concentrations of smoke particles in the bronchial epithelium by virtue of their association with a radionuclide is of considerable interest, regardless of considerations of radiation dose to the tissue, and would justify further experimental effort in that direction.

The question of the part played by physiological uptake of lead-210 in producing tissue concentrations of polonium-210 is still not completely settled^{21,22}. Evidence from work with experimental animals, as summarized by the International Commission on Radiological Protection⁵, indicates that the steady condition reached following prolonged chronic ingestion of an equilibrium mixture of lead-210, bismuth-210 and polonium-210 would be one in which lead-210 was considerably in excess of polonium-210; the predicted ²¹⁰Pb : ²¹⁰Po ratios for bone, liver and kidney being 400, 32 and 25, respectively. Values for this ratio between these figures and unity would still be consistent with the data from the International Commission on Radiological Protection if a fraction of the decay products of lead-210 was retained in the tissue where it had been formed, whereas values less than unity could only be explained by an active uptake of polonium-210 into the tissues (or, possibly, by redistribution of polonium-210 between different tissues²²). In fact, I have repeatedly found values for this ratio considerably less than unity in a number of soft tissues and consistently close to unity for bone. It appears, therefore, that, under the conditions we are studying, there may be a true uptake of polonium-210 into certain tissues independently of lead-210, while in bone the initial uptake is of lead-210 and this is followed by quantitative retention of decay products.

Although this article is primarily concerned with polonium-210 it is of interest that at least four other isotopes of polonium, all α -emitters, are normally present in the human body: polonium-218 (radium A), polonium-216 (thorium A), polonium-214 (radium C') and polonium-212 (thorium C'). Owing to their short half-lives, direct measurements of the tissue levels and distribution of these isotopes have not hitherto been possible, but experimental data obtained with rats²³ indicate that a dose rate to the kidney, due to polonium-218 and polonium-214, of the order of 1 mrad/year may result from continuous inhalation of radon at a concentration of 10^{-12} c./l. On the basis of the behaviour of polonium-210 the corresponding dose rate to the gonads may be expected to be of a similar order of magnitude.

Conclusion

The object of the work described in this article is to define the part played by polonium-210 in contributing to the biologically effective radiation dose received by man in a variety of environments. In the foregoing I have reported the gross concentrations of the nuclide that are found in human tissues, together with some of their variations with environmental conditions, and I have discussed the origin of the material and some information relating to its physiological and biochemical behaviour which suggests that its distribution within a tissue may be rather non-uniform. One major area of ignorance with which we are still faced, however, is in the basis for a comparison of the physical dose rates of high linear energy transfer radiation that we have measured with other,

predominantly low linear energy transfer, sources of background radiation; in other words, the problem of relative biological effectiveness at very low dose rates. In the absence of better evidence we may simply assume a relative biological effectiveness of 10 for the polonium-210 α -particle, in which case, by a convenient arithmetical accident, our tissue concentrations expressed as pc/kg become equivalent to mrem/year. This is almost certainly a very poor approximation to whatever the true position may be, and it should be pointed out that some recent reports²⁴⁻²⁶ have suggested that the relative biological effectiveness for high linear energy transfer radiation may become very large at low dose rates, tending towards values of 30 or more for the conditions of background radiation. On this basis polonium-210 would evidently constitute a major fraction of background dose to man, the total of which is generally considered to be of the order of 100 mrem/year.

I thank Dr. C. F. J. Baron, Dr. D. Haler, Dr. L. A. Barrer and Dr. F. D. Sowby for arranging the supply of post-mortem material; Prof. K. Liden, Dr. J. K. Miettinen, Dr. O. Paakola and Dr. P. M. Bird for the Arctic specimens; Prof. J. E. Roberts for the camel bones; Dr. I. W. Hughes and the British American Tobacco Co. for help in the experiments on cigarette smoking and tobaccos; and Dr. G. A. Stewart, of Messrs. Burroughs Wellcome and Co., for assistance with the measurements on insulins. I am indebted to several of my colleagues for their helpful advice, and to Mr. Howard Dawson for

carrying out most of the measurements. Finally, I thank Prof. W. V. Mayneord for his support and advice.

- ¹ Crooks, R. N., et al., *A.E.R.E.-R* 2952 (1959).
- ² Burton, W. M., and Stewart, N. G., *Nature*, **188**, 584 (1960).
- ³ Cambray, R. S., et al., *A.E.R.E.-R* 4392 (1963).
- ⁴ Patterson, R. L., and Lockhart, L. B., in *The Natural Radiation Environment*, edit. by Adams, J. A. S., and Lowder, W. M., 383 (Univ. Press, Chicago, 1964).
- ⁵ *I.C.R.P.—Report of Committee II* (Pergamon Press, 1959).
- ⁶ Hill, C. R., *Nature*, **187**, 211 (1960).
- ⁷ Cherry, R. D., *Nature*, **203**, 139 (1964).
- ⁸ Holtzman, R. B., *Health Phys.*, **9**, 385 (1963).
- ⁹ Holtzman, R. B., in *The Natural Radiation Environment*, edit. by Adams, J. A. S., and Lowder, W. M., 227 (Chicago Univ. Press, 1964).
- ¹⁰ Radford, E. P., and Hunt, V. R., *Science*, **143**, 247 (1964).
- ¹¹ Marsden, E., *Nature*, **203**, 230 (1964).
- ¹² Osborne, R. V., *Nature*, **199**, 295 (1963).
- ¹³ Stahlhofen, W., *Assessment of Radioactivity in Man*, **2**, 505 (International Atomic Energy Agency, Vienna, 1965).
- ¹⁴ Hurst, J. B., *Science*, **132**, 1666 (1960).
- ¹⁵ Segall, A., *Science*, **140**, 1337 (1963).
- ¹⁶ Little, J. B., et al., *Rad. Res.*, **22**, 209 (Abstract 119) (1964).
- ¹⁷ Fink, R. M., *Biological Studies with Polonium, Radium and Plutonium* (McGraw-Hill, 1950).
- ¹⁸ Lacassagne, A., and Lattès, J., *Bull. d'Hist. Appliquée à la Physiol. et à la Pathol.*, **1**, 279 (1924).
- ¹⁹ Thomas, R. G., *Rad. Res.*, Supp. **5**, 29 (1964).
- ²⁰ Feldman, I., and Saunor, P., *Rad. Res.*, Supp. **5**, 40 (1964).
- ²¹ Hill, C. R., *Health Phys.*, **9**, 952 (1963).
- ²² Holtzman, R. B., *Health Phys.*, **10**, 783 (1964).
- ²³ Morken, D. A., *Amer. Med. Assoc. Indust. Health*, **20**, 505 (1959).
- ²⁴ Neary, G. S., et al., *Intern. J. Rad. Biol.*, **6**, 127 (1963).
- ²⁵ Davies, D. R., and Bateman, J. L., *Nature*, **200**, 485 (1963).
- ²⁶ Batchelor, A. L., Phillips, R. J. S., and Searle, A. G., *Nature*, **201**, 207 (1964).
- ²⁷ Black, S. C., *Health Phys.*, **7**, 87 (1961).

PATTERN MEASUREMENT

By DR. A. WARD and G. W. McMASTER

Radiation Laboratory, University of Strathclyde

PATTERN measurement is a rapidly growing development which is likely to affect a number of very different fields of study to a much greater extent than has previously been the case. The term is used here to describe any technique in which an instrument (usually of an electronic and optical nature) is used to scan an image or a permanent photographic record of an image, store the information obtained from the scan and finally analyse this information in terms of a series of parameters the quantitative values of which describe the features of the image which are essential, or at least of interest.

Clearly such instruments merely carry out essentially the same function as the combination of the human eye and part of the human brain. Indeed, those engaged in the field of pattern measurement develop a very healthy respect for the flexibility, speed and selectiveness of the human eye and brain in recognizing patterns. Unfortunately, as we all know, the human eye and brain are subject to fatigue; the brain is easily distracted and finds it difficult to remember with complete clarity the details in different parts of the same photograph and in comparing a series of similar photographs.

Bearing in mind the enormous capacity of a photograph to record permanently and conveniently the essential data in an image, it is understandable that most instruments in pattern measurement are designed to scan photographs rather than the original image.

Progress at Strathclyde

Nearly three years ago research and development were begun here to produce a series of instruments for pattern measurement. After a number of abortive starts using electro-mechanical devices of a fairly elaborate nature, the first of these instruments has been completed.

The problem in all instruments of this type, or at least the main problem, is to build into the instrument a very

high degree of stability—short-term stability over periods less than a second and also long-term stability over periods of the order of days, weeks or even months. Unless such stability is present the advantages of the instrument over the human eye and brain are largely lost. There is simply no room for erratic unknown errors in the three processes of scanning, storage and analysis.

Other factors involved include the total number of points into which the photograph can be divided, the geometrical shape of the scan, the range of grain densities that may be encountered, the speed of the scan, the speed, capacity and permanency of storage of information obtained from the scan, the speed of extraction of this information and the flexibility and speed of its analysis. This does not exhaust all the practical factors which are encountered; for example, it is quite important to have visual monitors at different parts of the system, not only to check for satisfactory operation but also to permit satisfactory adjustment of the system to deal with particular types of images. To satisfy the demands of stability, speed and flexibility we have found it necessary to develop purely electronic/optical systems.

The particular instrument described here (Mark 1) is made up of three units which carry out the functions of scanning, storage and analysis respectively. The scanning unit, which will be described in a little more detail later, is an original instrument designed by ourselves in collaboration with General Electric Co., Ltd., who built it for us using modern, fully transistorized circuits. The storage unit is a Laben 512 channel analyser used in its multi-scaler mode of operation, and we exploit three of its facilities for recording the data stored in its magnetic memory unit. These three facilities are a paper-tape puncher, an electric typewriter and a 'Polaroid' camera, which photographs a convenient display of the stored data. The analysis unit is a Ferranti *Sirius* computer.

Scanning Unit

Returning to the scanning unit, this is essentially a flying-spot scanner in which a raster of successive spots of the face of a cathode-ray tube is focused on a transparency (the photograph being examined) in the form of a 35-mm slide. Each spot had a duration of 1/10 of a second, and a measuring photo-multiplier behind the transparency provides a current which is proportional to the amount of light passing through the particular point on the transparency. Circuitry in the instrument converts this analogue current into a digital output which can vary between 0 and 128 depending on the grain density at the point being examined. The darker the film at a point the smaller is the integer which represents the measure of the grain density at this point. The instrument therefore accommodates 128 shades of grey and facilities are provided for varying over wide limits the range of grain densities corresponding to these 128 shades. The lower limit of grain density corresponding to 128 can be varied independently. The raster of points on the face of the cathode-ray tube can have from 4 to 512 points on each line and from 4 to 256 lines in each complete scan. The photograph being examined can therefore be divided into a total number of points varying from 16 to rather more than 125,000. The size of the raster can be varied electronically, thus providing a convenient method of continuously variable magnification in the scanning procedure. The area being scanned can be varied over a factor of 256. For convenience in setting up operations, prior to the actual scanning procedure, a display cathode-ray tube is built into the instrument which provides a reproduction of the selected area of the photograph. This reproduction looks very like the picture obtained on a television monitor. A line grid drawn on the face on this display tube makes it possible to correlate what is seen visually on the display tube, when the instrument is used in the viewing mode of operation, with the output on, say, the paper-tape puncher or visual display of the results of a scan as stored by the Laben 512 channel analyser. As it is possible to move the raster independently in the vertical or horizontal directions, a particular part of the display seen in the viewing mode of operation can be selected for scanning. Much more detailed information on this instrument will be given elsewhere, as it embodies a number of circuits entirely concerned with the problem of stability. However, it may be worth mentioning here that the instrument is driven by a master oscillator in both the high-speed viewing mode of operation and in the slow-speed scan, and a second photo-multiplier is used which monitors continuously the brightness of the spot of light on the cathode-ray tube (which provides the raster) and through feed-back circuits ensures that the amount of light in the spot focused on the transparency remains very constant.

In using the mark I model for pattern measurement a total of thirty 35-mm slides can be loaded into the scanning unit and examined separately one after another. Having viewed a selected slide by operating the scanner in the viewing mode of operation, it is then scanned in a series of 512 points. The 512 integers, varying between 0 and 128, corresponding to the digital output of the scanning instrument, are stored in the magnetic memory unit of the Laben 512 channel analyser. After visual inspection of these 512 numbers of the display tube of the Laben, they are printed-out on the paper-tape puncher. These operations are repeated until the whole of the selected area on one 35-mm slide has been scanned, and stored permanently in the form of the paper-tape print-out. This entire procedure can then be repeated for successive 35-mm slides, the paper tapes being inserted into the Ferranti *Sirius* computer which we programme to analyse the data.

Applications

Clearly this technique can be used to measure patterns in a series of photographs, or even just one photograph,

in a wide variety of fields ranging from studies of chromosomes, a comparison of cancer and normal cells (see pp. 485 and 486 of this issue of *Nature*) to more detailed examination and classification of astronomical objects such as galaxies, Sun spots, the surface of the Moon and so on. These are the projects under way here at present, but it has been said that if an object can be photographed at all or if certain characteristics in a problem can be recorded photographically (for example autoradiographs or sweeps on a cathode-ray tube), then a suitable choice of a pattern measurement instrument may be of considerable use in extracting essential features of interest from the mass of data which are so easily recorded by a photograph. There is little doubt that instruments for pattern measurement will displace the unsupported human observer more and more as the years pass and techniques of measurement and problems being tackled become ever more ambitious and more sophisticated.

As an illustration of the operation of the instrument described here, Fig. 1 shows the result of a line scan across a photograph of the spiral nebula M51. Fig. 2 shows the corresponding appearance of the nebula as observed on the cathode-ray tube of the scanning unit operated in the viewing mode. The variations in grain density across the photograph of the galaxy are very striking. They show how all the information recorded in such a photograph can be transformed into an array of integers which can be permanently stored in the form of the paper-tape print-out and analysed at our convenience according to whatever programme is selected for the computer.

General Remarks

Perhaps the best way to think about pattern measurement is to consider the data, when inserted into the analysis.

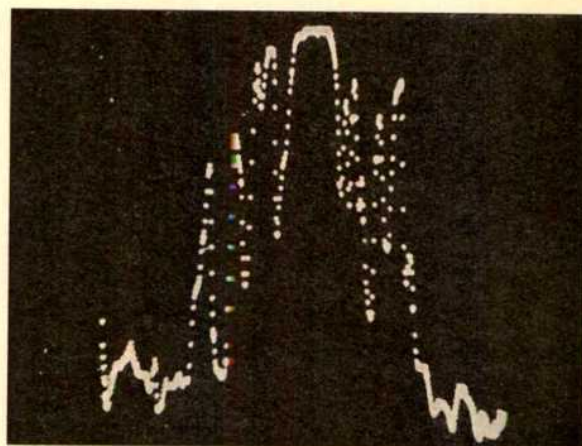


Fig 1

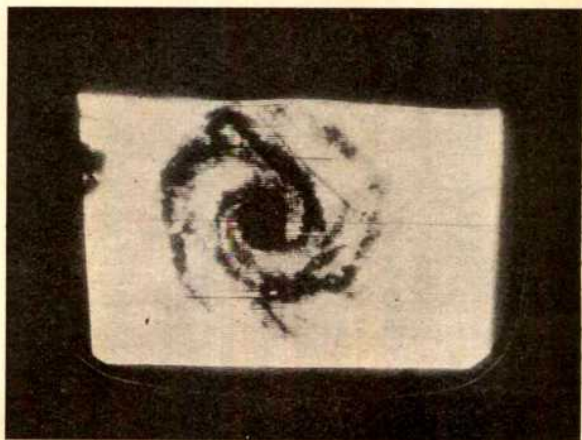


Fig. 2

ing computer, as a three-dimensional model of the original photograph or image. The original ordinary two dimensions are preserved by the geometry of the scan and the third dimension is the measured grain density. The function of the programme inserted into the computer is to 'look' at this three-dimensional model in a quantitative analytical way and measure values for parameters of interest. The word pattern reflects the fact that whatever the image is it will have a pattern which can conveniently be described in terms of a series of parameters which can be measured. The weakness of the human eye and brain is their inability to make precise measurements on these parameters and remember them sufficiently accurately for purposes of comparison. However, at least at present, the technique of pattern measurement still depends on the remarkable ability of the human eye and brain to recognize qualitatively the existence of parameters to describe the pattern of interest and thus make it possible to set up the appropriate programme for insertion in the computer.

Although devices for pattern measurement are not new developments, it is probably true to say that fairly recently, since the development of computer-type transistorized circuits and their widespread use, a number of instruments have appeared or are being developed which raise quite exciting possibilities when one considers, for example, the wealth of beautiful photographs obtained with great care and labour and now stored away in laboratories throughout the world. In fact, the development of pattern measurement makes it even more worth while straining to obtain even better photographic records.

We shall be pleased to hear from laboratories, here or abroad, interested in the possibility of collaboration in applying our instruments for pattern measurement to an analysis of the data recorded on existing or projected photographs.

It is our intention, funds permitting, to continue with the development of successive models with even wider facilities, faster speed of scanning and specialized computer and inspection facilities built into the original instruments. In this field, the continued development of genuinely useful instruments depends on the feed-back of experience gained by practical applications.

As a result of such feed-back we have come to the conclusion that, in general, there is no need to consider really large generalized computers even when pattern measurements are being made on complex images which require very large numbers of points to describe them adequately. The use of such computers is really only necessary if one insists on inserting all the information obtained, by scanning a complete image, before commencing the process of analysis. A study of our programme using the mark I instrument suggests strongly that it is worth considering pattern measurement instruments in which one, two or perhaps three lines only are scanned at a time and stored temporarily. This would be followed by immediate analysis using small specialized computer units to extract provisional values for the parameters of interest. These parameters are relatively few in number compared with the total number of points on one or two lines, and the provisional (or line) values of the parameters can be stored for subsequent analysis by a small final computer unit when all the image has been scanned and all the provisional values obtained. This separation of the computer analysis into two parts, provisional and final, is the major difference in our own work and similar efforts in the United States. It does not seem to introduce any great limitations for most images, it avoids the expense of time on large computers which can include the rental of land lines. Perhaps best of all, it opens up the real possibility of relatively inexpensive pattern measurement instruments for individual laboratories where the entire operations and the results are immediately in the hands of individual workers, thus allowing the all-important changes and adjustments which occur in any practical developing project.

This and other points are included in the projected instruments, now being designed here.

We acknowledge the provision of funds by the Department of Scientific and Industrial Research which enabled us to pursue this work, and the award of a D.S.I.R. research studentship to one of us (G. W. McM.). We also thank Mr. Donald Chadney of General Electric Co., Ltd., for his skilful choice of circuitry and components in the design and the successful construction of our present mark I instrument.

OBITUARIES

TWO PIONEERS OF PEST CONTROL

BY strange coincidence the two scientists who had the greatest influence on the development of the initially independent firm of Pest Control, Ltd., died within a few months of one another. Dr. W. E. Ripper crashed in the snow-covered mountains of Greece on March 21, 1965, while on a solo flight, though this fact was not known with certainty until his remains were discovered in June. Dr. E. Parry Jones died in hospital after a long illness, following a longer period of failing health, on June 30.

Both were trained as entomologists. Both gave the best years of their lives to control of pests in agriculture and particularly through their service to the same Company. Their abilities, service and successes were largely complementary. Both brought to bear talents which are essential for the development of a technically based industry. Ripper was essentially an initiator, a creator; sometimes ruthless, sometimes careless under his powerful inner drive to get things going. Parry Jones appeared far more conservative, but this was largely the response of a strongly self-disciplined man to the circumstances he had to face. Ripper's policy had brought the Company into a difficult financial position, and Parry Jones was insistent that, in the next phase of development, novelty and originality had to be kept within the framework of the commercially practicable.

The nature of the untimely death which overtook these men is in a strange sympathy with this difference between them. Ripper's friends had always advised him to forgo his passion for solo flying owing to deterioration of his eyesight, but he was an irrepressible enthusiast for physical as well as mental action, a keen skier and water-skier. He would perhaps have preferred to leave this world suddenly at the height of his powers. Parry Jones, also a keen sportsman in his younger days, had known himself to be in declining health for a long time owing to serious failure of lung capacity. He would not openly admit it, certainly never make a fuss about it, and strongly resented any tendency on the part of his colleagues to do so, but it was clear that he carefully conserved his physical energy and knew that he had to do so. Perhaps the discipline he had to apply to himself helped him to apply a similar discipline to the Company which he nursed round, after its acquisition by Fisons, Ltd., to become Fisons Pest Control, Ltd., from a position of considerable financial loss to one of sound financial profit.

These two men, in their different ways, were leaders in the field of applied science. Without the one the Company of Pest Control would never have started. Without the other it would not have survived the problems of adolescence. The death of both will be a great loss to many.

Dr. W. E. Ripper

Walter Eugen Ripper was born on January 24, 1906, in Austria, the son of Ministerialrat Max Ripper. He was a precocious child and a lot of his stimulus to scientific inquiry came from his father. He once told me that his earliest scientific publication was produced in his teens. He joined the United States Department of Agriculture as entomologist in 1931, working in Europe on the study of natural parasites for the control of pests introduced into the United States. He also acted as consultant to several large seed-growing estates in Austria and Hungary, devising special machinery for spraying unusual crops.

He realized that much good entomological research was being frustrated by the fact that only growers of very expensive crops could afford the necessary spray equipment. In 1938, at a congress in Berlin, he met Sir Guy A. K. Marshall, director of the Commonwealth Institute of Entomology, who was worried by the same difficulty. As a result, Walter Ripper settled in Cambridge, and in 1939 founded Pest Control, Ltd., of which he was managing director and Sir Guy was chairman.

Their aim was to bridge the gap between research and farming by providing a complete service, including machines, chemicals, labour and technical advice. By training, experience and temperament, Ripper was probably better equipped than anyone in Europe for such an enterprise. A man of tremendous physical and mental energy, he worked from 8 a.m. to midnight, seven days a week, driving himself sometimes to the verge of breakdown. A similar pace was expected of his staff, and it is a tribute to his power of inspiration that he was not disappointed.

For many people his drive to apply and exploit obscured an insatiable appetite for knowledge as such. Perhaps I saw more of this facet of a complex man than most. Except in the quiet *tête-à-tête* he would rarely admit ignorance, except, quite clearly, to himself, and then he would always take steps to correct it.

Ripper was always very quick to see the practical application of new research developments. In 1940 he introduced the weedkiller DNOC, which became mainly responsible for the disappearance of the picturesque sheets of red poppies which used to disfigure East Anglian cornfields. His first contract service in England was for the control of aphids by means of a portable nicotine fumigator. This gave a hitherto unobtainable degree of control, and without harm to predacious insects.

When the results of German research became available after the Second World War, Ripper was the first to seize on the idea of systemic insecticides, which could achieve selectivity and access to hidden insects without the need for special machines. His first introduction, 'Schraderin', was also selective, leaving predators to 'mop up' aphids which might survive to give rise to resistant strains. Ripper always thought in terms of 'tailor-made' insecticides, each designed to kill one pest selectively. However, the increasing strictness of regulations for the use of insecticides and the sheer cost of obtaining official approval for a product of relatively limited market forced him to abandon this ideal.

Bringing research results to the farm was very often an engineering problem, and Ripper devised many special machines for the purpose. Even before helicopters became available for civilian use he began to consider their use as air-borne spray machines, and was the first to develop commercial aerial crop-spraying in the United Kingdom.

Shortly after the take-over of his Company by Fisons, Ltd., in 1953, Ripper severed contact with it. It was rather inevitable that the impatient initiator should not be in sympathy with the conservative policy the new management felt necessary to adopt. He joined the Dow Chemical Co., with whom he had had many previous contacts, to found their British subsidiary, Dow Agro-

chemicals, Ltd., in King's Lynn, near to his second wife's large farm at Docking, on which he carried out many experiments. He left Dow to return to his interests in contract spraying, founding Crop Savia, Ltd., in the Sudan, and pursuing further the possibilities of 'robot' aircraft for crop spraying. At the time of his death he was giving much thought to the careful, technically supervised, use of insecticides to avoid undesirable side-effects, a subject that had always been of intense interest to him.

He married first, in 1938, Berthe Siedeck, by whom he had a son and two daughters, and second, in 1952, Nancy Deacon. His son and his second wife intend to continue some at least of his uncompleted work.

Dr. E. P. Jones

Dr. Elwyn Parry Jones, or "P. J.", as he was invariably known to his friends and colleagues, was born in 1907 in Barry, Glamorgan, and educated at Taunton School. He obtained a first-class honours degree in zoology at the University of Wales and continued his education at the Imperial College of Tropical Agriculture, Trinidad, and at the University of Edinburgh, where he gained a Ph.D.

After a short period as a lecturer in the University of Aberdeen, he was seconded to the British South Africa Co. as a research entomologist at the Mazoe Citrus Experimental Station, Rhodesia, where he worked on the control of pests and diseases of citrus crops, until the beginning of the Second World War. After service in the Royal Air Force in Rhodesia, he was invited by Ripper to become the first managing director of a newly formed subsidiary company of Pest Control, Ltd., in Africa, and he was responsible for getting this Company, Pest Control (Central Africa), Ltd., off the ground.

The major activity of this new Company was control of pests of the tobacco crop, but it was also concerned with maintaining public health, and under Parry Jones's guidance it grew and thrived. Being closely associated with tobacco growing, he came to realize that there was an urgent need for a research centre to study improved pest control techniques if the industry was to survive and prosper, and he succeeded in interesting the Rhodesian Government and the Rhodesia Tobacco Association in the formation of the Pest Control Research Scheme, in which his Company was a partner. This arrangement led to the setting up of the present Tobacco Research Board.

Although he was keenly interested in African affairs, he declined an invitation to enter Rhodesian politics, feeling he could serve the country and the pesticide industry better in the Company.

When he returned to the United Kingdom in 1953, he became a member of the board of the parent Company and, shortly afterwards, it was taken over by Fisons, Ltd., and became Fisons Pest Control, Ltd. In the same year, he was made technical general manager, and in this position he was responsible for the research work carried out at Chesterford Park Research Station, where important chemicals used in farming have been developed.

In 1958, Parry Jones was made joint managing director, and in 1961, chairman, and managing director. He was also chairman of Fisons Farmwork, Ltd., a member of the Fisons group main board and of the board of Fisons International Division.

He believed in long-term thinking, with an emphasis on a high level of research, and he considered the well-being of the staff to be of the greatest importance. He attached a high priority to education, and he took a great interest in the instructional courses organized by the Company for farmers, merchants, agricultural workers and advisers.

He saw the Company through its most difficult period. Wasteful marginal activities were pruned. Engineering research and development were abandoned, although the

contracting organization, separately managed as Fisons Farmwork, Ltd., was prosperously maintained. Concentration was on chemicals. Realizing that the search for new chemicals is becoming increasingly difficult and costly, he followed through the initiative of Avison Wormald, the first managing director under the Fisons régime, in negotiating development agreements with other organizations. Most important was the link-up with the Swiss giant, J. R. Geigy S.A., but this was followed by arrangements with Kureha Chemical Industries, Ltd., of Japan, and Boots Pure Drug Co., and successful licences were obtained from others for compounds which his own Company was well fitted to exploit.

Dr. Parry Jones leaves a widow, two sons and a daughter.
G. S. HARTLEY

Prof. K. R. L. Hall

PROF. RONALD HALL'S untimely death on July 14 of this year, at the early age of forty-seven, came as a shock to all who knew him, and not least to his colleagues in the Department of Psychology, in the University of Bristol, where he had been professor since 1959.

During the six years in which Prof. Hall held the chair in the University of Bristol, he had built up an extensive research programme for the comparative study of social behaviour in primates. He was especially interested in early learning and the development of social behaviour in monkeys and baboons. He wrote extensively on the comparison between monkey and ape behaviour, and felt strongly that, since the early writings of people such as Zuckerman and Carpenter, so little had been done to further field studies of a comparative type. He himself was as much concerned with laboratory experiments as with field studies, and he was also concerned with the systematic accumulation of data from all sources to contribute to a picture—detailed in depth—of the behaviour of the animals who come nearest in human evolution to man.

Prof. Hall's work ranged from detailed studies of a specific kind, such as the social vigilance behaviour in the Chacma baboon, to a study of the sexual activity of the same animal, and on to more general efforts to build up an integrated picture, ultimately, of human behaviour.

It was the problems of human behaviour that, in the end, he was working to unravel. His interests in animal studies dated from his period as professor of psychology at the University of Cape Town, which lasted from 1954 until 1959, when he returned to Bristol. Since 1959, he has achieved an international reputation for his work, and has by now published extensively in a wide range of journals. During 1962–63, he was a Fellow at the Centre for Advanced Study in the Behavioural Sciences at Palo Alto, California. He returned to East Africa in 1963 to carry out further ecological and behavioural studies of monkeys and to build more detail into his expanding theoretical framework of behaviour.

Kenneth Ronald Lambert Hall was the only son of Kenneth Lambert Hall, a former acting Governor and Commander-in-Chief of Nyasaland. He was educated at Cheltenham College and at Brasenose College, Oxford, where he took his first degree in Law. During the Second World War, he served in the Middle East and in Germany as a captain in the Royal Artillery, and later as a staff officer. After the War, he returned to Oxford to study psychology, and was awarded the degree of D.Phil. in 1949. In the same year he was appointed head of the Department of Experimental and Clinical Psychology in Bristol Mental Hospitals and was also part-time lecturer in the Department of Psychology at the University of Bristol. During this period his interests were primarily in experimental abnormal psychology, a field in which he built a considerable reputation before his appointment in Cape Town, and his change of primary interest to his ethological work.

He was an exceptional athlete, and as an undergraduate played rugby football for the Oxford University Greyhounds and was awarded a blue for boxing. He continued to play cricket, tennis, squash and golf until his recent illness.

His loss is inevitably a great blow to the development of ethology, and the understanding of animal behaviour, especially as viewed as a basis for a comparison with, and understanding of, human behaviour. He leaves behind him, however, a collection of substantial publications which must in the course of time be brought together into a single collection of his life work.

In 1941 he married Pauline Sophie Assinder, who survives him.
F. H. GEORGE

NEWS and VIEWS

Science Education Officer, British Council :

Mr. D. G. Chisman

MR. D. G. CHISMAN, education officer of the Royal Institute of Chemistry, has been appointed to a new post of science education officer at the London Headquarters of the British Council from January 1966. The Council is increasingly being called on to provide assistance in the expansion and reform of science teaching in schools overseas, particularly in the developing Commonwealth. It now has six science teaching specialists in East and West Africa whose function is to provide assistance with the reform of the curriculum and methods, and the adaptation to local needs and conditions of the results of recent and current British work in this sphere. The new post will serve as a point of reference for the Council's overseas officers, keep them in touch with up-to-date British trends and supply information on new equipment, text-books and audio-visual aids, as well as on new teaching methods. Mr. Chisman, after graduating in chemistry at King's College, London, took a postgraduate certificate of education, and had some years practical secondary school teaching experience before joining the Royal Institute of Chemistry. Since then, as a secretary to the British

Committee on Chemical Education and as a member of both the Consultative Committee for the Nuffield Chemistry Project and the Physical Science Committee of the Association for Science Education, he has been closely concerned with the study of the problems of school science teaching in Britain, and has also been involved with overseas problems as a consultant to the Organization for Economic Co-operation and Development and Unesco.

Zoology in the University of Newcastle upon Tyne :

Prof. A. D. Hobson

PROF. A. D. HOBSON retires from the chair of zoology in the University of Newcastle upon Tyne in September 1966, and is spending most of the year 1965–66 on sabbatical leave. He has been professor of zoology at Newcastle and director of the Dove Marine Laboratory since 1932, first under the title of Armstrong College, then King's College, and, since 1963, as the separate University of Newcastle upon Tyne. During his time at Newcastle he has seen the Department grow from a stage when it consisted of three lecturers, one of them half-time, to its present size of sixteen teaching staff. The closely associated Dove Marine Laboratory has also expanded during this period.

Prof. Hobson's early work was in experimental cytology and he was one of the group of young biologists concerned with the founding of the Society for Experimental Biology. During the Second World War, he worked on nematode physiology, particularly on cultural methods for *Ascaris*; his scholarly work on this subject is well known. Prof. Hobson's other great interest has been marine zoology and, in addition to his directorship of the Dove Marine Laboratory, he has been a member of both the North-East and the Northumberland Sea Fisheries Committees. Prof. Hobson has worked devotedly for the welfare of the Department of Zoology at Newcastle during this period of expansion and change. Many former students and colleagues in zoological work all over the world will wish him every happiness in his retirement.

Prof. R. B. Clark

DR. R. B. CLARK, who has been appointed to succeed Prof. A. D. Hobson, will carry on the tradition of work in marine biology so long established at the University of Newcastle. Dr. Clark started his career as a physical scientist, graduating from Chelsea Polytechnic in 1944 and taking up a post as scientific officer in the Road Research Laboratory of the Department of Scientific and Industrial Research. In 1947 he entered University College, Exeter, to read zoology with Prof. Harvey, gaining his special honours degree in 1950. From 1950 until 1956 he was assistant lecturer at Glasgow, though his tenure there was interrupted by periods spent in the University of Washington, Seattle, and at Berkeley, where he was assistant professor in the University of California from 1953 until 1955. He was appointed lecturer in zoology in the University of Bristol in 1956, and gained his London D.Sc. in 1965. His special interests in marine zoology have centred round the annelids, with particular reference to the polychaetes. Within this group, however, his work has ranged widely. He has contributed substantially to ecology and natural history, and his investigations on polychaete morphology undoubtedly stimulated his interest in the physical factors concerned in the evolution of invertebrates—illustrated in his recent book *Dynamics in Metazoan Evolution* (Oxford, 1965). Dr. Clark is probably best known for his extensive contributions to annelid neurosecretion and neuroendocrinology, where his work has been almost that of a pioneer in annelid physiology. More recently he and his students have published several papers on behaviour in polychaetes, with particular reference to the learning process. His interests will therefore admirably complement and extend those of the active group of zoologists now in the Department.

Metallurgy in Brunel College, London :

Prof. C. Bodsworth

DR. C. BODSWORTH, at present chief physical metallurgist at the Central Research Laboratories of Richard Thomas and Baldwins, Ltd., has been appointed to the newly established post of professor and head of the Department of Metallurgy at Brunel College of Advanced Technology. His early industrial training was with the United Steel Co., Ltd. In 1950, after three years War service with the Royal Corps of Signals, he graduated in metallurgy at the University of Sheffield. He was awarded an M.Met. in 1951 and a Ph.D. in 1957. From 1952 until 1964 he was a lecturer in the Department of Metallurgy in the University of Liverpool. Dr. Bodsworth's scientific interests have centred on metallurgical thermodynamics, and he has written two books on this subject. He has also been actively engaged in investigations of various aspects of phase transformations in ferrous materials.

Assistant for U.S. Medical Research Fellowship Analyses : Dr. E. C. Bracken

DR. EVERETT C. BRACKEN has been appointed assistant to the Chief, Research Fellowships Branch, National

Institute of General Medical Sciences, National Institutes of Health. As professional assistant to Dr. Frederick P. Ferguson, Dr. Bracken will be responsible for conducting analyses of National Institute of General Medical Sciences fellowship programmes to evaluate their effectiveness and to identify scientific areas requiring special emphasis. For the past six years, Dr. Bracken has been at the University of Oklahoma School of Medicine, serving as associate professor in the Microbiology Department since 1960, and as associate professor in the Pediatrics Department and director of the Infectious Diseases Laboratory, Children's Memorial Hospital, since 1959. Dr. Bracken was previously at Vanderbilt University, where he was an instructor and assistant professor in the Microbiology Department of the School of Medicine from 1954 until 1959, and a teaching assistant and instructor in the Biology Department from 1951 until 1954. He is a member of the American Society for Microbiology, the New York Academy of Sciences, the Electron Microscopy Society of America, the American Society for Experimental Pathology, the Southern Society for Pediatric Research, Phi Beta Kappa, and Sigma Xi.

Minister of Power's Advisory Council on Research and Development

MR. F. LEE, the Minister of Power, has appointed Sir Charles Sykes to the chairmanship of his Advisory Council in Research and Development. Sir Charles Sykes succeeds Lord Fleck, who has served as chairman of the Advisory Council since it was constituted in July 1960 and is now retiring. Sir Charles Sykes became a member of the Advisory Council on Research and Development on May 27, 1965. He is managing director of Thos. Firth and John Brown, Ltd., and chairman of Firth-Vickers Stainless Steel, Ltd. He served as a member of the Advisory Council for Scientific and Industrial Research from 1954 until 1956 and as a member of the Research Council of the Department of Scientific and Industrial Research from 1962 until its dissolution in March 1965. Lord Fleck served as chairman of the Minister of Power's Scientific Advisory Council from 1958 until 1960.

The terms of reference of the Advisory Council are:

- (1) To advise the Minister of Power on research and development in relation to his statutory duty of securing the effective and co-ordinated development of coal, petroleum and other sources of fuel and power in Great Britain, and of promoting economy and efficiency in the supply, distribution, use and consumption of fuel and power, whether produced in Great Britain or not.
- (2) To advise the Minister of new scientific and technical knowledge or applications of knowledge throughout the world, which in the opinion of the Council should be taken into account in the performance of his statutory duties.
- (3) To keep the whole field of fuel and power under continuous review with the object of identifying problems needing research and development and advising the Minister of these problems with the view of discussion with the industries concerned.

Other members of the Council are: Captain (E.) W. Gregson (deputy chairman), Mr. M. A. L. Banks, Prof. P. M. S. Blackett, Sir Charles Cawley, Mr. H. E. Collins, Sir Kenneth Hutchison, Sir Harry Melville, Mr. R. V. Moore, Dr. L. Rotherham and Prof. M. W. Thring. The assessors are Dr. E. Lee, Mr. H. T. Ramsay and Mr. H. S. Stephenson.

University Entrance Requirements in Britain

THE third edition of *A Compendium of University Entrance Requirements for First Degree Courses in the United Kingdom, 1965*, which has been compiled from material provided by the universities to the Committee of Vice-Chancellors and Principals, has been extensively revised and is more comprehensive than either of its

predecessors (Pp. 219. London: The Association of Commonwealth Universities, 1965. 11s. 6d.). Its coverage of combined honours courses has been extended and also expanded to include details of the entrance requirements of the ten colleges of advanced technology and the Heriot-Watt College, all of which are to have the status of university institutions. In addition, the tables include for the first time the entrance requirements of the Scottish universities, expressed in terms of the General Certificate of Education. In this edition the course requirements of the principal full-time first-degree courses offered by the universities of the United Kingdom are set out in terms of the General Certificate of Education in eighty tables, covering single honours, combined honours and general degree courses. The information provided in the text is supplemented by a self-indexing list of combined honours courses offered by fewer than four universities, and by notes on particular institutions the courses and requirements of which do not fall into the usual pattern. The compendium is not an official publication of the universities concerned and cannot be regarded as superseding their own publications, which are the ultimate authorities. The tables indicate that many universities formally require only two Advanced Level passes in the General Certificate of Education for most courses in arts, law and social studies (and for a few in science and technology), whereas other universities formally require three. Most candidates present three or more, which many university selectors regard as preferable. However, the pattern of academic entrance requirements may still be unduly complex, and it has been agreed that consideration of ways in which general and course requirements might be simplified will be one of the first tasks to be undertaken by the Standing Committee on University Entrance, a delegate body, established in June 1965, representative of the universities of the United Kingdom.

La Trobe University, Melbourne

LA TROBE UNIVERSITY, which will be the third University in the Melbourne metropolitan area, was established under an Act of the Parliament of Victoria on December 9, 1964. The site for the new University is an undeveloped tract of some 500 acres about 8 miles north-east of the city centre. Planning of the site works and initial buildings is proceeding and the University will open in March 1967. Planning and development of the University, in the present preparatory stage, are controlled by an Interim Council of twenty-five members under the chairmanship of J. R. A. Glenn, chairman and managing director of I.C.I.A.N.Z., Ltd. The principal officers appointed so far are: *Vice-Chancellor*, Dr. D. M. Myers (at present dean of the Faculty of Applied Science, University of British Columbia); *Librarian*, D. H. Borchardt. Applications have been requested for foundation chairs in English, history, philosophy, a modern European language, economics, sociology, politics, mathematics, physics, chemistry and biology; but so far no appointments have been made. The academic work of the new University is to be organized in schools of related disciplines. Each school will be the functional unit for teaching, research and administration in the disciplines which it embraces. Its field will be wider than that of a traditional department but narrower than that of a faculty. Student activities will be centred on colleges that will provide study, recreational, social, dining and some tutorial facilities for all members, and residential facilities for a minority of the members. All members of the academic, library and administrative staffs will also belong to the colleges.

Museums Journal

THE *Museums Journal* (65, No. 2; September 1965. 15s.) includes a number of interesting papers, in addition to a full report of the annual conference of the Museums Association in Dublin in June 1965. Dr. S. Dillon Ripley,

as secretary of the Smithsonian Institution, describes the Institution as a great museum centre; Mr. C. Douglas Deane gives details of the Field Museum and Nature Trail in Northern Ireland; and Mr. H. Wakefield gives much useful information to curators concerning the availability of purchase grants from the Victoria and Albert Museum, London, to provincial museums. Mr. N. Cook, president and keeper of the Guildhall Museum, London, dealt with many subjects in his address, chiefly those concerning the Museums Association in relation to Government grants. Dr. A. T. Lucas, director of the National Museum of Ireland, developed the theme of the role of the National Museum in the study of Irish social history, and Mr. R. Rowe spoke on "Art Museums and the Idea of Progress". At the annual meeting, Sir Frank Francis, director of the British Museum, was elected president for 1965-66, when the annual conference will be held in Sheffield during July 4-9.

British Museum : Department of Western Asiatic Antiquities

THE British Museum regrets to state that the Assyrian sculpture, mostly of Sennacherib and Ashurbanipal, from their palaces at Ninevah, which has been exhibited in the Assyrian Saloon and the Assyrian basement, will be for the most part unavailable for examination until further notice, owing to the rebuilding of the Assyrian Saloon and the insertion of a new floor.

Storage of Books

AN essay by P. W. Plumb, entitled "Central Library Storage of Books", which was awarded the Sevensma Prize, 1962, has been issued as *Library Association Pamphlet No. 24* (Pp. 57. London: Library Association, 1964. 16s.; L.A. members 12s.). It reviews experience gained in the United States, Britain and elsewhere, including the use of microfilm. There are brief appraisals of methods used at the British Museum Newspaper Library, the Storage Library of the University of London, the National Central Library and the National Lending Library for Science and Technology, as well as at the Bibliothèque Nationale Storage Library, and, in the United States, the New England Deposit Library, the Midwest Inter-Library Centre, and the Medical Library Center of New York. Mr. Plumb commends the systems of the National Central Library and the National Lending Library for Science and Technology, and concludes that a national scientific and technical lending library and one for the humanities and sociology in each country with similar but perfected aims and techniques would go far to solving the space problems of libraries. Certainly this suggestion, if applied, would help in avoiding the major disabilities apparent in many existing schemes and proposals.

Directory of Indian Scientific Periodicals

THE *Directory of Indian Scientific Periodicals, 1964*, with its 725 entries listing periodicals current to the end of 1963, including annual reports of scientific and technical institutions and other learned societies, is intended as a first step towards consolidating and servicing scientific communication in India (Compiled by G. K. Arora, S. Dutta, D. N. Gupta and H. N. Rangachar. Pp. 133. Delhi: Indian National Scientific Documentation Centre, 1965). The *Nisfor Guide to Indian Periodicals*, published at Poona in 1955-56, and a cyclostyled list of Indian scientific periodicals issued in 1960 laid some foundation for the present *Directory*. The entries are arranged according to the Universal Decimal Classification and there is an alphabetical index of titles. The periodicals are mainly in English, about half a dozen being bilingual with English as one language; there are 60 periodicals in Indian regional languages (33 in Hindi) and one in French. The *Directory* is well printed and well set out.

Volcanological Research in Britain

ON behalf of the Volcanological Research Committee of the Royal Society, the terms of reference of which are "to encourage, promote and co-ordinate the comprehensive study of volcanicity . . .", a booklet has been published to record the scope of researches being undertaken at present by universities and other scientific institutions in Britain on modern and ancient volcanoes (*United Kingdom Volcanological Research, 1965*. A summary of current volcanological studies with provisional plans for future years. Pp. 55. London: The Royal Society, 1965). The extent of the studies recorded is wide, embracing work at about a hundred localities outside the British Isles, described in close on 150 recent papers (inclusive of some reports of researches in experimental petrology). It is hoped to publish a further edition of the booklet in about two years' time when attention will be restricted to current investigations on Tertiary and Recent volcanicity.

Age Determinations of North American Rocks

A COMPILATION of the radiometric ages determined for rocks of the North American continent, complete up to May 1964, has recently been published by the Committee on Nuclear Science of the National Academy of Sciences—National Research Council in Washington (Publication 1276: *Geochronology of North America*. Nuclear Science Series—Report No. 41. Pp. v+315. Washington, D.C.: National Academy of Sciences—National Research Council, 1965. 6 dollars). In it, a brief introduction to the present status of geochronological methods, by Dr. G. W. Wetherill, is followed by a 270-page tabulation of results listed on a regional basis and by a bibliography of 144 items. The oldest well-dated rocks in North America bear ages of about 2,500–2,800 million years, and are found in an apparently continuous belt extending from Quebec through Ontario, Manitoba, Minnesota, North Dakota, Montana, and into Wyoming, as well as in the Great Slave Lake area of the North-West Territory. They are apparently much younger than the most ancient formations of southern Africa and of arctic Russia. Extensive recent data supplementary to the results in the American compilation have been published contemporaneously by the Geological Survey of Canada and are accompanied by a report by Dr. C. H. Stockwell which proposes a new time-stratigraphic classification and nomenclature for the rocks of the Canadian shield (Paper 64-17. Parts I and II. *Age Determinations and Geological Studies*. (I) Pp. iv+126. 75 cents. (II) Pp. iv+29. 75 cents. Ottawa: Queen's Printer, 1965). In this, the Archaean and Proterozoic are each identified as eons, with the latter divided into the Aphebian, Helikian (Palaeohelikian and Neohelikian) and Hadrynian eras, the lower boundaries of which are respectively marked by the Kenoran (2,390 m.y.), Hudsonian (1,640 m.y.; Elsonian at ?1,280 m.y.), and Grenville (880 m.y.) orogenies.

Soil Micro-fauna

THE formation of humus is a most important matter to foresters, not only because it helps to maintain proper conditions for tree growth, but also because it may bring about improvements to the quality of the trees and give greater productivity to the site. A big part is played by oribatids in the breakdown of the litter and its conversion into humus, and members of this super-family of the Acarinae occur in very large numbers in the soil fauna. In *Contribution à l'Etude Ecologique des Oribates de la Litière dans une Forêt de Moyenne-Belgique*, by Philippe Lebrun, we not only have an example of a very fine piece of research carried out in 13 months of field work, but also results presented in a full and yet concise manner, and set out with proper reference to the other factors affecting the biocenose (Institut Royal des

Sciences Naturelles de Belgique. Memoire No. 153. Pp. 96. Bruxelles: Institut Royal des Sciences Naturelles de Belgique, 1965). The published account is divided into three chapters. The first is an account of the locality factors. The research was carried out in an oak-wood on the south-eastern slopes of a small valley near Louvain in central Belgium in 1961–62. Information is given of the vegetation, soil, local climate, the water-holding capacity of the soil and the evapotranspiration. The second chapter is devoted to the synecology of the oribatids with the view of discovering the bonds existing between different groups of them. The final chapter deals with the autecology of the oribatids found on the site. The forester recognizes the great importance of these minute members of the animal kingdom, but it is not always remembered in what quantities they occur. In a Belgian oak-wood it is estimated that there are about 75,000 individuals per square metre of the upper soil and humus layers and even up to as many as 300,000 per square metre. Thus, their numbers alone present some difficulty to the research worker who, in this case, had to contend with 48 species in a sample population of 85,000 individuals. Philippe Lebrun has not only clearly described his fine piece of research but he has also taken into consideration, as is very proper but not always done, the various factors of the vegetation, soil and climate which contribute to the biocenose. Thus, the danger in such a work of omitting some important factor affecting the result has been avoided. The numerous histograms and graphs make for easy understanding of the various components of the investigation. Nothing seems to have been forgotten: for example, there is a histogram showing the precipitation reaching the soil compared with the actual precipitation. This is but one example of the detail to be found in a work which will be acclaimed by foresters and others interested in the soil micro-fauna.

Oil Pipeline over the Alps

It may not be generally realized that work is in progress on the colossal project of constructing a 40-in. diameter, 300 miles long, Trans-Alpine oil pipeline to convey oil from the Adriatic to the heart of Germany, as so far this scheme has received relatively little publicity. To a brief extent, this has now been remedied by an article entitled "Pipeline Over the Alps" (*Esso Magazine*, Esso Petroleum Company, London, Summer 1965). The route lies from Trieste, by Udine, Lienz, Kitzbuhel, Kufstein, Rosenheim, east of Munich to Ingolstadt. It necessitates the construction of three tunnels under the Alps, one known as the Plöcken Tunnel, between Udine and Lienz; one known as the Felber Tauern Tunnel, and another as the Hahnenkamm Tunnel, both between Lienz and Kitzbuhel. These are indeed major engineering feats in themselves, and the photographic illustrations included in the article, together with a panoramic map in colour, make this abundantly clear. This project will ultimately include the construction of a branch pipeline just north of the Plöcken Tunnel to link up with the Austrian State Refinery near Vienna. The Trans-Alpine pipeline is a venture in which several oil companies are taking part and in which the Esso Petroleum Company has the largest interest. However, because the pipeline has to pass through three separate countries, three separate companies have been formed for constructional and operational purposes in their respective areas. These companies are Deutsche Trans-Alpine Oelleitung G.m.b.H., Trans-Alpine Oelleitung in Österreich G.m.b.H., and Società Italiana per l'Oleodotto Trans-Alpino S.p.A. The German company is the co-ordinating company. Among the many practical problems concerned with such a project, apart from tunnelling and mechanical excavation in the high Alps, are the necessity to dredge the harbour at Trieste so that it can eventually accommodate oil tankers of 160,000 dead weight tons; setting storage tanks there on piles because

available land is a rocky hill site; construction of several thousand feet of piers into the Adriatic, so laying many connecting pipelines to the tank-farm. Involved also in the scheme is the building of five separate pumping stations, each equipped with two 4,000-horse-power electric centrifugal pumps required to lift hundreds of thousands of tons of oil from sea-level to one of the highest points at Felber Tauern. It would appear that completion of the main works and expectation that the new pipeline will be 'on stream' are likely in about two years' time, which will be a most remarkable achievement if realized.

University News:

Aberdeen

Dr. H. J. EVANS has been appointed to the newly created chair of genetics. The following lecturers have also been appointed: Dr. P. G. Jarvis (botany); J. F. Robbie (engineering); Dr. J. B. Wilson (geology); Dr. G. F. Burnett (agriculture).

Manchester

Dr. D. M. McDOWELL has been appointed to the chair of hydrodynamics and hydrology. The following lecturers have also been appointed: Dr. G. N. Smith (chemistry); W. McLewin (mathematics); Dr. A. R. Mainwaring (pathology); Dr. H. A. Lee (medicine).

Birmingham

THE following appointments have been made: *Lectureships*, Dr. C. J. Hooke (mechanical engineering); V. L. Moffat (engineering production); Dr. B. Sklarz (chemistry); Dr. J. H. Coote and Dr. A. R. Tindall (physiology); Dr. I. D. Green (medicine); Dr. N. R. Ling (experimental pathology); *Senior Fellowship*, Dr. J. M. Whittaker (pure mathematics); *Research Fellowships*, P. F. Adams, C. G. Drury and I. T. Franks (engineering production); K. J. Blackburn, C. J. Chesterton and J. Kendrick-Jones (biochemistry); S. F. Campbell, Dr. W. B. Hollyhead and M. A. Salam (chemistry); Dr. M. K. Das and Dr. M. M. Sadek (mechanical engineering); G. Immirzi and Dr. D. H. Lyth (mathematical physics); J. Jafar (physics); M. J. Pemberton (minerals engineering); M. W. Stanley (physical metallurgy); Dr. P. S. Hasleton (pathology); Dr. C. C. McCormick (experimental pathology).

Lalor Foundation Grants for 1966

THE Lalor Foundation has announced the programme of awards for 1966, which it is offering for support of research on the fundamental biochemical and physiological mechanisms concerned with fertility and the early stages of reproduction in various forms of life. The objectives are to further the knowledge and understanding of the basic phenomena involved and to extend and develop the possibilities for effective regulation and control. The awards are open to all nationalities and may range up to 8,000 dollars a year, depending on the scope and duration of the projects approved. Preference will be given to younger members of university and college faculties and staff, with an upper age-limit of forty-one years. The work may be carried on at the applicant's own institution or elsewhere. The Foundation will also grant post-doctorate summer or short-term research awards at the Marine Biological Laboratory at Woods Hole, Mass., or elsewhere for appropriate projects in the fields specified. These awards will normally not exceed 1,200 dollars for a single man or woman, 1,400 dollars for a married man working at his home institution, and 1,550 dollars for a married man with a principal programme at another institution. Further information and application forms can be obtained from the Lalor Foundation, 4400 Lancaster Pike, Wilmington, Delaware. The final date for receipt of executed applications, complete with supporting data, is January 15, 1966. Notification of appointment will be on or before March 15, 1966.

European Society for Comparative Endocrinology

At the third meeting of European comparative endocrinologists, held in Copenhagen during August 2-5, it was decided to form a European Society for Comparative Endocrinology. The following were elected as the first officers and council of the Society: *President*, H. Helle (Bristol); *Vice-President*, M. Fontaine (Paris); *Secretary and Treasurer*, P. G. W. J. van Oordt (Utrecht); *Honorary Editor of the Proceedings of the Society*, E. J. W. Barrington (Nottingham); *Council Members*, H. Herlant-Meewis (Brussels), C. Barker Jørgensen (Copenhagen), M. Lüscher (Bern), V. Mazzi (Turin) and V. J. A. Novák (Prague). Those interested in the aims of the Society are invited to write to the Secretary, Zoological Laboratory, State University of Utrecht, Janskerkhof 3, Utrecht.

Announcements

Dr. H. E. HUXLEY has been awarded the first William Bate Hardy Prize of the Cambridge Philosophical Society for his work on the structure of muscle. The William Bate Hardy Prize was established in 1964 by the Cambridge Philosophical Society to commemorate the centenary of the birth of Sir William Bate Hardy, a Fellow of the Society from 1889 until his death in 1934. It is awarded for the best original memoir, investigation or discovery by a member of the University of Cambridge in connexion with biological science published during the three years immediately preceding the award. The present value of the Prize is £200.

A SHORT course for librarians and information officers on "The Trade Literature Collection", arranged by Aslib, will be held at Aslib during November 25-26. Further information can be obtained from the Education Officer, Aslib, 3 Belgrave Square, London, S.W.1.

A SEMINAR on "Creativity in Science", arranged by the Science of Science Foundation, will be held at the Foundation on December 8. Further information can be obtained from the Science of Science Foundation, c/o the Ciba Foundation, 41 Portland Place, London, W.1.

THE second Australian conference on "Hydraulics and Fluid Mechanics" will be held at the University of Auckland during December 6-12. Further information can be obtained from the Convenor of the conference, School of Engineering, Ardmore College Post Office, University of Auckland, Auckland.

THE 1965 International Building Exhibition will be held at Olympia, London, during November 17-December 1. During the Exhibition, five conferences will be held: building for people; system building—can it be economic?; exporting building materials; the important role of building component manufacturers; the change to metric. Further information can be obtained from the Conference Secretary, the Building Exhibition, 11 Manchester Square, London, W.1.

A MEETING of the Biochemical Society will be held in the University of Manchester during November 11-12. The programme will include a colloquium on "Biosynthesis in Micro-organisms: Genetic and Environmental Factors"; a discussion meeting on "Biochemistry and Industry"; and the Colworth Medal Lecture on "The Role and Control of the Glyoxalate Cycle in *Escherichia coli*", to be delivered by Prof. H. L. Kornberg. Further information can be obtained from Prof. K. S. Dodgson, Department of Biochemistry, University College of South Wales and Monmouthshire, St. Andrew's Place, Cardiff.

ERRATUM. In Table 4 of the article entitled "Identification of Dinitro-octylphenols in Certain Commercial Fungicides", by Dr. A. H. M. Kirby and L. D. Hunter, which appeared on p. 189 of the October 9 issue of *Nature*, the last five lines should be amended to read as follows:

7-41(7g)	7-37g	7-26m	7-15(6 8se)	7-28(7 5t)	1-CH
1-91s {	1-89s {	1-85s	1-90s {	1-87s {	1-CH ₂
		1-88s			5-H
					3-H

HORTICULTURAL SCIENCE AT THE UNIVERSITY OF READING

THE diamond jubilee of the Horticultural Education Association was marked by a conference at the University of Reading during September 6-9. The object of the conference indicated by the theme, "Horticultural Science at the University of Reading", was to bring together contributions from a number of the branches of science that find a meeting point in the technology of horticulture. It was part of the success of the conference that it directed attention to the importance of border-line research between disciplines and to the desirability of combining pure and applied research.

Contributions from the Department of Horticulture were concerned with plant physiology and plant protection. Prof. O. V. S. Heath discussed methods of measuring stomatal aperture and pointed to the importance of a knowledge of stomatal physiology in attempts to control water loss from crop plants. Dr. H. Meidner demonstrated a portable and relatively cheap instrument for measuring the water content of soils by determination of their heat conductivity. One of the main advantages of the method was that the probe employed, unlike the porous block used in other techniques of soil moisture determination, did not have to equilibrate with water in the soil.

Papers by Dr. D. Vince and Dr. G. P. Harris were concerned with the photoperiodic control of flowering in long-day plants. Dr. Vince described experiments with different strains and species of *Lolium* that demonstrated a changing sensitivity to red and far-red radiation during a 16-h night. It appeared that in order to produce a maximum promotion of flowering by interruption of the night with red light, the timing of the interruption had to be varied according to the species or strain of *Lolium* used. The potentialities of detailed investigations of the photoperiodic responses of plants of horticultural importance were underlined in the paper by Dr. Harris on flowering in the glasshouse carnation. Out of a number of photoperiodic treatments tested, the use of lighting from tungsten-filament bulbs throughout the night was found to be most effective in promoting flowering under winter conditions. Applications of this observation to commercial practice were discussed. Dr. H. F. van Emden bridged the gap between plant physiology and entomology in a discussion of plant-insect relations. Results were presented indicating the role of the nitrogen status of the plant and particularly the soluble-nutritional content in controlling rates of aphid multiplication.

The Agricultural Research Council Unit of Flower Crop Physiology, which, under the direction of Prof. O. V. S.

Heath, is attached to the Department of Horticulture, was represented both in a comprehensive demonstration of equipment and in a paper read by Dr. A. P. Hughes. The main item of equipment in the Unit is 9 (later to be 12) growth cabinets designed by the National Institute of Agricultural Engineering and housed in a special hall with controlled ventilation. Dr. Hughes described the use of this equipment to investigate interactions of light intensity, temperature and carbon-dioxide concentration. He also presented results of experiments demonstrating effects of photoperiodic treatments on leaf expansion in a variety of plants and discussed the use of such treatments under conditions where light intensities are limiting to growth in dry weight.

In a contribution from the Department of Physiological Chemistry, Dr. K. A. Hassall discussed problems encountered in the use of crop protection chemicals including questions of toxicity, persistence and selectivity. Experimental results showed how the persistence and effectiveness of a pre-emergence weed-killer could vary with the amount of rain falling after application.

From the Department of Agricultural Botany there were papers on physiological ecology and plant breeding and genetics. Prof. A. H. Bunting described work on the water relations of plants growing on soils of the chalk downs near Reading and demonstrated the capacity of the chalk to retain water by reference to its pF -moisture content curve. The pore size distribution derived from this curve was shown to be similar to a distribution based on direct measurements of pore sizes in electronmicrographs. Dr. J. K. Jones and Mr. G. D. Rowley in contributions concerned with plant breeding both emphasized the value of using wild species as sources of genetic variation. Dr. Jones presented evidence that the sets of chromosomes in all species of strawberries were homologous, that it was possible to produce many inter-specific hybrids and to induce polyploidy and therefore to use most, if not all, of the species in breeding. Mr. Rowley showed how the derivation of cultivars of rose could be traced by crossing supposed parent species and underlined the importance of making provision for the conservation of species and cultivars that might not be of immediate commercial value.

This report indicates the varied nature of the papers presented; but the contributions had in common an immediate or potential value to horticulture. The conference provided members with the opportunity to examine the role of the University in the development of this subject.

G. P. HARRIS

THE WORLD HEALTH ORGANIZATION, 1964

A REMINDER that the control of communicable diseases is "still the most important health challenge facing mankind" is given by Dr. M. G. Candau, director-general of the World Health Organization, in his annual report for 1964*.

It is not, therefore, surprising that, notwithstanding the new developments in the work of the World Health Organization, the chapter on communicable diseases remains the longest in the volume. While some diseases have declined in public health and economic importance, others are still obstinate or present new problems.

* Official Records of the World Health Organization, No. 139: The Work of WHO. Annual Report of the Director-General to the World Health Assembly and to the United Nations. Pp. xiv+240. (Geneva: World Health Organization; London: H.M.S.O., 1965.) 4 Sw. francs; 6s. 8d.; 1.25 dollars.

Dr. Candau points to the increasing incidence of plague the heavy toll of cholera and smallpox, the continuing problems of malaria and tuberculosis, the upsurge in the incidence of syphilis and gonorrhoea. He lists, as the three main obstacles to effective control, lack of knowledge, insufficient international support for the Organization and financing of campaigns and, most formidable of all, lack of adequate health services in the countries most affected. Lasting success can be achieved only through the establishment of permanent and effective health services applying national health plans.

The experience of the Organization has shown that quick results cannot be expected; the countries most in need of assistance for national health planning are those where

statistics are scanty, the shortage of trained staff acute, and where multiple health problems compete for priority. Nevertheless, Dr. Candau is able to report progress in comprehensive health planning in a number of countries, for example, Africa, Latin America, and Asia—the areas presenting the greatest problems.

In campaigns against many of the communicable diseases, the use of research is a valuable adjunct to the work in the field. In its medical research programme, the Organization draws on the advice of leading experts in many special fields and now has the collaboration of laboratories in all parts of the world. The lists of the reference laboratories of the World Health Organization and of scientific meetings during the year provide some indication of the scope of the programme. The research programme is by no means restricted to the communicable diseases. The report contains, for example, accounts of the present studies on cancer (including the possible relation between viruses and cancer), cardiovascular diseases, and human genetics—a new field of activity for the World Health Organization (see *Nature*, 208, 230; 1965). The importance of immunology is stressed, and achievements are mentioned in the Organization's relatively new programme of immunological research, particularly in relation to parasitic diseases—a hitherto largely unexplored area of immunization in which further research has been planned on the advice of an expert committee.

Other topics dealt with in the "General Review", constituting Part 1, include malaria eradication, environmental health, public health services, health protection and promotion, education and training, biology and pharmacology, health statistics, publications, information,

and administration and finance. A final chapter outlines the work done in co-operation with the United Nations and related agencies, and with non-governmental organizations.

Part 2 deals with the health problems and programmes in each of the Organization's six regions—Africa, the Americas, south-east Asia, Europe, eastern Mediterranean and western Pacific. In each chapter, broad surveys of work in the region concerned are complemented by detailed descriptions of some of the typical projects in which the Organization is giving assistance. They include the preparation of a plan to be financed under the United Nations Special Fund for the metropolitan area of Accra in Ghana; the improvement of nursing education and services in Guatemala; a project in south-east Asia to increase the local production of freeze-dried smallpox vaccine, which is essential for the successful pursuit of the smallpox eradication campaign in tropical countries; malaria eradication in Pakistan; and, in the western Pacific region, the first regional conference of deans of medical schools. For the European region, one of the projects described is a symposium in Moscow on the toxicology of drugs and methods of ensuring the therapeutic safety of drugs.

The list of current projects contained in Part 3 is a reflexion of the Organization's varied activities and of their adaptation to the particular requirements of countries in the various regions.

Information given in the annexes includes membership of the Organization, its executive board and expert committees, the budget, the secretariat, fellowships, awards under the medical research programme, and the reference centres.

A COMPARISON OF EUROPEAN HEALTH SERVICES

ALL European countries have some form of administrative arrangements to deal with environmental health; for example, housing standards, water purity, sewage and refuse disposal, and food hygiene. Modern life has brought into prominence new environmental problems, largely arising from industrialization and urbanization. A special monograph on health services in Europe was prepared for the European Conference on Public Health Administration held by the World Health Organization Regional Office for Europe in Zagreb, Yugoslavia, in June 1964*.

Air pollution is one cause for concern. Already most industrialized European countries have special regulations giving local authorities a measure of control over industrial emissions. Czechoslovakia and other countries have established standards for the control of health hazards from this source, while in the Federal Republic of Germany there are trade regulations and a Civil Code. In the U.S.S.R., the Institute of General and Communal Hygiene of the Academy of Medicine is carrying out research on air pollution in the principal industrial centres. Maximum permissible concentrations for 20 pollutants commonly found in the air of industrial cities have already been worked out. In the United Kingdom, smokeless zones are being established under a Clean Air Act. In Spain, provision is made for air disinfection in hospitals and certain clinics, and special legislation has been created covering unhealthy and dangerous gases and smoke.

Noise, as a public health problem, is also being tackled. Bulgaria has imposed restrictions on it in industry and in transport. In Spain, what are called "silence campaigns"

have been carried out in the larger towns, where noise elimination is the concern of the municipal authorities and industrial boards. The U.S.S.R. has set up a Central State Health Inspectorate to deal with problems concerned with noise.

In many European countries, in accordance with the requirements of the International Commission on Radiological Protection, radiation hazards have been the subject of precautionary measures. In the United Kingdom, watch is kept on radioactivity in the air and in the soil by the Atomic Energy Authority in collaboration with the Medical and Agricultural Research Councils, and the Ministry of Housing and Local Government supervises the disposal of radioactive material. In Denmark, under an Act which has been in force since 1953, no radioactive material may be manufactured, owned or imported without special permission from the National Health Service, acting on behalf of the Ministry of the Interior; the Act lists safety precautions covering transport, storage, and use. Finland's Radiation Protection Act controls X-ray and other radiation installations, and Norway also has regulations covering the protection of personnel and the transport of radioactive material. Similar provisions exist in Czechoslovakia, Hungary, Ireland, Switzerland and Turkey. In the Federal Republic of Germany, the Max-Planck Institute of Biophysics carries out systematic investigations of radioactivity in ground water, rivers, and soil, and the meteorological stations make regular measurements of radioactivity in the atmosphere.

To-day, all countries in Europe have some arrangements for controlling the spread of infectious diseases, and the incidence of most of them has been on the wane for some time. Tuberculosis, however, still remains a cause for great concern. In the Federal Republic of Germany, several *Länder* have legislation providing for regular

* A limited number of copies of this document (reference *Euro* 268-4) are available to persons officially or professionally interested in the subject, on request from the World Health Organization Regional Office for Europe, Copenhagen

chest X-ray examinations. One result of this approach has been the discovery that the percentage of undiagnosed cases of tuberculosis is higher among older people. In Norway, the Ministry of Social Affairs has the power to enforce X-ray screening of part of, or all, the population, as well as to test for tuberculosis, and to give B.C.G. vaccination. The radiography services in Italy are aimed at detecting not only tuberculosis but also other respiratory diseases, cardiovascular diseases and lung tumours. Compulsory B.C.G. vaccination is practised in some countries, such as Yugoslavia. Tuberculosis is notifiable in most European countries, but not in The Netherlands, Portugal or Romania.

There is a good deal of variation in the regulations regarding immunization against such infections as smallpox, diphtheria, tetanus, and whooping cough. Smallpox vaccination is compulsory in most European countries, and it is prescribed by the International Sanitary Regulations. It is required, for example, in Czechoslovakia, Hungary, Turkey, the U.S.S.R., and Yugoslavia, but it is not compulsory in the United Kingdom. In Yugoslavia immunization against diphtheria and tetanus is also obligatory.

Some communicable diseases are confined to particular areas. In Europe, for example, trachoma is endemic only in the Mediterranean countries. Spain carries out trachoma control through a network of clinics helped by the World Health Organization and the United Nations International Children's Emergency Fund; in Greece, treatment and control are carried out through a chain of anti-trachoma dispensaries. In Italy provincial anti-trachoma associations have been set up in co-operation with public and school dispensaries, specialized hospital services, and district health visitor services. Turkey is gaining control over the disease through an increase in the number of dispensaries. Yugoslavia has made a successful attack on its foci of trachoma: senior medical students have carried out surveys among people in affected areas, and

suspects are called for examination by specialists. Typhus, formerly widespread in eastern Europe, is now under control, and in Romania success has been achieved through co-operation between Government, the sanitation and epidemiological services, and the public. Public instruction in control methods has been given by trade unions, co-operative services and the Red Cross organizations.

An important development in the control of communicable disease has been the recognition of the part that veterinary science can play against zoonoses. In Italy, for example, there is a Veterinary Division within the Ministry of Health, which deals with such diseases as brucellosis, canine rabies and glanders. In Hungary, the *sanepids* (sanitary and epidemiological stations) have established programmes for brucellosis control in agricultural undertakings, helminthiasis control among schoolchildren, and ancylostomiasis control among miners. In Spain close co-operation is maintained between the health authorities and the veterinary services in the control of brucellosis, tuberculosis transmitted by animals, and other zoonoses. Laboratory facilities, an essential element in the control of communicable diseases, are provided by the local *sanepids* in most eastern European countries, at the health offices in the Federal Republic of Germany and at the universities and hospitals in Austria. In England and Wales there is a national network of laboratories, operated on behalf of the Ministry of Health.

In Yugoslavia, the institutes of hygiene undertake special research projects, as well as routine examinations of public health specimens. This arrangement has been particularly helpful in special field campaigns against endemic syphilis and trachoma.

Other preventive services described in the monograph include food and drug control, maternal and child health, school health, occupational health, mental health and alcoholism, care of the aged, dental care, and health education.

ANTARCTIC AERONOMY AND ICE SHEETS

DURING the past ten years the amount of scientific data available from Antarctica has increased by an order of magnitude. The position has at last been reached when it is stimulating and useful to summarize knowledge in many disciplines in the form of maps. The United States, the U.S.S.R. and the United Kingdom all have projects in hand to publish scientific atlases of Antarctic Research.

The Antarctic Map Folio Series is being published by the American Geographical Society under a contract with the National Science Foundation. The price is most reasonable and indicates that considerable and well-justified financial support has been given by the National Science Foundation. The form of publication and the contents have been based on replies to lengthy questionnaires sent to many interested scientists. The series will consist of about twenty folios, each devoted to one subject or scientific discipline. Each folio consists of a series of maps or diagrams printed on one side of sheets usually 17 × 22 in. and several pages of text of 17 × 11 in.; some plates are also of this size. Each folio series is contained in a light cardboard folder. Four colours are used in the production of maps.

The first folio* deals with aeronomy and has been produced by members of the AVCO Corporation, Wilmington, Massachusetts. A brief text explains the purpose of the observations and the method of compilation. It also

includes brief outlines and maps of some data relevant to conjugate points and to the southern auroral zone. The definition of magnetic B and magnetic L values which is given is somewhat involved for the layman. The plates all show ionospheric conditions for 1957 or 1958, the period of maximum sunspot activity. The maps on a scale of about 1:100 million show the following parameters: f_oE and f_oF_1 at 0000 and 1200 U.T.; f_oF_2 at 3 h (U.T.) intervals for winter, equinoctial and summer conditions, the percentage of time that $f_oE_s > 5$ Mc/s, and magnetic B and L values at 0, 100, 200, 500, and 1,000 km. The folio should be useful for general reference purposes for scientists in contact with ionospheric problems, but a non-specialist seeking a general appreciation of this work would learn more from a general review article.

The second folio† on the physical characteristics of the Antarctic ice sheet has been compiled in university and other institutes. It deals with several topics of general geographical interest. The Antarctic maps at 1:13.6 million are at a scale suitable to the detailed knowledge of many regions. Use of four-colour printing brings out general distributions clearly. Where both colour shading and data figures are shown, as for net annual accumulation of snow and for mean annual temperatures, the effect is good, as one can see clearly the actual measurements and the degree of interpolation. However, the surface elevation and ice thickness maps show neither profile

* American Geographical Society. Antarctic Map Folio Series. Folio 1: *Aeronomical Maps for the Antarctic*. By R. Penndorf, T. M. Noel, G. F. Bourke, and M. A. Shea. Pp. 6+9 plates. (New York: American Geographical Society, 1964.) 3 dollars.

† Folio 2: *Physical Characteristics of the Antarctic Ice Sheet*. By C. R. Bentley, R. L. Cameron, C. Bull, K. Kojima and A. J. Gow. Pp. 10+10 plates. (New York: American Geographical Society, 1964.) 4 dollars.

lines nor points of observation, since the former are shown on a separate map. In theory one superimposes the maps on a light table as necessary—in practice a light table is not always available, and even if it is, the maps differ in placing in relation to the sheet edges, so that careful positioning is needed.

The maps give a reasonably full cover of material available in late 1963, but there are some omissions. Treatment of the Antarctic Peninsula by different compilers is inconsistent, since it is not always considered as part of the main ice sheet. Glaciological data from this region are both very variable and somewhat sparse, but ample information which is available on surface elevations has not been included. The text also states that meteorological observations have been obtained for one year or longer at twenty-one stations on the Antarctic Peninsula or off-lying islands, but the relevant map shows mean annual temperatures for only twelve of these stations. Reliable data for some of the missing stations are available from references given in the useful list of data sources which accompanies the text.

Another obvious omission is that the ice-thickness map does not show data from the French traverse of 1957–58 although the relevant reference is included with the text.

Studies of borehole data on temperatures, densities, crystal size and orientation, borehole closure and of stratigraphic profiles are shown diagrammatically on three data sheets. The introductory text is clear, and there is an excellent table setting out various over-snow traverses on the main continent, although the Antarctic Peninsula is again omitted.

For 7 dollars (3 dollars for Folio 1 and 4 dollars for Folio 2), the material supplied is good value and will provide a suitable reference source. Being in separate sheets, the maps can be revised and re-issued as necessary, and even in less than two years since they went to press, and under six months since their arrival in the United Kingdom, substantial additions to many of the maps could already be made. The intention in the United Kingdom and the U.S.S.R. to publish bound atlases at a definite time, rather than loose-sheet folios, has much to recommend it for convenience of librarians and general users. The maps are perhaps more important in supplying background information in fields related to a specialist's own studies, rather than to supply knowledge in his own field. Whatever the map, the specialist will almost always be aware of later data which are missing.

G. DE Q. ROBIN

OBSERVATIONS OF POLARIZED OH EMISSION

By DR. S. WEINREB, PROF. M. L. MEEKS and J. C. CARTER

Lincoln Laboratory, Massachusetts Institute of Technology, Lexington, Massachusetts

AND

PROF. A. H. BARRETT and A. E. E. ROGERS

Research Laboratory of Electronics, Massachusetts Institute of Technology, Cambridge, Massachusetts

IN a recent article Weaver, Williams, Dieter and Lum¹ reported strong microwave emission lines in the *HII* regions *W3*, *W49*, *W51*, *W75*, *NGC 6334* and *Orion A* at a frequency of approximately 1,665 Mc/s. In some cases the lines exhibited complex spectral structure, and in all cases the emission at 1,667 Mc/s bore little resemblance in intensity and/or structure to the 1,665 Mc/s emission. In particular, the emission in the region of *W3* was approximately 30° K at 1,665 Mc/s, while no emission was detected at 1,667 Mc/s. Weaver *et al.* concluded that the observations could not be entirely attributed to OH and postulated the existence of an unidentified microwave line which they called "mysterium".

In this article we wish to report observations of emission lines near the source *W3* which reveal that: (a) emission is present not only at 1,665 Mc/s but also at 1,667 Mc/s and 1,720 Mc/s with frequency spacings as predicted from the OH molecular spectrum²; (b) some of the emission at 1,665 Mc/s is linearly polarized by as much as 37 per cent; (c) the position of the maximum 1,665 Mc/s emission is displaced from *W3* by approximately 14 min of arc; (d) emission features as narrow as 1–1.5 kc/s have been observed. As yet we have not observed other regions. In our opinion, "mysterium" is anomalously excited OH.

The observations were conducted using the 120-ft. parabolic antenna of the Haystack Research Facility³ of Lincoln Laboratory, Massachusetts Institute of Technology, and a spectral-line autocorrelation radiometer. The radiometer is similar to that used in the initial discovery of the OH lines⁴; however, the present radiometer is an entirely new system with several improvements. A room-temperature parametric amplifier is incorporated in the radiometer, and a digital correlator allows the examination of the spectrum in band-widths which can be selected from 40 kc/s to 4 Mc/s. The frequency resolution is 1/40 of the band-width. A Univac 490 computer is

coupled directly to the radiometer output and produces the frequency spectrum immediately after the end of each pre-set integration time. The antenna beam-width is 23 min of arc and the overall system temperature is approximately 200° K. The measured continuum antenna temperatures were approximately 240° K on *Cassiopeia A* and approximately 12° K on *W3*.

Typical emission spectra at 1,665, 1,667 and 1,720 Mc/s are shown in Fig. 1 and the results of our observations are summarized in Table 1. Because of our ability to examine the spectra with a frequency resolution of 1 kc/s, more lines are reported in Table 1 than reported by Weaver *et al.* Most of our observations were also made at the position of maximum 1,665 Mc/s emission, $\alpha = 2^h 23^m 29^s$, $\delta = +61^\circ 38' 1''$ (1950.0), slightly displaced from *W3*. The region of strong emission at 1,665 Mc/s

Table 1. SUMMARY OF PRINCIPAL OH SPECTRAL FEATURES

Feature No.	Velocity (km/s)	Feature width (kc/s)*	Average antenna temp. (°K)	Polarization (per cent)	Position angle	Comments	
1,665 Mc/s, 1 kc/s resolution:							
1	-49.1	1.3 ± 0.3	6.1 ± 0.6	30 ± 12	145° ± 10°	Appears as two or more blended lines Line profile changes with feed position angle Line blended with adjacent lines	
2	-46.5	3.0 ± 0.4	14.3 ± 1.5	2 ± 6			
3	-45.2	3.0 ± 0.2	35.4 ± 3.0	37 ± 6	65° ± 4°		
4	-44.5	1.7 ± 0.3	7.0 ± 0.7	22 ± 16	107° ± 22°		
5	-43.7	1.6 ± 0.2	14.8 ± 1.5	22 ± 10	140° ± 16°	Spectrum not resolved due to poor signal-to-noise ratio	
6	-43.1	1.2 ± 0.4	3.1 ± 0.3	10 ± 10			
7	-41.7	1.6 ± 0.3	7.2 ± 0.7	16 ± 16			
1,667 Mc/s, 1 kc/s resolution:							
1	-45.5	3.0 ± 0.5	2.2 ± 0.3	10 ± 10			
2	-43.8	1.2 ± 0.5	1.3 ± 0.3				
3	-43.0	1.6 ± 0.5	1.7 ± 0.3				
1,720 Mc/s, 3 kc/s resolution:							
~ -44			0.85 ± 0.3				

* Feature widths given have instrumental width removed.

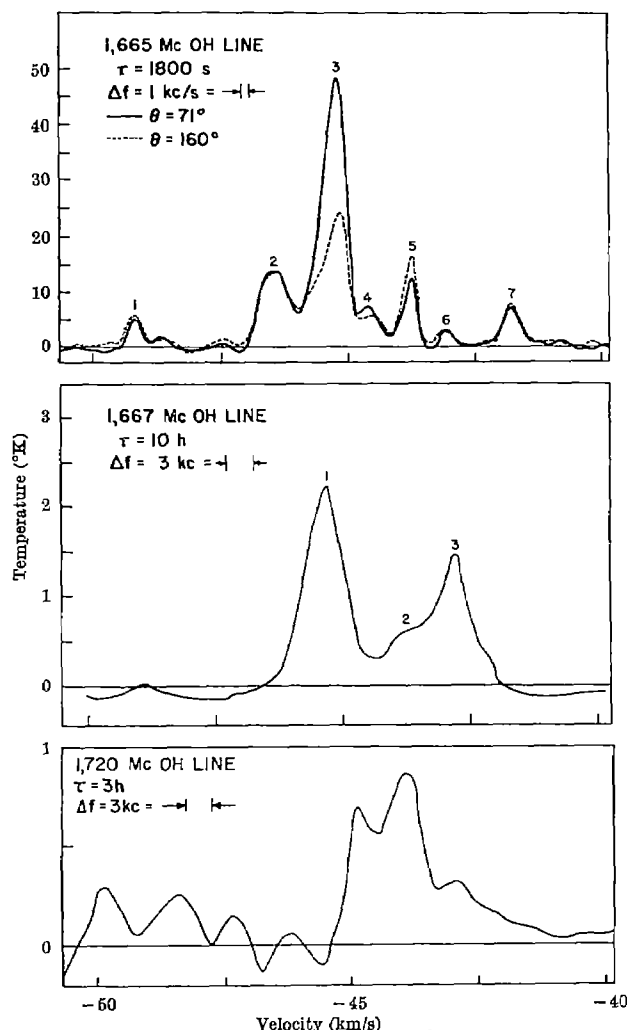


Fig. 1. Examples of OH-line spectra at 1,665, 1,667 and 1,720 Mc/s. The antenna temperatures are shown as a function of velocity relative to the local standard of rest. The data presented in Table 1 are, in some cases, of longer integration time and higher resolution than the examples presented in this figure.

appears to be of slightly greater angular extent than our beam-width, as adjudged by the intensity of the -45 km/s line. The values of Table 1 must be considered uncertain to the extent that blending of the lines occurs in some cases. Spectra with 1 kc/s resolution at various polarization angles reveal that some of the lines may be quite

complex and consist of two or more lines blended together. The observations also suggest that a small, but significant, amount of absorption may be present, particularly at 1,667 Mc/s, thereby making interpretation of the spectra difficult without further observations.

The observations clearly show that a large anomaly exists in the intensity ratios of the lines. For small optical depths, the intensities for the 1,665, 1,667 and 1,720 lines have theoretical ratios of 5:9:1 whereas our observations give approximately 50:2:1. The 1,612 Mc/s line was not detected during our limited search and its intensity appears to be less than the 1,720 Mc/s line. We observe a maximum antenna temperature of 50° K for the 1,665 Mc/s emission which implies, from considerations of antenna efficiency and beam filling, that the brightness temperature is in excess of 200° K. On the other hand, the narrowness of the lines implies maximum kinetic temperatures of 15° – 30° K. Therefore, the population distribution among the OH energy-levels must be established to a large degree by radiative processes and collisions are relatively ineffective.

The existence of strongly polarized OH emission is both surprising and difficult to explain. Possible polarization mechanisms are: (1) Zeeman effect; (2) Stark effect; (3) resonance scattering; (4) amplification, through a maser-type population inversion, of polarized background radiation. The approximate difference of 90° between the position angles of the -45.2 km/s line and the -43.7 and -49.1 km/s is characteristic of the Zeeman effect when viewed normal to the magnetic field. If the polarization is due to the Zeeman effect, magnetic fields of the order of 5×10^{-3} gauss are indicated. The Zeeman effect is unique among the four mechanisms listed above because it is the only mechanism which will give rise to circular polarization. Therefore, observations of this region with circular polarization are extremely important. Furthermore, if magnetic fields of the order of milligauss are present, 21-cm emission and absorption in the neighbourhood of W3 should be critically examined.

We thank G. H. Conant, jun., and P. P. Crowther and other contributions by the staff of the Haystack Research Facility for their assistance.

The work of two of the authors (A. H. B. and A. E. E. R.) was supported principally by the National Aeronautics and Space Administration (grant NsG-419). Lincoln Laboratory is a centre for research and development operated by Massachusetts Institute of Technology with the support of the U.S. Air Force.

¹ Weaver, H., Williams, D. R. W., Dieter, N. H., and Lum, W. T., *Nature*, **208**, 29 (1965).

² Radford, H. E., *Phys. Rev. Letters*, **13**, 534 (1952).

³ Weiss, H. G., *I E E E Spectrum*, **2**, 50 (1965).

⁴ Weinreb, S., Barrett, A. H., Meeks, M. L., and Henry, J. C., *Nature*, **200**, 820 (1963).

OH EMISSION IN THE DIRECTION OF RADIO SOURCE W49

By B. ZUCKERMAN, PROF. A. E. LILLEY and H. PENFIELD

Harvard College Observatory, Cambridge, Massachusetts

FOLLOWING the first detection of microwave lines of OH in emission near the source W49 by Gundermann¹ and by Goldstein and Gundermann² at Harvard University, similar emission was observed in a number of other galactic radio sources³. These initial investigations revealed a number of surprising results, particularly that the $F=(1 \rightarrow 1)$ 1,665 Mc/s transition has a greater intensity than the $F=(2 \rightarrow 2)$ 1,667 Mc/s transition, a reversal of the expected result.

In this article, we discuss recent detailed observations of OH emission in the direction of the source W49 and preliminary results for W51. We have obtained 3 kc/s resolution spectra of W49 at the OH frequencies of

1,665 Mc/s and 1,667 Mc/s with two radio telescopes: the Harvard University 60-ft. radio telescope and the 120-ft. Haystack telescope of the Lincoln Laboratory of the Massachusetts Institute of Technology⁴. These spectra have been quantitatively compared with each other. We have also obtained 1 kc/s resolution spectra at 1,665 and 1,667 Mc/s using the Haystack antenna. In view of the discovery of polarization of OH radiation in the source W3 by Weinreb, Meeks, Carter, Barrett and Rogers⁵, the W49 spectra have been checked for polarization and also for possible time variations. OH spectra in the region surrounding W49 have been obtained in an attempt to determine the angular size of the OH emitting

region and its position relative to the continuum source W49. Finally, using the results obtained on W49 and W51, we have carried out a preliminary investigation of the possibility that a spatial correlation exists between OH observed in emission and associated continuum sources.

The Harvard radio telescope is equipped with a cavity maser radiometer and a multi-channel filter bank composed of 10 units with available band-widths of 3, 25, 80 and 150 kc/s. At the OH frequencies, the antenna has a half-power beam-width of 52 min of arc and its aperture efficiency is 0.38. The mode of operation switches the radiometric system between the antenna input and a resistive comparative load maintained at approximately 4° K by immersion in liquid helium. The switching rate between the comparison load and the antenna is 3.5 c.p.s. An additive comparative noise source balances the system during operation. The total system noise is approximately 100° K. The characteristics of the Haystack OH spectral line system have been discussed by Weinreb *et al.*⁵

A quantitative comparison shows that the 3 kc/s resolution spectra for W49 obtained with the Harvard and the Haystack telescopes are practically identical in shape and differ only in amplitude. If an OH cloud and a continuum source are observed with two radio telescopes of different apertures, and if the cloud and source are small compared to the beam-widths of both telescopes, then the amplitude of each spectral feature should be proportional to the effective apertures of the telescopes. In this investigation, the ratio of effective apertures (Harvard/Haystack) is ≈ 0.38 . Using the total antenna temperatures at Harvard and at Haystack, we found that the observed ratios for the major spectral features at +17 km/s and +21 km/s (1,665 Mc/s transitions) are ~ 0.33 . If we take into account the known differences in effective filter shapes at Harvard and at Haystack, the observed ratio agrees quite well with the ratio of effective apertures.

We made a direct investigation of the angular size of the OH region responsible for the emission features at 1,665 and 1,667 Mc/s, by using 3 kc/s spectral resolution and the Haystack antenna. Comparison spectra were obtained for a number of positions close to W49. We were unable to detect any noticeable broadening of the Haystack beam, using the strong features at +17 and +21 km/s. Both the angular probe in position and the scaling of the spectral features imply that the region responsible for the OH emission is small with respect to the 23 min of arc beam-width of the Haystack antenna. The position of maximum OH emission lies at $\alpha = 19^h 7^m 55s$ and $\delta = 9^\circ 1'$ (1950.0).

Fig. 1 presents the spectrum of W49 obtained at the Haystack facility with a band-width resolution of 1 kc/s. The total integration time is 15 min. Numerous narrow spectral features are observed (~ 2 –3 kc/s at half maximum) and there is little correlation between the spectral shapes at 1,665 and 1,667 Mc/s.

The expected total line-width at half-power due to a thermally broadened cloud of OH is:

$$\Delta\nu \text{ (cycles/sec)} = 289\sqrt{T}$$

For the strongest features, the maxima observed in Fig. 1 are approaching the limit permitted by thermal broadening. However, since the OH emitting region is small in angular size, the apparent temperatures will continue to increase when observed by telescopes with larger effective areas and will exceed amplitudes $\sim 10^3$ °K. This argues for radiative excitation of the OH lines in the direction of W49, similar to the situation of W3 as reported by Weinreb *et al.*⁵. The continuum temperature which lies beneath the base-line in the two spectra in Fig. 1 is $T_c = 13^\circ \pm 1^\circ$ K.

In view of the polarization of the radiation from a similar source W3, as announced by Weinreb *et al.*⁵, we

have examined the 1,665 Mc/s peaks at radial velocities of +17 and +21 km/s with 3 kc/s and 1 kc/s band-width resolutions respectively for polarization effects. In each case, we made six measurements of the amplitude of the features, rotating the plane of polarization of the Haystack antenna feed system by approximately 30° between adjacent measurements. Using a definition of the percentage of polarization $p = 100 (T_1 - T_2)/(T_1 + T_2)$, where T_1 and T_2 are the maximum and minimum temperatures obtained with appropriate orthogonal positions of the antenna feed system, we conclude that the polarization of the features examined has an upper limit of 8 per cent.

We have examined our data for possible time variations in the +17 and +21 km/s features. There is nothing in the data to indicate that such an effect is present.

The prediction by Kardashev⁶ and the successful detection of several lines of excited hydrogen with transitions of the $n \rightarrow n-1$ type raise a question of possible confusion for OH spectral line research. Transitions of the $n \rightarrow n-1$ type occur throughout the region of the four OH lines when n takes on values between 155 and 160. However, calculations show that none of these hydrogen lines falls sufficiently close to the four OH lines to contaminate OH spectral analysis, and of the six lines that bracket the OH frequencies, none falls within 10 Mc/s of the OH lines.

The continuum source W49 has been studied by a number of workers. Bechis and Penfield⁷, utilizing the Haystack antenna at 8.25 Gc/s, have obtained a radio map of the region W49 clearly showing the two principal components of the source. Both components have angular widths of 3–4 min of arc and are separated by about 13 min of arc in right ascension. Data obtained at Green Bank⁸ at 750 Mc/s and at 1,400 Mc/s and at Haystack (8 Gc/s) suggest that one source is thermal and the other non-thermal (spectral index ≈ -0.6), their flux densities being equal at approximately 750 Mc/s. Weaver *et al.*⁹ noted that wherever OH had been found in emission (six sources) there was an HII region somewhere along the line of sight. If there is indeed an actual spatial correlation between continuum sources and OH emitting clouds, as our results on W49 and W51 suggest, then we can obtain a distance to the thermal component of W49 as well as to the clouds responsible for the OH spectra.

Akabane and Kerr⁹, utilizing the Parkes antenna, have attempted to deduce the distance to the source W49 by

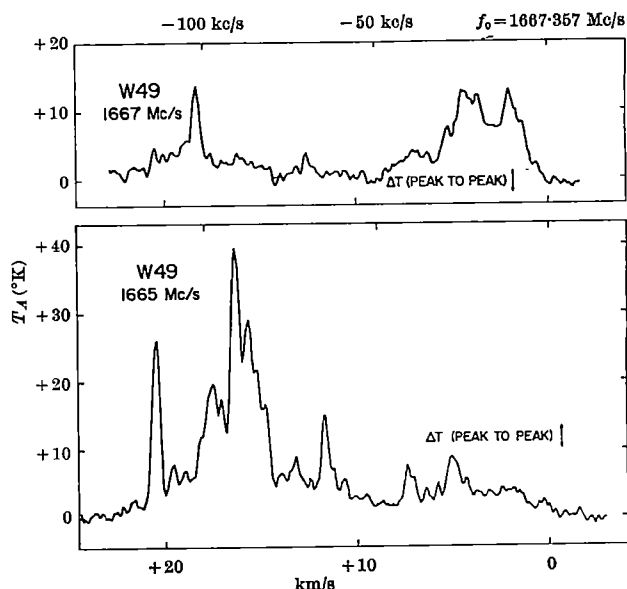


Fig. 1. Spectra of W49 obtained with a resolution of 1 kc/s and an integration time of 15 min. The abscissae are frequency and velocity with respect to the local standard of rest. The ordinates are antenna temperature. The above spectra were obtained for the direction $\alpha = 19^h 7^m 55s$, $\delta = 9^\circ 0'$ (1950)

examining the absorption at 21 cm of its continuum radiation due to intervening HI. From their work, it appears that the non-thermal component, located some 10 min of arc away from the galactic plane, was essentially out of their beam as they observed the thermal source on the galactic equator. For a Sun-centre distance of 10 kpc, they arrive at a probable distance of 15 kpc and a possible alternative distance of 7.5 kpc.

Utilizing the standard differential theory of galactic rotation, we have inferred that the OH clouds are approximately either 14 kpc or 1 kpc distant from the Sun. If we assume that the OH clouds are physically close to the HII region, then this unambiguously places the OH clouds and the thermal component of W49 at about 14 ± 1 kpc. We found that the position of the greatest line amplitude of the major features at 1,665 Mc/s and 1,667 Mc/s was within ± 1 min of arc of the thermal component of W49 and unrelated to the non-thermal component. This argues in favour of a close physical association of the OH clouds and the thermal source. With a close physical association, the thermal region could be the radiative source responsible for the OH line excitation.

A further argument for associating the OH and continuum sources arises from our 8.25 Gc/s studies at Haystack of the source W51, which also appears to be complex, consisting of four components. Although we have not yet had an opportunity to examine this region in detail, we find, with Hoglund and Mezger¹⁰, that the source at α (1950) = 19h 21m 20s, δ (1950) = $14^\circ 23'$ (component A) is the strongest one at 8.25 Gc/s. We have noted OH emission at 1,665 Mc/s in W51, using 3 kc/s resolution at Haystack. We easily distinguished between the Westerhout position and the component A position, their separation being 17 min of arc. At the Westerhout position, we found an OH signal which, if real, was barely

above the noise, whereas at the position of component A we noted a number of emission features; the strongest was about 10° K.

Additional indication that a spatial correlation may exist between the OH in emission and continuum sources comes from a study of radial velocities in W51. The OH velocity was +58 km/s, whereas from a study of two newly discovered lines of excited hydrogen¹¹ in this source, we found ~ 60 km/s for a thermal source located in W51. The observed thermal region and the OH clouds appear to lie at the point of maximum radial velocity along the line of sight in their direction in III, bII, which suggests that their distance is about 6.5 kpc.

Wherever OH has been found in emission there appears to be an associated EII region. Therefore, it appears profitable to search for excited hydrogen lines in areas where OH is discovered in emission.

We thank M. L. Meeks, S. Weinreb, A. E. E. Rogers, and J. C. Carter for their assistance. The programme in radio astronomy at Harvard is supported by the National Science Foundation. Lincoln Laboratory is a centre for research and development operated by the Massachusetts Institute of Technology with support of the U.S. Air Force.

¹ Gundermann, E., dissertation, Harvard Univ. (June 1965).

² Goldstein, S. J., and Gundermann, E. (to be published).

³ Weaver, H., Williams, D. R. W., Dieter, N. H., and Lum, W. T., *Nature*, 208, 29 (1965).

⁴ Weiss, H. G., *I.E.E.E. Spec. rum*, 2, 50 (1965).

⁵ Weinreb, S., Meeks, M. L., Carter, J. C., Barrett, A. H., and Rogers, A. E. E. (preceding communication).

⁶ Kardashev, N. S., *Soviet Astronomy, AJ* 3, 813 (1959).

⁷ Bechis, K., and Penfield, H., *Harvard Radio Astron. Rep.* No. 116 (August 1965).

⁸ Menon, T. K. (personal communication).

⁹ Akabane, K., and Kerr, F. W., *Austral. J. Phys.*, 18, 91 (1965).

¹⁰ Hoglund, B., and Mezger, P. G., *Science* (in the press).

¹¹ Lilley, A. E., Menzel, D. H., Penfield, H., and Zuckerman, B., *Harvard Radio Astro. Rep.* No. 11E (September 1965).

COMPARISON OF EXPERIMENTAL DATA OF THE EFFECTIVE ION MASS IN THE UPPER IONOSPHERE WITH A THEORETICAL STRUCTURE

By P. R. ARENDT

Institute for Exploratory Research, U.S. Army Electronics Command, Fort Monmouth, New Jersey

ELECTRON-DENSITY profiles obtained from evaluation of the readouts from the U.S.-Canadian topside-sounder satellite *Alouette* permit the measurement of the scale height in the upper ionosphere, from which information can be obtained¹⁻⁴ regarding the composition of the ionospheric regions. We centred our evaluation of the topside ionograms on the task of finding directly^{5,6} the exponential functions that govern the electron-density profiles. Thus, by the same process of numerical approximation, we obtain the electron density and the scale height. Therefore the computation reflects the main natural irregularities that are not smoothed out as by a differentiation of a density profile.

Our computation describes the electron density versus altitude profile in terms of an exponent α and the altitude h as follows:

$$N(h) = N_0 \exp(-(h-h_0)\alpha) \quad (1)$$

where N and N_0 are the electron densities at altitude h and h_0 respectively; the exponent α is a function of h . Under the assumptions mentioned below the exponent α can be considered the inverse electron-scale height $1/H$. Changes from region to region (of different but constant α) are made by our computation programme when the α for best data fit of a specific region has to be changed by more than the arbitrarily set 3 per cent. This setting results in an eventual uncertainty of the scale height by not more than 6-9 km within the altitude range we are going to consider.

Typical scale height versus altitude curves at daytime are given in Figs. 1-4. It is interesting to note a special fine-structure in the scale-height profiles. Very often data are presented in groups of constant exponent, thus reflecting a laminated structure (first observed by King¹). Each electron-density profile contains an inflexion point

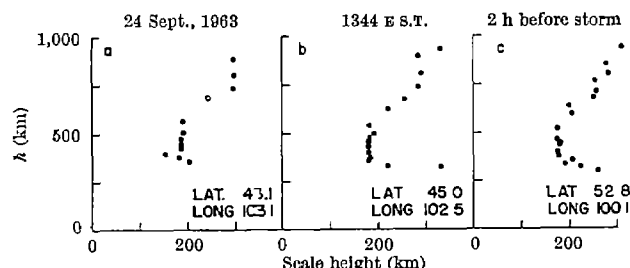


Fig. 1. Scale height derived directly from topside ionograms

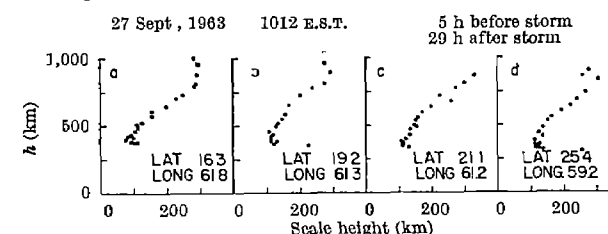


Fig. 2. Scale height derived directly from topside ionograms

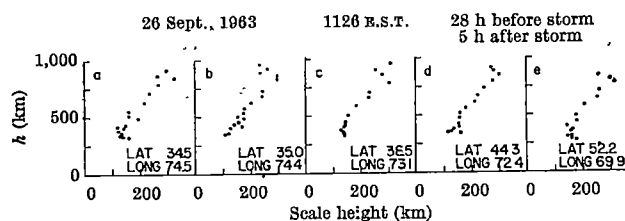


Fig. 3. Scale height derived directly from topside ionograms

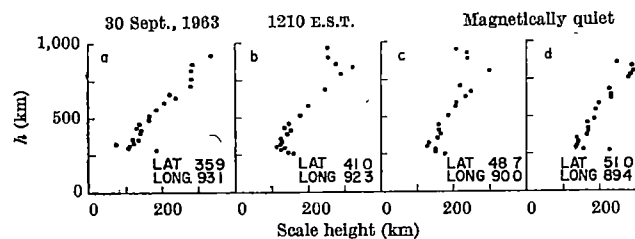


Fig. 4. Scale height derived directly from topside ionograms

where the curvature from the concave bending around f_oF_2 maximum changes to the convex curvature of exponential decay in the higher ionosphere. Obviously, a constant slope (that is, a constant exponent) will always be observed at the altitude of this inflexion. The altitude of inflexion is necessarily a function of the overall shape of the profile and might thus be related to seasonal, geographic, and other parameters. It appears to be rather pronounced at the magnetic equator¹. However, it is questionable whether a specific physical interpretation can be correlated to this inflexion point.

Our investigations⁶ of the altitude changes of typical plasma frequencies during magnetic activity have shown that the onset of a storm might be accompanied by a decrease in electron density. Such decreases may continue longer than the storm lasted as indicated by the local K index. It is plausible, therefore, that the slope of the scale-height profile is affected by magnetic events. The data shown in Fig. 1 (taken 2 h before a storm) and in Fig. 3 (taken 5 h after a storm) represent such magnetically disturbed conditions. A typical set of undisturbed data appears to be Fig. 4, which was taken more than a day after the last storm and more than ten days before the next storm. Comparison of Figs. 3 and 4 indicates that immediately after a storm the slope of the scale height is increased. During magnetically quiet periods (Fig. 4) the plateaux of constant exponent are repeated at nearly the same altitude during the passage of the satellite at the various points along its trajectory (36° – 51° N.). As indicated in Fig. 4 and Table 1, one can distinguish, during this passage for example, about five groups or plateaux, each of which is represented by a specific constant value. An average over the above latitude range taken during the same satellite passage results in five data pairs that can be considered representative for this latitude region at this specific time. Fig. 5 gives the end values from Table 1 in graphical form. The rectangles in Fig. 5 represent the spread of the single data. The lower three rectangles cover the entire range of the pertinent scale, indicating a really laminated structure that is less pronounced at higher altitudes partly because fewer data points are obtained there by our evaluation procedure. The combination of simultaneous data from various latitudes appears permissible in view of the fact

Table 1. AVERAGED SCALE HEIGHT
September 30, 1963. 1200 E.S.T.

Lat. Long.	Group 1		Group 2		Group 3		Group 4		Group 5	
	H	h	H	h	H	h	H	h	H	h
35-9 93-1	283	784	221	631	171	521	138	442	125	347
41-0 92-3	329	823	244	683	189	546	143	423	124	340
48-7 90-0	297	800	231	697	197	550	157	407	129	313
51-0 89-3	284	783	227	650	188	536	166	412	137	325
Averages	298	798	231	665	189	536	151	421	129	332

H , scale height; h , real height, both in km.

that the ionosphere above our region has been found very well stratified⁶ during magnetically quiet periods. The observed narrow spread of the data encourages the use of these data for a typical model (daytime, mid-latitude).

Considering the portions of the electron-density profile where constant exponents are observed, one might conclude that within such a range electrons and ions are each in specific isothermal equilibrium. In this case the inverse exponent is equivalent to the electron-scale height H :

$$\frac{1}{\alpha} = H = R(T_i + T_e)/Mg \quad (2)$$

Here R is the universal gas constant, T_i and T_e are the kinetic ion and electron temperatures, g is the acceleration of gravity and M is the effective ion mass (in atomic units); all parameters are a function of height; however, $T_i + T_e$ are considered constant for each region of constant $1/\alpha = H$. Since H and g are known, equation (2) reveals⁴ the ratio $(T_i + T_e)/M$ as a function of laminated altitude. The variation with altitude of equation (2) can be described by:

$$\frac{1}{M} \frac{dM}{dh} = \frac{1}{T_i + T_e} \frac{dT_i}{dh} - \frac{1}{H} \frac{dH}{dh} - \frac{1}{g} \frac{dg}{dh} \quad (3)$$

when it can be assumed that T_e is close to a constant value^{7,8} in the altitude range under consideration (300–1,000 km). The function $T_i(h)$ is not known exactly. Therefore, an empirical solution is suggested. A comparison of the possible magnitude of $\Delta M/M$ and $\Delta T_i/(T_i + T_e)$ as it can be obtained from the literature^{8,9} indicates that, for the altitude range in question, one might assume:

$$(1 \pm \epsilon) \frac{\Delta M}{M} = - \frac{\Delta T_i}{T_i + T_e} \quad (4)$$

where ϵ is a correction term which is also a function of altitude. The used numerical comparison of the terms in equation (4) is based on the neutral particle composition given by Harris and Priester⁹ for the mass data. This composition is not considered identical with the ion mass composition; however, the relative change in magnitude is assumed similar for neutrals and ions. A justification for equation (4) might be seen in the possibility that the lighter ions are more easily heated than the heavier ones by their interactions with the electrons. This effect will become more pronounced at higher altitudes.

Application of the empirical equation (4) permits a solution of (3) in case an assumption is made for the magnitude of $\epsilon(h)$. If we can assume $\epsilon = 0$ for the altitude interval in question we arrive at:

$$\left| \frac{dM}{dh} \right| = \frac{M}{2} \left\{ \frac{1}{H} \left| \frac{dH}{dh} \right| - \frac{1}{g} \left| \frac{dg}{dh} \right| \right\} \quad (5)$$

For $\epsilon \neq 0$ the above denominator reads $2 \pm \epsilon$ instead of 2.

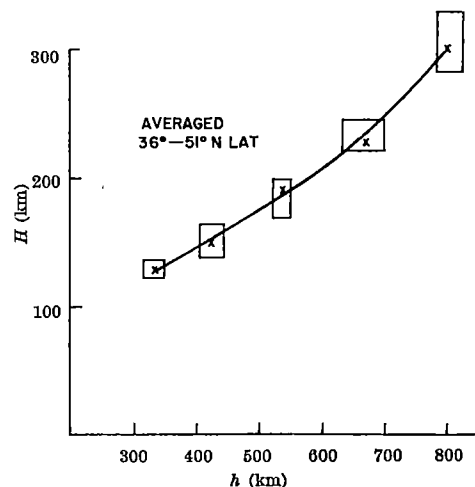


Fig. 5. Averaged scale height during magnetically quiet period

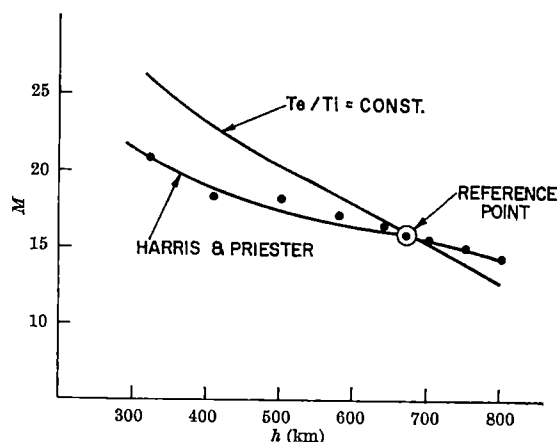


Fig. 6. Effective ion mass versus real height. Lower curve, Harris and Priester data for neutral atmosphere; upper curve, data from Fig. 5 converted for isothermal equilibrium of electrons and ions. Dots: data from Fig. 5 converted for non-isothermal conditions as indicated by equation (5)

Equation (5) can be numerically solved, for example, by insertion of data from Table 1 provided, however, that a reasonable value is obtained for an initial reference point M, h . For this reference we have chosen rather arbitrarily the value of the altitude that Harris and Priester ascribe to $M = 16$ for daytime (11 h L.M.T.) under consideration. This value is 670 km, to which we co-ordinate the experimental value of $H = 245$ km from Fig. 5. With these assumptions we obtain the data points for M as shown in

Fig. 6. For comparison the original curve from Harris and Priester is also shown in Fig. 6 as well as another evaluation of our scale-height data which was based on the assumption that both T_i and T_e were constant. Obviously, both evaluations are not correct. The upper curve deviates apparently too much towards higher M values. The good correlation of the curve described by the row of points in Fig. 6 with the Harris and Priester curve cannot be taken as finite establishment of the applied correction term $\varepsilon = 0$. However, it is taken as an indication that an application of equation (5) comes closer to the true composition than the assumption of a completely isothermal medium (T_i and $T_e/T_i = \text{constant}$).

Bauer has informed me that he has referred to a more realistic reference point derived from rocket probes for the ionic mass 16, which point is related to an average altitude of 300 km, that is near the inflexion point of the profile. Thus the dotted curve in Fig. 6 has to be moved downwards to show a variation of M between 16 and 7 in the range 300–800 km.

¹ King, J. W., *Nature*, **197**, c39 (1963).

² Bauer, S. J., and Blumle, L. J., *J. Geophys. Res.*, **69**, 3618 (1964).

³ Nelms, G. L., *Proc. Fourth Intern. Space Sci. Symp.* (June 1963).

⁴ Barrington, R. E., Belrose, J. S., and Nelms, G. L., *J. Geophys. Res.*, **70**, 1647 (1965).

⁵ Arendt, P. R., and Papaycinou, A., *USAF Tech. Rep. No. 2424, Fort Monmouth, New Jersey* (February 1964).

⁶ Arendt, P. R., and Papaycinou, A., *J. Geophys. Res.*, **70**, 15, 3675 (1965).

⁷ Evans, J. V., *J. Geophys. Res.*, **69**, 7, 1436 (1964).

⁸ Spencer, N. W., Brace, L. H., and Carigan, G. R., *J. Geophys. Res.*, **67**, 157 (1962).

⁹ Harris, I., and Priester, W., *Time-dependent Structure of the Upper Atmosphere*, Inst. Space Studies, Goddard Space Flight Center, NASA (April 12, 1962); presented at Caspar (1962); *J. Atmosph. Sci.*, **19**, 286 (1962).

DEPENDENCE OF THE PROPERTIES OF MOVING STRIATIONS IN THE PLASMA OF THE POSITIVE COLUMN ON SOME EXTERNAL AND INTERNAL PARAMETERS

By G. LAKATOS* and DR. J. BITÓ

Industrial Research Institute for Telecommunication Technique, Budapest

IN general, gas and vapour discharges occur associated with a number of oscillatory phenomena. Even under the simplest conditions, in the event of direct current discharges, a number of discharge anisotropies or instabilities in the state of equilibrium of the plasma can be demonstrated, which may lead to the generation of oscillations. Of these oscillations there is one which stands out because of its regularity. This is the so-called plasma oscillation which has been demonstrated and theoretically discussed by Langmuir and Tonks¹. These oscillations are characteristic of the given discharge plasmas; they accompany them all, and may be demonstrated in the positive column by means of a pair of Lecher-wires. Their frequency can be written:

$$n_e = \left(\frac{N_e \cdot e^2}{\pi m_e} \right)^{1/2} \quad (1)$$

where n_e is the frequency, N_e is the electron concentration, e the charge of electron, and m_e the electron mass.

The same relationship holds good for ions merely substituting the corresponding numerical values in the formula. For electron oscillations, using equation (1), a value of about 10^8 c/s is obtained for the frequency at an electron concentration of 10^{10} electrons/c.c. The frequency for ion oscillations, however, is much lower, due to the mass proportion and concentration assumed being smaller in certain instances.

In addition to these typical plasma oscillations (electron and ion oscillations), there is still another oscillation process

worth mentioning—a process which also regularly accompanies direct current discharges. This process is known as 'striation', and two specific forms of the process can be distinguished², namely the standing and moving striations.

As for the frequency, the study of striation does not require the rather complex techniques required for the so-called plasma oscillations, and for striations the frequency can be shown to be in the 10^2 – 10^4 -c/s range. Results published in the literature in this field have already been discussed by one of us in an earlier paper³.

A number of research workers are at present engaged on investigations of these striations, and are exploring their generation under various conditions. So far as the origins of the striations are concerned, no uniformly accepted theory has as yet been formulated. Certain research workers are investigating the processes occurring in the region of the electrodes for the anisotropy responsible for the phenomenon of striation—which can be demonstrated in the positive column and which is generally accompanied by variations in light. Others believe the origin of striation to be in the temporal changes of the basic processes of the positive column (excitation, diffusion, variation in the concentration of metastable atoms, recombination, ionization, etc.). Some are concerned with investigations of parameters influencing these striations^{2–4}, and by way of these studies are trying to draw conclusions as to their physical nature. Using the earlier findings of Szigeti⁵, one of us⁶ has succeeded in demonstrating the influences of certain internal plate-side parameters of the discharge tube on the moving striations within the positive column

* Now at the Research Institute for Electrical Industry.

of a low-pressure gas discharge. Studies are also being undertaken on influences external to the gas discharge tube which affect the moving striations. According to Yoshimoto *et al.*¹⁰ the velocity of the moving striations can be influenced by certain external conditions (ambient temperature, external resistance, etc.) in a critical manner. Pekarek¹¹ also mentions a weak influence of external circuits on the moving striations. Moreover, according to Nedospasov *et al.*¹², the external effects will, in addition to the velocity, influence the wave-length of the striations.

Donahue¹³ believes that for constant electrical and external parameters (temperature, geometry, etc.) the phenomenon of striation characteristic of direct current discharges can be considered as a superposition. In this case the moving striation is characterized by two space-charge waves moving in directions opposed to each other. In the discharge tube these space-charge waves move in conformity with the sign of the space-charge in opposing directions. Of these waves the negative space-charge wave composed mainly of electrons is characterized by a higher velocity.

The subject-matter of the tests to be described here was the investigation of the characteristic properties of these higher-speed space-charge waves at a variety of discharge currents. The instrumentation and set-up of the measurements performed during the tests were similar to those described by Donahue¹³. We have already described this set-up in an earlier paper, which deals with the pressure-dependence of the striation frequency and the speed of propagation³.

Striation in the direction of the axis of the positive column may be controlled by factors inside and outside the discharge in a number of ways^{3,4,5}. Thus, among other effects in the event of using Penning gas (that is, a gaseous mixture of mercury and argon), the process of striation is likely to be influenced by variations in the ambient temperature, through the variation of mercury-vapour pressure and the consequent variation of the concentration of the neutral atoms. It is a well-established fact that in the event of mercury vapour discharges the concentration of mercury atoms in the discharge is determined by the wall temperature of the discharge vessel through the partial pressure resulting from the saturated vapour pressure of the mercury.

Another object of the tests reported here was to study, using a Penning gas, the influence of certain external and internal parameters on the moving striations of the d.c. mercury vapour discharge, and to explain the dependence of the striations on these parameters. The following were included: as external parameters, the limiting resistance and ambient temperature; as internal parameters, the discharge curve.

No investigations of the external parameters of moving striations are known to us from the literature which have included studies of the dependence of temperature. Such tests would be justified by the aforementioned character of the mercury-vapour discharge in this type of d.c. discharge. For the theoretical description of the phenomenon of oscillation we accepted the calculations made by Kapzow¹⁵ for an equivalent circuitry, and followed the trend of thought of his calculations.

For purposes of measurement, we made use of the method introduced by Donahue¹³ and described by us in detail in an earlier paper³. Accordingly, in order to test the frequency of a moving striation, its nodes and light maxima, recourse was had to a method using photocells, and, by passing the signals of the photocell through an amplifier-stage, direct observations could be made on an oscilloscope screen. With the use of the oscilloscope the wave-length and frequency could be estimated (by means of Lissajoux curves). Furthermore, the light intensity of the striae formed during striation could be investigated along the discharge tube as a function of distance.

The discharge was supplied by a stabilized d.c. source while the discharge current was limited by symmetrically

arranged ohmic resistances in series. The discharge tube used on this occasion had a glass wall 1 mm thick; its length was 1,200 mm, and its outer diameter was 38 mm. After the appropriate vacuum treatment the tube was filled up with a mercury-argon gas mixture at a mercury column pressure of 3 mm, and about 60 mg mercury. To maintain the mercury vapour pressure at a constant value the discharge tube was placed in a water-jacket, and water was circulated continuously at a fixed rate. By using an ultra-thermostat, the temperature of the water responsible for temperature stabilization could be set at any value specified with an accuracy of $\pm 0.02^\circ \text{C}$. Tests began after a steady-state operating period of the discharge tube of 20 min, at specified predetermined stabilized temperatures of 20° , 25° and 40°C .

(a) *Influence of the external resistance on the moving striation.* When these tests were made the discharge tube was supplied from the stabilized d.c. source already mentioned, with an arc current of 100 m.amp, without specially heating the cathode. As an external limiting element during the first period of the tests a variable ohmic resistance was used which had negligible inductance. For the various values of the supply voltage (200, 300, 400 V) the external ohmic resistance was set so that during the tests the value of the current invariably remained 100 m.amp. By means of the measuring methods already described, and for a stabilized temperature of $25 \pm 0.02^\circ \text{C}$, the results shown in Fig. 1 were obtained. In Fig. 1 the dependence of the various characteristics on the value of the external ohmic resistance applied can be followed for an arc current of 100 m.amp. Curves are plotted for A , the amplitude of the light intensity variation, for v , the travelling speed of the moving striation, and for the resistance-dependence of the wave-length λ of the striation and the frequency ν . Of the two striation waves of different velocity detectable in the discharge, the parameters in the diagram in Fig. 1 apply to that travelling at the higher speed.

In the second set of tests, in addition to the ohmic resistance in series, an inductance coil, also in series, was used as an external influence. This resistance was alternately inserted or removed, as required. Its value was 1.5 henries, and the ohmic resistance was 10 ohms. With the inductance coil inserted in order to maintain the resultant ohmic resistance of the outer circuit on a constant value, the value of the original variable resistance had been reduced by 10 ohms. The effect of the inductance on the frequency of the moving striation for an arc current of 20 m.amp is shown in Table 1.

Table 1
Inductance coil
Inserted Removed
541 498

From Fig. 1 it can be seen that for an unchanged arc current the value of the external limiting ohmic resistance

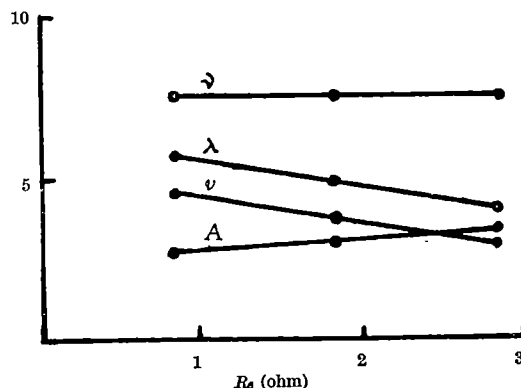


Fig. 1. The various parameters of moving striations as functions of the value of the applied external ohmic resistance for an arc current of 100 m.amp. A , Amplitude of the variation of light intensity in arbit units; v , travelling speed of the moving striation in 10^8 cm sec $^{-1}$; λ , wave-length in cm; ν , frequency in 10^3 c/s units

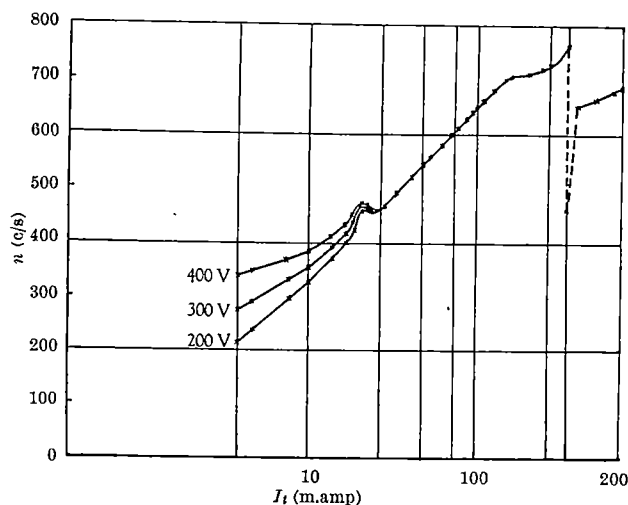


Fig. 2. Current dependence of frequency n of the moving striation for various supply voltages, 200, 300, 400 V; I_t , discharge current

will, in the range under test, influence the amplitude of the light variations in the moving striation, the speed of propagation and the wave-length of the moving striation; however, it will not affect its frequency. The conclusion may thus be drawn that in the range in question the internal parameters of the discharge will have a decisive effect on the frequency of the moving striation.

On the other hand, what Table 1 shows is, provided that the required conditions exist, the presence of the inductive member of the external limiting element affects the frequency of the moving striation, that is, in this case it is not the internal parameters of the discharge alone that determine the frequency.

(b) *Influence of the shape of the voltage versus current plot on the moving striation.* In this set of tests, by increasing the range of current, measurements were carried out in the discharge current range between 5 and 200 m.amp.

In Fig. 2 curves have been plotted for the current-dependence of the frequency of the moving striation at a stabilized temperature of 25° C, first in the current range of 20–200 m.amp, and then of 5–20 m.amp. As may be observed, the shapes of the two frequency characteristics differed fundamentally from one another in the two ranges. In the 20–200 m.amp range the frequency of the moving striation could be controlled mainly by means of the internal parameters of the discharge, whereas in the 5–20 m.amp range together with the internal parameters the external parameters were having a remarkable influence on the character of the current-dependency of the frequency.

From the results plotted in Fig. 2, and using the results of measurements made at 20° and 40° C, an empirical formula was derived for the current-dependence of a frequency n in the range between 20 and 70 m.amp:

$$n = n_c(T) + C(T) \log i_t/i_{0t} \quad (2)$$

where $i_{0t} = 20$ m.amp, i_t denotes the discharge current, n_0 is the frequency of the moving striation at i_{0t} , T is the temperature of the thermostating water-jacket, and $C(T)$ is a constant at the given temperature.

It could be shown that with rising temperature T , the value of frequency n_0 tended to drop, while the value of C increased.

A further conclusion drawn from the results was that with increasing vapour pressure the frequency was decreasing in the range between 5 and 40 m.amp, whereas it was rising at currents of 120 and 130 m.amp. It should be noted, however, that the curve plotted in Fig. 2 has a rather marked kink at 120 m.amp. The conclusion to be drawn from these results is that the concentration of the mercury atoms, as one of the internal parameters, had a slight effect on the frequency response of the moving striation.

The experimental results established for the current dependence of the light and current oscillations of the moving striation are shown in Figs. 3 and 4. As was observed by Bauer²¹ using a supply voltage of 160 V, the light oscillations had a relative maximum when the voltage-current plot had a maximum. With this observation in mind we investigated the problem of the current dependence of the light oscillations, the results of which are plotted in Fig. 3. The amplitude of the light variation, L_o , was a maximum at 50 m.amp, and measurements showed that this maximum was independent of the value of the supply voltage—a circumstance which seems to indicate that the effect was independent of the external resistance. A dependence could, however, be shown below the discharge current of 20 m.amp and above 70 m.amp.

The curve of the amplitude of the current variations has been plotted in Fig. 4, and for a discharge current

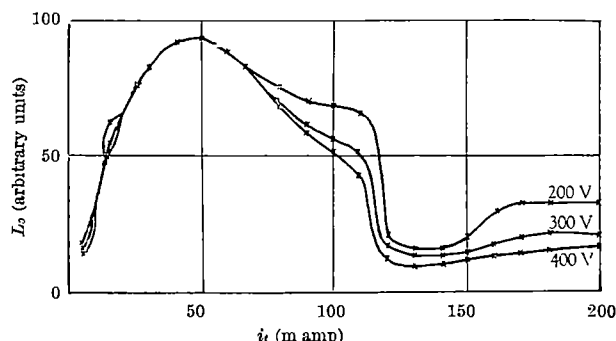


Fig. 3. The amplitude L_o of the variation of light intensity as the function of the discharge current i_t for various supply voltages

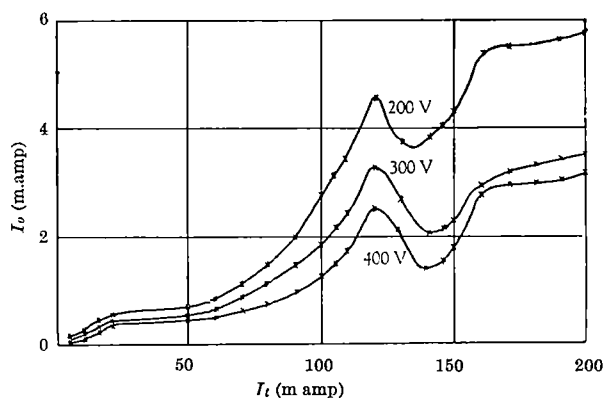


Fig. 4. The amplitude L_o of the current variation as the function of the discharge current

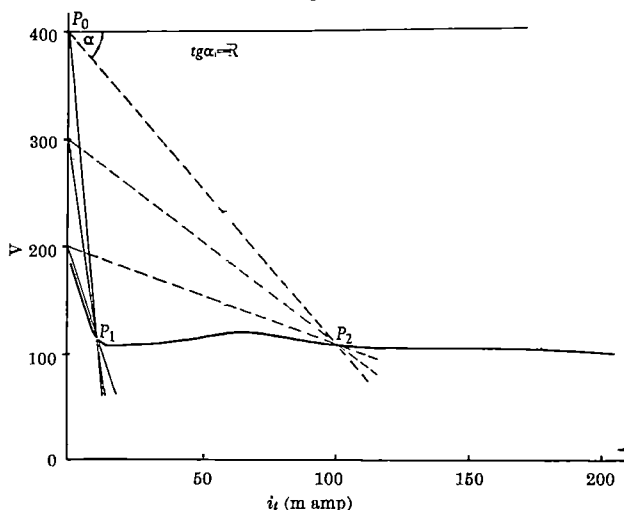


Fig. 5. Stabilization for various supply voltages

of 120 m.amp this curve has a maximum at the kink in the curve in Fig. 2.

In Fig. 5 the voltage versus current characteristic has been plotted for the gas-discharge tube under test, with the known resistance 'straights' associated with working points P_1 and P_2 . At working point P_1 a steep curve section is associated with the lower current intensities. The angle closed by the resistance 'straight' and its associated characteristic section changes here much more markedly than, for example, in the neighbourhood of working point P_2 . Consequently, for a given current intensity variation, a much greater voltage variation will arise, for example along the resistance 'straight' associated with the 400-V supply than along the resistance 'straight' belonging to the 200-V supply. We believe that this stronger voltage variation offers an explanation of why the frequency, as shown in Fig. 2, becomes dependent on the external parameters of the tube exactly at a current intensity of 20 m.amp corresponding to the working point P_1 in Fig. 5. This problem will receive a theoretical examination according to the method established by Kapzow¹⁵ in the following section.

(c) *Influence of the ambient temperature on the moving striation: theoretical study of the influence of external parameters.* In Fig. 6 the discharge current I_d and the burning tube voltage V_t of the discharge tube have been plotted at a stabilized wall temperature of 25° C. It may be observed that for low current intensities the slope of the curve is extremely steep and of a negative character.

The dependence of the frequency of the moving striation on the discharge current is shown in Fig. 2 for a wall temperature stabilized at 25° C. Measurements were made in the current range 5–200 m.amp. The external limiting resistance used in the current range 5–20 m.amp had an effect on the frequency of the striation⁴. A sharp kink in the frequency curve manifesting itself at the discharge current value of 120 m.amp could be found at all three test temperatures, that is, 20°, 25° and 40° C, for the same current. The curve plotted for the current-dependence of the striation frequency followed the trend of the curve plotted in Fig. 2 for a temperature of 25° also at the other two temperatures tested.

In Fig. 7 the temperature-dependence of the frequency of the moving striation is shown for a few values of the discharge current. For currents below 120 m.amp the temperature-dependence curves have a tendency to decline; with rising wall temperatures the frequency of the striations tends to decrease. At 120 m.amp—the current at which the kink occurs in the curve plotted in Fig. 2—the temperature-dependence curve shown in Fig. 7 inverts, and for higher currents the frequency of the striation rises with rising wall temperature.

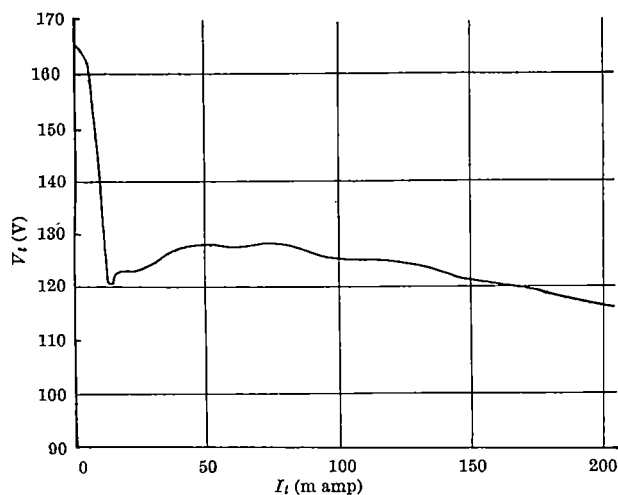


Fig. 6. Curve plotted for the discharge current, I_d , of the discharge tube versus the burning voltage, V_t .

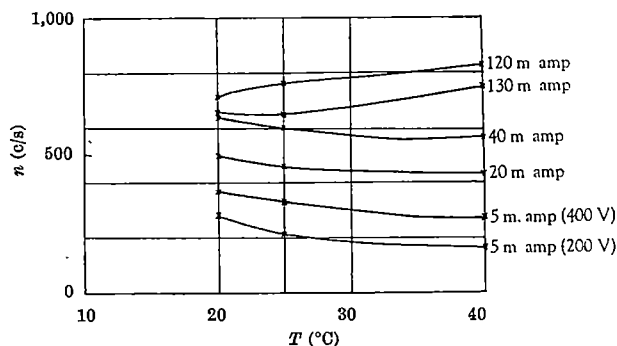


Fig. 7. Temperature-dependence of the frequency of the moving striation for specific discharge current values

As has been made clear earlier in this article, maintenance of the wall temperature at a predetermined value amounts to maintaining the concentration of the mercury atoms (which play a decisive part in the discharge) at a predetermined level. The argon atoms, owing to their higher excitation and ionization potentials, are only moderately represented in the basic processes of the discharge still in a steady-state balanced condition compared with the mercury atoms.

The values for vapour pressure and the number of mercury atoms at the various test temperatures are shown in Table 2 (ref. 7). The well-known striking growth of concentration of the mercury atoms occurs in the temperature range between 25° and 40° C.

	20	25	40
Vapour pressure of the mercury atoms (10^{-3} mm Hg)	1.201	1.85	6.07
Concentration of mercury atoms (atom/c.c.)	3.95×10^{13}	5.94×10^{13}	20×10^{13}

Before discussing the results obtained here, certain characteristic sections in the current versus voltage curve in Fig. 6 deserve special attention. For small currents the curve has a negative directional tangent, whereas for currents larger than 20 m.amp the curve is first positive and later negative.

The characteristics of the curve shown in Fig. 2 for the current dependence of the striation frequency can perhaps be explained with the characteristic properties described earlier and the influence of the internal parameters of the discharge. In this connexion, we outlined our opinions at the second Czechoslovak Conference on Electronics and Vacuum Physics⁴, and pointed out that, whereas in the current range up to 20 m.amp the external parameters, mainly the value of the limiting resistance, affected the striation frequency in a decisive manner, for current above 20 m.amp it was mainly the internal parameters which determined the striation frequency. We also made it clear that for a theoretical description of the influence of the external factors the equivalent circuitry developed by Kapzow offered a convenient method¹⁵. A demonstration of this method follows later.

In all curves describing the current-dependence of the frequencies (Fig. 2) there is a break point at 120 m.amp for all three temperatures. At this point the frequency drops by 200–300 c/s, and then for currents in excess of 120 m.amp the curve progresses between values corresponding to the original frequency value and the break point, in a closely linear, monotonously rising manner up to 200 m.amp. The temperature-dependence of this frequency breakdown is represented in Fig. 7 by the curve marked with the 120-m.amp parameter.

The theoretical relationship for the influence of the ambient temperature according to Fig. 7 can only be established after further investigations. However, even without any further investigations the statement may be ventured that there may be a definite relationship between the variation of the concentration of neutral

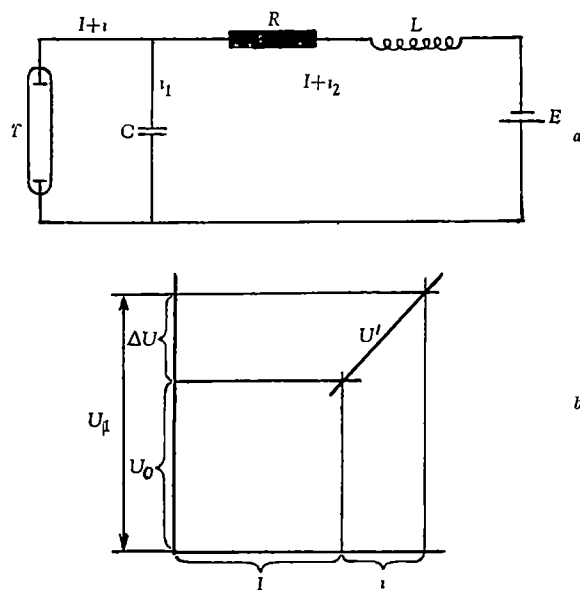


Fig. 8 (a) Equivalent circuitry for the electric circuit of the discharge.
(b) Auxiliary figure for the notations used in the deduction

mercury atoms in accordance with Table 1 and the influence of the ambient temperature. A consequence of this relationship is perhaps the drop in the striation frequency with the growing mercury atom concentration below the current intensity of 120 m.amp.

From the fact that with the increase in concentration of mercury atoms the frequency variation curve becomes inverted at 120 m.amp, it can be concluded that owing to the larger energy losses associated with the larger current rate beyond 120 m.amp a change can occur in the heat dissipation conditions in the discharge. This may, among other things, influence the trend in the temperature-dependence of the moving striation frequency.

The influence of temperature should be considered as an external factor at the formation of moving striation. As has already been explained⁴⁻⁶, in specific sections of the current range the external parameters resistance, capacity and induction of the electric circuit have a critical influence. A method for studying this influence has been provided by Kapzow¹⁵. Assuming an equivalent circuitry for the discharge circuit, he discusses the conditions for oscillation by solving the second-order differential equation for the discharge circuit.

Accordingly, for the present investigations, the electric circuit for the discharge can be described by the equivalent circuitry as shown in Fig. 8a, where C denotes the sum total capacity of the discharge circuit and the water-jacket surrounding the tube which affects the discharge, R is the sum total resistance of the external circuit, L is the sum total induction given by these resistances and other circuit members, and E is the voltage of the d.c. current source maintaining the discharge.

By applying Kirchhoff's law in the vibrationless, balanced state of the discharge the following relation is obtained:

$$E = I \cdot R + U_0 \quad (3)$$

where U_0 denotes the tube voltage at a current I .

If with the start of an oscillatory process of any kind the current of the discharge changed to $I + i$, then corresponding to the discharge characteristic of a slope U' in accordance with Fig. 8b, the voltage drop of discharge would change to a value U_1 . Owing to the oscillations now in full swing a current i_1 would flow through condenser C , and also the current flowing in the equivalent chain RL would vary by a value i_2 .

Similar to equation (3), the following circuit relation can be written:

$$E = U_1 + R(I + i_2) + L \frac{di_2}{dt} \quad (4)$$

$$\text{since } U_1 = U_0 + \Delta U \quad (5)$$

$$\text{and } \Delta U = U' \cdot i \quad (6)$$

$$\text{further } U_1 - U_0 = \Delta U = \frac{1}{C} \int i_1 \cdot dt \quad (7)$$

$$\text{and then } CU' i = \int i_1 \cdot dt \quad (8)$$

$$C \cdot U' \frac{di}{dt} = i_1 \quad (9)$$

With this substituted in equation (4), since

$$i_2 = i + i_1 \quad (10)$$

the relations:

$$E = U_0 + U' i + R \cdot I + R \cdot i + R \cdot i_1 + L \frac{di}{dt} + L \frac{di_1}{dt} \quad (11)$$

and

$$E = U_0 + U' i + R \cdot I + R \cdot i + RCU' \frac{di}{dt} + L \frac{di}{dt} + LCU' \frac{d^2 i}{dt^2} \quad (12)$$

will be obtained. After turning the equations into their standard or a differential equation of the form:

$$LCU' \frac{d^2 i}{dt^2} + (RCU' + L) \frac{di}{dt} + (R + U') i + \underbrace{RI + U_0 - E}_{=0} = 0 \quad (13)$$

$$\frac{d^2 i}{dt^2} + \frac{RCU' + L}{LCU'} \frac{di}{dt} + \frac{R + U'}{LCU'} i = 0 \quad (14)$$

will be obtained. After solving this equation according to:

$$i = A_1 \exp(r_1 t) + A_2 \exp(-r_2 t) \quad (15)$$

where A_1 and A_2 are constants, the following equation may be written for r :

$$r = -\frac{1}{2} \frac{RCU' + L}{LCU'} \pm \sqrt{\frac{1}{4} \left(\frac{RCU' + L}{LCU'} \right)^2 - \frac{R + U'}{LCU'}} \quad (16)$$

or in a reduced form:

$$r = -\frac{1}{2} \left(\frac{R}{L} + \frac{1}{CU'} \right) \pm \sqrt{\frac{1}{4} \left[\frac{R}{L} + \frac{1}{CU'} \right]^2 - \frac{1}{LC} \left[\frac{R}{U'} + 1 \right]} \quad (17)$$

(a) If the quantity under the root sign is positive, then the current variation is of an aperiodic nature, and r_1 and r_2 are real. In this event the condition for stability may be written:

$$r_1 < 0 \quad (18)$$

$$r_2 < 0 \quad (19)$$

This condition can be satisfied, first, if

$$\frac{R}{L} + \frac{1}{CU'} > 0 \quad (20)$$

and, secondly, if the absolute value of the first term under the root sign is smaller than the square of the first term. Otherwise a positive r would also be possible, that is, to satisfy conditions (18) and (19) it is required that:

$$\frac{1}{4} \left(\frac{R}{L} + \frac{1}{CU'} \right)^2 - \frac{1}{LC} \left(\frac{R}{U'} + 1 \right) < \left[\frac{1}{2} \left(\frac{R}{L} + \frac{1}{CU'} \right) \right]^2 \quad (21)$$

that is, the inequality:

$$\frac{1}{LC} \left(\frac{R}{U'} + 1 \right) > 0 \quad (22)$$

exists, since both L and C are positive, for real r_1 and r_2 the condition for stability is given in the form:

$$\frac{R}{U'} + 1 > 0 \quad (23)$$

(b) If the quantity under the root sign in equation (17) is negative, then a solution will be found in the form:

$$i = A_1 \exp(-\delta t + j\omega t) + A_2 \exp(-\delta t - j\omega t) \quad (24)$$

where A_1 and A_2 are constants, and

$$\delta = \frac{1}{2} \frac{R}{L} + \frac{1}{CU'} \quad (25)$$

$$j\omega = \sqrt{\frac{1}{4} \left(\frac{R}{L} + \frac{1}{CU'} \right)^2 - \frac{1}{LC} \left(1 + \frac{R}{U'} \right)} \quad (26)$$

The condition for stability is that $\delta > 0$, that is, the expression in (25) must also be greater than zero. Thus the inequality:

$$\frac{1}{2} \left(\frac{R}{L} + \frac{1}{CU'} \right) > 0 \quad (27)$$

should exist. If conditions (22) and (26) exist at the same time, then the external parameters will cause no instability in the discharge.

By using relation (26) the oscillation frequency in the neighbourhood of the current value corresponding to the working point may be given which might then become the generator of the moving striation.

With the values of the external circuit elements in (26) and the slope of the curve associated with the current value in question inserted, a quantitative relation will be obtained showing that the frequencies of the oscillations are in fact within the range between 100 and 1,000 c/s.

In the course of the experiments reported here the ohmic resistance, capacity, and inductance of the external electric circuit were determined for the purpose of these calculations. The latter inductance is due to the inductivity of the ohmic resistance.

As an external value the capacity of the water-jacket surrounding the discharge tube had to be taken into

account. With this capacity taken up in the calculation the values of all other capacitive elements of the circuit become negligible. In Table 3 the circuit parameters associated with a 50-m.amp discharge current and the value of the slope U' taken from Fig. 6 have been compiled.

Table 3				
R	L	C circuit	C jacket	U'
3,440 ohms	12×10^{-3} henries	14×10^{-12} farads	2.1×10^{-8} farads	150 ohms

The value of the frequencies is markedly influenced by the values of U' and R , whereas L varies only slightly, and C may in this case be considered a constant (water-jacket). Depending on the slope and the character of the curve a variety of frequency values will be obtained from equation (26) for the various currents. As for order of magnitude, these frequency values agree with the experimentally established frequencies.

It is thus obvious that external parameters (especially temperature) together with the members of the external electric circuit have a critical influence on the striation processes. In the course of pertinent investigations more extensive and far-reaching information may be obtained concerning the fundamental physical phenomena occurring in the plasma.

¹ Langmuir, I., and Tonks, L., *Phys. Rev.*, **33**, 195 (1929).

² Bitó, J., *Magyar Fizikai Folyóirat*, **10**, 303 (1962).

³ Lakatos, G., and Bitó, J., *Acta Phys.*, **13**, 2, 193 (1961).

⁴ Lakatos, G., and Bitó, J., paper given at the second Czech. Conference on Electronics and Vacuum Physics, 1962, *Czechoslov. J. Phys.* (in the press).

⁵ Lakatos, G., and Bitó, J., *Zh. T. F.*, **32**, 902 (1962).

⁶ Lakatos, G., and Bitó, J., *Acta Phys.*, **13**, 2, 245 (1961).

⁷ Uytendhoeven, W., in *Elektrische Gasentladungslampen (Electrical Gas Discharge Lamps)* (Springer, Berlin, 1938).

⁸ Szilgeti, G., and Bitó, J., *Acta Phys. Hung.*, **11**, 103 (1960).

⁹ Bitó, J., *Magyar Híradástechnika*, **11**, 23 (1960).

¹⁰ Yoshimoto, H., Sato, M., and Nakao, Y. J., *Phys. Soc. Jap.*, **13**, 734 (1958).

¹¹ Pekarek, L., *Czech. J. Phys.*, **8**, 32 (1958).

¹² Nedospasov, A. V., Pankova, D. J., and Konakh, V. F., *Zh. T. F.*, **30**, 125 (1960).

¹³ Donahue, T., and Dieke, G. H., *Phys. Rev.*, **81**, 248 (1951).

¹⁴ Bauer, G. T. (personal communication).

¹⁵ Kapzow, N. A., in *Elektrische Vorgänge in Gasen und im Vakuum (Electric Processes in Gases and in Vacuum)* (Deutscher Verlag der Wiss., Berlin, 1955).

KINETIC PARAMETERS FOR OXYGEN IN REACTIONS INVOLVING PURIFIED OXIDASES AND TISSUE SLICES

By DR. M. McCABE*

Department of Biochemistry, Institute of Diseases of the Chest, Brompton Hospital, Fulham Road, London

AND

DR. D. A. GILBERT

Chester Beatty Research Institute, Institute of Cancer Research, Royal Cancer Hospital, Fulham Road, London

HAYAISHI¹ has recently summarized the apparent Michaelis constants for oxygen of several oxidase enzymes. In only one of the papers cited (that by Longmuir²) was the time-course of the reactions considered in any detail. In order to assess the suitability of Longmuir's polarographic apparatus to our own work, we have examined the applicability of both integrated and differential rate methods for determining the kinetic parameters for oxygen from the time-course of two oxidase reactions. These procedures are basically inferior to those in which only initial rates are considered due, for example, to effects resulting from the accumulation of products. However, we have found that consistent results are obtainable by these methods, provided excess catalase is added to decom-

pose rapidly hydrogen peroxide formed during the reactions considered. The good reproducibility of the values obtained for the parameters invites a more frequent use of these methods especially when the supply of enzyme is limited and maximum information must be gleaned from each individual experiment. Application of these methods to the results obtained on the oxygen uptake by intact cells (cf. Longmuir³, Chance⁴, Bander and Kiese⁵) leads us to propose an alternative interpretation of published results on the kinetics of tissue respiration.

The apparatus used for these investigations was essentially as described by Longmuir², the cell volume being approximately 18 ml. The experimental technique adopted was as follows: a buffered solution of the primary substrate (in large excess) was equilibrated with air (or oxygen at up to 3 atmospheres pressure) at 37°C, this

* Present address: Physical Biochemistry Department, John Curtin School of Medical Research, Australian National University, Canberra.

being followed by the addition of excess catalase (final concentration approx. 5 $\mu\text{g}/\text{ml}$). The latter had no effect on the diffusion current. The mixture was then introduced into the air-tight polarographic cells (maintained at the same temperature) and the reactions were initiated by the injection of 0.2 ml. of the appropriate enzyme solution. Glucose oxidase was obtained from Boehringer and Soehne, Mannheim, Germany. This enzyme is specific for β -D-glucose, but, since the preparation contained a highly active epimerase, the β -D-glucose concentration was effectively that of the total D-glucose present. Pig kidney D-amino-acid oxidase was purchased from Seravac Laboratories, Ltd., Colnbrook, England, the substrate used being D-alanine.

Analysis of the results obtained with both glucose oxidase and D-amino-acid oxidase shows that, under the conditions described, the reactions conform to simple Michaelis-Menten kinetics except at extreme values of oxygen concentration. Thus, if the data are plotted in the form of the integrated rate equation:

$$\frac{S_0 - S}{t} = V_m + \frac{K_m}{t} \cdot \ln \left(\frac{S_0}{S} \right)$$

(where S_0 and S are the oxygen tensions at time zero and time t respectively, V_m is the theoretical limiting rate and K_m the apparent Michaelis constant), the graphs obtained are linear over a considerable range of oxygen pressures (for example, 30–700 mm mercury). From the intercepts and slope it is thus possible to calculate both V_m and K_m . Alternatively, but perhaps less accurately, it proved possible to determine the rate of the reaction at any given partial pressure of oxygen from the slope of the time-course trace and hence obtain Lineweaver-Burk plots. The kinetic parameters can then be obtained in the usual way. In addition it is possible to determine the apparent reaction order for oxygen from the slope,

n , of the $\log \left(\frac{V_m - V}{V} \right)$ versus $\log S$ plot (where V is

the rate at the oxygen tension S). This value should be unity for simple Michaelis-Menten kinetics. By these methods the following range of values were obtained from the data of five experiments with each enzyme.

Glucose oxidase: $V_m = 240$ – $270 \mu\text{l. O}_2/\text{min}$ (mean 250); K_m (oxygen) = 120–190 mm mercury (mean 150); $n = 0.97$ – 1.05 (mean 1.00) (0.05 M phosphate buffer, pH 6, 0.01 M potassium chloride, 0.05 M glucose; initial oxygen tension varied from air saturated to 1 atmosphere).

D-Amino-acid oxidase: $V_m = 85$ – $95 \mu\text{l. O}_2/\text{min}$ (mean 89); K_m (oxygen) = 120–130 mm mercury (mean 125); $n = 0.94$ – 1.03 (mean 0.98) (0.05 M phosphate buffer, pH 8, 0.01 M potassium chloride, 5×10^{-2} M D-alanine; initial oxygen tension varied from air saturated to 3 atmospheres).

The K_m values in both cases (equivalent to about 170 μM for D-amino-acid oxidase and 210 μM for glucose oxidase) are in good agreement with the values obtained by other methods (140–300 μM and 200 μM respectively^{6,7}).

Non-conformation at both high and low oxygen tensions is to be expected as a result of the inapplicability of the simple theoretical equations at extreme values. In addition the degree of accuracy of the analytical methods is also lower in these regions. The deviations from simple Michaelis-Menten kinetics observed at very high oxygen tensions appear, however, to be real and could be due to inhibitions by oxygen or to intermediate intra-enzymatic reactions (that is, those not directly involving oxygen), or to the primary substrate being rate-limiting.

The calculations described were also applied to results obtained with actively respiring rat liver slices (using succinate as substrate) in place of the purified oxidase systems. Again the kinetics of the uptake of oxygen conformed to simple Michaelis-Menten kinetics over a wide range of oxygen tensions. Measurements of this

kind have been conducted by several workers²⁻⁴; each of these have referred to the apparent reaction order for oxygen as being anomalous, with a non-integral value in the region of 1.3–1.4. It should be pointed out, however, that these workers all used the *observed* maximal rate for the value of V_m . If, on the other hand, one uses the *theoretical* value for V_m (for example, the value obtained from extrapolation of the Lineweaver-Burk plots) as required by the relevant equations, a reaction order of unity is obtained (Fig. 1). It should be noted that the linearity of the Lineweaver-Burk plot implies a reaction order of unity irrespective of the nature or value of the maximum velocity. In the demonstration of the reaction order by means of the log-log plot, however, the interpretation will be dependent on the value used for the maximum velocity. Similarly, if the observed and theoretical limiting rates are significantly different, then large errors may be incurred in the estimation of the K_m value from the oxygen concentration at which the respiration is half-maximal. The anomaly can thus be resolved on the basis that the observed and theoretical maximum rates are not necessarily equivalent. On this interpretation, it would appear that, under the conditions used, the reactions directly involving oxygen are not initially rate-limiting but become so when the tension falls below the so-called critical value. Chance⁴ has described how the tissue respiratory data can be explained on the basis of sequential reactions involving four cytochrome components and this rationale is unaffected by our interpretation of the data.

In addition, we wish to mention that the apparent Michaelis constants for oxygen which we have obtained for tissue respiration (200 mm mercury, about 300 μM) differ by as much as one or even two orders of magnitude from those hitherto reported for the reaction of oxygen with rat liver cytochrome oxidase¹. It thus seems necessary to contemplate the existence of other rate-determining factors.

Some anomalies have already been considered by Longmuir and Bourke^{8,9}. Initially they felt the discrepancy might be explained by a gradient of partial pressure of oxygen through tissue down which oxygen diffused from the outside to the respiratory site within the tissue. However, this required a value for the diffusion coefficient for oxygen in tissue some hundred-fold less than through water. In addition, their original hypothesis did not explain the paradoxical result that the thickness of the slice appeared to have little effect on the parameter of critical oxygen tension of the tissue: the existence of a diffusion gradient would require that the critical oxygen

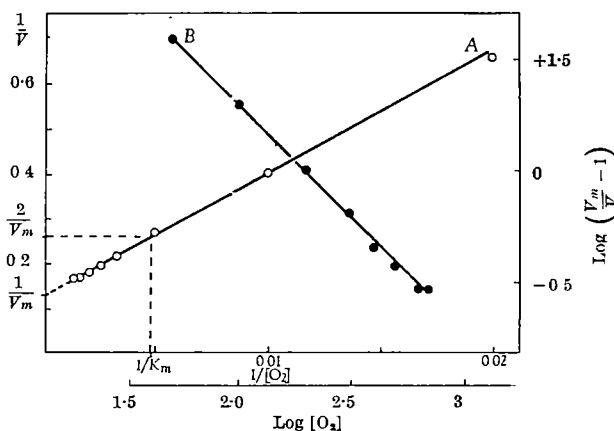


Fig. 1. Respiration of rat liver kinetic parameters for oxygen. A freshly prepared slice (0.4 mm thick) of rat liver was suspended in a medium (0.05 M phosphate buffer pH 7.4 0.05 M KCl 37° C) contained in a reaction cell of the Longmuir type. Respiration was initiated by injection of sodium succinate to a final concentration of 0.12 M. Curve A shows the conformation to simple Michaelis-Menten kinetics by means of the Lineweaver-Burk plot; this graph also indicates the method of determining the theoretical maximum rate (V_m , 73 $\mu\text{l}/\text{min}$) and the Michaelis constant (K_m , 220 mm mercury). The slope of the double logarithmic plot (curve B) gives the reaction order for oxygen (1.0).

tension should increase as the square of the slice thickness. Further examination of their experimental results led Longmuir and Bourke to the conclusion that diffusion could not be limiting, indeed the experimentally determined value of the diffusion coefficient for respiratory tissue appeared to be 10 times that for water. In addition, they considered that respiration of a slice might be limited by an oxidase with a low affinity for oxygen¹⁰.

The differences noted could arise from the existence of an active transport mechanism for oxygen within the cell. On the other hand, the results may merely reflect a marked dependence of the parameters on the conditions used, an alteration in the kinetic behaviour of the cytochrome oxidase as a result of cell disruption or perhaps the existence of different kinetic forms (isozymes) of the latter enzyme within a particular species.

We thank Dr. I. S. Longmuir for his advice and for use of facilities. One of us (D. A. G.) was supported by grants

to the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital) from the Medical Research Council and the British Empire Cancer Campaign for Research, and by Public Health Service research grant CA-03188-08 from the National Cancer Institute, U.S. Public Health Service. M. M. also was in receipt of a grant from the Medical Research Council.

¹ Hayashi, O., *Ann. Rev. Biochem.*, **31**, 25 (1962).

² Longmuir, I. S., *Biochem. J.*, **57**, 81 (1954).

³ Longmuir, I. S., *Biochem. J.*, **65**, 378 (1957).

⁴ Chance, B., *Fed. Proc.*, **16**, 671 (1957).

⁵ Bänder, A., and Kiese, M., *Arch. Exp. Path. Pharmac.*, **224**, 312 (1955).

⁶ Miyake, Y., Mochizuki, S., Yamano, T., and Aki, K., *Symp. Enzy. Chem. (Japan)*, **14**, 73 (1960).

⁷ Gibson, Q. H., Swoboda, E. P., and Massey, V., *J. Biol. Chem.*, **239**, 3927 (1964).

⁸ Longmuir, I. S., and Bourke, A., *Nature*, **184**, 634 (1959).

⁹ Longmuir, I. S., and Bourke, A., *Biochem. J.*, **76**, 225 (1960).

¹⁰ Longmuir, I. S., *Rec. Chem. Prog.*, **24**, 3 (1963).

URINARY METABOLITES IN THE ω -OXIDATION OF 2,4-DIMETHYLHEPTANOIC ACID

By DR. S. R. TANNENBAUM and PROF. S. A. MILLER

Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Massachusetts

WHILE examining the metabolism of 2,4-dimethylheptanoic acid-2-methyl-¹⁴C (DMHA) in the intact rat, it was found that approximately 20 per cent of the radioactive label was recoverable in the urine after 48 h, while more than 60 per cent was recoverable in respiratory carbon dioxide. (Manuscript in preparation. Normally, less than 1 per cent of the radioactivity from a carbon-14 labelled fatty acid is excreted in the urine.) An investigation of the nature of this radioactive material indicates that it may be the ω -oxidation product of the parent compound.

ω -Oxidation of fatty acids occurs when oxidation from the carboxyl end of the molecule is blocked, or when the acid is of medium chain-length^{1,2}. Tryding and Westoo examined a series of ¹⁴C-carboxyl labelled, methyl-substituted stearic acids³ which indicate that α -substitution has little overall effect on metabolism, while β - or α,α -disubstitution almost completely block β -oxidation. The demonstration of ω -oxidation *in vitro* in a cell-free system has also been reported⁴. On the other hand, there is no previous report concerning the metabolism of methyl-substituted fatty acids such as DMHA in which the substitution occurs on the α and γ carbon atoms.

Rats weighing 200 g were given a diet containing 10 per cent corn oil, and 0.2 per cent of DMHA-2-methyl-¹⁴C, specific activity 5×10^4 d.p.m./mg. The synthesis of the non-radioactive compound has been previously reported⁵. The radioactive label was incorporated through a minor variation of the published procedure which involved the condensation of diethyl 2-methylpentylmalonate⁶ with methyl-¹⁴C-iodide. The rats were housed in glass metabolism cages and urine was collected over a period of 48 h. The urine was acidified to pH 1, and continuously extracted with ether for 18 h with and without prior saponification with potassium hydroxide. Under these conditions, 93 per cent of the urine radioactivity (by liquid scintillation counting) could be recovered from the saponified urine, while only 57 per cent could be recovered from the unsaponified urine, indicating the presence of conjugated material.

Acids in the ether extract from the saponified urine were esterified with diazomethane⁶ and gas-chromatographed on a 2-meter column of 10 per cent diethyleneglycol succinate on acid-washed diatomaceous earth at a helium flow rate

Table 1. GAS CHROMATOGRAPHY OF ESTERIFIED ACIDIC COMPONENTS OF URINE OF RATS FED 2,4-DIMETHYLHEPTANOIC ACID

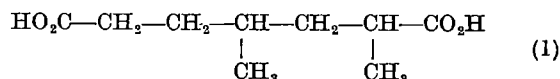
Peak	Retention time (min)*	Percentage of total urine radioactivity
A	76	21.8
B	70	35.5
C	56	7.4
Dimethyl succinate	16	2.3
Methyl 2,4-dimethylheptanoate	8.5	0

* A 2-meter column of 10 per cent diethyleneglycol succinate on acid-washed diatomaceous earth. Column temperature 100° C. Helium flow rate 60 ml./min.

of 50 ml./min. The chromatographic effluent corresponding to each major peak in the chromatogram was collected in glass capillaries immersed directly in liquid scintillation counting vials containing the scintillation liquid. Only three major peaks were found which did not correspond to a chromatogram of the urine of rats which had been fed the same diet without DMHA. These peaks were designated as shown in Table 1. None of these peaks had the same retention time as the methyl esters of a series of known compounds. (Methyl esters of: C-2, C-3, C-4, C-6, C-7, C-10 straight-chain dicarboxylic acids; methylmalonic, methylsuccinic, 2-methylglutaric, 3-hydroxybutyric, lactic, malic and citric acids. Ketoacids and fumaric acid are excluded, since they are not satisfactorily esterified and give non-volatile products with diazomethane.) The only compound which occurred in normal urine, and had a significant fraction of radioactivity, was dimethyl succinate (Table 1). No unmetabolized DMHA could be detected, although the method is suitable for identification of 5 μ g of this compound.

The materials corresponding to peak A, B, and C were trapped from the chromatograph in 1-ml. conical centrifuge tubes cooled with liquid nitrogen. Their infra-red spectra were determined in a Beckman IR-5 spectrophotometer using 0.05-mm cavity cells and a $5 \times$ KBr beam condenser. The spectra of peaks A and B were essentially identical both as pure liquids and in CCl₄ solution. This supports an assumption that they are diastereoisomers, as had previously been demonstrated for the parent compound, DMHA⁵. The spectra of A and B indicated that these compounds were esters. The transmission ratio of the carbonyl band at 5.75 μ to that of the C—H band at 3.75 μ was similar to that found in a series of dicarboxylic rather than monocarboxylic esters, suggesting that A and B

might be dicarboxylic esters. The presence of a band at 7.25μ also indicated the possibility of methyl groups on the chain⁷, particularly because the retention time did not correspond to that of a straight-chain compound. Although the specific activities of *A* and *B* could not be accurately determined owing to the very small amount of material available, on a volumetric basis, they appear to be in the range of 90 per cent of that of the parent compound, DMHA. It was tentatively assumed, therefore, that *A* and *B* were derived from DMHA via ω -oxidation and had the structure of 2,4-dimethylpimelic acid (1).



The mass spectrum of *B* was obtained with a CEC 21-130 mass spectrometer, and indicated fragment peaks typical of dicarboxylic esters^{8,9}. The peak at highest mass to charge ratio obtained was at *m/e* 185, and no discernible peak could be seen at *m/e* 216 where the parent mass ion (*M*) might be expected. It would not be unusual to find no parent mass ion for this type of compound, and the peak at *m/e* 185 probably corresponds to loss of a methoxyl ion (*M*-31). The base peak in the spectrum was at *m/e* 55 (C_4H_7), and corresponds to the branched paraffinic ion expected from the fragmentation pattern of (1). Other important peaks were found at *m/e* 88 (re-arrangement typical of 2-methyl substituted methyl esters (ref. 9)), *m/e* 59 (CO_2CH_3), and *m/e* 83 (C_4H_{11} , fragmentation at 1,2 and 5,6 with loss of H). No major peaks were detected that would not logically arise from (1). It is therefore tentatively concluded that (1) is the correct structure for *A* and *B*.

The infra-red spectrum of peak *C* was similar to that of DMHA, both in the position and intensity of the bands over the region of 3.3 – 9.5μ . The major differences in the spectra of *C* and DMHA are the presence in the spectrum of *C* of a strong band at 2.9μ and a medium intensity band at 10.6μ . The 2.9μ band is due to a hydroxy group and varies in position with dilution, as expected¹⁰, shifting to lower wave-lengths in more dilute solutions. The 10.6μ

band may indicate the type of substitution of the carbon bonded to the hydroxyl, but is of limited value¹⁰. Since only a trace of this compound was available, it was not possible to obtain any further information regarding its structure. On the basis of biological considerations, *C* might be the β -hydroxy derivative of DMHA, a compound which would be a normal intermediate of β -oxidation. Another possibility is that *C* is the ω -hydroxy derivative of DMHA, since it has recently been shown that the ω -hydroxy acid is an intermediate in ω -oxidation⁴.

It might have been expected *a priori* that fatty acids with alternate α -methyl substitution would be metabolized *in vivo*, via β -oxidation, with the same ease that has been demonstrated for fatty acids with a single methyl group in the 2 or 4 position³. Although 60 per cent of the ingested DMHA was oxidized to carbon dioxide via β -oxidation, the presence of 20 per cent of the radioactivity in urine might indicate that the alternate methyl groups along the chain present increased steric hindrance at one of the active sites in the β -oxidation enzyme system. Alternatively, it is possible that the extent of ω -oxidation is primarily a function of the chain-length of the compound—a hypothesis which remains to be tested.

This work was supported by contract Af 33(616)-6008 with the U.S. Air Force.

We thank Drs. Philip Issenberg and Emily Wick for their advice.

¹ Deuel, Jun., H. J., in *The Lipids*, Biochemistry, 3, 291 (Interscience Pub., Inc., New York, 1957).

² Weitzel, G., *Physiol. Chem.* 287, 254 (1951).

³ Tryding, N., and Westoo, G., in *Biochemical Problems of Lipids*, edit. by Popjak, G., and Le Breton, E., 193 (Interscience Pub., Inc., New York, 1956); *Acta Chem. Scand.*, 2, 427 (1957).

⁴ Wakabayashi, K., and ShEnazono, N., *Biochim. Biophys. Acta*, 70, 132 (1963).

⁵ Tannenbaum, S. R., and Wick, E. L., *J. Org. Chem.*, 27, 2650 (1962).

⁶ Roper, R., and Ma, T. S., *Microchem. J.*, 1, 245 (1957).

⁷ Bellamy, L. J., in *The Infra-red Spectra of Complex Molecules*, 21 (John Wiley and Sons, Inc., New York, 1959).

⁸ Kouray, R. E., Tuffley, B. L., and Yarborough, V. A., *Anal. Chem.*, 31, 1760 (1959).

⁹ Ryhage, R., and Stenhagen, E., in *Mass Spectrometry of Organic Ions*, edit. by McLafferty, F. W., 44 (Academic Press, New York, 1963).

¹⁰ Bellamy, L. J., in *The Infra-red Spectra of Complex Molecules*, 96 (John Wiley and Sons, Inc., New York, 1959).

'PINK SPOT' IN THE URINE OF SCHIZOPHRENICS

By R. E. BOURDILLON, PROF. C. A. CLARKE, A. PAULINE RIDGES and PROF. P. M. SHEPPARD, F.R.S.

Nuffield Unit of Medical Genetics, Department of Medicine, University of Liverpool

DR. P. HARPER*

Department of Psychological Medicine, University of Liverpool

AND

DR. SHIRLEY A. LESLIE

Psychiatric Registrar, Royal Liverpool Children's Hospital, Liverpool

SINCE Friedhoff and van Winkle¹ reported that they had isolated the compound 3,4-dimethoxyphenylethylamine (DMPE)—the 'pink spot'—from the urine of 15 out of 19 schizophrenics whereas they found it was absent in 14 mentally normal people, other workers have carried out similar investigations. Of these, Sen and McGeer², Kuehl *et al.*³ and Horwitt⁴ were confirmatory, Perry *et al.*⁵ and Faurbye and Pind⁶ were not, and Takesada *et al.*⁷ found that the compound was also present in nearly half their controls. In all these series the number of individuals tested was small, and moreover it appears that in none of them were the tests done in ignorance of the diagnosis. In the past many metabolic abnormalities have been described in schizophrenia,

but none has since been shown to have any fundamental bearing on the disease. It therefore seemed important to us to investigate the pink spot further, for if it could be shown to be causally related to schizophrenia this would provide important evidence favouring the abnormal methylation hypothesis as put forward by Osmond and Smythies⁸ and Harley-Mason⁹.

We have tested for the pink spot in the urine of 808 individuals to try to find out how often it is found: (a) in the different forms of schizophrenia; (b) in different types of mental disease; (c) in close relatives of schizophrenics; (d) in mentally normal people. To try to answer these problems, four experiments were planned:

Exps. I and II: Here two independent and 'blind' surveys were carried out in which separate workers ascertained the incidence of the pink spot using different

* Present address: c/o Research Facility, Rockland State Hospital, Orangeburg, New York.

methods for its detection and also different criteria for the assessment of schizophrenia.

Exp. III: A small series of family studies in which the close relatives of pink spot positive schizophrenic *propositi* were investigated.

Exp. IV: A survey of mentally normal individuals some of whom were apparently healthy in every way and some of whom were in-patients in the wards of two general hospitals.

Exp. I. The urines of 101 mental hospital in-patients, all 'possible schizophrenics', were tested with the primary object of finding the incidence of pink spot in the disease. The patients, who were drawn from three mental hospitals, consisted of acute and chronic cases and some had been off drug therapy for up to two months. All had at one time been given a provisional diagnosis of schizophrenia, and this and the drug therapy were known to the investigator (R. E. B.) who was testing for the pink spot. After he had assessed the presence or absence of this, each patient was interviewed and re-assessed by a psychiatrist (S. A. L.) who was ignorant of the pink spot finding. It was she who made the final assessment, and in about 17 per cent of the patients she thought the original diagnosis of schizophrenia was substantially in doubt; when this was so they were classed as non-schizophrenic. Where the diagnosis of schizophrenia was definitely upheld the patients were grouped into those who at any time had exhibited one or more of Schneider's first-rank symptoms¹⁰ and those who had not (these are referred to as Schneider positive and Schneider negative respectively). Afterwards the patients were also sub-divided into classical schizophrenics and those who showed only paranoid features without formal thought disorder or flattening of affect. The former for convenience are referred to as 'non-paranoid' and the latter as 'paranoid'. In five patients the information necessary for diagnosis was unobtainable either because the patients were unco-operative or because of language difficulties, and these five are classed as 'impossible to assess'.

The urine sample was, whenever possible, collected over 20 h, though in some cases shorter periods of collection proved more practicable. The extraction and the paper chromatography were carried out as described by Friedhoff and van Winkle¹ and this is referred to here as Method 1. Where there was marked interference by drugs the presence or absence of the pink spot sometimes had to be classed as 'impossible to assess'.

Because a much more detailed investigation would be necessary to ascertain that 3,4-DMPE was in fact being demonstrated we have, throughout this article, preferred to use the term 'pink spot' rather than 'presence of DMPE' in reporting the results.

These are given in Table 1 and the statistical analysis in Table 2. This consists of various comparisons using the χ^2 test or Fisher's two-tailed test for exact probability, whichever seemed the more appropriate. The control group were 149 mentally normal individuals (tested by Method 1) none of whom had the pink spot. Given also (Table 3) are the percentages of individuals with the pink spot within sub-classes.

Table 1. EXPERIMENT I
(a) Pink spot in relation to Schneider's first-rank symptoms

Schneider rating	Pink spot rating			Totals
	Positive	Negative	Impossible to assess	
Positive	35	11	6	52
Negative	7	16	4	27
Impossible to assess	4	0	1	5
Non-schizophrenic	0	16	1	17
Totals	46	43	12	101

(b) Pink spot in relation to non-paranoid and paranoid classification

Psychiatric assessment	Pink spot rating			Totals
	Positive	Negative	Impossible to assess	
Schneider positive { Non-paranoid	35	7	4	42
Paranoid	0	4	4	4
Schneider negative { Non-paranoid	5	5	10	20
Paranoid	2	11	13	26
Totals	42	27	69	138

Table 2. EXPERIMENT I. COMPARISON OF INCIDENCE OF PINK SPOT BETWEEN CLASSES

Comparison	χ^2	d.f.	Probability
Schneider positive with Schneider negative	11.57	1	<0.001
Schneider positive with non-schizophrenic	24.95	1	<0.001
Schneider positive with control			= 2.5×10^{-49}
Schneider negative with non-schizophrenic			= 0.029
Schneider negative with control			= 3.1×10^{-7}
Non-paranoid with paranoid	20.18	1	<0.001
Non-paranoid with non-schizophrenic	26.80	1	<0.001
Non-paranoid with control			= 8.06×10^{-32}
Paranoid with non-schizophrenic			N.S.
Paranoid with control			= 0.0099
Schneider positive with Schneider negative within non-paranoid			= 0.0388
Schneider positive with Schneider negative within paranoid			N.S.
Paranoid with non-paranoid within Schneider positive			= 0.0020
Paranoid with non-paranoid within Schneider negative			N.S.

Table 3. EXPERIMENT I. PERCENTAGE OF INDIVIDUALS WITH PINK SPOT WITHIN SUB-CLASSES

Sub-class	Percentage with pink spot
Schneider positive, non-paranoid	83.3 \pm 5.75
Schneider positive, paranoid	0.0
Schneider negative, non-paranoid	50 \pm 15.8
Schneider negative, paranoid	15.4 \pm 10.0

Exp. II. In the second experiment a different psychiatrist (P. H.) and biochemist (A. P. R.) worked together. The urines of 296 psychiatric in-patients drawn from two hospitals were investigated for the pink spot. The patients had often been in hospital for many years and included a larger proportion of chronic cases than in the first experiment. The samples of urine were analysed in ignorance both of the diagnosis and of the nature and quantity of drugs being received, so that a different part of the work was 'blind' from that in Exp. I. The psychiatrist selected the patients so as to include a variety of mental diseases, and he accepted the diagnosis given in the case-sheets and did not interview the patients personally as did the psychiatrist in Exp. I. He divided the cases into four groups: (a) 'non-paranoid' schizophrenics; (b) 'paranoid' schizophrenics; (c) schizophreniform syndromes (where although some features of schizophrenia were present it was uncertain whether or not the primary diagnosis was schizophrenia); (d) non-schizophrenics, which included such conditions as manic depressive psychosis, organic dementia and mental defect.

Since a different psychiatrist was scoring the cases and the diagnosis was assessed from the case sheets, the classes are probably not strictly comparable with those of Exp. I.

The chromatographic procedures were the same as those described by Friedhoff and van Winkle¹ (our 'Method 1'), but a different extraction procedure was used and the technique is referred to by us as 'Method 2'. A volume of 300–500 ml. of a 16-h overnight sample of urine was adjusted to pH 9.0 with 2 N sodium hydroxide. This was extracted three times with 100-ml. portions of 1,2-dichloroethane and the extracts dried over anhydrous sodium sulphate. The first extract was examined by ultra-violet absorption at 279 m μ . A peak at this value is a useful indicator of DMPE in the absence of interfering substances such as drug metabolites. The ultra-violet absorption spectrum helps particularly to distinguish DMPE from any closely related compounds which could not satisfactorily be resolved from it by the chromatographic procedures used. The three extracts were then combined and evaporated to dryness on a rotary evaporator. The residue was

Table 4. EXPERIMENT II. PINK SPOT ASSESSMENT IN 296 PSYCHIATRIC PATIENTS

Psychiatric assessment	Pink spot rating			Totals
	Present	Absent	Impossible to assess	
Non-paranoid schizophrenics	20	30	19	69
Paranoid schizophrenics	2	54	6	62
'Schizophreniform syndromes'	5	58	25	88
Non-schizophrenics	1	68	8	77
Totals	28	210	58	296

Table 5. EXPERIMENT II COMPARISON OF INCIDENCE OF PINK SPOT BETWEEN CLASSES

Comparison	χ^2	d.f.	Probability
Non-paranoid with paranoid	19.16	1	< 0.001
Non-paranoid with non-schizophrenic	27.05	1	< 0.001
Non-paranoid with control			$= 2.81 \times 10^{-18}$
Paranoid with non-schizophrenic			N.S.
Paranoid with control			N.S.

redissolved in dichloroethane for application to the chromatography paper. Authentic 3,4-dimethoxyphenylethylamine (K and K Laboratories) and mixed spots of urine extracts and authentic DMPE were applied alongside the urine extracts on the chromatography papers and the separation carried out.

The results of Exp. II are given in Table 4 and the statistical analysis in Table 5. The controls are 310 mentally normal individuals (tested by Method 2) one of whom had the pink spot. The results for the schizophreniform syndrome class have not been analysed because the group was probably very heterogeneous.

Exp. III. The pink spot was investigated in 20 close relatives of three schizophrenic propositi, all three of whom were found (by one or both of the two methods) to excrete the pink spot. None of the relatives was schizophrenic though some were reported as having other mental abnormalities.

The pink spot was not found in the urine of any of the close relatives, and these therefore are not significantly different from the control group of mentally normal individuals.

Exp. IV: Investigation of mentally normal individuals. These consisted of two groups. The first was made up of 265 healthy individuals, mostly undergraduates or university staff. The second was composed of 126 mentally normal in-patients, comprising 54 pre-operative and 20 post-operative cases, 10 patients with liver disease, 20 with chronic neurological states and 22 with general medical conditions. The pink spot was assessed by one or both of the two methods previously described and the series has acted as the control for the various comparisons.

All the controls were negative for the pink spot with the exception of one healthy individual, a woman aged 54. She suffered from migraine but was mentally normal and so was her family.

Comparison between Method 1 (Friedhoff and van Winkle) and Method 2 (ultra-violet + chromatography) in the investigation of the pink spot. In 133 people both methods for assessing the pink spot were used (Table 6).

Table 6 COMPARISON OF METHOD 1 AND METHOD 2 IN INVESTIGATION OF THE PINK SPOT

Friedhoff and van Winkle (Method 1)	Ultra-violet + chrom. (Method II)		Totals
	Pink spot positive	Pink spot negative	
Pink spot positive	12	7	19
Pink spot negative	2	112	114
Totals	14	119	133

Though there is a significant correlation, $P = 1.06 \times 10^{-10}$, between the results given by the two methods, there are discrepancies.

There are several reasons which might account for this non-concordance: (1) Method 2 may be less sensitive in detecting the pink spot (as opposed to DMPE); (2) slightly different substances may be being estimated; (3) one method may be more likely to produce undetectable false readings because of the presence of drugs; (4) there may be observer discrepancies. These possibilities are being investigated and will be the subject of a further article.

The association previously found between the pink spot and schizophrenia has been confirmed using large numbers. The results of Exp. I give compelling evidence that its presence is particularly associated with Schneider-positive individuals. It is much rarer in those who are Schneider negative and rarer still (if present at all) in non-schizophrenics.

In Exp. I the same patients have also been classified as to whether or not they only have paranoid features. The presence of the pink spot is particularly associated with the non-paranoid group but is significantly higher among the paranoid group than among the mentally normal controls.

When the individuals are classified as being Schneider positive and non-paranoid this sub-class has a significantly higher incidence of the pink spot than does any other group (83.3 ± 5.75 per cent). Thus to predict the presence of the pink spot the double classification is the most efficient.

Five individuals were so seriously disturbed that they could not be interviewed or assessed on the Schneider rating. The fact that four of them showed the pink spot suggests that these were true Schneider-positive non-paranoid cases as judged by our other results.

The second experiment using a different method for detecting the pink spot again shows the very strong association between the presence of the pink spot and 'non-paranoid' schizophrenia. The lower frequency of the pink spot may be due to any of the four reasons mentioned above and/or to the cases being less florid than in Exp. I. However, not enough have been scored by the Schneider rating to show whether the association is particularly with the Schneider-positive 'non-paranoid' group although the data show a strong tendency in this direction.

The evidence from the two experiments suggests that the pink spot is highly associated with schizophrenia and is not often present in other forms of mental disorder or in close relatives or in controls. Moreover, from our survey it seems highly improbable that diet and institution life play any part in producing the pink spot. An important problem is whether the abnormal metabolite precedes or is a result of the disease. The family data point to the latter conclusion, but it is possible that the pink spot only appears shortly before the disorder manifests itself, and that more refined techniques might demonstrate it in sibs. It would be interesting to follow pink-spot-positive patients with florid schizophrenia during remissions since these data might indicate whether the acuteness of the disease was associated with the quantity of pink spot material. If it did, this might explain its absence in sibs. The possibility has to be considered that drugs might be the cause of the pink spot. This is refuted by our data. Thus the relationship between schizophrenia and pink spot is at least as marked in those who had been off drugs for two weeks or more as in those who had not. Moreover, the non-schizophrenics on drugs showed no elevation of the frequency of the pink spot over those not on drugs. However, a complicating factor in using the pink spot for diagnosis is that drugs interfere both with the chromatography and with the ultra-violet absorption and it is difficult to obtain patients (particularly acute cases) who are not on drug therapy.

We thank Dr. B. Finkleman, Prof. F. J. Fish, Dr. A. V. de P. Kelly, Dr. I. Leveson, and Dr. B. Ward for allowing us to examine and test their patients, and the staffs of the hospitals concerned for help in the collection of samples. We also thank the Hon. Miriam Rothschild for obtaining the co-operation of certain of the individuals investigated.

¹ Friedhoff, A. J., and van Winkle, E., *Nature*, **194**, 897 (1962).

² Sen, N. P., and McGeer, P. H., *Biochem. Biophys. Res. Comm.*, **14**, 227 (1964).

³ Kuehl, F. A., Hichens, M., Osmond, R. E., Meisinger, M. A. P., Gale, P. H., Cirillo, V. J., and Brink, E. G., *Nature*, **203**, 154 (1964).

⁴ Horwitz, M. K., contribution to discussion at *Symp. Amine Metabolism in Schizophrenia*, Atlantic City, April 1965 (in the press).

⁵ Perry, T. L., Hansen, S., and Macintyre, L., *Nature*, **202**, 519 (1964).

⁶ Faurbye, A., and Pind, K., *Acta Psychiat. Scand.*, **40**, 540 (1964).

⁷ Takesada, M., Kakimoto, Y., Sano, I., and Kaneko, Z., *Nature*, **199**, 203 (1963).

⁸ Osmond, H., and Smythies, L., *J. Ment. Sci.*, **98**, 309 (1952).

⁹ Harley-Mason, J., *J. Ment. Sci.*, **98**, 313 (1952).

¹⁰ Schneider, K., *Fortschr. Neurol. Psych.*, **25**, 487 (1957).

INTERFERON APPEARANCE STIMULATED BY ENDOTOXIN, BACTERIA, OR VIRUSES IN MICE PRE-TREATED WITH *Escherichia coli* ENDOTOXIN OR INFECTED WITH *Mycobacterium tuberculosis*

By PROF. JULIUS S. YOUNGNER and PROF. WARREN R. STINEBRING

Department of Microbiology, School of Medicine, University of Pittsburgh, Pittsburgh

WHEN mice are injected intravenously with bacteria or bacterial endotoxin an inhibitor indistinguishable from virus-induced interferon appears in the circulation¹. *Salmonella typhimurium*, *Serratia marcescens*, and endotoxin from *Escherichia coli* produce peak-levels of circulating interferon at about 2 h after injection. Maximum interferon-levels following injection of *Brucella abortus* or Newcastle disease virus (NDV) are reached at about 12 h. To elucidate the mechanisms of the different time patterns of appearance of interferon, experimental procedures known to alter the reactivity of animals to the lethal or pyrogenic effects of endotoxin were investigated. Repeated exposure to endotoxin reduces the responsiveness of animals to subsequent injection of endotoxin, while prior infection with *Mycobacterium tuberculosis* is known to increase the reactivity of the host to endotoxin. These phenomena have been termed 'tolerance' and 'hyper-reactivity' in the literature dealing with endotoxin². In connexion with the investigations reported here, which primarily deal with interferon production rather than lethality or pyrogenicity, it seems more appropriate to use the term 'hyporeactivity' in place of 'tolerance'. This article deals with the modification of interferon appearance in the circulation which results from prior treatment of mice with endotoxin or infection with tubercle bacilli.

Methods for the inoculation and bleeding of mice, for the assay of interferon in L-cells using the plaque-reduction method with vesicular stomatitis virus (VSV), and for the preparation and standardization of inocula containing *B. abortus* or NDV have been described in detail previously^{1,3}. The endotoxins used were prepared from different strains of *E. coli* (0111:B4, 0113, or K235) and had similar potencies when measured by LD₅₀ determinations or interferon appearance in the plasma. Interferon titres were expressed as the dilution of plasma which would reduce the plaque count to 50 per cent of that of the controls and were calculated by probit analysis.

Preliminary studies showed that mice given 250 µg of endotoxin intravenously exhibited the usual pattern of peak interferon levels in the plasma 2 h later¹; however, a second dose of endotoxin given 48 h later failed to produce circulating interferon. To further document and extend this observation, the following experiment was performed using challenge with NDV as well as endotoxin. A large group of mice was injected intravenously with 125 µg of endotoxin. Forty-eight hours later these animals were challenged intravenously with either a second dose of 125 µg of endotoxin or with 5×10^7 plaque-forming units of NDV. Control mice which had received no prior endotoxin were also challenged with endotoxin or NDV as described. Because of the different time patterns of appearance of interferon, blood samples were obtained at 2, 4 and 6 h in the case of endotoxin challenge and at 2, 6 and 12 h from animals injected with NDV. The results presented in Fig. 1 show that prior treatment with endotoxin completely eliminated the characteristic appearance of interferon which followed endotoxin inoculation of untreated mice. In the case of NDV challenge, mice treated 48 h previously with endotoxin showed a markedly depressed interferon titre compared to the untreated controls. The untreated mice showed significant interferon

levels 2 h after injection of NDV. However, mice pre-treated with endotoxin did not produce a detectable level of interferon until 6 h after stimulation. At 12 h after inoculation of NDV, the interferon titre in control mice was 1:2,500, while in mice pretreated with endotoxin the titre had reached only 1:140. It is of interest to note that, although the levels of circulating interferon in the two groups of mice were significantly different, the rate of interferon increase after 6 h was the same.

Another experiment was performed in which mice were injected with different amounts (50 or 250 µg) of endotoxin and challenged 48 h later with a large dose (1,000 µg) of endotoxin, *B. abortus*, or decreasing amounts of NDV. The results obtained are summarized in Table 1. It is

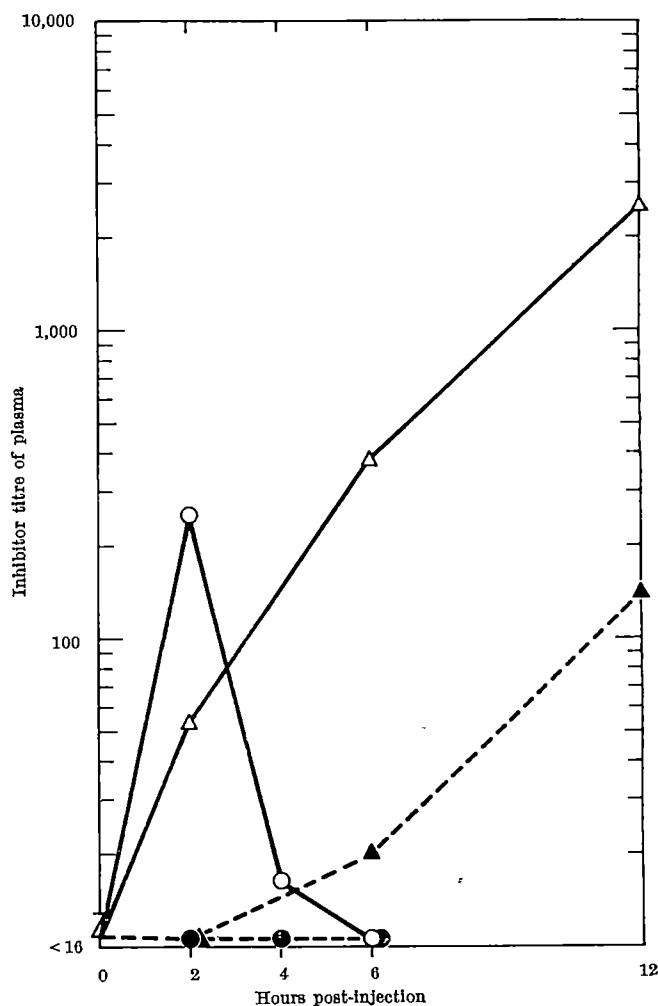


Fig. 1. Effect of prior injection with endotoxin on interferon titres of plasma pools from mice at different times after intravenous injection of endotoxin or Newcastle disease virus (NDV). (Groups injected as follows: O, no prior treatment, 125 µg endotoxin; ●, pretreatment with 125 µg endotoxin 48 h before 125 µg endotoxin; Δ, no prior treatment, 5×10^7 plaque-forming units of NDV; ▲, pretreatment with 125 µg endotoxin 48 h before 5×10^7 plaque-forming units of NDV)

clear that both doses of endotoxin produced hyporeactivity although the higher dose (250 μ g) was much more effective. In addition, decreased interferon responses were demonstrable in animals challenged with *B. abortus* as well as with endotoxin and NDV. It should be noted that no significant change in lethality of endotoxin was found in mice 48 h after the original dose of endotoxin.

The possibility was considered that circulating humoral factors present 48 h after endotoxin injection were responsible for the hyporeactive state. To test this in the case of endotoxin, 50 mice were injected intravenously with 250 μ g of endotoxin; 48 h later, the survivors were bled and plasma collected and pooled. Before transferring this plasma pool to recipient mice, tests of the plasma pool showed that no residual interferon was detectable, eliminating the possibility of transfer of interferon to the recipients. Ten uninoculated mice received intraperitoneally 1 ml. of the donor plasma pool. Six hours later these mice and control mice were injected with 1,000 μ g of endotoxin; 2 h later they were bled to determine the interferon content of their plasmas. Control mice had received plasma from untreated mice rather than from mice treated with endotoxin. No difference was noted in the interferon content of plasmas from the two groups of animals following the endotoxin challenge. These results tend to rule out the possibility of a circulating humoral factor which might mediate the lack of response to the second dose of endotoxin.

Table 1. APPEARANCE OF INTERFERON FOLLOWING INJECTION OF ENDOTOXIN, *B. abortus*, AND NDV IN CONTROL MICE AND IN MICE INJECTED 48 h PREVIOUSLY WITH A SINGLE DOSE OF *E. coli* ENDOTOXIN

Challenge of mice at 48 h with	Interferon titre of plasma of mice injected 48 h previously with:		
	Saline (0.1 ml. i.v.)	50 μ g endotoxin (i.v.)	250 μ g endotoxin (i.v.)
Endotoxin (1,000 μ g)	1,750†	45	<16
<i>B. abortus</i> (1.5 $\times 10^8$ viable units)	560†	70	<16
NDV (4.1 $\times 10^8$ P.F.U.)	1,400†	1,100	330
NDV (4.1 $\times 10^7$ P.F.U.)	860	350	200
NDV (4.1 $\times 10^6$ P.F.U.)	50	55	<16
Saline (0.1 ml. i.v.)	<16	—	—

* Mice challenged with endotoxin were bled 2 h after injection.

† Mice challenged with *B. abortus* or NDV were bled 10 h after injection. The lot of endotoxin used in this experiment had an LD_{50} of 420 μ g.

Previous experience using passively transferred anti-NDV chicken serum in mice showed that interferon did not appear following NDV injection into recipients with circulating antibodies (Table 2). To test for the presence of virus-neutralizing substances in mice which had been rendered hyporeactive to endotoxin and NDV, the following was done. Mice were injected with 125 μ g of endotoxin (Fig. 1); 48 h later they were bled without challenge and their plasmas were tested for NDV-neutralizing activity by a plaque-reduction method using chick embryo fibroblast cultures. No NDV-neutralizing activity was found. This result eliminated antibody as the mediating factor in decreased reactivity to NDV.

Table 2. EFFECT OF PASSIVE IMMUNIZATION OF MICE WITH ANTI-NDV SERUM ON INTERFERON TITRES FOLLOWING INTRAVENOUS INJECTION OF NDV

Treatment	Interferon titre of plasma at	
	4 h	12 h
Anti-NDV chicken serum†	<16†	<64†
Normal chicken serum*	256	>4,096

* One ml. serum injected intraperitoneally 18 h prior to challenge with 5×10^7 plaque-forming units of NDV.

† Plasma from passively immunized mice prior to challenge with NDV showed a neutralizing titre of 1:1,250 by the plaque-inhibition method using chick embryo fibroblasts as host cells.

‡ Lowest dilution tested.

In order to determine whether or not prior injection of NDV would result in decreased interferon stimulation by endotoxin, a large group of mice was injected intravenously with 2×10^8 plaque-forming units of NDV. The titre of interferon in representative animals was determined using plasmas obtained 2, 6, 12, 24, 48 and 72 h after inoculation of virus. At 48, 72 and 144 h after

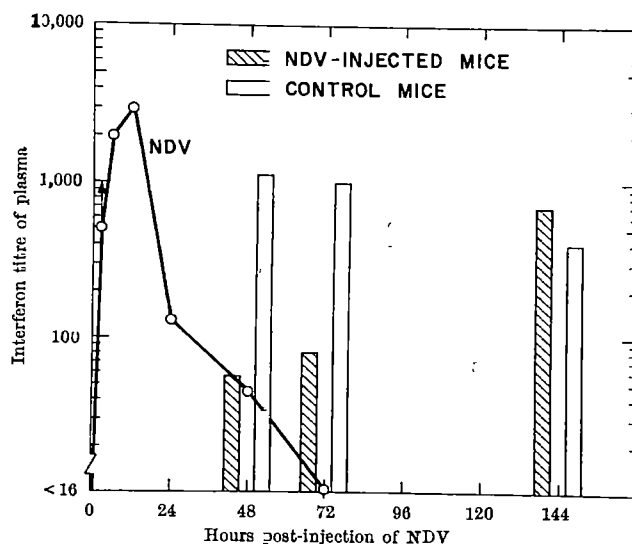


Fig. 2. Influence of prior injection of NDV on interferon response of mice to endotoxin. Interferon titres following injection of 2×10^8 plaque-forming units of NDV (O); a challenge dose of 500 μ g of endotoxin was given at 48, 72, and 144 h and plasmas were collected 2 h later (see bars)

the single dose of NDV, virus-treated and control mice were injected intravenously with 500 μ g of endotoxin; 2 h later these animals were bled and their plasmas tested for interferon. The results of this experiment, presented in Fig. 2, show that NDV effectively decreased interferon response to endotoxin. This decreased response to endotoxin did not disappear until between 72 and 144 h after injection of NDV. It should be noted that 48 h after virus injection there was a residual interferon titre of 1:45 in the plasma and no additional interferon was detected after endotoxin inoculation; in control mice the same dose of endotoxin produced a titre of 1:1,100. At 72 h after NDV inoculation, when no detectable residual interferon was present in the circulation, there was about 90 per cent inhibition of interferon appearance in virus-treated mice as compared with controls. By 144 h after NDV injection, full reactivity to endotoxin had returned. The preceding experiments show: (1) that the decreased appearance of interferon in hyporeactive animals is not limited to the material used to produce this state; (2) that no detectable humoral factor is responsible for the decreased interferon response induced by the injection of endotoxin or NDV.

Hyper-reactivity, the enhanced state of responsiveness to the lethal and pyrogenic effects of endotoxin, is known to occur in mice infected with attenuated tubercle bacilli⁴. Results are presented here to show that infection with *M. tuberculosis*, strain B.C.G., increases the reactivity of mice to endotoxin when measured by interferon response as well as by lethality. Mice were infected intravenously with 0.1 mg of a 10-day culture of B.C.G. grown in a modified Dubos-Middlebrook medium⁵. Fourteen days later, LD_{50} determinations with endotoxin were made using infected and un-infected mice. In addition, groups of infected and control mice received different doses of endotoxin, and interferon determinations were made with plasmas

Table 3. INFLUENCE OF HYPER-REACTIVITY FOLLOWING BCG INFECTION ON INTERFERON TITRES FOLLOWING DIFFERENT DOSES OF *E. coli* ENDOTOXIN

Group	LD_{50} of endotoxin	Amount of endotoxin used for interferon stimulation		Interferon titre of plasma at 2 h
		(μ g)	(LD_{50})	
B.C.G.-infected mice (infected 14 days prior to testing)	23 μ g	32	1.4	>4,096
		3.2	0.14	2,300
		0.32	0.014	120
		0.032	0.0014	<16
Un-infected mice	540 μ g	500	0.9	>2,048
		50	0.09	200
		5	0.009	200
		0.5	0.0009	30

obtained from bleedings 2 h later. The results are shown in Table 3. The mice infected with B.C.G. showed a 23-fold increase in sensitivity to the lethal effects of endotoxin. Associated with this, infected mice demonstrated a markedly enhanced ability to produce interferon following decreasing doses of endotoxin. For example, in infected mice 3.2 µg of endotoxin produced the same interferon titre as 500 µg in un-infected animals. Additional experiments, in which the degree of hyper-reactivity varied depending on the duration of infection, confirmed the correlation between heightened interferon-levels and increased sensitivity to the lethal effects of endotoxin.

The appearance of interferon in the circulation following the injection of *B. abortus* and NDV was tested in B.C.G.-infected mice which were hyper-reactive to the lethal and interferon-stimulating effects of endotoxin. No significant differences were found in the responses of infected and control mice to *B. abortus* and NDV over a wide dose-range.

The experiments which have been described indicate that the appearance of interferon in the circulation of mice can be affected by procedures which alter responsiveness to the lethal and pyrogenic effects of endotoxin. Hypo-reactivity produced with endotoxin inhibited responsiveness, as measured by interferon-levels, to endotoxin, *B. abortus*, and NDV; moreover, prior inoculation of

NDV markedly reduced interferon stimulation by endotoxin. These results indicate the likelihood that the same cells, probably phagocytic, are involved in the release of interferon following both types of stimulation, despite the different time patterns of appearance of interferon in the circulation¹.

In the case of hyper-reactivity to endotoxin elicited by infection with tubercle bacilli, it is apparent that the increased lethality of endotoxin is accompanied by an increased capacity of small doses of endotoxin to elicit interferon. The lack of a parallel increase in response to different doses of *B. abortus* or NDV in B.C.G.-infected mice is not explained by the results presented here and is being investigated further.

We thank Nancy Arnold, Velma Axelrod, Marion Kelly, Theodora Koshar, Gretchen McIntyre and Gloria Simon for their assistance. Endotoxin was provided through the generosity of Dr. A. I. Braude (University of Pittsburgh) and Dr. F. McIntire (Abbott Laboratories).

The work was supported by grants AI-03561 and AI-06264 from the U.S. National Institutes of Health.

¹ Stinebring, W. R., and Youngner, J. S., *Nature*, **204**, 712 (1964)

² Landy, M., and Braun, W., ed., *Bacterial Endotoxins* (Institute of Microbiology, Rutgers, The State University, New Brunswick, N.J., 1964).

³ Youngner, J. S., and Stinebring, W. R., *Science*, **144**, 1022 (1964).

⁴ Suter, E., *Trans. New York Acad. Sci.*, Ser. II, **24**, 281 (1962)

⁵ Dubos, R., and Middlebrook, G., *Amer. Rev. Tuberc.*, **56**, 334 (1947).

ALLOGENEIC INHIBITION OF TUMOUR CELLS BY *IN VITRO* CONTACT WITH CELLS CONTAINING FOREIGN H-2 ANTIGENS

By K. E. HELLSTRÖM, I. HELLSTRÖM and C. BERGHEDEN

Institute for Tumour Biology, Karolinska Institutet Medical School, Stockholm

HOMOZYGOUS tumour cells grow in a higher frequency and after a shorter latency period when small cell numbers are transplanted to syngeneic hosts than to 'semi-syngeneic' F_1 hybrids¹⁻³. This phenomenon has been designated by the term 'syngeneic preference' in relation to the homozygous host and 'allogeneic inhibition' with reference to the F_1 hybrid host⁴.

Allogeneic inhibition could not be explained by a host-versus-graft reaction against hypothetical antigens which might have been present in the homozygous cells and hosts but absent from the F_1 hybrid hosts, since pre-irradiation of the F_1 animals did not abolish the difference and since pretreatment of the F_1 hybrids prior to grafting with normal or tumour cells of the homozygous strain did not influence allogeneic inhibition⁵. Tissue culture experiments showed that cell extracts or homogenates containing foreign histocompatibility antigens inhibited the growth of explanted target cells, suggesting that allogeneic inhibition *in vivo* might be due to the contact between the grafted tumour cells and the foreign isoantigens of the host^{5,6}. This hypothesis was subjected to further test in the present article. Neutralization experiments were made in which lymphoma cells were exposed *in vitro* to spleen or tumour cells, containing foreign H-2 antigens, and their outgrowth in syngeneic hosts was measured after subsequent implantation. Furthermore, tissue culture experiments were performed, in which explanted tumour cells were followed for the development of cytopathic changes after exposure to lymphoma cells, containing H-2 antigens of the syngeneic or allogeneic type.

Neutralization experiments. Four isoantigenic variant sub-lines were used, all derived from the *LNSF* lymphoma (of $A/Sn \times A.SW F_1$ hybrid origin). Three lines, designated *LNSFSC*, *LNSFSD* and *LNSFSE*, had been selected from *LNSF* for compatibility with the *A.SW* parental strain, and permanently lacked detectable H-2

antigens specific for the *A* strain⁵. One line, *LNSFAD*, had been selected from *LNSF* for compatibility with strain *A*. It lacked detectable H-2 antigens that were specific to the *A.SW* strain⁵. The variant lines were propagated by serial transfer in syngeneic mice (*A.SW* or *A*, respectively) during 1-10 passages.

The technique used for the neutralization tests was a modification of one described by Klein and Sjogren⁷. *A.SW*-compatible variant tumours (*LNSFSC*, *LNSFSD* or *LNSFSE*) were gathered from syngeneic (*A.SW*) mice and cell suspensions were prepared mechanically and diluted in balanced salt solution to give a concentration of 10^4 or 10^5 cells per ml. Similar suspensions were prepared from the spleens of adult, untreated mice of various genotypes (*A*, *A.BY*, *A.SW*, *CBA*, *A.SW* \times *A F_1*, *A.SW* \times *A.BY F_1*, *A.SW* \times *CBA F_1*). Among these strains *A*, *A.SW* and *A.BY* are congenic resistant lines, differing at the H-2 locus⁸. Within each experiment spleens were taken from mice of the same sex (mostly virgin females), weight and age. After washing, the spleen cells were diluted to a final concentration of 10^6 blue unstained cells/ml. In half the experiments they were irradiated *in vitro* immediately after preparation of the cell suspension with an X-ray dose of 1,000 r. (or in two experiments up to 10,000 r.). Tumour and spleen cells were mixed in equal volumes and Wellcome phytohaemagglutinin (PHA) added in a final concentration of 1:100 to establish a close contact between the tumour and spleen cells^{9,10}. The mixtures were thoroughly shaken and incubated for 45 min at 37° C. Afterwards they were inoculated subcutaneously to adult *A.SW* mice to yield a total number of 10^3 or 10^4 tumour cells inoculated per mouse. The recipient mice had been irradiated with 350 r. immediately before inoculation in order to decrease their own immunological reactivity. They were inspected 2-3 times weekly for tumour development, three diameters

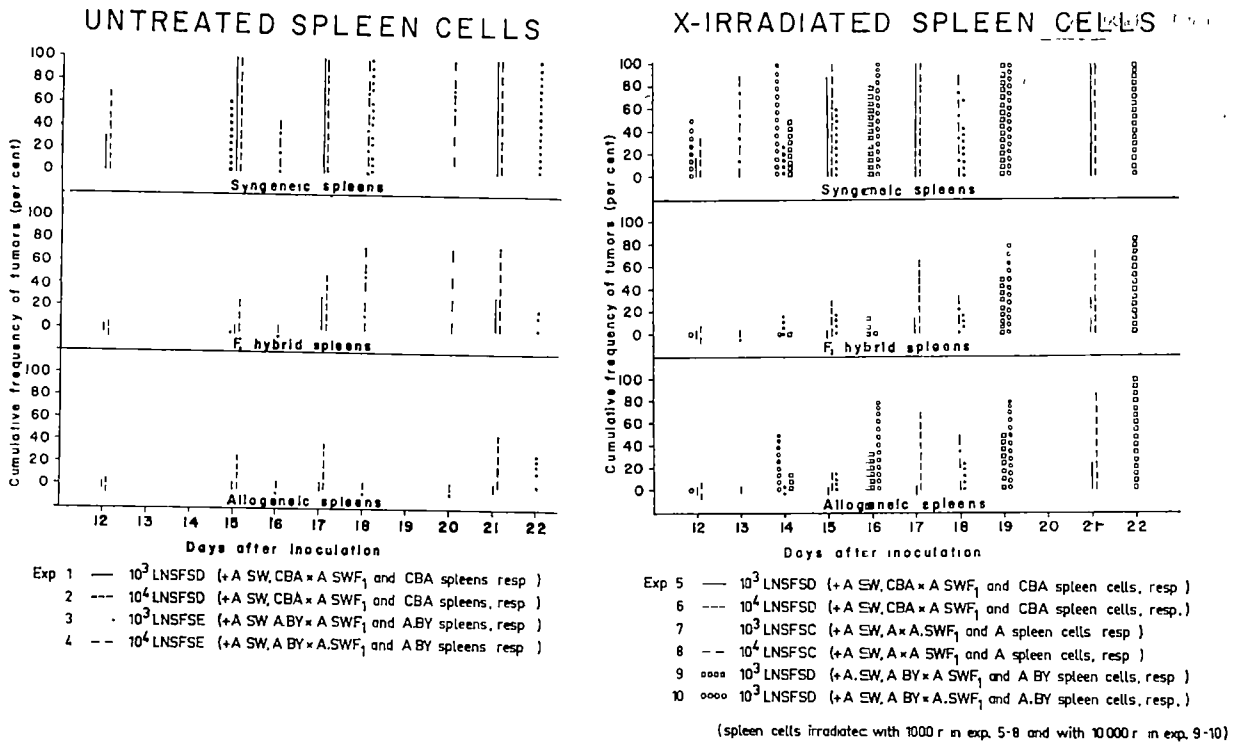


Fig. 1. Neutralization of lymphoma cell outgrowth in syngeneic mice, pre-irradiated with 350 r, after exposure to untreated or X-irradiated spleen cells and phytohaemagglutinin *in vitro*. In each group of experiments 6-8 mice were inoculated

of any tumours appearing being measured and the geometric means calculated.

Fig. 1 shows that allogeneic and F_1 hybrid spleen cells inhibited the outgrowth of the lymphoma lines, as compared to syngeneic spleen cells. When unirradiated spleen cells were added, F_1 hybrid cells were slightly less effective than allogeneic cells. Pre-irradiation of the spleen cells minimized this difference by decreasing the inhibitory effect of the allogeneic cells but scarcely affecting the effect of F_1 hybrid cells.

The demonstration of allogeneic inhibition after contact of tumour cells with spleen cells, carrying foreign $H-2$ isoantigens, led to experiments where the spleen cells had been replaced with tumour cells. This was done in order to test the possibility that neutralization as observed after addition of spleen cells was due to an immunological reaction of the added cells not sensitive to X-irradiation. Experiments were performed in a similar manner as when spleen cells were added. Two variant lines derived from *LNSF* were used, namely, *LNSFAD*, compatible with strain *A* and lacking $H-2$ antigens of *A.SW* origin, and *LNSFSE*, compatible with *A.SW* and lacking $H-2$ antigens of *A* origin. In each experiment, suspensions containing 10^4 or 10^5 untreated lymphoma cells of one type were mixed with an equal volume containing 10^8 lymphoma cells of the other type which had been rendered incapable of multiplication by X-irradiation with 15,000 r.; in controls, irradiated lymphoma cells of the syngeneic type were added. After incubation in the presence of PHA the mixtures were inoculated in mice, syngeneic to the untreated cells, 6-8 mice being inoculated in each experimental group. In part of the experiments, the irradiated cells were ultrasonically disintegrated before they were added to the untreated lymphoma cells.

A total of four neutralization experiments were performed with added lymphoma cells (or cell extracts) and gave similar results, as exemplified by the experiment presented in Fig. 2. It appears that exposure of *LNSFAD* cells (*A*-compatible) to *LNSFSE* cells (*A.SW*-compatible)

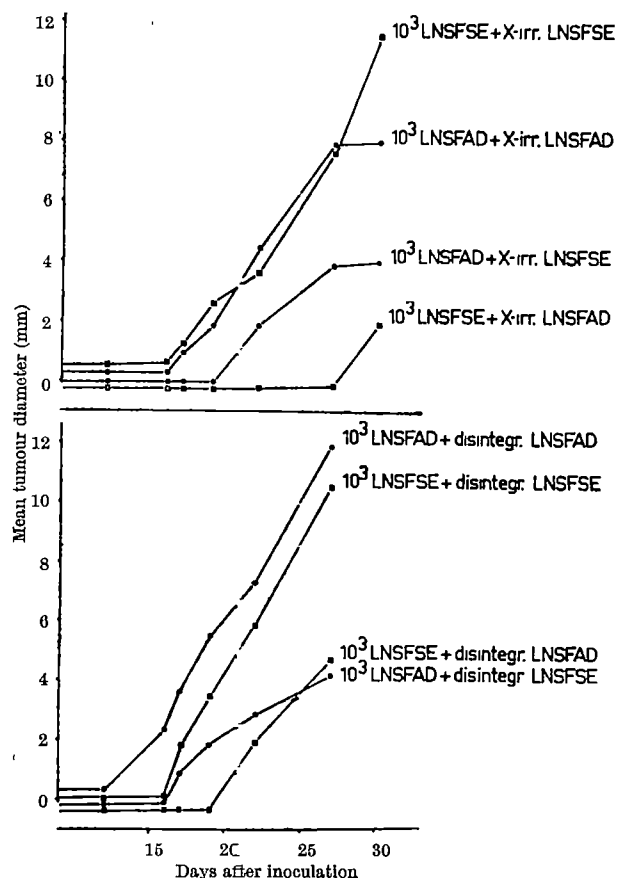


Fig. 2. Neutralization of outgrowth of lymphoma variants *LNSFAD* and *LNSFSE* in syngeneic (*A* and *A.SW*, resp.) mice after exposure to X-irradiated (15,000 r.) intact and ultrasonically disintegrated *LNSFAD* and *LNSFSE* cells. In each group of experiments 6 mice were inoculated

Table 1. RECORDS OF CULTURES EXPOSED TO LYMPHOMA VARIANT CELLS CONTAINING *H-2* ISOANTIGENS OF THE SYNGENEIC OR ALLOGENEIC TYPE

Exp. No.	Explanted tumour		Days after addit. of lymphoma cells	Records of cultures given*						Ratio of records with syng : allog. ly. c.†		
				LNSFAD (A-comp.)†			LNSFSD (A.SW-comp)†					
	Designation	Strain of origin		2 × 10 ⁷	10 ⁷	5 × 10 ⁶	2 × 10 ⁷	10 ⁷	5 × 10 ⁶	2 × 10 ⁷	10 ⁷	5 × 10 ⁶
1	S1A	A	4		3.3	3.7		2.0	3.0		1.7	1.2
			5		2.7	3.3		1.3	2.3		2.1	1.4
			7		2.3	3.0		0.3	2.0		7.7	1.5
	MWE	A.SW	4		1.0	1.3		2.3	3.7		2.3	2.8
			5		1.0	1.0		2.3	3.0		2.3	3.0
2	S1A	A	9		0.3	0.7		5.0	5.0		16.6	7.1
			3		4.0	4.0		1.0	2.0		4.0	2.0
			6		4.0	4.3		1.0	2.0		4.0	2.2
	MWE	A.SW	2		2.0	3.0		3.0	3.0		1.5	1.0
			3		2.0	2.3		2.6	4.0		1.3	1.7
3	S1A	A	6		2.0	3.0		3.0	4.0		1.5	1.3
			3		2.7	3.0		1.5	2.5		1.8	1.2
			4			4.0			2.5			1.6
	MWE	A.SW	3	2.0	4.0		3.5	4.0		1.8	1.0	
			4	2.0	4.0		4.0	5.0		2.0	1.3	
4	YAA-C1-C3	A	3	4.3	4.0	5.0	0.5	4.0	4.8	8.6	1.0	1.0
			6	4.0	4.0	5.0	0.5	0.5	3.5	8.0	8.0	1.4
5	YAA-C1-C3	A	3	2.5	3.0		1.0	3.0		2.5	1.0	
			5	2.0	4.0		1.0	3.0		2.0	1.3	
			10	3.0	5.0		1.0	4.0		3.0	1.3	

* Cultures were observed for development of cytopathic changes after addition of 2 × 10⁷, 10⁷ and 5 × 10⁶ syngeneic or allogeneic lymphoma cells and compared with untreated control cultures, consisting of monolayers of apparently healthy cells. They were recorded as follows: 5, treated cultures better than untreated controls; 4, no difference between treated cultures and untreated controls, 3, approximately 25 per cent of cells destroyed; 2, approximately 50 per cent of cells destroyed; 1, only a few cells surviving; 0, all cells destroyed. The Table shows the mean of records given to a minimum of 3 Petri dishes which had been exposed to the same treatment.

† *LNSFAD* and *LNSFSD* cells were gathered from A × A.SW F₁.

‡ Ratios were calculated between records with added syngeneic as compared to allogeneic lymphoma cells (allog. ly. c.).

inhibited the outgrowth of *LNSFAD* and that growth of *LNSFSE* was inhibited after contact with intact or disintegrated *LNSFAD* cells.

The neutralization experiments demonstrate that differences between homozygous and F₁ hybrid mice with regard to general host factors such as endocrine balance, immunological reactivity or hybrid vigour cannot account for allogeneic inhibition. The finding that tumour outgrowth was inhibited, both by pre-irradiated F₁ hybrid spleen cells and by intact or disintegrated lymphoma cells carrying foreign *H-2* antigens, gives increased weight to the hypothesis that allogeneic inhibition depends on a close contact between cells carrying different histocompatibility antigens, and thus having a different surface structure⁶. This is also strengthened by (unpublished) experiments where the inhibitory effect of added allogeneic or F₁ hybrid cells was abolished if their *H-2* antigens were specifically covered with antibodies.

Tissue culture experiments. *In vitro* experiments were performed in order to definitely exclude all influence of the host animal on neutralization of tumour growth as observed after addition of cells carrying foreign *H-2* antigens. Transplanted tumours of A or A.SW origin were explanted and the cultures were exposed to added lymphoma cells from A- or A.SW-compatible variant sub-lines, selected from *LNSF*. Three kinds of tumour cells were explanted: S1A, MWE and YAA-C1-C3. S1A is a mammary carcinoma of A origin, MWE a sarcoma of A.SW origin and YAA-C1-C3 a cloned lymphoma of A origin¹¹. Explantation was made into 50-mm 'Falcon' plastic Petri dishes, each Petri dish being seeded with 3 × 10⁶ tumour cells. About 24 h after seeding, the culture medium was decanted and suspended lymphoma variant cells of the *LNSFAD* (A-compatible) or *LNSFSD* (A.SW-compatible) lines were added in 0.2-ml. volumes to each Petri dish, the lymphoma suspensions containing between 2.5 × 10⁷ and 10⁸ cells per ml. In addition, 0.2 ml. PHA, diluted 1:20, was added. After 45 min incubation at 37° C, 4-ml. culture medium, containing 20 per cent heat-inactivated calf serum in Earle's solution, was added to each Petri dish, and the dishes were incubated in a 5 per cent carbon dioxide-air atmosphere. The Petri dishes were followed for appearance of cytopathic changes, which were recorded as stated in the footnote to Table 1. A minimum of three Petri dishes were used for each kind of treatment. All dishes were given coded numbers at the time of addition of the

lymphoma cells, the code being unknown to the persons observing the cultures.

Findings are presented in Table 1. Addition of lymphoma cells containing *H-2* antigens, allogeneic to the explants, consistently produced cytopathic changes in the explanted cells with very small variation between individual Petri dishes, exposed to the same type and number of lymphoma cells. The fact that both lymphoma lines (*LNSFAD* and *LNSFSD*) were gathered from A × A.SW F₁ hybrid mice and tested on tumours for which they were syngeneic and allogeneic, respectively, excludes influence of normal stroma cells of host origin on the results. It follows that the cytopathic changes detected after exposure of explanted cells to allogeneic lymphoma variants are scarcely a consequence of immune reactions of the added cells, unless the assumption is made that the lymphoma cells are capable of immunological reactivity; the fact that neither pre-irradiation nor ultrasonic disintegration of the lymphoma cells abrogated their ability to neutralize tumour growth *in vivo* excludes the latter possibility. Both neutralization and tissue culture experiments therefore strongly support the hypothesis that allogeneic inhibition of tumour growth is a consequence of target cell contact with foreign *H-2* isoantigens⁶. It is suggested that allogeneic inhibition may function as a surveillance mechanism, capable of eliminating cellular variants, the (*H-2*) antigenicity of which differs from the general pattern within a given tissue, without necessary engagement of immunologically competent cells.

This work was supported by grants from the Swedish Medical Research Council and the Swedish Cancer Society, and by grant C-3700 to Prof. G. Klein from the National Cancer Institute, U.S. Public Health Service. We thank Prof. G. Klein for his advice and Mrs. Solveig Humla, Mrs. Britta Jonsson, Miss Maj Britt Sondell and Miss Cecilia Wijnbladh for their assistance.

¹ Hellström, K. E., *Nature*, **199**, 614 (1963).

² Hellström, K. E., *Science*, **143**, 477 (1964).

³ Snell, G. D., and Stevens, L. C., *Immunology*, **4**, 366 (1961).

⁴ Hellström, K. E., and Möller, G., *Prog. Allergy*, **9**, 158 (1965).

⁵ Hellström, K. E., and Hellström, I., in *Isoantigens and Cell Interactions* (Wistar Institute Mon., in the press).

⁶ Hellström, K. E., Hellström, I., and Haughton, G., *Nature*, **204**, 661 (1964).

⁷ Klein, E., and Sjögren, H. O., *Cancer Res.*, **20**, 452 (1960).

⁸ Snell, G. D., *J. Nat. Cancer Inst.*, **21**, 843 (1958).

⁹ Holm, G., Werner, B., and Perlmann, P., *Nature*, **203**, 841 (1964).

¹⁰ Möller, E., *Science*, **147**, 873 (1965).

¹¹ Sjögren, H. O., and Hellström, I., *Exp. Cell Res.* (in the press).

SPECIFIC UPTAKE IN CORPORA LUTEA OF A NON-STEROID SUBSTANCE WITH ANTI-GESTAGENIC PROPERTIES

By ÅKE HANNGREN, NIELS EINER-JENSEN and SVEN ULLBERG

Department of Medicine, Karolinska Hospital, and Department of Pharmacology, Royal Veterinary College, Stockholm, Sweden

CERTAIN unsymmetrical diphenylalkenes have been found to possess the ability to interfere with the vaginal oestrous reaction induced by oestradiol benzoate^{1,2}. Among these substances, *bis*(*p*-hydroxyphenyl)-cyclohexylidenemethane (compound 'F6060', Fig. 1) has an oestrogenic activity only about 1/1,000 that of oestradiol benzoate.

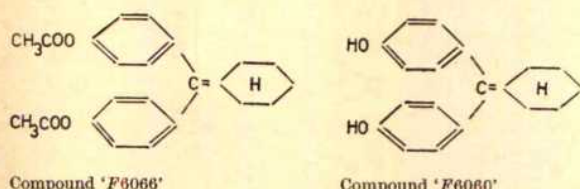


Fig. 1

In clinical investigations it was found that the acetylated form of the compound (Compound 'F6066') had an effect on prostatic cancer similar to conventional oestrogens but without the feminizing side-effects³. In postmenopausal women a depression of the output of oestradiol-17 β and changes in the relation between excreted FSH and LH were found⁴.

By determining the body distribution of the compound it was hoped to gain further information regarding their site of action. The substances were labelled with ¹⁴C at the α -carbon⁵. The specific activity was about 10 μ C/mg.

The compounds, which were synthesized by Ferrosan, Ltd., Sweden, were dissolved in absolute alcohol and mixed with water containing 'Tween 80' (5 per cent).

Female mice, non-pregnant and pregnant in a late stage of gestation, were used. A dose of 0.8 mg corresponding to 8 μ C was given intravenously. The mice were killed by rapid freezing at the following intervals after injection: 5 min, 20 min, 1 h, 4 h and 24 h.

20- μ sections at various levels through the whole animal were taken at -10°C. The sections were freeze-dried and pressed against Gevaert 'Structurix D 7' X-ray film⁶.

The autoradiograms showed a remarkably strong and selective accumulation of radioactivity in corpora lutea after only 5 min (Fig. 2). This accumulation was still prominent after 20 min (Fig. 3) and then decreased successively but was still apparent after 24 h (Fig. 4). It was most pronounced in the pregnant mice. In non-pregnant mice the accumulation in corpora lutea varied, probably owing to different functional states.

A selective accumulation was also noticed in part of the placenta. The activity was confined to the visceral yolk sac epithelium and was seen from 1 to 24 h (Fig. 4) with a peak at 4 h. From this time and during the whole observation period it showed the highest activity in the body. A detailed description of the autoradiographic results will be published elsewhere.

The influence of 'F6066' on early pregnancy was tested in rats and rabbits. Groups of six female rats were caged together with males, two females to one male. On the first and second day the females were injected subcutaneously with an oil solution of 'F6066'. The injections were then repeated three times at weekly intervals. If pregnancy was observed the animals were killed just be-

Table 1. ANTI-FERTILITY IN RATS

	No. of pregnancies per 6 animals	No. of fully developed foetuses per 6 animals
Olive oil	5	51
1 mg/kg/day 'F6066'	4	41
5 mg/kg 'F6066'	1	11
25 mg/kg 'F6066'	0	0

fore parturition. On the day of the last injection the males were removed and 3 weeks later all females were killed for examination of possible pregnancy and number of living foetuses. The results are presented in Table 1. The number of living foetuses decreased with increased dose. The highest dose used was <1.5 per cent of the acute LD₅₀.

In a modified Clauberg test, immature female rabbits (600-800 g) were primed with 0.5 μ C oestradiol benzoate for 7 days. From days 8 to 14 they were all injected with 0.2 mg progesterone in olive oil, subcutaneously, and half of them also received 'F6066' simultaneously, on days 8-14 or days 13-14.

In the Clauberg test, injections of oestrogens produce a proliferation of the endometrium. Progesterone injections

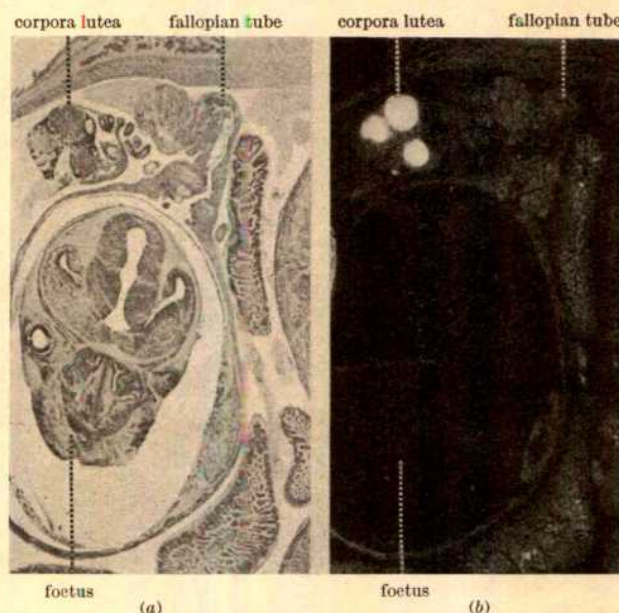


Fig. 2. Autoradiogram (b) showing the distribution of ¹⁴C-'F6060' in ovary, fallopian tube and pregnant uterus 5 min after intravenous injection. Corresponding tissue section (a) is stained by haemalum-eosin. Note the very high concentration in corpora lutea. No activity in the foetus

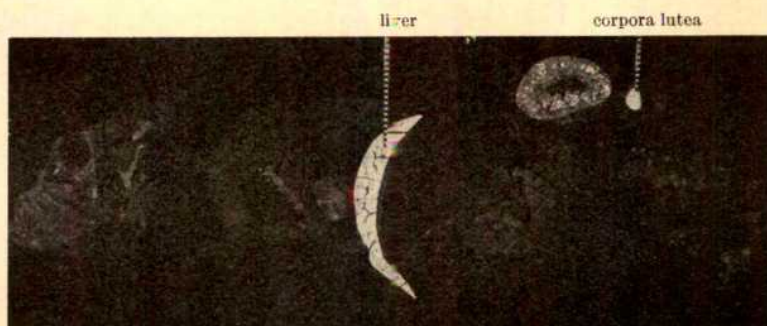


Fig. 3. Whole-body autoradiogram from a pregnant mouse 20 min after intravenous injection of ¹⁴C-'F6066'. Note high radioactivity in corpora lutea and liver

transform this to a secretory endometrium. Simultaneous administration of 1 mg 'F6066' from days 8 to 14 prevented the development of the secretory endometrium. If 5 mg 'F6066' was injected on days 13 and 14, when the secretory phase was established, a regression could be seen (Figs. 5 and 6).

The present investigation has shown that the substances have: (1) a selective and high accumulation in corpora lutea; (2) an anti-fertility activity in rats; and (3) an anti-progesterone effect in the Clauberg test.

The strong affinity for corpora lutea is unique and may indicate an interference with progesterone production. The lack of accumulation in some corpora lutea may indicate that the affinity is not related to the cell-type as such but to the intensity of hormone production.

On the other hand, the Clauberg test shows that the substances interfere with the effect of injected progesterone, that is, have a peripheral effect on a target organ. It is known that natural oestrogens and stilboestrol have similar properties. Thus the observed effect may be due to the weak oestrogenic activity. In the anti-fertility tests, however, the autopsies after administration of 'F6066' did not show the large volume of sanguinary fluid in the uterine cavity that is often seen after stilboestrol-induced abortion.

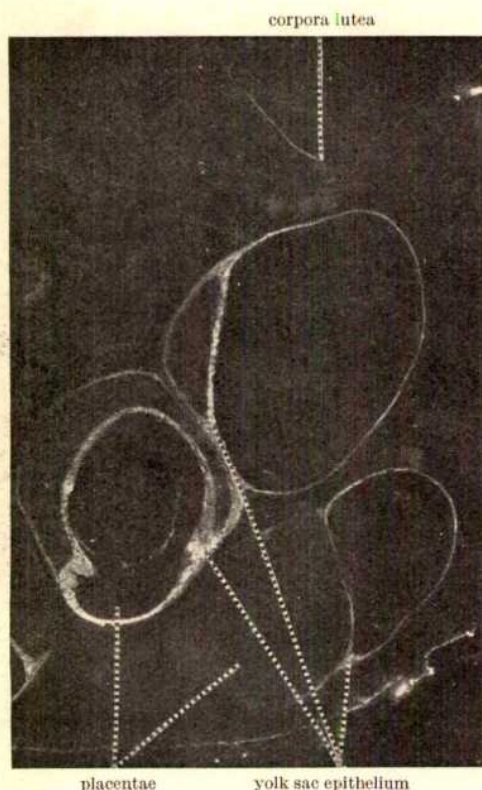


Fig. 4. Autoradiogram showing the distribution of ^{14}C -'F6060' in a pregnant mouse 24 h after intravenous injection. Note high radioactivity in the visceral yolk sac epithelium. Radioactivity is seen in 5 corpora lutea. Three placentae and foetuses show no radioactivity.



Fig. 5. Endometrium of rabbit uterus on day 15 after administration of 0.5 µg oestradiol benzoate on days 1-7, and 0.2 mg progesterone on days 8-14.

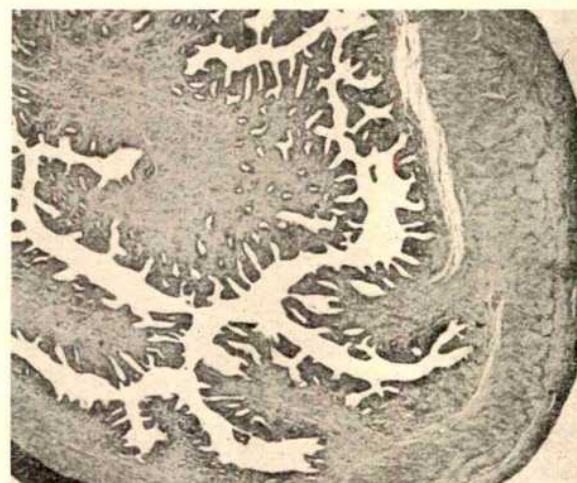


Fig. 6. Endometrium of rabbit uterus on day 15 after administration of 0.5 µg oestradiol benzoate on days 1-7, 0.2 mg progesterone on days 8-14 and 5.0 mg 'F6066' on days 13 and 14.

The observations made may indicate interference with gestagen production as well as an inhibition of the gestagen effect on target organs. The accumulation found in the yolk-sac placenta may be explained as a foetal excretory event, but interference with progesterone synthesis may also be considered.

Laboratory equipment used in this investigation was kindly supplied by Ragnar och Torsten Söderbergs stiftelser.

¹ Miquel, J. F., Bárány, E. H., and Müller, W., *Arch. Intern. Pharmacodyn.*, **127**, 262 (1958).

² Miquel, J. F., Wählstam, H., Olsson, K., and Sundbeck, B., *J. Med. Chem.*, **6**, 774 (1963).

³ Nylander, G., and Terner, N., *Urologia*, **271**, 1 (1964).

⁴ Persson, B. H., *Acta Soc. Med., Upsal.*, **1** (1964).

⁵ Karlsson, L., and Sundbeck, B. (in preparation).

⁶ Ullberg, S., *Proc. Second U.N. Intern. Conf. Peaceful Uses of Atomic Energy*, **24**, 248 (1958).

AN INSECT 'BITOMETER'

By PHILIP KASHIN and HAROLD G. WAKELEY

Life Sciences Research Division, IIT Research Institute, Chicago, Illinois

THE methods at present used to examine biting by mosquitoes are chiefly limited to direct visual observation^{1,2}, or to the measurement of engorgement by changes in weight or radioactive tracer intake after the mosquito has bitten³⁻⁵. In determining the attractant

or repellent properties of a compound based on the method of engorgement, there is no way to determine whether a mosquito has penetrated into and salivated in the skin of a host unless engorgement occurs. The degree of repellency attributed to a compound can be erroneously

sessed if a mosquito penetrates the skin of a host and salivates, but does not engorge. On the other hand, the method of direct visual observation is laborious, uncertain, and in many cases impractical. Since salivation is the factor by which mosquito-borne disease is disseminated, the measure of repellency of a compound by the degree of engorgement is seriously limited.

Through a new assay procedure recently developed in our laboratories, the various phases of the mosquito bite from the initial penetration of the mouth-parts to final withdrawal can be detected electronically.

It was reasoned that the contact between mosquito and its host could be used to form an integral part of a series electrical circuit, where the insertion and withdrawal of the mosquito's mouth-parts in relation to the host could be used as the making and breaking switch in the circuit.

Since it is impracticable to connect a wire directly to a mosquito, a 50- or 100-mesh bronze screen is used to separate the mosquito from its host. The screen covers a 1-pint round ice-cream carton (or glass container if simultaneous visual observation is desired).

Female *Aedes aegypti* L. are placed into the container. The screen is connected by a soldered lead to resistances totaling 16 M Ω , and then to the negative pole of a 1.35-V standard mercury cell (Mallory mercury battery, RM-1-R). The positive pole of the battery is connected to the input of a suitable recording instrument. The recorder is a Sanborn model 320 two-channel general-purpose recorder (Sanborn Co., Waltham, Massachusetts).

The circuit passes through the recorder and is carried by another lead from the recorder to the tail of an anaesthetized mouse (the host), where it is embedded via a hypodermic needle soldered on to the lead. The mouse is placed on top of the bronze screen, belly down. Contact of the mouse's paws with the screen is carefully avoided. The hair on the mouse's body is sufficient to insulate the mouse from the screen, and no current flows. The entire circuit is thus connected in series (Fig. 1). The wiring diagram of the box which contains the resistors and the battery is shown in Fig. 2.

When a mosquito bites the mouse, the mosquito holds on to the wire screen with its legs and passes its proboscis through the spaces of the mesh and into the mouse. When the skin of the mouse is penetrated, the circuit between the mouse and the screen is completed through the mosquito. The resulting flow of current is registered by a displacement of the writing stylus of the recorder. The current flows continuously so long as the mosquito

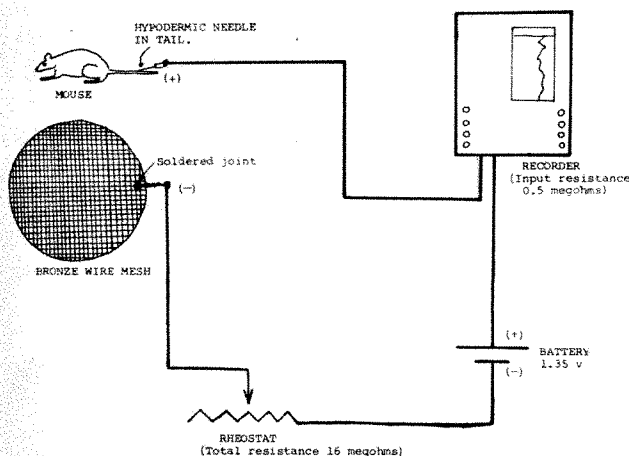


Fig. 1. Circuit diagram of apparatus

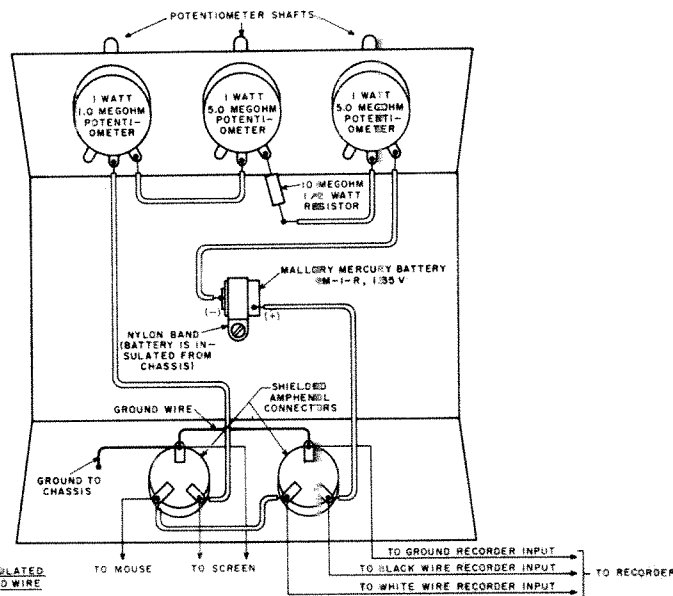


Fig. 2. Wiring diagram of resistor-battery box

is completing the circuit, and the stylus is displaced from the base line (point of no current flow) on the recorder chart paper for the entire time the mosquito is biting. When the proboscis is withdrawn, the circuit is broken. The writing stylus immediately returns to the base line, indicating that virtually infinite resistance has been re-established between the mouse and the bronze mesh.

The question of whether the flow of an electric current could change a mosquito's behaviour or create artefacts in the system was considered. A calculation taking into account the total resistance of the circuit and the total voltage available showed that a maximum of 8.2×10^{-8} amp is available in the circuit, assuming no resistance between the mouse and the mesh. The average displacement of the stylus is 10 mm from the base line when a single mosquito bites a mouse through the screen. In calibrating the recorder (which was always set at its highest sensitivity level of 0.5 mV/mm and maximum gain), it was observed that a displacement of 3.4 mm from the base line is equivalent to 1 mV. A displacement of 10 mm (equivalent to an average single mosquito bite), therefore, indicates a voltage drop of 2.94 mV across the recorder.

From the known input resistance of the recorder (0.5 M Ω), and the measured voltage drop across the recorder when a mosquito bites, it can be shown that approximately 6.0×10^{-8} amp flows through a mosquito at the time of the bite. This is probably an over-estimation, since the resistance of the mosquito is not taken into account. This is an extremely small quantity of electricity, and is probably no more than the static electrical charge that the insect attains during free flight. We therefore consider that no artefacts that might affect a mosquito's activities are introduced by this flow of electrical current. We could not detect changes in a mosquito's behaviour even when all resistances were removed from the circuit. A value of 16 M Ω of resistance between the mesh and the battery seemed to give the most uniform recordings.

The question of whether biting was the only activity that could cause a departure from the base line in the recording was also considered. When male *Aedes aegypti* L. mosquitoes or female mosquitoes whose proboscises were amputated were placed in the mesh-covered container, no displacement from the base line was observed at any time. We therefore conclude that it is only the biting female mosquito which causes displacement.

A visual observation and an electronic recording were made simultaneously during the course of a single mosquito bite. The patterns recorded during the observed

probing activities of the mosquito were generally irregular (left side of Fig. 3). When the mosquito was observed to be in the biting attitude, the chart recording was much more regular (right side of Fig. 3). Engorgement was observed during the more regular phase of the recording. When the mosquito was fully engorged after about 1.5 min the proboscis was withdrawn from the mouse and the stylus immediately returned to the base line on the chart recording (Fig. 4).

When about 50 mosquitoes were placed in the container and permitted to bite simultaneously, the pattern was very irregular (Fig. 5). The departure from the base line was greater than seen with a single mosquito bite. This greater departure is expected, since the resistance between the mouse and the mesh during the biting of a group of mosquitoes is less than during the biting of a single mosquito. However, proportionality of stylus displacement could not be detected for the bites of succeeding mosquitoes. The first mosquito that bites changes the resistance of the system from an infinite to a finite value. Subsequent bites do not cause as large a change, and proportionality should therefore not be expected. In effect, when a group of mosquitoes bite, a number of miniature parallel circuits are set up between the mouse

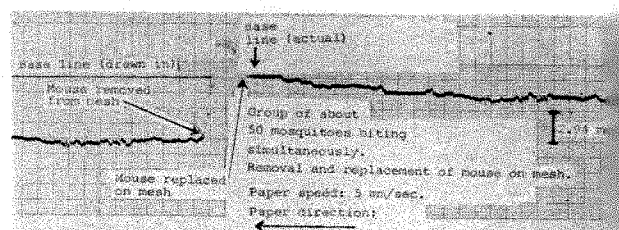


Fig. 6. Removal and replacement of mouse on mesh during biting by a group of mosquitoes

and the mesh. Assuming the resistances of all the mosquitoes are equal, each biting mosquito should draw the same quantity of current. In terms of amps, then, proportionality and additivity might be expected as the number of parallel units (biting mosquitoes) in the circuit increases. We are at present examining methods to quantify the apparatus so that the number of insects biting simultaneously can be ascertained.

During the recording of a group of mosquitoes biting simultaneously, the mouse was removed from the mesh causing all the probosces to be withdrawn from the mouse, and then replaced. Fig. 6 shows that, after the mouse was replaced on the mesh, the base line reverted to its original position. It was displaced again about 1.5 sec later when the mosquitoes resumed their attack on the mouse.

By changing the mesh size of the screen or the host animal, the apparatus can be adapted to determining the biting activities of many species of biting and blood sucking insects, and the attractant, repellent and insecticidal properties of various compounds can be determined. By allowing the recorder to run overnight, or for a longer period of time, the duration of effective activity of a compound can be automatically monitored. The recorder is also capable of portable operation with a battery converter and can therefore probably be adapted for operation under actual field conditions.

This work was supported by the U.S. Army Medical Research and Development Command, Department of the Army, under research contract DA-49-193-MD-2281.

Note added in proof. A paper by McLean and Kinsey (*Nature*, 202, 1358; 1964) describing a technique for recording electronically aphid feeding and salivation has recently come to our attention. Though similar in concept, the instrumentation, electrical circuitry, applications, and sensitivities of these techniques are different.

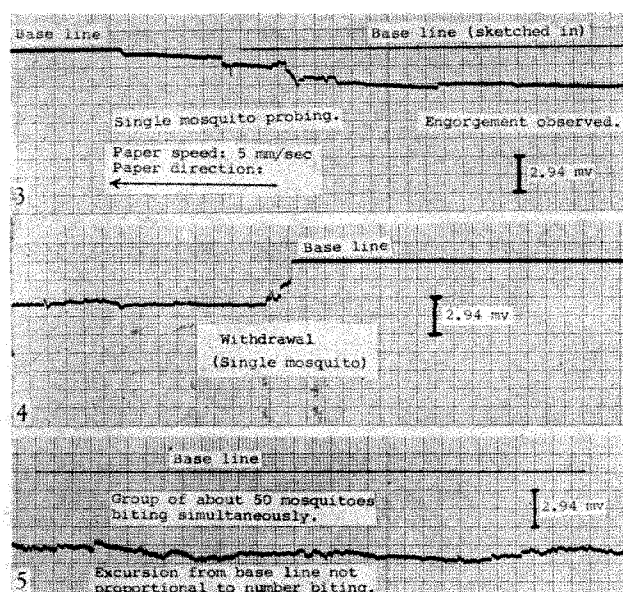


Fig. 3. (Top). Probing (left) and engorgement (right); single mosquito

Fig. 4. (Middle). Withdrawal of mosquito from mouse and return of stylus to original base line. Paper speed: 5 mm/sec. Paper direction: right to left

Fig. 5. (Bottom). Many mosquitoes biting mouse simultaneously. Paper speed: 5 mm/sec. Paper direction: right to left

¹ Gordon, R. M., and Lumsden, W. H. R., *Ann. Trop. Med.*, **33**, 259 (1939).

² Griffiths, R. B., and Gordon, R. M., *Ann. Trop. Med.*, **46**, 311 (1952).

³ Bar-Zeev, M., and Schmidt, C. H., *J. Econ. Entomol.*, **52**, 268 (1959).

⁴ Boormal, J. P. T., *Ann. Trop. Med. Parasitol.*, **54**, 8 (1960).

⁵ Lal, H., Gnocchio, S., and Hawrylewicz, E. J., *Proc. Soc. Exp. Biol. and Med.*, **113**, 770 (1963).

SYNTHESIS OF RIBOSOMAL RNA IN SYNCHRONIZED HELA CELLS

By PROF. MATTHEW D. SCHARFF and DR. ELLIOTT ROBBINS

Departments of Cell Biology, Medicine, Neurology and Microbiology, Albert Einstein College of Medicine, Bronx, New York

WHILE the synthesis and metabolism of ribosomal RNA in randomly dividing cultures have been described¹⁻⁵, it is not known whether these processes occur similarly in all stages of the growth cycle. Several investigators working with individual cells have demonstrated that the rate of RNA synthesis increases progressively throughout interphase⁶⁻⁸; however, in order to study the synthesis of the different molecular species of RNA large numbers of synchronized cells are necessary. Using a method of synchronization^{6,9} which avoids the

ambiguities introduced by employing physical and chemical inhibitors, we have related the synthesis of high molecular weight and ribosomal RNA to various stages of the cell's life-cycle.

Suspension cultures of synchronized S3 HeLa were prepared by preferentially detaching mitotic cells from monolayers grown in Eagle's spinner medium¹⁰ supplemented with 3.5 per cent calf and 3.5 per cent foetal calf serum. Rates of synthesis of total RNA, DNA or protein were obtained by labelling 0.5-ml. aliquots of synchronized

cells in suspension for 30 min with either 0.2 μ Ci of 14 C-uridine (30 mc./mmole), 0.5 μ Ci of 14 C-thymidine (30 mc./mmole), or 1 μ Ci of a mixture of 14 C-threonine, valine and leucylalanine (100–300 mc./mmole) (New England Nuclear Corporation). The incorporation of isotope was stopped by chilling, the cells washed free of serum with Earle's saline, and the pellets treated with 5 per cent trichloroacetic acid (TCA). The acid-precipitable material was collected on Millipore filters and analysed for radioactivity in a low background gas flow counter (Nuclear-Chicago). All determinations were carried out in duplicate with a replication error of less than 10 per cent.

To characterize the species of RNA being formed, cells were exposed to 14 C-uridine for either 20 min or 2 h, washed in Earle's salts, resuspended in hypotonic buffer (0.2 M NaCl, 10^{-2} M tris-HCl pH 7.4, 1.5×10^{-3} M $MgCl_2$) and disrupted with 1 per cent sodium dodecyl sulphate (SDS). In some experiments cytoplasm was separated from nuclei by Dounce homogenization following hypotonic shock^{11,12}. Unlabelled HeLa cell cytoplasm was added to provide optical density markers and the lysates were analysed by centrifugation at 20° C through 15–30 per cent linear sucrose gradients containing 0.5 per cent SDS, 0.1 M NaCl and 5×10^{-3} M tris-HCl pH 7.4. The ultra-violet absorbance at 260 m μ was monitored with a recording spectrophotometer (Gilford) and acid-precipitable radioactivity was determined by making samples 12 per cent with TCA and processing them as already described here. Alternatively, cells were washed, resuspended in hypotonic buffer, added to HeLa cell cytoplasm, and the RNA extracted with hot phenol and SDS¹. While both techniques have given essentially the same results if less than 10^6 cells are used, extraction with SDS alone is more convenient and gives more reproducible data.

The metaphase index was determined by sedimenting a small aliquot of cells and counting the fraction in metaphase.

The life-cycle of mammalian cells has been divided into the interval between mitosis and DNA synthesis (G_1), the period of DNA synthesis (S), and the interval between DNA synthesis and mitosis (G_2)¹³. In order to relate the rates of synthesis of high molecular weight and ribosomal RNA to these stages, it was important to establish the rate of total RNA synthesis throughout interphase and its relationship to the synthesis of DNA and protein in our strain of HeLa S3 (Fig. 1). In general, the progressive increase in the rate of total RNA synthesis throughout interphase as well as the character and duration of the DNA synthesis curve confirm the findings which have been reported for single cells^{6–8}. The depression of RNA and protein synthesis in the presence of a high mitotic index immediately after synchronization (55 per cent of the cells in metaphase, 45 per cent in anaphase or telophase) and between 14 and 17 h, is

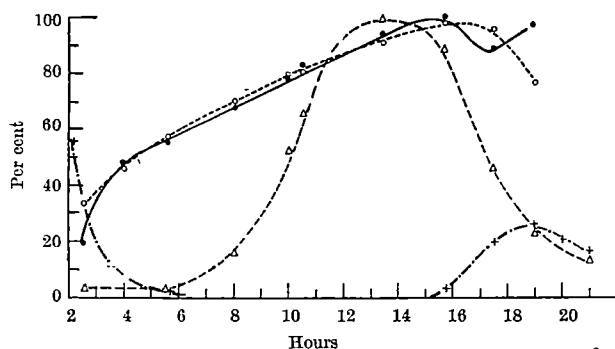


Fig. 1. Synthesis of RNA, DNA and protein. 10^6 cells in 0.5 ml. aliquots were incubated at the indicated times with 14 C-uridine, 14 C-thymidine or a mixture of 14 C-valine, 14 C-threonine and 14 C-phenylalanine for 30 min and processed as described in the text. The RNA data have not been corrected for the 8–10 per cent of the acid-precipitable 14 C-uridine counts which are found in DNA during the period of significant DNA synthesis. x, Metaphase index; Δ , 14 C-thymidine incorporation; \bullet , 14 C-uridine incorporation; \circ , 14 C-amino-acid incorporation.

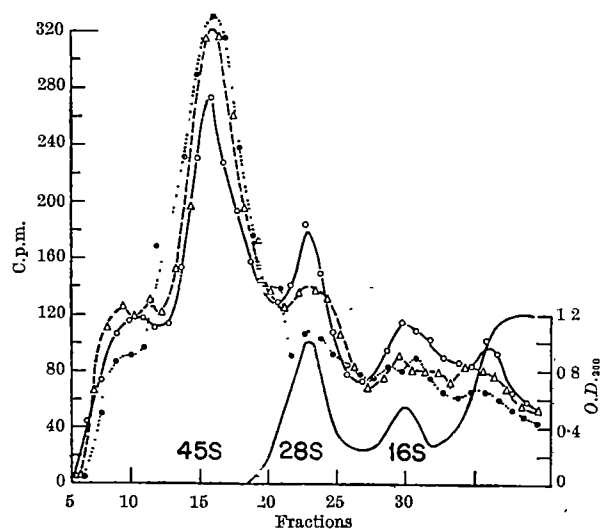


Fig. 2. Synthesis of 45S RNA in G_1 , S and G_2 . Separate preparations of synchronized HeLa cells were collected at 2, 8 and 15 h prior to labelling so as to allow simultaneous processing of G_1 , S and G_2 populations. Four ml. of cells at 2×10^6 cells/ml. were incubated with 5 μ Ci 14 C-uridine for 20 min. Following washing, the cells were mixed with 1 ml. of unlabelled HeLa cell cytoplasm from 2×10^6 cells. The mixture was extracted with 1 per cent SDS and layered on linear sucrose gradients which were centrifuged for 14 h at 19,000 r.p.m. in the SW 25 swinging bucket rotor. \circ , G_1 ; Δ , S ; \bullet , G_2 .

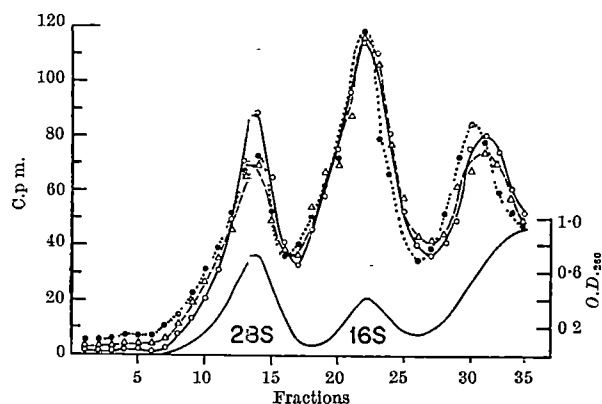


Fig. 3. Synthesis of cytoplasmic RNA. Cells from G_1 , S and G_2 were prepared as described in the legend of Fig. 2. Two ml. from each stage was exposed to 2.5 μ Ci 14 C-uridine for 2 h, washed, and mixed with 2×10^7 unlabelled HeLa cells. Cytoplasm was prepared as described in the text and following extraction with 1 per cent SDS analysed on sucrose gradients centrifuged at 23,000 r.p.m. for 16 h. \circ , G_1 ; Δ , S ; \bullet , G_2 .

consistent with autoradiographic investigations^{14–17} and with our recent observation that the number of polysomes are greatly decreased during mitosis¹⁸.

Since mitosis lasts 30–45 min, an appreciable mitotic index persisting for several hours indicates the loss of synchrony which characteristically occurs during one division cycle. It is clear that this loss of synchrony makes it impossible to obtain a uniform population of cells which have completed DNA synthesis but have not yet entered mitosis (G_2). Because of this, cell populations which we will call G_2 are in fact contaminated with significant numbers of cells which are still synthesizing DNA or in mitosis, as well as a few which have begun a second interphase.

In randomly dividing cultures of mammalian cells most of the RNA synthesized is ribosomal RNA. Its synthesis begins with the appearance in the nucleus of a high molecular weight RNA with a sedimentation value of approximately 45S. After 20–30 min exposure to 14 C-uridine, small amounts of newly synthesized 28S and 16S RNA are also detectable and with longer labelling times 28S and 16S RNA are found in the cytoplasm in association with the 60S and 40S ribosomal sub-units respectively.

The 16S RNA enters the cytoplasm more rapidly than the 28S RNA¹⁻⁵.

Figs. 2 and 3 illustrate that these events occur at all stages in the cell-cycle. Fig. 2 shows the results of exposing cells to ¹⁴C-uridine for 20 min during G1, s and G2. For more convenient comparison of the relative quantities of the different RNA species, the data have been 'normalized' so that there are the same number of total counts per gradient. Thus, while there is less total RNA synthesized per ml. of cells in G1 than in s, this is not apparent from the curves as drawn. As can be seen, cells in G1, s and G2 synthesized primarily high molecular weight RNA during the first 20 min of exposure to label, although there was also a small amount of newly synthesized RNA in the 28S and 16S areas. In the experiment illustrated there is relatively less newly synthesized 45S RNA and more 28S and 16S RNA in G1 than in s and G2, but these differences are variable and of unknown significance; the precise height of the peak, however, is of less significance than the fact that 45S RNA is made in large quantity throughout interphase. Analysis of the cytoplasm alone (Fig. 3) further emphasizes the constant pattern of RNA synthesis during the growth cycle; after 2 h exposure to ¹⁴C-uridine, 28S and 16S ribosomal RNA as well as 4S transfer RNA are present in the same relative amounts at all stages; 16S RNA was consistently present in larger amounts due to its more rapid entry into the cytoplasm. Similar results were obtained when other time points in G1 and s were examined.

Since it has been shown that RNA is not synthesized on replicating DNA^{14,19}, it is important to consider whether a decrease in either total or ribosomal RNA synthesis should be detectable during s in synchronized HeLa cells. During the time of maximum thymidine incorporation (11.5-12 h), only 5 per cent of the total DNA was synthesized. If the replication of the 400 cistrons responsible for synthesis of ribosomal RNA²⁰ were a random process, it is clear that we would expect to see, at the most, a parallel 5 per cent drop in ribosomal RNA synthesis; this is essentially our experimental error. Even if the replication of these cistrons were not a random process and occurred during a short interval, lack of perfect cell synchrony would make detection of a decrease in RNA

synthesis difficult in our experiment; however, analysis of single cells using short exposures to label and autoradiography might resolve this question. It should be noted that while Reiter and Littlefield²¹ reported a decrease in nuclear RNA synthesis during s in chick fibroblast cells synchronized with FUDR, it does not appear that their results are applicable to untreated HeLa cells.

Perhaps more interesting is the absence of a significant increase in RNA synthesis during s. This clearly implies that either the newly synthesized DNA does not act as a template for RNA synthesis or that the pre-existing DNA is progressively repressed.

We thank Dr. A. Zetterberg for allowing us to see his manuscripts prior to publication.

This work was supported by grants from the U. S. National Science Foundation (GB 984), the U. S. National Institutes of Health (AI 05231, AI 04153, GM 11558 and GM 12182) and the A.C.S. (E-379). Both authors are recipients of career development awards from the National Institutes of Health.

¹ Scherrer, K., Latham, H., and Darnell, J. E., *Proc. U.S. Nat. Acad. Sci.* **49**, 240 (1963).

² Girard, M., Penman, S., and Darnell, J. E., *Proc. U.S. Nat. Acad. Sci.*, **5**, 205 (1964).

³ Girard, M., Latham, H., Penman, S., and Darnell, J. E., *J. Mol. Biol.*, **187** (1965).

⁴ Perry, R. P., *Proc. U.S. Nat. Acad. Sci.*, **48**, 2179 (1962).

⁵ Rake, A. V., and Graham, A. F., *Biophys. J.*, **4**, 267 (1964).

⁶ Terasima, T., and Tolmach, L. J., *Exp. Cell Res.*, **30**, 344 (1963).

⁷ Seed, S., *Nature*, **192**, 944 (1963).

⁸ Killander, D., and Zetterberg, A. (personal communication).

⁹ Robbins, E., and Marcus, P. I., *Science*, **144**, 1152 (1964).

¹⁰ Eagle, H., *Science*, **130**, 432 (1959).

¹¹ Penman, S., Scherrer, K., Becker, Y., and Darnell, J. E., *Proc. U.S. Nat. Acad. Sci.*, **49**, 654 (1963).

¹² Scharff, M. D., Shatkin, H. J., and Levintow, L., *Proc. U.S. Nat. Acad. Sci.* **50**, 686 (1963).

¹³ Howard, A., and Pele, S. R., *Heredity*, Supp. **6**, 261 (1953).

¹⁴ Taylor, S. H., *Ann. N.Y. Acad. Sci.*, **90**, 409 (1960).

¹⁵ Prescott, D. M., and Bender, M. A., *Exp. Cell Res.*, **26**, 260 (1962).

¹⁶ Feinendegen, L. E., and Bond, V. P., *Exp. Cell Res.*, **30**, 393 (1963).

¹⁷ Konrad, C. G., *J. Cell Biol.*, **19**, 287 (1963).

¹⁸ Robbins, E., and Scharff, M. D., *Fed. Proc.*, **24**, 445 (1965).

¹⁹ Prescott, D. M., and Kimball, R. F., *Proc. U.S. Nat. Acad. Sci.*, **47**, 686 (1961).

²⁰ McConkey, E. H., and Hopkins, J. W., *Proc. U.S. Nat. Acad. Sci.*, **51**, 1197 (1964).

²¹ Reiter, J. M., and Littlefield, J. W., *Biochim. Biophys. Acta*, **80**, 562 (1964).

HETEROGENEOUS BINDING OF ACRIDINE ORANGE BY POLYRIBONUCLEOTIDES

By PROF. R. F. BEERS, jun., and G. ARMILEI

Department of Radiological Sciences, School of Hygiene and Public Health, Johns Hopkins University, Baltimore

ACRIDINE orange (AO) binding of polyribonucleotides results in extensive stacking of the dye molecules in linear arrays along the polymer chain¹⁻³. This is attributed to a highly co-operative influence of neighbouring bound dye molecules. The dissociation constant for the first dye molecule is larger than that of the second or subsequent adjacent dye molecules. If the discrepancy between the first and subsequent dissociation constants is great enough, a given polymer chain becomes 100 per cent saturated with the dye before a second polymer chain begins to bind the dye.

Evidence for this quantum type of dye binding has been obtained in adsorption isotherm investigations with *Micrococcus lysodeikticus* and *Bacillus cereus*⁴. The fraction of cells stained orange by the dye (10 per cent of dry weight) is proportional to the amount of dye bound by a given suspension of cells, that is, the percentage saturation of the bacterial suspension is equal to the

percentage of bacterial cells saturated 100 per cent by the dye.

The molecular weight of a polynucleotide-dye complex 100 per cent saturated with AO is approximately twice that of the free polymer. Density gradient ultracentrifugation should demonstrate an increase in mobility of the polymer saturated with dye and, indeed, separate the dye-polymer complexes from the free polymer molecules⁵. Polyadenylic, polyuridylic and polycytidylic acids were purchased from Miles Chemical Co., Elkhart, Indiana. Polyadenylic acid saturated 50 per cent with AO was centrifuged in a sucrose gradient at 63,500g for 17.5 h. Fig. 1 shows the distribution pattern of the polymer (260 mμ) and of the dye (465 mμ). The slowest component, which is essentially free of the dye, has the same mobility as that of the control sample of polyadenylic acid. The faster component with a mobility approximately twice that of polyadenylic acid corresponds to the distribution

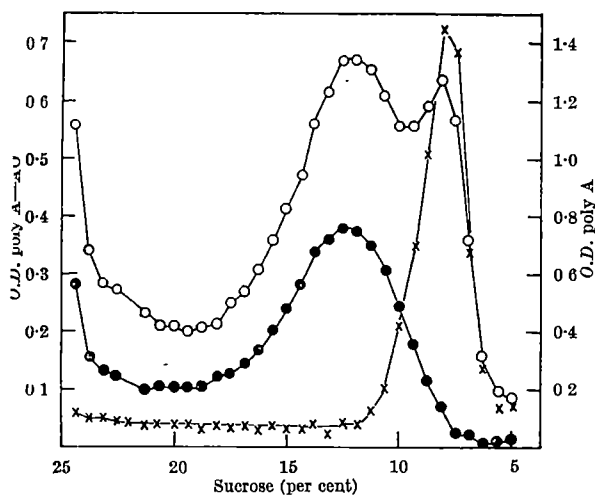


Fig. 1. Density-gradient ultracentrifugation of poly A saturated 50 per cent with acridine orange. One ml. each of 1 mM poly A and 0.5 mM acridine orange in 0.5 M *tris*, pH 7.5, were mixed and layered in a sucrose gradient 5–25 per cent. The preparation was centrifuged at 63,500g for 17.5 h. Control was performed under identical conditions without the dye. One-ml. aliquots were moved from the bottom of the centrifuge tube for measurement of their respective optical densities at 260 mμ and 465 mμ. ○, Optical density of poly A-acridine orange mixture at 260 mμ; ●, optical density of poly A-acridine orange mixture at 465 mμ; ×, optical density of poly A control at 260 mμ.

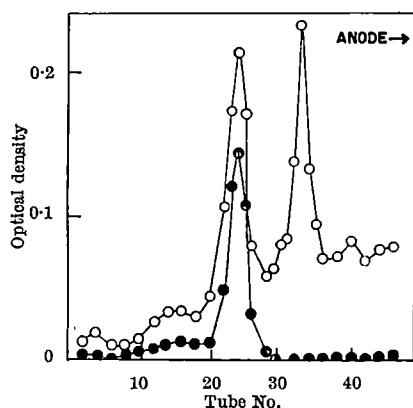


Fig. 2. Density-gradient electrophoresis of poly A saturated 50 per cent with acridine orange. One ml. sample containing 8×10^{-3} M poly A, 4×10^{-3} M acridine orange and 5×10^{-3} M phosphate buffer, pH 7.5, in 4 per cent sucrose was layered on a sucrose gradient column 4–25 per cent in 5×10^{-3} M phosphate buffer, pH 7.3. Electrophoretic run lasted 3 h at 2 m.amp and 20° C. One-ml. aliquots were removed for measurement of their respective optical densities at 260 mμ and 465 mμ. ○, 260 mμ; ●, 465 mμ.

pattern of the dye and, in fact, is 100 per cent saturated with AO. A third component sedimenting to the bottom of the centrifuge tube is an aggregate of the dye-saturated polymers. Similar results have been obtained with polyuridylic and polycytidylic acids. The fraction of polymer molecules saturated with the dye is always proportional to the amount of dye present.

Resolution of copolymers by this method has not been successful, although the peak of dye distribution measured at 465 mμ is always in advance of that of the polymer measured at 260 mμ. The observed mobility of the dye-polymer mixture increases with the degree of saturation of the polymer mixture. Copolymers were synthesized by the action of polynucleotide phosphorylase prepared from *Micrococcus lysodeikticus* cells.

Because AO is a singly charged cation at the pH ranges employed in these studies (pH 7–8), the fully saturated polymer should have a zero net charge. Therefore, it should be possible to separate the polymer components by electrophoresis. Density gradient electrophoresis studies were performed in a water-jacketed column modified after the apparatus described by Olivera *et al.*⁶ Pre-

liminary studies with zinc-free dye failed to demonstrate any separation of the components. However, with the zinc-dye complex (net charge +3) the polymer can be separated into two components. The faster component demonstrates a bright green fluorescence and the slower component a bright orange fluorescence. The former corresponds to Complex II, the latter to Complex I (ref. 2). Fig. 2 shows the distribution pattern of polyadenylic acid 50 per cent saturated with acridine orange electrophoresed in a sucrose gradient. The slower component is 100 per cent saturated with the dye. The addition of $MgCl_2$ at concentrations comparable with those of the dye reduced or abolished the resolution of the peaks. In contrast, zinc increases the resolution of the peaks.

Fig. 3 gives the distribution pattern of a copolymer of adenylic and uridylic acids with a one-to-one base ratio. Similar results have been obtained with yeast RNA. However, the extent of dye binding by the slower component is considerably less than 100 per cent.

Equal molar mixtures of polyadenylic acid, polyuridylic acid and AO, heated to 190° briefly and cooled slowly when electrophoresed in the sucrose gradient, fractionate into two components (Fig. 4). The faster component contains 80 per cent polyadenylic acid, indicating that the dye has a stronger affinity for polyadenylic acid than for polyuridylic acid.

These results suggest that the capacity of a particular copolymer to bind the dye may be a function of its base composition or sequence. To test this hypothesis, an equi-molar mixture of two copolymers having 24.5 and 86.5 per cent adenylic acid, respectively, was electrophoresed with equi-molar AO in the sucrose gradient. The faster component free of the dye had an adenylic acid content of only 47.5 per cent, indicating that the copolymer containing the higher fraction of adenylic acid preferentially bound the dye.

Similar results have been obtained with methylene blue and acriflavine. Acriflavine appears to provide a better separation of the polymer complexes. Moreover, the fully saturated dye-polymer complex does not migrate from the origin.

These investigations clearly establish for the first time the quantized nature of acridine orange binding by polyribonucleotides containing one to four different bases. Even with RNA there is no evidence of any significant

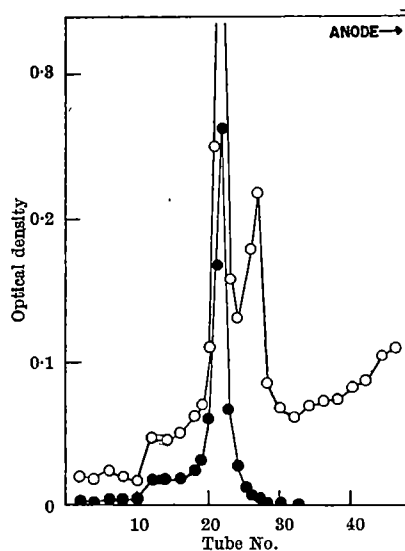


Fig. 3. Density-gradient electrophoresis of poly AU saturated 50 per cent with acridine orange. One ml. sample of 9×10^{-3} poly AU (1:1 base ratio), 4.5×10^{-3} M acridine orange, and 5×10^{-3} M phosphate buffer, pH 7.5, in 4 per cent sucrose was applied to electrophoretic column. Electrophoretic run lasted 1 h at 3 m.amp and 4° C. Experimental conditions were the same as in Fig. 2. ○, 260 mμ; ●, 465 mμ.

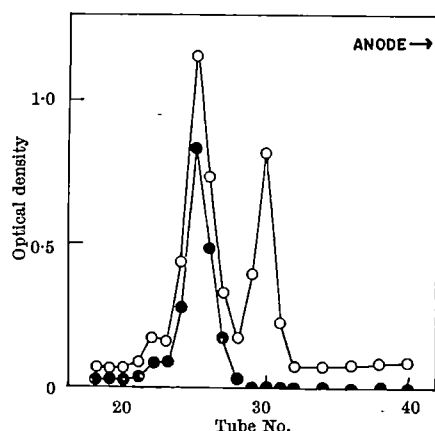


Fig. 4. Density-gradient electrophoresis of equi-molar mixtures of poly A, poly U and acridine orange. One-ml. sample containing 0.36 mM each of poly A, poly U and acridine orange in 4 mM phosphate buffer, pH 7.5, was heated briefly to 100° in a water-bath and cooled slowly to 4° before applying to the sucrose gradient. Electrophoretic run lasted 3 h at 3 m. amp and 4° C. Experimental conditions were the same as in Fig. 2. ○, 260 mμ; ●, 465 mμ.

random dye binding in which the dimer of the dye predominates³.

Because of these findings the effects of the dye on the kinetics of polynucleotide phosphorylase must be re-interpreted⁷. In particular the mechanism by which the fully saturated dye-polymer complex stimulates the polymerization reaction apparently involves more than a simple electrostatic effect as postulated elsewhere. The dye titration studies with various polyribonucleotides^{2,3} also

must be re-interpreted on the basis of a quantized scheme of dye binding.

The second significant observation is the dependence of the dye binding on the base ratios and possible base sequences. This suggests the possibility of using the dye as a reagent for fractionating RNA according to the base composition of the polynucleotides.

The persistent negative electrostatic charge of fully saturated AO-polymer complexes is an unexpected finding which may be accounted for by the presence of a zeta potential of the dye-polymer aggregate. These aggregates are formed reversibly but can be readily demonstrated by density gradient ultracentrifugation. Visual inspection of the electrophoretic column suggests the presence of aggregates. If this is the case the dye-polymer complexes are not in solution but exist as the solid component of a two-phase system. This would provide a physical basis for the existence of a zeta potential on the surface of these aggregates. Attempts to reverse the charge of the complexes by the addition of equi-molar Zn^{2+} were unsuccessful. During the electrophoretic run the Zn^{2+} contained in the dye migrated to the cathode, whereas the dye-polymer complex migrated to the anode.

This work was supported by Research Program Project grant GM 10802-02, U.S. National Institute of General Medical Sciences.

¹ Steiner, R. F., and Beers, jun., R. F., *Arch. Biochem. Biophys.*, **81**, 75 (1959).

² Beers, jun., R. F., *Ann. N.Y. Acad. Sci.*, **81**, 645 (1959).

³ Bradley, D. F., and Wolfe, M. K., *Proc. U.S. Nat. Acad. Sci.*, **45**, 944 (1959).

⁴ Beers, jun., R. F., *J. Bact.*, **88**, 1249 (1964).

⁵ Beers, jun., R. F., *Sixth Intern. Cong. Biochem. Abstr.*, Sect. I, 43 (1964).

⁶ Olivera, B. M., Balne, P., and Davidson, N. J., *Biopolymers*, **2**, 245 (1964).

⁷ Beers, jun., R. F., *J. Biol. Chem.*, **235**, 726 (1960).

NUCLEOTIDE PEPTIDES IN YEAST

By DR. D. J. MILLIN* and M. J. SALTmarsh-ANDREW

Brewing Industry Research Foundation, Nutfield, Redhill, Surrey

THE occurrence of complexes containing nucleotides and amino-acids has recently been reviewed¹. The best known of these are the amino-acid derivatives of transfer-RNA and the corresponding peptide derivatives now regarded as ribosomal-bound intermediates in protein synthesis^{2,3}. Complex mixtures of less-well-characterized compounds of low molecular weight are present in aqueous-ethanolic and trichloroacetic acid extracts of yeasts⁴⁻¹¹ and similar mixtures have been reported from other micro-organisms, green plants and various mammalian tissues^{1,12-14}.

Davies and Harris^{9,10} isolated several compounds from yeast and assigned structures involving phosphoanhydride bonds between the 5'-phosphate of uridylic acid and the carboxyl group of alanine. The extracts obtained from yeast were clearly very complex^{10,11}, but many of the components had broadly similar amino-acid and nucleotide composition and reduced periodate rapidly. They were thus thought to possess 5'-phosphoanhydride bonds. After mild treatment with alkali the ability of these mixtures to reduce periodate increased. This was interpreted as indicative of the presence of nucleotide peptides possessing ester bonds between the 2'- or 3'-hydroxyl group of ribose and the carboxyl group of an amino-acid¹⁵. Similar structures had been suggested by Koningsberger *et al.*^{4,6}.

During re-investigation of these preparations, attempts were made to correlate nucleotide (calculated as uridylic acid from the extinction at 260 mμ), ribose (determined by the method of Mejbbaum¹⁶ after bromination of the sample¹⁷), amount of periodate reduced¹⁵ and number of

alkali-labile 'activated' bonds. The latter were estimated by reaction with hydroxylamine at neutral pH before and after mild alkaline hydrolysis followed by determination of the hydroxamate formed¹⁸. The values thus obtained did not exceed 3 μmole/g yeast in any of the samples tested. The nucleotide- and ribose-levels were always in reasonable agreement and in excess of the alkali-labile 'activated' bonds. The initial uptake of periodate was, however, erratic and invariably in excess of the total ribose content. In some samples even the increment in consumption of periodate resulting from mild alkaline hydrolysis was greater than the total ribose in the sample. As purification proceeded, the correlation improved, but only after numerous procedures were the results observed from some fractions consistent with ribose being the only moiety oxidized by periodate. These results indicated the desirability of characterizing further components of the mixture, in particular a 2'- or 3'-ribose ester. One such class of compounds might be expected with a free 5'-phosphate group. These would be relatively acidic and were therefore sought in that small proportion of nucleotide-peptide concentrate which was retained by 'Dowex-1' (formate) in the presence of 0.1 M sodium acetate buffer, pH 5 (ref. 10), a fraction hitherto not investigated in detail.

Elution of the 'Dowex-1' (formate) column with 0.1 N formic acid and subsequent rechromatography of poorly resolved regions on the same resin gave rise to thirteen distinct fractions. Each of these separated into two to four further fractions when passed through 'Sephadex G-25'. Certain of the latter, which absorbed ultra-violet light, reacted with ninhydrin and exhibited an increased ability to reduce periodate after mild treatment with alkali, were

* Present address: Research Laboratories, Brooke Bond Tea, Ltd., Blounts Court, Sonning Common, Reading, Berkshire.

lected for detailed examination. One such investigation described in some detail as illustrative of the results obtained.

The sample had λ_{\max} 255 m μ , λ_{\min} 235 m μ and appeared as a single ninhydrin-positive spot with coincident absorption of ultra-violet light on thin-layer chromatography on cellulose in three solvents (0.1 N hydrochloric acid, solvent *a*; propan-2-ol-water, 7:3 v/v, solvent *b*; saturated ammonium sulphate-0.1 M sodium acetate H 7.5-propan-2-ol, 40:9:1 v/v). It failed to move or separate on paper electrophoresis (0.1 M sodium acetate buffer, pH 4, 36 V/cm). After treatment at pH 10 for 16 h at room temperature it was possible to separate the ninhydrin positive and ultra-violet absorbing material by paper chromatography (solvent *b*).

The peptide moiety was eluted and purified by paper electrophoresis and chromatography before being hydrolysed (6 N hydrochloric acid, 100°, 16 h, sealed tube). Amino-acids, determined by the method of Spackman, Stein and Moore¹⁸, were present in the hydrolysate in the approximate ratio glutamic acid:glycine:serine:alanine, 2:1:1 (Table 1) together with traces of aspartic acid, threonine and lysine. Reaction of the original material with 'dansyl' chloride²⁰ and subsequent hydrolysis yielded a single derivative tentatively identified as dansyl-alanine by chromatography²¹.

Table 1. ANALYTICAL DATA ON A SELECTED NUCLEOTIDE PEPTIDE FRACTION

	Moles/mole ribose
Nucleotide (calculated as G)	1.2
Periodate consumed: (a) Initially	0.9
(b) After hydrolysis at pH 10	1.03
That is, ribose-ester bonds	0.13
Amino-acids: Glu	0.063
Gly	0.05
Ser	0.026
Ala	0.022
That is, total peptide	c. 0.024

The nucleotide moiety was also subjected to hydrolysis (0.3 N potassium hydroxide, 37°, 16 h) after elution from the paper. Paper chromatography of the products (*n*-butanol-acetic acid-water; 4:1:1 v/v) revealed two components (*A* and *B*, $R_F = 0.2$ and 0.38 respectively) with distinctive ultra-violet absorption spectra (Table 2). Neither component could be identified when compared with all the available authentic bases or nucleoside monophosphates either by paper or thin-layer chromatography or by electrophoresis. Component *A* was then subjected to acid hydrolysis (pH 2, 100°, 10 min) and the products chromatographed on paper (*n*-propanol-ethyl acetate-water, 7:1:2 v/v) adjacent to unhydrolysed component *A*. On developing with silver nitrate it was apparent that a hexose had been released by acid hydrolysis. This was identified as galactose, together with traces of glucose and mannose, by paper chromatography (pyridine-ethyl acetate-water, 4:10:3 v/v, and methyl ethyl ketone-acetic acid-water, 9:1:1 v/v saturated with boric acid) and electrophoresis (0.1 M sodium borate buffer, pH 10, 22 V/cm). Finally, the nucleotide remaining

after acid hydrolysis was tentatively identified as guanosine 5'-phosphate by eluting from chromatograms of the hydrolysate and re-running on paper (propan-2-ol-6 N hydrochloric acid, 17:8 v/v; solvent *c*). Similar treatment of component *B* failed to reveal any acid-labile carbohydrate. The spectral characteristics and chromatographic behaviour (Table 2) of this component therefore suggested that it was a methylated guanosine²².

From the data enumerated so far, it is tempting to suggest a structure for this nucleotide peptide such as:

Galactose-pGpMeG-peptide

Indeed, a rather similar glucose-cytidylic acid-peptide having a qualitatively identical amino-acid composition has recently been isolated from chlamydospores of oat smut²³. The quantitative data collected in Table 1 are, however, not in agreement with such a conclusion. One might reasonably expect to recover at least 20-30 per cent of the peptide after eluting and re-running several times on paper. In this event there is reasonable agreement between the amounts of peptide and material which reduces periodate released by cleavage at pH 10. There is, however, far too little of either ribose-ester bonds or peptide when compared with the total nucleotide content. Furthermore, phosphate analyses were not in accord with this view. Perhaps the best interpretation is that the fraction is a nucleotide derivative of galactose some 5-10 per cent of which bears a peptide attached by a ribose-ester bond.

It is clear that neither the techniques of separation nor the criteria of homogeneity so far used have been adequate. Thus the possibility that the fraction may be considerably more complex cannot be entirely excluded in view of the known strong association of guanine derivatives²⁴ and the recently reported occurrence of xanthosine monophosphate galactose in close association with nucleotide peptides from *Escherichia coli*¹².

Several other apparently homogeneous ester-linked components were also examined in detail, but in no case were satisfactory quantitative data obtained to support the qualitative findings. In addition, several other fractions from the 'Sephadex' columns which were clearly heterogeneous were fractionated further on DEAE-cellulose according to Tomlinson and Tener²⁵. Invariably, in addition to fractions exhibiting typical nucleotide spectra, several other components were obtained with λ_{\max} 225-230 m μ ; 321 m μ , λ_{\min} 267 (pH 2 and 7), and λ_{\max} 232-233 m μ ; 308-310 m μ , λ_{\min} 264 (pH 9). These latter were not investigated closely, but it was established that they reduced periodate very rapidly. Compounds with these spectral characteristics were also found in considerable amounts in the whole nucleotide-peptide concentrates.

The presence of bound hexoses and materials which react readily with periodate, other than the nucleotide peptides themselves, invalidates the measurement¹⁵ of the proportions of anhydride and ester-linked compounds in these nucleotide peptide concentrates. Thus, the reported changes in these compounds accompanying physiological changes in the cells^{14,23-28} cannot be regarded as having any quantitative significance or affording any indication as to the metabolic significance of nucleotide peptides.

One class of nucleotide peptides having ribose-ester bonds might arise by the action of ribonuclease on ribosomal-bound transfer-RNA peptides^{3,29} during the extraction procedure or, in cell-free systems at least, by degradation of the transfer-RNA-amino-acid prior to peptide bond formation, a process which inhibits protein synthesis and presumably releases nucleotide peptides³⁰. The nucleotide composition of such components should reflect the characteristic terminal sequence of transfer-RNA. Such products would not be intermediates in protein synthesis but might provide an explanation of the apparent connexion between nucleotide peptides and protein synthesis³¹⁻³⁴. Although investigations of the

Table 2. SPECTRAL AND CHROMATOGRAPHIC CHARACTERISTICS OF NUCLEOTIDES DERIVED FROM AN INDIVIDUAL YEAST NUCLEOTIDE PEPTIDE FRACTION

Component	<i>A</i>			<i>B</i>		
pH	2	7	11	2	7	11
Max	252	245	268	256	257	257
	271-4 (infl.)	265-70 (infl.)				
$E_{250\text{m}\mu}/E_{260\text{m}\mu}$	1.2	1.1	0.95	0.95	0.99	0.99
$E_{260\text{m}\mu}/E_{280\text{m}\mu}$	0.63	0.79	0.83	0.23	0.74	0.66
%Guanosine						
Cellulose thin layer, solvent <i>a</i>	2.34			0.85		
solvent <i>b</i>	0.79			1.38		
No. 1 paper, solvent <i>c</i>	1.4			0.8		

incorporation of radioactive amino-acids into protein by systems derived from yeast and dependent on either ribosomes³⁵ or a heavier particulate fraction³² have indicated that this incorporation is accompanied by passage of radioactivity into materials which possess the characteristics of nucleotide peptides^{32,34}, kinetic evidence has failed to demonstrate an intermediary role for these substances in protein synthesis in either system³⁴. From the extract of the heavier particulate fraction the nucleotides obtained by alkaline degradation may well have been characteristic of terminal fragments of transfer-RNA³². However, it has not proved possible to isolate, from either system, sufficient radioactive material in an adequate state of purity to elucidate the nature of the bond between nucleotide and peptide³⁴. No nucleotide peptides having structures compatible with terminal fragments of transfer-RNA have, as yet, been adequately characterized and, to judge from the overall nucleotide composition of the preparations^{1,11}, it seems likely that only a relatively small fraction could be so derived. Perhaps the most reasonable explanation of an apparent intermediary role in protein synthesis in living cells³³, which is unconfirmed in cell-free systems, is that certain nucleotide peptides are involved in the elaboration of structures which require the integrity of the cell, such as membrane, mitochondria, or cell walls²⁸.

Although fragments of transfer-RNA amino-acid derivatives inhibit protein synthesis³⁰, it is not obvious that the analogous peptide derivative could have the similar but apparently specific action required to explain the repression of the induced synthesis of α -glucosidase observed by Koningsberger *et al.*³⁶. We have also examined the latter phenomenon using a strain of *Saccharomyces cerevisiae* (National Collection of Yeast Cultures No. 366). Cells in early log phase and protoplasts derived therefrom showed an induction pattern almost identical with that of stationary phase cells of this strain³⁷. Initially, in the presence of rather crude preparations containing nucleotide peptides our observations were very similar to those of Koningsberger *et al.*³⁶. Attempts to fractionate these preparations by the techniques already outlined here, however, afforded rather confusing results, several fractions exhibiting similar activity being obtained. Repeated passage through 'Sephadex G-25' and final removal of traces of salts on ion retardation resin ('Bio-Rad. AG11A8') led to a gradual loss in activity, suggesting either that the active material is rather labile or that it is not, in fact, nucleotide peptide. One of the most surprising observations was that one fraction which had been kept cold throughout the preparative manipulations and exhibited only very slight activity was able to inhibit completely the synthesis of maltase in protoplasts after it had been warmed at 60° for 10 min.

We also have independent evidence that may indicate the presence of substances having repressor-like activity in the readily extractable pool of low-molecular-weight substances from yeast. The sequential induction of the components of the maltose utilizing system in a further strain of *Saccharomyces cerevisiae* (N.C.Y.C. 240 M), in which maltase activity only became apparent some 3.5 h after the addition of the inducer, was described earlier³⁷. It has since been found that this behaviour is dependent on the temperature at which the cells are washed prior to addition of the inducer. The delayed induction, in fact, occurs only after the cells have been washed at 0°–5°, whereas washing at 20–25° results in induction within less than 2 h. The latter effect can, however, be largely offset by an increase in the concentration of glucose in the inducing medium. One possible explanation is that washing at the higher temperature removes substances with repressor-like activity. Freeze-dried washings are, however, without effect on the induction in whole cells or protoplasts when observed manometrically. Nevertheless, it is possible that the repressor is degraded on excretion by enzymes located in the cell wall³⁸. Indeed, this could

also explain the observation³⁶ that repressor activity can be demonstrated on protoplasts but not on whole cells. These observations may, perhaps, be connected with the phenomenon of shock excretion of amino-acids as nucleotides³⁹ which is enhanced by elevated temperature. Conceivably, some of the excretion products may be derived from nucleotide peptides; indeed, it is not impossible that one function of shock excretion is removal of repressor substances prior to an adjustment of enzyme levels in response to a change of environment. Again these ideas, however, radioactivity from nucleotide peptides obtained from yeast grown in the presence of radioactive amino-acids failed to enter either cells or protoplasts to any significant degree⁴⁰, and a synthetic nucleotide peptide⁴⁰ linked by a 5'-phosphoanhydride bond failed to support yeast growth despite the fact that the tetrapeptide moiety alone was able to serve as a nitrogen source⁴¹.

The precise significance of these results remains uncertain. There seems little doubt that low-molecular-weight substances having a repressor-like activity exist in yeast, but whether this observation is distinct from that of catabolic repression and is more specifically associated with nucleotide peptides seems rather doubtful. The variety of structures which have been observed make it unlikely that one can assign a single function to nucleotide peptides. Some may fulfil a genuine intermediary role in the assembly of cellular structures. The picture is a complex one and, for further systematic study, require the application of additional separation techniques and the development of more rigid criteria of homogeneity.

We thank Dr. A. H. Cook for his advice.

- ¹ Bogdanov, A. A., *Uspekhi Sovremennoi Biol.*, **55**, 321 (1963).
- ² Watson, J. D., *Bull. Soc. Chim. Biol.*, **46**, 1399 (1964).
- ³ Gilbert, W., *J. Mol. Biol.*, **6**, 389 (1963).
- ⁴ Koningsberger, V. V., van der Grinten, C. O., and Overbeek, J. Th. G., *Biochim. Biophys. Acta*, **26**, 483 (1957).
- ⁵ van der Grinten, C. O., thesis, Utrecht (1959).
- ⁶ Koningsberger, V. V., in *Protein Biosynthesis*, edit. by Harris, R. J. C., 207 (Academic Press, London, 1961).
- ⁷ Harris, G., Davies, J. W., and Parsons, R., *Nature*, **182**, 1565 (1958).
- ⁸ Hase, E., Mihara, S., Otsuka, H., and Tamura, H., *Arch. Biochem. Biophys.*, **83**, 170 (1959).
- ⁹ Davies, J. W., and Harris, G., *Proc. Roy. Soc., B*, **151**, 537 (1960).
- ¹⁰ Davies, J. W., and Harris, G., *Biochim. Biophys. Acta*, **45**, 28, 39 (1960).
- ¹¹ Davies, J. W., Harris, G., and Neal, G. E., *Biochim. Biophys. Acta*, **51**, 95 (1961).
- ¹² Khanina, M. F., Venkstern, T. V., and Baev, A. A., *Biokhimiya*, **29**, 142 (1964).
- ¹³ Matheson, N. A., and Syngé, R. L. M., *Biochem. J.*, **92**, 48P (1964).
- ¹⁴ Wiseman, A., *Organisation for Protein Biosynthesis*, Chap. 11 (Blackwell, Oxford, 1965).
- ¹⁵ Harris, G., and Wiseman, A., *Biochim. Biophys. Acta*, **55**, 374 (1962).
- ¹⁶ Meibaum, W., *Z. Physiol. Chem.*, **258**, 117 (1959).
- ¹⁷ Massart, L., and Hoste, J., *Biochim. Biophys. Acta*, **1**, 83 (1947).
- ¹⁸ Briggs, D. E., and Millin, D. J., *Nature*, **208**, 287 (1965).
- ¹⁹ Spackman, D. H., Stein, W. H., and Moore, S., *Anal. Chem.*, **30**, 1190 (1958).
- ²⁰ Gray, W. R., and Hartley, B. S., *Biochem. J.*, **89**, 59P (1963).
- ²¹ Boulton, A. A., and Bush, I. E., *Biochem. J.*, **92**, 11P (1964).
- ²² Smith, J. D., and Dunn, D. B., *Biochem. J.*, **72**, 204 (1959).
- ²³ Elnaghy, M. A., and Nordin, F., *Biochem. Biophys. Res. Comm.*, **18**, 501 (1965).
- ²⁴ Pierce, J. G., and Zubkoff, P. L., *Biochim. Biophys. Acta*, **87**, 343 (1964).
- ²⁵ Tomlinson, R. V., and Tener, G. M., *Biochemistry*, **2**, 697 (1963).
- ²⁶ Harris, G., and Wiseman, A., *Biochim. Biophys. Acta*, **55**, 775 (1962).
- ²⁷ Harris, G., and Wiseman, A., *Biochim. Biophys. Acta*, **61**, 395 (1962).
- ²⁸ Harris, G., and Wiseman, A., *Biochim. Biophys. Acta*, **61**, 478 (1962).
- ²⁹ Halvorsen, H. O., Kihara, H. K., and Young, R. J., *Proc. Eighth Intern. Congress Microbiol., Montreal*, **21** (abstract A 37) (1962).
- ³⁰ Takanami, M., *Proc. U.S. Nat. Acad. Sci.*, **52**, 1271 (1964).
- ³¹ Harris, G., and Neal, G. E., *Biochim. Biophys. Acta*, **47**, 122 (1961).
- ³² Cooper, A. H., Harris, G., and Neal, G. E., *Biochim. Biophys. Acta*, **61**, 573 (1962).
- ³³ Cooper, A. H., Harris, G., Neal, G. E., and Wiseman, A., *Biochim. Biophys. Acta*, **68**, 68 (1963).
- ³⁴ Cooper, A. H., and Millin, D. J. (unpublished observations).
- ³⁵ Cooper, A. H., and Millin, D. J., *Biochem. J.*, **92**, 61P (1964).
- ³⁶ Van Dam, G. J. W., Slavenburg, J. H., and Koningsberger, V. V., *Proc. Acad. Sci. Amsterdam*, **B**, **67**, 314 (1964); *Biochem. J.*, **92**, 48P (1964).
- ³⁷ Harris, G., and Millin, D. J., *Biochem. J.*, **88**, 89 (1963).
- ³⁸ Neu, H. C., and Heppel, L. A., *Biochem. Biophys. Res. Comm.*, **17**, 215 (1964).
- ³⁹ Lewis, M. J., and Phaff, H. J., *J. Bact.*, **87**, 1389 (1964); **89**, 960 (1965).
- ⁴⁰ Harris, G., and MacWilliam, I. C., *J. Chem. Soc.*, 2053 (1961).
- ⁴¹ Harris, G., and MacWilliam, I. C. (to be published).

A MOLECULAR MODEL FOR A SODIUM PUMP

By DR. L. J. OPIT and DR. J. S. CHARNOCK

Cell Physiology Research Unit, Department of Surgery, Queen Elizabeth Hospital, Woodville, South Australia

ALTHOUGH there are considerable experimental data relating to the exclusion of Na^+ and accumulation of K^+ in living cells, our knowledge of the mechanism is rudimentary and the molecular basis for this phenomenon remains unknown. Excellent reviews^{1,2} stressing these deficiencies continue to be published. From the work of Skou, Post, Glynn and Whittam during 1957-61 there is strong evidence that the enzyme system known as Na^+ and K^+ dependent adenosine triphosphatase is closely associated with the active transport of sodium and potassium ions across cellular membranes.

In summary, the salient characteristics which relate this enzyme system to the movement of Na^+ and K^+ are:

(1) The enzyme system is membrane bound, and virtually ubiquitous in animal cells^{3,5,7}.

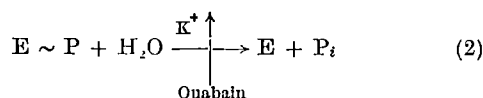
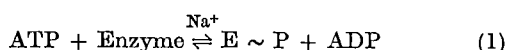
(2) It requires the presence of both Na^+ and K^+ ions for activation—a well-known requirement for ion transport in whole cell or tissue systems *in vitro*⁶⁻⁸.

(3) In the erythrocyte, enzyme activation occurs only when Na^+ is on the 'inside' and K^+ is on the 'outside' of the cell^{9,10}.

(4) Na^+ and K^+ activated ATPase is specifically inhibited by the cardiac glycoside ouabain, known to be a potent inhibitor of cation transport both *in vivo* and *in vitro*^{9,7,11}.

(5) Kinetic examination of the reaction *in vitro* shows activation by substrate and ions, and inhibition by ouabain at concentrations compatible with 'physiological conditions'^{7,12}.

Studies by Charnock and Post^{13,14}, Rose¹⁵ and others^{1,16} have suggested the following reaction mechanism for the hydrolysis of ATP by this enzyme in the presence of Mg^{++} , Na^+ and K^+ :



The initial reaction step consists of the rapid reversible formation of a phosphorylated complex. This step is stimulated by Na^+ ions, and no other ion appears able to replace Na^+ . The formation of this complex is followed by an almost irreversible decomposition to P_i and free enzyme. This latter reaction is stimulated by K^+ but not Na^+ ions; K^+ can be replaced with either NH_4^+ , Li^+ , Rb^+ or Cs^+ . It is this decomposition which is inhibited by the cardiac glycoside ouabain. The chemical nature of the phosphorylated complex is not yet known, although it seems certain that it is phosphoprotein and not phospholipid¹³⁻¹⁷. Kinetic data from this laboratory also support this general reaction mechanism. The K_m for ATP is 2.8 mM; ADP acts as an inhibitor. Little consistent inhibition is apparent with concentrations of phosphate as high as 30 mM (ref. 12).

Although the details of the reaction mechanism of this membrane-bound ATPase have been extensively studied, the means by which this enzyme effects a vectorial transport of Na^+ and K^+ is unknown. Further advances in understanding the mechanism of Na^+ and K^+ transport

therefore require information about how such an ATPase might transport Na^+ against the concentration gradient from the inside of the cell, coupled with the movement of K^+ in the opposite direction. The purpose of this article is to present a molecular model for the directional transport of these ions based on what is known of the ATPase reaction mechanism, and which is consistent with knowledge about Na^+ and K^+ transport characteristics in the whole cell or tissue.

It has been generally accepted that the ATPase enzyme itself is membrane bound. We have recently obtained evidence that the enzyme preparation obtained from guinea-pig kidney cortex consists entirely of bilaminar membrane structures, many of which are vesicular, and which are thought to be derived from the endoplasmic reticulum of the kidney cells¹⁸.

We propose, therefore, to develop the model on the basis of these bilaminar membrane fragments and take as our starting point the configuration of the unit membrane proposed by Danielli. This unit contains a double protein layer, with lipid interposed. We will consider the inner facing protein structure to contain the ATPase enzyme. This belief is supported by the observations of Whittam that hydrolysis of ATP only occurs in the red cell ghost when the ATP is intracellular¹⁰.

If, in the intact cell, these enzyme membrane fragments were associated with cell surface or its prolongations into the cell and the endoplasmic reticulum¹⁹⁻²¹, then it is reasonable to suppose that the outer protein surface is directed toward the extracellular compartment.

The inner protein structure containing the ATPase must now be visualized as a highly ordered, polarizable chain, with many surface orientated cationic and anionic groups. Among the groups are situated a much smaller number of amino-acid groups that constitute the active centre of the ATPase enzyme itself (Fig. 1). The justification for this type of approach to biologically active protein structure has been developed in some detail by Ling²², who refers to the active centre as the cardinal site.

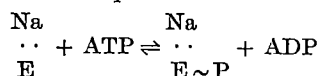
In the first state of the protein enzyme which we will consider, the model presupposes that the fixed anionic sites will show no strong preference for either Na^+ ions or K^+ ions, but will form associated ion pairs with either ion. The proportion of ion pairs formed by Na^+ will depend on the Na^+ concentration relative to the K^+ concentration. To initiate operation of the 'sodium pump' a critical number of the protein anionic sites must be occupied by sodium ion pairs. Thus we propose to start with the concept that the operation of the 'pumping cycle' has an inherent feed-back control. The enzyme cycle is stimulated by raising Na^+ concentration and depressed as this concentration falls and the K^+ concentration rises. As a result of sufficient sodium ion pair formation, there is a redistribution of electron density throughout the macro-molecule, and this occurs as a result of both direct and indirect interaction between Na^+ and the protein.

As in the protein fixed-charge model developed by Ling²², it is considered that the short-range interactions predominate in importance, and contribute in particular to the difference between the sodium and potassium

protein ion pairs. The inductive effect of the cation on the fixed-charge system will be additive and hence the effect of the sodium ion will bear a relation to the number of fixed anionic sites occupied by sodium ions.

Because the protein will also contain surface orientated cationic groups it will also form associated ion pairs with anions at these sites. The interaction between anion and the protein will also effect the induction of charge by cation-anionic site ion pairs. Thus when sodium and potassium are present as fluoride salts *in vitro* the ATPase activity is virtually abolished¹². In this model we imply that the highly electro-negative fluoride anion is capable of preventing the inductive changes produced by Na⁺ adsorption and which are necessary to initiate operation of the enzyme.

The redistribution of electron density induced by sodium ion pair formation is focused in the neighbourhood of the cardinal sites, and, as a result, this centre now reacts more actively with the substrate (ATP) to form the phosphorylated intermediate (Fig. 1). This step corresponds to the biochemical reaction sequence:



As a result of the phosphorylation of the cardinal site, there is a further inductive effect, and a redistribution of electron density along the polarizable chain of the protein. This produces a folding or deformation of the protein as well as a change in the adsorption energy of the fixed anionic site.

The deformation of the enzyme results, in the model, in a rotation outwards of many of the fixed anionic sites, but

leaves the phosphorylated site 'anchored' and still inward facing (Fig. 2). There are many ways in which the deformation might produce such a change in orientation of the molecule. We consider there is evidence for believing that the whole membrane structure is concerned in the transport process, although not directly part of the enzyme.

It is interesting to note that a recent paper by Tanaka and Abood has suggested that lecithin is required to restore activity to this enzyme after treatment with deoxycholate²³. We would interpret this finding as suggesting that the lipid is a necessary part of the enzyme system, although not involved in the chemical reaction itself.

The deformation suggested, therefore, implies unfolding, or lengthening the inner enzyme protein chain with subsequent rotation of the membrane fragment about the central lipid core. For a membrane unit of 1000 Å long and 60 Å wide, and assuming that the axis of rotation is in the centre of the lipid core, a change in length of about 50 Å would be required to produce a single 27° spiral.

Because of the adsorption energy changes associated with this phosphorylation and subsequent enzyme deformation, a marked preference for K⁺ is now exhibited by the cation binding groups, and the Na⁺ at these sites is exchanged for K⁺ (Fig. 2). The previous rotation of the membrane now implies that this exchange occurs extracellularly, hence Na⁺ has been transported from the intracellular to the extracellular environment. The adsorption of K⁺ on to these sites while the macro molecule is in this state of 'electron strain' again redistributes the electron density. In the region of the

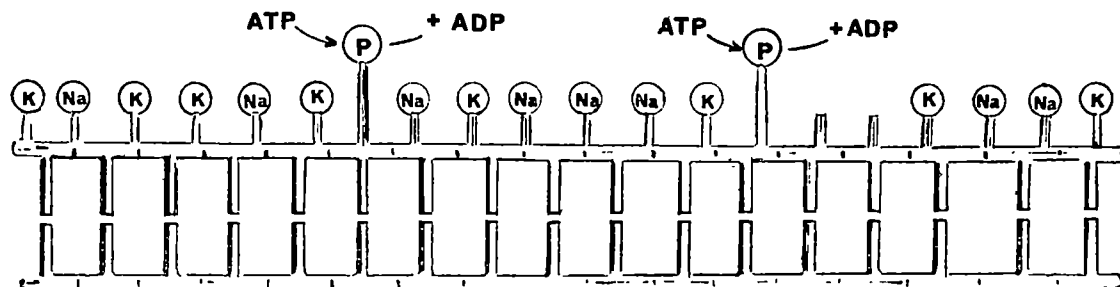


Fig. 1. Diagrammatic representation of a unit segment of membrane in which the horizontal sections depict the inner and outer protein layers, and the vertical broken units between the protein represent the central phospholipid complex described by Danielli. Projecting from the inner coil are shown the surface orientated anionic groups of the protein with which Na⁺ and K⁺ have formed associated ion pairs. The longer vertical projections represent the amino-acid groups which constitute the active centre of the enzyme. In this figure sufficient sodium ion pairs are considered to have formed to allow the active centre to react with ATP, and form the phosphorylated intermediate. Although the figure shows ATP reacting directly at the cardinal site it has long been established that magnesium ions are required for this reaction Mg²⁺ has been omitted from the figure for clarity.

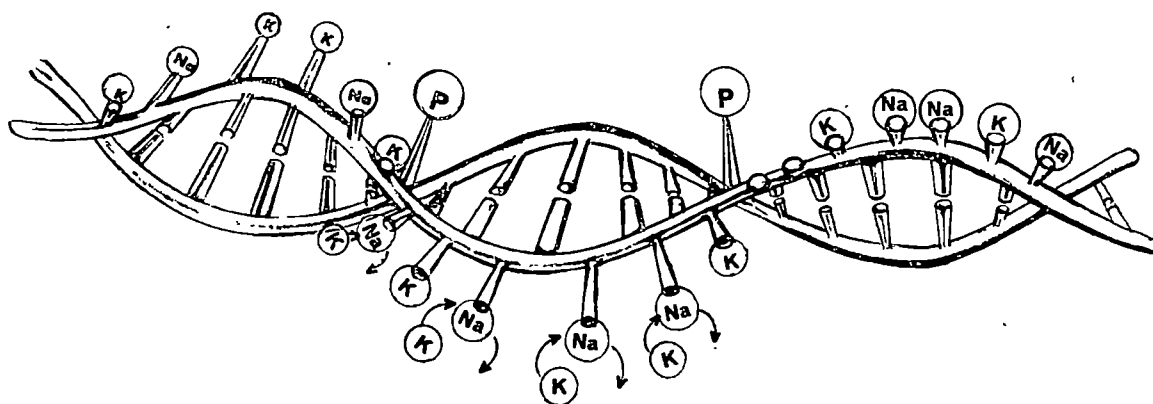


Fig. 2. Representation of the postulated effects of phosphorylation of the active centre of the enzyme. Elongation of the inner protein surface has produced rotation of these protein molecules about the centre of the lipid core. The site of the phosphorylated active centre is shown as resisting this rotation. The surface orientated anionic groups with their associated ions have rotated outwards following the elongation and twisting of the protein molecule. K⁺ for Na⁺ exchange is now shown to be taking place at sites of Na⁺ adsorption to these groups.

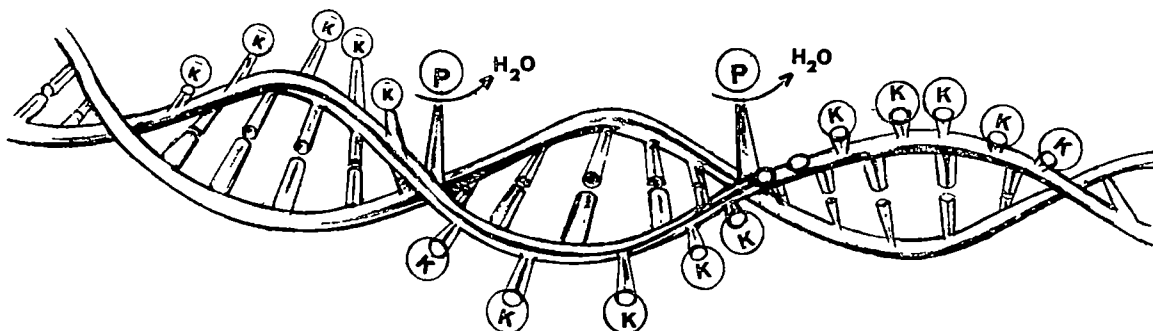
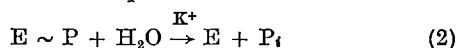


Fig. 3. Diagram showing that, following the K^+ for Na^+ exchange illustrated in Fig. 2, the inductive changes of electron density which take place in the elongated protein molecule lead to changes of field strength in the region of the phosphorylated active centre. As a result of these changes the phosphorylated centre is now readily attacked by water with cleavage of phosphate from the protein.

ardinal sites this alteration in electron distribution renders the phosphate groups more vulnerable to attack by water and cleavage of the phosphate bond occurs with the liberation of orthophosphate.

The liberation of phosphate from the phosphoprotein allows the molecule to return to its initial shape, and as a result the anionic binding sites with their adsorbed K^+ state inwards toward the cell centre. Within the cell some of these adsorbed K^+ ions are now displaced by Na^+ and the cycle of reaction begins again.

These last hypothetical steps correspond with the second step of enzyme reaction sequence:



Thus the cycle results in net transfer of Na^+ from inside the cell to the outside, coupled with the transport of K^+ in the opposite direction, during which process ATP is hydrolysed to ADP and orthophosphate.

Because there is evidence that the site of ouabain inhibition is at reaction (2) in the sequence given above¹³, it seems unlikely that ouabain acts directly at the active centre of the ATPase enzyme. Ouabain does not prevent the formation of the phosphoprotein intermediate but appears to prevent its decomposition^{13,14}.

For the toxicity and action of cardiac glycosides on ion transport certain features of cardiac glycoside molecule appear essential^{24,25}. These features include a *cis*-configuration of the *C-D* ring function, the presence of a β -hydroxy group at C_{14} and an unsaturated lactone ring at C_{17} . A conformational representation of ouabain is shown in Fig. 4. Although the orientation of the molecule

will depend on the positions adopted by the lactone ring at C_{17} and the sugar moiety at C_3 , as both groups are free to rotate, a conformation could exist where the ketone group and the β -hydroxyl at C_{14} face away from the same surface of the ouabain molecule. When oriented in this position, these groups might form by hydrogen-bonding the primary attachment of the ouabain molecule to the surface of the ATPase enzyme.

In addition, a cluster of OH groups exist both on the AB rings of the aglycone and on the sugar moiety of ouabain. These groups could also form hydrogen-bonds with the protein enzyme surface. Attachment of ouabain to the protein enzyme by the separated clusters of OH-groups would act, in the model presented in this paper, by maintaining the deformed state of the membrane shown in Figs. 2 and 3. This interaction would prevent both release of phosphate from the active centre, and operation of the deformation-restoration cycle required to move Na^+ and K^+ ions in the directions required.

In the absence of direct experimental evidence, such an explanation for the mechanism of ouabain inhibition must remain entirely speculative.

The model presented in this article is an attempt to combine data about the ' Na^+ + K^+ activated ATPase' into a consistent model which seeks to explain the means by which such an enzyme could produce vectorial Na^+ and K^+ transport.

A useful model should be fragile, and ought to suggest the appropriate tests as to its own validity. We believe this model suggests at least two general questions about ' Na^+ + K^+ activated ATPase' with respect to its capacity to transport these ions.

First, Do transition states of the enzyme exist with selective Na^+ or K^+ binding capacity? The model, in fact, suggests that selective E^+ binding occurs when the enzyme is in the phosphorylated state (Fig. 3). Recently an answer to this question has been sought using a histochemical electron microscope technique. With this we have been able to show what is believed to be evidence of markedly increased Cs^+ binding by the enzyme membrane during the existence of the phosphoprotein transition state¹⁶. Because Cs^+ can replace K^+ completely in the enzyme reaction^{2,6} we believe that this implies that K^+ binding will occur under the experimental conditions that demonstrated Cs^+ binding. As Cs^+ has a greater electron density than K^+ it is consequently easier to visualize using the electron microscope.

This evidence has necessitated the inclusion of a further step in the reaction sequence to account for a potassium exchange.

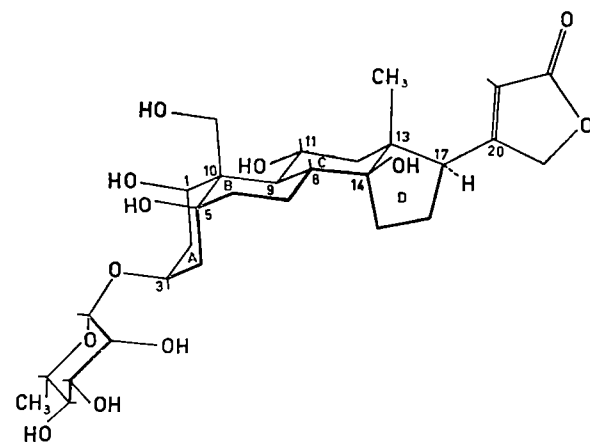
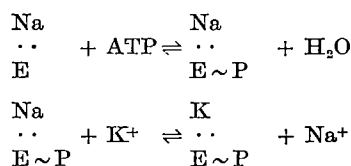
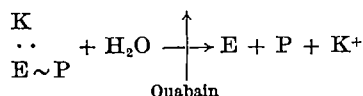


Fig. 4. Conformational representation of ouabain (3-O- β -D-rhamno-pyranosyl-1,3,3,5,11a,14,19-hexahydroxy-5 β -cardo-20(22)-enolide). We are indebted to Dr J. Mills, C S I R.O. Division of Biochemistry and General Nutrition, Adelaide, for this interpretation of the structure. Rotation of the rhamnose molecule will produce a configuration in which the undersurface of the molecule is hydrophobic, and the upper surface contains projecting OH and =O groups. We envisage that these groups form hydrogen bonds with the extended surface of the inner membrane protein containing the ATPase.



A second question which the model poses is: Does a measurable or detectable degree of molecular deformation occur during or following the formation of the phosphoprotein? One approach to the solution of this question requires direct biophysical examination by such techniques as X-ray diffraction, flow birefringence or light scattering. We have recently been able to show that the particulate enzyme gives a clear orientated X-ray diffraction pattern although as yet we have obtained insufficient resolution to determine the presence of significant deformation²⁶.

A more indirect approach would be to attempt an estimate of entropy change associated with the hydrolysis of ATP by this enzyme. The model predicts that the entropy values should be high for the ouabain-sensitive Na⁺ and K⁺ ATPase, and much less for the ouabain-insensitive ATPase which is always associated with our preparations.

Other workers interested in 'Na⁺ + K⁺ activated ATPase' have speculated about the mechanism of ion transport by this enzyme. Some authors have considered that either ATP itself, or the phosphate group of the labile phosphoprotein, acts directly as the ion carrier²⁷. However, most investigators have treated the enzyme as a polyanionic macromolecule the ion selectivity of which is determined by the turnover of labile phosphate groups^{1,2}. Eisenmann²⁸ has evolved characteristics for such a selective ion binding system which is based on the field strength of the anionic site, and Ling²² has developed this type of approach with considerable rigour as the basis of specialized protein function in general.

Working with the 'Na⁺ + K⁺ activated ATPase' from the electric organ of the electrophorus, Albers, Fahn and Koval²⁹ have suggested a model with spatial separation of three enzyme steps. Their model, however, suggests that Na⁺ and K⁺ movement is coupled with the translocation of phosphate from inside the cell, but fails to account, however, for the return of the phosphate to the cell.

All these models have sought to explain the changing selectivity of binding sites associated with the enzyme

'ion carriers', but none of them has attempted to include an explanation of the vectorial nature of the ion movement, or the highly selective inhibition of both the enzyme and ion transport by the cardiac glycosides. Although the model presented in this article will undoubtedly be proved to be inconsistent as new facts emerge, we believe that it will help to suggest the experiments necessary to uncover these new facts.

- ¹ Judah, J. D., and Ahmed, K., *Biol. Rev.*, **39**, 160 (1964).
- ² Skou, J. C., *Prog. Biophys. Chem.*, **14**, 131 (1964).
- ³ Skou, J. C., *Biochim. Biophys. Acta*, **58**, 314 (1962).
- ⁴ Bonting, S. L., Simon, K. A., and Hawkins, N. M., *Arch. Biochem. Biophys.*, **95**, 416 (1961).
- ⁵ Taylor, C. B., *Biochim. Biophys. Acta*, **60**, 437 (1962).
- ⁶ Skou, J. C., *Biochim. Biophys. Acta*, **42**, 6 (1960).
- ⁷ Post, R. L., Merritt, C. R., Kinsolving, C. R., and Albright, C. D., *J. Biochem.*, **235**, 1796 (1960).
- ⁸ Charnock, J. S., and Post, R. L., *Austral. J. Exp. Biol. and Med. Sci.*, **41**, 547 (1963).
- ⁹ Glynn, I. M., *J. Physiol.*, **180**, 18p (1961).
- ¹⁰ Whitlam, R., *Biochem. J.*, **84**, 110 (1962).
- ¹¹ Schatzmann, H. J., *Biochim. Biophys. Acta*, **94**, 89 (1965).
- ¹² Charnock, J. S., and Opit, L. J., *Biochim. Biophys. Acta* (submitted for publication).
- ¹³ Charnock, J. S., and Post, R. L., *Nature*, **199**, 910 (1963).
- ¹⁴ Charnock, J. S., Rosenthal, A. S., and Post, R. L., *Austral. J. Exp. Biol. and Med. Sci.*, **41**, 675 (1963).
- ¹⁵ Rose, S. P. R., *Nature*, **199**, 375 (1963).
- ¹⁶ Judah, J. D., Ahmed, K., and McLean, A. E. M., *Biochim. Biophys. Acta*, **66**, 472 (1962).
- ¹⁷ Glynn, I. M., Slayman, C. W., Eichberg, J., and Dawson, R. M. C., *Biochem. J.*, **84**, 692 (1965).
- ¹⁸ Charnock, J. S., Casley-Smith, J. R., and Opit, L. J., *J. Cell Biol.* (submitted for publication).
- ¹⁹ Siekevitz, P., and Palade, G. E., *J. Biophys. Biochem. Cytol.*, **4**, 203 (1958).
- ²⁰ Hanzon, V., and Toschi, G., *Exp. Cell Res.*, **18**, 256 (1959).
- ²¹ Siekevitz, P., in *Ciba Found. Symp. Regulation of Cell Metabolism*, edit by Wolstenholme, G. E. W., and O'Connor, C. M., 17 (Little, Brown and Co., Boston, 1959).
- ²² Ling, G. N., *A Physical Theory of the Living State* (Blaisdell, New York, 1962).
- ²³ Tanaka, R., and Abood, L. J., *Arch. Biochem. Biophys.*, **108**, 47 (1964).
- ²⁴ Fieser, L. F., and Fieser, M., *Steroids*, 768 (Reinhold Publishing Co., N.Y., 1959).
- ²⁵ Wright, S. B., *The Metabolism of Cardiac Glycosides*, 368 (American Lecture Series, Charles C. Thomas, Springfield, Illinois, 1960).
- ²⁶ Opit, L. J., and Medlin, H. (unpublished data).
- ²⁷ McIlwain, H., in *Ciba Found. Symp. Enzymes and Drug Action*, 170, edit. by de Reuck, A. V. S., and Mongar, J. L. (J. and A. Churchill Ltd., London, 1962).
- ²⁸ Eisenmann, G., in *Membrane Transport and Metabolism*, edit. by Kleinzeller, A., and Kotyk, A., 163 (Academic Press, London, 1961).
- ²⁹ Albers, R. W., Fahn, S., and Koval, G. J., *Proc. U.S. Nat. Acad. Sci.*, **50**, 474 (1963).

Relictogobius kryzhanovskii AND THE PENETRATION OF MEDITERRANEAN GOBIES INTO THE BLACK SEA

By DR. P. J. MILLER

Department of Zoology, University of Glasgow

THE Ponto-Caspian seas of southern Russia are of particular interest to the European ichthyologist in their diverse fauna, with its endemic components, and its relationships to that of the Mediterranean. In addition, nomenclatural problems involving Mediterranean species have resulted from systematic work on this fauna. Fortunately, detailed reviews of both the hydrography and zoogeography of the Black and Caspian Seas have recently become available in English¹⁻³ together with several comprehensive lists of the fishes of these seas⁴⁻⁶. Both seas, and their tributary drainage systems, are populated in varying part by endemic 'Caspian' (= 'Sarmatic' auctt.) faunal elements of more or less stenohaline brackish-water or freshwater animals. The phyletic ancestors of these species evolved from ancient Mediterranean stock with onset of brackish conditions in the vast Sarmatic basin of the South Russian geosyncline

after this had become separated in the Upper Miocene from the Mediterranean region of the disappearing Tethys Sea. The establishment of the present connexion between the Black Sea and Mediterranean in the late Pleistocene (post-Würmian^{3,7}) led to an increase in salinity of what had previously constituted the semi-fresh Novo-Euxine stage in the history of the Black Sea basin. In consequence, the originally widespread Caspian fauna of the latter now survives as ecological relicts in environments of lower salinity provided by coastal lagoons ('limans'), estuaries of rivers, and the Sea of Azov, and has been largely replaced in coastal and open waters by Mediterranean immigrants which have entered the Black Sea probably within the past 12,000 years⁷. Although there are many Caspian forms restricted in distribution to the Caspian Sea, the existing Caspian fauna of the Black Sea exhibits little endemism above the subspecific level,

wer than 5 per cent of the species being absent from the Caspian Sea. This has been attributed to the effect of an earlier Pleistocene (Early Würm/Main interstadial¹, or Riss/Würm interglacial²) ingress of Mediterranean waters into the Black Sea basin, when the Caspian fauna of the then hyposaline Ancient Euxine stage of this basin is believed to have been almost completely eliminated from the ensuing marine Karangat era³. After the closure of this connexion with the onset of the main Würmian glaciation^{4,5}, salinity of the Black Sea basin became once more reduced and a Caspian fauna, mostly identical in species with the present one, was re-established throughout the sea by immigration from that persisting in the previously isolated basin of the Caspian Khvalynsk stage (via a temporary river, flowing westward, along the Manych depression)³.

In the modern Black Sea, gobiid fishes form significant elements in both the Caspian and Mediterranean components of the fauna⁴⁻⁶. Among those at present included in the former is the monotypic genus *Relictogobius* Pchelina⁸, represented by *R. kryzhanovskii* Pchelina, known from the type locality, a saline lagoon (20 ‰), closely walled off from the sea, on the Abrau Peninsula, near Novorossiysk, U.S.S.R. (44° 42' N., 37° 45' E.)⁸, as well as from Sochi, to the south-east (43° 35' N., 39° 40' E.)¹⁰, and from the Gulf of Varna, on the Bulgarian coast (43° 13' N., 27° 55' E.)⁹. *Relictogobius* has recently been listed as the only non-freshwater fish genus endemic to the Black Sea⁸. This is in marked contrast to the other 'Caspian' Black Sea gobiids which occur also in the Caspian basin and the populations of which found in the two regions are differentiated into no more than sub-species^{3,10}. The Caspian Sea also contains several bizarre genera and species of gobiid fishes lacking in the Black Sea¹⁰. Had the existence of *Relictogobius* not been apparently overlooked, its level of differentiation suggested by generic status could have provided evidence for persistence through the marine Karangat era of Ancient Euxine forms the survival of which into the Novo-Euxine phase has been the subject of controversy^{2,3}.

However, while compiling a check-list of European gobies (unpublished), I was impressed by the similarity between published illustrations^{8,9} of *R. kryzhanovskii* and specimens of the widely distributed Mediterranean *Gobius quadrivittatus* Steindachner, hitherto unrecorded from the Black Sea. Although the type specimens of the former were destroyed at Novorossiysk during the Second World War¹¹, the original detailed account leaves no doubt that the two species are identical. Both are small (up to 65.5 mm), elongate gobiids, with about 60-70 rows of cycloid scales in lateral series, a tubular posterior nostril, naked nape and breast, canine teeth, no free pectoral fin-rays, and in coloration show an intricate pattern of dark vermiculate markings over the head, numerous vertical bars across the flanks, and three pale transverse bands across the back, the broadest and most conspicuously demarcated being across the posterior nape and pectoral bases. The modified lateral-line system on the head lacks the cephalic canal ρ^1 - ρ^2 (nomenclature of Sanzo¹²), but shows a seventh transverse sub-orbital row of sensory papillae, extension of the most posterior inferior segment of the sub-orbital series towards preopercular-mandibular row e, and approach or union in dorsal midline of occipital rows o. *Gobius quadrivittatus* was made the type of a new sub-genus (*Chromogobius*) of *Gobius* L. by De Buen¹³. Suitably expanded in definition to include another Mediterranean form (identified by me as *Gobius depressus* var. *zebrata* Kolombatovic), this taxon could well rank as a separate genus, for which *Relictogobius* Pchelina 1939 becomes a junior subjective synonym of *Chromogobius* De Buen 1930.

Chromogobius quadrivittatus thus joins the increasing list of Mediterranean gobiids recognized as having penetrated into the Black Sea. These also now include *Gobius niger*

L., *G. paganellus* L., *G. cobitis* Pallas (= *G. capito* C. & V.), *G. buccichi* Steinbachner, *G. ophioccephalus* Pallas, *Pomatoschistus minutus elongatus* (Canestrini), *P. leopardinus* (Nordmann) [*P. microps leopardinus* auct., but specifically distinct from the true *P. microps* (Krøyer)], and *Aphia minuta* (Risso)^{4,5,10,14}. All these species have in common an inshore habitat, several are known to tolerate brackish conditions, and all are widely distributed throughout the Mediterranean, with five out of nine extending outside the Mediterranean into the colder Atlantic Boreal region. In their euryhalinity and eurythermality (to be inferred from their wide geographical range), these gobies, when opportunity arose for colonization, would have been pre-adapted for life in the present Black Sea, with its broad temperature fluctuations in shallow waters and markedly lower salinity than is found in neighbouring parts of the Mediterranean². In common with most of the other Mediterranean fishes which have permanently established themselves in the Black Sea, the gobies have not formed sub-species peculiar to the latter area, although local differentiation in the case of *Pomatoschistus leopardinus* is reported from the hypersaline lakes (41.8-44.3 ‰) at Eforia on the coast of Romania¹⁵. The presumably more stenotopic offshore gobiids of the Mediterranean (such as *Lesueurigobius*, *Deltentosteus*, *Buenia*, *Odondeubenia*, *Pomatoschistus* spp., and *Cratilogobius*, etc.) have not been recorded from the Black Sea. In parts of the Black Sea comparable to their known bathymetric range (25-300 m)¹⁶, the values for temperature and salinity are appreciably less than those of the Mediterranean, and there is increasing deoxygenation and hydrogen sulphide pollution with depth^{2,17}, so that entry of these forms would seem to be effectively prevented by an array of ecological barriers. A reverse tendency for 'Caspian' gobiids to extend their range outside the Black Sea and its drainage area (including the Sea of Marmora) appears to be restricted to the brackish and freshwater *Proterorhinus marmoratus* (Pallas), found in rivers entering the northern Aegean Sea^{4,5,18}, and *Knipowitschia lencoraria* (Kessler) [= *Pomatoschistus caucasicus* (Kawrajsky) Berg], known from the mouth of the R. Yaila (Samothrace) and lagoons near Kavala, northern Greece (40° 56' N., 24° 25' E.)¹⁴. Spread of certain Caspian gobiids of Black Sea rivers into the Baltic Sea through Dnieper-Bug, Nieman, Dvina and Don-Volga-Ladoga canal connexions may also be expected^{3,18}.

I thank Dr. R. W. Hey, Geology Department, Cambridge, for advice on Ponto-Caspian successions. Useful accounts of Russian work on the biostratigraphy of Quaternary marine deposits in the Caspian, Black and Mediterranean Seas and also on the general correlation of local Quaternary sequences in Europe and the Ponto-Caspian region have recently appeared in English^{19,20}.

¹ Caspers, H., *Mem. Geol. Soc. Amer.*, No. 87, 1, 801 (1957).

² Zenkevitch, L., *Biology of the Seas of the U.S.S.R.* (London, 1963).

³ Mordukhai-Boltovskoy, F. I., *Intern. Rev. Hydrobiol.*, 49, 139 (1964).

⁴ Slastenenko, E. P., *The Fishes of the Black Sea Basin* [in Turkish] (Istanbul, 1955-1956).

⁵ Slastenenko, E. P., *Hydrobiologia*, 14, 177 (1959).

⁶ Nalbant, T., *Bull. Inst. Cerc. Proiect. Pisc.*, An. 21, 73 (1962).

⁷ Flint, R. F., *Glacial and Pleistocene Geology* (New York, 1957).

⁸ Pchelina, Z. M., *C.R. Acad. Sci., U.S.S.R.*, 23, 586 (1939).

⁹ Georgiev, J. M., *Bull. Inst. Pisc. Pch.*, Varna, 1, 141 (1961).

¹⁰ Berg, L. S., *Freshwater Fishes of the U.S.S.R. and Neighbouring Countries*, 8 (Moscow, 1949).

¹¹ Personal communication from R. S. Poljakowa, Scientific Secretary, Zoological Institute, Academy of Sciences, Leningrad.

¹² Sanzo, L., *Mitt. Zool. Sta. Neapel*, 20, 249 (1911).

¹³ De Buen, F., *Bull. Soc. Sci. Nat. Maroc*, 10, 120 (1930).

¹⁴ Georgiev, J. M., *Bull. Inst. Pisc. Pch.*, Varna, 4, 189 (1964).

¹⁵ Pora, E. A., Bănărescu, P., Rosca, D., and Wittenberger, C., *Bul. Sti. Acad. Rep. Rom., Sect. Biol.*, 8, 203 (1954).

¹⁶ Page, L., *Rep. Danish Oceanogr. Exped. Medit.*, 1908-1910, 2, A3 (1918).

¹⁷ Ekman, S., *Zoogeography of the Sea* (London, 1953).

¹⁸ Mordukhai-Boltovskoy, F. I., *Hydrobiologia*, 23, 159 (1964).

¹⁹ Fedorov, P. V., *Rep. Sixt. Intern. Cong. Quaternary*, Warsaw, 1961, 77 (1964).

²⁰ Gromov, V. I., Krasnov, I. I., Nikiforova, K. V., and Shancer, E. V., *Rep. Sixt. Intern. Cong. Quaternary*, Warsaw, 1961, 95 (1964).

LETTERS TO THE EDITOR

ASTROPHYSICS

Ionosphere, and X-ray Images of the Sun

RUSSELL^{1,2} has obtained X-ray photographs of the Sun at an epoch close to the recent minimum in solar activity which occurred late in 1964 and which preceded the present rising phase of the new solar cycle. The photographs were taken from a high-altitude rocket, and the quality and resolution are sufficiently good to allow limb brightening to be clearly distinguished from several small active regions that were present on the disk. The photographs seem to suggest that, in contrast to the brightening at the limbs, there was also some darkening at the north and south poles.

These directly made photographs provide at least qualitative confirmation of the solar brightness distribution derived by me³ from ionospheric observations made during the previous solar minimum in 1954. This distribution was based on measurements of the changes in electron density, in the ionosphere, that were caused by the total solar eclipse of June 30, 1954. On the day of the eclipse, the Sun was exceptionally quiet and no active area of any importance was visible on the disk. Analysis of the ionospheric data led to the conclusion that, on this occasion, 82 per cent of the radiation could be attributed to an extended source which covered the entire disk and which was uniformly bright except for some darkening near the north and south poles. The remaining radiation (18 per cent) was attributed to limb brightening, although the slight asymmetry which was observed suggests that there may have been a very small active area near the west limb.

When ionospheric data are used to determine the brightness of the limbs relative to the uniform background, the degree of brightness which results depends on the value assumed for the effective recombination coefficient, α' , in the expression:

$$J(t) = \left[\frac{1}{\alpha'} \frac{dNe}{dt} + Ne^2 \right] \sec \chi$$

where $J(t)$ is proportional to the intensity of the incident radiation, Ne is the electron density at the peak of the E or F_1 layer, and χ is the zenith angle of the Sun⁴. The accuracy of the calculated brightness distribution is limited mainly by the uncertainty in the value of α' .

It seems likely that the new X-ray photographs will give good quantitative information about the actual degree of limb brightening near the 1964 minimum, and it is probably reasonable to assume that the brightening during the 1954 minimum cannot have been very different. If this assumption is correct, it would be possible to use the 1954 ionospheric data, in conjunction with the 1964 value for limb brightening, in a new determination of α' . The result would be of considerable interest because the numerical value and the precise significance of α' have long been subjects for controversy.

Russell also refers to the presence of sources of X-radiation which extend outwards beyond the visible limb of the Sun, as predicted theoretically by Elwert⁵. A knowledge of the rapidity with which the intensity decreases with distance from the limb would allow estimates to be made of the amount of residual X-radiation which is present during the total phase of a solar eclipse. This information also could be used in the determination of α' from ionospheric data obtained during total eclipses, and

its use for this purpose has been discussed on previous occasions^{6,7}.

C. M. MINNIS

International Years of the Quiet Sun Secretariat,
6 Cornwall Terrace,
London, N.W.1.

¹ Russell, P. C., *Nature*, 205, 684 (1965).

² Russell, P. C., *Nature*, 206, 281 (1965).

³ Minnis, C. M., *J. Atmos. Terr. Phys.*, 12, 266 (1958).

⁴ Minnis, C. M., *J. Atmos. Terr. Phys.*, 6, 91 (1955).

⁵ Elwert, G., *J. Atmos. Terr. Phys.*, 12, 187 (1958).

⁶ Minnis, C. M., in *Solar Eclipses and the Ionosphere*, edit. by Beynon, W. J. G. 204 (Pergamon Press, London, 1956).

⁷ Minnis, C. M., *J. Atmos. Terr. Phys.*, 12, 272 (1958).

Radiation Balance of Jupiter and Saturn

It is frequently stated^{1,2} that the radiation balance of Jupiter presents a significant problem in that the temperature derived for the cloud tops ($\sim 165^\circ \text{K}$) is considerably higher than the value which would be achieved by a rapidly rotating body having Jupiter's characteristic in simple equilibrium with solar radiation ($\sim 125^\circ \text{K}$). This discrepancy has been cited as possible evidence for thermal radiation from the interior of the planet; that is it has been suggested that Jupiter might still be cooling down from an early, high-temperature phase³. The role of the atmosphere in maintaining the thermal balance has generally been disregarded since it has been assumed that the known constituents were all transparent in the wavelength region of importance (20–40 μ). The situation for Saturn is very similar; the temperatures are simply somewhat lower. A number of recent findings when considered together appear to require a revision of these ideas.

Trafton⁴ has pointed out that the pressure-induced dipole absorption of H_2 may produce significant absorption in the case of Jupiter. He finds that temperatures of the order of 147°K are not difficult to maintain by an atmospheric greenhouse effect depending only on this absorber. Owen⁵ has reviewed a number of measurements of the temperature of Jupiter and has analysed one of the methane bands in the planet's spectrum to obtain a value of $200^\circ \pm 25^\circ \text{K}$. The conclusion of this investigation was that there appeared to be strong evidence for temperatures in the range 175° – 200°K in and above the cloud layer which is envisaged as considerably more complex than a simple flat surface. It thus seems necessary to invoke another absorber if the atmosphere is to provide the necessary opacity.

The way out of this dilemma appears to be provided by some recent laboratory studies of ammonia. Walsh⁶ has obtained spectra of ammonia in the region 20–35 μ at a number of path-lengths, with the gas subjected to various degrees of pressure broadening by N_2 . A series of his tracings for an equivalent path-length of about 12 m atm. of ammonia at three different pressures of N_2 is reproduced in Fig. 1. It is evident that, even with no broadening by N_2 , the transparency in this region is very low. In Jupiter's atmosphere, of course, the temperature will be lower than the laboratory value, so the population in the higher rotational levels (which are responsible for the absorption shown in Fig. 1) will be diminished. On the other hand, the atmospheric pressure within the region of the clouds is almost certainly in excess of 76 cm mercury and, while the broadening gases will be H_2 and He instead of N_2 , the susceptibility of ammonia to pressure broadening is so great that at the expected pressures the absorption

ould lie within the range displayed by the three curves Fig. 1. Whether this will indeed be sufficient to produce a required greenhouse effect can only be demonstrated by a more careful treatment which includes the absorption of other wave-lengths. The spectrum of appropriately large amounts of methane should also be examined to see whether the band at 44μ may be a significant source of acity.

Saturn presents a rather different problem. Following Dunham⁶, it is generally assumed that Saturn's atmosphere also contains ammonia. However, recent examinations of the spectrum of this planet have failed to reveal the absorption bands of this gas⁷, and an alternative explanation for Dunham's identification of ammonia in terms of an overlapping methane band has been adduced⁸. Furthermore, a recent temperature measurement by Low⁹ of a wave-length of 10μ has led to a value of 93°K , well below the earlier results of $\sim 130^\circ\text{K}$. At 90°K , the vapor pressure of ammonia is only $10^{-4}\mu$ mercury, suggesting that its spectroscopic detection is extremely unlikely. Hence the low temperature and the absence of ammonia absorption are consistent.

It thus seems highly probable that the 93° temperature refers to some level of the atmosphere within the cloud layer, that is, the thermal radiation measured at 10μ is coming from cloud particles rather than from the ν_2 absorption band of ammonia as in the case of Jupiter. There may be some radiation from higher rotational lines of the nearest strong methane band at 7.7μ . A model of the detailed structure and composition of the cloud layer will have to await additional observations, but it seems likely that the absence of convective phenomena, given by the large latent heat of sublimation of ammonia (41 cal/g mole at 198°K) may be responsible for the greater visual uniformity of the disk of Saturn compared with that of Jupiter. While it is inevitable that clouds consisting of ammonia crystals will form at some atmospheric level, the low temperature of the upper atmosphere of Saturn also permits the deposition of methane, suggesting a possible explanation for the difference in the polarization of Jupiter and Saturn reported by Dollfus¹⁰. In this view, the atmosphere above the level of visual opacity in the case of Jupiter is charged with ammonia crystals, while in the much colder atmosphere of Saturn these are replaced by crystals of methane. The difference in the polarization of the two planets could then arise from the difference in the index of refraction of the two kinds of crystals or a difference in their mean size.

The consistency established by the low temperature and absence of ammonia in the atmosphere of Saturn has interesting implications for the foregoing argument. Saturn's atmosphere is known to contain methane and hydrogen in somewhat greater amounts than the atmosphere of Jupiter. (The increase is due to the lower level of the cloud layer in the atmosphere of Saturn.) Yet the measured temperature is very close to the radiation

equilibrium value ($\sim 90^\circ\text{K}$), and apparently refers to a region in the atmosphere very close to the visually observed cloud layer. In other words, in the absence of a detectable quantity of gaseous ammonia, a Jovian-type atmosphere does not seem to have sufficient opacity to produce a large greenhouse effect. Thus either Jupiter is, in fact, still cooling down (while Saturn is not), or it is the presence of a substantial amount of ammonia in its atmosphere which leads to the observed high temperatures. The laboratory evidence presented in Fig. 1 suggests that the latter alternative must be seriously considered before resorting to the internal heat source suggested by the former.

TOBIAS OWEN

Astro Sciences Center,
ITT Research Institute,
Chicago 16, Illinois.

THOMAS E. WALSH

Radio Corporation of America Laboratories,
Princeton, New Jersey.

- ¹ Urey, H. C., in *Handbuch der Physik*, edit. by Flugge, S., 52, 400 (Springer Verlag, 1959).
- ² Opik, E. J., *Icarus*, 1, 200 (1962).
- ³ Trafton, L., *Astrophys. J.*, 14C, 1340 (1964).
- ⁴ Owen, T., *Astrophys. J.*, 141, 4-4 (1965).
- ⁵ Walsh, T. E., *Prog. Rep. O.N.T.C. Contract NON. 2481 (58)*, Johns Hopkins University (1963).
- ⁶ Dunham, T., in *The Atmospheres of the Earth and Planets*, edit. by Kuiper, G. P. (Univ. Chicago Press, 1952).
- ⁷ Spinrad, H., *App. Optics*, 3, 18- (1964).
- ⁸ Owen, T., *Astrophys. J.* (in the press).
- ⁹ Low, F., *Astron. J.*, 69, 550 (1964).
- ¹⁰ Dollfus, A., in *The Solar System*, 3, *Planets and Satellites*, edit. by Kuiper, G. P., and Middlehurst, B. I., 394 (Univ. Chicago Press, 1961).

GEOPHYSICS

Daily Variation of Cosmic Rays

ONE of the interesting properties of cosmic radiation of intermediate energies, 1-100 GeV, is the daily variation of intensity measured at the Earth. This variation, of average amplitude $\sim 0.3-0.4$ per cent, appears largely diurnal in character although harmonic analysis reveals a semidiurnal component. The maximum intensity occurs, on the average, about 1700 L.T. although the phase can vary considerably.

An understanding of the mechanism producing the variation has increased in the past few years with the discovery of the solar wind, the development of a model of the large-scale magnetic field configuration in the inner solar system, and the use of computers to achieve detailed information about the trajectories of charged particles in the geomagnetic field. Several theories have been put forward in recent months to account for the observed anisotropy in terms of the (overtaking) streaming of the cosmic ray gas parallel to the Earth's motion around the Sun. On the other hand, Sarabhai *et al.*¹ have suggested

that part of the daily variation is due to removal of particles from the morning side of the Earth due to scattering by magnetic irregularities. This communication proposes an alternative mechanism for such removal.

Several investigators have calculated particle trajectories under the influence of the Earth's magnetic field to ascertain 'asymptotic' directions of such particles, as a function of rigidity, outside this influence. By projecting a negative particle from the Earth (re-creating the history of a positive particle arriving at the Earth) and following its trajectory until the path leaves the

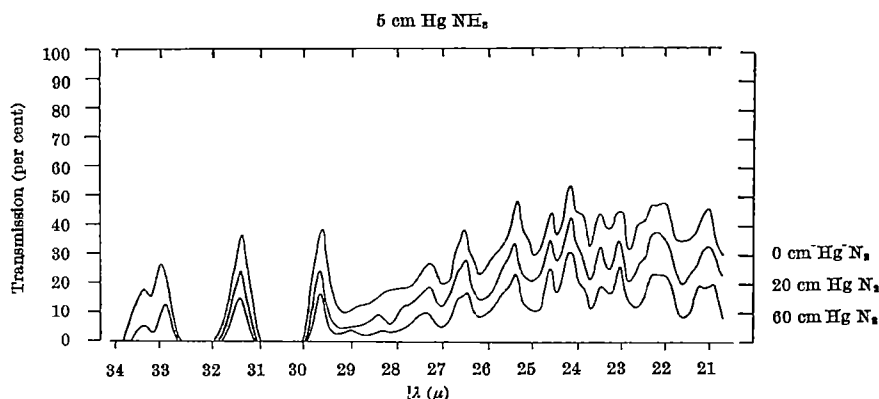


Fig. 1. Absorption spectra of ammonia subjected to pressure broadening by nitrogen (ref. 5)

magnetosphere, orbit calculations have been made in detail for protons entering this region of influence of the Earth. Allowing for the energy spread of primary particles capable of contributing effectively to neutron monitor counting rates, and for atmospheric effects, protons may enter the magnetosphere within an appreciable cone of directions prior to detection on the Earth at any location. The cone will, however, be restricted to equatorial latitudes for low and middle latitude detectors.

Outside the magnetosphere, however, the magnetic field has a value of about 3 or 4γ, and is usually considered to be oriented in the basic pattern of an Archimedes spiral centred on the Sun. When the cone looks up the Archimedes spiral, most of the detected primaries will have mirrored between the Sun and the Earth, assuming that the Alfvén guiding-centre approximation is valid and ignoring magnetic irregularities in the spiral. Cosmic ray primaries approaching the Earth from the antisolar direction up the spiral will be largely isotropically distributed in direction. (We should, perhaps, emphasize that in this model the majority of particles detected at the Earth by neutron monitors are confined to the spiral arm.) Passing the Earth, those the pitch angles of which are large will mirror near the Earth and then pass outwards along the spiral. For smaller pitch angles the mirror points will be closer to the Sun. For very small pitch angles, however, the mirror points will be in the Sun and the particles will be lost. Using the subscripts E and S for values at the Earth and Sun respectively, the critical value for the pitch angle α_E is given, assuming the conservation of magnetic moment, by:

$$\frac{\sin^2 \alpha_E}{\sin^2 \alpha_S} = \frac{B_E}{B_S}$$

that is, $\sin \alpha_E = (B_E/B_S)^{1/2} \approx 5 \times 10^{-3}$ assuming $B_r \propto (\text{distance from Sun})^{-2}$, that is, $\alpha_E \approx 5 \times 10^{-3}$ rad. Assuming an isotropic pitch angle distribution at the Earth, the number of particles absorbed by the Sun will be:

$$\Delta N = \frac{N}{4\pi} \int_0^{2\pi} d\phi \int_0^{\alpha_E} \sin \theta d\theta \approx 10^{-5} N$$

where N is the number density of particles. The free space anisotropy should therefore be of the order of 0.001 per cent. Neutron monitors, however, detect primaries from restricted angle of approach. For Sulphur Mountain the effective solid angle is about 2×10^{-2} sterad. Hence the detected flux:

$$F \approx \frac{2 \times 10^{-2}}{4\pi} \cdot NV \approx 1.7 \times 10^{-3} NV$$

where V is the velocity.

Thus $\Delta F/F = \Delta N \cdot V/F \approx 0.6$ per cent when asymptotic direction of approach coincides with Sun-Earth field line. This value, however, overestimates the magnitude of the anisotropy since the particles, differing rigidities, absorbed by the Sun would not have contributed with equal effectiveness to the direct response of the detector. On the other hand, if B_r should vary more slowly than $1/r^2$, the effect will be enhanced.

The effect of the cosmic-ray gas streaming past Earth due to co-rotation of the gas with the Sun has been estimated by Axford² and Parker³ to be of the order of 0.4–0.7 per cent. The predicted minimum due to this effect is at 0600 L.T. The solar absorption effect will, however, be directed up the spiral, 45° west of the Earth-Sun line. The resultant will therefore be the vector sum of the two effects. Figs. 1a and b show the frequency occurrence of the experimentally observed anisotropies polar diagrams for 1964 for selected days at Sulphur Mountain and Calgary respectively (counting rates 10^6 and $2.5 \times 10^5/\text{h}$ respectively). Selection of the data was based only on internal consistency between the data from 154 days are included. It is clear from the plots that the minimum direction is centred on 60° west of the Earth-Sun line. The averaged amplitudes of the minimum for the data taken together are 0.56 ± 0.01 per cent and 0.56 ± 0.01 per cent for Sulphur Mountain and Calgary respectively. Both direction and magnitude of the observed minimum are in fair agreement with the vector sum of the two effects considered, taking the lower value of 0.4 per cent for the streaming mechanism.

The absorption mechanism suggested by the authors is restricted by the following considerations:

(1) The gyroradii of the participating particles must be less than the scale size of irregularities in the magnetic field. This being so, the guiding centre approximation remains valid. When the gyroradii are of the same magnitude as the irregularities, however, the particles are scattered by the latter, and the fraction that reaches the Sun will be small.

The gyroradii of protons the pitch angles of which are $\leq 5 \times 10^{-3}$ radians in a field $B_E = 4\gamma$ and the energies of which are 1, 10 and 100 GeV are less than 5.5×10^3 , 4.5×10^4 and 4.0×10^6 km respectively.

Assuming the spiral field suggested by Parker³ and the constancy of $(\sin^2 \alpha)/B$, it follows that the gyroradius of a proton decreases almost linearly with decreasing distance from the Sun. Measurements by Explorer

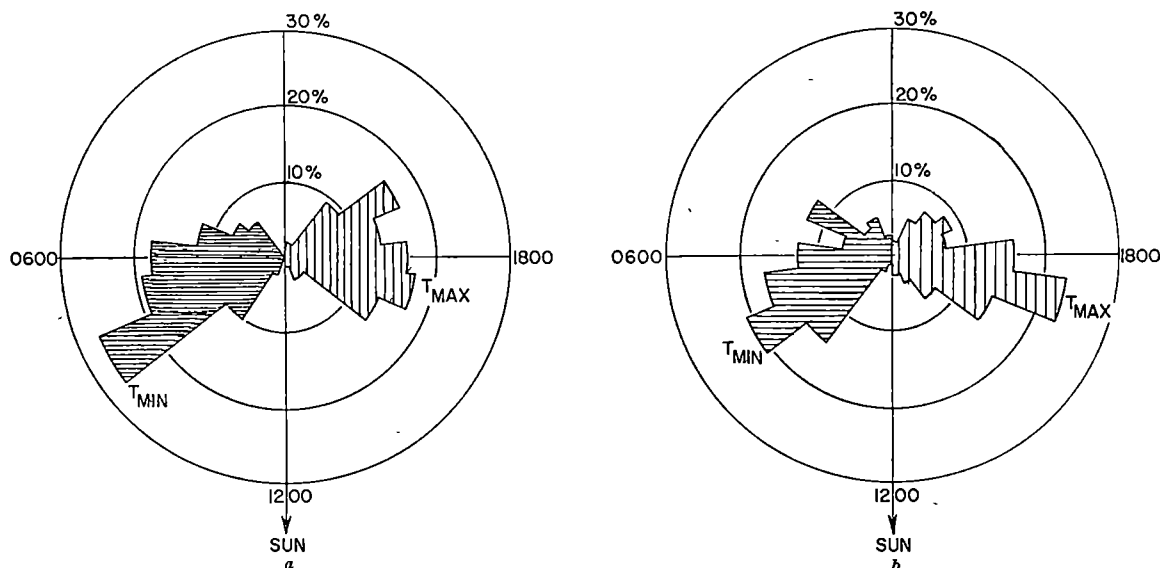


Fig. 1. Direction-frequency plot of T_{\max} and T_{\min} for the daily variation in neutron intensity for 154 days during 1964 at (a) Sulphur Mountain and (b) Calgary, Canada

VIII⁵ indicate periods in which magnetic irregularities 10^5 – 10^8 km exist. If 10^5 km is the minimum extent the fluctuations, then the mechanism will be operative and will be independent of energy. Should smaller irregularities occur an energy dependence would be expected.

(2) The direction of minimum intensity will be that of the field lines at any instant, and since their direction changes over periods of a few minutes to a few hours $10^5 \sim 10^7$ km 450 km/sec] there will be a broadening of the minimum and perhaps a difference in the recorded time of minimum for stations whose cones of acceptance do not greatly differ.

(3) Changes in direction of the 'average' spiral field on a day-to-day basis will be responsible for part of the spread in direction of T_{min} as observed in Figs. 1a and b. Such fluctuations would be related to changes in solar wind velocity.

J. B. MERCER
B. G. WILSON

Department of Physics,
University of Alberta, Calgary, Canada.

Sarabhai, V., Pal, G. L., and Wada, M., *Nature*, **206**, 703 (1965).

Axford, W. I., *Planetary and Space Sci.*, **13**, 115 (1965).

Parker, E. N., *Planetary and Space Sci.*, **12**, 735 (1964).

Parker, E. N., *J. Geophys. Res.*, **69**, 1755 (1964).

Ness, N. F., Scarce, C. S., and Seek, J. B., *J. Geophys. Res.*, **69**, 3531 (1964).

Frequency Spectrum of Doppler-shifted Whistler-mode Signals

To further our knowledge of very-low-frequency signals received via the whistler mode of propagation we have constructed a 25-channel spectrum analyser. Other work^{1,2} has shown the existence of apparent Doppler shifts on these signals of up to two or three parts in 10^5 of the carrier frequency; but the equipment used did not allow the variations of doppler shift with time to be resolved to better than 30 min. It sometimes appeared that there could be large positive and negative shifts occurring within a single 30-min period.

The new equipment consists of 25 filters, of the coherent detector-low pass amplifier type, in which the filter is tuned to the reference frequency supplied to the coherent detector and the band-width is determined by the low-pass amplifier. Use of this type of circuitry reduces the problem of the alignment of a bank of closely spaced filters to one of frequency synthesis, which in this case is done electro-mechanically. One filter is at the nominal carrier frequency and the others are disposed about this, 12 above and 12 below at a nominal spacing of 2 parts in 10^5 of the carrier frequency. Each channel has a band-width of approximately 0.5 parts in 10^5 .

In the short time that the equipment has been operating whistler-mode activity has been seen fairly frequently at off-sets of several parts in 10^5 . Occasionally events such as those shown in Fig. 1 are seen in which the Doppler shifts are oscillatory in nature. This is shown more clearly in Fig. 2 where the median frequency shift is plotted as a function of time. It is probable that it was events of this nature

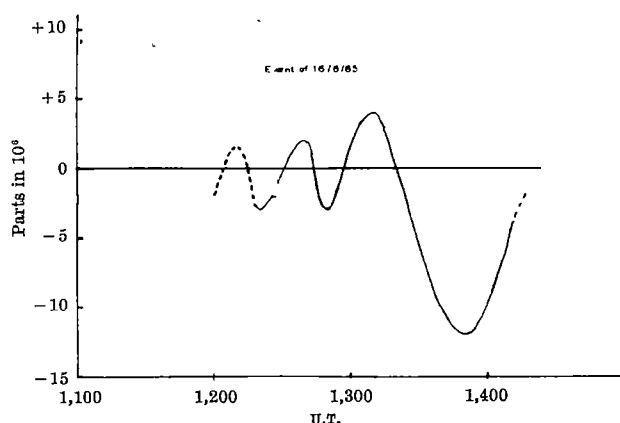


Fig. 2

which, when recorded on the original equipment, indicated the possibility of simultaneous positive and negative Doppler shifts.

The signals in this case are from station NPG at Seattle on a frequency of 18.6 kc/s. It will be noticed that there is a short period of 'key-up' followed by a period of 'key-down' just prior to the beginning of each hour. The loop aerial was turned to obtain a minimum of the direct or ionospheric signal, this being done to limit the spill-over of this component into the filters adjacent to the centre frequency. The large-amplitude slow-period beat on the centre channel is the result of a perfect null not being obtained. The period of the beat indicates the difference between the carrier frequency, as received, and the local standard.

R. A. MORRIS
N. POLETTI

Physics and Engineering Laboratory,
Department of Scientific and Industrial Research,
Lower Hutt, New Zealand.

¹ Crombie, D. D., McNeill, F. A., and Allcock, G. M., *J. Geophys. Res.*, **68**, 6229 (1963).

² McNeill, F. A., and Allan, A. H., *J. Geophys. Res.*, **69**, 3989 (1964).

GEOCHEMISTRY

Radioactive Elements in the Continental Crust

THE radioactive elements are particularly useful as indicators of the chemical composition of the deeper parts of the crust. Thorium, uranium, and potassium show a strong concentration towards the surface of the Earth and are enriched in the continental crust. The abundance of thorium, uranium and potassium can be estimated by two independent methods; a physical method based on heat-flow data, and a geochemical method based on geological premises. These estimates differ by a factor of two (Table 1). The estimates for the heat-flow data are from Clark and Ringwood¹, and are based on a 37-km-thick two-layer crust. The upper layer is 16 km thick, resting on a layer 21 km thick. Calculations

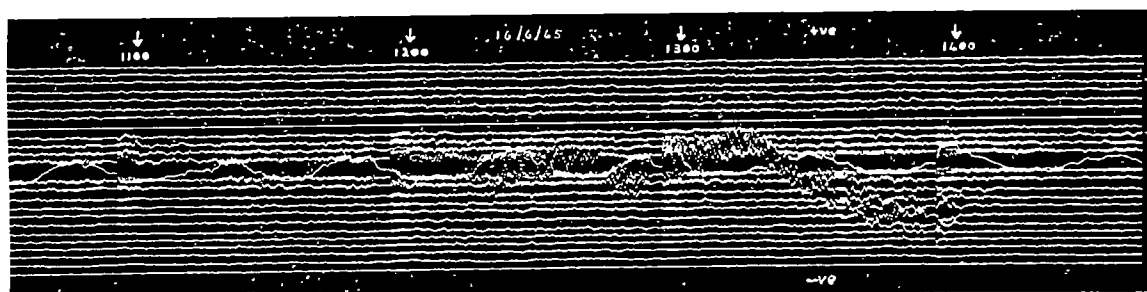


Fig. 1

Table 1. ESTIMATES OF THORIUM, URANIUM AND POTASSIUM ABUNDANCES IN REGIONS OF THE CONTINENTAL CRUST

Th (p.p.m.)	U (p.p.m.)	K (per cent)	Ref.
11.4 ± 2	3.0 ± 0.6		Geochemical estimates
10.0	2.8	2.6	Adams <i>et al.</i> ¹² ; geochemical balance; abundance in crust exposed to weathering
9.6	2.7	2.09	Heier and Rogers ¹³ ; correlation between Th, U, and K in magmatic rocks; K figure adopted from MacDonald ¹⁴ (continental crust)
9.0	2.3	2.55	Taylor ² ; 1:1 ratio of basalt and granite (continental crust)
8.8	2.2	2.35	Clark <i>et al.</i> ³ ; median of granodiorites
5.3	1.4	1.5	1:1 ratio of mafic and felsic rocks (data from Clark <i>et al.</i> ³)
4.6	1.12	1.84	1:1 ratio of basic rocks and granodiorite (data from Clark <i>et al.</i> ³)
3.2	0.81	1.81	Estimates from heat flow
2.5	0.64	1.05	Clark and Ringwood ¹ ; surface heat flow 1.5 $\mu\text{cal}/\text{cm}^2 \text{ sec}$; average of 37 km crust
			Surface heat flow 1.2 $\mu\text{cal}/\text{cm}^2 \text{ sec}$
			Surface heat flow 1.0 $\mu\text{cal}/\text{cm}^2 \text{ sec}$

Table 2. ESTIMATES OF THORIUM, URANIUM AND POTASSIUM ABUNDANCES IN DIFFERENT REGIONS OF THE CONTINENTAL CRUST

Abundances derived from heat flow data (after Clark and Ringwood (ref. 1))	Surface heat flow ($\mu\text{cal}/\text{cm}^2 \text{ sec}$)	Depth interval (km)	U (p.p.m.)	Th (p.p.m.)	K (per cent)
Shield	1.0	0-16	1.00	4.00	1.63
		16-37	0.37	1.48	0.61
Continental	1.2	0-16	1.32	5.28	2.15
		16-37	0.42	1.68	0.69
Continental	1.5	0-16	1.87	7.48	3.05
		16-37	0.57	2.28	0.93

lations of the concentrations of thorium, uranium and potassium within each layer for different heat flows are given in Table 2. It is noteworthy that the concentrations of the radioactive elements in the 16-km layer in regions with high heat flow are comparable with the geochemical estimates made on the basis of the new abundance data by Clark *et al.*³. The concentrations calculated for the lower layer are similar to those in basalts. This could indicate that this layer has a basaltic composition. However, metamorphic processes, including generation of granite magma, and the cycling of elements within the crust could also account for the low concentrations of radioactive elements in the deep crust³⁻⁵. These processes would result in a more mafic average composition of the lower crust as compared with the higher crust³⁻⁵. There is also some indication⁶ that high-grade metamorphic rocks, especially those of granulite facies, have lower concentrations of thorium and uranium than magmatic rocks of comparable chemical composition, so that the upward concentration of thorium and uranium in the crust could be more marked than that of potassium.

Crustal abundances in general are derived from premises similar to those used to obtain the geochemical estimates of uranium and thorium in Table 1. It is possible, therefore, that we are at present overestimating many of the volatile and 'granitophile' elements in the crust, and that the average continental crust is more mafic than present estimates^{8,9}. It is worth noting that Clarke¹⁰ and Goldschmidt¹¹ restricted their estimates to the composition of a 10-mile-thick upper crust.

K. S. HEIER

Department of Geophysics and Geochemistry,
Australian National University, Canberra.¹ Clark, Jun., S. P., and Ringwood, A. E., *Rev. Geophys.*, **2**, 35 (1964).² Clark, Jun., S. P., Peterman, Z. E., and Heier, K. S., *Geol. Soc. Amer. Spec. Paper* (in the press).³ Heier, K. S., *Nature*, **202**, 477 (1964).⁴ Heier, K. S., *Geol. Foren. (Stockholm) Förhändl.*, **87**, 249 (1965).⁵ Heier, K. S., and Adams, J. A. S., *Geochim. Cosmochim. Acta*, **29**, 53 (1965).⁶ Bowen, N. L., *Geol. Soc. Amer. Bull.*, **58**, 263 (1947).⁷ Ramberg, H., *J. Geol.*, **56**, 448 (1948).⁸ Poldervaart, A., *Geol. Soc. Amer. Spec. Paper*, **62**, 119 (1955).⁹ Taylor, S. R., *Geochim. Cosmochim. Acta*, **28**, 1273 (1964).¹⁰ Clarke, F. W., *U.S. Geol. Surv. Bull.*, **770** (1924).¹¹ Goldschmidt, V. M., *Fortsch. Min. Krist. Petrog.*, **17**, 112 (1933).¹² Adams, J. A. S., Osmond, J. K., and Rogers, J. J. W., *Physics and Chemistry of the Earth*, **3**, 299 (1959).¹³ Heier, K. S., and Rogers, J. J. W., *Geochim. Cosmochim. Acta*, **27**, 137 (1963).¹⁴ MacDonald, G. J. F., in *Res. in Geochemistry*, edit. by Abelson, P. H., **476** (1959).

PHYSICS

Sub-millimetre Dispersion and Rotational Line Strengths of the Hydrogen Halides

To obtain the integrated absorption strength of narrow spectral line by direct spectrophotometric measurement high resolution is necessary and the resulting profile must be corrected for finite instrumental resolution. As an alternative, dispersion data at much lower resolution may be used to obtain line strengths, and we report some measurements on a few of the low J rotational transition in the halogen halides.

Table 1. SUB-MILLIMETRE ROTATIONAL LINE STRENGTHS IN THE HYDROGEN HALIDES

Rotational transition $J \rightarrow (J+1)$	HCl k_i (cm ⁻¹)	HCl A_i^0 (cm ⁻² atm ⁻¹)	HBr k_i (cm ⁻¹)	HBr A_i^0 (cm ⁻² atm ⁻¹)	HI k_i (cm ⁻¹)	HI A_i^0 (cm ⁻² atm ⁻¹)
0 → 1	20.88	1.73 ± 0.34	16.70	0.24 ± 0.02	—	—
1 → 2	41.76	8.25 ± 0.49	33.40	2.13 ± 0.05	25.70	0.42 ± 0.02
2 → 3	—	—	50.10	5.39 ± 0.30	38.54	1.10 ± 0.3
3 → 4	—	—	—	—	51.36	2.72 ± 0.0

We have used the method of Fourier refractometry¹ to obtain the refractive index of gaseous HCl, HBr and HI in the region 10–55 cm⁻¹ at a resolution of 2 cm⁻¹. The gases were carefully dried before introduction into the cell placed in one arm of a Michelson interferometer. Dispersive interferograms were recorded for various gas pressures up to about 500 mm mercury at temperatures near 293° K, and after Fourier transformation the difference $n(k) - \bar{n}$ between the refractive index $n(k)$ at wave number k and the mean \bar{n} over the radiation band was calculated² from:

$$n(k) - \bar{n} = \frac{1}{4\pi k t} \left\{ \arctan \frac{Q(k)}{P(k)} + \pi m \right\} \quad (1)$$

where $P(k)$ and $Q(k)$ are respectively the cosine and sine Fourier transforms of the interferogram function, m is an integer, and t is the cell length (20.3 cm). A typical set of results for HCl at three pressures is given in Fig. 1, in which the anomalous dispersion associated with the lines $J = 0 \rightarrow 1$ and $J = 1 \rightarrow 2$ is seen. The data were reduced to 760 mm mercury pressure and 300° K temperature using a standard form^{3,4}, and from these the strength per atmosphere A_i^0 of a line at wave-number k_i was calculated using the relation⁴:

$$A_i^0 = \frac{2\pi^2}{f_i(k', k'')} \left\{ n_i^2(k') - n_i^2(k'') \right\} - \sum_{j \neq i} \frac{A_j^0 f_j(k', k'')}{f_i(k', k'')} \quad (2)$$

between the refractive index difference at two wave-numbers k' , k'' ($k' < k_i < k''$) and the absorption strength. We have written:

$$f_i(k', k'') = \left\{ \frac{1}{k_i^2 - k'^2} + \frac{1}{k_i^2 - k''^2} \right\} \quad (3)$$

The experimental index values determined near any given transition k_i include the significant contributions of

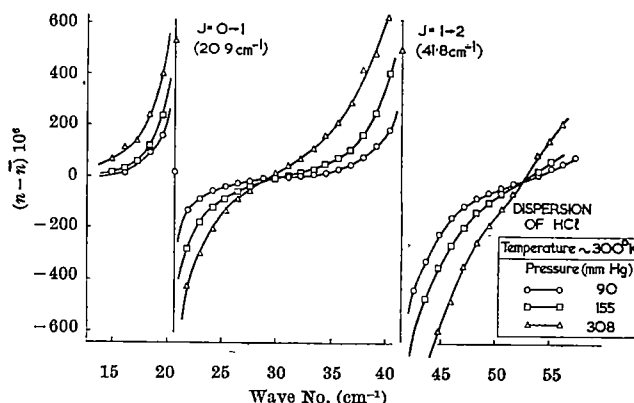


Fig. 1. The sub-millimetre dispersion of gaseous HCl in the region of the $J = 0 \rightarrow 1$ and $J = 1 \rightarrow 2$ rotational lines. Each point represents the average of two independent measurements made at the pressure stated, and the curves join the experimental data points. Cell-length is 20.3 cm.

ighbouring transitions k_j and account is taken of these using the experimental refractive index data in equation (2) and including the term under the summation. The various A_j^2 , A_j^3 are found by an iterative method since they are all initially unknown. Details of the experimental method and calculation will be given elsewhere. Using our index data and some recently published values of the rotational constants⁵ to calculate the k_j in equation (2), we find the values for the line strengths given in Table 1.

J. E. CHAMBERLAIN
H. A. GEBBIE

Molecular Science Division,
National Physical Laboratory,
Teddington, Middlesex.

Chamberlain, J. E., Gibbs, J. E., and Gebbie, H. A., *Nature*, **198**, 874 (1963).
Chamberlain, J. E., Findlay, F. D., and Gebbie, H. A., *Applied Optics* (to be published)

Schurh, B., and Rollefson, R., *J. Chem. Phys.*, **28**, 1089 (1957)

Chamberlain, J. E., *Infra-red Physics* (to be published).

Jones, G., and Gordy, W., *Phys. Rev.*, **136**, A1229 (1964).

CRYSTALLOGRAPHY

X-ray Studies on Highly Pure Magnesium Oxide

HIGH-PURITY (99.999 per cent) magnesium oxide was prepared by the thermal decomposition of pure magnesium oxalate dihydrate. Details of the preparation, analyses and thermal decomposition of the magnesium oxalate have been given elsewhere^{1,2}. Prior to the X-ray examination, the magnesium oxide was recrystallized by firing as a pressed disk at 1,500° C for 24 h. X-ray powder diffraction data were obtained by means of a 19 cm diameter camera using copper K_α -radiation with a nickel filter, and standard procedures. The observed spacings are given in Table 1.

Table 1. X-RAY DATA FOR PURE MgO

hkl	I/I_0	d (Å)
111	4	2.438
200	10	2.108
220	10	1.4899
311	4	1.2695
222	6	1.2155
400	3	1.0529
400	2	1.0529
331	4	0.9682
331	1	0.9681
420	4	0.9418
420	2	0.9418
422	4	0.8594
422	2	0.8594
511	2	0.8103
511	1	0.8104

Table 2. COMPARISON OF THE LATTICE CONSTANTS OF MgO

Material	Lattice constant (Å)
MgO, data of Swanson and Tatge (ref. 4)	4.213
MgO, present study	4.2119 ± 0.0005

The accurate lattice constant of the cubic magnesium oxide was determined by the method of Nelson and Riley³, using sodium chloride as an internal standard. The result obtained is given in Table 2 together with the value given by Swanson and Tatge⁴, for comparison.

This work was supported by the Steelley Organization Research Department. I thank Dr. A. J. E. Welch for initiating and supervising the work.

R. A. BROWN*

Inorganic Research Laboratories,
Imperial College of Science and
Technology,
London.

* Present address: Chemical and Physical Laboratory, Radio Corporation of America, Lancaster, Pennsylvania.

¹ Brown, R. A., thesis, Univ. London (1963).

² Brown, R. A., *Amer. Ceram. Soc., Bull.*, **44** (6), 483 (1965).

³ Nelson, J. B., and Riley, D. P., *Proc. Phys. Soc.*, **57**, 160 (1945).

⁴ Swanson, H. E., and Tatge, E., *J. C. Fel. Reports*, NBS (1949).

CHEMISTRY

Adsorption and the Differential Capacitance of the Electrical Double-layer at Platinum/Halide Metal Interfaces

MEASUREMENTS of the differential capacitance of the electrical double-layer at an LiCl-KCl eutectic melt/platinum interface at 450° C have been made by Laitinen and Roe¹ over a range of potentials from -0.34 V to -2.11 V versus a Pt/Pt²⁺ (1 M) reference electrode. Their curve of capacitance versus potential can be roughly divided into two regions: an anodic region of comparatively high specific capacitance ($\sim 100 \mu\text{F cm}^{-2}$) and a cathodic region of low specific capacitance ($\sim 50 \mu\text{F cm}^{-2}$), the transition between them occurring at approximately -0.8 V to -1.0 V. The high values of capacitance were attributed^{1,2} to the specific adsorption of chloride ions at potentials which are presumably anodic to the point of zero charge of this interface.

Double-layer capacitance versus potential curves for other metal/melt interfaces are quite unlike the previously published curves for platinum^{1,2,4} in that they are symmetrical about a well-defined minimum at the potential of zero charge (silver, thallium and tin are exceptions to this rule in that they exhibit a step in the cathodic branch⁵). Although it appears that the halide ions are surface active, because, for example, the addition of iodide ions to a chloride melt produces an increase in double-layer capacitance⁶, the increase is the same in both the anodic and cathodic branches of the curve for a given $|e|$ (the magnitude of the rational potential).

The present work suggests that the double-layer capacitance of a platinum/melt interface is dependent on the well-known existence of oxide layers on platinum surfaces⁷, and on the specific adsorption of platinum ions at concentrations determined by the redox potential of the melt.

The capacitance of the double-layer at a flame-polished, platinum hemisphere microelectrode under certain well-defined conditions (see following) falls to a minimum at approximately -0.8 V versus a Pt/Pt²⁺ (1 M) reference electrode (Fig. 1, curve a). In the region from -0.6 V to -0.8 V the capacitance varies with time so that it is

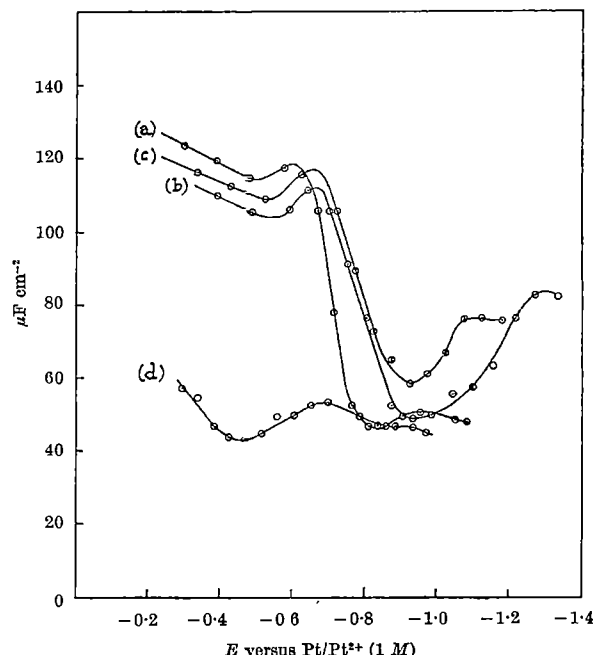


Fig. 1. The differential double-layer capacitance at platinum microelectrode/LiCl-KCl eutectic melt interfaces at 450° C. (a) Pure melt; (b) $\sim 10^{-6}$ M Pt²⁺; (c) $\sim 10^{-5}$ M Pt²⁺; (d) pure melt

necessary to allow up to 10 min for the system to come to equilibrium at each value of potential. If this procedure is not adopted, the curve becomes less well-defined and shows a marked similarity to the curves obtained by other workers^{1,2,4}, provided that the potential is changed in a cathodic direction. If the potential is changed in an anodic direction and time is not allowed for the system to come to equilibrium, the higher values of capacitance are never attained (Fig. 1, curve *d*). Under equilibrium conditions curve (*a*) in Fig. 1 can be reproduced without any difficulty whatever the direction of potential change; provided that the potential of the platinum electrode is not taken too far into the cathodic region, where there is risk of surface modification by, for example, the deposition of alkali metal.

The slow change of capacitance with time at potentials in the range -0.6 V to -0.8 V can be attributed to the desorption of chemisorbed oxygen atoms or the cathodic stripping of a stoichiometric oxide from the platinum surface. This process is reversed as the electrode potential is returned through the -0.8 V to -0.6 V region, because of the presence of low concentrations of oxide ions even in highly purified melts in glass apparatus⁸ (purified by vacuum pre-electrolysis and filtration⁹). It is significant that the adsorption process is slower than the desorption process, reflecting not only the irreversibility of the process but also the inaccessibility of the oxide ions present at low concentration in the melt.

If the platinum surface is completely stripped over the range -0.6 V to -0.8 V, then this latter value (the point of minimum capacitance) is the zero charge potential for the system (the average value calculated from a number of experiments is -0.83 ± 0.02 V versus the Pt/Pt²⁺ (1 M) reference electrode). This value agrees rather well with the proposition that the difference between the standard electrode potential and the potential of zero charge of an M/M^{2+} couple is in the range of 0.5 V– 0.9 V¹⁰.

The addition of very small quantities of platinum ions to the melt by anodic dissolution of a platinum counter-electrode produces a number of changes in the capacitance versus potential curve (Fig. 1, curves *b* and *c*), the most interesting of which is the formation of a better defined minimum and the shift of this minimum to more cathodic

potentials. This suggests that platinum ions are adsorbed on to the electrode surface as negatively charged species perhaps as a square-planar PtCl₄²⁻ complex ion, which particularly suited to adsorption on a plane surface can lead to a more compact structure for the double-layer. The minimum capacitance in curves (*b*) and (*c*) is indeed increased over that of the pure melt even for the very lowest concentration of platinum ions and this is typical of an adsorption phenomenon. This effect could also arise from a change in the electrode surface following the electrodeposition of platinum metal. However, curve (*a*) in Fig. 2 indicates that this is not so. This curve was obtained with the previously used microelectrode introduced into a fresh melt without prior surface treatment. The minimum capacitance was reproduced within the accuracy of the method (± 3 per cent) and the addition of platinum ions (in somewhat higher concentrations than before) produced the characteristic increase at the minimum together with the cathodic shift (Fig. 2, curve *b*).

A method has been developed to make these experiments possible, the details of which are published elsewhere¹⁰. A fast rise-time (10^{-8} sec) constant current pulse is applied to the electrode system, and measurements are made of the resulting over-potential/time transient before the faradaic process interferes, that is, within 2×10^{-7} sec of the initiation of the pulse¹¹. This method¹² has been modified¹⁰ in the present work to enable measurements to be made at electrode potentials other than the equilibrium redox potential of the system. It is possible, therefore, to measure a true double-layer capacitance (free from pseudocapacitance) even in the presence of a fast electron transfer process, and hence detect the effects of the adsorption of the electroactive species.

This work is supported by the Central Electricity Generating Board.

A. D. GRAVES
D. INMAN

Department of Chemistry,
Northampton College of Advanced Technology,
St. John Street, London, E.C.1.

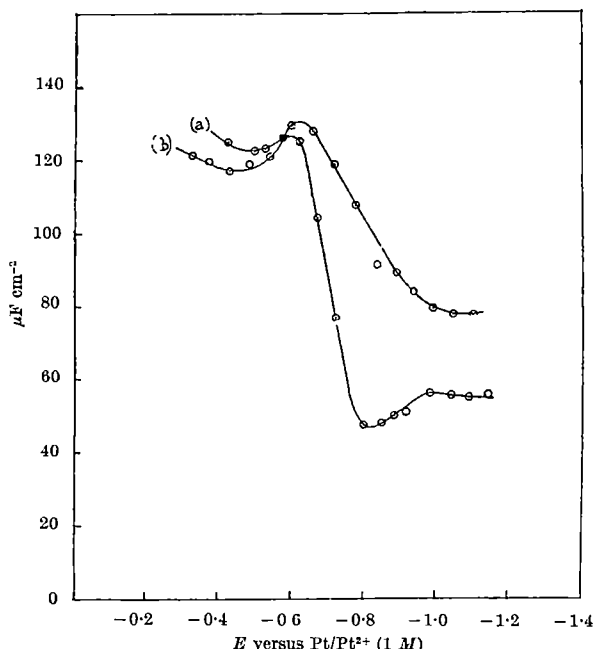


Fig. 2. Double-layer capacitance values obtained with the previously used microelectrode introduced into a fresh melt without prior surface treatment. (*a*) Pure melt; (*b*) $\sim 10^{-4}$ M Pt²⁺

- ¹ Laitinen, H. A., and Roe, D. K., *Coll. Czech. Chem. Comm.*, **25**, 3065 (1960).
- ² Hills, G. J., and Johnson, K. E., *J. Electrochem. Soc.*, **108**, 1013 (1961).
- ³ Ukshe, E. A., Bukun, N. G., Leikis, D. I., and Frumkin, A. N., *Electrochim. Acta*, **9**, 431 (1964).
- ⁴ Laitinen, H. A., and Gaur, H. C., *J. Electrochem. Soc.*, **104**, 730 (1957).
- ⁵ Ukshe, E. A., Bukun, N. G., and Leikis, D. I., *Russ. J. Phys. Chem.*, **36**, 1260 (1962).
- ⁶ Ukshe, E. A., Bukun, N. G., and Leikis, D. I., *Bull. Acad. Sci. U.S.S.R.; Div. Chem. Sci.*, No 1, 25 (1963).
- ⁷ Lingane, J. J., *J. Electroanal. Chem.*, **2**, 296 (1961).
- ⁸ Littlewood, R., *J. Electrochem. Soc.*, **109**, 525 (1962).
- ⁹ Inman, D., Graves, A. D., and Wrench, N. S. (to be published).
- ¹⁰ Graves, A. D., Hills, G. J., and Inman, D., in *Advances in Electrochemistry and Electrochemical Engineering*, edit. by Delahay, P., and Tobias, C. W., **4** (1965).
- ¹¹ Inman, D., Bockris, J. O'M., and Blomgren, E., *J. Electroanal. Chem.*, **2**, 508 (1961).
- ¹² Blomgren, E., Inman, D., and Bockris, J. O'M., *Rev. Sci. Instr.*, **32**, 11 (1961).

Evidence of Thermal and Hot Processes in the 'Hot-zone' produced by Copper-64 Recoil Atoms in Solids

ALTHOUGH the mechanism of recoil atom reactions in solids has been studied by a number of authors, some important problems still remain unsolved. For example, thermal and hot processes were discriminated in the recoil atom reactions in the gaseous or liquid system¹, but this is not clear in solids. Harbottle and Sutin² have proposed a concept of chemical reactions in the 'hot-zone'; however, this concept was somewhat ambiguous from the point of view of the presence of thermal and hot processes in the 'hot-zone'. In the course of studying hot-atom chemistry of copper-64 recoil atoms in α - and β -copper phthalocyanines, we have found evidence of thermal and hot processes in the 'hot-zone' in solids.

The hot-atom chemistry of copper phthalocyanine has been investigated by Herr and Gotte³, while the effects of crystal structure on the initial retention and on the thermal annealing rate have been investigated by Cook⁴ and by ourselves⁵.

Purified α - and β -copper phthalocyanines were checked by the X-ray diffraction method. Neutron irradiation of the target materials was carried out in a nuclear reactor JIGA Mark II at Rikkyo University (neutron flux: $\times 10^{11}$ n/cm²/sec). An irradiated sample was dissolved in cooled sulphuric acid ($\sim -20^\circ\text{C}$), and the solution poured into ice water to precipitate copper phthalocyanine. Afterwards the precipitate was filtered, and the precipitate and the filtrate separately counted by means of a Baird-atomic single channel γ -ray spectrometer (crystal: aI, 1.75 in. \times 2 in.).

The relationship between the initial retention and irradiation temperature is shown in Fig. 1. To avoid any effects of thermal and radiation annealing during irradiation, all retention values have been corrected for irradiation time $t = 0$. A remarkable difference between α - and β -crystals can be seen in Fig. 1. The initial retention in β -crystals at low temperature $R_{\beta 0}$ is much higher than that of α -crystals $R_{\alpha 0}$. The shape of the curve of β -crystals is different from that of α -crystals. This sensitivity of the initial retention in β -crystals seems to be closely related to the rate of increase of the retention on annealing. Table 1 shows initial annealing rates in α - and β -crystals at various temperatures. A remarkable difference between α - and β -crystals can again be seen.

Table 1. COMPARISON OF INITIAL ANNEALING RATES IN α - AND β -CRYSTALS

Annealing temperature	Initial annealing rate α	Initial annealing rate β	α/β
87°C	0.25%	4.0%	1/16
139°C	1.0	20	1/20
198°C	3.4	50	1/15
	Mean		1/17

According to Harbottle's model², hot-zone reactions proceed in a very short time after the recoil event, and therefore they are not completed in a 'hot-zone', but continue on thermal annealing. In other words, a reaction in a 'hot-zone' will be succeeded by substantially the same kind of reaction at the recoil site on thermal annealing. If this is true, the relationship of annealing rate between α - and β -crystals must hold good for that of 'hot-zone' reactions between α - and β -crystals (at least in the thermal range of the 'hot-zone'). On this assumption, we can simply go back to 'hot-zone' reactions from thermal annealing as is shown schematically in Fig. 2(a). At the crossing-point of two lines the quantity R_h ($=5.0$ per cent) can be obtained. R_h represents reactions which complete in the 'hot-zone' and is considered to be contributed by hot processes. In this way we can estimate the percentage of thermal processes and hot processes. Fig. 2(b) shows the results obtained. The apparent insensitivity of the retention in α -crystals to temperature, and the sensitivity of the retention in β -crystals to temperature, can be easily

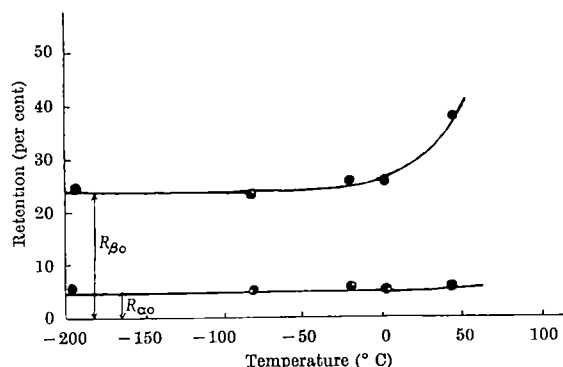


Fig. 1. Relationship between initial retention and irradiation temperature

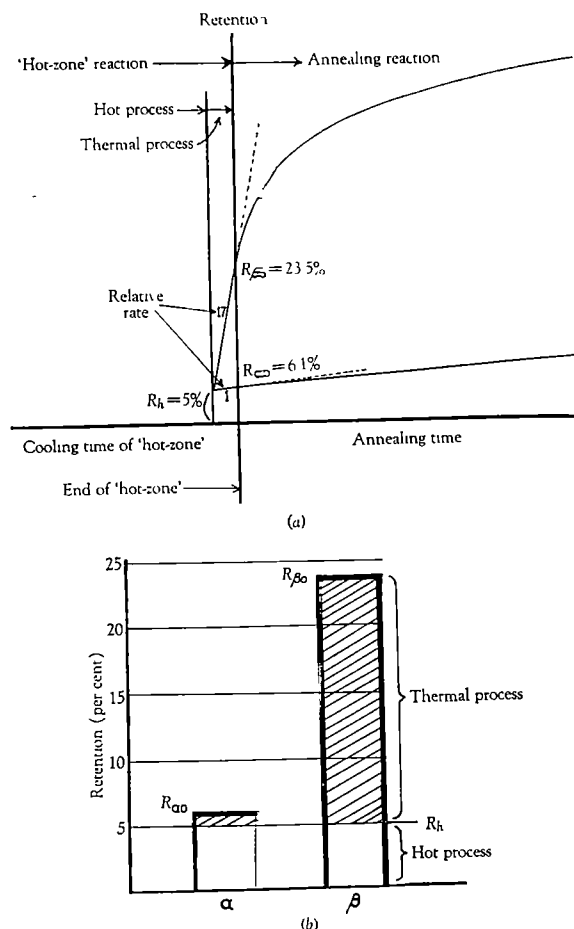


Fig. 2. Estimation of thermal and hot processes in the 'hot-zone'. (a) Methods of analysis. (b) Results

understood by considering the extent of the contribution of the hot process to the retentions. This kind of analysis is limited to the polymorphic crystals in which structure-sensitive reactions are concerned at the present stage. Although this method is not very accurate (small corrective terms will be discussed elsewhere), it makes it possible to estimate thermal and hot processes which in solids it was hitherto difficult to discriminate between.

We thank Dr. Shibata for his help.

KENJI YOSHIHARA
HIROSHI EBIHARA

Japan Atomic Energy Research Institute,
Tokai, Ibaraki, Japan.

¹ Willard, J. E., *Ann. Rev. Nucl. Sci.*, **3**, 193 (1953).

² Harbottle, G., and Suttn, N., *J. Phys. Chem.*, **62**, 1344 (1958).

³ Herr, W., and Gotte, H., *Z. Naturforsch.*, **5a**, 629 (1950).

⁴ Cook, G. B., *J. Inorg. Nucl. Chem.*, **14**, 301 (1960).

⁵ Yoshihara, K., and Ebihara, H., *Radiochim. Acta*, **2**, 219 (1964)

Electron Spin Resonance in X-irradiated Oriented Deoxyribonucleic Acid

SEVERAL papers dealing with electron spin resonance investigations of irradiated deoxyribonucleic acid (DNA) and its constituents have appeared in the literature¹⁻⁵. In all cases, however, unoriented powder samples have been used. In this communication we wish to report some preliminary findings made with an oriented sample of calf-thymus DNA.

Fibres of the DNA were drawn under controlled humidity conditions (66 per cent relative humidity) and wound on to a 'Spectrosil' grade quartz plate which could be mounted inside a tube⁶ of the same material, enabling the fibres to

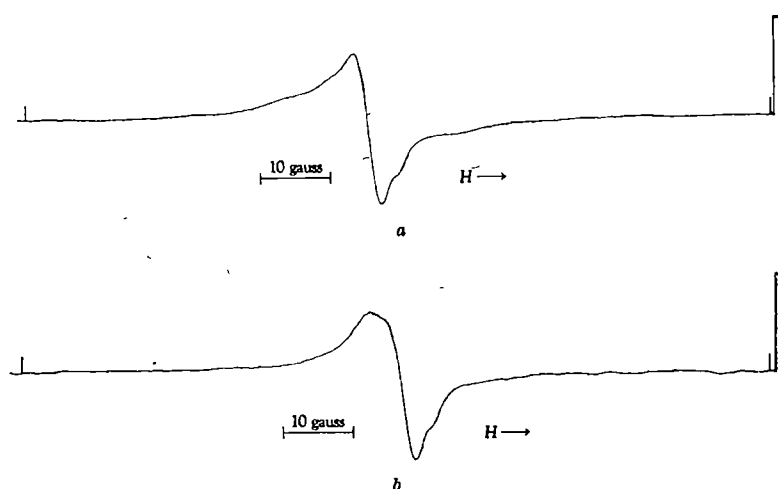


Fig. 1. Electron spin resonance spectra of dry calf thymus DNA fibres X-irradiated at room temperature. *a*, Fibres parallel to the magnetic field; *b*, fibres normal to the magnetic field.

be irradiated in an inert atmosphere of known humidity and afterwards transferred to the electron spin resonance spectrometer. The latter was of conventional design, using 100 kc/s modulation and first derivative presentation.

Fig. 1 shows spectra obtained after irradiating the fibres (weight 10 mg) at 0 per cent relative humidity under nitrogen with 250 kV X-rays to a dose of about 1 Mrad at room temperature. Spectra are shown with the magnetic field both parallel and normal to the fibres. The spectrum for the parallel position is approximately symmetric (line width between points of maximum slope ≈ 4 gauss), while that for the normal position is somewhat broader and asymmetric. Spectra were recorded over the dose range 0–1.5 Mrad, but were in all cases similar to those of Fig. 1. After the maximum dose (1.5 Mrad) had been given, the radical concentration was estimated to be 5.6×10^{18} free spins g^{-1} corresponding to a yield $G \approx 6$ (radicals/100 eV). The radicals showed little evidence of decay over several weeks, the fibres being stored under dry nitrogen at room temperature.

It will be noticed that the spectra are very different from those obtained by Ehrenberg *et al.*³ for γ -irradiated calf-thymus DNA in two respects. First, our spectra show only a faint trace of hyperfine structure and, secondly, the line width, measured between points of maximum slope, is much less than those reported in the literature. However, when an amorphous sample of the same batch of DNA was irradiated under similar conditions, a spectrum⁷ showing a narrow central line (maximum slope width ≈ 6 gauss) and well-resolved hyperfine lines was obtained.

An attempt was made to anneal the radicals by immersing the quartz sample tube in warm water for 10 min at successively higher temperatures. Between 60° and 70° C a 50 per cent decrease in radical concentration occurred, while at 80° C there was evidence of the appearance of hyperfine lines, but these were always of low intensity.

When the tube containing the (dry) DNA fibres was opened to the atmosphere, no change in signal was observed after 30 min. The signal disappeared, however, after leaving the fibres exposed to the atmosphere overnight. It has previously been reported that the decay of radiation-produced electron spin resonance signals in DNA appears to depend on the water content³.

Since DNA is believed to have a helical structure, we consider the expected angular variation of the spectrum of a system of helically distributed radicals. Assuming that the free radical sites have the same orientation as in the undamaged helix, the g -value parallel to the helix axis will be the same for each member of a population of

repeated radical species irrespective of the helical rotation operation. With the fibre normal to the helix one has, in general, a distribution of g -values depending on the magnitudes and inclinations to the helix axis of the principal g -values of the radical species. If the g -tensor has cylindrical symmetry, a spread of g -values is, of course, obtained only if the unique direction does not coincide with the helix axis. Further consideration of the perpendicular fibre case shows that, although the spread of g -values produces an overall broadening effect, this alone will not give an asymmetric spectrum provided that the fractional change in g -value is very small.

Similar considerations apply to the hyperfine coupling tensors in that single values are split into splittings occur along the helix axis. The parallel field case is analogous to that found in single crystals when site-splittings are absent. Averaging of the spectrum occurs in the perpendicular case when one or more of the tensors exhibits anisotropy in this direction, but the spectrum becomes asymmetric only when hyperfine anisotropy is accompanied by g -value anisotropy. It can be shown, for example, that only an anisotropic doublet is present, the maximum absorption of the smeared-out spectrum is displaced towards the low-field side if the direction of the smallest g -value in the perpendicular plane coincides with that of the larger hyperfine splitting.

The foregoing considerations are independent of the exact nature of the radical sites. We can now consider what types of radical are present. As mentioned previously the spectrum for the parallel orientation shows only slight resolution of components even though the best resolution is expected for this direction. One factor to be considered is the degree of orientation of the radicals within the helices and of the helices within the fibres themselves. If only one type of radical were present, the lack of resolution could be accounted for by poor orientation. However, we should then expect a distribution of g -values leading to an asymmetric spectrum. The observed spectrum is nearly symmetric. If we suppose that several radical species are present, the overlapping hyperfine structure characteristic of each producing a poorly-resolved resultant, the g -values must lie within narrow limits to account for the relative lack of asymmetry. This would be the case if the radicals were π -electron systems located on the bases.

Now polarized infra-red investigations⁸ have shown that, at high humidities at least, the base planes in DNA are nearly normal to the helix axis. Theoretical considerations¹⁰ show that the g -values of such radicals normal to the plane of the π -electron system should be nearly equal to the free spin value, with slightly larger in-plane values. Reported experimental data¹¹ confirm this. The two in-plane values are usually unequal. On this picture we expect all the π -electron radicals to contribute nearly the same g -value along the helix axis so that a nearly symmetric spectrum results. At low water contents the bases are tilted off the helix axis; but even if this were by as much as 30° the g -value normal to the base planes would still make the largest contribution along the helix axis.

If this picture is correct the asymmetry of the perpendicular spectrum presumably arises both from asymmetry in contributions from any one radical species due to simultaneous g -value and hyperfine anisotropy as discussed above, and asymmetry arising from differences in the mean in-plane g -values of the various species supposed to be present. In practice, we cannot distinguish these two effects.

One of us (J. P. E.) thanks the British Empire Cancer Campaign for Research for a maintenance grant. We also

Thank Mr. Paul Cooper of the Department of Biophysics, King's College, London, for supplies of DNA.

J. P. ELLIOTT
S. J. WYARD

Department of Physics,
Guy's Hospital Medical School,
London, S.E.1.

- Shields, H., and Gordy, W., *Proc. U.S. Nat. Acad. Sci.*, **45**, 269 (1950).
Alexander, P., Lett, J. J., and Ormerod, M. G., *Biochim. Biophys. Acta*, **51**, 207 (1961).
Ehrenberg, A., Ehrenberg, L., and Löfroth, G., *Nature*, **200**, 376 (1963).
Müller, A., *Intern. J. Rad. Biol.*, **6**, 137 (1963).
Salovey, R., Shulman, R. G., and Walsh, W. M., *J. Chem. Phys.*, **39**, 839 (1963).
Elliott, J. P., *J. Sci. Instrum.* (in the press).
Cook, J. B., Ph.D. thesis, London (1965).
Crick, F. H. C., and Watson, J. D., *Proc. Roy. Soc., A*, **223**, 80 (1954).
Bradbury, E. M., Price, W. C., and Wilkinson, G. R., *J. Mol. Phys.*, **3**, 301 (1961).
McConnell, H. M., and Robertson, R. E., *J. Phys. Chem.*, **61**, 1018 (1957).
Morton, J. R., *Chem. Rev.*, **64**, 453 (1964).

At the moment all these parameters are being examined with the view of obtaining quantitative definitions of the various classifications of cervical cells.

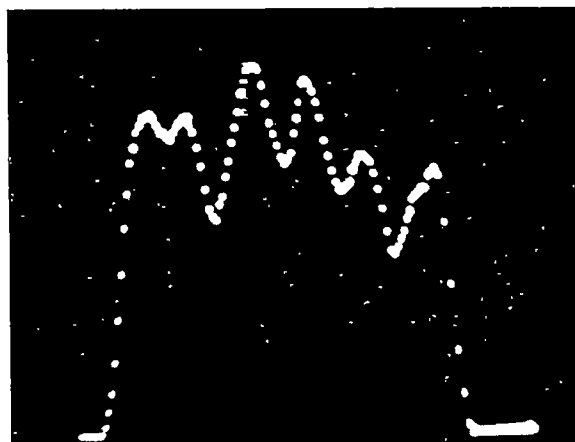


Fig. 1

BIOPHYSICS

Computer Recognition of Cervical Cell Nuclei

COMPUTER programmes which process data obtained by scanning 35-mm films of individual cervical cells are being tested in an attempt to obtain a quantitative definition of the differences between normal and malignant cells. The scanning instrument is of an electro-optical nature and has a digital output^{1,2}. It scans the photographic film in an array of points with each point yielding a number (between 0 and 128) the value of which depends on the emulsion density. The scan resolution can be varied from 4 lines each with 4 points to 256 lines each with 512 points. A typical single line scan (with 128 points) across a cervical cell nucleus is seen in Fig. 1. Normally these data are punched out automatically on paper tape, suitable for input to a Ferranti Sirius computer.

Having transformed the cell into an array of numbers, the problem now is to extract from it parameters which show significant differences between normal and malignant cells. It is essential for the solution of this problem that the original geometry of the cell nuclei be preserved. This has now been achieved in spite of the difficulties encountered when a cytoplasm is present.

The successful preservation of the geometry is illustrated by the graphs in Figs. 2 and 3, which were obtained after the computer had processed data obtained from scanning a normal cell and a 'malignant' cell. The object was to obtain a measure of the apparent thickness of the nuclear surface membrane. As a check on the validity of the computer programme the same sets of data were processed manually. These results are plotted beside the computer's selections.

The results for the nuclear surface membrane are taken from only a small part of the computer programme which is designed to extract a number of parameters characteristic of the cell. It was because of the ease of visual display of the nuclear surface membrane that it was selected for presentation here.

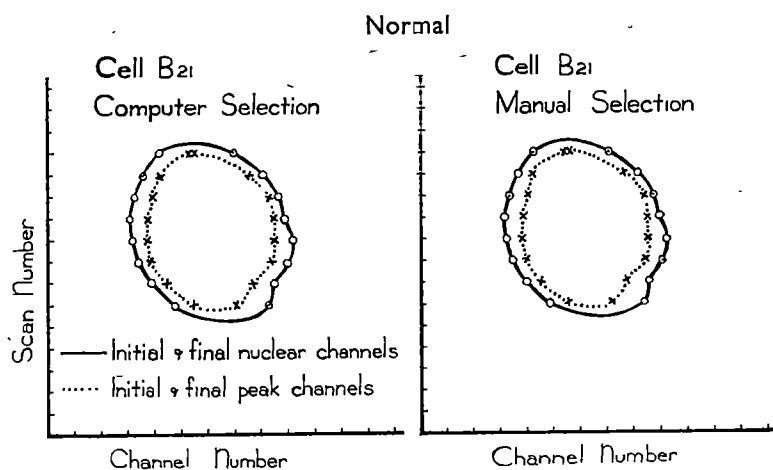


Fig. 2

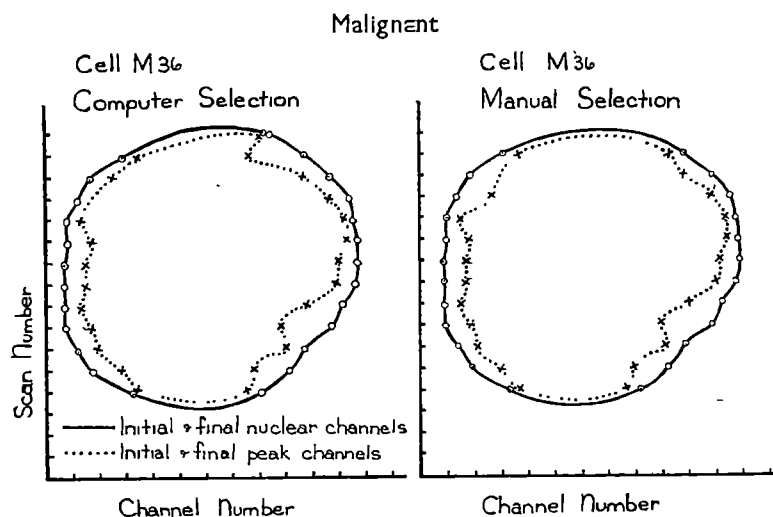


Fig. 3

I thank Dr. A. Ward for his advice and Mr. John Gray for assistance.

Radiation Laboratory,
University of Strathclyde.

G. W. McMASTER

¹ Ward, A. and McMaster, G. W., p. 428 of this issue of *Nature*.

² McMaster, G. W., "A Technique for the Digital Scanning of Normal and Malignant Cervical Cells", paper presented at the Northern Cancer Res. Group's Conf., Sheffield, during April 8-9, 1965.

Distribution of Deoxyribonucleic Acid along Human Chromosomes

To the physicist the structure of the 46 normal human chromosomes is a problem analogous to that of atomic structure with as much fundamental significance. Although the appearance of artefacts and deviations resulting from variations in the technique of preparing samples of human chromosomes introduces, at this time, a degree of error into the results, it is possible to consider the measurement of the distribution of deoxyribonucleic acid (DNA) along the chromosome as essentially a purely physical problem of structure.

Elsewhere the electronic instrumentation involved in this work has been described in some detail¹⁻⁴. Briefly, the equipment consists essentially of a flying-spot scanner which scans a 35-mm transparency of (in this case) a human chromosome and converts the image into an array of numbers representing the grain densities involved. In the work described here the scan consists of 16 lines each containing 32 points and the grain density on any one point is represented by an integral number lying anywhere between 0 and 128. That is to say, the image of the human chromosome is scanned with 128 shades of grey.

The decision to limit the scan to a matrix of 16×32 was made for technical reasons only, and it is hoped later to increase this matrix as the equipment is improved. Nevertheless, even at present, using a 16×32 matrix and bearing in mind the limitations in the image due to diffraction and artefacts, the present matrix appears to be of considerable use in discovering the DNA distribution along the chromosomes. It is anticipated that, as the techniques of preparing the human chromosomes for this work steadily improve and artefacts are reduced and as the measuring equipment improves, it will be possible to make a more and more carefully detailed study of the DNA distribution, that is to say the physical structure of the human chromosome. The results described here may be regarded as a preliminary survey indicating the likely final development of the technique. Eventually, when the structure of the normal human chromosomes has been discovered in sufficient detail, the technique will be applied to a study of human chromosomes associated with different physiological conditions in human beings, particularly those involving malignant growths.

For convenience, chromosomes 4 and 12 were selected for study, as they are obviously different when viewed by the human eye. The transparencies of the chromosomes were inserted into the scanning instrument and the images of each of the chromosomes were transformed into a 16×32 matrix of numbers. These numbers were observed visually on a display tube as a check on the scanning procedure and then punched out on paper-tape. The paper-tapes were analysed by a Ferranti *Sirius* computer programmed to provide two forms of output data. The first form consists of a geometrical print-out, an array

of crosses indicating when a number was above an arbitrarily selected background-level. The result of such a print-out is shown in Fig. 1 for chromosomes 4 and 12. The second form in which the data appear from the *Sirius* computer consists of a series of 32 numbers; each of these numbers represents the sum total of all the numbers appearing in that particular vertical column of the original matrix. That is to say, these numbers represent the total DNA at a particular point along the chromosome, the distance along the chromosome being determined by which of the particular columns is being considered. This type of analysis is particularly interesting, as a graph of these 32 numbers gives essentially the DNA distribution along the chromosome, and typical results for chromosomes 4 and 12 are shown in Fig. 2.

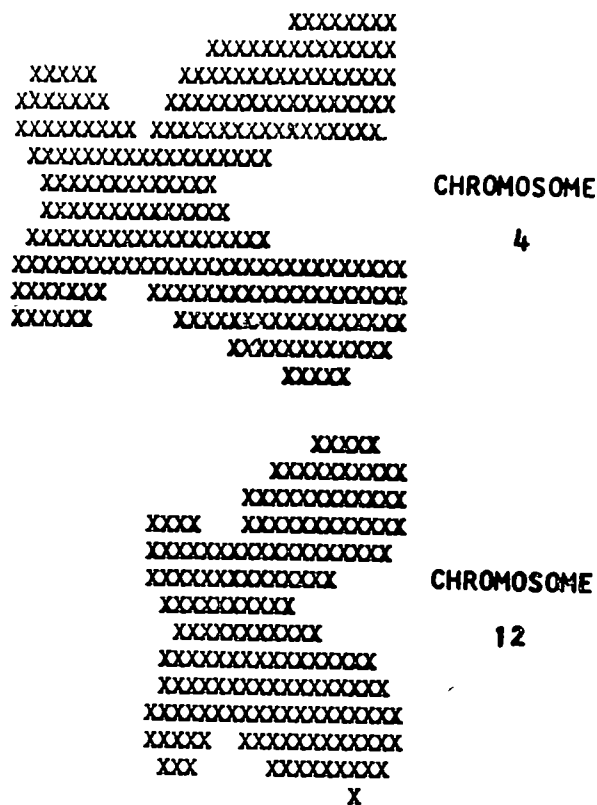


Fig. 1

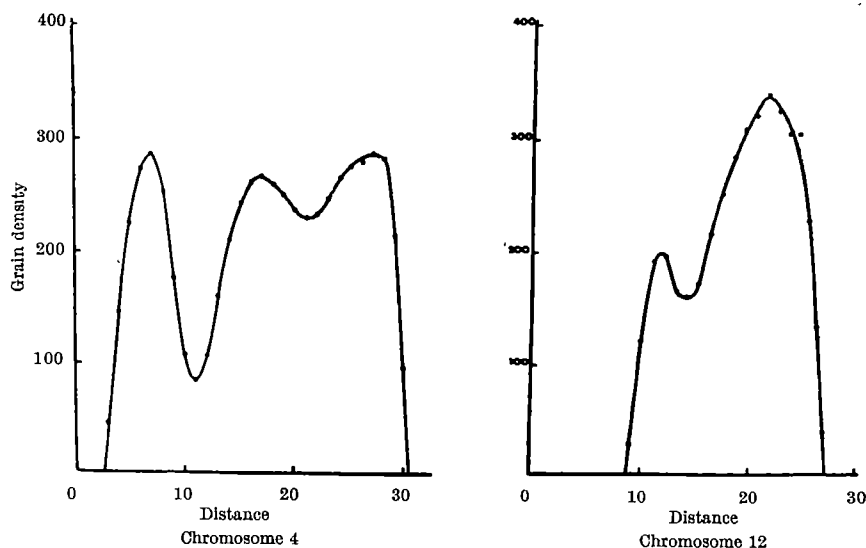


Fig. 2

It can be seen from Fig. 2 that the variation in DNA distribution along the two chromosomes is quite striking though it should be stated that these are preliminary results and there is some reason to believe that the staining used may in itself tend to be taken up with variable efficiency along the chromosome and therefore the stain density, which is of course the quantity being measured, is not a very accurate representation of the DNA distribution. This factor of the best choice of stain is being investigated at this laboratory, and it is hoped to find a stain which is sufficiently dark for scanning purposes and which also is taken up uniformly by the DNA present. Other results on chromosomes 4 and 12 show essentially the same type of structure as indicated in Fig. 2, and a programme is now under way to discover the essential features of the DNA distribution along all the 46 normal human chromosomes.

This work was made possible by research grants from the Medical Research Council and the Department of Scientific and Industrial Research. I thank Mr. J. Gray, Miss L. Robertson and Mr. G. Fraser, head of the Computer Unit at the University of Strathclyde, for their help.

A. WARD

Radiation Laboratory,
University of Strathclyde.

*Ward, A., and McMaster, G. W., p. 428 of this issue of *Nature*.

*McMaster, G. W., preceding communication.

*McMaster, G. W., "A Technique for the Digital Scanning of Normal and Malignant Cervical Cells", paper presented at the Northern Cancer Research Group's Conf., Sheffield, during April 8-9, 1965.

McMaster, G. W., "Quantitative Scanning of Cervical Cells", Proc. Seventeenth Brit. Cong. Obstet. Gynaecol., Glasgow, during July 5-7, 1965.

BIOCHEMISTRY

Secretion of Newly Synthesized Insulin *in vitro*

Up to the present, there has been little information available concerning the rate at which newly synthesized protein hormones may be secreted, following the stimulation of endocrine glands. In recent work on the synthesis of insulin within pancreas slices, *in vitro*, it became of interest to determine whether or not newly synthesized insulin may be released directly into the incubation medium, or alternatively whether or not release of insulin might be delayed following its accumulation within β -granules. It has already been suggested by Lacy¹ that release of insulin from β -cells in response to glucose involves the movement of these granules to the cell surface, where they are released into blood vessels.

In the experiments to be reported, insulin was labelled by incorporation of label into pancreas slices *in vitro*, and its release into media containing glucose studied. When rabbit pancreas slices are incubated in physiological media, such as Krebs-Ringer bicarbonate, there is a small release of insulin at low glucose concentrations, though this is very greatly increased when the sugar concentration is raised. The increased release of insulin has been shown to reflect, at least qualitatively, the physiological behaviour of β -cells *in vivo*². To label the insulin, rabbit pancreas slices were incubated for 1 h with tritiated leucine of high specific activity by methods which have already been described in detail elsewhere^{3,4}. The incubation medium was then poured off and the pancreas slices washed thoroughly with fresh medium to remove excess radioactivity. The slices were then incubated for 30 min in a medium containing low concentrations of glucose (50 mg/100 ml.). For the final 1-h incubation phase, the medium was again discarded and the pancreas slices divided into two parts, one being incubated in a medium of low glucose concentration (50 mg/100 ml.), the other being incubated in medium containing a much higher concentration of glucose (250 mg/100 ml.). The medium was gassed with 95 per cent oxygen and 5 per cent carbon dioxide before each incubation phase. Insulin in the medium

derived from the final incubation phase was assayed by an immunological method⁵, and isolated from the medium so that its specific radioactivity might be determined.

The isolation of labelled insulin in the medium was carried out by an immunological technique, based on the precipitation of an insulin-antibody complex by means of an anti- γ -globulin. For this purpose, the whole medium was dialysed against distilled water and freeze dried. The proteins were then dissolved in a small volume of 0.003 M hydrochloric acid, and allowed to react with 0.5 ml. of a potent anti-serum made by repeated injection of crystalline ox insulin into guinea-pigs⁶. The whole γ -globulin fraction was then precipitated by the addition of a rabbit-anti-guinea-pig γ -globulin serum. The precipitate obtained was spun off, and after washing with saline, the insulin was regenerated from it by acid, and further purified by two-dimensional chromatography and electrophoresis on paper as previously described⁴. Insulin regenerated was assayed in the extract by immunoassay. The results of two representative incubations are shown in Table 1.

Table 1

Exp.	Insulin release into medium in final incubation phase (mU/flask/h)		Specific radioactivity of insulin extracted from medium (c.p.m./ μ g)	
	Low glucose	High glucose	Low glucose	High glucose
1	6.48	≈ 4	1,040	567
2	7.36	≈ 1	912	552

It will be seen that when pancreas slices are incubated in a medium containing a low concentration of glucose for 1.5 to 2.5 h after the start of labelling, there is some release of labelled insulin. However, when the final phase of incubation is in a medium of high glucose concentration, insulin release from the slices is increased several-fold while the specific activity of the insulin falls markedly. Very recently, similar results have been obtained from experiments in which the medium was extracted with acidic ethanol and the crude insulin precipitated with ether-ethanol⁷ before purification by the method already described here. In two such experiments the ratio of insulin output in the high glucose medium to that in medium of low glucose content was 4.08:1, while the ratio of specific activities of the insulin extracted was 0.35:1. It seems, therefore, that the greater part of the insulin secreted in response to a glucose stimulus has not been synthesized in the preceding 1.5-2.5 h. These results are in marked contrast with those obtained for the secretion from liver of newly synthesized albumin which is known not to be stored in granules⁸.

In pancreatic acinar tissue there is evidence from autoradiographic studies that newly synthesized secretory proteins appear first in the rough surfaced elements of the endoplasmic reticulum, then in the Golgi complex and finally after 1 h in the zymogen granules, from which they begin to be discharged 2 h after synthesis⁹. From the results described here, it seems possible there may be analogies between the secretion of newly synthesized insulin and the discharge of recently made secretory proteins.

We thank the Medical Research Council, the British Diabetic Association and British Insulin Manufacturers for financial assistance for various aspects of this work.

S. L. HOWELL
D. G. PARRY
K. W. TAYLOR

Department of Medicine,
King's College Hospital Medical School,
Denmark Hill, London, S.E.5.

¹ Lacy, P. E., *Diabetes*, **11**, 509 (1962).

² Coore, H. G., and Randle, P. J., *Biochem. J.*, **93**, 66 (1964).

³ Mallory, A., Smith, G. H., and Taylor, K. W., *Biochem. J.*, **91**, 484 (1964).

⁴ Taylor, K. W., Gardner, G., Parry, D. G., and Jones, V. E., *Biochim. Biophys. Acta*, **100**, 521 (1965).

⁵ Hales, C. N., and Randle, P. J., *Biochem. J.*, **88**, 137 (1963).

⁶ Jones, V. E., and Cunliffe, A. C., *Nature*, **192**, 136 (1961).

⁷ Grodsky, G., and Tarver, H., *Nature*, **177**, 223 (1958).

⁸ Peters, Jun., T., *J. Biol. Chem.*, **237**, 1186 (1962).

⁹ Caro, L. G., and Palade, G. E., *J. Cell. Biol.*, **20**, 473 (1964).

Hormonal Control of Lipid Concentration in Rat Heart and Gastrocnemius

In perfused rat heart and isolated rat diaphragm the rate of release and oxidation of fatty acids from muscle glycerides is increased by alloxan-diabetes¹. These changes in diabetes involve actions of growth hormone and corticosteroids in insulin-deficient rats because they are not seen in muscles of hypophysectomized diabetic rats unless the animals are treated with growth hormone and cortisol¹. As part of an investigation of the mechanisms of these changes we have investigated the effects of diabetes, hypophysectomy and treatment with growth hormone and cortisol on the concentrations of glycerides and phospholipids in rat heart and gastrocnemius (the latter as a representative skeletal muscle). The methods and procedure were as follows.

Rat hearts were perfused for 10 min with medium containing glucose (5.5 mM) and insulin (0.1 unit/ml.), and the excised ventricles frozen with a tissue clamp and powdered in a percussion mortar². Gastrocnemius muscle was frozen in acetone-solid carbon dioxide mixture and powdered after 10 min of hind-limb perfusion through the abdominal aorta with the foregoing medium containing glucose (11 mM) but no insulin. These perfusions remove blood and blood lipids. Details of induction of alloxan-diabetes and treatment with growth hormone and cortisol were as described elsewhere². The frozen muscle powder was extracted with chloroform/methanol (2:1 v/v) (15–30 ml./g) in a Potter homogenizer, allowed to stand at 0° for 2 h and centrifuged. The supernatant was shaken with 0.3 vol. of 4 mM magnesium chloride and phases separated by centrifugation after 30 min. The chloroform phase was evaporated to dryness at 70° and the residue taken up in about 7 ml. of chloroform and a sample (0.5 ml.) taken for determination of phospholipid-P (ref. 3), after evaporation of chloroform and oxidation with 0.15 ml. concentrated H₂SO₄/HClO₄ (2:1 v/v). The remainder was shaken with 0.5 g silicic acid to remove phospholipid⁴ (confirmed by assay of phospholipid-P). A sample of supernatant (4 ml.) was evaporated to dryness and glycerides separated by thin-layer chromatography on 'Silica Gel G' (Merck) using as solvent petroleum ether (fraction 40°–60°)/di-ethyl ether/methanol/acetic acid (90:20:2:3 v/v). As markers crude triolein (British Drug Houses, Ltd.) containing mono- and di-olein, and pure tripalmitin and mono-olein (Hormel Institute, University of Minnesota), were used (about 0.5 µmole). The *R_F* values of the separated classes were: mono-glycerides 0.05; diglycerides 0.45; triglycerides 0.90. The spots, located by markers, were scraped from the plate and extracted twice with 2 ml. of chloroform/methanol (2:1 v/v). The extract was evaporated to dryness at 70°, saponified with 0.5 ml. 4 per cent potassium hydroxide in 95 per cent ethanol for 30 min at 60°, acidified with 0.5 ml. 10 per cent HClO₄, neutralized with saturated KHCO₃, and KClO₄ separated by centrifugation at 0°. Glycerol was assayed enzymatically in the supernatant⁵. Recoveries (using 1-[¹⁴C]-palmitate labelled tripalmitin from the Radiochemical Centre, Amersham, added to extracts of heart) were in excess of 90 per cent. Recovery of mono-olein was in excess of 80 per cent. Contamination with phospholipid-P was less than 0.05 per cent.

The results are shown in Table 1. In the glyceride series the concentration of triglyceride was greatly in excess of that of di- and mono-glyceride, the concentration ratios being 140:1:6 in normal rat heart and 120:1:4 in normal rat gastrocnemius. These values indicate that loss of the first fatty acid may limit triglyceride breakdown in muscle. The concentration of triglyceride in heart and gastrocnemius was significantly increased by alloxan-diabetes. In hypophysectomized rats alloxan-diabetes failed to increase the triglyceride concentration unless the animals were treated with growth hormone

Table 1. GLYCERIDE AND PHOSPHOLIPID CONCENTRATIONS IN RAT HEART AND GASTROCNEMIUS

Rat	Tissue	Concentration µmoles/g dry wt. (Mean ± S.E.)			
		Triglyceride	Diglyceride	Monoglyceride	Phospholipid
Normal (6)	Heart	13.6 ± 1.4	0.1	0.6	184 ± 8
Alloxan-diabetic (6)		* 28.2 ± 1.9	0.6	0.7	163 ± 8
Hypophysectomized (6)		10.3 ± 1.3	0.4	0.7	143 ± 4
Hypophysectomized diabetic (6)		10.6 ± 0.9	0.3	0.7	154 ± 4
Hypophysectomized diabetic treated with growth hormone and cortisol (4)		* 22.9 ± 3.7	—	—	142 ± 5
Normal (8)	Gastrocnemius	12 ± 1.5	0.1	0.4	64 ± 2
Alloxan-diabetic (8)		26 ± 2.7	0.4	0.5	63 ± 1

* *P* < 0.01 versus control. No. of muscle samples given in parentheses.

and cortisol. The concentration of diglyceride (measured on pooled extracts) may also have been increased in alloxan-diabetes whereas the concentrations of mono-glyceride and phospholipids appeared to be unchanged. When hearts from normal rats were perfused for 60 min without substrate the triglyceride concentration fell from 18.7 ± 0.8 (8) to 8.7 ± 0.7 (8) (µmoles/g dry wt.; mean ± S.E. with number of hearts). No significant change in phospholipid was detected (186 ± 6–171 ± 7).

These observations lead us to conclude that heart muscle triglyceride is broken down during perfusion, thus providing fatty acid for oxidation⁶. Evidence for the location of this glyceride in muscle cells has been presented^{4,7}. The concentration of triglyceride, but not that of phospholipid, is increased by actions of growth hormone and cortisol in an insulin-deficient rat. Loss of the first fatty acid from triglyceride appears to limit breakdown in both normal and diabetic rats. The role of increased triglyceride concentration in accelerating lipolysis in diabetic muscle¹ and the source of muscle triglyceride (that is, whether derived from plasma glycerol or plasma non-esterified fatty acids) is under investigation.

We thank Mr. R. O. Law for help with perfusion of hind limbs and the British Diabetic Association, the Medical Research Council and the Royal Society for grants.

R. M. DENTON
P. J. RANDLE

Department of Biochemistry,
University of Bristol.

¹ Garland, P. B., and Randle, P. J., *Biochem. J.*, **93**, 678 (1964).

² Newsholme, E. A., and Randle, P. J., *Biochem. J.*, **93**, 641 (1964).

³ Lindberg, O., and Ernster, L., *Meth. Biochem. Anal.*, **3**, 1 (1956).

⁴ Carlson, L. A., *J. Atherosclerosis Res.*, **3**, 334 (1963).

⁵ Garland, P. B., and Randle, P. J., *Nature*, **196**, 987 (1962).

⁶ Garland, P. B., Newsholme, E. A., and Randle, P. J., *Biochem. J.*, **93**, 661 (1964).

⁷ Orth, D. N., and Morgan, H. E., *J. Cell. Biol.*, **15**, 509 (1962).

Acrylamide-gel Electrophoresis of β-Lactoglobulins stored in Solutions at pH 8.7

NATIVE β-lactoglobulins-A and -B, mol. wt. 35,500, can reversibly dissociate into sub-units of mol. wt. 17,750 in acidic¹ or alkaline² solutions. Aggregation of β-lactoglobulin-A to a tetramer structure has been observed¹ in solutions of pH 3.5–5.2 stored at 4° C. Irreversible denaturation of β-lactoglobulin occurs on heating³ neutral solutions above 65° C or on storage^{4,5} in alkaline solution at 25° (slowly in the pH range 8–9.5, more rapidly in solutions of higher pH). This denaturation was shown by changes in iso-electric solubility, optical rotation and light scattering; Tiselius electrophoresis in 'Veronal' (pH 8.4) buffer separated one, or occasionally two, faster-migrating components in addition to native β-lactoglobulin. An initial reversible splitting followed by irreversible aggregation was postulated³, but the nature and number of products formed were not studied further. Bingham, Krugman and Estermann⁶ recently noted that stored samples of β-lactoglobulin were separated by acrylamide disc electrophoresis⁷ into five bands whereas the fresh material split into only two bands; they gave no indication

the pH or composition of the β -lactoglobulin samples. This communication reports the progressive formation of at least eleven new species when β -lactoglobulin-A or -B or -A/B is stored in alkaline solution.

Twice-crystallized β -lactoglobulin-A and -B genetic variants were separately prepared⁸ from the milks of individually-typed Ayrshire cows⁹, and the A/B mixture from pooled herd milk, and each was freeze-dried. Each was electrophoretically pure (Fig. 1, a, b, c). The protein (0 mg) was dissolved in buffer solution (1 ml.) consisting of 0.076 M tris titrated to pH 8.7 with HCl and containing 0.10 M NaCl, and stored for several days at 2° C. Aliquots were removed daily for electrophoretic examination.

Sample solutions containing 100 μ g of β -lactoglobulin were separated by vertical disc electrophoresis⁷ in 11 per cent acrylamide-gel rods. The gels were prepared in 63 \times mm glass tubes using 'Cyanogum 41' (a mixture of acrylamide and methylenebisacrylamide; B.D.H., Ltd., Poole, England) dissolved in 0.076 M tris titrated to pH 8.6 with citric acid. The electrode tanks contained 0.3 M acetic acid titrated to pH 8.5 with sodium hydroxide¹⁰. It was not necessary to use the system⁷ of additional 'spacer' and 'sample' gels of weaker acrylamide concentration polymerized on top of the main gel column; the sample solution, made more dense by addition of 10 per cent sucrose and made visible by a trace of bromophenol blue, was layered directly on to the flat top of the 11 per cent acrylamide gel underneath the tank buffer solution. After electrophoresis for about 1 h at a constant current of 4 n.amp per gel rod, the rods were stained with amido black and electrolytically de-stained. The gel rods were stored in 7 per cent acetic acid, and photographed under strong back-lighting.

The electrophoretic patterns revealed a progressive formation of slower-migrating protein bands as the storage period increased. After one day about three new bands were visible (Fig. 1, d, e, f) and after seven days there were at least eleven sharp, slower-migrating bands. The β -A and β -B and β A/B mixture appeared to behave similarly, giving equal numbers of new bands at the same rate. After seven days storage nearly all the original β -

lactoglobulin bands had disappeared (Fig. 1, g, h, i) from the alkaline solution, but a control sample of 1 per cent β -lactoglobulin in 0.076 M tris titrated to pH 7.0 with hydrochloric acid still showed only the original (fast) β -lactoglobulin band and no new bands. Portions of 7-day-old alkaline solutions were titrated to pH 6.0 and examined after storage at this pH for several days further, but their electrophoretic patterns remained identical to those of the 7-day-old alkaline solution. This shows that the formation of the new species is induced by the high pH and is not reversible by subsequent acidification. Because of the molecular-sieving effect of 11 per cent acrylamide gels¹¹, it is probable that the slower-migrating bands represent progressively higher molecular weight aggregates formed slowly from either the original β -lactoglobulin molecules (mol. wt. 35,500) or their half-units. The amount of aggregation did not cause precipitation from the pH 8.7 solutions even after storage for several weeks, and no protein material remained on the origin of the gel rod after electrophoresis.

Probably the new species result from alkaline oxidation of cysteine residues, linking adjacent β -lactoglobulin molecules by a disulphide bond. The similarity of heat- and alkali-induced aggregation is now being investigated. The foregoing results emphasize that β -lactoglobulin or whey protein solutions should always be freshly prepared for routine electrophoretic examination and not stored in alkaline solution.

I thank Mr. M. Rogers for his help.

P. AKROYD

Unilever Research Laboratory,
Colworth House, Bedford.

- ¹ Timasheff, S. N., and Townend, R., *J. Dairy Sci.*, **45**, 259 (1962).
- ² Georges, C., Guinand, S., and Tonnelat, J., *Biochim. Biophys. Acta*, **59**, 737 (1962).
- ³ Stauff, J., and Uhlein, E., *Kolloid. Zeit.*, **143**, 1 (1955).
- ⁴ Groves, M. L., Hipp, N. J., and McMeekin, T. L., *J. Amer. Chem. Soc.*, **73**, 2790 (1951).
- ⁵ Nozaki, Y., Bunville, L. G., and Tanford, C., *J. Amer. Chem. Soc.*, **81**, 5523 (1959).
- ⁶ Bingham, W. E., Krugman, S. L., and Estermann, E. F., *Nature*, **202**, 923 (1964).
- ⁷ Ornstein, L., and Davis, B. J., *Disc Electrophoresis* (preprint by Distillation Products Industries, Rochester 3, N.Y., 1962).
- ⁸ Aschaffenburg, R., and Drewry, J., *Biochem. J.*, **65**, 273 (1957).
- ⁹ Aschaffenburg, R., *Biochim. Biophys. Acta*, **82**, 188 (1964).
- ¹⁰ Poulik, M. D., *Nature*, **180**, 1477 (1957).
- ¹¹ Hjerten, S., *J. Chromatog.*, **11**, 66 (1963).

Haemoglobin Polymorphism in the Cod (*Gadus morrhua*): a Single Peptide Difference

HAEMOGLOBIN of the cod (*Gadus morrhua*), when submitted to agar-gel electrophoresis at pH 7.3, shows a clear polymorphism.

Using this technique Sick¹ showed the existence of three patterns (Fig. 1) suggesting a single gene control for the fast bands Hb I1 and Hb I2. Furthermore, he found the

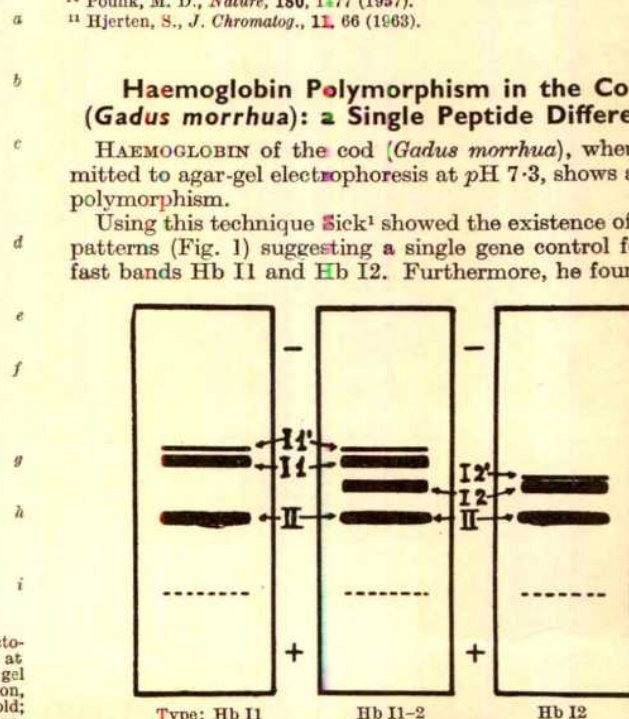


Fig. 1. Patterns showing progressive aggregation of a 1 per cent β -lactoglobulins-A, -B or -A/B mixture on storage in tris buffer (pH 8.7) at 2° C. Disc electrophoresis was performed in 11 per cent acrylamide gel rods (details given in text). a, β -A solution, fresh made; b, β -B solution, fresh made; c, β -A/B solution, fresh made; d, β -A solution, 1 day old; e, β -B solution, 1 day old; f, β -A/B solution, 1 day old; g, β -A solution, 7 days old; h, β -B solution, 7 days old; i, β -A/B solution, 7 days old. The sample application point is at the left-hand side of the photographs.

Fig. 1. Tracings of patterns of cod haemoglobin subjected to agar-gel electrophoresis, phosphate buffer 0.02 M, pH 7.3

change in position is indeed small, the substitution, if any, should involve two neutral amino-acids. A preliminary analysis seems to suggest that this is so. (2) What is the relationship between the aforementioned 'core' peptides and the 'extra' one in Hb II chymotryptic digest? The answers will come only from sequence analysis of the free peptides.

This work was carried out in the Laboratory of the Department of Paediatrics of the University of Groningen. We thank Prof. J. P. H. Jonxis for his help and advice, and Miss du Vigny, Royal Institute for Fishery Research, Muiden, Holland, for the blood samples.

M. C. RATTAZZI

Instituut voor Anthropogenetica,
Universiteit, Leiden, Holland.

C. PIK

Kinderkliniek, Universiteit,
Groningen, Holland.

ick, K., *Nature*, **192**, 894 (1961).

anson, M. L., and Mirsky, A. E., *J. Gen. Physiol.*, **13**, 469 (1930).

ngram, V. M., *Biochim. Biophys. Acta*, **28**, 539 (1958).

agliani, C., *Biochim. Biophys. Acta*, **48**, 392 (1961).

ngram, V. M., and Stratton, A. O. W., *Biochim. Biophys. Acta*, **62**, 445 (1962).

Iunt, J. A., and Ingram, V. M., *Biochim. Biophys. Acta*, **28**, 546 (1958).

Factors affecting the Amount and Composition of the Serum Seromucoid Fraction

THE glycoprotein-rich seromucoid fraction¹ is isolated by first precipitating most serum proteins with perchloric acid—seromucoid being precipitated from the filtrate with phosphotungstic acid. The solubility of certain glycoproteins in perchloric acid is assumed due to covalently bound hexose, hexosamine, fucose and sialic acid residues. Thus, seromucoid contains perchloric acid-soluble rosomucoid, Zn- α_2 -glycoprotein, haemopexin, the haptoglobins and 3.5 S α_1 -glycoprotein² with total carbohydrate and sialic acid contents above 8.0 and 3.3 per cent respectively. Insoluble glycoproteins, absent from seromucoid, include α_2 -macroglobulins, ceruloplasmin and transferrin with less carbohydrate content. Seromucoid also contains small amounts of co-precipitated albumin, pre-albumin and γ -globulins which, when pure, are perchloric acid-insoluble.

If a causal relationship were to exist between glycoprotein solubility and carbohydrate content, then removal of carbohydrate residues from glycoproteins should decrease their solubility in perchloric acid and lead to decreased seromucoid precipitation. Since terminally-bound sialic acid residues on glycoproteins can be selectively removed by neuraminidase, the effect of this carbohydrate on glycoprotein solubility in 0.6 M perchloric acid has been investigated.

Normal human serum, containing 60 mg glycoprotein-bound sialic acid/100 ml., was used; the seromucoid-level was 103 and 16.7 mg/100 ml., expressed in terms of protein and hexose contents respectively. Seromucoid was precipitated³ before and after treatment of serum with neuraminidase (*E.C.* 3.2.1.18), the source of which was receptor destroying enzyme (R.D.E.) from *Vibrio cholerae* (Burroughs Wellcome and Co., London). To 10 ml. of serum were added 10 ml. of 0.2 M acetate buffer (pH 5.5, containing 10^{-2} M calcium acetate) and 3 ml. 0.1 N hydrochloric acid, the final pH being 5.5. To 4-ml. aliquots were added 2 ml. R.D.E. These mixtures were incubated at 37°. At varying time intervals (0–24 h) 24.8 ml. 0.9 per cent sodium chloride was added to each mixture, and free sialic acid estimated in 0.2 ml. aliquots⁴. To 30 ml. of the remaining mixture were added 14.96 ml. of 1.8 M perchloric acid; the precipitated proteins were separated by filtration through No. 50 Whatman filter paper after 10 min. The seromucoid fraction was then precipitated from 32 ml. of the filtrate with 6.4 ml. of phosphotungstic acid reagent (5 per cent, w/v, in 2 N hydrochloric acid), and measured turbidimetrically at 400 m μ after standing

for 15 min. The fraction was then centrifuged down, washed with 10 ml. 95 per cent (v/v) ethanol, dissolved in 4 ml. 0.1 N sodium hydroxide, and estimated for hexose and protein⁵. As is seen in Fig. 1, the enzymatic removal of sialic acid from serum glycoproteins was accompanied by decreased seromucoid precipitation. Sialic acid residues evidently confer hydropathic properties on some serum glycoproteins, their removal decreasing glycoprotein solubility in perchloric acid, thus decreasing the amount of seromucoid precipitated from the perchloric acid filtrate by phosphotungstic acid. This effect was particularly noticeable when seromucoid was estimated turbidimetrically⁶.

Glycoproteins with decreased perchloric acid-solubility following sialic acid removal are probably relatively low in carbohydrate content since purified orosomucoid, containing 40 and 12 per cent total carbohydrate and sialic acid respectively, remained perchloric acid-soluble after the complete removal of sialic acid, presumably because the amounts of hexose and hexosamine remaining were sufficiently high to maintain solubility.

The presence in seromucoid of serum proteins which are normally perchloric acid-insoluble may result from the 'protective' properties of glycoproteins⁷. The protective effect of seromucoid on the precipitation of albumin by perchloric acid was therefore investigated, using seromucoid (containing 4 per cent sialic acid) isolated from normal serum by the precipitation method described in Fig. 1. To five tubes containing albumin (0.8 mg), dissolved in 0.1 ml. 0.9 per cent sodium chloride, were added increasing amounts of seromucoid (0–1 mg), dissolved in 0.1 ml. 0.05 N sodium hydroxide. To each tube was added 3 ml. phosphate buffer (0.2 M, pH 7.4) followed by 1.6 ml. 1.8 M perchloric acid; the albumin precipitated was measured turbidimetrically at 400 m μ after 10 min. From Fig. 2, it is evident that seromucoid protected albumin from precipitation by perchloric acid.

Serum glycoproteins function as protective colloids, probably enabling small amounts of normally insoluble substances, such as albumin, pre-albumin and γ -globulins, to remain soluble on the addition to serum of perchloric acid, addition of phosphotungstic acid to perchloric acid filtrates causing them to precipitate with the seromucoid fraction. The presence of these normally insoluble substances in seromucoid, therefore, depends on the amount and protective efficiency of serum glycoproteins, which may vary in pathological conditions.

Factors affecting seromucoid precipitation are of some importance, since these levels vary in disease⁸ and are claimed to be of value in cancer diagnosis and in assessing response to treatment⁹. This is particularly relevant in

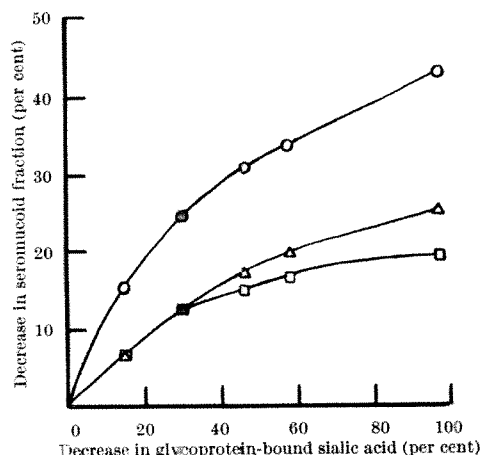


Fig. 1. Decreased precipitation of seromucoid following the removal of sialic acid from serum glycoproteins. Seromucoid-levels were determined turbidimetrically (○), and by estimation of seromucoid protein (△) and hexose (□) contents. Results are expressed as the percentage decrease in the seromucoid fraction following the progressive enzymatic removal of sialic acid from serum glycoproteins.

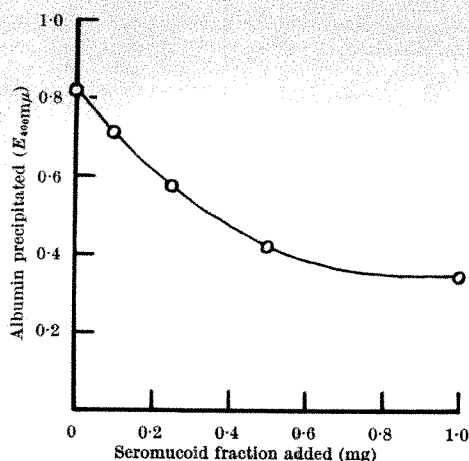


Fig. 2. Protective effect of seromucoid on the precipitation of albumin by 0.6 M perchloric acid. Albumin was estimated turbidimetrically at 400 mμ.

view of recent reports that orosomucoid, isolated from the serum of patients with certain diseases, is sialic acid-deficient^{10,11}. If other serum glycoproteins also lack sialic acid, low seromucoid-levels may result due to decreased glycoprotein solubility in perchloric acid.

Whether the low seromucoid-levels observed in patients with parenchymatous liver diseases¹² result from defective synthesis in the liver¹³ producing sialic acid-deficient, perchloric acid-insoluble glycoproteins must await further knowledge of the composition of these substances in abnormal sera, the perchloric acid-solubility and protective properties of which appear dependent on the nature and amount of covalently bound carbohydrates.

A. J. ANDERSON

Department of Pathology,
Institute of Orthopaedics,
University of London.

¹ Winzler, R. J., Devor, A. W., Mehl, J. W., and Smyth, I. M., *J. Clin. Invest.*, **27**, 609 (1948).

² Schultze, H. E., Heide, K., and Haupt, H., *Clin. Chim. Acta*, **7**, 854 (1962).

³ Winzler, R. J., *Methods of Biochem. Anal.*, **2**, 279 (1955).

⁴ Warren, L., *J. Biol. Chem.*, **234**, 1971 (1959).

⁵ Anderson, A. J., *Biochem. J.*, **88**, 460 (1963).

⁶ de la Huerza, J., Dubin, A., Kushner, D. S., Dyniewicz, H. A., and Popper, H., *J. Lab. Clin. Med.*, **47**, 403 (1956).

⁷ MacLagan, N. F., and Anderson, A. J., in *Ciba Found. Symp. Chemistry and Biology of Mucopolysaccharides*, edit. by Wolstenholme, G. E. W., and O'Connor, M., 268 (J. and A. Churchill, London, 1958).

⁸ Lockey, E., Anderson, A. J., and MacLagan, N. F., *Brit. J. Cancer*, **10**, 209 (1956).

⁹ Cameron, E., Campbell, A., and Plenderleith, W., *Scot. Med. J.*, **6**, 301 (1961).

¹⁰ Schmid, K., Burke, J. F., Debray-Sachs, M., and Tokita, K., *Nature*, **204**, 75 (1964).

¹¹ Whitehead, P. H., *Proc. Assoc. Clin. Biochem.*, **3**, 247 (1965).

¹² Cameron, E., Campbell, A., and Plenderleith, W., *Scot. Med. J.*, **6**, 308 (1961).

¹³ Shetlar, M. R., Capps, J. C., and Hern, D. L., *Biochim. Biophys. Acta*, **83**, 93 (1964).

PHYSIOLOGY

Tubular Site of Urate Secretion in the Rabbit

In the rabbit a pronounced secretion of urate during infusion of urate has been shown¹. In the Dalmatian dog secretion of urate may be readily demonstrated, while re-absorption of urate prevails in the mongrel (that is, non-Dalmatian) dog. A proximal re-absorption of urate in the mongrel dog has consistently been observed by the stop-flow method²⁻⁴, whereas secretion has been found at this site in the Dalmatian dog^{2,4}. Yü *et al.*⁴ have suggested that a weak secretion of urate also occurs in the distal tubule. We have examined the site of urate secretion in the rabbit by the stop-flow method and with the aid of labelled urate.

The experiments were conducted on male animals anaesthetized intravenously with 45 mg 'Nembutal'. Respiration was maintained throughout the experiment by a respiration pump, and polyethylene catheters were inserted in the jugular vein and carotid artery on one side, and in the right ureter. The stop-flow procedure was similar to the method of Malvin *et al.*⁵. An infusion containing creatinine (0.5 per cent), mannitol (5 per cent) and in some instances urate (0.1 per cent), dissolved in physiological saline, was given through the venous cannula at a rate of 2.3 ml./min. One minute before releasing the occlusion, a solution containing 250 mμ inulin and either 50 mg urate or 15 μc. ⁸⁻¹⁴C-urate was injected over a period of 30 sec. Urate was analysed by an enzymatic method⁶. Radioactivity was measured by a Packard 'Tri-Carb' scintillation counter after dissolving 50 μl. of urine in 5 ml. of Bray's mixture⁷.

Fig. 1 gives the results of an experiment in which 50 mg of urate was injected post-occlusively. The concentration of Na⁺, urate, and inulin has been plotted as function of the amount of urine collected after the stop-flow period. Corresponding to the dip in the Na⁺ curve (which marks the distal part of the nephron), the urate concentration increases sharply. The concentration of urate reaches a peak value after collection of c. 4 ml. of urine. It can be seen from Fig. 1 that an increase in urate concentration occurs just before the appearance of inulin (the inulin indicates formation of new glomerular filtrate after the stop-flow period). The experiment thus shows a pronounced permeation of urate across the proximal tubules.

In the experiment shown in Fig. 2 urate was infused during the whole experiment, and probenecid—which inhibits urate secretion in the rabbit—was administered intravenously in a dose of 100 mg/kg prior to the stop-flow period. At the end of the occlusion period labelled urate was infused along with inulin. It appears from Fig. 2 that radioactivity in samples originating from the distal tubule is very small. An increased number of counts is

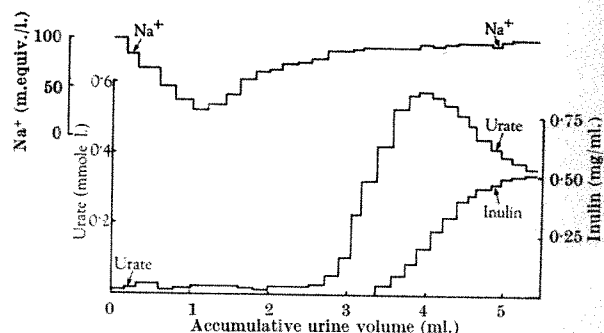


Fig. 1. The stop-flow pattern after injection of 50 mg urate and 250 mg inulin post-occlusively.

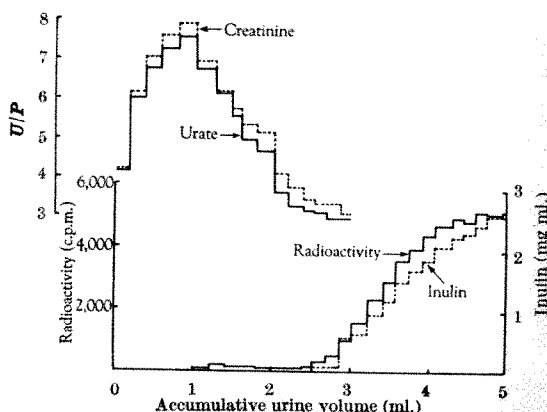


Fig. 2. The stop-flow pattern after injection of 15 μc. urate post-occlusively. Urate was infused during the whole experiment, and 100 mg probenecid was given intravenously prior to the stop-flow period.

served concomitantly with the excretion of inulin in the urine. It is seen that the rise in the 'radioactivity rve' is only slightly greater than that for the inulin rve. On the basis of these findings it may be concluded that probenecid strongly inhibits an active transport of urate from the plasma to the tubular fluid in the proximal tubules.

The two curves on the left side of the diagram show a lot of the U/P values of chemically determined urate and creatinine. A pronounced rise in the U/P values for urate and creatinine is observed in samples from the distal tubule. Creatinine may be used as an approximate measure of glomerular filtration rate in the rabbit, and the increase in the distal concentrations of creatinine during stop-flow conditions is due to re-absorption of water following the re-absorption of Na^+ . The U/P values for urate are a little below the corresponding ones for creatinine. A similar relationship between the excretion of urate and creatinine after administration of probenecid is found under free-flow conditions, and so it must be concluded that the re-absorption of water in the distal part of the nephron accounts entirely for the increased concentration of urate during the stop-flow period. No net transport of urate can be demonstrated, and since the distal tubule is practically impermeable to the radioactive urate, infused during the occlusion, urate has either moved out of or into the tubular fluid of the distal part of the nephron.

The experiments thus show that the secretion of urate in the rabbit is localized to the proximal tubules and may be almost completely inhibited by administration of probenecid. In contrast, the distal part of the nephron is virtually impermeable to urate.

JESPER V. MØLLER

Department of Biochemistry,
University of Aarhus,
Denmark.

- Poulsen, H., and Praetorius, E., *Acta Pharmacol. and Toxicol.*, **10**, 271 (1954).
 Kessler, R. H., Hierholzer, K., and Gurd, R. S., *Amer. J. Physiol.*, **197**, 601 (1959).
 Latham, W., Davis, B. B., and Rodnan, G. P., *Amer. J. Physiol.*, **199**, 9 (1960).
 Yü, T. F., Berger, L., Kupfer, S., and Gutman, A. B., *Amer. J. Physiol.*, **199**, 1199 (1960).
 Malvin, R. L., Wilde, W. S., and Sullivan, L. P., *Amer. J. Physiol.*, **194**, 135 (1958).
 Praetorius, E., and Poulsen, H. E., *Scand. J. Clin. and Lab. Invest.*, **5**, 273 (1953).
 Bray, G. A., *Anal. Biochem.*, **1**, 279 (1960).

Response of Melanocytes of Dermis and Epidermis to Lightening Agents

THE frog has been prominent in investigations of hormonal control of human skin colour. The excised skin of *Rana pipiens* is sensitive to α -melanocyte stimulating hormone (α -MSH); less than 10^{-11} g added to 20 ml. of Ringer's solution containing the specimen produces darkening. The responsiveness of frog skin to MSH led to its use in an *in vitro* frog skin assay¹ which played an important part in the isolation and identification of α - and β -MSH². These peptides when administered systemically darken human skin^{3,4}. The use of the frog skin assay led also to the isolation of an indole, melatonin, which is synthesized in the pineal⁵. When added to frog skin previously darkened with MSH, melatonin was found to be a very potent lightening agent. Other compounds sharing this ability to lighten MSH darkened frog skin are nor-epinephrine and acetylcholine⁶.

It is the purpose of this communication to contrast the effect of these lightening agents on dermal and epidermal melanocytes.

Frogs were decapitated, the skins removed and washed in four changes of frog-Ringer's solution for 3 h before use. Skins were mounted on rings, placed in beakers containing

20 ml. Ringer's solution and reflectance (skin colour) was measured with a 'Photovolt' model 610 reflectance meter as described¹.

Specimens were fixed in 10 per cent formol-saline for 30 min, then incubated with 0.5 per cent trypsin (1 : 250 Difco) for 10 min at 30°. The specimen was then rinsed and, under observation with a dissecting microscope, the epidermis was peeled from the dermis.

Examination of the epidermis in an area of a dark skin marking revealed contracted melanocytes with a rare projection (Fig. 1). Five units of MSH, added to the specimen 60 min before fixation, effected the expansion of epidermal melanocytes to arborized, dendritic forms with long-branched processes, morphologically indistinguishable from mammalian melanocytes (Fig. 2).

Melatonin (2×10^{-3} μ g) added to a skin darkened for 60 min with 5 units of MSH produced almost complete lightening (increased reflectance) to a pre-MSH treatment value within 20 min. The epidermal melanocytes (Fig. 3), however, were identical with epidermal melanocytes of the MSH-darkened skin.

This lack of response of frog epidermal melanocytes to melatonin is consistent with its lack of effect on human skin colour⁷ and guinea-pig epidermal melanocytes⁸.

Dermal melanocytes from the preceding specimens were found to be contracted and compact before the addition of

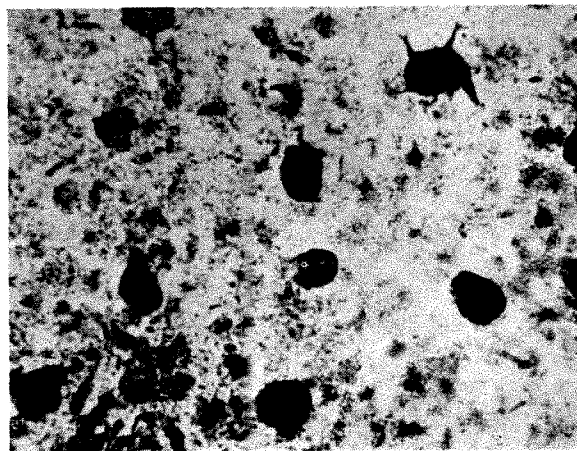


Fig. 1. Epidermis from skin of *Rana pipiens* which has been fixed and separated from dermis as indicated in text. The specimen was cleared in xylene and mounted on a glass slide. The skin was washed for 3 h in Ringer's solution before fixation. The melanocytes are dense and contracted. A rare dendritic process is filled with melanin granules. Notice the extra-melanocytic melanin granules in the epidermis. ($\times 468$)

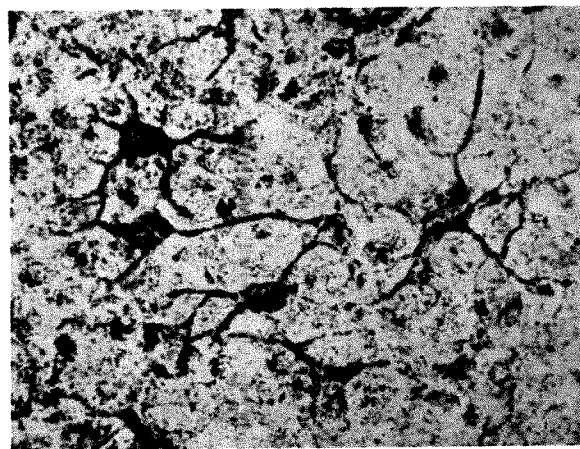


Fig. 2. Epidermis from a specimen exposed to 5 units of MSH for 60 min before fixation. The cell bodies are less dense than in Fig. 1. Melanin granules fill the delicate dendritic processes. ($\times 468$)

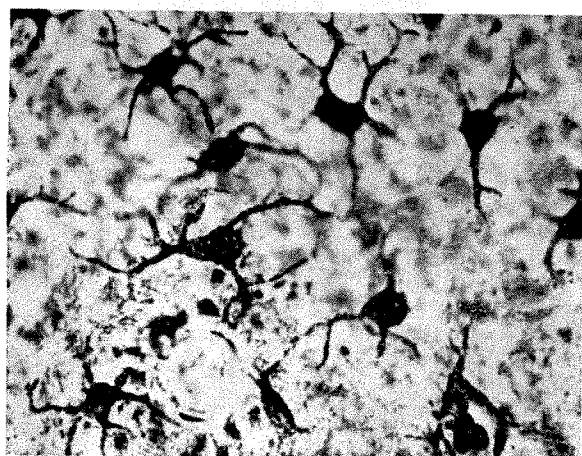


Fig. 3. Epidermis from a specimen exposed to 5 units of MSH for 60 min to which melatonin 2×10^{-3} μ g was then added 20 min before fixation. The epidermal melanocytes are identical with those fixed in formalin before treatment with melatonin (Fig. 2). ($\times 468$)

MSH. MSH effected the expansion of dermal melanocytes to stellate, dense forms. The addition of melatonin to the specimen darkened with MSH caused the dermal melanocytes to contract to the pre-MSH state.

Two other agents, acetylcholine and norepinephrine, are powerful lightening agents for MSH-darkened frog skin⁶. Acetylcholine (5×10^{-3} M) or norepinephrine (5×10^{-4} M) when added to specimens darkened with MSH produced 90 per cent lightening (increased reflectance) and contraction of the dermal melanocytes to the pre-MSH state. Neither acetylcholine nor norepinephrine, however, caused any morphological alteration in epidermal melanocytes previously expanded by MSH.

It is apparent from these observations that dermal melanocytes have a qualitatively different response to melatonin, acetylcholine and norepinephrine from epidermal melanocytes. Both populations of melanocytes respond similarly with melanin granule dispersion to MSH⁹.

This work was supported by U.S. Public Health Service grants AM 08073, T7-AM-1003, and CA-04679.

JOSEPH MCGUIRE
HALVOR MÖLLER

Section of Dermatology,
Department of Medicine,
Yale-New Haven Medical Center,
New Haven, Connecticut.

¹ Shizume, K., Lerner, A. B., and Fitzpatrick, T. B., *Endocrinology*, **54**, 553 (1954).

² Lee, T. H., and Lerner, A. B., *J. Biol. Chem.*, **221**, 943 (1956).

³ Lerner, A. B., and McGuire, J. S., *Nature*, **189**, 176 (1961).

⁴ Lerner, A. B., and McGuire, J. S., *N. Eng. J. Med.*, **270**, 639 (1964).

⁵ Lerner, A. B., Case, J. D., Takahashi, Y., Lee, T. H., and Mori, W., *J. Amer. Chem. Soc.*, **80**, 2587 (1958).

⁶ Wright, R. M., and Lerner, A. B., *Endocrinology*, **66**, 599 (1960).

⁷ Lerner, A. B., and McGuire, J. S. (unpublished observations).

⁸ Snell, R. S., *J. Invest. Derm.*, **44**, 273 (1965).

⁹ McGuire, J. S., and Möller, H., *Endocrinology* (in the press).

Role of the Gut in Synthesizing the Protein Component of Low-density (Beta) Lipoprotein

SYNTHESIS of the protein component of human low-density lipoprotein (LDL), and elaboration of these lipoproteins from lipid and protein constituents, are regarded as occurring in the liver¹. In the Bassen-Kornzweig syndrome, absence of LDL is associated with steatorrhea and changes in small-gut histology². Salt *et al.*³ have speculated that the gut wall might be one site of lipoprotein synthesis. Isselbacher and Budz⁴ have shown that rat intestinal mucosa does synthesize lipoproteins. In rats, cholesterol synthesis occurs in the gut⁵. In human beings, 70 per cent of plasma cholesterol is carried by

LDL, and if gut wall synthesis of cholesterol is shown occur in man, the possibility again arises that a proportion of human LDL might also be elaborated in the intestinal mucosa. LDL newly synthesized by the gut could reach the general circulation via lacteal-lymphatic pathway or directly through the portal venous system.

The opportunity to investigate possible synthesis and transport of LDL by the lymphatic pathway arose in two patients with chyluria secondary to filarial obstructive of upper abdominal and thoracic lymphatics. Lymph angiography in these patients had revealed free communication between distended lymphatics in the upper abdomen, including the area occupied by the base of the small gut mesentery, and the right renal pelvis. Proteinuria in the two patients averaged 13.0 g and 5.0 g a day respectively. This total included approximately 800 mg and 300 mg a day of LDL, determined by gel-diffusion precipitin assay⁶, and by dextran sulphate precipitation.

Turnover of labelled LDL was followed in these patients by a method previously reported⁸. Total LDL was isolated from the serum of each patient, labelled with iodine-125 in the peptide component, and returned to the patient by intravenous injection. Serum LDL was assayed in duplicate by dextran sulphate precipitation and the concentration of LDL in urine measured by modified procedure using large volumes of urine and additional Ca^{++} . Specific activities were then calculated for plasma and for urine on the assumption that a radioactivity entering a trichloroacetic acid precipitate was associated with LDL. Data from the turnover investigations are shown in Table 1.

During the first 3 h of each investigation, a diuresis was produced by oral intake of fluid, including milk. In both patients, free radioiodide was present in urine passed 20 min after the intravenous injection, but protein-bound radioactivity was first detected in urine formed after 90 min in both patients. This interval of 90 min agreed with the time of appearance of T_{1824} -albumin in urine following intravenous injection of 6.78 mg of Evans blue into these two patients as a separate investigation, and contrasted with the rapid appearance of T_{1824} -albumin in patients with nephrotic syndrome. The time-courses for specific activity of plasma and urine are shown in Fig. 1. Specific activity figures for urine samples are plotted at the midpoint of the successive collection periods, which were short early in the investigations and over 24 h periods in the latter stages. Plasma specific activity declined steadily during the investigation. Urinary specific activity rose, during the 4-12-h (overnight) collection period, to reach a maximum value of the same order as that of the plasma, and then declined in parallel with plasma specific activity.

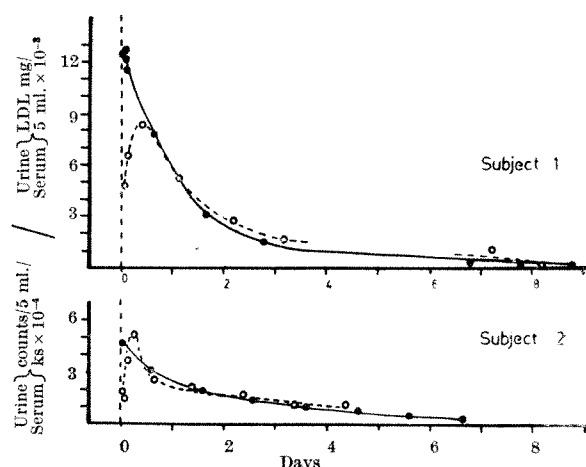


Fig. 1. Specific activity of ^{125}I -LDL in serum and urine of the two patients, expressed as counts per mg of total LDL in 5-ml. quantities of serum or urine. 24-h urine collections were incomplete in subject 1 over the interval 2.7-6.7 days. ●, Serum; ○, urine

Table 1. TURNOVER RESULTS

Subject	Sex	Age	Wt. (kg)	Plasma volume (ml/kg)	Mean serum LDL level (mg/100 ml.)	Serum $t_{1/2}$ 125 I-LDL (days)	IV LDL pool (mg/kg)	U/P ratio (%) [*]	Corrected U/P ratio (%) [†]	Uncorrected catabolic rate serum LDL (mg/kg/day) [‡]	Corrected catabolic rates [§]	% of total 125 I-LDL distributed extra-vascularly [¶]
Patient 1	F	34	75	36.1	530	2.5	192	47.5	35.9	91.2	68.9	19
Patient 2	M	61	87	50.0	570	2.8	285	31.8	29.9	90.6	85.2	32
Range of values obtained in 7 normal men and 6 normal women for 131 I or 125 I-LDL studies						2.1	112	—	24.2	—	44	12
						to	to	—	ot	—	to	to
						3.7	353	—	54.4	—	138	40

Ratio 24 h 125 I excretion to mean plasma radioactivity (ref. 12).

U/P ratio recalculated, after correction for protein bound 125 I (125 I-LDL) in urine; that is, using only free 125 I excretion.

Calculated from uncorrected U/P ratio.

Calculated from corrected U/P ratio.

From multi-compartmental analysis by method of Matthews (ref. 12).

Turnover investigations have revealed a small extra-vascular distribution of LDL peptide in human subjects^{9,10}. In animals there is evidence for transfer of LDL from plasma to hepatic lymph¹¹. Human abdominal lymphatics may therefore contain some LDL from hepatic lymph besides transporting LDL which has passed to the extravascular compartment from blood capillaries. During a 125 I-LDL investigation, the fall in specific activity of plasma LDL is due to catabolism of labelled LDL with immediate excretion of iodine-125, and dilution of the remaining 125 I-LDL by newly synthesized, unlabelled LDL. Regardless of catabolism, LDL returning to the blood vascular compartment via abdominal lymphatics would be expected to show the same specific activity as plasma unless newly synthesized apoprotein was added to LDL in the lymphatics from gut tissues or possibly from the liver.

In our two patients specific activity in abdominal lymph over 4–12 h to equal that of plasma, and then maintained this equilibrium over successive 24-h periods. This finding argues against entry of newly synthesized LDL peptide into abdominal lymphatics. The results recorded here do not exclude LDL elaboration by the gut with direct passage of newly synthesized lipoprotein into the portal venous system. This possibility could be explored by the 125 I-LDL technique only if portal venous blood could be sampled over an adequate period and its specific activity compared with that of peripheral blood.

P. J. SCOTT

C. C. WINTERBOURN

Medical Unit, Auckland Hospital,
Auckland, New Zealand.

¹ Cornwell, D. G., and Kruger, F. A., *J. Lipid Res.*, **2**, 110 (1961).

² Isselbacher, K. J., Scheig, R., Plotkin, G. R., and Caulfield, J. B., *Medicine* (Baltimore), **43**, 347 (1964).

³ Salt, H. B., Wolff, O. H., Lloyd, J. K., Fosbrooke, A. S., Cameron, A. H., and Hubble, D. V., *Lancet*, ii, 325 (1960).

⁴ Isselbacher, K. J., and Budz, D. M., *Nature*, **200**, 364 (1963).

⁵ Fliegelson, E. B., Drake, J. W., and Recant, L., *J. Lab. Clin. Med.*, **50**, 437 (1957).

⁶ Soothill, J. F., *J. Lab. Clin. Med.*, **59**, 859 (1962).

⁷ Walton, K. W., and Scott, P. J., *J. Clin. Path.*, **17**, 627 (1964).

⁸ Scott, P. J., Dykes, P. W., Davies, J. L., Walton, K. W., in *Biochemical Problems of Lipids*, edit. by Frazer, A. C., 318 (Elsevier Publishing Co., Amsterdam, 1963).

⁹ Gitlin, D., Cornwell, D. G., Nakasato, D., Oneley, J. L., Hughes, W. L., and Janeway, C. A., *J. Clin. Invest.*, **37**, 172 (1958).

¹⁰ Walton, K. W., Scott, P. J., Dykes, P. W., and Davies, J. L., *Clin. Sci.* (submitted for publication).

¹¹ Courtice, F. C., Woolley, G., and Garlick, D. G., *Austral. J. Exp. Biol. and Med. Sci.*, **40**, 111 (1962).

¹² Matthews, C. M. E., *Phys. in Med. Biol.*, **2**, 36 (1957).

Evidence for Pre-synaptic Inhibition in the Lateral Geniculate Body

SZENTAGOTHAI¹ reported the presence of axo-axonic endings on fibres entering the lateral geniculate body from the retina. Bizzi and Brooks² have shown that in the unanaesthetized, unrestrained cat irregular groups of waves could be recorded simultaneously from the pontine

reticular formation and the lateral geniculate body during the desynchronized phase of sleep. The latter waves could no longer be recorded in the same conditions if the optic fibres were allowed to degenerate³. Recently, Andersen, Brooks, Eccles and Sears⁴ have produced evidence pointing to the existence of presynaptic inhibition in the ventrobasal thalamus on stimulation of peripheral nerves.

Since axo-axonic endings have been demonstrated in the lateral geniculate body and the experiments by Bizzi and Boller³ may be taken to indicate that the slow waves in the lateral geniculate body could be presynaptic in origin, an investigation has been carried out in order to see if there is any evidence for presynaptic inhibition in the same nucleus.

The experiments were performed on cats in which a complete transection of the brain stem immediately rostral to the exit of the trigeminal nerve had been made ('mid-pontine pre-trigeminal preparation'⁵) under ether anaesthesia. A micro-electrode was inserted into the lateral geniculate body and used to stimulate the optic fibres entering this nucleus. The antidromic response to this stimulus was recorded either from a bipolar concentric electrode inserted into the optic chiasm, or from the optic nerve after enucleation. The presence of any change in the terminal parts of the optic nerve fibres was assessed by any change in the excitability of these fibres near the microelectrode tip, and hence change in the size of the antidromic response⁶.

It has been found that after a brief tetanus to the mesencephalic reticular formation or to the visual cortex the antidromic volley recorded either from the chiasm or from the optic nerve was increased in size. Examples of the increase in excitability of the optic nerve fibres after a tetanus of 8 shocks (with an interval of 3.2 msec) had been applied to the reticular formation and visual cortex are shown in Figs. 1A and B respectively. It can be seen that the maximum increase in excitability occurred around 50 msec after the start of the tetanus and the effect of the tetanus lasted for approximately 250 msec. In addition, after stimulation of the reticular formation with a short tetanus, a negative wave could be recorded from the optic nerve, which resembled the dorsal root potential⁷. This latter wave was found to increase in size with a decrease in the body temperature of the animal, and to decrease in size with an increase in the rate of presentation of the tetanus. An example of the slow wave recorded from the optic nerve to a tetanus of 8 shocks with an interval of 3.2 msec to the mesencephalic reticular formation is shown in Fig. 1C. Often, especially at low temperature, spikes were seen to be superimposed on the summit of the slow wave; these spikes could also be recorded from the optic chiasm. The spikes may correspond to the dorsal root reflex⁸. Comparison of Figs. 1A and C shows that the increase in excitability and the slow wave of the optic nerve ran a similar time-course. That this slow wave was probably not an envelope of

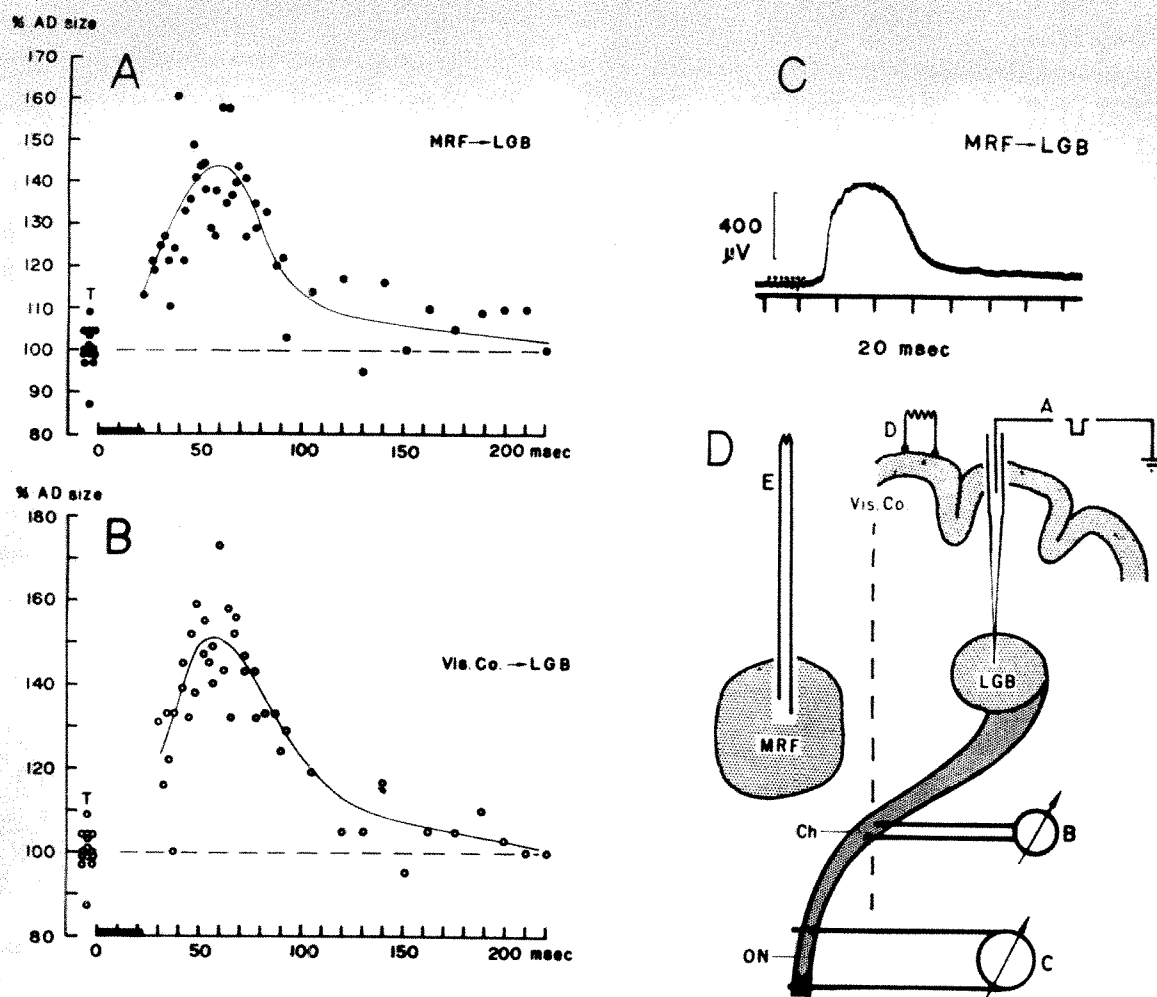


Fig. 1. Evidence for depolarization in optic nerve fibres by stimulation of the mesencephalic reticular formation and visual cortex. The experimental arrangement is shown diagrammatically in D. The micropipette used for stimulating is placed in the lateral geniculate body (LGB); the electrodes for recording the antidromic response were either in the chiasm (Ch) or on the optic nerve (ON). The graphs show the effect on the percentage amplitude of the antidromic response (AD) at various times after a brief tetanus (8 shocks with a duration of 1 msec and a separation of 3.2 msec) applied at a rate of 1/5 sec to the mesencephalic reticular formation (A) and the visual cortex (B). Each point is the average of 5 observations. The smooth curves were obtained by averaging the observations in each 50 msec period and drawing the curves through these points. Both graphs were obtained from the same animal in which the stimuli were applied alternately to the mesencephalic reticular formation and the visual cortex. The slow wave recorded monophasically from the optic nerve after stimulation of the mesencephalic reticular formation in an animal with a rectal temperature of 32° C is shown in C.

centrifugally conducted impulses has been shown by stimulating the chiasm with a single electric shock at various times during the slow wave: no evidence of occlusion between the action potential, evoked by the chiasm stimulus, and slow wave was obtained.

Using the size of the slow wave as an indicator of the effectiveness of the tetanus applied to the brain stem, it has been found that the regions giving the biggest slow wave are centred around the central tegmental tract.

The increased excitability of the optic fibres, produced by stimulation of the mesencephalic reticular formation and visual cortex, can be taken to indicate that both structures exert a presynaptic inhibitory control on the transmission through the lateral geniculate body, according to previous investigations¹¹. The possibility that the increased excitability of the optic nerve fibres may be passively produced by current flowing in a potential field due to activity elicited in nearby structures exists and cannot be overlooked. However, the fact that the time-course of the increased excitability, and of the slow wave, in the optic nerve have the long duration characteristic of the presynaptic inhibition shown in the spinal cord, and cuneate nucleus after cortical stimulation^{9,10} seems to indicate that the phenomenon we observe is due to a true presynaptic inhibition.

This work was supported by the Office of Scientific Research OAR, through the European Office, Aerospace Research, U.S. Air Force, under grant AF-EOAR 63-9.

A. ANGEL*
F. MAGNI
P. STRATA

Istituto di Fisiologia dell'Università di Pisa,
Centro di Neurofisiologia e
Gruppo d'Elettrofisiologia del C.N.R.,
Sezione di Pisa, Italy.

* Wellcome Research Fellow.

- ¹ Szentágothai, J., in *Information Processing in Nervous System*, 3, 119, edit. by Gerard, R. W., and Dwyer, J. W. (Excerpta Medica, Amsterdam, 1962).
- ² Bizzi, E., and Brooks, D. C., *Arch. Ital. Biol.*, **101**, 666 (1963).
- ³ Bizzi, E., and Boller, F. (personal communication).
- ⁴ Andersen, P., Brooks, C. McC., Eccles, J. C., and Sears, T. A., *J. Physiol.*, **174**, 348 (1964).
- ⁵ Batini, C., Palestini, M., Moruzzi, G., Rossi, G. F., and Zanchetti, A., *Arch. Ital. Biol.*, **97**, 1 (1959).
- ⁶ Wall, P., *J. Physiol.*, **142**, 1 (1958).
- ⁷ Barron, D. H., and Matthews, B. H. C., *J. Physiol.*, **92**, 276 (1938).
- ⁸ Toennies, J. F., *J. Neurophysiol.*, **1**, 378 (1938).
- ⁹ Andersen, P., Eccles, J. C., and Sears, T. A., *J. Neurophysiol.*, **27**, 63 (1964).
- ¹⁰ Andersen, P., Eccles, J. C., Schmidt, R. F., and Yokota, T., *J. Neurophysiol.*, **27**, 92 (1964).
- ¹¹ Eccles, J. C., *The Physiology of Synapses* (Springer-Verlag, Berlin, 1964).

HAEMATOLOGY

Chronic Granulocytic Leukaemia : Demonstration of the Philadelphia Chromosome in Cultures of Spleen Cells

AN abnormal marker chromosome in chronic granulocytic leukaemia was described by Nowell and Hungerford^{1,2} and its occurrence widely confirmed³⁻⁷. This marker, the Philadelphia chromosome (Ph¹), occurs in almost all typical cases of chronic granulocytic leukaemia and appears to be an acquired abnormality specific for the disease. The Ph¹ is derived from a chromosome of pair 21 by loss of a variable proportion of the long arms.

It has been shown⁸ that in untreated chronic granulocytic leukaemia all the dividing cells in direct preparations of bone marrow are Ph¹-positive. Apparently, megakaryocytes and erythroid precursors as well as granulocyte precursors carry the Ph¹. As haematological abnormalities are usually confined to the granulocytic series, it has been suggested⁹ that the genetic material lost from the Ph¹ has immediate relevance to leucopoiesis only. It is postulated⁸ that the erythroid, granulocytic, and megakaryocytic series have a common stem cell and the original deletion to produce the Ph¹ occurs in this stem-line. However, peripheral blood cultures in untreated cases yield mixed populations of Ph¹-positive and negative cells^{4,11}. As the dividing cells in cultures of normal blood are almost certainly of lymphoid origin¹⁰, it appears that the blood lymphocytes are Ph¹-negative and therefore must originate from a separate stem-line. Apart from cells of bone marrow origin and lymphocytes in the peripheral blood, cultured subcutaneous fibroblasts have been examined for the presence of the Ph¹ chromosome with negative results³. Only in the spleen are cells of both lymphocytic and bone marrow stem-lines to be expected to occur together, and so it is particularly important to study the cytogenetics of that organ in chronic granulocytic leukaemia. To our knowledge no such investigations have been reported. We have developed a method¹² for the cytogenetic study of lymphoid tissue; this technique was applied to spleen to obtain the results here reported.

The patient was a married woman, aged twenty-nine. Chronic granulocytic leukaemia, diagnosed 24 months previously, had been treated with busulphan, splenic irradiation, 6-mercaptopurine and cyclophosphamide. Resistance to all therapy had arisen and the disease had undergone acute transformation. Splenectomy was performed for massive splenomegaly and intractable thrombocytopenia. Spleenic cell suspensions were cultured: (a) for 16 h without phytohaemagglutinin, (b) for 84 h with added phytohaemagglutinin. The chromosome count distribution was:

Chromosome No.	<45	45	46	47	48	92-93	Totals
No. of cells (a)	7	8	26	5	1	0	47
(b)	7	12	53	16	0	2	90

On analysis, the dominant cell line was diploid and Ph¹-positive. A second line with 47 chromosomes due to the presence of a second Ph¹ was also identified. Excluding technically unsuitable cells, results of analysis as regards the presence of the Ph¹ chromosome were:

	Cells analysed	Ph ¹ +	Ph ¹ ++	Ph ¹ -	Status uncertain
Preparation (a)	41	29	7	1	4
(b)	73	57	14	1	1

Thus Ph¹ negative cells accounted for only 2.4 per cent of the cells in 16-h culture and 1.4 per cent in 84-h culture. Splenic sections showed loss of normal architecture and absence of lymphocyte aggregations. A differential count of 500 cells in a splenic imprint showed 4 per cent of lymphocytes in a population of leukaemic blast cells and promyelocytes.

Thus almost all dividing cells from this patient's spleen were Ph¹ positive. This result, to be expected in the 16-h

unstimulated culture, is surprising in the 84-h culture with phytohaemagglutinin, where Ph¹-negative cells of lymphoid origin might well be prominent. Possibly these cells failed to divide; however, splenic lymphocytes have been successfully cultured in this laboratory and elsewhere¹³. The initial small number of lymphocytes relative to leukaemic blast cells might explain the rarity of Ph¹-negative mitoses, but studies have shown that in cultures with phytohaemagglutinin normal cells may outstrip leukaemic cells¹⁴⁻¹⁶. A better hypothesis is that in advanced chronic granulocytic leukaemia all splenic stem cells, possibly including lymphocytic precursors, carry the Ph¹.

The presence of two Ph¹ chromosomes per cell has previously been observed in the chronic phase of chronic granulocytic leukaemia¹⁷ and also in the phase of acute transformation¹⁸⁻²⁰, when it is more common. Further investigations of splenic cytogenetics in chronic granulocytic leukaemia during the chronic phase and after acute transformation are desirable.

A. S. D. SPIERS
A. G. BAIKIE

University of Melbourne
Department of Medicine,
St. Vincent's Hospital,
Melbourne, Australia.

¹ Nowell, P. C., and Hungerford, D. A., *J. Nat. Cancer Inst.*, **25**, 85 (1960).

² Nowell, P. C., and Hungerford, D. A., *Science*, **132**, 1497 (1960).

³ Baikie, A. G., Court Brown, W. M., Buckton, K. E., Harnden, D. G., Jacobs, P. A., and Tough, I. M., *Nature*, **188**, 1165 (1960).

⁴ Tough, I. M., Court Brown, W. M., Baikie, A. G., Buckton, K. E., Harnden, D. G., Jacobs, P. A., King, M. J., and McBride, J. A., *Lancet*, **i**, 411 (1961).

⁵ Kinlough, M. A., and Robson, H. N., *Brit. Med. J.*, **ii**, 1052 (1961).

⁶ Adams, A., Fitzgerald, P. H., and Gunz, F. W., *Brit. Med. J.*, **ii**, 1474 (1961).

⁷ Fitzgerald, P. H., Adams, A., and Gunz, F. W., *Blood*, **21**, 183 (1963).

⁸ Tough, I. M., Jacobs, P. A., Court Brown, W. M., Baikie, A. G., and Williamson, E. B. D., *Lancet*, **i**, 844 (1963).

⁹ Baikie, A. G., *Lancet*, **i**, 556 (1964).

¹⁰ MacKinnon, A. A., Stohlman, F., and Brecher, G., *Blood*, **19**, 349 (1962).

¹¹ Whang, J., Frei, E., Tjio, J. H., Carbone, P. P., and Brecher, G., *Blood*, **22**, 864 (1963).

¹² Spiers, A. S. D., and Baikie, A. G. (in preparation).

¹³ Baker, M. C., and Atkin, N. E., *Lancet*, **i**, 1164 (1963).

¹⁴ Nowell, P. C., *Exp. Cell Res.*, **19**, 267 (1961).

¹⁵ Tough, I. M., Court Brown, W. M., Baikie, A. G., Buckton, K. E., Harnden, D. G., Jacobs, P. A., and Williams, J. A., *Lancet*, **ii**, 115 (1962).

¹⁶ Sandberg, A. A., Ishihara, T., Crosswhite, L. H., and Hauschka, T. S., *Cancer Res.*, **22**, 748 (1962).

¹⁷ Dougan, L., and Woodliff, H. J., *Nature*, **205**, 405 (1965).

¹⁸ Kemp, N. H., Stafford, J. L., and Tanner, R., *Brit. Med. J.*, **i**, 1010 (1964).

¹⁹ Hammond, F., Quaglino, D., and Hayhoe, F. G. J., *Brit. Med. J.*, **i**, 1275 (1964).

²⁰ Spiers, A. S. D., and Baikie, A. G. (in preparation).

Stable Messenger RNA in Nucleated Erythrocytes

THE mammalian reticulocyte is capable of synthesizing haemoglobin for many hours after its nucleus has been extruded. Since the nucleus is the site of messenger-RNA synthesis¹, these cells must be using stable messenger for continued protein synthesis. In contrast to the red blood cells of mammals, erythrocytes of lower vertebrates and invertebrates are almost always nucleated. With the nucleus present these cells could conceivably synthesize protein on short-lived messenger templates. In order to determine whether nucleated red blood cells utilize short- or long-lived messenger-RNA, representative avian and reptilian erythrocytes were investigated by stopping DNA-dependent RNA synthesis with actinomycin D and following the course of protein synthesis in the functionally 'enucleated' cells.

Reticulocytosis was produced in young chickens and in adult turtles (*Pseudemys elegans*) by repeated bleedings; 15-day chick embryo erythrocytes (about 100 per cent reticulocytes) were used with identical results. Reticulocytes in turtle blood samples numbered about 10 per cent and in chick blood about 30 per cent. The red cells were separated by centrifugation, washed, and incubated in Waymouth medium with penicillin and streptomycin in

a 5 per cent atmosphere of carbon dioxide. Chick cells were incubated at 37°, turtle cells at 30°. Parallel incubations were done with control red cells and with red cell mixtures containing 10 µg/ml. actinomycin D. Aliquots were removed at intervals, washed, and incubated for 30 min with 5 µc./ml. L-leucine-¹⁴C (sp. act. 220 mc./mM) in saline. The cells were precipitated with 5 per cent trichloroacetic acid (TCA), plated on 'Millipore' filters and counted in a gas flow counter. Each experiment was repeated at least twice. To ensure that actinomycin stopped messenger-RNA synthesis persisting in circulating cells, experiments were performed in which reticulocytes were incubated in nutrient medium with 0.5 µc./ml. uridine-¹⁴C (sp. act. 25 mc./mM) for 10 min. The culture was then divided and 10 µg/ml. actinomycin added to one portion. The synthesis of RNA (uridine counts precipitable with cold 5 per cent TCA) was shown to stop within 10 min for chick cells and within 20 min for turtle cells.

Experiments such as those in Fig. 1 demonstrated that protein synthesis continued for at least 24 h in the absence of new messenger-RNA production. The life-span of the messenger was similar in both the chicken and in the turtle erythrocytes. The results do not show that protein synthesis in these red blood cells is equivalent to haemoglobin synthesis, but haemoglobin is the major protein product of the mammalian reticulocyte², and it is likely for these cells as well.

The fact that there is long-lived messenger-RNA in the chicken and turtle erythrocytes plus the evidence of Cameron and Prescott³ that maturation of the chick erythrocyte nucleus is associated with cessation of RNA synthesis indicate that in the maturing erythrocyte the nucleus is inactive so far as transfer of genetic information is concerned.

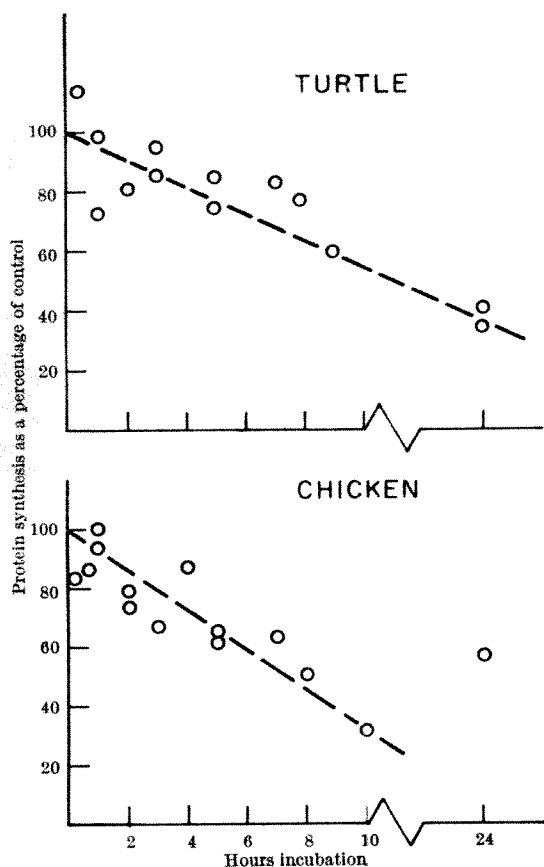


Fig. 1. Persistence *in vitro* of protein synthesis (above) in turtle erythrocytes (incubated at 30°) and (below) in chicken erythrocytes (incubated at 37°) after stopping messenger-RNA synthesis with actinomycin D

This work was supported by a grant to Prof. Eugene Bell from the U.S. National Science Foundation (GB-6400) by a postdoctoral research scholarship of the American Cancer Society (R. B. S.), and by an established investigatorship of the American Heart Association (R. A. M.).

ROBERT B. SCOTT
RONALD A. MALT

Department of Biology,
Massachusetts Institute of Technology,
Cambridge, Massachusetts.

* Present address: Department of Medicine, Medical College of Virginia, Richmond, Virginia.

† Present address: Surgical Services, Massachusetts General Hospital, Boston, Massachusetts.

¹ Girard, M., Latham, H., Penman, S., and Darnell, J. E., *J. Mol. Biol.* **187** (1965).

² Kruh, J., and Borsook, H., *J. Biol. Chem.*, **220**, 905 (1956).

³ Cameron, I. L., and Prescott, D. M., *Exp. Cell Res.*, **30**, 609 (1963).

IMMUNOLOGY

Serological Relationship between Galactans from Normal Bovine Lung and from *Mycoplasma mycoides*

A GALACTAN (pneumogalactan) isolated from bovine lung^{1,2} precipitated 28 per cent of pneumococcal polysaccharide type XIV antiserum³. This suggested that bovine polysaccharides have galactose residues of similar linkage possibly β -1:6, β -1:3 or β -1:3:6⁴, and agreed with the postulated structures of type XIV pneumococcal polysaccharide⁴ and of lung galactan².

Recently it was reported^{5,6} that a galactan was isolated from *Mycoplasma mycoides*, the causative organism of contagious bovine pleuropneumonia (CBPP). It was decided, therefore, to determine whether a serological relationship existed between the galactans from normal bovine lung and from this organism causing lung lesions in cattle.

Purified pneumogalactan was kindly supplied by Hoffman-La Roche, Inc., Nutley 10, New Jersey. Since the isolation method of pneumogalactan is not described, we extracted, with hot phenol⁷, an acetone powder of bovine lung obtained from the U.S.A., a country free of CBPP (California Corporation for Biochemical Research, 3625 Medford St., Los Angeles); this material is referred to as normal bovine lung extract. *M. mycoides* cells were used for preparation of antiserum (x') in sheep⁸. Sera from animals naturally infected with CBPP were also used (sera Nos. 21 and 42). As our attempts to produce antisera against pneumogalactan have so far failed, the results presented here are of experiments with pneumogalactan, *M. mycoides* antigens and antisera against *M. mycoides*.

Table 1 shows the results of complement-fixation, indirect haemagglutination⁹ and tube agglutination tests using *M. mycoides* antigen with immune sera and sera absorbed with excess pneumogalactan. These results show that the pneumogalactan absorbs a considerable proportion of the relevant antibodies against *M. mycoides*.

In the agar-gel precipitin test, serum against *M. mycoides* (x') gave homologous lines with hot-phenol-extracted carbohydrate from *M. mycoides*¹⁰, pneumogalactan, and normal bovine lung extract. Sera 21, 42 and x' formed precipitin lines with hot-phenol-extracted carbohydrate; after absorption with 30 mg of pneumogalactan no lines were formed with either serum 21 or 42 and only very faint lines were produced with serum x'.

Table 1. RECIPROCAL TITRES OF *M. mycoides* ANTISERA, BEFORE AND AFTER ABSORPTION WITH PNEUMOGALACTAN, AGAINST *M. mycoides* ANTIGEN

<i>M. mycoides</i> antiserum	Complement fixation	Indirect haemagglutination	Agglutination
x' before absorption	80	640	640
x' after absorption	10	80	80
'21' before absorption	80	320	80
'21' after absorption	20	80	40
'42' before absorption	160	320	320
'42' after absorption	20	80	40

Animals naturally infected with CBPP (Nos. 21 and 42) gave positive skin test reactions¹¹ with both hot-phenol-extracted carbohydrate from *M. mycoides* and pneumolactan.

The cross-reactivity of pneumogalactan and the galactan from *M. mycoides* indicates that both these polymers have the common linkages. We are now investigating the possibility that the pneumogalactan plays a part in the pathogenesis of CBPP, either in an allergic type reaction, by localizing the organisms in the lung through an antibody-antigen reaction. It would also be of interest to determine whether human lung contains a galactan which might cross-react with the pneumococcal polysaccharides. This work was partly financed by the United States of America Agency for International Development under the terms of CCTA/AID Joint Project 16 for research on contagious bovine pleuropneumonia.

M. SHIFFRINE*
R. N. GOURLAY

East African Veterinary Research Organization,
Muguga,
P.O. Box 32,
Kikuyu, Kenya.

* U.S. Department of Agriculture, Agricultural Research Service, on leave from University of California, Davis.

Wolfson, M. L., Weisblat, D. I., Karabinos, J. V., and Keller, O., *Arch. Biochem.*, **14**, 1 (1947).

Wolfson, M. L., Sutherland, G., and Schlanowitz, M., *J. Amer. Chem. Soc.*, **74**, 4883 (1952).

Heidelberger, M., Dische, Z., Neely, W. B., and Wolfson, M. L., *J. Amer. Chem. Soc.*, **77**, 3511 (1955).

Stacey, M., and Barker, S. A., *Polysaccharides of Micro-organisms* (Clarendon Press, Oxford, 1960).

Plackett, P., and Buttery, S. H., *Nature*, **182**, 1236 (1958).

Buttery, S. H., and Plackett, P., *J. Gen. Microbiol.*, **23**, 357 (1960).

Westphal, O., Luderitz, O., and Bister, F., *Z. Naturf.*, **7B**, 148 (1952).

Gourlay, R. N., *Res. Vet. Sci.*, **5**, 473 (1964).

Cottew, G. S., *Austral. Vet. J.*, **36**, 54 (1960).

Shiffrine, M., and Gourlay, R. N., *J. Comp. Path.*, 1965 (in the press).

Gourlay, R. N., *J. Comp. Path.*, **74**, 286 (1964).

RADIOBIOLOGY

Influence of Oxygen and Cysteine on the Radical State of X-irradiated Lyophilized Liver

Blyumenfel'd and Kalmanson¹ as well as Gordy² and Miyagawa³ have already reported that radicals are formed in lyophilized rat liver by ionizing radiation using electron spin resonance techniques. Moreover, Gordy and Miyagawa have shown that oxygen and cysteine, which was allowed to diffuse into the liver before freeze-drying, alter the electron spin resonance spectra. This report describes investigations on X-irradiated mouse liver. The study also includes preparations of liver in which solutions of cysteine were injected before freeze-drying. To some extent we obtained results different from those described by the foregoing authors.

Livers freshly removed from mice were frozen at 77° K in an untreated state and subsequently lyophilized. Preparations containing cysteine were obtained by injecting 0.5 ml. of a cysteine solution (300 mg cysteine-HCl/ml. H₂O) into the freshly-removed organ. Then the liver was frozen at 77° K and lyophilized. The preparations were ground (bulk volume about 330 cm³/100 g) and irradiated with X-rays (200 kV, half-value layer: 0.64 mm copper, dose-rate: 920 r./min, dose: 100 kr.) under vacuum (about 10⁻³ torr) and in air respectively at room temperature. The electron spin resonance measurements were carried out under the same conditions, with an X-band spectrometer. Technical details were as already described in a previous publication⁴.

Samples of non-irradiated liver show a signal (5×10^{16} radicals/g), which possesses the structure of an unresolved doublet. The 'blank' signal looks like the dotted spectrum

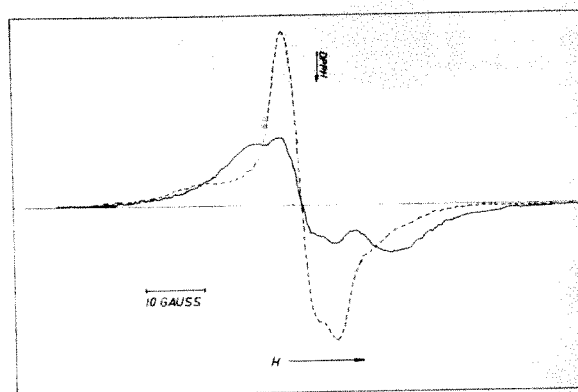


Fig. 1. Electron spin resonance spectra of X-irradiated lyophilized liver. The solid line gives the resonance of a sample irradiated and measured in vacuum, and the dotted line shows the resonance of the same sample measured 20 min later after admission of air.

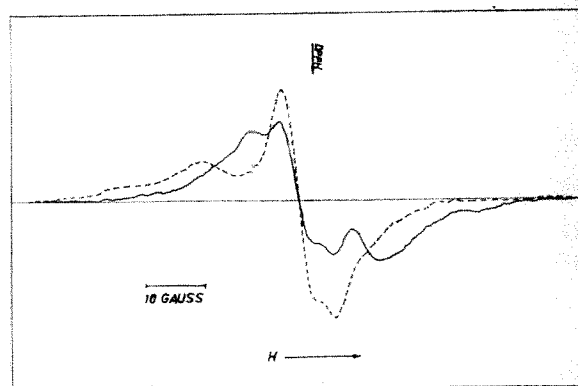


Fig. 2. Electron spin resonance spectra of X-irradiated lyophilized liver treated with cysteine. The solid line gives the resonance of a sample irradiated and measured in vacuum, and the dotted line shows the resonance of the same sample measured 20 min later after admission of air.

in Fig. 1 but does not correspond with the glycylglycine-type signal⁵ because of its smaller breadth. A touch of moisture leads to a rapid decay of the blank. Irradiation of the liver preparation with and without cysteine respectively in air produces an increase of the 'blank' signal without a change of its structure.

Irradiations of the samples with and without cysteine respectively under vacuum conditions produce in both cases the same electron spin resonance spectra (compare the solid spectra in Figs. 1 and 2). The 'blank' signal is slightly increased by the irradiation and overlapped by a second broad signal. With regard to the 'blank' signal the radiation yield amounts to $G=3.0$ per 100 eV absorbed X-ray energy. The increase of the doublet by irradiation may be due to oxygen residues in the sample tube.

An admission of air to the substances irradiated under vacuum immediately leads to a change of the electron spin resonance spectra. The alteration produces somewhat different spectra in the samples with and without cysteine (see dotted spectra in Figs. 1 and 2). In both cases the broad signal is quenched and at the same time the doublet is increased. This process continues for some hours after admission of air, because the air has to penetrate the whole sample by diffusion. Immediately after admission of air only about 80 per cent of the initial radical concentration can be measured. In the preparation with cysteine the increase of the doublet is not so large as in that without cysteine. On the other hand, an additional signal is formed in the region of lower magnetic field strength. This may be due to a sulphur radical, although it is not the typical 'cysteine-cystine-spectrum'.

Warming the irradiated sample in the evacuated state to 353° K for 10 min will only quench the broad signal; the signal height of the doublet is not altered all the time.

Only if the sample is stored for several hours at this high temperature does the radical concentration of the doublet also decrease.

These investigations show that atmospheric oxygen is able to induce a radical shift without external energy feed. Similarly, we have also found a transposition of X-ray-induced radicals from a carbon to a sulphur atom in a dimeric form of homocysteine-thiolactone, in experiments which we hope to report soon.

MITSUYUKI ABE*
HANS MÖNIG
RUPRECHT KOCH

Radiologisches Institut,
Universität Freiburg im Breisgau,
Germany.

* Present address: Department of Radiology and Faculty of Medicine, Kyoto University, Japan.

¹ Blyumenfel'd, L. A., and Kalmanson, A. E., *Biofizika*, **2**, 552 (1957).

² Gordy, W., *Rad. Res.*, Suppl. 1, 491 (1959).

³ Gordy, W., and Miyagawa, I., *Rad. Res.*, **12**, 211 (1960).

⁴ Mönig, H., and Koch, R., *Nature*, **202**, 289 (1964).

⁵ Gordy, W., and Shields, H., *Rad. Res.*, **9**, 611 (1958).

'Cell Killing' in Radiobiology

ONE of the most significant advances in cellular radiobiology was the development by Puck and Marcus¹ of a technique by means of which the survival from irradiation of animal cells in culture was placed on a quantitative basis. Survival has usually been expressed arbitrarily as the ability of an irradiated cell to produce, over a 10–20-day period, a macroscopic clone of descendants comprising at least 50 'normal' (that is, non-giant) cells.

The loss of this capability is at present described in a variety of ways: 'cellular death', 'cellular lethality', 'reproductive death', or 'reproductive incapacity'²; 'inhibition of clone formation', 'damage to reproductive capacity', or 'inhibition of unlimited proliferation'³; 'lethal events' or 'killing'⁴; 'reproductive cell killing', 'loss of reproductive integrity' or 'cell killing'⁵; 'effect on ability to proliferate'⁶; 'loss of proliferative capacity'⁷; 'loss of proliferative integrity', or 'lethality'⁸.

May one now enter a plea for a return to the succinct terminology of Puck and Marcus? These authors pointed out the analogy between their technique with irradiated HeLa cells and those with irradiated bacteria. They also showed that the term 'cell killing' had long had a specific meaning in microbiology, referring only to the ability of an individual cell to produce a macroscopic colony. In the absence of full knowledge of the mechanisms involved in the prevention of clone formation by irradiation, it is difficult to describe the phenomenon without ambiguity. But the use of a standard phrase in all reports of radiobiological investigations involving single cell plating techniques would seem to be a step in the right direction; and 'cell killing' is proposed as being the most suitable choice.

D. M. ROBINSON

Medical Research Council,
Radiobiological Research Unit,
Harwell,
Berkshire.

¹ Puck, T. T., and Marcus, P. I., *J. Exp. Med.*, **103**, 653 (1956).

² Greenblatt, C. L., *Intern. J. Rad. Biol.*, **4**, 185 (1961).

³ Barendsen, G. W., in *The Initial Effects of Ionizing Radiation on Cells*, edit. by Harris, R. J. C., 183 (New York: Academic Press, 1961).

⁴ Taylor, J. H., *J. Cell. Comp. Physiol.*, **62**, Suppl. 1, 149, 150 (1963).

⁵ Tolmach, L. J., *J. Cell. Comp. Physiol.*, **62**, Suppl. 1, 150 (1963).

⁶ Archambeau, J. D., Drew, R. M., and Robertson, J. S., *The Biological Effects of Neutron and Proton Irradiations*, **1**, 343 (Vienna: Intern. Atomic Energy Agency, 1964).

⁷ Whitmore, G. F., and Till, J. E., *Ann. Rev. Nucl. Sci.*, **14**, 347 (1964).

⁸ Elkind, M. M., and Sinclair, W. K., in *Current Topics in Radiation Research*, edit. by Ebert, M., and Howard, A., **1**, 165 (Amsterdam: New Holland Publishing Co., 1965).

BIOLOGY

Ultrastructure of the Antigen-retaining Reticulum of Lymph Node Follicles as shown by High-resolution Autoradiography

PREVIOUS investigations in this laboratory^{1–4} have described the localization of antigens in lymphoid follicles. Much circumstantial evidence was presented for the existence in follicles of a highly specialized fine dendritic web of macrophage fibrils responsible for antigen trapping and retention. Light microscopy alone, however, provided inadequate proof for the unequivocal demonstration of the structure of this web. The electron microscopic investigation reported here has defined more precisely the sites of localization of *Salmonella* flagellar antigen in the subpopliteal lymph node.

Whole flagella of *Salmonella adelaide* were labelled *in vitro* with iodine-125 by the direct oxidation method of Hunter and Greenwood as has been described elsewhere⁵. Ten-week-old normal Wistar rats were injected in one hind-foot pad with 20 µg flagella containing 1 mc. iodine-125 (0.7 g atom iodine-125 per 30,000 flagella). The draining popliteal lymph node was removed one to five days later, diced into pieces of about 2 mm³, fixed in cold buffered OsO₄ (ref. 6), dehydrated in ethanol and embedded in 'Araldite'. Throughout the fixation and dehydration, less than 5 per cent of the node's total radioactivity was lost⁷, despite the large area of exposed surfaces.

A section 2 µ thick, embracing the total available area of tissue, was cut from each block and mounted on a gelatine-coated slide for normal autoradiography with Kodak NTB-2 emulsion. To facilitate subsequent staining with methyl green-pyronin, the 'Araldite' was removed from the section with sodium methoxide⁸ before the emulsion was applied. Exposure for one day was sufficient to allow the detection of labelled follicles under 100-fold magnification. The position of a heavily labelled follicle was noted on the autoradiograph, the 'Araldite' block oriented appropriately and then trimmed to the size of the specified area comprising a follicle and some perifollicular tissue. Other blocks were prepared to contain typical areas of heavily labelled lymph node medulla. Ultra-thin sections displaying silver to pale gold coloration were cut with glass knives on a Huxley ultramicrotome and mounted on collodion coated slides⁹. Matching sections (0.5 µ) were cut to enable precise light microscopic autoradiographic correlation studies to be made.

The technique of high-resolution autoradiography developed by Salpeter and Bachmann⁹ with Kodak NTB-2 emulsion was used with the following modifications: (1) the slides were fire-polished¹⁰ before being coated with collodion; (2) sections were either unstained, or were stained for 0.5–2 h (before or after mounting) with 1 per cent aqueous uranyl acetate; (3) the emulsion was applied by dipping the slides into a Coplin jar containing diluted⁹ NTB-2 emulsion. After exposure for 1–3 months the slides were developed by passing them through the following solutions: 'Dektol' (Kodak, diluted 1/2) at 24° C, 1 min; 3 per cent CH₃COOH, 10 sec; water, 5 sec; 'Amfix' (May and Baker, Australia), 30 sec; water, 2 min. Finally, the slides were soaked in distilled water for 1 h or overnight before the collodion film was scored around the area of the sections and then stripped from the glass. Sections were mounted on either annular copper grids of 1-mm internal diameter¹¹ or NEW 100 'Athene' grid (Smethurst High-Light, Ltd., England). A Siemens UM100E4 electron microscope was used with a 50-µ objective aperture and was operated at 60 kV. Specimens were photographed at magnifications ranging from × 1,250 to × 7,200.

The specificity of the developed grains was established by locating the label on serial sections. With the development régime used, the grains appeared as round or

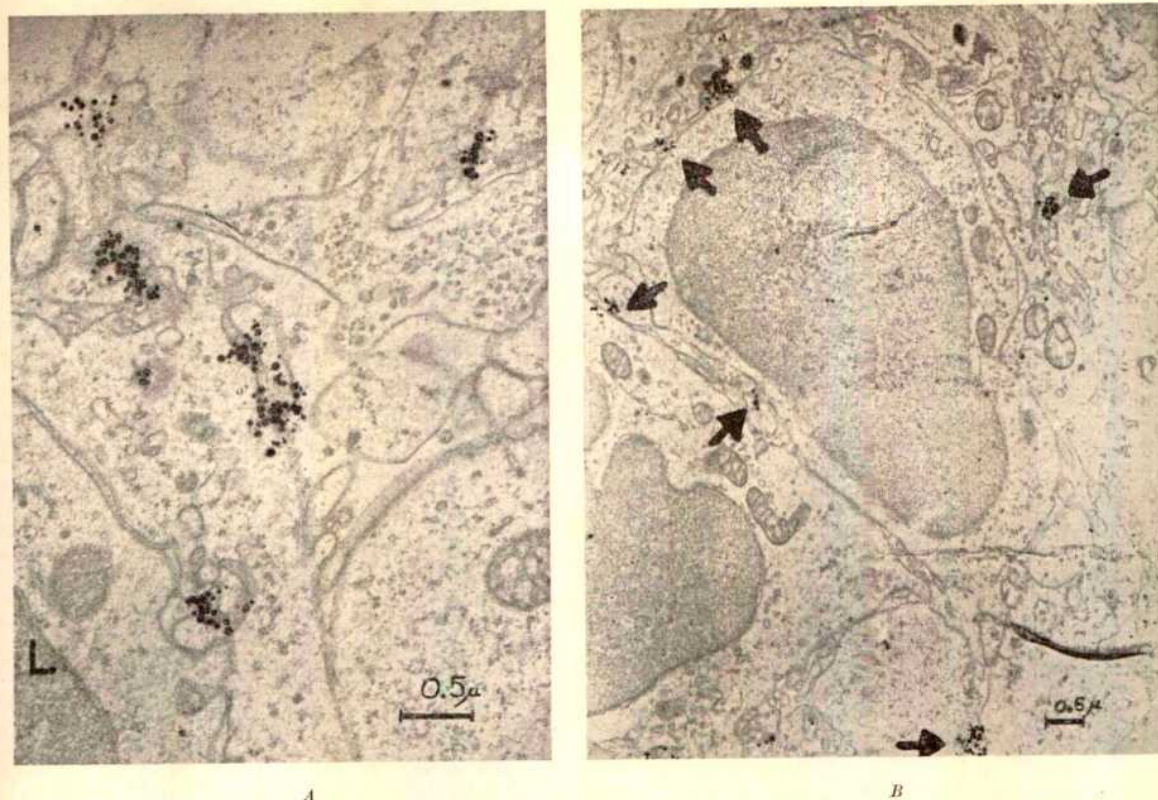


Fig. 1. Electron microscopic autoradiographs of a primary follicle from the popliteal lymph node of a rat injected with ^{125}I -labelled *Salmonella* flagella. A, Note the heavy label associated with fine cytoplasmic processes. L, Lymphocyte. ($\times c. 19,260$). B, Magnification $\times 11,300$. A lymphocyte surrounded by membrane-associated clumps of label.

comma-shaped dots (diam. $400 \pm 200 \text{ \AA}$), rather than as spirals. Significant background labelling was not seen, so that two or more grains at the one location could be identified with some confidence as specific label. By making a photo-montage of the complete section, the exact nature and location of labelled cells could be correlated with the more familiar and characteristic appearance of the matching autoradiograph in the light microscope.

The results showed that label in primary lymphoid follicles was indeed associated with fine cytoplasmic processes lying between the lymphoid cells (Fig. 1), thus confirming the previous findings and speculations⁴. The processes appeared to be long, fine, convoluted extensions of dendritic reticular cells. The label showed close association with the cytoplasmic membrane of these processes, a fact that was more readily apparent from inspection of areas where the density of grains was lower than in Fig. 1. The lack of label of the body of reticular cells and of the broader processes emanating from them (Fig. 1A) suggested that the antigen was preferentially located at the terminal arboreal regions of the dendritic processes.

The association between labelled dendritic processes and adjacent lymphocytes was extremely close (Fig. 1B). In some cases the contact was so intimate that it was impossible to determine whether label emanated from the process of a reticular cell or from the cytoplasmic membranes of the lymphocyte itself. The labelled dendritic processes were distinct from the cytoplasmic extensions which often ensheathed clumps of collagen fibrils. Furthermore, the dendrites did not appear to emanate from the 'tingible body' macrophages¹² which contained no obvious intracellular label.

The findings in the lymph node medulla were strikingly different. Here the label was located almost exclusively in the cytoplasm of typical macrophages (Fig. 2), much of it being associated with large granules and vacuoles. There was no evidence of label specifically associated with cytoplasmic processes or cell membranes.

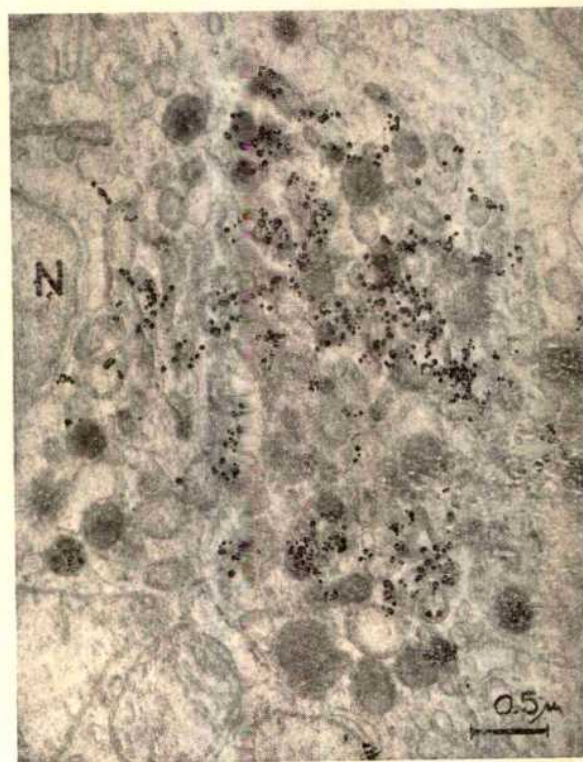


Fig. 2. Portion of a medullary macrophage showing intracellular label predominantly associated with electron dense granules. N, Nucleus of the macrophage. ($\times c. 19,300$)

We conclude that there are two basic mechanisms for antigen retention in lymph nodes. In the lymphoid follicles there is a mechanism whereby antigen becomes

associated with the surface of dendritic processes of reticular cells, where it comes in contact with lymphocytes. By contrast, in the medullary sinuses antigen is retained intracellularly in the inclusions of typical phagocytic cells. The distribution of label in lymphoid follicles leads us to suggest that the term 'phagocytic reticulum' which we have previously used⁴ in relation to this area should be replaced by the term 'antigen-retaining reticulum'. It is tempting to speculate that the two patterns of lymph node antigen distribution reflect different facets of the immune processes.

We thank Prof. G. J. V. Nossal and Dr. G. L. Ada for their advice and Mr. J. Pye for performing the iodinations. This work was supported by grants from the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service (AI-0-3958), to Prof. Nossal, and from the National Health and Medical Research Council, Australia. Some batches of iodine-125 were a gift of Glaxo Laboratories, England.

JUDITH MITCHELL
A. ABBOT

Walter and Eliza Hall Institute,
Melbourne, Australia.

¹ Ada, G. L., Nossal, G. J. V., Pye, J., and Abbot, A., *Nature*, **199**, 1257, Part II (1963).

² Nossal, G. J. V., Ada, G. L., and Austin, C. M., *Austral. J. Exp. Biol. and Med. Sci.*, **42**, 311 (1964).

³ Ada, G. L., Nossal, G. J. V., and Austin, C. M., *Austral. J. Exp. Biol. and Med. Sci.*, **42**, 331 (1964).

⁴ Miller, J. J., and Nossal, G. J. V., *J. Exp. Med.*, **120**, 1075 (1964).

⁵ Ada, G. L., Nossal, G. J. V., and Pye, J., *Austral. J. Exp. Biol. and Med. Sci.*, **42**, 295 (1964).

⁶ Palade, G. E., *J. Exp. Med.*, **95**, 285 (1952).

⁷ Mitchell, J. (submitted for publication to *J. Immunol.*).

⁸ Mayor, H. D., Hampton, J. C., and Rosario, B., *J. Biophys. Biochem. Cytol.*, **9**, 909 (1961).

⁹ Salpeter, M. M., and Bachmann, L., *J. Cell Biol.*, **22**, 469 (1964).

¹⁰ Luft, J. (personal communication).

¹¹ Dowell, W. C. T., *Fourth Intern. Conf. Electron Microscopy*, Berlin, 1958, 1, 375 (1960).

¹² Fleming, W., *Arch. Mikr. Anat.*, **24**, 550 (1885).

Zinc and Other Metallic Ions as Hatching Agents for the Beet Cyst Nematode, *Heterodera schachtii* Schm.

SOME inorganic salts stimulate eggs of the beet cyst nematode, *Heterodera schachtii* Schm., to hatch, but less effectively than diffusate from sugar-beet roots¹⁻³. The effective ions are potential oxidizing agents. We have extended hatching tests to some metallic ions not previously tried, including Ba²⁺, Al³⁺, Pb²⁺, MoO₄⁻, Mn²⁺, Co²⁺, Zn²⁺ and Cd²⁺, and have re-tested some salts (MgCl₂, KCl, NaCl, HgCl₂ and FeCl₃). Copper, previously tested as the sulphate, was tested as the chloride, and chlorides of the other metals were included in tests wherever possible. All salts were first tested at a concentration of ~3 mM in water and those with some activity were tested again over a range of concentrations. The hatch of eggs (Table 1) is expressed as a hatch rating³:

$$\frac{H_s - H_w}{H_d - H_w} \times 100$$

where H_s is the hatch in the substance, H_d is the hatch in beet root diffusate and H_w is the hatch in distilled water, and also as a percentage of the total number of eggs in the cysts.

Zinc sulphate, zinc nitrate and zinc and cadmium chlorides proved potent hatching agents; twelve other salts were moderately or weakly active, and ten were inactive or inhibitory. The zinc salts and cadmium chloride are more effective than the other inorganic hatching agents for *H. schachtii*³ although neither anion nor cation is a potential oxidizing agent. The activity of these two chlorides (zinc and cadmium) was not caused by hydrochloric acid formed by hydrolysis, because hydrochloric acid itself is only moderately active at its optimum concentration.

Table 1. THE ABILITY OF VARIOUS METALLIC SALTS TO STIMULATE HATCHING OF *H. schachtii* EGGS EXPRESSED AS A HATCH RATING AND ALSO AS A PERCENTAGE OF THE TOTAL NUMBER OF EGGS IN THE CYSTS

Based on total hatch after three weeks from three batches of 100 cysts

Compound	Concentration (mM)	Hatch rating	Percentage hatch
Zinc chloride	4*	180†	64
Cadmium chloride	0.6*	177	56
Zinc sulphate	2	127	55
Zinc nitrate	2	113	45
Ammonium molybdate	3*	82	38
Lead acetate	3*	75	23
Aluminium chloride	3*	69	32
Manganese chloride	3*	66	32
Cobalt chloride	0.6*	60	31
Ferrous sulphate	2	59	23
Zinc acetate	2	57	27
Cadmium sulphate	0.6*	56	34
Barium chloride	3*	54	27
Calcium chloride	3*	53	18
Cadmium nitrate	0.6*	31	22
Ferrous ammonium sulphate	1	26	16
Lead nitrate	2*	22	20
Potassium chloride	3	19	11
Ferric chloride	0.6*	16	20
Sodium chloride	3	15	11
Cupric chloride	0.6*	4	20
Ammonium nitrate	8	-14‡	10
Ammonium sulphate	4	-15	10
Magnesium chloride	3	-30	14
Sodium nitrate	6	-88	1
Mercuric chloride	0.6*	-95	1

* Optimum concentration in a dilution series.

† Bold type indicates hatch as good as or better than in root diffusate which is 100 ± 10.

‡ Negative sign indicates percentage inhibition compared with the hatch in water, which is 0 ± 10.

Because of its potency in causing *H. schachtii* eggs to hatch, we tested the ability of zinc chloride to hatch eggs of other *Heterodera* species: *H. avenae*, *H. carotae*, *H. cruciferae*, *H. glycines*, *H. goettingiana*, *H. rostochiensis*, *H. tabacum* and *H. trifolii*. With *H. avenae*, where the number of eggs which hatch in oat-root diffusate and in water is the same, zinc chloride inhibited hatching slightly. With *H. goettingiana*, where only very few eggs hatch either in water or in pea-root diffusate *in vitro*, a few more larvae hatched out in zinc chloride. With all the other species, zinc chloride caused more eggs to hatch than did water, and with most of these species hatching was as great as or greater than that in the root diffusate from their host plants. As with other agents that stimulate hatching, it is not known how Zn²⁺ and Cd²⁺ ions act.

A. J. CLARKE

AUDREY M. SHEPHERD

Rothamsted Experimental Station,
Harpenden, Hertfordshire.

¹ Rademacher, R., and Schmidt, O., *Arch. PflBau*, **10**, 237 (1933).

² Wallace, H. R., *Ann. App. Biol.*, **44**, 274 (1956).

³ Clarke, A. J., and Shepherd, A. M., *Nematologica*, **10**, 431 (1964).

Failure of the Zona Reaction in Five Pig Eggs

In most mammals which have been examined, the first spermatozoon that penetrates into the egg stimulates the egg to undergo a reaction which prevents subsequent spermatozoa from traversing the zona pellucida¹. This phenomenon is called the 'zona reaction'. In the pig following the zona reaction, spermatozoa can still penetrate into the zona but they normally cannot traverse it². However, eggs aged for several hours before exposure to the first spermatozoon sometimes fail to elicit the zona reaction and several spermatozoa enter the vitellus (polyspermy) to become male pronuclei (polyandry)³.

In the course of examining several hundred recently fertilized pig eggs, we observed five eggs which did not exhibit a zona reaction. The first of these came from a litter of nine eggs recovered about 8 h after ovulation. Eight of these were at normal stages while one egg (egg A) was unusual in that it contained two spermatozoa in the perivitelline space (Fig. 1). After the egg was fixed in acetic-alcohol and stained with orcein, a vesicular nucleus was revealed (Fig. 2). Hancock⁴ reported that out of 1,677 pig eggs examined, three had vesicular nuclei. The second egg that had not undergone a zona reaction came from a litter of five eggs recovered 54 h after insemination

four of these were normal, fertilized, four-celled eggs while the fifth (egg B) was one-celled and contained more than twenty spermatozoa in the perivitelline space (Fig. 3). Unfortunately, egg B was lost during fixation so that its nuclear status could not be determined. Eggs D, and E (Figs. 4, 5, and 6) were recovered 10–16 h after ovulation and were found to contain 10–15 accessory sperm in the perivitelline space and to have vesicular nuclei. We are not aware of any other recorded observation describing spermatozoa in the perivitelline space of a pig egg.

Pig eggs, and most other mammalian eggs examined, are normally in second metaphase at the time of ovulation. Polge and Dziuk⁵ found twenty-eight eggs with vesicular nuclei during a study of 1,472 recently fertilized pig eggs, and nine of these twenty-eight each had a spermatozoon in the cytoplasm but pronuclei had not formed, such as is shown in Fig. 6. Some eggs, therefore, fail either to receive or to respond to the signal for initiation of meiotic development and maturation prior to ovulation. Eggs A, C, D and E had vesicular nuclei and therefore were not fully mature. This suggests that as an egg matures meiotically it concomitantly develops its ability to elicit a zona reaction. This hypothesis could be tested by transferring follicular

eggs at various stages of maturation into the oviduct of recently mated sows. After an appropriate time-interval these eggs would be recovered and examined to see whether spermatozoa are present in the perivitelline space.

P. J. DZIUK

Animal Science Department,
University of Illinois,
Urbana.

Z. DICKMANN

Department of Obstetrics and Gynecology,
Vanderbilt University, Nashville, Tennessee.

¹ Braden, A. W. H., Austin, C. R., and David, H. A., *Austral. J. Biol. Sci.*, **7**, 391 (1954).

² Dickmann, Z., and Dziuk, P. J., *J. Exp. Biol.*, **41**, 603 (1964).

³ Hancock, J. L., *Animal Prod.*, **1**, 103 (1959).

⁴ Hancock, J. L., *J. Reprod. Fertil.*, **2**, 307 (1961).

⁵ Polge, C., and Dziuk, P. J., *J. Reprod. Fertil.*, **9**, 357 (1965).

Effects of (2-Chloroethyl)trimethylammonium Chloride and Gibberellic Acid on Growth, Fruit Bud Formation and Frost Resistance in One-year-old Pear Trees

THE growth retardant *N*-dimethylaminosuccinamic acid (B-995) has been shown to modify growth in various fruit trees^{1–3}.

In experiments at East Malling, another growth retardant, (2-chloroethyl)trimethylammonium chloride (CCC), has shown similar effects on pear trees. A single spray with 1 per cent CCC, applied on May 25, 1964, to 1-year-old pear trees of the variety 'Williams' Bon Chrétien', halved the total new shoot-length and greatly increased the number of fruit buds. These effects were obtained regardless of any pre-treatment of the trees with gibberellic acid (GA).

Four sets of ten trees were sprayed with GA at 500 p.p.m. on the following dates: (A), April 18, April 29 and May 4, 1964; (B), April 29 and May 4; (C), April 29 only; (D), April 18 only. A fifth set (E) was left unsprayed. On May 25, half the trees in each set were sprayed with 1 per cent CCC (treatment F).

The results are shown in Figs. 1–3. The GA sprays had no effect on primary shoot growth, but the triple GA spray (treatment A) increased both secondary and axillary shoot growth. The single CCC spray dramatically reduced shoot growth, regardless of whether or not GA had previously been applied (Fig. 1). Fruit bud formation was reduced by triple GA spray, but greatly increased by the CCC spray in every case (Fig. 2). Fig. 3 shows the appearance of trees of the most contrasting treatments E, EF, and A in the following spring.

On April 26, 1965, when the trees were in full bloom two trees of each treatment were exposed to a freezing cycle,

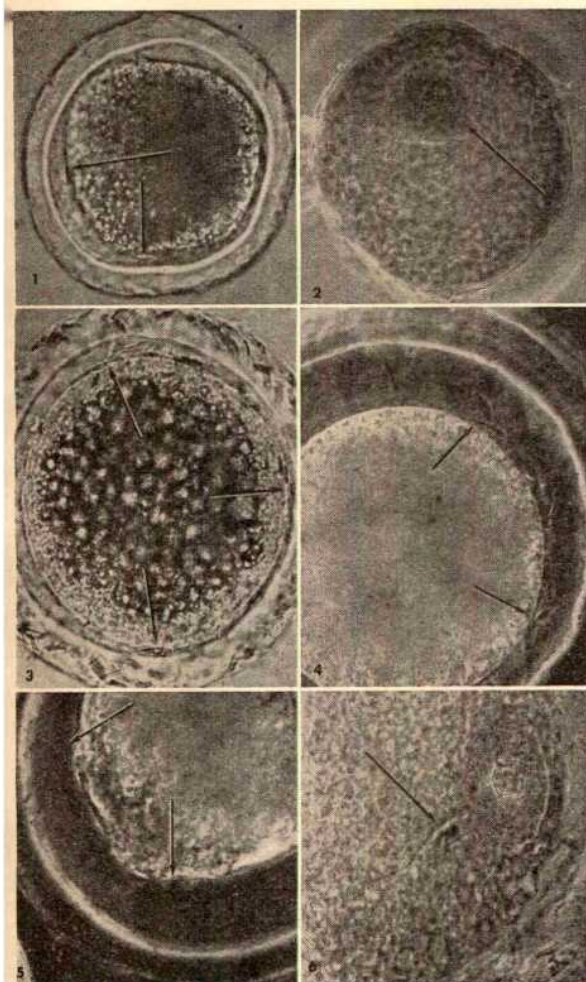


Fig. 1. Egg A with spermatozoa in perivitelline space (arrows) (fresh whole-mount; $\times c. 225$)

Fig. 2. Egg A showing vesicular nucleus (arrow) (fixed and stained; $\times c. 125$)

Fig. 3. Egg B showing several spermatozoa in perivitelline space (arrows) (fresh whole-mount, $\times c. 280$)

Figs. 4 and 5. Eggs C and D showing spermatozoa in perivitelline space (arrows) (fresh whole-mounts, $\times c. 280$)

Fig. 6. Egg E showing penetrated spermatozoon (arrow) near vesicular nucleus (fixed and stained, $\times c. 280$)

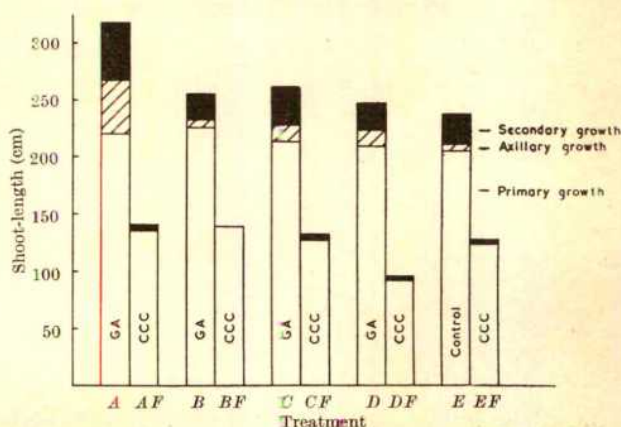


Fig. 1. Effect of GA and CCC on shoot growth of 'Williams' BC'

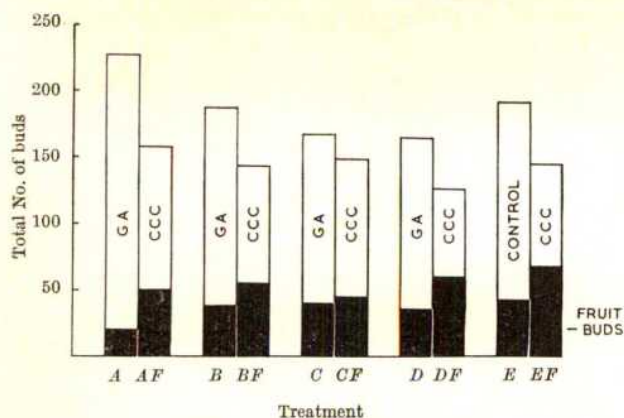


Fig. 2. Effect of GA and CCC on fruit bud formation of 'Williams' BC'

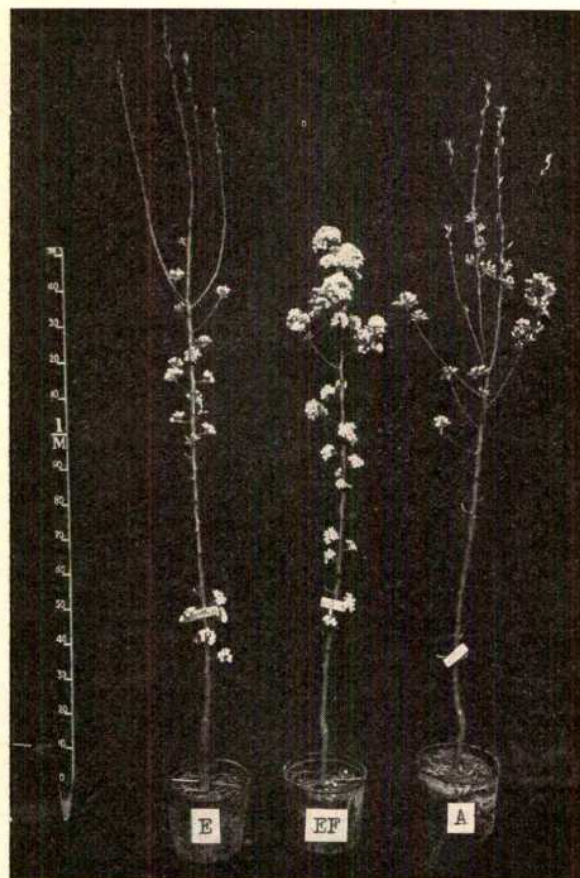


Fig. 3. 'Williams' Bon Chrétien' pear, a year after receiving treatments: E, unsprayed control; EF, 1 per cent CCC on May 25, 1964; A, GA 500 p.p.m. on 3 occasions. Photo, April 21, 1965

giving a temperature of -3.5°C for 15 min. The results (Table 1) showed that blossoms on the trees receiving a CCC spray in 1964 had a greatly increased frost resistance. The cell size of blossoms is being investigated.

Table 1. EFFECT OF CCC SPRAY IN MAY 1964 ON FROST DAMAGE TO FLOWERS IN 1965

Treatments	(Percentage flowers killed)				Mean
	A	B	C	D	
Without CCC	80	85	93	54	81
With CCC	45	44	54	67	50

Growth retardants, used alone and in conjunction with gibberellins, open exciting new possibilities in modifying

and controlling growth and fruitfulness of trees, and in the control of irregular bearing.

IRENA MODLIBOWSKA

East Malling Research Station,
East Malling, Maidstone, Kent.

¹ Batjer, L. P., Williams, M. W., and Martin, G. C., *Proc. Wash. St. Hort. Assoc.*, **59**, 107 (1963).

² Brooks, H. J., *Nature*, **203**, 1303 (1964).

³ Jonkers, H., *Fruittelt*, **54**, 878 (1964).

Anatomical Changes associated with Juvenile-to-Mature Growth Phase Transition in *Hedera*

THE morphological change which accompanies a juvenile to-mature transition in various plant species has received considerable attention in recent years¹⁻⁷. Some workers have attempted to relate biochemical^{8,9} and anatomical factors to those changes which are so striking in certain plants as to give the impression that the mature phase is an entirely different species. These studies confirm the findings of Beakbane¹¹ and extend her hypothesis concerning adventitious rooting.

Leaf, stem, root, and vegetative bud tissue was taken from actively growing juvenile, transitional, and mature phase plants of ivy (*Hedera helix*) and prepared for microscopic examination. In addition, flower buds of mature plants were studied. Stem sections were taken 2 cm below the apex. All tissues were prepared by dehydration in the tertiary butyl alcohol series, embedding in paraffin, staining with safranin-fast green, and sectioning with rotary microtome at 10μ .

Tissues were examined for characters which might be associated with a change in growth phase. No gross anatomical differences were noted in the leaves, roots, or buds of juvenile and mature tissues. However, one striking difference was observed in the cross-section of the stem. In addition to the anticipated greater lignification of tissues in the mature stem, a discontinuous ring of fibres encircled the phloem in these plants (Fig. 1, top). Phloem fibres were extremely rare in juvenile stems (Fig. 1, bottom). Transitional stems, beginning to show some mature morphology, possessed a few fibres which became more numerous as the maturing plant progressed in age.

Previous reports of difficulty in the vegetative propagation of mature *Hedera*^{9,12} resemble those reported by Beakbane¹¹ in various woody plants. She has shown that shoots of difficult-to-root plants often have a high degree of differentiation into fibres and sclereids. In 'Conference' pear an almost continuous ring of thick-walled fibres encircled the phloem. She found an inverse relationship between ease of rooting and continuity of the ring. Stoutemyer¹⁰ found that the mature phase stem in apple contained more pericyclic fibres than the juvenile form. Differences in response of cuttings from non-flowering and flowering plants have been correlated with differences in anatomical structure, the flowering stem containing a higher proportion of xylem¹³. Flowering of seedlings was also found to be associated with the structure of the stem. Struckmeyer¹⁴ showed that the cell walls of stems of various flowering plants had become greatly thickened, and changes in anatomical structure occurred early in the development of plants placed in an environment favourable to the formation of flower primordia.

This experiment supports Beakbane's suggestion that an increased difficulty in rooting is associated with increased fibre development in mature stems. Also supporting Beakbane, it is difficult to conceive that such a correlation could explain the rooting problem strictly on the basis of a mechanical barrier, since in no case observed in *Hedera* was the fibre ring continuous so as to form a lignified cylinder. Beakbane also refers to difficult-to-root plants, such as *Pittosporum*, in which no sclerenchyma is present. Recent experiments by Sachs, Loreti, and De Bie¹⁵ showed no simple relationship between density and

VIROLOGY

Milker's Nodule Virus Infections in Dorset and their Similarity to Orf

BETWEEN January 1964 and March 1965 seven farm workers on six Dorset farms developed lesions on the hand or forearm which were diagnosed as clinically typical orf infections. Material from each patient was examined by electron microscopy and tissue culture as previously described^{1,2}. In each instance pox-virus particles were seen which in morphology and size were identical with orf virus³. In tissue culture four specimens produced the same cytopathic changes as orf virus and serial passage of three virus strains was obtained in primary human amnion and rhesus monkey kidney. No difference in behaviour was noted between these strains and orf virus strains previously grown from humans infected from sheep.

Orf virus infection of sheep is well known as contagious pustular dermatitis (CPD). None of our seven patients had been in contact with sheep, but all had milked cows within three weeks of onset of their lesions. Orf infection of cattle has not been reported, so these human infections would have been diagnosed as milker's nodules or pseudocowpox if the animal source of infection had been considered of prime importance⁴⁻⁷.

Investigation of the six dairy herds revealed teat lesions on some animals in all six herds. Two distinct types of lesion are recognizable: (1) *Benign or chronic*. This is a mild erythema of the teat which is quickly followed by profuse scabbing. The scabs are soft, scurfy and yellowish-grey and are often rubbed off at milking. The teat surface becomes corrugated and prone to develop chaps and sores. The lesions are painless throughout their course and persist for several months. This type of lesion seldom affects many cows in a herd. (2) *Acute*. An erythematous eruption of the teat develops into a small vesicle or pustule which ruptures within 48 h. Prolific scab formation follows rapidly, some of the scabs being very large. Pain is only noticeable before scab formation, and indeed, scabs can be removed with no response by the cow. Granulation develops beneath the scab until the lesion becomes elevated some 2-3 mm above the general teat surface, and varies in diameter from 0.5 to 2.5 cm. The lesions heal from the centre, and, when the scab drops or is rubbed off, a characteristic horseshoe-shaped ring of minute scabs at the circumference is left. Each lesion takes 7-10 days to this stage. A small wart-like granuloma then remains, marked out in the early days by the horseshoe ring, and persists for many months, several of these making the teat very coarse. Spread of the lesions from cow to cow appears to be slow, and 5-10 per cent of the herd is usually affected at any one time. Freshly calving animals and those recently entering the herd are most often affected. Two herds had an epidemic of the infection which appeared a few weeks after their arrival from a different county.

Scabs were collected when present and small biopsies were made of the other types of lesion. As shown in Table 1, identical pox-virus particles were found in the

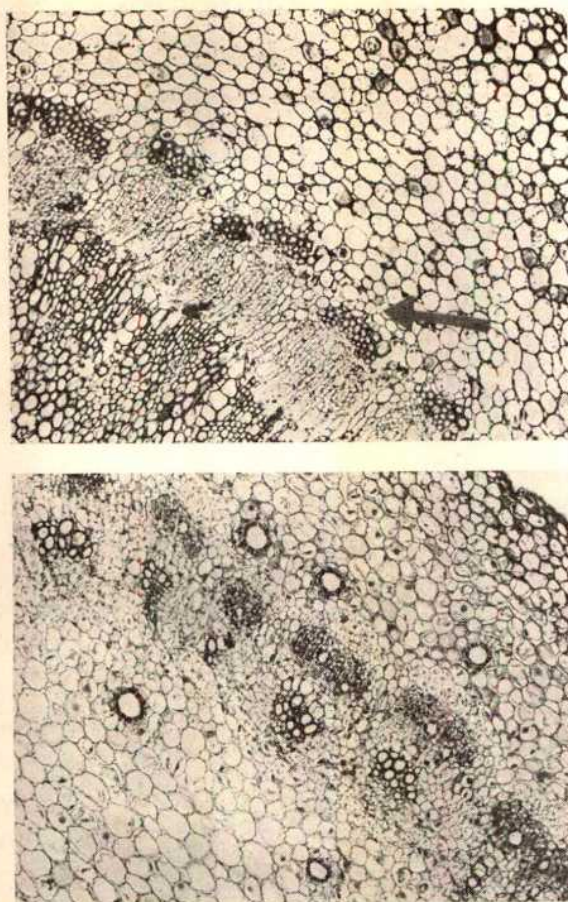


Fig. 1. Cross-sections of mature (upper) and juvenile (lower) stem of *Hedera helix*. Arrow points to fibres peripheral to the phloem in the mature stem.

continuity of sclerenchyma and ease of rooting in seven varieties of olive cuttings. Indeed, if the correlation described by Beakbane, and supported by the present work, is significant, a chemical involvement must be assumed.

One other point deserves mention concerning *Hedera* morphology and anatomy. As the ivy proceeds from the juvenile to mature growth phase, changes in leaf shape, stem shape, anthocyanin content, formation of aerial roots, and phyllotaxy occur. In addition, growth habit changes from a distinct plagiotropic to an orthotropic form. Probably the strengthening of stems, and change to a shrubby habit, may be associated with the increased shoot lignification and development of fibres. The additional shoot strength imparted by the fibres is apparently sufficient to change *Hedera* from a vining to an upright growth habit.

J. R. GOODIN*

Department of Agricultural Sciences,
University of California,
Los Angeles.

* Present address: Department of Agronomy, University of California, Riverside.

- ¹ Allsopp, A., *Nature*, **184**, 1575 (1959).
- ² Doorenbos, J., *Proc. Kon. Ned. Akad. Wetensch.*, Ser. C, **57**, 99 (1954).
- ³ Fisher, J. F. J., *Nature*, **173**, 406 (1954).
- ⁴ Goodin, J. R., and Stoutemyer, V. T., *Nature*, **192**, 677 (1961).
- ⁵ Hudson, J. P., and Williams, I. M., *Nature*, **175**, 814 (1955).
- ⁶ Robbins, W. R., *Amer. J. Bot.*, **44**, 743 (1957).
- ⁷ Robbins, W. R., *Amer. J. Bot.*, **46**, 485 (1960).
- ⁸ Goodin, J. R., Ph.D. thesis, University of California, Los Angeles (1963).
- ⁹ Hess, C. E., Ph.D. thesis, Cornell University, Ithaca, N.Y. (1957).
- ¹⁰ Stoutemyer, V. T., *Iowa Agric. Exp. Sta. Res. Bull.*, **220**, 308 (1937).
- ¹¹ Beakbane, A. Beryl, *Nature*, **192**, 954 (1961).
- ¹² Stoutemyer, V. T., Britt, O. K., and Goodin, J. R., *Proc. Amer. Soc. Hort. Sci.*, **77**, 552 (1961).
- ¹³ Struckmeyer, B. E., and Roberts, R. H., *Amer. J. Bot.*, **26**, 694 (1939).
- ¹⁴ Struckmeyer, B. E., *Bot. Gaz.*, **103**, 182 (1941).
- ¹⁵ Sachs, R. M., Loreti, F., and De Bie, J., *Calif. Agric.*, **18**, 4 (1964).

Table 1. TISSUE CULTURE IN PRIMARY HUMAN AMNION, SECONDARY MONKEY KIDNEY AND PRIMARY BOVINE TESTIS

Farm	Farm worker	Method of examination	Microscopy	Culture	Passages	No. of cattle examined	No. positive Microscopy	Culture
1	R.T.	Positive	Positive	2+	3	3	3	3
2	S.T.	"	"	4+	0	1	1	1
3	M.J.P.	"	"	None	1	1	1	1
4	J.M.P.	"	"	3+	4	3	3	3
5	A.M.	"	Negative	-	4	4	N.D.	N.D.
6	Miss C.	"	"	-	4	4	N.D.	N.D.
6	A.H.	"	N.D.	-	6	4	N.D.	N.D.

Passages given are number attempted. The virus from M.J.P. produced typical cytopathic changes but failed to passage. The others showed no evidence of decreasing infectivity when passage was discontinued.

N.D., not done.

five herds from which specimens were obtained and growth in tissue culture was successful. The virus behaved in every way like the human strains. Orf infection of lambs and ewes is enzootic in Dorset, the virus having been isolated by us both from sheep and from shepherds. The possibility of transfer of orf infection from sheep to cattle was considered, but no evidence of such transfer was found. In order to find out how common teat lesions might be among dairy herds in West Dorset which were not associated with human infections, 16 herds were inspected. Teat lesions were found in some animals in every herd. At least four presumptive human infections were also found although too late for laboratory investigation. Confirmation of the bovine infections was possible in 14 of the 16 herds. As only one specimen was examined from each of the two negative herds, this does not exclude their infection. Of 48 animals examined, 36 had the pox-virus infection, 5 bovine warts and 7 animals were negative. The infection is therefore enzootic. No marked seasonal variation was evident. Lesions of the mouth in calves have not been seen, but few calves were suckled for more than three days because these were dairy herds.

It has been found that electron microscopy is a quicker and more reliable method than tissue culture for examination of this kind of material. Bacterial and fungal contamination of the specimen is heavy and the virus grows slowly. When only small numbers of virus particles are present successful culture is far less likely, as shown in Table 2. No positive results were obtained with cultures when microscopy was negative. Microscopy is therefore an invaluable tool for this type of field investigation and could be applied on a large scale provided that adequate cultural confirmation is included.

Comparison of the growth of cattle and sheep virus strains in a variety of tissue culture cells is shown in Table 3. No consistent differences were observed in the range of cells affected, in the type of cytopathic change, or in the number of successful passages possible. Both viruses have a tendency to die out and the differences shown appear to be no greater than between different strains of either source. Growth in primary cells is more reliable and yields more virus than in continuous cell lines. Most strains have passed 10 or more times in primary cells without dying out.

There are several aspects of the work presented that seem worth consideration.

Table 2. COMPARISON OF NUMBER OF BOVINE POXVIRUS PARTICLES SEEN BY MICROSCOPY WITH CULTURAL RESULT

Virus particles per grid square*	Tissue culture	
	Positive	Negative
1,000-	1	0
100-1,000	10	0
10-100	8	6
1-10	6	4
0	0	12

* A standard 2.30 mm electron microscope grid has squares of approximately $100\mu \times 100\mu$.

(1) *Human lesions.* Infection with this bovine virus and ovine orf can produce a variety of clinical lesions man from multiple vesicles to the classical single indurated nodule. When the animal source is unknown, or doubt, it is not possible in our experience to differentiate between ovine and bovine infections. A wider experience might enable this, but all the dermatologists we have consulted so far have agreed that differential diagnosis is based on knowledge of the animal source. The shepherds are said to have orf whereas a similar lesion on a cowman is called a milker's nodule. We feel that thorough comparison of the lesions is likely to show the same pathology irrespective of the source of infection.

(2) *Bovine lesions.* This infection of dairy cattle hitherto unsuspected, is clearly enzootic in Dorset. The it came to our notice by the appearance of the human infections is suggestive of a higher recent incidence perhaps due to changes in farming, as three infections were from two herds with epizootic infection in consequence of being recently moved into the county.

There is no information at present on the incidence of infection elsewhere in Britain, but it is probably widespread and might be the 'spurious cowpox' described by Jenner in 1799 (ref. 6).

Teat lesions in dairy cattle tend to be regarded as due to minor trauma, chapping in cold weather, etc. But it is worth consideration that even trivial lesions are prone to secondary bacterial infection and this is one way in which bacterial infection of a teat could occur which might lead to mastitis.

(3) *The relations of the bovine virus.* The poxvirus are divisible into two groups by morphology. The large group have the vaccinia type of structure, the smaller one that described for orf^{3,8}. We refer to the latter as the orf group because of the antiquity of the word 'orf' that is Old English *hreo*: rough, scabby⁹, and orf-quail cattle plague¹⁰, and the fact that this morphological type was first described by Abdussalam and Cosslett in 1957 (ref. 8) for ovine virus, in preference to the suggested use of 'paravaccinia' group recently proposed by Peters *et al.*¹¹.

So far the orf structural group includes the viruses of orf or contagious pustular dermatitis^{3,8,12}, milker's nodule or pseudocowpox virus^{6,7}, bovine papular stomatitis virus^{13,14}, chamois contagious ecthyma^{15,16} and that described recently by Pournaki *et al.* as bovine X or BX virus¹⁷. We consider that our bovine virus is synonymous with those described as milker's nodule, pseudocowpox and BX. Its relationship to bovine papular stomatitis (BPS) virus remains to be determined. Plowright and Ferris¹⁸ consider BPS to be synonymous with the proliferative stomatitis described in the United States by Olson and Palionis¹⁹ and several other stomatitis agents. It is noteworthy that two of the American workers developed milker's nodes on their hands, and material from these injected into calves produced stoma-

Table 3. GROWTH IN TISSUE CULTURE

	Ovine strains				Bovine strains			
	Sheep	Human			Cattle		Human	
	CPD†	5957	25772	19510	24120	4390	21917	1637
Primary cell cultures								
Bovine testis	++	++	++	++	++	++	++	++
Human amnion	++	++	++	+	++	++	+	++
Monkey kidney	++	++	++	++	++	±	++	++
Cell lines								
HeLa strain 232	+	+	±	+	+	+	+	+
HeLa strain MK 2	±	±	±	±	±	+	±	±
Mouse ascites tumour*	—	++ (> 7)	±	—	±	++ (> 7)	±	±
Rabbit RK 13	—	±	±	±	±	±	±	±
KB	—	±	±	±	±	±	±	±
Mouse L cells	—	+	—	—	—	—	—	—
BHK 21 (hamster kidney)	—	±	—	—	—	—	—	—

++, Indefinite passage possible with cytopathic effect (CPE); +n, limited number of passages (last passage number with appreciable CPE indicated); ±, non-transmissible CPE from initial inoculum; —, no change.

* *In vitro* adapted Bandinelli strain.

† CPD is the Wellcome Laboratories vaccine strain.

itis. BPS strains are not readily available in Britain and we have been unable to find evidence of natural infections.

The relationship of the bovine strains to orf and the other animal viruses will remain conjectural until further work, especially serology, is complete, but on present evidence their similarity appears so close as to make separation difficult.

We thank Mr. G. H. C. Ellis, Mr. W. MacKinnon, Mr. J. Hall and Mr. M. H. Woodford for their help, and Prof. J. D. Boyd for enabling us to use the electron microscopy facilities of the Department of Anatomy, Cambridge.

J. NAGINGTON

Public Health Laboratory,
Cambridge.

G. H. TEE

Public Health Laboratory,
Dorchester.

J. S. SMITH

Dorchester.

¹ Nagington, J., *Brit. Med. J.*, ii, 1499 (1964).

² Nagington, J., and Whittle, C. H., *Brit. Med. J.*, ii, 1324 (1961).

³ Nagington, J., and Horne, R. W., *Virology*, 16, 248 (1962).

⁴ Becker, F. T., *J. Amer. Med. Assoc.*, 115, 2140 (1940).

⁵ Nomland, R., and McKee, A. P., *Arch. Dermat. and Syph.*, 65, 663 (1952).

⁶ Friedman-Klen, A. E., Rowe, W. P., and Banfield, W. G., *Science*, 140, 1335 (1963).

⁷ Moscovici, C., Cohen, E. P., Sanders, J., and Delong, S. S., *Science*, 141, 915 (1963).

⁸ Abdussalam, M., and Cosslett, V. E., *J. Comp. Path. Therap.*, 67, 145 (1957).

⁹ Webster's Third International Dictionary: "Orf".

¹⁰ Oxford English Dictionary: "Orf".

¹¹ Peters, D., Müller, G., and Büttner, D., *Virology*, 23, 609 (1964).

¹² Nagington, J., Newton, A. A., and Horne, R. W., *Virology*, 23, 461 (1964).

¹³ Reczko, E., *Zentr. Bakteriolog. Abt. 1 Orig.*, 169, 425 (1957).

¹⁴ Nagington, J., Plowright, W., and Horne, R. W., *Virology*, 17, 361 (1962).

¹⁵ Grausgruber, W., *Zentr. Bakteriolog. Abt. 1 Orig.*, 195, 175 (1964).

¹⁶ Gerstl, F., *Zentr. Bakteriolog. Abt. 1 Orig.*, 195, 182 (1964).

¹⁷ Pournaki, R., Vieuchange, J., Lépine, P., and Fasquelle, R., *Ann. Inst. Pasteur*, 107, 173 (1964).

¹⁸ Plowright, W., and Ferris, R. D., *Vet. Rec.*, 71, 718 (1959).

¹⁹ Olson, C., and Palouis, T., *J. Amer. Vet. Med. Assoc.*, 123, 419 (1953).

Plant Tumour Virus in Arthropod Host: Microcrystal Formation

THE wound tumour virus (WTV) belongs to a group of plant pathogenic viruses that infect not only plants but also their respective arthropod carriers¹. The virus has been detected in extracts of an insect vector and found to multiply in this vector, the clover leafhopper *Agallia constricta* van Duzee². Purified preparations of WTV from plant and insect hosts permitted the determination of the particle size and its morphology³. Purified WTV particles, about 600 Å in diameter, have the shape of an icosahedron with a surface of 92 sub-units, about 75 Å in diameter⁴. The virus core stains heavily with uranyl acetate and comprises about 20 per cent of the volume of the particles. It consists of a double-stranded RNA⁵. The virus reaches considerable concentrations in extracts from insects and plant tumours, but the infection of the insect host is not apparent⁶.

Until we undertook this investigation, it was not known whether WTV particles could be visualized in tissues of an insect vector *in situ*. The presence, in the cell cytoplasm of both healthy and virus-infected insects, of numerous spherical particles makes it extremely difficult to differentiate between normal cell constituents and virus, unless special circumstances arise which make identification possible.

Clover leafhoppers, *A. constricta*, were confined for 4 weeks to sweet clover (*Melilotus officinalis* L.) plants infected with WTV. Afterward, whole abdomens were dissected and fixed for 90 min in a 6 per cent glutaraldehyde and insect Ringer solution. This was followed by washing with 3 changes of distilled water for 30 min each, and further fixation in 2 per cent osmium tetroxide

for 60 min. After dehydration in graded ethanol, the pieces were embedded in methacrylate or epoxy resin. Ultrathin sections were cut with a Porter-Blum MT-1 microtome, using a diamond knife for epoxy, and a glass knife for methacrylate resin. The sections, approximately 25–50 mμ thick, were double-stained (for epoxy embedded materials) with 6 per cent uranyl acetate for 60 min and with lead for 15 min⁷. Electron micrographs were taken with a Siemens Elmiskop I, with 80 kV accelerating voltage.

Although the size and shape of WTV were known from purified preparations, the identification of isolated particles would have presented a difficult task. Fortunately, striking accumulations of particles, forming crystalline arrays, were encountered in the cytoplasm of muscle and fat body cells. Since these particles, found in crystalline arrangement, resembled in size and shape WTV particles, and since such particles were never observed in non-infected insects, it was concluded that the regular accumulations were WTV microcrystals. This conclusion was further confirmed by the finding of particles of the same size and morphology in WTV-infected plants, particularly in root and stem tumours of diseased sweet clover plants, and in WTV enations of *Rumex acetosa* and enlarged veins of WTV-infected *Trifolium incarnatum*⁸, but not in healthy plants of the same species. Similar regular arrangements and distances between particles of the same size as found in the microcrystals were observed by Whitecomb (unpublished results) in a cross-section of a pellet of purified WTV.

Fig. 1 shows one of the striking accumulations of WTV microcrystals in a single fat body cell of *A. constricta*. Individual microcrystals of compactly packed WTV particles were also encountered within microfibrils of muscle fibres, as shown in Figs. 2 and 3. These electron micrographs of microcrystalline virus inclusions indicate the regular, three-dimensional arrangement of the particles. When the microcrystals were found in fat body and muscle cells, it was not known whether they also occur in other organs of the arthropod host. The time-consuming part of our work was the subsequent identification of particles, particularly of individual ones or of very small WTV accumulations⁹. The finding of virus microcrystals, on the other hand, turned out to be easier than was anticipated. It should be pointed out that the size of the WTV inclusions is smaller than, or similar to, mitochondria, which makes their structural identification difficult under a light microscope.

The diameter of the virus particles within a microcrystal was calculated as 58–60 mμ. These measurements were made on epoxy embedded materials, including central darker areas surrounded by less dense regions.

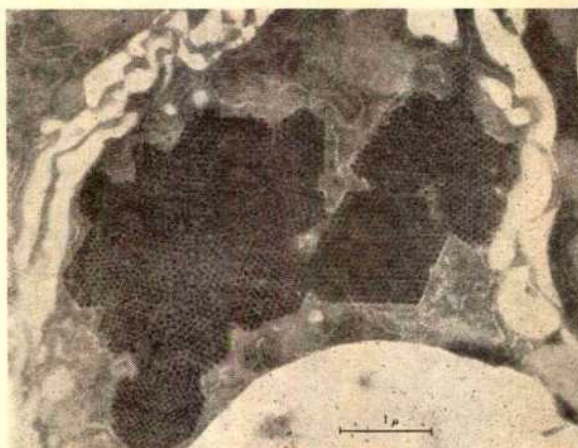


Fig. 1. Crystalline inclusions (microcrystals) of wound tumour virus (WTV) in the cytoplasm of a fat body cell of *A. constricta*.

Parsons⁹ summarized the differences in size between isolated virus particles and those obtained from ultrathin sections of animal viruses. It was known that methacrylate polymerization causes considerable shrinkage and distortion¹⁰. Therefore, the diameter of virus particles in methacrylate-embedded sections was expected to be smaller than the diameter of purified WTV particles. This was confirmed in the results shown in the legends to Figs. 2, 3 and 4. Centre-to-centre measurements resulted in different average diameters, when the measurements were made from different directions.

Electron micrographs of some WTV microcrystals clearly showed the regularly repeated pattern of darker and lighter particles (Fig. 4), corresponding with the denser and less dense masses of regularly arranged units. Obviously, in such sections, the cutting face of the knife was not parallel to the surface of the WTV microcrystal. The darker particles have a central core, while the less dark ones are devoid of the core.

The nature of the crystal formation is not clear. It is feasible to assume that they represent the result of virus multiplication at the site where they are found. In the

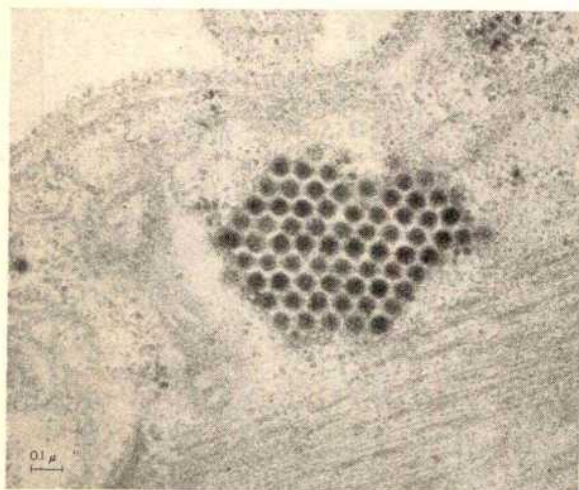


Fig. 2. Cross-section through a WTV microcrystal within microfilaments of a muscle cell of *A. constricta*. Epoxy resin embedding. Average diameters of 42 particles were: first direction (8 particles): 66.8 mμ; second direction (6 particles): 66.0 mμ; third direction (8 particles): 57.5 mμ; fourth direction (6 particles): 57.6 mμ; fifth direction (8 particles): 61.7 mμ; sixth direction (6 particles): 61.9 mμ. Single particles inside crystal (3 particles): 59.4 mμ single particle, outside microcrystal: 57.8 mμ

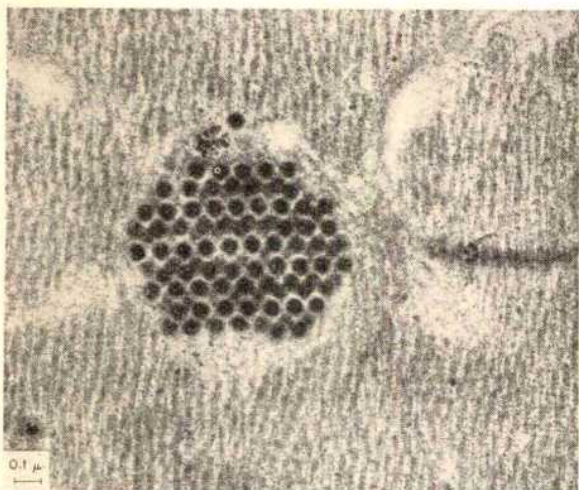


Fig. 3. Cross-section through a WTV microcrystal in *A. constricta* muscle cell. Epoxy resin embedding. Average diameters of 9 particles were: first direction—70.5 mμ; second direction—72.5 mμ; third direction—69.5 mμ. Single particle inside microcrystal measured 63.0 mμ × 59.6 mμ

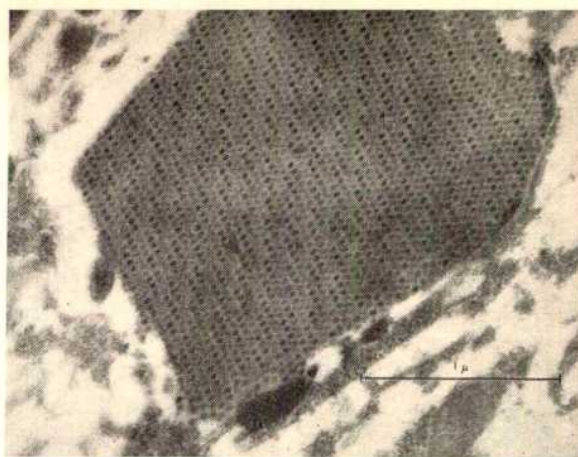


Fig. 4. Cross-section of a WTV microcrystal, embedded in methacrylate resin. Average diameters of 20 particles were: first direction—45 mμ; second direction—35 mμ. Average diameters of 10 particles in a loose cluster within same fat body cell were 57 mμ and 38 mμ for the two directions

case of animal viruses in animal host cells, such a hypothesis had been put forward by several workers^{11,12}. However, some virus particles are not arranged regularly in the cytoplasm¹³. While it appears likely that virus multiplication occurred in the cytoplasm of the cells in which the microcrystals were encountered, these regular arrangements of particles could also represent the final stage of storage and accumulation of particles that have multiplied earlier in a viroplasmic matrix situated at another part of the cell.

The novel and unusual feature of the present findings is the formation of microcrystals of a plant-pathogenic virus in an animal host. So far as the authors are aware, this is the first such account. Regular arrangements of rice dwarf virus have also been reported in an insect vector¹², but electron micrographs of that virus did not suggest the formation of a crystalline three-dimensional structure. It should be pointed out that WTV microcrystals were also found in some cells of infected plants, alongside of large accumulations of virus particles and irregularly scattered WTV⁷.

This work was supported in part by grants CA-07453 from the National Cancer Institute and AI-04290 from the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service, and by grant GB-1199 from the U.S. National Science Foundation, Washington, D.C.

EISHIRO SHIKATA*
KARL MARAMOROSCH

Boyce Thompson Institute for Plant Research,
Yonkers 3, New York.

* On leave from the Department of Botany, Hokkaido University, Sapporo, Japan.

¹ Black, L. M., *Amer. J. Bot.*, **32**, 408 (1945); in *The Viruses*, edit. by Burnet, F. M., and Stanley, W. M., **2**, 157 (Academic Press, New York, 1959). Maramorosch, K., in *Plant Virology*, edit. by Corbett, M. K., and Sisler, H. D., 175 (Univ. Florida Press, Gainesville, 1964).

² Maramorosch, K., Brakke, M. K., and Black, L. M., *Science*, **110**, 162 (1949). Black, L. M., and Brakke, M. K., *Phytopath.*, **42**, 269 (1952).

³ Brakke, M. K., Vatter, A. E., and Black, L. M., *Brookhaven Symp. Biol.*, **6**, 137 (1954). Maramorosch, K., and Jensen, D. D., *Ann. Rev. Microbiol.*, **17**, 495 (1963).

⁴ Bills, R. F., and Hall, C. E., *Virology*, **17**, 123 (1962).

⁵ Black, L. M., and Markham, Roy, *Neth. J. Plant Pathol.*, **69**, 215 (1963). Gornat, P. J., and Tamm, I., *Proc. U.S. Nat. Acad. Sci.*, **50**, 878 (1963).

⁶ Karnovsky, M. J., *J. Biophys. Biochem. Cytol.*, **11**, 729 (1961).

⁷ Shikata, E., and Maramorosch, K., *J. Nat. Cancer Inst.* (in the press).

⁸ Shikata, E., and Maramorosch, K., *Virology* (in the press).

⁹ Parsons, D. F., in *Techniques in Experimental Virology*, edit. by Harris, R. J. C., 381 (Academic Press, New York, 1964).

¹⁰ Fengel, D., and Balser, K., *Naturwiss.*, **51**, 538 (1964).

¹¹ Morgan, C., Howe, C., and Rose, H. M., *Virology*, **9**, 145 (1959). Fogh, J., and Stuart, jun., D. C., *ibid.*, **9**, 705 (1959). Shikata, E., Orenski, S. W., Hirumi, H., Mitsuhashi, J., and Maramorosch, K., *ibid.*, **23**, 441 (1964).

¹² Fukushi, T., Shikata, E., and Kimura, I., *Virology*, **18**, 192 (1962).

¹³ Dales, S., and Franklin, R. M., *J. Cell. Biol.*, **14**, 281 (1962).

VETERINARY SCIENCE

Development of *Eimeria tenella* in Avian Embryos

MORE than 300 species of the genus *Eimeria* have been recorded from birds and mammals¹. Where details of the life-cycles are known, only two species appear to develop in sites other than the intestinal tract. These species, *E. stiedae* and *E. truncata*, occur in the liver of the rabbit and the kidney of the goose respectively and are not known to develop at other sites. Infection of hosts normally occurs after the introduction of sporulated oocysts by mouth and the development of species, other than *E. stiedae* and *E. truncata*, is confined to the intestine. *E. tenella* usually shows a rigid preference for the caecal mucosa of the domestic fowl although, in common with the other species of *Eimeria* in the fowl, the sporozoites are released in the small intestine. When this parasite is introduced by either the intravenous or intraperitoneal routes developing stages can only be located in the caeca²⁻⁴.

This rigid site selection does not necessarily apply to *E. necatrix* or *E. brunetti* as sporozoites introduced directly into the caeca invade the walls and complete their life-cycles in the usual way^{5,6}. The present investigation shows that *E. tenella* can develop in the chorioallantoic membrane of the chick embryo.

Sporozoites were released from the oocysts by using a modification of an *in vitro* excystation method⁷. In pre-

liminary experiments, sporozoites injected into the allantoic cavity or intravenously caused the early deaths of ten-day-old embryos. Deaths usually occurred immediately after the intravenous injection of sporozoites whereas deaths occurred up to 24 h after allantoic injection and were associated with heavy bacterial contamination. To control contamination, the oocyst suspensions were incubated at 30° C for 24 h in peptone broth containing 50 i.u. penicillin and streptomycin per ml. This procedure was followed by a 1 h treatment in 1:500 'Chlorox' solution and finally in 2 per cent formalin solution. The oocysts were washed in sterile 0.9 per cent sodium chloride between each treatment and then excysted. The findings following the inoculation of sporozoites are summarized in Table 1.

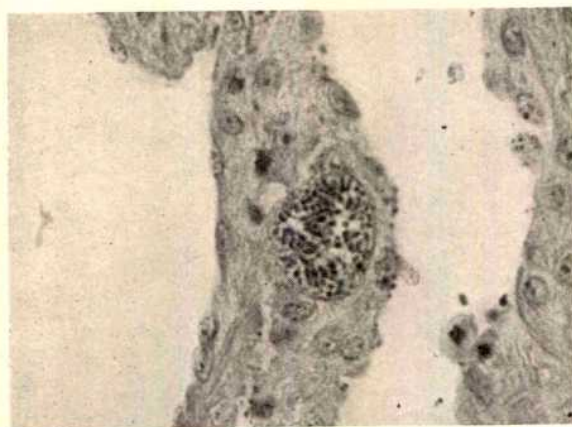
Sporozoites injected into the allantoic cavities of ten-day-old embryos produced infections and fewer early deaths than in six-day-old embryos. Intravenous inoculation of sporozoites produced no developing stages. Intramniotic inoculation was highly lethal and sudden deaths occurred associated with multiple haemorrhages.

In the infections which occurred from injections of sporozoites into the allantoic cavity parasites were only detected in the chorioallantoic membranes and not in the embryo itself. Immature and mature schizonts were seen in increasing numbers in histological sections of the chorioallantoic membranes on days 4, 5, 6 and 7 and numerous merozoites were found in the allantoic fluid on days 6 and 7. Schizonts, morphologically similar to those found previously, were also readily seen on days 8 and 9.

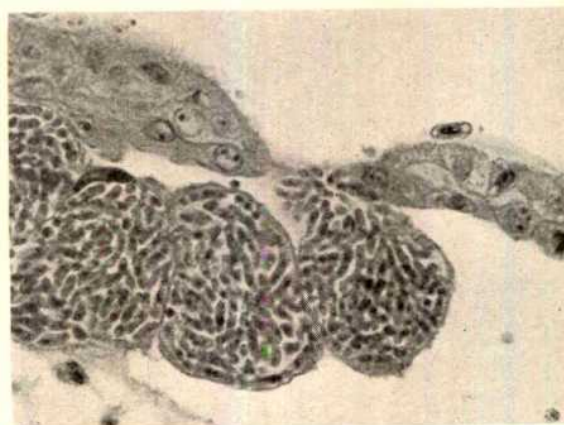
Table 1. THE EFFECT OF INTRODUCING SPOROZOITES OF *E. tenella* INTO AVIAN EMBRYOS BY DIFFERENT ROUTES

Number of sporozoites inoculated (10 ³)	Volume inoculated (ml.)	Site of injection	Age of embryos	Number injected	Early deaths		Findings
					No.	Time	
140	0.1	Intravenous	11	6	5	Within 72 h	One survivor, killed day 7, no parasites
140	0.1	Allantois	11	6	3	Within 72 h	In 2 survivors schizonts and oocysts present in C.A.M.* on days 5 and 10 after infection. One survivor negative on day 10
24-38	0.05-0.08	Intravenous	5	9	2	2-24 h	All negative for parasites when examined between 5 and 11 days after inoculation
48-240	0.1-0.5	Amnion	10	8	8	Within 24 h	Multiple haemorrhages
108	0.1	Allantois	6	12	7	Within 48 h	In 3 survivors schizonts were found in the C.A.M. on days 4, 5 and 7 after inoculation. No parasites were found in embryos examined on days 8 and 9 after inoculation
64	0.05	Allantois	10	20	1	Within 24 h	The C.A.M.'s of all embryos were positive for parasites when examined between days 4 and 11 of infection. Schizonts were found from days 4-10, gametocytes and oocysts from days 7-11. Nine deaths occurred on day 7; numerous schizonts were seen in the C.A.M. and numerous free merozoites were seen in the allantoic fluid

* Chorioallantoic membrane.

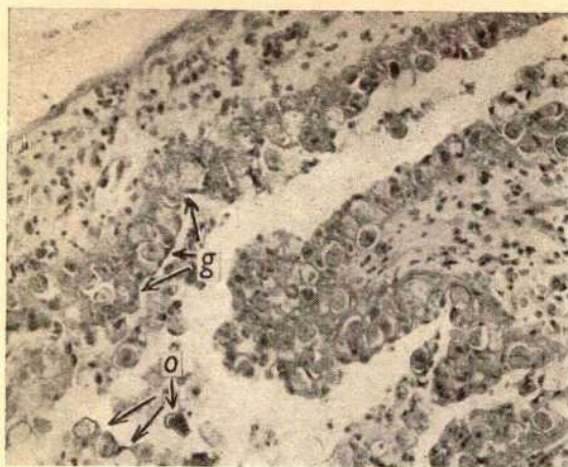


(a)

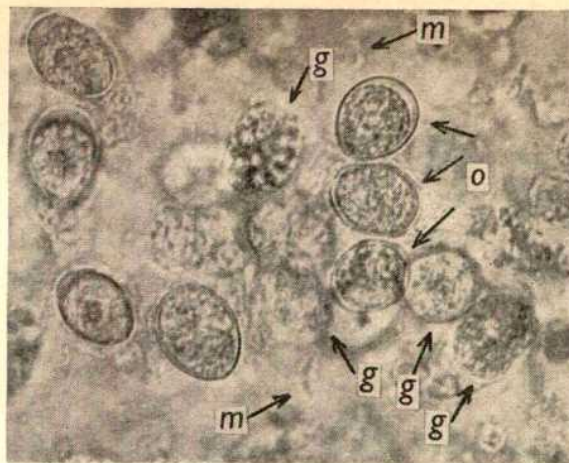


(b)

Fig. 1. Mature schizonts in chorioallantoic membranes; sections stained by haematoxylin and eosin ($\times 600$). (a) Schizont from 14-day-old embryo 4 days after inoculation; (b) schizonts from 16-day-old embryo 6 days after inoculation



(a)



(b)

Fig. 2. (a) Gametocytes (g) in epithelial cells of the chorioallantoic membrane and oocysts (o) free in the allantois of a 20-day-old embryo, 10 days after inoculation with sporozoites. Stained by haematoxylin and eosin ($\times 210$). (b) Oocysts (o), gametocytes (g) and merozoites (m) in the same embryo. Unstained preparation ($\times 600$).

These schizonts were similar to the second generation schizonts of *E. tenella* in the caeca of hatched chickens on days 5 and 6 after receiving sporulated oocysts *per os*. The nine embryos found dead on day 7 after inoculation were presumed to be due to the very large numbers of schizonts in the chorioallantoic membrane and haemorrhage which occurred in the allantoic cavities. Gametocytes and oocysts were found in small numbers on day 7 and were found in increasing numbers until day 11. Oocysts were found free in the allantoic fluid and in necrotic material sloughed off from the chorioallantoic membranes. Photomicrographs of the different stages observed are shown in Figs. 1 and 2. The oocysts recovered from a ten-day-old infection sporulated normally and produced typical caecal infections when inoculated into one-week-old chickens.

The results show that the life-cycle of *Eimeria tenella* can occur at a site other than the caeca of the chicken and this may have important consequences. The life-cycle appears to be slightly delayed (large numbers of oocysts occurring after day 9 of the infection) and the phase of schizogony extended.

Maintenance of the avian *Eimeria* normally necessitates frequent passaging in fowls kept in strict isolation. The growth of *E. tenella* in the chick embryo may provide a more convenient and less expensive alternative. It is possible that other species of *Eimeria* also develop in embryos. An extension of this work is proceeding with other species of *Eimeria* of the fowl and *E. stiedae* of the rabbit. The method may be of use in the evaluation of anti-parasitic substances and, in addition, provides bacteria-free material for immunological investigations. Of particular interest is the apparent prolongation of schizogony of the parasite and the opportunity now afforded for studying the parasite in a host in which resistance is minimal⁶.

P. L. LONG

Houghton Poultry Research Station,
Houghton,
Huntingdon.

¹ Pellerdy, L. P., *Catalogue of Eimeriidae (Protozoa; Sporozoa)* (Hungarian Academy of Sciences, 1963).

² Sharma, N. N., and Reid, W. M., *J. Parasit.*, **48**, Sect. 2, 33 (1962).

³ Davies, S. F. M., and Joyner, L. P., *Nature*, **194**, 996 (1962).

⁴ Long, P. L., and Rose, M. E., *Expl. Parasit.*, **18**, 1 (1965).

⁵ Horton-Smith, C., *Proc. First Intern. Parasitol. Cong., Rome, 1964* (in the press).

⁶ Horton-Smith, C., and Long, P. L., *Parasitology*, **55**, 401 (1965).

⁷ Farr, M. M., and Doran, D. J., *J. Protozool.*, **9**, 403 (1962).

⁸ Burnet, F. M., Stone, J. D., and Edney, M., *Austral. J. Exp. Biol. Med. Sci.*, **28**, 291 (1950).

MISCELLANEOUS

Effect of Certain Types of Paper on Sexual Maturation of the Insect *Pyrrhocoris apterus*

ON reading the article entitled "Juvenile Hormone Activity for the Bug, *Pyrrhocoris apterus*", by Sláma and Williams¹, I was struck by a likely correlation.

These authors report that this European insect fails to undergo normal sexual maturation in the presence of certain American papers (*New York Times*, *Wall Street Journal*, *Boston Globe*, *Science* and *Scientific American*), while British papers (*The Times* and *Nature*) had no inhibitory effect on the attainment of normal sexual maturity by this insect.

The active principle in the paper was found to be heat stable (100° C) and insoluble in water, but soluble in methanol, acetone, ether and petroleum ether. The authors ascertained that balsam fir, hemlock and yew contained the active principle, while red spruce, European larch and southern pine possessed very little activity.

The heart wood of Douglas fir contains dihydroquercetin as was first shown by Pew², and this is well known in the American wood pulping industry, for this substance interferes with pulping and tends to oxidize to quercetin, which is yellow on account of its cinnamoyl resonance; this spoils the appearance of the paper, so steps are taken to prevent its oxidation.

Moewus³ showed that quercetin occupies a central position in the development of sexuality in the primitive biflagellate alga *Chlamydomonas eugametos*, being a precursor of isorhamnetin required in the female gametes and of peonin required in the males.

It might therefore be profitable for entomologists to ascertain whether the inhibitory substance may not be dihydroquercetin, which could well interfere with sexual maturation if quercetin should be a step in the pathway to sexual maturity in these insects, as it is in *Chlamydomonas eugametos*.

C. A. B. CLEMETSON

Department of Obstetrics and Gynecology,
University of California Medical Center,
San Francisco,
California.

¹ Sláma, K., and Williams, M., *Proc. U.S. Nat. Acad. Sci.*, **54**, 411 (1965).

² Pew, J. C., *J. Amer. Chem. Soc.*, **70**, 3031 (1948).

³ Moewus, F., *Ann. New York Acad. Sci.*, **61**, 660 (1955).

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, November 1

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 9.30 a.m. and 2.15 p.m.—Colloquium on "HS 803 (Early Bird) and the Post Office Earth Station at Goonhilly".

PLASTICS INSTITUTE, PLASTICS PROPERTIES DISCUSSION CIRCLE (at the Mandeville Hotel, Mandeville Place, London, W.1), at 3 p.m.—Mr. D. R. Reid. "Ageing".

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 4 p.m.—Dr. D. A. Price Evans: "Genetics and Drug Idiosyncrasy".*

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. J. Ralph Audy (U.S.A.): "Red Mites and Typhus. I. Scrub-itch and the Ecologist".*

UNIVERSITY OF LONDON (in the Chemistry Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. D. J. Millen: "Molecular Measurements" (Inaugural Lecture).*

INSTITUTION OF MECHANICAL ENGINEERS, MEDICAL ENGINEERING WORKING PARTY (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Informal Discussion on "The Doctor's View of Engineering: The Engineer's View of Medicine".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 6 p.m.—"The Island" (film with introduction by Prof. C. A. Fisher).

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dame Kathleen Lonsdale, D.B.E., F.R.S.: "Crystallography as a Research Tool in Chemistry" (Jubilee Memorial Lecture).

Tuesday, November 2

UNIVERSITY OF LONDON (at Imperial College of Science and Technology, London, S.W.7), at 1.30 p.m.—Mr. E. F. Schumacher: "Guides to Action: Is There a Universal Teaching of Mankind?"*

UNIVERSITY OF LONDON (at the Institute of Obstetrics and Gynaecology, Hammersmith Hospital, London, W.12), at 3 p.m.—Prof. P. Polani: "Human Chromosomes and their Abnormalities (II)".*

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, London, S.W.1), at 5.30 p.m.—Mr. J. A. Banks: Presidential Address.

ROYAL INSTITUTION, LIBRARY CIRCLE (at 21 Albemarle Street, London, W.1), at 5.30 p.m.—Dr. H. D. Anthony: "The Study of Scenery".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. J. B. Kimmith: "Angiology of the Human Lymph System". (Fourth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation).*

UNIVERSITY OF LONDON (in the Large Physics Lecture Theatre, Westfield College, Kidderpore Avenue, London, N.W.3), at 5.30 p.m.—Dr. J. Sicher (Czechoslovak Academy of Science): "The Steric Structure of Medium Ring Compounds and Approaches to Conformational Analysis".*

UNIVERSITY OF LONDON (at Queen Mary College, Mile End Road, London, E.1), at 6 p.m.—Prof. W. R. Sears (University of Cornell): "Fundamental Ideas". (First of four lectures on "Magneto-Fluid Dynamics").*

PLASTICS INSTITUTE, LONDON SECTION (at the Wellcome Building, Euston Road, London, N.W.1), at 6.30 p.m.—Mr. H. Birtles: "The Government Services for Exporters".

Tuesday, November 2—Wednesday, November 3

INSTITUTION OF MECHANICAL ENGINEERS, HYDRAULIC PLANT AND MACHINERY GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1)—Symposium on "Surges in Pipelines".

PLASTICS INSTITUTE (at the Connaught Rooms, Great Queen Street, London, W.C.2)—Conference on "Automatic Control for Plastics Processing".

Wednesday, November 3

BIOMETRIC SOCIETY—BRITISH REGION (at the Wellcome Building, Euston Road, London, N.W.1), at 2.30 p.m.—Symposium on "Non-Parametric Methods". Principal Speakers: Mr. F. Sprent, Prof. F. Armitage, Mr. I. D. Hill and Dr. G. B. Wetherill.

COLOUR GROUP (Great Britain) (in the Physics Department, Imperial College, Prince Consort Road, London, S.W.7), at 3 p.m.—Mr. A. C. Hardy: "The Colour Coordination of Tiles and other Factory Coloured Products for the Building Industry in relation to BS 2660"; Mr. F. Malkin: "An Experiment in the Colour Tolerance of Glazed Wall Tiles".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. P. A. Bradley and Mr. C. Clarke: "Atmospheric Radio Noise and Signals Received on Directional Aerials at High Frequencies"; Mr. C. Clarke, Mr. P. A. Bradley and Mr. D. E. Mortimer: "Characteristics in Atmospheric Radio Noise Observed at Singapore".

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "The Distribution and Application of Electricity in Building Sites".

ROYAL METEOROLOGICAL SOCIETY (at 49 Cromwell Road, London, S.W.7), at 5.30 p.m.—Mr. M. H. Freeman: "Long Range Weather Forecasting".

ROYAL MICROSCOPICAL SOCIETY (at the Royal Society, Burlington House, Piccadilly, London, W.1), at 5.30 p.m.—Mr. R. W. Horne: "Electron Microscopy on the Macromolecular Level".

SOCIETY OF NON-DESTRUCTIVE EXAMINATION (in the Weir Lecture Hall, Royal Institution of Naval Architects, 10 Upper Belgrave Street, London, W.1), at 5.30 p.m.—Mr. B. E. Byrne: "The Selection, Training and Briefing of N.D.T. Personnel" (Third Croxson Memorial Lecture).

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. J. Ralph Audy (U.S.A.): "Red Mites and Typhus. II. Akamushi: The Red Mites of Japan".*

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, ELECTRO-ACOUSTICS GROUP (at 9 Bedford Square, London, W.C.1), at 6 p.m.—Dr. B. L. Clarkson: "High Intensity Acoustic Noise Sources".

Thursday, November 4

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 10.30 a.m.—Discussion Meeting on "Advanced Methods of Energy Conversion—Magnetohydrodynamic Power Generation" organized by Dr. L. Rotherham, F.R.S.

SOCIETY OF CHEMICAL INDUSTRY, MICROBIOLOGY GROUP (at the School of Pharmacy, 29 Brunswick Square, London, W.C.1), at 2.30 p.m.—Meeting on "Recent Developments in Microbiology in the Beverage Industries".

UNIVERSITY OF LONDON (at King's College Hospital Medical School, Denmark Hill, London, S.E.5), at 4.30 p.m.—Prof. H. Rahn (U.S.A.): "The Physiology of the Diving Women of Korea and Japan".*

INSTITUTION OF ELECTRICAL ENGINEERS (joint meeting with the Institution of Electronic and Radio Engineers, at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "The Selection of Topics for Research in Electrical and Electronic Engineering" opened by Sir Robert Cockburn, K.B.E., Dr. E. Eastwood, C.B.E., Prof. Sir Willis Jackson, F.R.S., and Dr. L. Rotherham, F.R.S.

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Mr. D. C. Phillips: "The Structure of Lysozyme". (Fifth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation).*

UNIVERSITY OF LONDON (in the Large Physics Lecture Theatre, Westfield College, Kidderpore Avenue, London, N.W.3), at 5.30 p.m.—Dr. J. Sicher (Czechoslovak Academy of Science): "Conformational Equilibria in Disubstituted Cyclohexanes".*

INSTITUTE OF REFRIGERATION (at the National College for Heating, Ventilating, Refrigeration and Fan Engineering, Southwark Bridge Road, London, S.E.1), at 6 p.m.—Dr. H. N. Daglish: "Applications of Low Temperatures in Satellite Communications".

INSTITUTION OF MECHANICAL ENGINEERS, STEAM PLANT GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "The Relative Merits of Once-Through Versus Other Types of Boiler Circulation".

UNIVERSITY OF LONDON (at Queen Mary College, Mile End Road, London, E.1), at 6 p.m.—Prof. W. R. Sears (University of Cornell): "Steady Flow". (Second of four lectures on "Magneto-Fluid Dynamics").*

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (joint meeting with the Brunel College Chemical Society, at Brunel College, Woodlands Avenue, London, W.3), at 7 p.m.—Mr. M. C. Hyde: "Collecting Information on the Chemical Industry".

Friday, November 5

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. R. W. Cannon, Mr. G. C. Rider and Mr. D. Wilkinson: "Operational Experience with Tropospheric Scatter Systems".

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (joint meeting with the Fine Chemicals Group, at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. W. Gerrard: "Studies in Boron Chemistry: Organic Analogues of Heterocycles".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Dr. D. C. Phillips: "The Structure and Function of Lysozyme".

Saturday, November 6

INNER LONDON EDUCATION AUTHORITY (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Mr. Michael Boorer: "Rhinoecroses".*

Monday, November 8

PLASTICS INSTITUTE, PLASTICS PROPERTIES DISCUSSION CIRCLE (at the Mandeville Hotel, Mandeville Place, London, W.1), at 3 p.m.—Mr. R. A. Horsley: "Creep Properties of Thermoplastics".

SOCIETY OF CHEMICAL INDUSTRY, COLLOID AND SURFACE CHEMISTRY GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Mr. G. P. C. Chambers: "The Colloidal and Surface Properties of Clay Minerals".

INSTITUTION OF ELECTRICAL ENGINEERS, CONTROL AND AUTOMATION DIVISION (at Hatfield College of Technology, Hatfield), at 6 p.m.—Discussion on "Is Laboratory Work Really Necessary?" opened by Dr. K. R. Sturley.

INSTITUTION OF MECHANICAL ENGINEERS, NUCLEAR ENERGY GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Fuel Element Behaviour in Gas-Cooled Reactors".

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, W.C.2), at 6.30 p.m.—Mr. A. G. Page: "Some Manufacturing and Processing Techniques in the Electronics Industry".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Lord Rennell of Rodd: "Heinrich Barth and the Opening Up of Central Africa".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

RESEARCH ASSISTANT or RESEARCH FELLOW (graduate in physics or engineering) in THE DEPARTMENT OF CIVIL ENGINEERING, to work on problems of creep in concrete—The Registrar, King's College (University of London), Strand, London, W.C.2 (November 5).

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

RESEARCH ASSISTANT (with a degree in mathematics, physics or chemistry and preferably some postgraduate experience) IN THE CHEMISTRY DEPARTMENT, to help in the devising and running of computer programmes for large problems in crystallography and theoretical chemistry under the supervision of Prof. D. W. J. Cruickshank—The Secretary of the University Court, University of Glasgow, Glasgow, W.2 (November 5).

ASSISTANT LIBRARIAN (university graduate and chartered librarian)—The Principal, Lanchester College of Technology, Priory Street, Coventry (November 8).

GRADUATE IN THE DEPARTMENT OF BOTANY for work on the physiology of insect-pathogenic fungi—The Registrar, University Senate House, Bristol, 2 (November 8).

RESEARCH ASSISTANT (preferably with a degree in engineering) IN THE DEPARTMENT OF MECHANICAL ENGINEERING IN THE FACULTY OF SCIENCE, to operate research equipment engaged in investigation of combustion in reciprocating engines, to help in analysis of observations, and to assist in the development of instrumentation—The Registrar, The University, Manchester, 13, quoting Ref. 185/65 (November 10).

LECTURER (graduate with some years of teaching experience at degree level, and preferably an interest in control systems work or in computing) TO ASSIST IN THE FACULTY OF TECHNOLOGY—The Academic Registrar, Enfield College of Technology, Enfield, Middlesex (November 12).

SPECIAL TERMINABLE ASSISTANTSHIP IN APPLIED PSYCHOLOGY (full-time post) with duties in either industrial or educational psychology—The Secretary, University College, Cork, Republic of Ireland (November 12).

SUPERINTENDENT (graduate in physics, preferably with experience of administration in industry, in the government service or in universities) OF LABORATORIES IN THE DEPARTMENT OF PHYSICS—The Registrar, University College of Swansea, Singleton Park, Swansea (November 12).

LECTURER IN MEDICAL PHYSICS—The Registrar, The University, Manchester, 13, quoting Ref. 188/65 (November 13).

READER IN STATISTICS AT THE LONDON SCHOOL OF ECONOMICS—The Academic Registrar, University of London, Senate House, London, W.C.1 (November 18).

LECTURERS/ASSISTANT LECTURERS (3) IN THE DEPARTMENT OF PURE MATHEMATICS—The Registrar, University College of Wales, Aberystwyth (November 19).

CHAIR OF PURE MATHEMATICS—The Registrar, University of Newcastle upon Tyne, 6, Kensington Terrace, Newcastle upon Tyne 2 (November 20).

LECTURER IN PHILOSOPHY—The Secretary of the University Court, University of Glasgow, Glasgow, W.2 (November 20).

LECTURER IN SOCIAL ANTHROPOLOGY—The Secretary, The Queen's University, Belfast, Northern Ireland (November 20).

READER, SENIOR LECTURER, LECTURER OR ASSISTANT LECTURER IN PHYSICS IN THE SCHOOL OF MATHEMATICS AND PHYSICS—The Registrar, University of East Anglia, Earlham Hall, Norwich, NOR 88G (November 20).

CHAIR OF ORGANIC CHEMISTRY at the University of Tasmania, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 22).

CHAIR OF MICROBIOLOGY IN THE FACULTY OF MEDICINE—The Secretary, The Queen's University, Belfast, Northern Ireland (November 24).

CHAIR OF BIOCHEMISTRY—The Secretary of the University Court, The University, Glasgow (November 26).

CHAIR OF PHYSIOLOGY—The Secretary of the University Court, The University, Glasgow (November 26).

CHAIR OF SOCIAL AND PREVENTIVE MEDICINE at the University of the West Indies—The Secretary, Senate Committee on Higher Education Overseas, University of London, Senate House, London, W.C.1 (November 29).

LECTURER (preferably with special interests and qualifications in terrestrial zoology with emphasis on entomology, especially the ecology of insects) IN ZOOLOGY at Victoria University of Wellington, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, November 30).

LECTURER (with a distinguished academic record and preferably with a research interest in one of the following fields: vertebrate physiology, embryology or tissue culture, cell biology, invertebrate zoology, other than entomology or marine biology) IN THE DEPARTMENT OF ZOOLOGY, School of Biological Sciences, University of New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 30).

LECTURERS (2) (one preferably theoretical reactor physicist with experience in modern computational techniques, and the other well qualified in the fields of fluid dynamics and heat transfer) IN THE DEPARTMENT OF NUCLEAR ENGINEERING—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (November 30).

LECTURER (Grade II) IN THE DEPARTMENT OF SOCIOLOGY—The Registrar, University Senate House, Bristol, 2 (December 1).

TEMPORARY LECTURER IN ZOOLOGY at the University of Western Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, December 4).

SENIOR FELLOW OR FELLOW; and a SENIOR RESEARCH FELLOW OR RESEARCH FELLOW IN THE DEPARTMENT OF MATHEMATICS, Institute of Advanced Studies, Australian National University—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (December 6).

SENIOR LECTURER/LECTURER IN THE SCHOOL OF TEXTILE TECHNOLOGY, University of New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, December 12).

ENTOMOLOGIST (national of the United Kingdom or the Republic of Ireland, with a B.Sc. degree and preferably experience of African conditions) in Bechuanaland, to undertake a survey of the cotton, maize and sorghum pests and make recommendations as to their control—The Appointments Officer, Ministry of Overseas Development, Room 301, Eland House, Stag Place, London, S.W.1, quoting Ref. 213/19/06.

HEAD OF THE MATHEMATICS DEPARTMENT—The Master, Dulwich College, London, S.E.21.

LECTURER (with a good honours degree in zoology and preferably a higher degree)—The Registrar, Regional College of Technology, Leicester.

RESEARCH ASSISTANT IN THE DEPARTMENT OF AGRICULTURAL BOTANY for work on soil microbiology—The Registrar, University College of Wales, Aberystwyth.

TECHNICIAN/SENIOR TECHNICIAN IN THE DEPARTMENT OF BIOLOGICAL SCIENCES—The Secretary, Bradford Institute of Technology, Bradford 7.

The Royal Institute of Chemistry. Lecture Series 1964. No. 3: Orbital Degeneracy and Spin Resonance of Free-Radical Ions. By Dr. A. Carrington. (Meldola Medal Lecture 1964.) P. 22. 4s. No. 4: Relaxation Methods for Studying Very Rapid Reactions in Solution. By Dr. D. N. Hagne. Pp. 16 4s. No. 5: The Chemistry of Carbenes. By Dr. A. Ledwith. Pp. 66. 6s. (London: The Royal Institute of Chemistry.) [149]

British Broadcasting Corporation. B.B.C. Engineering Monograph No. 58: Sine-Squared Pulse and Bar Testing in Colour Television. Part 1: Pulse and Bar Testing of the Chrominance Channel. Part 2: An Augmented Pulse and Bar Waveform for Testing the Complete Colour Signal. By L. E. Weaver. Pp. 27. (London: British Broadcasting Corporation, 1965.) 6s. [149]

The Royal Observatory, Edinburgh. Publications—Vol. 3, No. 9: The Cygnus II Association. I. Intercomparison of Photometrics with the Edinburgh and Hamburg Schmidt Telescopes. By L. C. Lawrence and V. C. Reddish. Pp. 279-309. (Edinburgh and London: H.M. Stationery Office, 1965.) 9s. net. [149]

London and Home Counties Regional Advisory Council for Technological Education. Engineering Education in the Region. Ninth edition. P. 48. (London: London and Home Counties Regional Advisory Council for Technological Education, 1965.) 5s. [149]

Sixth Report from the Estimates Committee together with the Minutes of the Evidence taken before Sub-Committee E and Appendices, Session 1964-65—Recruitment to the Civil Service. Pp. xxxviii+269. (London: H.M. Stationery Office, 1965.) 26s. net. [149]

Library Association Pamphlet No. 24: Central Library Storage of Books. By P. W. Plumb. Pp. 57. (London: Library Association, 1965.) 16s.; (L.A. members 12s.) [149]

Standing Committee on National Parks. Study No. 1: Afforestation in National Parks—a Policy for Tree Planting and Management in National Parks. Pp. 13. 1s. Study No. 2: The Future of National Parks and the Countryside. Pp. 17. 1s. (London: Standing Committee on National Parks of the Council for the Preservation of Rural England and the Protection of Rural Wales, 1965.) [149]

Other Countries

United States Department of the Interior: Geological Survey. Bulletin 1133-G: Geology and Hydrology of the Hartford Research Center, CANEL Site, Middletown, Connecticut. By J. A. Baker, S. M. Lang and M. P. Thomas. Pp. iv+42+plates 1 and 2. Bulletin 1199-F: Bauxite Deposits of the Springvale District, Georgia. By Lorin D. Clark. Pp. iii+24+plate 1. Water-Supply Paper 1578: Geology and Ground-Water Resources of the Bristol-Plainville-Southington Area, Connecticut. By A. M. La Sala, Jr. Pp. iv+70+plates 1-3. Water-Supply Paper 1615-G: Testing Procedures and Results of Studies of Artificial Recharge in the Grand Prairie Region, Arkansas. By R. T. Sniegocki, F. H. Bayler, Kyle Engler, and J. W. Stephens. Pp. iv+56. 25 cents. Water-Supply Paper 1649: Water for Oregon. By K. N. Phillips, R. C. Newcomb, H. A. Swenson, and L. B. Laird. Pp. x+150. 60 cents. Water-Supply Paper 1772: Geology and Ground-Water Resources of Prowers County, Colorado. By Paul T. Voegeli, Sr. and Lloyd A. Hershey. Pp. vi+101+plates 1-8. Water-Supply Paper 1788: Ground-Water Resources and Geology of Niobrara County, Wyoming. By Harold A. Whitcomb. With a section on Chemical Quality of the Ground Water by T. Ray Cummings. Pp. v+101+plates 1-3. (Washington, D.C.: Government Printing Office, 1965.) [149]

United States Department of the Interior: Geological Survey. Water-Supply Paper 1790-B: Summary of Floods in the United States During 1960. By J. O. Rostvedt. Pp. vi+147. 45 cents. Water-Supply Paper 1809-C: Ground Water in the Upper Star Valley, Wyoming. By Eugene H. Walker. Pp. iv+27+plate 1. 15 cents. Water-Supply Paper 1809-G: Ground-Water Resources on North Park and Middle Park, Colorado—a Reconnaissance. By Paul T. Voegeli, Sr. Pp. iv+54+plates 1 and 2. Professional Paper 491-A: Introduction, Spread and Area Extent of Saltcedar (*Tamarix*) in the Western States. By T. W. Robinson. Pp. iii+12+plate 1. Professional Paper 498-B: Variations in Chemical Character of Water in the Englishtown Formation, New Jersey. By Paul R. Seaber. Pp. iii+35. 30 cents. (Washington, D.C.: Government Printing Office, 1965.) [149]

The Law in Nature and the Dynamics of Openness. By Ernest L. Remits. Pp. iv+96. (Ottawa: The Runge Press, Ltd., 1965.) [149]

World Health Organization. Protection of the Public in the Event of Radiation Accidents. (Proceedings of a Seminar jointly sponsored by the Food and Agriculture Organization of the United Nations, the International Atomic Energy Agency, and the World Health Organization, Geneva, 18-22 November 1963.) Pp. 370. (Geneva: World Health Organization; London: H.M. Stationery Office, 1965.) 22 Sw. francs; 37s. 6d.; 7.25 dollars. [149]

Annals of the New York Academy of Sciences. Vol. 126, Article 1: Viral Diseases of Poikilothermic Vertebrates. By M. H. Attleberger and 71 other authors. Pp. 1-680. (New York: New York Academy of Sciences, 1965.) 12 dollars [149]

Proceedings of the Preliminary Meeting on College Level Mathematics Education, Katada, 1964. (Under the auspices of the U.S.-Japan Program on Scientific Co-operation.) Pp. xi+96. (Tokyo: Japan Society for the Promotion of Science, 1965.) [149]

Editorial and Publishing Offices of "NATURE"

MACMILLAN (JOURNALS) LIMITED

4 LITTLE ESSEX STREET, LONDON, W.C.2

Telephone Number. Temple Bar 6633. Telegrams: Phusis London W.C.2

Annual subscription: Inland £14, Overseas £13 15s., payable in advance, postage paid to any part of the world.

Advertisements only should be addressed to

T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2

Telephone Number: Holborn 4743

Registered as a newspaper at the General Post Office

Copyright © Macmillan (Journals) Limited, October 30, 1965

NATURE

No. 5010

SATURDAY, NOVEMBER 6, 1965

Vol. 208

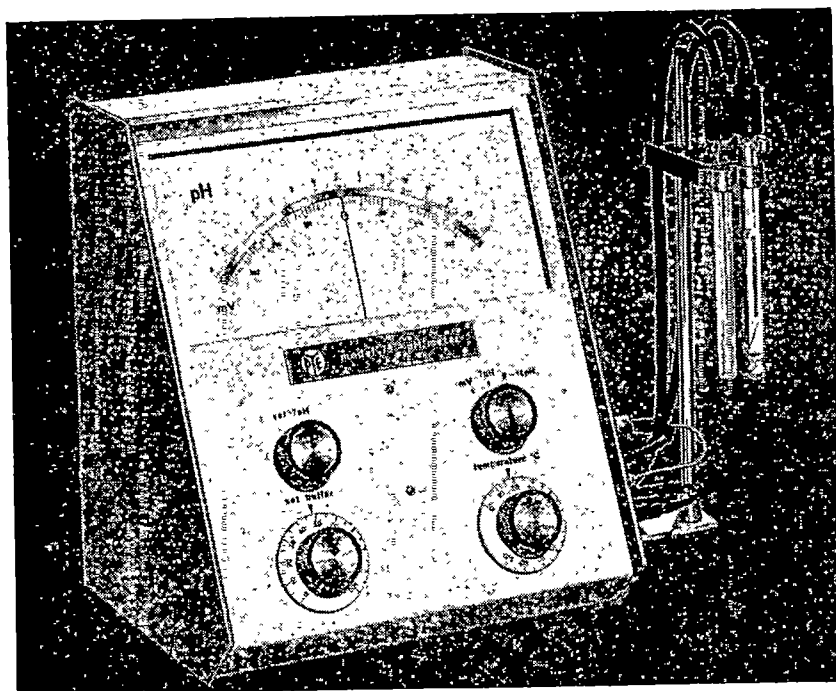
Published by
MACMILLAN (JOURNALS) LTD.

CONTENTS

	Page
THE ROAD TO PEACE	513
NATURALLY OCCURRING PHENOLIC COMPOUNDS. By Sir Robert Robinson, O.M., F.R.S.	515
HIGH-PRESSURE PHYSICS. By Dr. R. S. Bradley	515
SPACE DYNAMICS. By D. G. King-Hele	516
MATERIALS AT HIGH TEMPERATURES. By Dr. E. H. Andrews	517
THE WORLD OF FISH. By Dr. L. Harrison Matthews, F.R.S.	517
PURPOSE AND MEANING OF A UNIVERSITY. By R. Brightman	518
MIMICRY AND THE EVOLUTION OF ANIMAL COMMUNICATION. By Dr. W. Wickler	519
THE JUVENILE HORMONE. By Sir Vincent Wigglesworth, C.B.E., F.R.S.	522
OBITUARIES :	
DR. H. R. MARSTON, F.R.S. By Dr. E. G. Holmes	524
PROF. ARTHUR HOLMES, F.R.S. By Prof. F. H. Stewart, F.R.S.	525
SIR ERNEST THOMAS FISK. By Sir Lionel Hooke	526
NEWS AND VIEWS	527
USE OF PIGS IN BIOMEDICAL RESEARCH. By Dr. L. K. Bustad and R. O. McClellan	531
NATURAL SELECTION AND TRANSMISSIBLE DISEASE. By Prof. N. A. Barnicot	535
A SIMPLE ASSESSMENT OF PARTITION DATA FOR CORRELATING STRUCTURE AND BIOLOGICAL ACTIVITY USING THIN-LAYER CHROMATOGRAPHY. By Dr. C. B. C. Boyce and Dr. B. V. Milborrow	537
SPECTRAL DENSITY FUNCTIONS OF HYDROMAGNETIC EMISSIONS AT HIGH LATITUDE. By Dr. Alv Egeland	539
COINCIDENCE OF MAGNETIC DISTURBANCES WITH LOCAL EARTHQUAKES RECORDED FROM THE ETHIOPIAN RIFT SYSTEM. By Prof. Pierre Gouin	541
RELATIVE SIMILARITIES IN ONE DIMENSION. By Prof. J. W. Carmichael, Prof. R. S. Julius and Dr. P. M. D. Martin	544
A.C. RESISTIVITY OF ANODIC OXIDE FILMS ON VALVE METALS. By Dr. G. C. Wood and C. Pearson	547
COLOUR OF 'PURE' WATER. By Dr. John E. Tyler	549
RELATIONSHIP BETWEEN THE ELECTROMYOGRAM AND THE FORCE OF THE ISOMETRIC REFLEX RESPONSE OF NORMAL HUMAN SUBJECTS. By Alex M. Clarke	551
PURINE RICH REGULATORY SEQUENCES IN MESSENGER RIBONUCLEIC ACID. By Prof. Roy H. Doi	552
CHANGES IN THE t-RNA METHYLATING ENZYMES DURING INSECT METAMORPHOSIS. By Dr. B. S. Baliga, Prof. P. R. Srinivasan and Prof. Ernest Borek	555
INFLUENCE OF HETEROLOGOUS HISTONE ON THE SYNTHESIS OF CELLULAR AND VIRAL RIBONUCLEIC ACID AND PRODUCTION OF HAEMAGGLUTININ. By A. G. Bukrinskaya, A. K. Gitelman and I. M. Shapiro	557
SUPPRESSION OF THE PUPILLARY LIGHT REFLEX IN BINOCULAR RIVALRY AND SACCADIC SUPPRESSION. By M. Lorber, Dr. B. L. Zuber and Dr. L. Stark	558
FUNCTIONAL SIGNIFICANCE OF SWEAT GLANDS AND SEBACEOUS GLANDS IN SEALS. By John K. Ling	560
INTERFEROMETRIC ESTIMATION OF EFFECTS OF TESTOSTERONE AND METHANDROSTENOLONE ON THE DRY MASS OF VARIOUS RAT MUSCLES. By Dr. W. Staubli and Dr. T. Okada	562
A PHAGOCYTIC FUNCTION OF HASSALL'S CORPUSCLES. By Dr. J. N. Blau	564
ANDROGEN-OESTROGEN-INDUCED TUMOURS: LACTIC DEHYDROGENASE RELATIVE TO CHAET-EPITHELIOMA INDUCTION IN THE SYRIAN HAMSTER. By Prof. F. T. Algard, A. H. Dodge and Prof. H. Kirkman	567
TITRATION CURVES DEPICTING CENTRAL STIMULANT, DEPRESSANT AND DUAL STIMULANT-DEPRESSANT ACTION OF GLUTARIMIDE HOMOLOGUES IN MICE. By Dr. A. Shulman, Glenda M. Laycock and J. A. Henry	568
BREEDING A PELAGIC COPEPOD, <i>Euterpina acutifrons</i> (Dana), IN THE LABORATORY. By Heinz W. Neunes and Gian-Franco Pongolini	571
OPTICAL ROTATORY DISPERSION OF A CHLOROPLAST PREPARATION. By Dr. Bacon Ee	573

(Continued overleaf)

Pye model 79 pH meter



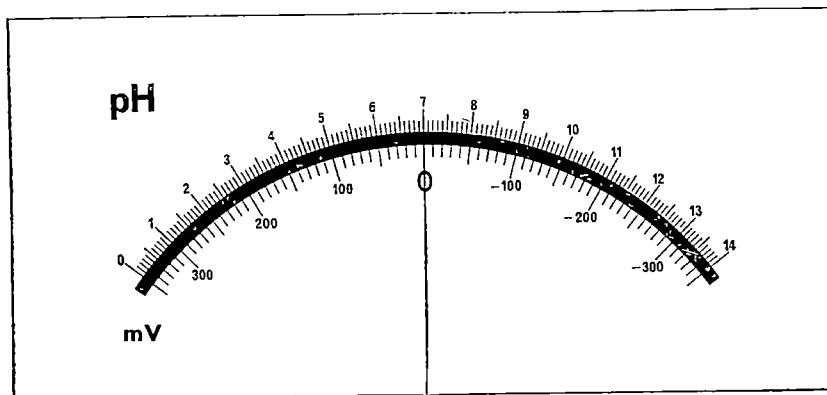
The Pye Model 79 pH Meter is a new instrument based on new principles. Moderate in cost and robust in construction, it provides exceptionally good accuracy and discrimination. It sets new standards in this class of measuring instrument for stability and freedom from effects of power supply variations.

Based upon the Pye taut band microammeter and a specially developed transistorised power stabiliser, the Model 79 represents a major advance in general purpose pH meter design.

Ranges of measurement are 0 to 14 pH or +350 to -350 mV which are covered on a 7 inch scale. With a precise millivolt measuring range, the Model 79 has many applications as a high impedance millivoltmeter.

Facilities are provided for both automatic and manual temperature compensation for slope and zero shift throughout the range of 0 to 100°C. A calibrated buffer control can be used to indicate electrode deterioration. An output of 5-0-5 mV is available for a potentiometric recorder.

The Model 79 represents exceptional value for the modern chemical laboratory. Please write for full details.

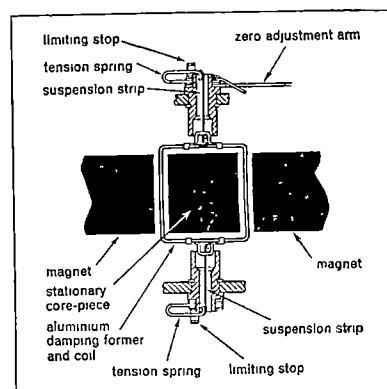


Accessories

The Model 79 is supplied with an integral electrode clamp and a Pye Ingold combined glass/reference electrode which has a measuring range of 0 to 13 pH, a temperature range of -5 to +70°C (intermittent use to 100°C) and an electrical null point of $E_0.7$ for long-term stability. Additional accessories include a nickel resistance thermometer, a laboratory stand, a wide range of $E_0.7$ electrodes, etc

Clear Scale

The fascia of the Model 79 is at an ideal viewing angle and parallax errors are eliminated by the knife-edge pointer and backing-mirror on the meter. These features, combined with bold and well-defined calibration on the unusually wide scale, enable readings to be taken to 0.02 pH without difficulty.



Taut Band Meter

Special features of the Pye taut band, or pivotless, panel meter are its invulnerability to damage by heavy electrical overloads and its remarkable freedom from mechanical hysteresis, friction, sticking and deterioration. This gives very high sensitivity coupled with high accuracy and long term stability. The Pye suspension system employs strong incorrodible gold alloy strip

LETTERS TO THE EDITOR

	Page		Page
ASTROPHYSICS		Prolonged Administration of Atropine or Histamine in a Silicone Rubber Implant.—Paul Bass, R. A. Purdon and J. N. Wiley	
Rotational Period of the Planet Mercury.—Dr. G. Colombo	575	Facilitation as a Function of Temporal Spacing of Stimuli in Intracranial Self-stimulation.—Prof. J. A. Deutsch and R. Collins	591
ASTRONOMY		IMMUNOLOGY	
Elementary Nebulosity in the Vicinity of the Cetus Arc.—Dr. J. Meaburn	575	A Specific Agglutinin in the Snail <i>Otala (Helix) lactea</i> .—Prof. William C. Boyd and Rebecca Brown	593
PHYSICS		Differentiation between Cytophilic Antibody and Opsonin by a Macrophage Phagocytic System.—W. E. Parish	594
Parathéodory's Principle and the Kelvin Statement of the Second Law.—J. Dunning-Davies	576	BIOLOGY	
Ionizing Radiation: a Potential Lightning Hazard?—P. J. Gillespie	577	Signal Value of the Genital Tassel in the Male <i>Tilapia macrochir</i> Blgr. (Pisces: Cichlidae).—Dr. W. Wickler	595
GEOLOGY		Emission of Feulgen-positive Particles during the <i>in vitro</i> Maturation of Toad Oocytes.—Prof. J. Brachet	596
Observations on the Mont Pelée Eruption of 1902 and their Significance to Ordovician Welded Tuffs of County Mayo, Eire.—Dr. J. McManus	578	Wheel-running Preferences of <i>Peromyscus</i> .—Prof. J. Lee Kavanau and Prof D. H. Brant	597
Origin of Fossil 'Zoophycos'.—Dr. Miroslav Plička	579	Methylcholanthrene Carcinogenesis influenced by Vegetable Extracts.—V. Vendég	598
METALLURGY		ANATOMY	
Dispersion-softened Zinc Alloys.—W. H. McCarthy, Prof. J. C. Shyne and Prof. O. D. Sherby	579	Sex Differences in the Rate of Tissue Regeneration in the Rabbit's Ear.—Dr. J. Joseph and Dr. Mary Dyson	599
CHEMISTRY		Possible Role of Neuroglia.—Prof. C. P. Wendell-Smith and Prof. M. J. Blunt; Dr. L. Hertz	600
Structure of a Diolefin Complex of Platinum(II) $C_4H_{14}O_3PtCl_2$.—D. Gibson, C. Oldham, Prof. J. Lewis, D. Lawton, Prof. R. Mason and G. B. Robertson	580	ENTOMOLOGY	
Ultra-purification by Separation of Aerosol Particles.—Dr. Stefan Behrendt	581	An Improved Method for obtaining, <i>in vitro</i> , Uniform Cell Monolayer Sheets from Tissues of the Tick, <i>Hyalomma dromedarii</i> (Ixodidae).—Dr. M. G. R. Varma and Miss Wendy Wallers	602
Polyamide Layer Chromatography of Oestrogens.—Yih Chih Tung and Prof. Kung-Tsung Wang	581	MICROBIOLOGY	
Slow Inversion at the Nitrogen Atom in Certain Amides.—Dr. T. H. Siddall and Dr. C. A. Prohaska	582	Occurrence of Dimorphic Forms of <i>Mycobacterium phlei</i> .—D. E. S. Stewart-Tull	603
BIOCHEMISTRY		VIROLOGY	
Proteins of Wheat Flour.—Dr. C. B. Coulson and Dr. A. K. Sim	583	Propagation and Properties of Hepatitis Virus.—Dr. Z. F. Ch. Kachani	605
Tumour-promoting Agents in Unburned Cigarette Tobacco.—Dr. Fred G. Book, Raymond J. Shamberger and Huston K. Myers	584	'Ring-forms' of Reovirus Particles.—Dr. Frederick J. Grose, Prof. Maurice H. Bernstein and Dr. A. Martin Lerner	606
Synthesis of Ribonucleic Acid by <i>Escherichia coli</i> during Inhibition by Azetidine-2-carboxylic Acid.—Dr. Joan M. Midgley and Dr. D. G. Wild	585	GENETICS	
'Lipid A' Component from the Cell-walls of <i>Pseudomonas aeruginosa</i> .—Dr. K. Clarke, Dr. G. W. Gray and D. A. Reaveley	586	'Complement Fractionation' in a Natural Hybrid between <i>Rubus procerus</i> Muell. and <i>R. laciniatus</i> Willd.—Dr. R. K. Bammi	608
Isozymes of Arylamidase (Aminopeptidase): Source of Blood Aminoacyl Naphthylamidase in the Rat.—Dr. Benito Monis	587	SOIL SCIENCE	
Evidence for the Release of Individual Amino-acids from the Resting Human Forearm.—Dr. D. R. London, Dr. T. H. Foley and C. G. Webb	588	Thermistor Hygrometer for determining the Free Energy of Moisture in Unsaturated Soils.—B. G. Richards	608
PHYSIOLOGY		Photographic Reproduction of Soil Fabric Patterns.—D. Lafeber and M. Kurbanovic	609
Quantum Energy Specificity of Simple Photoreceptor Units.—Prof. William W. Dawson	589		
Presynaptic Effects of Potassium Ion on the Mammalian Neuromuscular Junction.—Dr. Rodney L. Parsons, Prof. William W. Hofmann and Prof. George A. Feigen	590		

AGAROSE

A neutral galactose polymer obtained from Agar by a unique method using reagents, having no toxicity.

- * Negligible electroendosmotic properties.
- * High gel strength.
- * Non-toxic.
- * Minimum adsorption properties.

This product and many ENZYMES and RELATED BIOCHEMICALS of the highest possible integrity are produced entirely in our own laboratories.

Send for descriptive brochure

SERAVAC LABORATORIES (PTY.) LTD.

Moneyrow Green, Holypport,
Maidenhead, Berks., England.

The latest of **GRIFFIN'S**

STATISTICAL MONOGRAPHS & COURSES

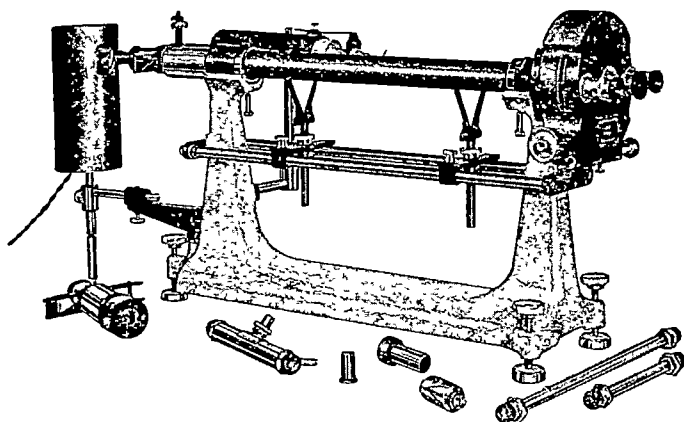
- | | | |
|----|--|-----|
| | <i>Kendall & Moran</i> | |
| 10 | GEOMETRICAL PROBABILITY | 28s |
| 11 | <i>Wegner</i> AN INTRODUCTION
TO SYMBOLIC PROGRAMMING | 38s |
| 12 | <i>David</i> THE METHOD OF
PAIRED COMPARISONS | 28s |
| 13 | <i>Buckland</i> STATISTICAL ASSESSMENT
OF THE LIFE CHARACTERISTIC | 24s |
| 14 | <i>Lukacs & Laha</i> APPLICATIONS
OF CHARACTERISTIC FUNCTIONS | 45s |
| 15 | <i>Geary & McCarthy</i> ELEMENTS
OF LINEAR PROGRAMMING | 30s |
| 16 | <i>Godwin</i> INEQUALITIES ON
DISTRIBUTION FUNCTIONS | 24s |
| 17 | <i>Keilson</i> GREEN'S FUNCTION
METHODS IN PROBABILITY THEORY | 40s |

CHARLES GRIFFIN & COMPANY LIMITED
42 DRURY LANE, LONDON W.C.2



PRECISION INSTRUMENTS

Polarimeter Model A



This instrument gives the highest degree of accuracy with divided circle on glass reading to 0.01 degree.

Circle centre on ball bearings • Easily removable trough • Divided drum replacing verniers • Stationary observation telescope • Circle reader enlarging image of scale • Slow motion adjustment always in action • Suitable, with additions, for measurement of rotatory dispersion.

This polarimeter has been supplied in large numbers both in this country and abroad and has always been acclaimed as being of advanced design and workmanship. Please ask for particulars "N".

SOLE DISTRIBUTORS IN U.K. OF SCHOTT INTERFERENCE FILTERS

Bellingham & Stanley Limited

71 HORNSEY RISE, LONDON, N.19. PHONE: ARCHWAY 2270

THE ROAD TO PEACE

IT is now more than twenty years since the United Nations Charter was signed on June 26, 1945, at the San Francisco Conference, but although the anniversary was duly observed at San Francisco the dominant note was gloom rather than rejoicing. The problem of paying for past and future operations of the United Nations was still unsolved and was overshadowed by the continuing clash over Vietnam. Moreover, when on October 4 Pope Paul addressed the General Assembly of the United Nations, while neither of these had been resolved, the conflict in Kashmir had added to immediate anxieties.

Little wonder is it that there should be deep concern over the future of the United Nations, quite apart from any disappointment over failure to fulfil the high hopes entertained twenty years ago. While it is indeed appropriate that we should re-examine the structure of the Organization itself and seek the causes of weaknesses which in practice have developed, it is no less important to consider the changed circumstances and conditions in which the United Nations as an organization operates to-day, compared with those existing when it was established. It is true that the repeated failure to reach agreement on the revision of the Charter has proved a great hindrance and disappointment—this is especially so since, more clearly than the Covenant of the League of Nations, the Charter provides expressly for its own revision and modification in the light of experience.

Some of the changed conditions, indeed, are to be found in the United Nations itself. While China remains outside, the fifty nations represented at Dumbarton Oaks have grown to 114. The increase does not represent any substantial new areas or populations, but the proliferation of former dependencies into independent States with their own representation. The change, moreover, has been largely within the past decade: equatorial middle Africa is now represented, for example, by 30 independent Black States, and of these only two were independent before 1956, and 19 have been created since 1960.

It is natural enough that a strong spirit of nationalism should run among the new nations, but nationalism goes ill with international statesmanship and co-operation. Further, the new nations are inexperienced as yet in world affairs: perhaps the most serious charge that could be levelled against the former Colonial Powers is that they did so little, and that late, to prepare their dependencies for independence, even when, as with Britain, the goal had long been accepted. This comparative political immaturity of the new nations constitutes a great danger, as it can tend to political irresponsibility. The resources required to support the United Nations and make its policies effective remain, by and large, with the Great Powers.

That position has to be recognized and it should be noted, in passing, that this extends also to scientific policy, as has been recently emphasized (*Nature*, 201, 327; 1964). The influence of science, however, is even more significant in two other factors which have transformed the world since the San Francisco Conference. The first is the appearance of the nuclear bomb, which

was only used for the first time well over a month after that Conference. The decision to use the bomb introduced a new and unforeseen factor into the world situation, to which adequate thought was certainly not given by statesmen, although it would appear that some scientists did so. The second factor, and like unto the first, is the increasing pace of technological change of all kinds, of which a noteworthy instance is to be seen in the developments leading up to the *Early Bird* satellite transmission, which allowed Pope Paul's address to the General Assembly to be seen and heard, as he delivered it, thousands of miles away.

Development and change on this scale and at this rate must profoundly affect the relations between nations and the institutions through which those relations are organized. There has indeed been a remarkable growth in the activities of the various international scientific unions and the organizations through which those activities are conducted; so much so, that considerable expansion in the resources provided for the International Council of Scientific Unions is imperative if those activities are not to be circumscribed. The advent of nuclear warfare has intensified problems of disarmament; and its control, quite apart from all the psychological consequences and the problem of preventing the spread of nuclear weapons, is probably one of the most urgent and important problems of our times.

All this, however, has not made the United Nations redundant but more essential than ever if mankind is to survive. There is no other such viable international body reflecting the growing interdependence of the world and providing on an adequate scale the framework for the immense variety of inter-relations to-day. If it were dissolved it would be imperative to create immediately just such another institution as the alternative to world chaos. That this is realized is one hopeful feature in a gloomy situation.

Consider first, however, some of the consequences and implications of these scientific and technological developments of the past twenty years. The advent of nuclear weapons has increased the complexities of any steps towards disarmament, while at the same time it has undoubtedly had a restraining effect through the widespread realization that mankind cannot risk being involved in a nuclear war. If this problem could be solved the prospect of nuclear power has much to offer the world, though probably to a different extent in different parts of the world.

Here, however, we see that the real issue is the use which mankind makes of the new powers that have been placed at our disposal, whether they are used for good or for ill. The improvements in speed of travel and communication which, for example, enabled the Pope to reach New York so swiftly and allowed him to be seen and heard by millions when addressing the General Assembly, can be used to enable the Security Council to meet swiftly to deal with any emergency—if its members so desire. The techniques of television or broadcasting can be used thus for purposes of goodwill and to promote public understanding: they can equally be the sounding

board for propaganda, for misrepresentation and for fostering ill-will.

Finally, it should not be forgotten that the United Nations was founded deliberately as a democratic institution and such institutions make high demands on those who belong to them. They cannot function without discipline and fair-mindedness and a respect for the rights of others. The eruption into the General Assembly of a considerable number of those who, as yet, have had little experience of practising those qualities, of fostering the self-discipline, cannot but produce strains, misunderstanding, and failure to equate power and responsibility, and to forget that who wills the end must will the means. Time and experience are required to develop the qualities to use a democratic institution and to make it work effectively. If the demands made on the patience and sympathy of the old and more experienced democracies may sometimes seem heavy, if not excessive, it is at least encouraging that the new nations should be eager to use the United Nations as their main instrument and platform. That way there is at least hope.

If then the United Nations has ceased to be what its founders expected it to be, there is another side. In those twenty years of disappointment and frustration rather than failure have come the new opportunities provided by nuclear power, the exploration of outer space, the development of the backward parts of Africa and Asia, the acceleration, if not transformation, of communications; these opportunities which call imperatively for some sort of co-ordinating body have not been entirely missed by the United Nations. With some political failures there has been an immense advance in expert collaboration, within which to-day the International Council of Scientific Unions functions, and of which it is well aware. Some of this activity may be humdrum, but it is none the less vital, and the operations of the International Atomic Energy Agency, of the International Labour Organization, of the Food and Agriculture Organization, of the World Health Organization and of the United Nations Educational, Scientific and Cultural Organization are widely known. Some of them are indeed direct descendants of organs of the League of Nations and testify to the greater ease of international collaboration in technical as opposed to political matters. In fact, it is largely the influence of scientists that has led to what slender progress has yet been made in the control of nuclear weapons.

That is one important reason for welcoming the emphasis laid on technical assistance and the attention being given to the formulation of scientific policies for the developing nations. It is true that the spirit of the Lagos Conference on the organization of research and training in Africa in relation to the study, conservation and utilization of natural resources (*Nature*, 205, 1141; 1965) was deeply disappointing as well as unrealistic. It gave little indication that Dr. S. Dedijer's wise counsel was receiving the attention it deserved. Nevertheless, the fact remains that the developing nations are using the United Nations as a means of obtaining help from the richer countries without becoming open to political pressures and also of securing important reforms in the system of world trade.

In this last-mentioned connexion, the solidarity of some 75 nations at the United Nations conference on trade and development should not be overlooked, nor indeed the skilful diplomacy of Britain which contributed so much to avoid a split and to help the United Nations to retain its chance of being a principal instrument for

solving a large and explosive world problem. Since then, a most constructive report has come from the Advisory Committee on the Application of Science and Technology to Development. This body was established by the Economic and Social Council in January 1964 to follow up the work of the Geneva Conference in the previous year on the Application of Science and Technology for the Benefit of the Less Developed Countries (*Nature*, 207, 897; 1965). Here again are ample opportunities for constructive work, given goodwill and determined co-operation.

All this amounts to really impressive opportunities, and especially for Britain. Certainly, it should not be forgotten how much Britain has already contributed since the organization was founded. She is, in fact, the second largest contributor in cash and exceeds even the United States in the provision of experts who have served with the United Nations in the technical fields. This summer Britain gave a further lead with an unconditional gift of ten million dollars to help the United Nations out of its financial difficulties. However much more Britain might or should be doing, it is no service to peace to disparage or make party political capital of what she has hitherto done.

Where perhaps Britain—and others—have failed is in the tepid support given to the International Co-operation Year and the Development Decade. Half-way through that decade the target of 151 million dollars set in 1961 is still unachieved. Neither Britain nor the other members are really using the United Nations to do the jobs for which it was designed and where political obstacles are unsubstantial. That is why it is not enough for the emphasis to be placed on peace and peace-loving. We need not underrate the influence of Pope Paul's noble address, which may well extend far beyond his own Church or even Christendom. Where the way forward may seem at the moment to be blocked, we should be using to the full the opportunities for advance open elsewhere, and especially for bringing together in practical effort or consultation men and women of different nationalities.

Here the very rapidity of advance since the Charter was drafted at Dumbarton Oaks and then signed at San Francisco places a greater responsibility on the shoulders of the scientist and technologist. The opportunities which are theirs to-day are immensely greater, and though time may still be required before the effect of their co-operation reaches up to the highest levels, it can already begin to lay firm foundations for wider and deeper understanding. The Pugwash Conferences have at least indicated some of the possibilities. The challenge, moreover, is to professional institutions as well as to individual scientists and technologists. Their colleagues in the new nations will require the support and guidance which such institutions in the older countries can give if they are to organize themselves so as to influence effectively the Governments of their own countries and shape policy on lines that will promote the fullest development and use of indigenous resources. There are still large opportunities of personal service overseas which can contribute to international goodwill and understanding, and there is the responsibility that rests on all citizens to see that Britain's contribution to technical assistance and in other fields is generous.

If it is true that Britain should be leading the United Nations, part of that leadership must be contributed from the ranks of scientists and technologists. This is not to deny that realistic and constructive thought must

be given to the outstanding difficulties. Peace-keeping operations in the future will not always be agreed by everyone, and when some members have an objection to a particular operation, it is absurd and impractical to insist that they should be financially responsible for it. Moreover, while from time to time proposals will no doubt be advanced like those recently made for a world centre for medical research or an administrative staff college, which are of dubious merit on professional grounds, it should always be remembered that success in one place or field will lead to demands to use the United Nations and its agencies for others. All countries should be wary of squandering on dubious projects their resources and especially those of the United Nations, but where the effort is worth while, their response should be whole-hearted, imaginative and sustained. That is the road to peace.

NATURALLY OCCURRING PHENOLIC COMPOUNDS

Biochemistry of Phenolic Compounds

Edited by J. B. Harborne. Pp. xi+618. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1964.) 126s.

BIOCHEMISTRY of *Phenolic Compounds* is a literary symposium which enjoys the advantages, and suffers the defects, of the type. It has been possible to gather together contributions from experts in their respective fields, hailing chiefly from the United States and the United Kingdom, with two Australian authors. But these are of unequal merit, ranging from a rather scrappy and ill-arranged selection of examples to well-ordered short monographs. For this reason the work is unlikely to be read *in toto* by students, though it must prove of the greatest value to research workers in any of the special fields discussed.

Few recent extensions of the organic chemistry and biochemistry of natural products have been more surprising than those recorded in relation to plant phenolics. Perhaps it was too readily assumed that these regions had been rather fully explored and that, in any event, their inhabitants were not particularly interesting. If so, both assumptions were quite incorrect. The variety and significance of phenolic substances is now seen to be great indeed.

This change of attitude is primarily due to the introduction of new techniques, particularly the chromatographic separations, which have injected a new precision into descriptive subjects such as those treated under anthoxanthins and anthocyanins. The results of surveys made with the use of these clearer glasses have given us not only a more accurate idea of composition, but have also provided taxonomic data and helped notably with certain genetic problems.

Chapters of fundamental importance to the worker in the chemistry of natural products are those of Margaret K. Seikel on the isolation and identification of phenolic compounds in biological materials, of J. B. Harborne and N. W. Simmonds on the natural distribution of phenolic aglycones, and of J. B. Harborne on the natural distribution of phenolic glycosides.

The first-mentioned covers the topic from *A* to *Z* and is an admirable account of it. The only useful tip, known to me, and not here mentioned, is the formation of good crystalline derivatives by combination of polyhydric phenols with *N*-methylstrychnine or *N*-methylbrucine.

The two chapters of which the editor is author and part-author are central to the theme, since without them the edifice could not have been constructed. The brilliant

pioneering work of Bate-Smith at Cambridge must be recalled in this connexion. An example among the anthocyanins will serve to show the kind of progress made. Many of these pigments are well-characterized by their properties, including habit of crystallization. This is the case with chrysanthemin and pelargonin, to take two clear examples. But mecocyanin, isolated by Willstätter and Weil (1917) from flowers of *Papaver rhoeas* L., is by no means so well defined. Its discoverers showed that it hydrolyses with formation of cyanidin and two molecules of glucose. When chrysanthemin was synthesized, it became clear that mecocyanin must be a cyanidin 3-bioside, because its colour reactions were as near as possible identical with those of chrysanthemin (cyanidin 3-glucoside). Accordingly in 1934 the 3-cellobioside, maltoside, lactoside and gentiobioside were synthesized. These pigments closely resembled each other but showed slightly different distribution ratios between partially miscible solvents. The gentiobioside and mecocyanin came closest. But later the chromatography of carefully hydrolysed mecocyanin showed that the sugar was sophorose, that is 2-glucosidylglucose instead of the 6-derivative. Sophorose was first isolated in 1940.

The advances in chromatographic technique led at once to their application to genetic problems and, as R. E. Alston shows, the increase of visibility on the road, while clearing the way, has opened up fresh vistas. It cannot, however, be claimed that much progress has been made towards a real understanding of the basis of heredity in these fields. The facts have been clarified and arranged and this is an important preliminary to future generalization.

Useful summaries of what is known are also contained in two chapters on the metabolism of phenolics. This is, however, a part of general biochemistry and it seems somewhat artificial to concentrate on the phenolic group in all cases.

More specific to the subject-matter are the four highly interesting chapters on aspects of the biosynthesis of phenolic substances. The major pathways, recorded by A. C. Neish, are those starting from acetate (malonate), shikimic acid (or both) and the isopentane route. The part played by each is evaluated as the result of application of various experimental methods, of which the most important is the use of isotopic tracers. The general reader as well as the specialist will be fascinated by the tale that is unfolded. S. A. Brown describes the use of these and other methods to elucidate the biosynthesis of tannins and lignin. The work mentioned has not only solved the problem set but also has reflected light on the structure of these important substances themselves.

The final chapters on phenolics in relation to their pharmacology, physiology, pathology, and even taste are doubtless of high interest but look as if they had been added for the sake of completeness.

This compendium, with some faults and many virtues, is primarily of interest to chemists and biochemists and will be a standard work of reference for them for many years to come. Its production reaches the high standard which we have been led to expect from the publishers. There are few errors, the structural formulae being especially clear and accurate. ROBERT ROBINSON

HIGH-PRESSURE PHYSICS

Physics of High Pressures and the Condensed Phase
- Edited by A. van Itterbeek. Pp. xv+598. (Amsterdam: North-Holland Publishing Company, 1965.) 140s.

THE growing interest in high-pressure studies has been reflected in recent years in a number of books, but there is no text dealing in such detail with the particular aspect chosen in this collection of articles by different

authors. The title of the book is somewhat misleading since most of the chapters deal with systems at low temperatures and moderately high pressures, and not all the topics are relevant to the condensed phase, for example, the articles on the spectra of gases. The standard is high, at the level of appeal to research workers. Since the chapters appear to be independent, with little cross-reference, they will be reviewed *seriatim*.

Chapter 1, by L. Deffet and L. Lialine, on "High Pressure Techniques in General", is limited to a rather perfunctory account of methods of closure and manometric devices, followed by a good summary of theory and observation on the elastic equilibrium and plastic flow of thick-walled cylinders. Ultra-high pressure techniques are not considered.

The mechanical properties of metals at low temperatures are treated by J. F. Watson, J. L. Christian and A. Hurlich in Chapter 2. This field has recently become important in relation to cryogenic work on missiles, hydrogen bubble chambers, etc. After a brief summary of theoretical principles the major part of this chapter consists of a detailed report on the behaviour of various alloys.

Chapter 3, by J. M. H. Levelt Sengers, consists of a critical appraisal of the experimental methods for the determination of the equation of state of gases at low temperatures and at pressures greater than a few atmospheres. An unusual feature is the report, about a page for each, on ten research centres throughout the world. A repeat is given of an account of manometric devices discussed in Chapter 1.

Chapter 4, by O. Verbeke and A. van Itterbeek, on "The Equation of State of Gases", is confined in the experimental section to work done at Leuven, Belgium (already reported on two pages in Chapter 3). This section is followed by a useful survey of data for the liquid vapour pressures of hydrogen, neon, oxygen, nitrogen, argon and methane and valuable compilations of thermodynamic data. In correlating these data more use could have been made of the principle of corresponding states. The final section comprises a summary of the various theories of the liquid state, including quantum liquids, and some comparison with observation (the authors state (p. 155) that the Monte Carlo method has not yet been applied to the liquid phase).

J. W. Stewart (Chapter 5) tackles the properties of "solidified gases" (that is, those substances which are gases under normal conditions) at high pressure and at temperatures less than 200° K, covering melting curves, compressibility, phase equilibria (surprisingly different for CH₄ and CD₄), etc. There is a useful summary of theories of melting.

After Chapter 4, with 23 pages on the theory of liquids, it is somewhat surprising to revert to this topic in Chapter 6 by A. Münster. However, the treatment is more sophisticated, although relaxation phenomena are omitted. Considerable attention is directed to the Monte Carlo method. As is usual in this rather unsatisfactory field, examples are limited to simple non-polar molecules.

Liquid and solid helium are rightly given separate chapters (respectively by R. H. Sherman, Chapter 8, and J. S. Dugdale, Chapter 9), since the quantum behaviour and choice of isotopes give a rich field of study. The bizarre properties of helium are excellently treated in these two chapters; the most compressible of solids, helium is often taken as a model substance from which the properties of other substances may be inferred in inaccessible regions. J. S. Dugdale rightly warns research workers against too slavish an adherence to this principle. Much remains to be done in this field; for example, why is an open body-centred cubic structure adopted near to 0° K?

"The Velocity of Sound in Dense Fluids" by W. van Dael and A. van Itterbeek (Chapter 7) and "Transport Phenomena" by O. van Paemel (Chapter 10) treat kinetic phenomena in fluids, often at low temperatures. The former chapter has the more detailed theoretical analysis.

Chapters 11 and 12, respectively by B. Oksengorn, H. Vu and B. Vodar on "Optical Aspects of High Pressure Investigations" and by J. P. Colpa on "Induced Absorption in the Infra-red", might have been combined; indeed the last-mentioned author devotes some space to the work of Vodar *et al.* already treated in Chapter 11. These chapters are concerned with molecular interactions in gases and their effect on spectral transitions, and such optical topics as the effect of pressure on the refractive index, rotatory dispersion, etc., are omitted.

The final two chapters on superconductivity (M. Levy and J. L. Olsen) and the electrical properties of metals and semiconductors (G. Landwehr) are, perhaps, somewhat out of place in this volume since the range of pressure is so much greater, extending to 600,000 atm. for measurements of conductivity, and only the former chapter is confined to low temperatures; nevertheless they are both useful summaries.

The treatment throughout is authoritative and the production excellent (although an author index would have been useful and the subject index is inadequate). In spite of the repetition, the occasional infelicities in the English, and the high price, this book can be recommended to all high-pressure workers, as it is packed with useful information, although much of the book is concerned with a rather limited range of molecules, the lighter inert gases and a few others of simple structure. The region of moderately high pressure, with which most of the book is concerned, provides a very satisfying study, since high precision is possible in the measurements and theory need not be unduly stretched. The editor is to be congratulated on bringing together this collection of articles which should stimulate further research—much remains to be done, for example, on the critical state.

R. S. BRADLEY

SPACE DYNAMICS

Dynamics of Rockets and Satellites

Edited by G. V. Groves. Pp. xi+313. (Amsterdam: North-Holland Publishing Company, 1965.) 80s.

THIS volume is a record of the lectures at the international summer school on the "Dynamics of Rockets and Satellites" held at Cambridge in July 1963. The book begins with a short section on the equations of motion of a rocket, by D. S. Carton. This is nicely balanced in the next chapter by an account of an actual design, the European Launcher Development Organization's launching vehicle, by H. G. R. Robinson. Chapters 3 and 4 return to mathematics, with J. M. J. Kooy discussing the dynamics of controlled rocket launching, and E. Stiefel dealing with many-body problems, as applied to interplanetary and circumlunar flight. In Chapter 5, R. H. Giese outlines the essentials of satellite tracking and orbit determination.

The next two chapters are concerned with orbital perturbations. M. J. Davies derives Lagrange's planetary equations and shows how they can be used for determining the effect of the atmosphere on orbits. W. M. Kaula propounds an elegant and generalized method for evaluating gravitational perturbations and also touches on the effects of radiation pressure, which have so far proved resistant to elegant formulation. The theme of the final two chapters is the rotational motion of a satellite about its centre of gravity. W. G. Hughes discusses attitude stabilization, including the torques acting, the methods of sensing attitude and control actuation. The subject of the last and longest chapter, by A. J. Sarnecki, is the dynamics of rigid-body rotation: a thorough and illuminating exposition of vectors and tensors is followed by a description of the rotational motion of typical satellites.

Most of the individual contributions are of a high standard and the complete book forms a valuable conspectus of space dynamics. In organizing the conference

Dr. Groves chose both subjects and lecturers judiciously; and in editing the book he has managed to avoid many of the perils of multiple authorship, though some overlapping among the contributions inevitably remains. The book would form a sound basis for an academic postgraduate course on space dynamics: it is, however, too advanced for an undergraduate course, and some of the contributions are of greater academic than practical interest. The book is well printed and the layout is pleasing.

D. G. KING-HELE

MATERIALS AT HIGH TEMPERATURES

Thermal Stress

Edited by Dr. P. P. Benham and Dr. Russell Hoyle. Pp. x+382. (London: Sir Isaac Pitman and Sons, Ltd., 1964.) 90s. net.

Plenum Press Handbooks of High Temperature Materials

No. 1. Materials Index

By Peter T. B. Shaffer. Pp. xx+740. 17.50 dollars.

No. 2. Properties Index

By G. V. Samsonov. Translated from the Russian. Pp. xii+418. 22.50 dollars.

No. 3. Thermal Radiative Properties

By W. D. Wood, H. W. Deem and C. F. Lucks. Pp. 470. 17.50 dollars. (New York: Plenum Press, 1964.)

THERMAL Stress is a symposium based on a conference held at the Imperial College of Science and Technology, London, in 1962, on the effects of temperature on the stresses within a body and on the properties of the component materials. It is divided into three parts, the first giving a résumé of the theoretical aspects of thermal stress, the second concerning thermal cycling and creep but also including a chapter on materials at high temperature, and the third detailing some industrial examples of thermal stress problems and their solution.

Like most published symposia, the book can be criticized for a lack of balance and completeness in dealing with its subject. The treatment of the high-temperature properties of materials, for example, is cursory, occupying only two out of twenty chapters, and would probably have been better omitted altogether. Regarded as a collection of papers, however, the book performs its function of presenting the thinking and results of those active in the field.

The *Plenum Press Handbooks of High Temperature Materials* provide, in three volumes, a wealth of data on those metals and ceramics that are used or usable at elevated temperatures. Volume 1 lists more than five hundred different ceramic materials such as carbides, borides, nitrides, sulphides, phosphides, silicides, and oxides, giving for each such structural, chemical, mechanical, thermal and physical data as could be culled from the sources consulted, these being mainly U.S. Government reports. The data available about a given substance vary enormously and the reader may be frequently disappointed in not finding the information for which he seeks. The book does not claim, however, to be exhaustive, and the author, Dr. P. T. B. Shaffer, is to be complimented on providing a good start to the classification of high-temperature materials and their properties.

Volume 2 in the series is the translation of a Russian work by Prof. G. V. Samsonov in which data on refractory materials are classified according to properties rather than materials. It has a brief but useful introduction followed by data on structure, composition, density and thermal stability. Chapter 2 deals with thermal properties of all kinds, and the succeeding chapters cover in turn the electrical, optical, mechanical, chemical and refractory

characteristics of these materials. Oxides are not covered in this book as they have been dealt with elsewhere. A final set of tables gives examples of the application of refractory compounds. This work is more exhaustive than Volume 1 with more than 1,300 references compared with less than 500 in the latter, but the two volumes are clearly complementary and together provide an indispensable source of collected data.

Volume 3 deals with the thermal radiative properties of high-temperature materials and contains data on titanium and its alloys, stainless steel, iron, nickel and cobalt and their alloys, chromium, columbium, molybdenum, tantalum and tungsten with their alloys, coated materials of many kinds, ceramics and graphites. For each material the data provided include a graph of emittance (spectral or total) against temperature (given unfortunately in degrees Fahrenheit) together with information on the methods and conditions of measurement and, of course, the reference. In many cases graphs of normal spectral emittance against wave-length at some reference temperatures are also supplied. There are introductory chapters on "Fundamentals and Definitions" and on "Methods of Measurement", the latter being a brief survey backed up by thirty references to the literature.

E. H. ANDREWS

THE WORLD OF FISH

A Draught of Fishes

By Dr. F. D. Ommañney. Pp. 254+23 photographs. (London: Longmans, Green and Co., Ltd., 1965.) 36s. net.

SOME twenty-three thousand species of fish inhabit the waters of the world, more than those of all the other vertebrates put together. Their habitat covers more than 70 per cent of the surface of the globe, and ranges from the sunshine and surf of the shore to the black death-like calm of the abyss. The fishes—using the word in its widest vernacular sense—are the oldest class among the living vertebrates, and have had many million years more than the other classes to evolve in adaptation to their infinitely varied environment; though time has apparently stood still for many of them since the Carboniferous, natural selection must yet be acting on the combinations and permutations in the gene pool of others. This great host, not only of species but also of individuals, provides a subject well suited to such an accomplished writer as Dr. Ommañney, who has spent many years in research on improving the yield of commercial fisheries in many parts of the world.

The book opens with a chapter sketching in the oceanographic background of the fish's world and of the fisheries that exploit its wealth. The various forms of fishing gear and their methods of use are then described, from the latest European V-D trawl, and the ships equipped with radar and fish-finding echo-sounders that use it, to the simple traps and fish-weirs so commonly used on tropical coasts—not that simple "fixed engines" are extinct in Europe, for the wicker putchers still used in the Severn Estuary can be little different from the fish traps no doubt fished there when Julius Caesar first landed, or perhaps in neolithic times or even earlier. Dr. Ommañney intersperses his chapters on such subjects as shoaling, the colours of fishes, the abyss and the inshore waters, fresh waters, and the plankton feeding fishes, with others describing the different researches on specific fisheries, and the exploratory voyages undertaken in their prosecution, with which he has been associated. The last are among the best in the book, for his vivid style conveys the essential flavour of life in a trawler on the northern grounds off Iceland and Bear Island or with the tunny men of Brittany in the Bay of Biscay. The fisherman's world is

poles apart from the ordered routine of the landsman, who can have no inkling of its distance from his way of life; but, rough and uncomfortable as the life of a fisherman may be, the ceaseless struggle against the unending hostility of the elements and the ever-changing beauty, probably seldom consciously appreciated, of their infinite variety bring a primitive satisfaction that drives men to sea again and again in spite of all sea-born vows to swallow the anchor for good.

The last two chapters discuss the possibility of farming the sea, and the future of fisheries and man. The artificial cultivation of fresh-water fishes has a long history and a bright future, but no more than the first tentative experiments have yet been made in cultivating marine fishes—the artificial rearing of fry, the transplantation of young fish to rich feeding grounds, and the fertilization of semi-enclosed areas of the sea. One wonders whether pouring the untreated sewage of much of northern Europe into the North Sea can have had any effect in making it one of the finest fishing grounds of the world, or whether the suggested ploughing of the bottom to stimulate the production of the benthos which forms the diet of so many food fishes could increase the harvest. The human population explosion results in an ever-increasing demand for food protein; Dr. Ommanney speculates about the possibilities of satisfying the demand with the products of the sea, and on the effects of the radioactive wastes that we dump into the depths in 'leak proof' containers when inevitably they do leak. "There seems to be no limit to what man may achieve with his technology and his formidable know-how. . . . Nevertheless, the abuse of his own know-how threatens his very existence and already casts a lengthening shadow which reaches even to the uttermost depths of the sea."

Dr. Ommanney's book will be enjoyed equally by the professional biologist and by the layman for whom it is primarily intended. It is well illustrated with coloured and monochrome photographic plates and with numerous line drawings of excellent quality. There are, however, several howlers that a more careful final revision might have avoided; the Weberian ossicles have certainly not become the hearing bones of the ear of higher animals (p. 45), the claspers of elasmobranchs are not, as Agassiz mistakenly supposed, "pressed together so that their grooved inner faces form a tube along which the sperm flows" but are inserted singly into the female passage (p. 73); no carefully measured Basking shark has reached "a length of 40 feet", 30 ft. is nearer the mark (p. 79); the marine lancelet *Branchiostoma* is not the same creature as the "sand lance", which is the sand eel *Ammodytes* (p. 197), and Fig. 19 purporting to show gill rakers is a failure. These regrettable lapses form the few shadows that contrast with the brilliance of the rest of the book.

L. HARRISON MATTHEWS

PURPOSE AND MEANING OF A UNIVERSITY

The Modern University

By Prof. G. L. Brook. Pp. 192. (London: André Deutsch, 1965.) 25s. net.

PROF. G. L. BROOK, Smith professor of English language and medieval English literature in the University of Manchester and a Pro-Vice-Chancellor, has written an account of university problems to-day similar to that of 'Bruce Truscott' some twenty years ago. There is the same ease of expression but more authority as well as balance about the whole book. It is a little disappointing, therefore, that the production of such an admirable book should fall considerably short of what was possible in the midst of the War at a third of the price.

Prof. Brook's opening chapter gives a brief discussion of the purpose of a university, and this, together with the

following chapter on entrance requirements, and an appendix on the organization of a modern university, should make plain to prospective students, and their parents, much that sometimes perplexes them. In the succeeding chapters he writes on students and on lectures and tutorials with a refreshing sanity and humour, as well as with balance; his remarks could be disconcerting to the extremist. In general, however, the most valuable chapters in the book are those which deal with books, with professors and lecturers and with academic freedom. The first will be welcomed by those urging more generous provision for university libraries. Librarians should be encouraged by the clear understanding shown of their difficulties and also of the problem of book-buying by students. The second corrects a misleading impression conveyed by 'Bruce Truscott' and should assist in giving a clearer understanding outside the university of the problems involved in the relations between research and teaching. Together with the final chapter on expansion, it brings down to earth some of the wilder statements and arguments of those who seek to determine university expansion by service to political doctrine rather than by reference to public needs and the real nature or purpose of a university.

For the chapter on academic freedom alone *The Modern University* would be welcome. Prof. Brook's exposition of the issues, including those aspects arising from the relations of university teachers with one another and the amount of freedom that students should enjoy, could scarcely be improved. He sees the danger at present as so real that our universities will do well to keep the very considerable freedom they have, and he believes that the chief threat comes from well-disposed persons, genuinely interested in education but who value other things more highly. The threat from outside is largely the result of the success of universities, and Prof. Brook shows clearly how the ear-marked grant can act in this way. The greatest single external threat to academic freedom to-day is the dependence on the State for so large a proportion of the university's income. The greatest internal threat is indifference among university teachers, or the practice of 'taking it for granted'; here he includes the fact that the scholar is reluctant to take his or her share in administration. Nor does he believe that the University Grants Committee will by itself suffice. For the defence of academic freedom it is necessary to convince public opinion that the State ought not to exercise the power it possesses. He adds that accordingly maintenance of a high standard of administrative efficiency under academic control is a most important safeguard of academic freedom, that many important decisions in university affairs should not rest entirely in the hands of one man, and that students, as well as teachers, have rights to be safeguarded.

Prof. Brook closes this chapter on a note of responsibility addressed particularly to the university teacher. That is the keynote of his final chapter, which includes many frank but uncomfortable words for student as well as teacher and administrator. He brings a timely pragmatic point of view to bear on the whole problem of expansion, arguing that the important question is whether potential students can profit by a university education sufficient to justify the considerable expenditure of time and money involved. He reminds us of the limits set to expansion by the availability of university teachers of the right calibre, and that the number of students who could profit from some other form of higher education is very much larger than the number of those whose proper place is in a university. He has constructive suggestions for breaking down existing barriers between school and university teaching. The whole tone of the book is a complete answer to those who accuse the university of being conservative, out of date and unwilling to face modern opportunities. Prof. Brook is to be congratulated on a most readable and timely book. R. BRIGHTMAN

MIMICRY AND THE EVOLUTION OF ANIMAL COMMUNICATION

By DR. W. WICKLER

Max-Planck-Institut für Verhaltensphysiologie, Seewiesen u. Starnberg, Germany

DURING the hundred years since Bates's discovery of mimicry in 1862, countless numbers of mere suggestions, experimental analyses and intense discussions about the evolution, the consequences and even the existence of mimicry have been compiled. This literature will not be reviewed here. Instead, I would like to suggest that the study of deceptive signals and a re-defined mimicry-concept offer a strict method for reconstructing the phylogeny of social signals and that we have to study mimicry carefully with respect to the evolution of animal communication. This makes it necessary to reconsider the concept of mimicry under the aspects of modern ethology.

As early as 1890, Poulton drew a distinction between defensive or protective mimicry, as illustrated by wasp-like beetles, moths, flies, etc., in which the mimic is preyed on by a predator, and aggressive mimicry, in which the mimicking species is itself the predator and preys on some animal which is not afraid of the mimicked species. This is the case, for example, in the mimicry between the wrasse, *Labroides dimidiatus*, and the blenny, *Aspidontus taeniatus*¹, which I have studied in greater detail², because *Aspidontus* mimics even behavioural characters of its model. *Labroides* removes ectoparasites from the bodies, fins and buccal and gill cavities of other fish. These fish often show special 'invitation postures' and the cleaner occasionally displays in a sort of dance in which the caudal fin is spread and the posterior part of the fish oscillates up and down. Size, form, coloration, and even the dancing of the cleaner are simulated by *Aspidontus*, who thus succeeds in eliciting the invitation posture from other fish. Being a fin-eater, however, he then attacks instead of cleaning them.

Another case classified as aggressive mimicry is that of the eggs and young of parasitic birds which mimic the signals serving as species recognition marks and/or eliciting brood care in the host species. The orchids of the genus *Ophrys*, the flowers of which mimic the social releasers of female Hymenoptera and elicit sexual behaviour in the males, fall into the same category; they offer no nectar but only the opportunity for pseudocopulation to the male insects, who thus transfer their pollen.

Essential for both types of mimicry is the deceived partner, one of the normal reactions of which is 'misused'. This may be a flight reaction or a blocking of feeding in protective mimicry, and quite a number of different reactions in so-called aggressive mimicry. Often a feeding reaction is exploited as by the angler fishes, but many other behaviour patterns may also be exploited, such as the need for being cleaned (by *Aspidontus*), the copulatory reaction (by *Ophrys*), or the care of the young (by the various brood parasites). These examples show (1) that in spite of some existing mimicry-definitions no predator need be involved in a mimicry system, and (2) that the terms 'defensive', 'protective', and 'aggressive' are inadequate to cover the diversity of signals and reactions misused in mimicry. Moreover, though colours and forms of organs and movements as optical markers are

most conspicuous to us, scents and sounds may be mimicked also, as Kullenberg³ has shown in some orchids which even imitate the sexual odours of hymenopteran females, and as Nicolai⁴ has shown in the whydahs that imitate the calls and songs of their host species. Therefore, instead of referring to pseudosematic or deceptive colours we should rather speak of deceptive stimuli or signals. Since the deceived partner may be responding as predator, or mate, or parent, or cleaning customer, it will be called simply the reacting signal-receiver or the receiver.

Mimicry, then, can be defined in the following way:

(1) A signal is emitted by two different signal-senders (S_1 , S_2) which have at least one signal-receiver (R) in common that reacts similarly to both of them.

(2) One of the senders is called a model, the other a mimic (and the whole model-mimic-receiver system a case of mimicry), if it is profitable (+) for the receiver to give the reaction to one of the senders, but unprofitable (-) to react in the same way towards the other. That means that if the signals from the two senders could be distinguished by the receiver, individual experience and/or selection would favour different reactions. This is shown, for example, by the cleaner mimicry: *Aspidontus* deceives mainly younger fish, at least some of which then learn to distinguish between model and mimic and to avoid the latter. Constant learning by the signal-receiver results in a strong selection pressure against detectable differences between model and mimic.

(3) It suffices to define model and mimic as follows: the sender the signal of which elicits a reaction which has negative selective consequences for the receiver (which would be selected against if given to this sender only), that is the sender by which the receiver is deceived, is called the mimic, the other the model. These relationships may be symbolized thus:

$$S_1 + R - S_2$$

The selective consequences for the receiver of responding to the model are always positive (the reaction would disappear if it were at all unfavourable to the receiver). The mimic—if a living creature—always has a selective advantage (+) in releasing the reaction from the receiver (an unfavourable signal will disappear, too). If S_2 is the mimic, our formula automatically turns into:

$$S_- + R - +S_2$$

(It is, of course, a matter of convenience whether one chooses the reaction or the signal as a reference unit; one could equally as well have considered the selective consequences for the receiver in receiving the signal from both senders and for them in emitting it.)

The selective consequence for the model eliciting and obtaining the reaction from the receiver may: (a) be absent, if the model is an inanimate object; (b) be negative, if the model is a worm, for example, usually eaten by the receiver and mimicked by another predator in order to catch the receiver; (c) be positive as in the wasp (who remains alive if it is avoided by the predator), or in the

cleaner (who feeds on parasites harmful to other fish), or in those hymenopteran females, the male-attracting signals of which are mimicked by certain orchids.

The simple definition given here overcomes the difficulties arising from some recent characterizations of model and mimic. It is irrelevant which of the two was first present. Poulton postulated that model and mimic should be sympatric. They need not be sympatric, however, but must only have a signal-receiver in common: a model might be in Africa and its mimic in Europe (or vice versa), functionally connected by a migratory bird. Another postulate was that mimics must be less numerous than their models. This means, correctly stated, that the receiver has to meet the mimic less often than the model, and is based on the assumption that one experience with the model has the same after-effect, the same 'weight' as has one with the mimic. This need not be so, however; in fact, the negative experience seems usually to be the stronger one. This negative experience may result from an encounter with the model (a wasp) or with the mimic (for example, the *Aspidontus*). In the latter case one would suppose that the mimic in fact has to be less numerous than the model², but there might very well be more wasp mimics than wasps.

Assuming that a predator learns to reject a conspicuously coloured protected animal from a single experience and—as in imprinting—without forgetting it, then the model-species has to sacrifice one individual per predator regardless of how many mimics there may be. Of course, the protective power of the model, that is the advantage of being its mimic, is reduced with increasing numbers of mimics, because the predator will eat larger numbers of them before his first encounter with the model. Furthermore, if the signal receiver reacts towards the signal via an 'innate releasing mechanism' (IRM) which cannot be modified by experience, each individual of the receiver species will be fully deceived by the mimic even if it never meets a model (this seems to be the case in some of the female-mimicking orchids). Thus, whether or not the reaction is eliminated in phylogeny depends on its usefulness to the species in other contexts.

In all cases the selective value for the mimetic characters increases, among other things, with the number of successful deceptions from one deception per receiver on. The orchids (and other mimicking flowers) are the only mimics known to me, however, that need at least two deceptions per receiver—in order to undergo cross-pollination. The disadvantage for the deceived species should, therefore, be minimized, as it is by the orchid-flower allowing only attempts at copulation and thereby parasitizing the strong reward offered by true copulations with a female. So, finally, the type of reaction given by the receiver has also to be taken into consideration.

Signal Standardization and Müllerian Mimicry

It will have become clear already that even fundamental characteristics of mimicry are determined mainly by behavioural properties of the signal-receiver and that, therefore, a precise knowledge of the identity of the receiver and a thorough ethological study of its behaviour are indispensable for the understanding of mimicry. Additional arguments for this will come from the following considerations which will show, moreover, that mimicry is not sharply distinct from, but gradually merges into, other sender-receiver communication systems.

First, since palatability is a matter of degree, there might possibly exist a whole range of increasing distastefulness even in the mimics, model and mimic in the extreme case being equally unpalatable and sharing the same warning coloration. This stage is called Müllerian mimicry which in our notation would be:

$$S_1 + +R + +S_2$$

This is no true mimicry, however, since nobody is deceived

and it is impossible to differentiate between model and mimic. But there is still another difficulty: all individuals of a given wasp-species look alike and all are equally protected. Yet this is not usually called Müllerian mimicry, simply because the signals were not independently, convergently evolved. Since, however, the male wasps have no protective properties⁵ but retain their group-specific warning coloration, this clearly is Batesian mimicry, although model and mimic are conspecifics and their signals are homologous. Convergence of the signal-characters, therefore, is essential only for the so-called Müllerian mimicry, and thus Müllerian mimicry is distinguished from other cases of signal standardization.

Summing up, the typical (Batesian) mimicry merges into Müllerian mimicry if the difference between the selective consequences for the receiver of reacting similarly to S_1 and S_2 diminishes; and by homology of the signal characters it further merges into general signal standardization and such problems as that of typical intensity⁶.

Secondly, insects may be protectively coloured by resembling a wasp or a twig, the first case being called mimicry, the latter mimesis. The difference again lies within the signal-receiver. If the mimetic signal does not release any reaction in the receiver, it falls under mimesis. This is illustrated by De Ruiter's neat experiments with stick caterpillars which by virtue of their close resemblance to twigs are protected against insect-eating birds⁷. As soon as there are too many caterpillars, however, the bird develops an interest in them and then also attacks real twigs. In our notation this would be:

$$S_1 - +R - -S_2$$

S_1 being the caterpillar, S_2 the twig and (as shown by R 's minus) the mimic. If then one positive experience of R with the caterpillar had the same weight as a negative one with a twig (and the signal remains unchanged) it will depend on the relative abundance of caterpillars and twigs whether all twigs are mistakenly exterminated or R 's feeding reaction towards twig-like objects disappears, changing our formula into:

$$S_1 + -R + +S_2$$

so that now the caterpillar (S_1) becomes the mimic.

This again illustrates the importance of R 's reaction to decide correctly which is the model. This example further shows how easily an object (here the twig) may quite involuntarily become a mimic. This is further well illustrated by the common 'farming' relationship between ants and aphids. The siphones and cauda of the aphid correspond respectively to the bases of the antennae and labium of the ant, and the aphid's abdomen is mistaken by the ants for the head of a fellow ant, thereby eliciting the food-begging response which is identical with milking. Saturated ants in their turn even try to feed the abdomens of the aphids⁸. Aphid-species with reduced abdominal siphones use their hind legs as antennae-dummies, the movements elicited being originally defensive movements. This is exactly the way in which mimicry arises. Mimetic characters need not have evolved under the selection pressure of mimicking; in fact, their earliest evolutionary stages could not even have been brought about in this way. All the cases that I have been able to study so far could be traced back to an incipient stage of deceptive resemblance borne as a pre-adaptive atelic by-product of pre-existing species-specific characters, thus providing a point of attack for selection pressure. The 'dancing' of *Aspidontus* mentioned already originated as an up-and-down movement of the head which occurs in all blennies during a conflict between the tendencies to approach an object and to flee from it. This may occur in stationary fish as nodding or be superimposed on swimming, becoming a sort of dance. These movements usually have a special social meaning in threat or court-

ship, but in *Aspidontus* they are socially meaningless and so could become specialized as an interspecific signal². That the characters of the mimicking signal secondarily evolve further under natural selection is usually admitted, though sometimes doubted, as is even the mere existence of positive selective consequences resulting from the deception of a communication partner. The simple reason for these doubts is that in order to analyse and understand a given mimicry-system one needs a rather comprehensive knowledge of morphology, behaviour, ecology and their mutual relationships in animals usually belonging taxonomically to different classes of the animal kingdom (for example, wasps (Hymenoptera)—flies (Diptera)—insect-eating amphibians, reptiles, birds and small mammals). To reconstruct the evolution of such a complicated communication system, one also needs a detailed acquaintance with a larger group of forms related to each of the animals involved. But, in fact, we are far from having all these data.

Intra-specific Mimicry

That positive selective consequences do result from the deception of a communication partner is, however, shown automatically by deceptive signals that have evolved intraspecifically. In the small characoid fish *Corynopoma rrisei* the operculum of the male is elongated into a thin whitish stalk terminating in a small blackish plate. This is raised during courtship together with the operculum, and by its jerky movement resembles an edible object. This the female tries to grasp, and so comes into close contact with the male. Here mimic and receiver belong to one species. There are other cases (for example, the mimicking orchids or the parasitic whydahs) in which model and receiver belong to the same species. However, the safest conclusions can be drawn from intraspecific mimicry, in which model, mimic and receiver all belong to one and the same species. The best-known such example is the use of egg-dummies by the males of mouth-brooding *Haplochromis* species³, a group of African cichlid-fish. These dummies are conspicuous yellow or orange spots near the base of the anal fin which precisely resemble the eggs of the particular species. They are displayed to the female during courtship and during fertilizing and elicit her reaction of snapping up eggs. During courtship they make the male more attractive to the female. In spawning they assure fertilization of the eggs, which are taken into the female's mouth immediately after they have been laid and before the male is able to fertilize them: in snapping at the 'egg spots' with her lips, the female inhales the sperm, thus fertilizing the eggs within her mouth.

Here the specific female behaviour pattern of gathering eggs into the mouth is elicited alternately by model and mimic several times in succession with intervals of only about 1–2 sec, and within the same spawning pit. This offers ideal opportunities for comparison, since environmental factors as well as the internal states of the animals remain roughly constant. Moreover, the undeceived and the deceived reactions together form a functional unit, the deception clearly having positive selective consequences for the species. Nevertheless, the receiver's minus towards the mimic in our formula remains correct, since the individual clearly is deceived.

Another example of intraspecific mimicry is offered by some Old World monkeys, especially the Hamadryas baboon, the females of which during oestrus display conspicuous swellings of the ano-genital region, which is presented to the males as an invitation to copulate. Presenting, however, serves still another social function, since it is used by both sexes as an important appeasement or 'greeting' ceremony towards higher-ranking group members. The males, in order that their presenting be as effective as that of the females, imitate the females' swelling by a conspicuously red coloration of their hind-quarters as a dummy oestrous-marking^{10,11}.

The Theoretical Importance of Deceptive Signals

To reconstruct the evolution of a case of intraspecific mimicry as a special communication system is relatively simple for one needs detailed knowledge of but one rather narrow taxonomic unit. This is important, because mimicry is an indispensable step in studying the evolution of animal communication. This study usually starts from conspicuously elaborated signals, which postulate a signal-receiver interested in them. The receiver will practically always have undergone a special moulding towards optimal receiving of the signal. One must, however, follow up the mutual adaptations of the sender and the receiver separately. This is easily done so far as the evolution of a reaction or a receiving mechanism is concerned in all predators that try to find their prey, and in all prey-animals that try to escape an approaching predator. The 'suppression' of signals may be studied in predators that try to sneak up on a prey unnoticed. However, the 'elaboration' of a signal (in which the receiver must, of course be interested) can only be studied without co-adaptive interference from the receiver where the sender has a one-sided interest in the signal, which then can only be an imitation of either a signal developed in their common interest by the receiver and another signal-sender or a signal emitted by another signal-sender and only made use of by the receiver in his own interest. Both cases, by the definition given, are called mimicry. An additional advantage is that we know the model as the final stage towards which the mimic will evolve (so far as the signal characters are concerned), thus indicating the trend in evolution that is still operating and that probably will further elaborate the mimetic signals.

Finally, we may briefly consider social signals in general. It is legitimate to treat them as structures, even if they are motor-patterns¹². Evolutionary 'new' structures are merely modifications of preceding structures; "the emergence of a new structure is normally due to the acquisition of a new function by an existing structure"¹³. In the same way the egg-signal in *Haplochromis*, which originally belonged to the context of brood-care, has taken over the new functions first of bringing the gametes together and, secondly, even of bringing the parents together; and it has given rise to a new structure, the egg-dummies. If all signals have emerged during evolution at one time or another as 'novelties' one may question for what they might have been mistaken *in statu nascendi* by their respective signal-receivers. To treat signals generally as having passed through an original mimetic stage renders studies of mimicry valuable tools for students of the phylogeny of signals and of animal communication. In this way I have found that another cichlid fish, *Tilapia macrochir*, deposits spermatophores: I considered the male's conspicuous genital tassel as an imitation of something which finally turned out to be the spermatophore¹⁴. It should be added, however, that it is not as yet quite clear whether the spermatophore truly is the model, because both tassel and spermatophore may be mimics of a still unknown model. Finally, there is the possibility of chain-mimicry, that is, of secondarily mimicking a mimic; however, research in this field is still in a very early phase.

¹ Randall, J. E., *Pacific Science*, 12, 327 (1958). Eibl-Eibesfeldt, I., *Z. Tierpsychol.*, 16, 19 (1959).

² Wickler, W., *Z. Tierpsychol.*, 20, 657 (1963).

³ Kullenberg, B., *Zool. Bidr. Uppsala*, 31, 253 (1956); *Svensk Bot. Tidsk.*, 50, 25 (1956).

⁴ Nicolai, J., *Z. Tierpsychol.*, 21, 129 (1964).

⁵ Liepelt, W., *Zool. Jb. Physiol.*, 70, 167 (1963).

⁶ Morris, D., *Behaviour*, 11, 1 (1957).

⁷ De Ruiter, L., *Behaviour*, 4, 222 (1952).

⁸ Klotz, W., *Biol. Zbl.*, 78, 863 (1959).

⁹ Wickler, W., *Nature*, 194, 1092 (1962); *Z. Tierpsychol.*, 19, 129 (1962).

¹⁰ Wickler, W., *Naturwiss.*, 50, 581 (1963).

¹¹ Wickler, W., *Naturwiss.*, 52, 535 (1965).

¹² Wickler, W., *Fortschr. Zool.*, 3, 303 (1961).

¹³ Mayr, E., in *Evolution after Darwin*, 1 (University of Chicago Press, 1960).

¹⁴ Wickler, W., p. 595 of this issue of *Nature*.

THE JUVENILE HORMONE*

By SIR VINCENT WIGGLESWORTH, C.B.E., F.R.S.

Department of Zoology, University of Cambridge

IN choosing this topic for the opening address to the third conference of European Comparative Endocrinologists, I had in mind the consideration of the juvenile hormone in general terms—as an agent with properties that must be of interest to all endocrinologists. I shall not therefore attempt a complete or specialized survey of this very complex subject, but will select only a limited number of points for discussion.

A Morphogenetic Hormone

The juvenile hormone is a morphogenetic hormone—one of the most outstanding morphogenetic hormones known. The existence of a hormone responsible for maintaining larval characters was first revealed by the fact that decapitation in young *Rhodnius* larvae causes premature metamorphosis¹. Parabiosis showed that this hormone was freely circulating in the blood; and by removing progressively more and more of the anterior parts of the head from the insects used in the parabiosis experiments, it was shown that the hormone came not from the brain but from the corpus allatum—an endocrine organ that buds off from the ectoderm at the base of the mouthparts and comes to lie just behind the brain¹. This conclusion was confirmed by the implantation of corpora allata from young (3rd- or 4th-stage) larvae into 5th-stage larvae. These 5th-stage larvae then moulted not into adults but into giant 6th-stage larvae².

The juvenile hormone acts directly on the epidermal cells responsible for laying down the cuticle at moulting: restricted local application of the hormone results in a restricted local patch of larval cuticle in an otherwise adult insect³. It is possible, for example, to produce adult insects with one larval wing³. The self-same cell has the capacity for laying down larval cuticle or adult cuticle. That is most clearly shown in the sensory bristles or hairs: the trichogen cells which form the bristles persist from one stage to the next; in the presence of a large amount of juvenile hormone these cells lay down larval-type bristles; in the absence of the hormone they lay down adult-type bristles, and with intermediate amounts of juvenile hormone, bristles of intermediate type are developed⁴. Under natural conditions intermediate forms do not commonly occur. There is a strong tendency for the insect to develop either larval or adult characters.

Experiments by many authors have shown that the juvenile hormone controls metamorphosis in insects of all kinds. In holometabolous insects, which have a pupal stage between the larva and the adult, the juvenile hormone again controls the morphogenetic change: a large amount of the hormone ensures retention or re-development of larval characters; absence of the hormone results in metamorphosis to the adult; the presence of a very small amount of juvenile hormone leads to the appearance of the pupal form⁴. This result not only confirms the importance of the juvenile hormone in controlling morphogenesis, but it illustrates in a most striking way a point often made by C. M. Child: that the same inductor substance can evoke totally different results depending on its concentration or the timing of its action.

Here again intermediate forms rarely occur in Nature. But they can be induced experimentally: if the corpus allatum is removed from the last-stage larva of the honey-bee⁵ or of the giant silkworm *Hyalophora*⁶ the supply of

juvenile hormone falls below the level necessary to produce the pupa, and monstrous forms intermediate between pupa and adult develop. Some caterpillars pass through a regular succession of morphological stages in successive instars. These forms also seem to be regulated by levels of juvenile hormone secretion—but the detailed evidence for this has not yet been fully worked out⁷.

The Nature of Hormone-controlled Metamorphosis

It has long been recognized that the effect of the juvenile hormone is to control the realization or suppression of inborn potencies. In other words, it brings about 'gene switching'. In this regard it resembles the inductor substances which control morphogenesis in different parts of the body during differentiation; and the factors which lead to the differences in form of different individuals in environmentally induced polymorphism⁸. Indeed, there are a number of polymorphic changes in which there is evidence that the level of juvenile hormone activity may itself be involved, notably (i) the change over from the 'solitary' to the 'gregarious' form in locusts⁹; (ii) the switch from apterous to alate forms among parthenogenetic aphids¹⁰; (iii) the production of the soldier caste in termites^{11,12}.

Just how and where the juvenile hormone is acting is not known. Twelve years ago I wrote "it is a matter for discussion whether the simultaneous inheritance of the dual potentialities for larval and adult differentiation within these societies of cells is by way of the nucleus or cytoplasm or both"¹³. Eight years ago I submitted that the juvenile hormone "controls the manifestation of alternative genetically controlled forms" and I suggested that "it is possible to conceive it as being concerned in the regulation of permeability relations within the cells—in such a way that the gene-controlled enzyme system responsible for larval characters is brought increasingly into action when the juvenile hormone is present"¹⁴.

The position is unchanged to-day—except that tissue-specific puffing patterns in the chromosomes of *Drosophila*¹⁵, on one hand, and the theories of enzyme induction in bacteria as developed by Jacob and Monod¹⁶ *et al.*, on the other, render us much more prepared to accept the idea of a primary action of the hormone at the level of the gene. Experimental evidence has yet to come.

Reversal of Metamorphosis

The question was early raised whether the genetic system responsible for the production of larval characters was still capable of re-activation by the juvenile hormone in the adult insect. In other words, whether metamorphosis can be reversed. Of course, this can only be tested by making the adult moult again by exposing it artificially to the moulting hormone.

In general it can be said that in most adult tissues it is not possible to induce any reversal of metamorphosis. But there are certain undoubted examples of such reversal: in the abdominal cuticle of *Rhodnius*¹⁷ and of *Oncopeltus*¹⁸, in the thoracic cuticle of the earwig *Anisulabis*, where the 'ecdysial line' characteristic of the larva can be re-induced in a moulting adult¹⁹, and in the integument of Lepidoptera where larval cuticle can be re-induced in fragments of pupal and imaginal integument^{20,21}.

Reversal of metamorphosis is an abnormal phenomenon. There has been no selection for its occurrence and it is

* Substance of an opening address to the third conference of European Comparative Endocrinologists held in Copenhagen during August 1965.

not surprising that it occurs with difficulty. Likewise the production of stages intermediate between the normal stages in metamorphosis is an abnormal phenomenon and selection will have acted against it.

Time of Action of the Juvenile Hormone

The most effective moment for exposing the tissues to the juvenile hormone seems to be just before they begin the synthetic activities characteristic of the larval stage. Hormone administered too early is less effective—presumably because it is broken down in metabolism before the time for its action has arrived^{22,23}.

But there are certain effects which are induced in the cells long before they become manifest. A very small dose of juvenile hormone administered soon after feeding in the 5th-stage larva of *Rhodnius* has no effect on the type of cuticle laid down: normal adult cuticle is produced over the abdomen. But it does have the effect of ensuring the survival of the trichogen cells (almost all of which normally break down and disappear from the dorsum of the abdomen during metamorphosis), so that an excessive number of hairs appears on the abdomen of the adult²³.

The juvenile hormone has a comparable effect on the survival of the thoracic gland. The thoracic gland (the source of the moulting hormone) normally undergoes autolysis in *Rhodnius* within 24 h after moulting to the adult. This is a response to some hormonal factor in the newly moulted adult. But if the gland has been exposed to juvenile hormone during the pre-moulting period, it does not respond in this way and fails to undergo autolysis²⁴. (The breakdown of certain muscles after metamorphosis in the silkworm *Hyalophora cecropia* has recently been shown to be regulated in much the same way²⁵.)

Influence of the Juvenile Hormone on Behaviour

In certain caterpillars, behaviour is different in the final stage before pupation than it is before moulting in the earlier larval stages. The wax moth *Galleria* spins a tough cocoon before pupation, a flimsy web before a larval moult²⁶. The sphingid *Mimas tiliae* crawls down the tree-trunk to the soil before pupation, but rests on the foliage before each larval moult²⁷. These differences result from the presence or absence of juvenile hormone and are attributed to a direct effect on the nervous system. But the possibility remains that they could result from a nervous feed-back effect from other organs, for example from the distended silk glands.

Gonadotrophic Effects of the Juvenile Hormone

Secretion of the juvenile hormone ceases in *Rhodnius* in the 5th-stage larva before metamorphosis, but begins again in the adult. The hormone is then necessary for yolk formation in the female and for the full activity of the accessory glands in the male, which serve to produce the spermatophores to enclose the sperm². In *Hyalophora cecropia*, in which the eggs are developed during the pupal stage, very little juvenile hormone is secreted in the adult female, but large amounts are produced in the adult male²⁸, which secretes a succession of spermatophores.

The precise nature of the gonadotrophic effect of the juvenile hormone is uncertain. The position is complicated by the fact that in some insects the secretion from the neuro-secretory cells in the brain seems to be more important than the juvenile hormone in ensuring yolk production. In the case of *Rhodnius*, G. C. Coles²⁹ concluded that the juvenile hormone acts on the fat body cells and serves to activate those components of the gene system which lead to the synthesis of the specific proteins that are discharged into the blood and are taken up by the oocytes and added to the yolk. In the male locust, *Schistocerca*, T. R. Odhiambo³⁰ considers that the juvenile hormone activates the many systems concerned in protein synthesis in the nuclei of the accessory glands.

Metabolic Action of the Juvenile Hormone

The juvenile hormone is often said to be a 'metabolic hormone'—by which is meant that it sets going metabolic processes, either of synthesis or of combustion, whether or not these are required for the working body. I am sceptical about the existence of metabolic hormones in this sense; I think they are unphysiological. I fancy that in most cases of this kind the hormone is setting in motion some physiological process, and the observed changes in metabolism are feed-back effects. (But, of course, abnormally large doses of hormones may have an abnormal pharmacological effect on metabolism which has little relation to their normal influence in the body.)

A well-known example of a metabolic effect of the juvenile hormone is the accumulation of reserves of fat and glycogen in the fat body of the grasshopper *Melanoplus* when the corpora allata are removed³¹. But Odhiambo³² has shown that in the locust *Schistocerca* the juvenile hormone acts on the central nervous system and causes continuous activity. After allatectomy the insect continues to feed normally but it becomes inactive; consequently reserves pile up. The accumulation of reserves is not a feed-back effect from the gonads; the same effects are seen in males with or without their accessory glands.

In the blowfly *Calliphora*³³ and in the cockroach *Leucophaea*³⁴ the corpora allata are necessary to maintain the normal high level of oxygen consumption. But here again the juvenile hormone may be initiating some physiological activity (perhaps the synthesis of ovarian proteins) which demands increased oxygen consumption. In these cases it is not known whether nervous or muscular activity is changed. In the bug *Pyrhocoris* the effect of the corpus allatum on metabolism is seen only if the gonads are present: it appears to be a feed-back effect from the activated ovaries which are demanding nutrients³⁵. Likewise in *Rhodnius*, the accelerated rate of digestion in the presence of the corpus allatum is a feed-back effect from the developing ovaries³⁶.

In *Leptinotarsa* the adult female goes into diapause when the juvenile hormone is absent or the corpora allata are removed. The rate of metabolism falls to a very low level and egg development ceases. In this state the thoracic muscles and their mitochondria degenerate almost completely. When juvenile hormone is supplied everything is restored: the beetles become active, the ovaries develop, muscles and sarcosomes are fully regenerated³⁷. The metabolic effects are profound—but just where the juvenile hormone is acting is not known.

It is, of course, self-evident that hormones can influence the body only by bringing about chemical changes in the cells. In this sense they are always 'metabolic hormones'. Within a few hours the moulting hormone restores nucleoprotein synthesis in dormant epidermal cells of *Rhodnius*¹⁴; the juvenile hormone seems specifically to induce the synthesis of yolk proteins in the fat body of *Rhodnius*²⁹; as Gilbert³⁸ has recently shown, the juvenile hormone will induce the fat body of the cockroach *Leucophaea* to synthesize ovarian lipids. But these, and many similar effects, are elements in a pattern of development evoked by the hormone. They are not simply quantitative changes in metabolism unrelated to growth requirements—as is commonly implied by the expression 'metabolic hormone'.

Chemical Nature of the Juvenile Hormone

The large accumulation of juvenile hormone in the adult moth of *Hyalophora cecropia* provided a source of active extract. Ether extracts from the abdomen of the male moth gave an orange-coloured oil rich in juvenile hormone activity²⁸. This material was utilized to develop methods of assay^{3,22} and methods for concentrating the active principle by countercurrent separation²². These procedures were applied to extracts from many sources

and have shown that material with roughly the same partition properties, and with juvenile hormone activity, is widely spread throughout the animal and plant kingdoms: in the tissues of invertebrates and vertebrates, in higher plants, some bacteria and yeasts.

The material extracted from two of these sources, namely from the excrement of the mealworm, *Tenebrio*, and from yeast, was examined chemically by Schmialek³⁹; the active principle was isolated and shown to be a mixture of *trans-trans*-farnesol and its aldehyde farnesal. The question arises whether the natural juvenile hormone in the insect has any relation to farnesol or is of a totally different nature.

At the present time the answer to this question is not known. Farnesol will certainly reproduce all the morphogenetic and gonadotrophic effects of the juvenile hormone in *Rhodnius*⁴⁰. It is particularly effective if its stability is increased by blocking the alcohol end of the molecule in the form of an ether (for example, farnesyl methyl ether) or as the farnesyl acetone⁴¹.

In the form of the methyl ether, a 0.06 per cent solution of the active *trans-trans* isomer is far more effective than the natural extract from *cecropia*. 1.2 µg will cause a partial retention of larval characters in *Rhodnius*—a dose of 5.2 µg/g of body-weight²³. In *Antheraea*, 20,000 µg of the natural extract from *cecropia* will cause a partial retention of pupal characters—a dose of 4,000 µg/g of body-weight⁴².

By repeated partition with methyl alcohol, chromatographic separation on silicic acid columns, followed by the crystallization of impurities, a 'non-crystalline fraction' has been isolated which will produce this same effect in *Antheraea* at a dose of 5 µg, that is, 1 µg/g of body-weight⁴². Accepting these results at their face value this product is about five times as effective as farnesyl methyl ether in *Rhodnius*.

Further attempted purification by gas-liquid chromatography leads to heat destruction of the natural substance, but one fraction isolated in this way had an activity twelve times that of the 'non-crystalline fraction'⁴². Meyer, Schneiderman and Gilbert⁴³ report similar results, showing pyrolytic breakdown of the material in the gas-liquid chromatography column, but with some highly active fractions. Røller, Bjerke and McShan⁴⁴ have isolated a well-defined active substance which is certainly not farnesol.

The significance of these results is uncertain. Assay methods for material that is exerting its action over a period of many days of development, and which is continuously being broken down in metabolism, have no very precise meaning. (By modifying slightly the means of administering the material I have recently repeated the results on *Rhodnius* as described here with half the earlier dose, that is, with about 2.5 µg of the active isomer of

farnesyl methyl ether per gram of body-weight.) At the present time farnesol derivatives are the only compounds of known chemical composition with juvenile hormone activity. They were identified as the substance with juvenile hormone activity in the extracts of non-insect material. They have been found in the extracts of *cecropia* and other silkworms. Until other known compounds have been isolated, I am inclined to hold to the provisional hypothesis that the active group in the juvenile hormone is indeed the triple isoprene unit of farnesol, and that this exists in the natural hormone in some form that has not yet been defined.

- ¹ Wigglesworth, V. B., *Quart. J. Micro. Sci.*, **77**, 191 (1934).
- ² Wigglesworth, V. B., *Quart. J. Micro. Sci.*, **79**, 91 (1936).
- ³ Wigglesworth, V. B., *J. Insect Physiol.*, **2**, 73 (1958).
- ⁴ Piepho, H., *Verh. Dtsch. Zoo. Ges.* (Wilhelmshaven), **62** (1951).
- ⁵ Schaller, F., *Bull. Soc. Zoo. Fr.*, **77**, 195 (1952).
- ⁶ Williams, C. M., *Biol. Bull., Woods Hole*, **121**, 572 (1961).
- ⁷ Staal, G. B., *Entom. Bericht.*, **25**, 34 (1955).
- ⁸ Wigglesworth, V. B., *The Physiology of Insect Metamorphosis* (Cambridge University Press, 1954).
- ⁹ Joly, P., *Insectes Sociaux*, **3**, 17 (1956).
- ¹⁰ Lees, A. D., *Symp. Roy. Entomol. Soc. Lond.*, **1** (Insect Polymorphism), 68 (1961).
- ¹¹ Kalsner, P., *Naturwiss.*, **42**, 303 (1955).
- ¹² Lüscher, M., *Naturwiss.*, **45**, 69 (1958).
- ¹³ Wigglesworth, V. B., *J. Embryol. Exp. Morph.*, **1**, 269 (1953).
- ¹⁴ Wigglesworth, V. B., *Symp. Soc. Exp. Biol.*, **11**, 204 (1957).
- ¹⁵ Beermann, W., *Chromosoma*, **5**, 138 (1952).
- ¹⁶ Jacob J., and Monod, J., *Cold Spring Harbor Symp. Quant. Biol.*, **26**, 193 (1961).
- ¹⁷ Wigglesworth, V. B., *Naturwiss.*, **27**, 301 (1939).
- ¹⁸ Lawrence, P., thesis, Univ. Camb. (1965).
- ¹⁹ Ozeki, K., *Sci. Papers Coll. Gen. Educ. Univ. Tokyo*, **11**, 102 (1961).
- ²⁰ Piepho, H., *Naturwiss.*, **27**, 301 (1939).
- ²¹ Piepho, H., and Meyer, H., *Biol. Zbl.*, **70**, 252 (1951).
- ²² Gilbert, L. I., and Schneiderman, H. A., *Trans. Amer. Micro. Soc.*, **79**, 38 (1960).
- ²³ Wigglesworth, V. B., *J. Insect Physiol.*, **9**, 105 (1963).
- ²⁴ Wigglesworth, V. B., *J. Exp. Biol.*, **32**, 485 (1955).
- ²⁵ Lockshin, R. A., and Williams, C. M., *J. Insect Physiol.*, **10**, 642 (1965).
- ²⁶ Piepho, H., *Z. Tierpsychol.*, **7**, 424 (1950).
- ²⁷ Piepho, H., Böden, E., and Holz, I., *Z. Tierpsychol.*, **17**, 261 (1960).
- ²⁸ Williams, C. M., *Nature*, **178**, 212 (1956).
- ²⁹ Coles, G. C., *Nature*, **203**, 323 (1964).
- ³⁰ Odhiambo, T. R., thesis, Univ. Cambridge (1965).
- ³¹ Pfeiffer, L. W., *J. Exp. Zool.*, **99**, 183 (1945).
- ³² Odhiambo, T. R., *Nature*, **207**, 1314 (1965).
- ³³ Thomsen, E., *J. Exp. Biol.*, **26**, 137 (1949).
- ³⁴ Sägerser, H., *J. Insect Physiol.*, **5**, 204 (1960).
- ³⁵ Novák, V. J. A., Slama, K., and Wenig, K., *The Ontogeny of Insects*, Symposium in Prague 1959, 147 (1961).
- ³⁶ Wigglesworth, V. B., *J. Exp. Biol.*, **25**, 1 (1948).
- ³⁷ Stegwee, D., *J. Insect Physiol.*, **10**, 97 (1964).
- ³⁸ Gilbert, L. I. (unpublished results).
- ³⁹ Schmialek, P., *Z. Naturf.*, **16b**, 461 (1961).
- ⁴⁰ Wigglesworth, V. B., *J. Insect Physiol.*, **7**, 73 (1961).
- ⁴¹ Schmialek, P., *Z. Naturf.*, **18b**, 516 (1963).
- ⁴² Williams, C. M., and Law, J. H., *J. Insect Physiol.*, **11**, 569 (1965).
- ⁴³ Meyer, A. S., Schneiderman, H. A., and Gilbert, L. I., *Nature*, **206**, 272 (1965).
- ⁴⁴ Røller, H., Bjerke, J. S., and McShan, W. H., *J. Insect Physiol.*, **11**, 1185 (1965).

OBITUARIES

Dr. H. R. Marston, F.R.S.

DR. HEDLEY RALPH MARSTON died in Adelaide on August 25, the day before his sixty-fifth birthday, and the day on which he was to retire from his post of chief of the Division of Biochemistry and General Nutrition of the Australian Commonwealth Scientific and Industrial Research Organization. He was one of three brothers born in Bordertown, South Australia; his father was a Civil Servant. The family moved to Adelaide where he attended Unley High School. A school-fellow, Mark (now Sir Mark) Oliphant, attained high scientific distinction and remained a life-long friend. In 1934 Marston married Kathleen Nelly Spooner, who survives him. They had no children.

There were two rather unusual features about Marston's career. From first to last he held appointments in Adelaide; and his first degree was conferred on him *honoris causa*, some thirty-three years after his first academic appointment. When an undergraduate student of chemistry in the University of Adelaide, he chanced to meet, during a country walk, a stranger with whom he fell into conversation. This proved to be Brailsford Robertson, newly arrived to take up the chair of physiology and biochemistry in the University. Within a few days, Marston had joined Robertson's department as demonstrator, and during the next seven years assisted him in organizing the teaching of biochemistry and physiology in the University. In 1926, Marston sat for the B.Sc. examination in biochemistry and physiology and headed the honours list; but he was

then so fully engaged in research and teaching that he was unwilling to retrace his steps in order to comply with the regulations necessary for taking a degree. This rather orthodox entry to the academic world in some degree calls that of Gowland Hopkins, who was first an articulated pupil to a public analyst: he moved to Guy's Hospital Medical School, where he took a medical degree, but only a time snatched from research and teaching commitments. It may be that academic life will be the poorer now that insistence on 'paper qualifications' has made such unusual shortcuts all but impossible. During these early years, Marston received much help from Archibald Watson, a professor of anatomy, of whom he always spoke with affection, as well as from Brailsford Robertson.

His first paper adumbrated the importance of the mitochondria as centres of enzyme activities—a concept since fully confirmed. In other papers he showed for the first time that the mammary gland of the monotreme echidna indeed secrete true milk, described the first micro-method for the determination of guanidine and methyl guanidine, and published work on the sterol isolated from the 'giant toad stool'.

In 1927 he joined the Division of Animal Nutrition of the (then) C.S.I.R., newly formed under Brailsford Robertson. He was in charge of the Division between Robertson's death in 1929, and the appointment of Sir Charles Martin as chief in 1930. A close bond grew up with Martin, and when he returned to England in 1932, Marston was appointed chief nutrition officer, and placed in charge of the Nutrition Laboratory, which became part of the Division of Animal Health until 1944, when the Division of Biochemistry and General Nutrition was established with Marston as its first chief. During this period the researches of Marston and his colleagues covered a wide field and had important practical applications. While he always insisted that advances in practice depended on an understanding of the underlying natural phenomena, he nevertheless tried to ensure that the practical means of applying scientific principles were available. Among other subjects, the work included valuable fundamental researches on the animal's requirements for protein for the manufacture of skin and wool and on the special need for sulphur-containing amino-acids (now, but not then, common knowledge). He early observed the effect of copper on keratinization and hence on the quality of wool. Other work dealt with the requirements of the sheep for phosphorus, and of the effects on it of thyroidectomy.

In 1936 he was invited by Hopkins to spend a year in the Biochemical Laboratory, University of Cambridge. There he continued his work on carbohydrate fermentation by ruminal micro-organisms, and interested Joseph Racker in general problems of ruminal function, with most fruitful results.

During the Second World War he furnished, with his colleagues, valuable information to the Army on available human rations, and compiled highly useful dietary tables. After the War he worked on the basic problems of energy transactions in ruminants. The work for which he is most widely known can be traced to its beginnings in 1933, when he became interested in 'Coast disease'. Considerable stretches of land, among them the 'ninety mile desert' near his birthplace, would support neither useful crops nor domestic stock. This was (wrongly) ascribed to phosphorus deficiency. Marston and his colleagues showed that phosphorus was adequate, but that there was a lack of cobalt and copper, and in certain areas of zinc, and that traces of such elements would permit the normal growth of both plants and animals. These fundamental discoveries proved the key to the solution of difficulties encountered by agriculturists and pastoralists in other continents besides Australia, and gave impetus to the conception of the importance of heavy metals in biological processes which is still growing. Marston was able to show that cobalt was effective only after it had been incorporated into vitamin B₁₂ by ruminal

micro-organisms, and that at least one of its functions in the body was that of co-enzyme in certain isomeric changes. The story must now be left to other hands to unfold: that there are further chapters is certain.

Marston was widely read, and widely travelled, both in Europe and the United States, with a fund of reminiscences of people and places. In 1957 he was elected D.Sc. *honoris causa* (his first degree) by the Australian National University, and in 1959 to a D.Sc. of the University of Adelaide *ad eundem gradum*. He was invited to lecture on the work of the Division to the Royal Society of London in 1948, and was elected Fellow in 1949. In 1951, Sir Mark Oliphant and he were active in the foundation of the Australian Academy of Sciences, of which they were original members. He numbered among his close friends not only scientific colleagues such as Martin, Hopkins, Rivett, Oliphant and E. V. McCollum, but also famous artists, and leaders in the industrial world such as W. S. Robinson in Australia and J. L. Pratt and Robert Kleberg, jun., in the United States. Perhaps from such contacts sprang some of his sympathy with practical problems.

E. G. HOLMES

Prof. Arthur Holmes, F.R.S.

ARTHUR HOLMES, emeritus professor of geology and mineralogy in the University of Edinburgh, died on September 20 at the age of seventy-five at his home in Putney. His brilliant research on the wider problems of geology has earned him his place as one of the great Earth scientists of the century.

Holmes was born at Hebburn, near Newcastle-upon-Tyne, in 1890. He graduated at the Imperial College of Science and Technology in 1910 and undertook an expedition to Mozambique in 1911. Here an attack of blackwater fever nearly ended his career (there was in fact an announcement of his death in the local paper), but he came back to rejoin the staff of Imperial College until 1921. He then joined the Yomah Oil Co. in Burma as chief geologist; but, fortunately for the future of geological science, he returned in 1924 to become head of the newly created Department of Geology in the University of Durham, which under his leadership rapidly gained an international reputation for petrological research. In 1943 he was appointed regius professor of geology in the University of Edinburgh, which was honoured with his occupation of the chair for thirteen years, and with his presence as emeritus professor for six years after he retired in 1956.

Prof. Holmes has exercised a profound influence on almost all branches of geology, not only through his own remarkable researches but also by his beautifully written text-books and the stimulation of the research qualities of his students. His output of publications has been prodigious—nearly 200, including several famous text-books such as *The Age of the Earth*, *Nomenclature of Petrology*, *Petrographic Methods and Calculations* and *Principles of Physical Geology*. He has made a great number of contributions to our knowledge of the geology of many parts of the world, from his home district in the north of England to Africa, India and elsewhere; but the descriptive aspect of his work has been, far more than with most men, merely a prelude to his remarkable work on the wider genetic problems of the science. He went deeply into the major questions of the origin of igneous rocks, partly in association with his accomplished and eminent wife, Dr. Doris Reynolds. He was never a man to shun controversy, and his work on the origin of the alkaline rocks of Africa and elsewhere, on the thorny problems of granites and their associates, on the origin and development of magmas, and on the role of metasomatism in rock genesis, has broadened the vision of igneous and metamorphic petrologists all over the world.

During his school-days, Holmes's inquiring mind had questioned the magic date for the Creation, 4004 B.C., in Archbishop Ussher's chronology for the book of *Genesis*,

and in the sixth form his interest in geology was awakened by an enlightened schoolmaster who encouraged him to read Kelvin and Suess. He was thirteen years of age when Rutherford realized that radioactivity might provide a means for determining the actual ages of minerals, and he was an undergraduate at Imperial College when this idea bore fruit, and Boltwood (using lead ratios) and then Strutt (using helium content) approximately dated a number of uranium-bearing minerals. Working in Strutt's laboratory, Holmes became one of the pioneers in this field, and since that time he has been one of the leaders in applying the results of radioactive methods to the dating of rocks. He took a major part in the construction of a geological time-scale in terms of millions of years—considering both post- and pre-Cambrian rocks and the age of the Earth itself.

Holmes's major work was not confined to igneous and metamorphic petrology and geological time. His contributions to the geophysical aspects of geology are classic: the nature of orogenic and epeirogenic activity, convection currents in the Earth's mantle, continental drift, radioactivity in geology—one could go on for a long time listing the aspects of geology to which he has made major contributions. In fact there is scarcely a main branch of the subject, other than palaeontology, with which his name is not associated. Much of his thinking was on the grand scale, applied to the broad philosophical aspects of the Earth sciences.

Holmes's distinguished career received international acclaim. He was elected Corresponding, Foreign or Honorary Member of the Geological Societies of America, Belgium, and Stockholm, and the Academies of Science of the United States, Sweden, the Netherlands, and France. He was awarded the Murchison and Wollaston Medals of the Geological Society of London, the Penrose Medal of the Geological Society of America, the Fourmarier Medal of the Royal Academy of Belgium, and the Makdougall-Brisbane Prize of the Royal Society of Edinburgh. In 1964 he shared with Prof. P. Eskola of Helsinki the Vetlesen Prize of Columbia University, New York, for outstanding achievement in science relating to "a clearer understanding of the Earth, its history or its relation to the universe". He was a Fellow of the Imperial College of Science and Technology and an Honorary Doctor of Laws of the University of Edinburgh.

In spite of many years of poor health, Holmes continued his creative work until the end. The second edition (largely re-written and greatly enlarged) of his *Principles of Physical Geology*, published this year, is a masterpiece of lucid writing and shows a breadth of knowledge and vision which few men possess.

Those fortunate enough to have known Prof. Holmes found him a quiet man of extraordinary charm and unfailing kindness, and will remember with affection and gratitude the help and advice which he gave so readily. In 1914 he married Margaret Howe, and they had one son. His wife died in 1938, and he later married Dr. Doris Reynolds, herself a distinguished geologist.

F. H. STEWART

Sir Ernest Thomas Fisk

WITH the death of Sir Ernest Thomas Fisk at his home in Sydney on July 8, at the age of seventy-eight, the world lost a man who made a conspicuous contribution to the development of wireless communication.

Born at Sunbury-on-Thames, England, on August 8, 1886, he entered the Marconi Company's training school in 1906 and came to Australia in 1911 as resident engineer of a branch which the Company had opened in Sydney. He submitted to the Australian Government proposals for the setting up of a number of wireless stations, but his proposals were not accepted.

In the same year, the Australasian Wireless Company was formed with Australian capital to acquire and operate

the patent rights of the Telefunken system. Two years later, Amalgamated Wireless (Australasia), Ltd., was inaugurated, and the rights of the Marconi and Telefunken systems were acquired. Fisk became technical and general manager, and in 1917, managing director.

In England in 1916 Fisk arranged with Marconi to conduct a series of tests, and in the following year erected an experimental receiving station at Pymble near Sydney. Tests were conducted there with the view of determining the best type of circuit to use for long-wave long-distance reception.

Later, Fisk transferred the station to Wahroong another Sydney suburb, and began experiments with the Caernarvon station in Wales early in 1918. When the tests were satisfactory, arrangements were made for public demonstration of direct wireless communication between England and Australia.

Messages to Australian newspapers were sent from Caernarvon by the then Australian Prime Minister, Mr. W. M. Hughes, and the then Australian Minister for the Navy, Sir Joseph Cook, who were in England at that time. These, the first direct wireless messages between the two countries, were received at Fisk's Wahroonga station and delivered to the Sydney Press.

Later, the experimental receiving station was transferred to Koo-wee-rup, in Victoria, where the scope of the work was extended, new apparatus was installed and continuous day and night observations made of signals from all high-powered stations in Europe and the United States.

The Koo-wee-rup experiments established that wireless waves from England to Australia followed the course of the greatest darkness—a discovery which ultimately was of immense value when aerials for the short-wave service were being designed.

As an outcome of successful experiments, Fisk, on behalf of Amalgamated Wireless in conjunction with the Marconi Company, in 1921 offered to establish a direct wireless service between Australia and Great Britain and to transmit messages at rates one-third less than existing cable charges. The proposal, which was strongly opposed by exponents of a relay system, found champions in Mr. W. M. Hughes and General Smuts of South Africa. They opposed the relay system and made it clear at the Imperial Conference in 1921 that their respective countries would proceed with direct communication.

Strengthened by the positive attitude of Mr. Hughes, Fisk persisted with his advocacy of a direct service, and in March 1922, on behalf of Amalgamated Wireless signed an agreement with the Australian Government under which the Company was to establish and maintain direct wireless communication between Australia and the United Kingdom and Canada, and to assume control of existing coastal and island wireless stations. The Government acquired 50 per cent of the shares in the Company plus one to give it a bare majority, the remainder of the shares being held by private shareholders.

Fisk was not daunted by the refusal of the British Government to grant a licence to the Marconi Company to erect a high-power station in England. The experiments were continued, and in January 1924 the first transmission of short-wave signals was received from the Marconi station at Poldhu, Cornwall. In August of that year, a fresh agreement was entered into with Amalgamated Wireless to erect short-wave transmitters with directional aerials to provide service to Great Britain and to Canada when corresponding stations were built in those countries. Fisk's foresight and initiative were rewarded in 1927 with the opening of the Beam Service to the United Kingdom and in 1928 to Canada.

Commenting that the Company had fought against powerful opposition, Royal Commissioners who investigated broadcasting within Australia reported to the Federal Parliament in 1927 that the Company, Fisk and his officers were "entitled to great credit for the establish-

ment of the Beam system". They commented that "... was largely due to the technical ability and persistence of its managing director that it ultimately prevailed and that Australia has the benefit of an up-to-date and extremely rapid means of communication with Great Britain...".

In the field of Australian broadcasting, Fisk was a pioneer. He gave the first demonstration of wireless telephony in Australia in August 1919. Gramophone music played into a wireless transmitter was received in

the lecture room of the Royal Society of New South Wales, several city blocks away. A year later he arranged a complete broadcast concert to a large audience in Parliament House, Melbourne.

He was foundation president of the Institution of Radio Engineers Australia and one of the earliest members of the Wireless Institute of Australia. He continued as chairman of directors of Amalgamated Wireless until 1945.

L. A. HOOKE

NEWS and VIEWS

Royal Society Research Professorship: Prof. W. S. Fyfe

PROF. W. S. FYFE, professor of geology in the University of California, Berkeley, has been appointed to a Royal Society research professorship. He will work at the University of Manchester and will take up his appointment in January 1966. Prof. Fyfe, who was born in New Zealand and has held appointments at the University of Otago and the University of California, is well known for his work in the field of experimental mineralogy and petrology.

Anglo-French Military Aircraft: Ministry of Aviation:

Mr. J. A. Hamilton

MR. J. A. HAMILTON, formerly of the Royal Aircraft Establishment, Farnborough, has been appointed project director for the *Jaguar* strike/trainer and the variable-geometry combat aircraft to be developed jointly by the United Kingdom and France. He will be responsible within the Ministry of Aviation for the management of the two projects, acting in concert with corresponding directors in the French Direction Technique des Constructions Aeronautiques. Mr. Hamilton was educated in Scotland at Penicuik and Lasswade Secondary Schools; he graduated in engineering from the University of Edinburgh in 1943 and then joined the Marine Aircraft Experimental Establishment. He was engaged initially on the development of anti-submarine weapons and later became responsible for all flight research within the Establishment. In 1951 he joined the Aerodynamics Department of the Royal Aircraft Establishment and in 1952 he was appointed head of the Free-flight Division. During his stay at Farnborough he promoted the development of methods for conducting aerodynamic research using rocket-propelled test vehicles, eventually co-operating with the Australian Weapons Research Establishment to extend the technique into the régime of hypersonic flight. Immediately before transfer to his present post he was head of the Projects Division of the Aerodynamics Department, and in this capacity responsible for the preliminary study of future military and civil aircraft.

Special Merit Promotions at the National Physical Laboratory:

Mr. H. H. Pearcey

It has recently been announced that Mr. H. H. Pearcey, of the Aerodynamics Division, National Physical Laboratory, has had a special merit promotion to deputy chief scientific officer. Mr. Pearcey is an internationally recognized authority in several branches of fluid dynamics, particularly the interaction between shock waves and boundary layers and the many intricate features of transonic, supersonic and separated flows. He has successfully used the results of his research in the progressive development of swept-wing aircraft for cruising at high subsonic, transonic and supersonic speeds by synthesizing methods for the design of the wing section and by evolving advanced section shapes. His 'peaky' type of aerofoil, with isentropic supersonic compressions, and the work that he initiated and supervised on aerofoils with thick trailing edges, including the use of automatic ventilation

to reduce the base drag, are well known. So also is his important discovery of the intrinsic connexion between the variation of static pressure at the trailing edge of a wing and the effects of shock-induced and other types of boundary-layer separation. His suggestion that this could be used to predict the threshold of aircraft buffeting has been widely exploited. Mr. Pearcey also made a classic investigation of methods for suppressing the separations that cause the buffeting and other undesirable aerodynamic characteristics. In particular, he demonstrated how the properties of vortex generators can be utilized in this respect and has been instrumental in applying them to numerous aircraft, thus effecting many significant improvements in aerodynamic behaviour and in safety at both high and low speeds. During the course of his work, Mr. Pearcey has made vital contributions to the development of experimental methods for research in high-speed flow and has played a leading part in planning, designing and commissioning the fine range of high-speed wind-tunnels now operating in the Aerodynamics Division of the National Physical Laboratory. Since 1958 he has been responsible for the High-speed Flow Group at the Laboratory; in 1963 he was awarded the Bronze Medal of the Royal Aeronautical Society, for his contributions to aerodynamics, and gained the Wolfe Award of the Department of Scientific and Industrial Research, for an outstanding contribution to the Department's research programme.

Dr. R. C. Lock

DR. R. C. LOCK, of the Aerodynamics Division, National Physical Laboratory, has been promoted to senior principal scientific officer (special merit). Dr. Lock is perhaps best known in aviation circles for his outstanding theoretical and experimental research that has produced aerodynamic design methods which render the concept of shock-free, low-drag flow—derived from the abstract infinite yawed wing—a reality for the finite-wing/fuselage combinations of practical aircraft, even for supersonic flight speeds. His papers on this subject are well established and extensively used by aircraft designers; one, on wing planform design, won the Royal Aeronautical Society's Edward Busk Memorial Prize for 1962. Dr. Lock is a leading figure in swept-wing research and plays a prominent part in several programmes in this and related fields that are co-ordinated between the National Physical Laboratory and other establishments in the United Kingdom, Europe and the Commonwealth. However, his reputation is more widely based than this. Already, before joining the Laboratory in 1954, he had had a brilliant career at Cambridge, and this had been interrupted at the end of the War by a brief but successful spell at the Royal Aircraft Establishment working on problems of aircraft design. At Cambridge he won the University Mayhew Prize for the best applied mathematics candidate in the Mathematics Tripos, Part III, and was awarded a research fellowship at Gonville and Caius College during the tenure of which he made important contributions in the fields of hydrodynamic stability and magnetohydrodynamics. At the National Physical Laboratory he first turned his attention to in-

volved problems of supersonic wing theory and wing/body interference, which led on to his work on swept wings, to his complementary experimental programme and to his active interest in the application of numerical methods and modern digital computers to outstanding problems in fluid dynamics. It is of considerable interest to note that Dr. Lock's work is in direct line of descent from the work that his father, the late C. N. H. Lock, pioneered at the National Physical Laboratory in the 1930's.

National Instrument Service in Britain

THE Science Research Council, in collaboration with the Ministry of Technology and the Office of Scientific and Technical Information, set up earlier this year a Panel under the chairmanship of Prof. G. Porter (University of Sheffield) to consider the feasibility and economics of a centralized national instrument service. A number of expensive, physicochemical instruments could provide a rapid service of routine measurements, on a repayment basis, for all scientists, such as those in Government, industrial and university laboratories. This service would enable physical-analytical techniques to be more widely available than at present and would thereby encourage the use of new instrumental techniques. Such a centralized arrangement would have additional advantages, particularly if it were associated with an interpretation service and data-store facilities. The Panel is hoping to find out whether such a service would be an effective means of meeting more economically the growing needs of research scientists for routine measurements on increasingly expensive research instruments. A description of some services which might be offered is being circulated, together with a brief questionnaire, to a cross-section of potential customers. The answers received will enable an assessment to be made of the need for services of this kind and of their economic viability. When this assessment has been thoroughly studied, the Panel will make recommendations to the Science Research Council regarding any future action.

Cambridge Engineering Freshmen

It is too early to know the state of this year's entry of students into the technological faculties of all universities and colleges of advanced technology, but a fact which should cheer industry is that at the University of Cambridge the number of freshmen entering the Engineering Department is 304, the highest in any year since the Mechanical Sciences Tripos was established in 1892. The exceptional quality of these men is indicated by the fact that 208 of them have been admitted to the fast stream which enables them to complete the course leading to the Mechanical Sciences Tripos Part I after two years of study instead of the normal three. As recently as 1960 the rate of freshmen entering the fast stream was only about one-third of the total intake.

I.C.S.U. Bulletin

The *I.C.S.U. Bulletin* (No. 4; July 1965), issued by the International Council of Scientific Unions, contains a brief account of the third meeting of the Executive Committee of the International Council of Scientific Unions, to which was presented a report on the Scientific Committee on Oceanic Research. There has been a steady increase in the activities of this Committee, which now has twenty-seven national adhering institutions. A report was also presented from the Scientific Committee on Antarctic Research, and a discussion on the work of this Committee directed attention to the near extinction of some species in Antarctica due to efficient and intensive fishing operations, and it was suggested that the restricted areas around Antarctica should be extended from 3 to 10 miles. The Executive Committee agreed that immediate action was necessary to prevent certain whales, particularly the blue whale, from becoming extinct. It also

discussed at length the increasing importance of solar terrestrial physics and the activities of the Inter-Union Commission on Solar Terrestrial Relations, its terms of reference and membership. A report was also presented from the Working Group on Relations with Developing Countries, and the suggestion was made that scientists from advanced countries should be encouraged to undertake missions in the developing countries. Scientists from the latter countries should be assisted to enable them to attend international meetings. The *Bulletin* also contains news from the scientific unions, including reports on: the work of the Committee on Atmospheric Sciences; the work of the Naples Zoological Station; the International Union of Biochemistry; and other reports from the Scientific Committee on Oceanic Research, the Committee on Space Research, the Federation of Astronomical and Geophysical Services, the Special Committee for the International Years of the Quiet Sun, and on the International Committee for Geophysics. There is also a list of publications, and the usual calendar of meetings for August 1965–January 1966.

International Paper Size

THE proposed change-over to the metric system in Britain will necessarily take a long time for full realization and it will pose many practical problems, both industrial and domestic. Not the least of these is the effect on paper sizes and printing, where tradition is likely to die very hard. If the proposed scheme of the metric-based 'International Paper Sizes' is adopted, it will completely revolutionize the present practice of the printing and publishing trades, with consequent repercussions in commerce, professional and private customs, particularly where books, pamphlets, display leaflets, stationery and envelopes are concerned, to mention only a few of the items involved. The keyword behind the conception of 'International Paper Sizes', as it is known, is simplification and rationalization. It means "... the introduction of a completely new range of paper sizes, from small to large, each based upon a metric measure and each related, in a logical sequence and proportion, to the rest of the range". One result, perhaps not unwelcome, will be the replacement of the time-honoured words 'quarto', 'crown', 'octavo', etc., by a numerical code, which will be universally understood. An impartial and non-technical exposition of the definitions, advantages and disadvantages of the 'International Paper Sizes' system has recently been published in the form of a booklet entitled *Paper at Work Number 4: International Paper Sizes* (A Series of Spicer Guides. Pp. 8. London: Spicers, Ltd., 1965). Included in this booklet is a full-sized A1 sheet (23.4 in. × 33.1 in., which is half the A0 size of 1 square metre. This A1 sheet is folded to demonstrate physically the full sizes of A2, A3, A4 and A5 and their relationship to each other. This example adequately illustrates the new code; the system is based on three series of sizes—all of the same proportion—designated A, B and C. The A series is perhaps the most widely used, for example, for stationery and general leaflet printing; the B series is intended primarily for larger printed items such as posters, wall charts, etc.; the C series, in conjunction with some of the B sizes, is intended for envelopes. In the ultimate adoption of this 'International Paper Size' system there are undoubtedly benefits of cost, convenience, international standardization and simplification. The disadvantages also have to be faced, but in the long run should not prove insuperable; for example, some printers' machinery may not be geared to handle 'International Paper Size' with optimum productivity; also office filing, filing equipment, addressing machines, etc. may not be suitable for dealing with 'International Paper Size' sizes; but these hazards will be overcome eventually and meanwhile everybody concerned has time for consideration and thoughtful reappraisal of the important

issues involved. Finally, the vital concern of all industry in Britain at the present time is export and it is here that 'International Paper Size' has come into its own. Twenty-six countries are using the system for commercial and technical paperwork; these include Austria, Belgium, Denmark, Finland, Germany, Holland, Italy, Norway, Portugal, Spain, Sweden and Switzerland.

Millboard Manufacture

THE main raw material in the manufacture of millboard is waste paper, and this, like many of the other commodities used in the process, has considerably increased in price since the end of 1964. Coupled with increased wages and overheads, and the fact that many overtime hours have had to be worked in the factory at Bourne End, Bucks., due to permanent under-staffing, the price structure of the products of Jackson's Millboard and Fibre Co., Ltd., has been correspondingly increased. These and some other interesting items are disclosed in the statement of the chairman (Mr. M. W. Jackson) at the seventy-fourth annual general meeting on September 29, 1965 (*Jackson's Millboard and Fibre Co., Ltd., Directors' Report and Accounts, 1965*. Pp. 12. London: Jackson's Millboard and Fibre Co., Ltd., 1965). The first fully automated intermittent board-making machine in Britain was installed in the Company's main factory in 1962. In this connexion, Mr. Jackson says: "In my report in September 1964 I referred to the purchase of the board making unit from Norway, and this installation is now on the point of completion. We have also modernized one of our existing board-making units at present concerned with the production of leatherboard, and this makes our second automatic machine. It is hoped that well before the end of the year we shall have three automatic board-making units in operation at Bourne End". These innovations should help considerably to mitigate price increases and expand the Company's export trade, an important item. But in spite of all efforts to improve efficiency, including expenditure on modern plant as described, "... the increases in operating costs over recent months are more than we are able to bear, if we are to show a reasonable return". Thus, even an advanced state of automation in manufacture, such as is in process of achievement in this factory, would not appear to be a panacea for all the economic ills in this industry at the present time. It can only be hoped that Mr. Jackson's somewhat gloomy foreboding will be dispelled by events this year in the shape of increased turnover and export business, especially in the Far East, where interesting results of overseas visits appear already to be bearing fruit.

Particle Size Measurement

A REPORT of a meeting, held in November 1963, at the British Ceramic Research Association, Stoke-on-Trent, to consider particle size measurement, has recently been published by the British Ceramic Society on behalf of the European Ceramic Association (Pp. 20. British Ceramic Society, Shelton House, Stoke-on-Trent, August 1965. 5s.). Measurement of particle size and interpretation of results to practical advantage are common problems in the ceramic and many other industries. Recent years have seen many improvements in traditional methods of measurement, such as using the microscope and the technique of sedimentation, but methods based on new principles have also appeared. It is pointed out, however, that unfortunately none of the latter has universal application, and so diversity of method increases. At the meeting four prepared contributions formed a basis for discussion: a review of the purpose of analysis and problems involved as seen by the British Ceramic Research Association; a report on a recent classification of methods undertaken by a sub-committee of the Society for Analytical Chemistry; an assessment of the Coulter

counter as a means of analysis of some ceramic materials; and an account of size analysis of clays within the sub-micron range. On the subject of appraisal of methods of particle size measurement now commonly publicized, some interesting results are revealed from replies to a questionnaire sent to industrial concerns, universities and colleges of technology. The questions concerned methods actually in use, type of material analysed, size-range, purpose of measurement (routine control, research, etc.). In so far as replies from 69 respondents may at this stage be considered representative, cautiously interpreted, they are certainly indicative, and fall into line with what might well be anticipated as the trend of things to-day. Summarily, sieving is the most frequently used single method; the Andreasen pipette method follows closely; sedimentation methods are generally popular; elutriation is relatively seldom used; gratitudes are frequently used, so also is the Coulter counter, while permeability methods receive a fair share of support. Of the 74 methods of particle size measurement in use and listed in the classification, 24 were not used by the respondents. Of the 69 replies received, 32 respondents used one method or another in routine control operations, 61 in research or development work. It is planned to organize comparative tests of some of the better-known methods on a large scale; possibly this may take the form of analysis by different laboratories of the same sample by the same method, or the analysis by the same laboratory of the same sample by different methods.

Surface Active Agents

SURFACE active agents are playing an increasingly important part in the manufacture of many diverse products in use in everyday life. These vary from materials such as oil paints and plastics, to duplicating inks and deodorants. These agents are incorporated in industrial and domestic preparations not only for their 'point of use' effect, for example, in shampoos, paint removers, etc., but also to facilitate manufacture, as in the case of emulsion paints, or to promote consistency in products such as cosmetic creams. The problem of determining the type of surface active agent present in a variety of different preparations is one constantly facing many analytical and industrial chemists, some of whom, by long experience, are experts in this field. Others, however, possibly the majority, have limited experience of these agents, and the apparatus requisite to their analysis. For the latter group, particularly, a most useful handbook has recently been published entitled *Surface Active Agents: their Extraction, Characterisation and Determination*, by Maurice Bell (Pp. 24. Leeds: Glovers (Chemicals), Ltd., 1965. 5s.). It is shown in this booklet that, in spite of the wide range of products of which surface active agents are important constituents, it is possible to divide such materials into a comparatively small number of groups: emulsions (oil-in-water or water-in-oil); aqueous based (that is, dissolved in water or mixtures of water and water-miscible solvents); non-aqueous based (dissolved or dispersed in oils, fats or organic solvents); and dry based (adsorbed on or admixed with powdered inorganic or organic materials, or similar in tableted or cake form). With this classification in mind, the various techniques and tests are considered under the appropriate headings: separation of the isolated surface active agents; characterization of the isolated surface active agents; quantitative examination of surface active agents; control of surface active agent concentration; chemical methods of control. The directions throughout, although to some extent summarized, are clear, concise and practical; if further detailed information on any procedure is required, then the list of references included will help. Domestic soap and detergent powders, washing-up liquids, and the like, are excluded from this booklet. They are the subject of special investigations and analytical schemes described

by other authors, to whom appropriate references are quoted. This little monograph is to be commended. It is the first of a series which it is the intention of the publishers to issue from time to time on various aspects of surface chemistry.

A Fresh Approach to the Problem of Allergy

G. B. West and J. M. Harris of the Department of Pharmacology, School of Pharmacy, University of London, have shown that rats secured from one Wistar albino colony are resistant to the first injection of dextran or egg-white, and that they fail to develop gross oedema of the extremities (the so-called anaphylactoid reaction) (*Ann. N.Y. Acad. Sci.*, 118, Article 8: *Pharmacogenetics—a Fresh Approach to the Problem of Allergy*. Pp. 439–452. New York: New York Academy of Sciences, 1964). Selective breeding experiments showed, first, that this non-reactivity is an autosomal recessive character, and secondly, that it is not linked with the colour genes for black or yellow. Pure stocks of non-reactor rats were established for both albino and non-albino strains, confirming that the anaphylactoid reaction is genetically controlled. Egg-white allergy in man is also partly inherited and also produces severe oedema of the exposed parts. It will be important, therefore, to determine the cause of non-reactivity of rats to egg-white and dextran, particularly as some genetical polymorphisms in man are known to produce biochemical differences.

Soil and Landscape at Archaeological Sites

It is not unusual for the pedologist to be concerned in the study of former occupation sites, and an interesting paper on the examination of soil profiles, and a suggested chronology of events in such a neighbourhood, has been prepared by P. H. Walker (*Records of the Australian Museum*, 26, No. 7; June 12, 1964: *Soil and Landscape History in the Vicinity of Archaeological Sites at Glen Davis, New South Wales*. Pp. 247–264+plates 25–27. Sydney: The Australian Museum, 1964. 6s.). The sites are in shelters formed from large erratic sandstone boulders on the lower slopes of the Capertree River Valley, Australia, where they are characterized by several hundred feet of intensively weathered coarse sandstone talus with narrow terraces. The debris on the upslope side of boulders reveals the past movement of the superficial deposits. The country rock is highly quartzose Triassic sandstone displaying strong current bedding. The deposits of the cave floors are relatively uniform and unstratified, and contain few rock fragments in comparison with the soil outside; but, according to modal particle size, are derived from the sandstone rock, are less sorted than the river deposits and have arisen from the deposition of coarse material in a sheltered position. Artefacts are found in the loose, sandy layers of the soil, which is unique in the locality. The soils inside and outside the caves are, however, to be regarded as minor surface disturbances of short duration in the development of the valley.

Gairdner Foundation Awards

THE 1965 Gairdner Foundation annual awards, valued at 5,000 dollars, have recently been awarded to: Dr. Charles P. LeBlond, head of the Department of Anatomy, McGill University, Montreal, for development of the technique of autoradiography; Dr. Jerome W. Conn, University Hospital, Ann Arbor, Michigan, for his pioneering work on the clinical significance of aldosterone; Dr. R. R. A. Coombs, Department of Pathology, University of Cambridge, for his work on antibody on the surface of red blood cells; Dr. Charles E. Dent, University College Hospital, London, for his work on paper chromatography leading to the discovery of diseases caused by imbalance of amino-acids; Dr. Daniel J. McCarty, Hahnemann Medical College and Hospital, Philadelphia, for his work on the causes of gout and rheumatoid arthritis; Sir Horace Smirk, University of Otago Medical School,

Dunedin, New Zealand, for his work on the application of nerve-blocking drugs in the treatment of high blood pressure. The Foundation was established in 1957 by J. A. Gairdner, the Toronto industrialist. Annual award of 5,000 dollars are bestowed on those who "have made contributions to the conquest of disease and the relief of human suffering".

David Anderson-Berry Prize, 1966

A DAVID ANDERSON-BERRY MEDAL, together with a sum of money, will be awarded by the Council of the Royal Society of Edinburgh during 1966, for recent work on the effects of X-rays and other forms of radiation on living tissues. Applications and proposals on behalf of others should be addressed to the General Secretary Royal Society of Edinburgh, 22–24 George Street, Edinburgh 2, from whom further information can be obtained. The closing date for applications will be March 31, 1966.

Announcements

PROF. O. V. S. HEATH, at present professor of horticulture in the University of Reading and honorary director of the Unit of Flower Crop Physiology of the Agricultural Research Council, has been appointed to fill a vacancy in the membership of the Agricultural Research Council, which has arisen on the retirement, after ten years' service, of Sir Hans Krebs.

THE sixth seminar on "Electrochemistry" will be held at the Central Electrochemical Research Institute, Karaikudi, during December 26–29. Further information can be obtained from Dr. K. S. Rajagopalan, Central Electrochemical Research Institute, Karaikudi 3, Madras State.

SEVERAL oceanographic meetings and conferences, sponsored by the Institute of Marine Science, will be held at Miami Beach during November 11–24. Further information can be obtained from the Institute of Marine Science, University of Miami, 1 Rickenbacker Causeway, Miami 49, Florida.

AN international conference on "Radiological Protection in the Industrial Use of Radioisotopes", organized by the Société Française de Radioprotection, will be held in Paris during December 13–15. Further information can be obtained from the Secretary General, Société Française de Radioprotection, Boîte Postale 3, Fontenay aux Roses, Seine.

THE winter meeting of the British Biophysical Society, on "The Conformation of Biological Macromolecules", will be held at Queen Elizabeth College, University of London, during December 20–22. Further information can be obtained from Prof. R. E. Burge, Department of Physics, Queen Elizabeth College, Campden Hill Road, London, W.8.

THE International Institute for Conservation is planning to hold a conference on "Chemicals in Museums: Quality, Care and Safe-handling" at the Institute of Archaeology, in the University of London, on January 27, 1966. The speakers invited to take part will include a factory inspector, the medical adviser to the Trades Union Congress, a representative from the firm of Hopkin and Williams, and a member of the Government Laboratory. Further information can be obtained from Miss Gedyne, Institute of Archaeology, 31 Gordon Street, London, W.1.

AN international conference on the "Lymphatic System", sponsored jointly by the Committee on Shock of the National Academy of Sciences–National Research Council and the Tulane University School of Medicine, will be held in New Orleans during December 12–14. Further information can be obtained from Prof. H. S. Mayerson, Department of Physiology, School of Medicine, Tulane University, New Orleans.

USE OF PIGS IN BIOMEDICAL RESEARCH

By DR. L. K. BUSTAD

Biology Department, Pacific Northwest Laboratory, Battelle Memorial Institute, Richland, Washington

AND

R. O. McCLELLAN

Medical Research Branch, Division of Biology and Medicine, U.S. Atomic Energy Commission, Washington, D.C.

THE first symposium devoted exclusively to the basis for and extent of utilization of pigs in biomedical research was held at the Pacific Northwest Laboratory, Richland, Washington, during July 19–21, under the sponsorship of the U.S. Atomic Energy Commission and the Battelle Memorial Institute (contract AT(45-1)1830). About 150 scientists from Canada, Denmark, France, Germany, Great Britain, Switzerland and the United States joined scientists of Battelle Memorial Institute's Pacific Northwest Laboratory (a U.S. Atomic Energy Commission facility formerly operated as the Hanford Laboratories by the General Electric Co.) for three days of formal presentations and panel discussions.

The symposium was divided into four general categories: (I) comparative anatomical and physiological characteristics; (II) biomedical applications of pigs and miniature pigs; (III) laboratory management practices; (IV) miniature pig development programmes. Session and panel chairmen were: D. L. Anderson, Division of Biology and Medicine, U.S. Atomic Energy Commission; T. J. Cunha, University of Florida; D. K. Detweiler, University of Pennsylvania; H. W. Dunne, Pennsylvania State University; D. C. England, Oregon State University; T. Gillman, Institute of Animal Physiology, Babraham, Cambridge; W. Montagna, Oregon Regional Primate Research Center; L. E. Mount, Institute of Animal Physiology, Babraham, Cambridge; and J. H. Rust, University of Chicago.

D. F. Cox, Iowa State University, reviewed pig genetics, pointing out that with the exception of information on the inheritance of blood antigens of pigs, knowledge of the genetics of inherited traits which are controlled by a few genetic factors is limited. He noted that domestic pigs provide some unique opportunities for research because of the vast array of genetic variation both between and within the various breeds.

Inheritance investigations described by J. Moustgaard and M. Hesselholt, Royal Veterinary and Agriculture College, Copenhagen, have shown that the presence or absence of antigenic factors on the surface of pig erythrocytes is genetically controlled by allele genes belonging to fourteen chromosomal loci. Blood group systems have been established and designated by letters A B C E F G H I J K L M N and O.

R. A. McFeely, University of Pennsylvania, presented recent work on pig cytogenetics. The pig appears particularly well suited for cytogenetic investigations because it has only 38 chromosomes, which can be readily paired and grouped in a manner not unlike human chromosomes. Of particular interest are studies in pigs with intersex conditions where chromosome analysis in several cases have shown that the role of the Y chromosome in the determination of sex may be somewhat different from that in man and the mouse.

D. Smidt *et al.*, University of Göttingen, reported on their investigations of reproduction using miniature pigs. They obtained fertilized ova by flushing oviducts and transferred the eggs from miniature sows to other miniature sows and reciprocally between miniature and

Landrace sows. The embryo implantation rate was 25 per cent, and 50 per cent of these were carried to term. The size of the sow influenced the birth weight and the proportions of the pigs. The weight differential persisted until they were about 6 weeks old.

Birthe Palludan, Royal Veterinary and Agricultural College, Copenhagen, presented an interesting, comprehensive review of teratological studies in pigs with avitaminosis-A. A number of malformations were observed, the most frequent being microphthalmia. No malformations were seen if vitamin A was administered to vitamin A-deficient sows before the 16th–17th day of gestation. Administration of large doses of vitamin A to pregnant sows caused eye anomalies and malformations of the heart similar to those caused by vitamin A deficiency. She also observed that thalidomide administered at high levels to pregnant pigs caused retarded development of the palate, heart, lungs, alimentary tract, and urogenital system.

L. E. Mount, Institute of Animal Physiology, Cambridge, described the patterns of heat production and heat loss in the new-born pig, relative to the pig's environment, and discussed some similarities to those observed in the human new-born.

S. L. Hansard, Louisiana State University, described extensive investigations of placental transfer and foetal utilization of absorbed minerals. He noted that absorbed minerals passed freely from dam to foetus at a rate which was inversely proportional to ionic size, but that the cations iron, calcium, phosphorus, and zinc traversed the placenta more rapidly than the anions iodine and phosphorus.

Several papers described work in the areas of dental and skeletal research. E. B. Jump and M. E. Weaver, University of Oregon Dental School, who pioneered the use of miniature pigs in dental research, described some advantages and limitations of this species. They noted that the pig masticates with both incision and trituration and is unique among the common laboratory animals in having a long period of deciduous and transitional dentition. This latter feature permits experimental investigations on many dental problems afflicting children. Their preliminary work demonstrates the suitability of miniature pigs for clinical experiments in restorative dentistry, orthodontics, periodontics, and the pathology and therapeutics of the tooth pulp. An interesting, but unexplained, difference they noted between man and pig was that the latter produces larger amounts of dental calculi without experiencing the periodontal pathology associated with dental calculus in man.

A paper by H. D. Dellmann and R. C. McClure, University of Missouri, described skull measurements to establish a co-ordinate system for stereotaxic placement in the brain of the pig. Their results suggest the possibility of developing a stereotaxic atlas for pigs even though breed and strain differences exist.

F. A. Spurrell, University of Minnesota, presented results obtained by him and his associates, W. J. L. Felts and L. A. Baudin, on the development of osteons in

pig and man, and the effect of dietary calcium restrictions. Cortical bone development in miniature pigs was compared and found similar to that in the standard domestic pig.

The structure of bones of starved pigs was evaluated by C. W. M. Pratt in a paper he presented with R. A. McCance, University of Cambridge. Newly weaned pigs were subjected to severe undernourishment for periods up to 1 year, maintaining their weight at 3–8 kg.

Their bones, although showing no specific pathology, were structurally distinctive, being unlike either normal growing bone or miniature mature bone. On the basis of calcium to collagen ratio, the bone appeared to be hypercalcified. Recovery of these undernourished animals occurred when they were allowed unlimited food. All bones ultimately reached close to their expected length, although mild deformities of the shaft appeared in some bones.

Only one paper on renal physiology was presented. In this paper, T. W. Nielsen with C. A. Maaske, University of Colorado, and N. H. Booth, Colorado State University, reported on their work on standard renal function tests performed on conscious unanaesthetized pigs. Endogenous creatinine, insulin, PAH, and osmotic clearances were determined. They reported that the pig has predominantly short-looped nephrons (97 per cent), compared with 86 per cent for man, whereas in the dog nearly 100 per cent of the nephrons are long. The ability of the pig to concentrate urine was also comparable to man. The pig, however, appeared to be insensitive to urea loading, and acetylation of the PAH in the pig probably compromises its use for clearance measurements using this material.

Several papers discussed gastrointestinal function and nutrition. D. F. Magee, University of Washington School of Medicine, described investigations of pancreatic secretion in pigs, which he found to have several advantages over dogs. He found that the volume of juice secreted is directly related to the hydrogen ion concentration in the duodenum of pH 7–1 and (unlike the dog) pepsin, oleic acid and olive oil all increased the output of amylase and lipase in the juice. Above pH 7, inhibition of secretion was noted. The pancreas seems to be involved in the regulation of duodenal pH on both sides of neutrality.

In a paper by T. W. Perry *et al.*, Purdue University, oesophago-gastric ulcers observed sporadically in pigs were described and were considered similar to those found in man. A high incidence of these ulcers could be produced by feeding corn, gelatinized corn, wheat, barley, or oats. Dietary additives such as α -tocopherol, menadione, vitamin A, antibiotics, copper, and methionine were without effect in preventing the oesophago-gastric ulcers.

W. G. Huber and R. F. Wallin, University of Illinois, analysed the gastric secretion from pigs bearing Heidenhain pouches or simple gastric fistulae. Differences were noted in pH, free acid, chloride, pepsin and histamine, between the two types of surgically prepared pigs; however, no significant difference was observed in total acid secretion. Difficulty was encountered in maintaining electrolyte balance in the pigs with the Heidenhain pouches.

Work on the sex-related differences in body composition in the pig and human infant was described by L. J. Filer *et al.*, University of Iowa. Filer noted that information on body composition of human infants is almost entirely restricted to that which may be accumulated by indirect methods of examination. He proposed that the growing pig be used as a model for comparisons to the human infant, permitting investigations on the influence of age, sex and diet on the rate of growth and body composition during infancy.

The application of pigs in investigations of severe protein malnutrition was discussed by W. G. Pond *et al.*, Cornell University. They found that when pigs were weaned at 3 weeks of age to dry, low-protein diets, bio-

chemical and anatomical changes occurred which resembled kwashiorkor in human infants. When a 3 per cent protein, low-fat diet was fed, the pigs remained active and alert and failed to develop the severe liver pathology and oedema seen in pigs fed a 3 per cent protein, high-fat diet.

B. C. Johnson, University of Illinois, also reported on the results of undernutrition of pigs, with emphasis on enzymatic and cardiovascular effects of starvation and re-feeding. Apparent irreversible damage was produced in myocardium as well as arteries and arterioles as a result of stresses of re-feeding following starvation. Johnson noted diastolic hypertension after only two starvation-re-feeding episodes.

E. R. Miller and D. E. Ullrey described cardiovascular effects in baby pigs with dietary deficiencies. Thiamine deficient pigs consistently exhibited bradycardia, sinus arrhythmia, and atrioventricular blocks. In recent years pigs have been increasingly used in cardiovascular research. In deference to this widespread interest, an evening panel was devoted entirely to cardiovascular research. D. K. Detweiler, University of Pennsylvania, who chaired this panel, reviewed the growing utilization of pigs in cardiovascular investigations, especially in North America and Germany. W. von Engelhardt, School of Veterinary Medicine, Hanover, presented a comprehensive review of pig cardiovascular physiology.

H. Luginbuhl, a Swiss scientist now at the University of Pennsylvania, discussed spontaneous atherosclerosis in pigs. Although it has been known for a decade that pigs develop lesions comparable to those of the pre-atheromatous phase in human atherosclerosis, Luginbuhl is the first to report on a large series of aged pigs (ranging from 8 to 14 years old). In these pigs, pre-atheromatous changes progressed to the formation of atheroma in the aorta, iliac, cerebral and coronary arteries of several animals. Luginbuhl identified all tissue elements constituting atheroma of man, and described for the first time the detection of complicated atherosclerotic lesions with ulceration and thrombus formation in pigs. Areas of cerebral occlusion were comparable to those in man.

H. C. Rowsell *et al.*, Ontario Veterinary College, reported on some of their investigations of the effects of dietary substances found in the diet of man on the development of atherosclerosis in pigs. Their work has shown that diets high in butter, egg yolk and lard plus cholesterol increased the amount of atherosclerosis and thrombosis in pigs. Rowsell *et al.* observed that, in endothelial preparations from pigs as young as 2 weeks of age, deposits rich in platelets occurred around vessel orifices and bifurcations in a topography and pattern similar to early atherosclerosis. Pigs were found to be ideally suited for the examination of the interactions of the vessel wall, the blood platelet, and blood coagulation.

Several papers were devoted to experiments involving heart surgery. C. A. Maaske, together with N. H. Booth and T. W. Nielsen, University of Colorado and Colorado State University, described experimentally induced cardiac failure in pigs. By partially occluding the main pulmonary artery of the pig, they produced a slow, progressive, right-sided, congestive heart failure accompanied by signs of clinical heart failure. They noted that induction of congestive heart failure in pigs was particularly significant, since cardiac failure was induced by a single surgical manoeuvre, while in dogs, multiple assaults or series of surgical manoeuvres are required to produce comparable results. At necropsy, grossly distended central veins, right atrial and ventricular dilatation and hypertrophy, hepatomegaly, and ascites were observed in affected animals.

G. D. Lumb, Warner-Lambert Research Institute, Canada, also reported on investigations of experimentally induced cardiac failure in pigs. Taking advantage of the similarity of the distribution of the coronary arteries of pig and man, he ligated the coronary branches supplying

the atrioventricular node and bundle of His, and then examined the degree of collateral circulation that ensued and the beneficial effects of medication.

D. C. Sawyer and H. L. Stone, U.S. Air Force School of Aerospace Medicine, reported on techniques and results of thoracic surgery for implantation of sensing devices for monitoring cardiac output and other parameters. They presented a coloured film on their surgical technique for implanting electromagnetic flow sensors placed around the ascending aorta. Implants remained functional more than 40 days. They reported values of 5.3 l./min for cardiac output, a stroke volume of 41 cm³/beat and a heart-rate of 124 beats/min in unanaesthetized pigs trained to recline quietly on a laboratory table. The major reason for their choice of miniature pigs over the dog for these investigations was the high incidence of aortic rupture in surgically prepared dogs.

In a more fundamental investigation, N. H. Booth with H. E. Bredeck and R. A. Herin, Colorado State University, reported on their work on the baro- and chemo-receptor reflex mechanism in pigs. H. Hoernicke, University of Hanover, closed the cardiovascular panel by reviewing the Berlin symposium on circulation in pigs. His review was largely concerned with *hertzsdod*, a cardiac disease in pigs of considerable economic importance in Germany. Differences in cardiovascular performance between domestic and wild pigs were also discussed. He noted that body-temperature loss appears to be an important factor in exercise tolerance by domestic pigs.

Normal haematological and biochemical parameters of miniature pigs were described by A. S. Tegeris *et al.*, Food and Drug Administration, and by R. O. McClellan *et al.*, Battelle-Northwest. Their papers provided extensive information on more than 30 parameters. Both papers emphasized the need for dynamic investigations in evaluating haematological and biochemical changes. McClellan emphasized the dynamic changes which appear to be age-related.

Another very interesting application of pigs is in certain immunological investigations, for which they seem uniquely suited. D. Segre, University of Illinois, stated that the immunological incompetence of colostrum-deprived baby pigs could be overcome by the administration of the antigen mixed with minute amounts of specific antibody or with large quantities of normal γ -globulin. By such investigations, support was generated for the natural selection theory of antibody formation, which holds that the antigen-antibody complex rather than the antigen alone constitutes the proper antigenic stimulus. The results presented by Y. B. Kim and by D. W. Watson differed somewhat from those of Segre. These workers, together with S. G. Bradley, University of Minnesota, found that "immunologic virgin" pigs appear to be immunologically competent, as manifested by their excellent response to antigenic stimuli—a single intraperitoneal injection of 10¹² particles of actinophage MSP-2. They found that germ-free, colostrum-deprived miniature piglets taken by hysterectomy 3–5 days before term were free of detectable immunoglobulins and antibodies if great care was exercised in preventing contamination of the new-born pig, with any foreign contaminant such as dam's blood. Of related interest also to immunologists was a paper by D. E. Ullrey, C. H. Long and E. R. Miller, Michigan State University, in which they investigated the absorption of intact proteins from the first milk of the mother for protection of the new-born pig. Colostrum-deprived pigs were used to establish that intact protein could be absorbed from the intestine after birth. This was done by feeding either a protein-free or protein-containing purified diet labelled with fluorescein isothiocyanate. One of their interests is to determine the mechanism of "gut closure", and in this regard they suggested that we may not have to wait very long for the clarification of this mechanism. It means for readily 'opening' and 'closing' the gut to γ -globulin-size

particles could be developed, it would represent a significant fundamental and applied technique for those interested in areas such as immunization and anaphylaxis.

Standard pigs are commonly afflicted with an arthritic condition that is of economic concern to pig producers. G. M. Neher and J. M. Carter, Purdue University, suggested that this naturally occurring arthritis of pigs bears many similarities to rheumatoid arthritis in man, although the aetiological agent is not common to both species. They observed that the disease could become active at any stage, and complete remissions were common in the early stages. Their work suggested that hypersensitivity is involved in the aetiology of the disease. R. W. Moore pointed out that at Texas A and M they have produced a mycoplasma arthritis in pigs which may be a superior model for rheumatoid arthritis in man.

F. D. Klopfer related the results of some of his investigations of visual learning in pigs at Washington State University. He found that unless special procedures are used in rearing very young pigs, they do not learn readily to solve visual discrimination problems for food reward. The effective procedures appear to be those preventing the strong development of position responses in feeding, so as to permit the development of visual discrimination while feeding. Using discrimination learning procedures, Klopfer found it was possible to demonstrate wave-length discrimination in these animals and to determine the photopic and scotopic visibility functions.

Pigs have been used quite extensively in radiobiological investigations; examples of present research in this area were reviewed in several papers. U.S. Naval Radiological Defense Laboratory scientists, in a paper presented by N. P. Page, described the recovery pattern of pigs given a large sub-lethal dose of X-irradiation. They found the acute $LD_{50/30}$ (mid-line air dose) of 8- to 9-month-old gilts was about 400 r. for 1 mVp. X-rays. Recovery from the effects of 238–265 r. exposures was estimated by re-determining the LD_{50} at various times after their initial exposure. The animals had recovered from 48 per cent of the initial injury by 3 days, and by 7 days the majority of animals appeared to have recovered completely. In fact, the data suggested that some of the animals had become radio-resistant. By 20 days the LD_{50} was about 170 per cent of the LD_{50} of the unconditioned animals.

In another paper on the effects of external whole-body radiation, D. G. Brown, University of Tennessee Agriculture Research Laboratory, reported on investigations of the late effects of pigs exposed to 15–700 rads of mixed neutron γ -radiation from a nuclear detonation 8 years ago. About 44 per cent of the irradiated pigs still survive compared with 69 per cent of the controls. The increased mortality in the irradiated pigs is attributed to gastrointestinal alteration and neoplasia.

R. O. McClellan reviewed the use of pigs in radionuclide toxicity investigations describing long-term examinations of the effects of daily ingestion of radiostrontium, investigations of the gastrointestinal absorption of plutonium and SNAF radionuclides, and metabolism and effects of plutonium deposited in the skin. He emphasized the importance of the size of the miniature pig, gastrointestinal tract and relatively long life-span as pertinent factors in favouring the use of the pig because of the importance of these criteria when results may be extrapolated to man. Of special interest was the reported high incidence of haematopoietic tissue neoplasms in miniature pigs ingesting large quantities of strontium-90.

For many years workers have regarded the skin of pigs as being very similar to human skin; it is only recently that careful, thorough, anatomical assessment has been accomplished by one of the outstanding authorities on skin, William Montagna. In a brief review of his work, Montagna stated that although pig skin shares some anatomical and histochemical features with that of man, it is distinctly different. The skin of both is characterized

by a sparse hair coat, a thick epidermis with a well-differentiated under-sculpture, a dermis which has a well-differentiated papillary body, and most noteworthy, a large elastic tissue content. In contrast to man, the pig dermis is poorly vascularized and the sebaceous glands contain much alkaline phosphatase. In view of his observations, Montagna recommended that caution should be exercised in suggesting that there is a strong resemblance between the two skins.

In his interesting paper on pig skin, G. D. Weinstein, University of Miami, found the kinetics of epidermal proliferation to be similar in man and pig. Using tritiated thymidine labelling, Weinstein showed that labelled cells in the epidermal basal layer had a transit time through the viable epidermis of 14 and 13 days in the pig and human epidermis, respectively. The total turn-over time of pig epidermis is about 30 days, while in human epidermis it is 27 or 28 days.

T. Gillman presented a film describing work on histogenesis of healing incised and excised wounds which he has performed with his associate, L. Ordman, at the Institute of Animal Physiology, Cambridge. Young pigs have been his principal subjects. Gillman stressed the indispensability of three-dimensional analysis of histogenesis. Sutures, he noted, evoked marked reactions especially in epidermis and skin appendages transected by the suture needle. Tape closure utilizing micropore (Minnesota Mining and Manufacturing Co.) was found highly effective in eliminating these complications and in permitting perfect healing without wound disruption. The results of this work demonstrate that widely accepted descriptions of healing cutaneous wounds are incorrect and therefore require revision.

Two papers described the results of radiation exposure of pig skin. In the first paper, J. O. Archambeau *et al.*, Brookhaven National Laboratory, reported on the use of pig skin for evaluating the effects of ionizing radiations. They concluded that the histology and radiation geometry of pig skin resembled the human sufficiently to warrant its use as an *in vivo* system for comparing effects of different types of ionizing radiations and extrapolating these results to man.

In a paper by the late L. A. George and L. K. Bustad, Battelle-Northwest, gross observations of early and late changes in pig, sheep and rabbit skin after acute exposures to phosphorus-32 or strontium-90 plaques were described. The pattern of early response observed in pigs resembled generally that described for man.

One day of the symposium was set aside for panel discussion of laboratory management and miniature pig development. L. E. Mount, Institute of Animal Physiology, Cambridge, was chairman of the panel on housing and handling. The members of this panel were: D. F. Cox, Iowa State University; A. Dettmers, University of Minnesota; F. L. Earl, Food and Drug Administration; R. L. Murphree, University of Tennessee (AEC); and R. O. McClellan, U.S. Atomic Energy Commission. The panel reviewed experimental pig units at their sites, and in this connexion made recommendations on housing, including floor space, air movement, temperature, feeding, water, and farrowing facilities, and on techniques in handling, including bleeding and anaesthesia.

S. G. Hastings, N. H. Booth and M. L. Hopwood, Colorado State University, submitted a paper on general anaesthesia for thoracic surgery procedures, and G. L. Waxler and C. K. Whitehair, Michigan State University, read a paper on techniques for using germ-free pigs in biomedical research. J. J. Landy, University of Miami, gave a brief report on his work with delivery and maintenance of germ-free pigs and the application of these procedures in human surgery.

Another panel discussed nutrition of pigs. The panel included the chairman, T. J. Cunha, University of Florida; A. Dettmers, University of Minnesota; L. K. Bustad for V. G. Horstman, Battelle-Northwest; L. J. Filer, Ross

Laboratories; E. G. Hill, University of Minnesota; C. K. Whitehair, Michigan State University; and J. P. Bowland, University of Alberta.

In addition, E. G. Hill submitted a paper on fatty-acid composition of miniature pig tissue lipids, and J. P. Bowland submitted a review of his extensive work on the composition of pig milk.

The third panel discussion was on disease and disease control. The chairman of the panel was H. W. Dunne, Pennsylvania State University. The panel members were: D. G. Brown, University of Tennessee (AEC); F. L. Earl, Food and Drug Administration; A. G. Edward, University of California, Davis; R. A. Griesemer, Ohio State University; R. O. McClellan, U.S. Atomic Energy Commission, for H. A. Ragan, of Battelle-Northwest; and M. J. Twiehaus, University of Nebraska. The panel reviewed the disease problems encountered and most likely to be encountered in experimental pig herds, and recommended an effective disease control programme for experimental pig herds and for the use of pigs in the laboratory.

D. C. England, Oregon State University, was the chairman of a panel on miniature pig development. The panel members were: W. E. Rempel, University of Minnesota; L. Welch, University of Nebraska; D. Smidt, Göttingen, Germany; J. C. Taylor, U.S. Department of Agriculture; P. Nizza, Fontenay-aux-Roses (Seine), France; and L. K. Bustad, Battelle-Northwest. The panel discussed the concept of miniature pig development and implementation of the concept, and defined essential features of existing herds.

In his introductory statement, D. C. England recalled that in 1949 the Hormel Institute of the University of Minnesota began the first project to develop a breed of miniature pigs specifically for use in biomedical research. Medical personnel of the Mayo Foundation gave added impetus, since it recognized the need for a convenient experimental animal that would better meet certain anatomical and physiological demands. The objectives of the University of Minnesota programme were to develop pigs with a mature size small enough to be easily handled and maintained, with normal physiological characteristics.

In the review of the various herds under development it was clear that one had an assortment of strains from which to choose. Several herds are white-skinned and have adult body-weights about the size of man.

An interesting recent introduction of the Labco pig, a gentle, sparsely haired pig obtained from Mexico by H. Quick, of Homewood, Illinois, into Hanford miniature pig was demonstrated. Many of the offspring were white and had sparse hair coats. The 'Labco' miniature and the 'Vitavet' lab. miniature developed by F. Conder, of Marion, Indiana, represent the only private development of miniature pigs which have come to our attention.

A promise of some very small stock from Nepal was reviewed by W. Rempel, University of Minnesota, who showed pictures of some pigs that weighed only about 10 lb. at one year of age. This is even smaller than the Vietnamese pigs which were imported by Haring *et al.* into Göttingen, Germany, and crossed with German 'Landrace' and 'Hormel' miniatures in their laboratory.

It appears that two classes of miniature pigs will be established on the basis of predicted need. Not only is there a real demand for pigs which have an adult weight less than 30 kg, but also for one that as an adult weighs about 70 kg, similar to the so-called standard man and to the weight of most of the miniature pigs now being used.

Collectively, the papers presented at the symposium reflect the present state of our knowledge on the applicability of pigs in biomedical research and establish the pig as an important and very promising experimental animal.

With the development of a standard 60-lb. pig which may well be available during the next decade, many pigs

will assume a major role in research laboratories. In fact, an appreciable increase in usage is predicted before this lower weight is realized.

Increased use of pigs and miniature pigs in the areas of genetics, nutrition, gastrointestinal and renal physiology,

immunology, toxicology, radiobiology, and fundamental and clinical cardiovascular physiology seems assured. Additional information is needed to provide a firm basis for a broader assessment of the future role of pigs in biomedical research.

NATURAL SELECTION AND TRANSMISSIBLE DISEASE

AN informal meeting on "Natural Selection and Transmissible Disease", arranged on behalf of the Human Adaptability Sub-Committee of the International Biological Programme (IBP), was held at the Ciba Foundation on July 21-22, 1965. In his introduction, Prof. J. S. Weiner said that there were now some thirty national IBP committees and it was envisaged that the programme would include both world-wide surveys on blood groups and other genetical traits and also more intensive multidisciplinary studies on selected populations. Genetical constitution in relation to disease had been adopted as an IBP topic, but so far there had been no discussion on the prospects of research or the methods to be used.

Speaking on "The Genetical Analysis of Disease Susceptibility", Prof. L. S. Penrose gave examples of inherited disease resistance in plants and animals, and pointed out that genetical analysis of this phenomenon in man was much more difficult. It was particularly difficult to disentangle the effects of inborn and acquired immunity and this made the interpretation of familial concentrations of such diseases as tuberculosis and acute rheumatism uncertain. Traditional beliefs about the differing disease susceptibility of racial groups might often be mistaken, and attempts to correlate body-build with disease susceptibility had not been convincing. Haldane initiated the modern approach in 1932, when he suggested that thalassaemia might protect against malaria, and Allison had provided evidence for this in the case of sickle-cell trait. In African peoples with high sickling rates, eradication of malaria could lead to a detectable fall of sickling frequency in three or four generations. Theoretical treatment of gene frequency dynamics was more complex for sex-linked genes and where several interacting alleles exist in the same population. He suggested that the effects of chromosomal abnormalities induced by viruses on susceptibility to other diseases might be worth investigating.

In the discussion, Prof. Penrose stressed the advantages of examining single-gene traits and questioned the value of twin-studies. Prof. Vogel thought that multifactorial characters should not be ignored and that useful data could be obtained from twins examined throughout their life-span. Dr. Kirk, Dr. Allison and Prof. Penrose discussed the interpretation of gene frequency differences between West Africans and American Negroes. Dr. Roberts suggested that genetical data might be collected on volunteers experimentally infected with the common cold. The relationship between susceptibility to disease and clinical severity was discussed by Sir Graham Wilson, Prof. Stuart-Harris, Dr. Omerod and Prof. Vogel.

Prof. C. H. Stuart-Harris spoke on the epidemiology of virus diseases. He said that their epidemiological behaviour was similar to that of bacterial diseases. Some, like poliomyelitis, were confined to man; others, like rabies, affected man by chance and did not spread; while others, which are spread by insect vectors, are maintained in the population by a cycle of transmission. Some virus diseases have been established for many centuries, and epidemics of these may be due to changes in virus virulence, host susceptibility or conditions influencing transmission. Viruses are well known to be plastic in the laboratory, but as yet it has not been proved that virulence changes underlie major epidemics. Viruses live in very close association with cells, and genetical variations in

the host tissues would be expected to be important in susceptibility. Mouse strains differing in resistance to certain viruses such as yellow-fever had been bred and inter-specific differences in susceptibility are well known. He gave examples of the varying relationship between attack-rates and severity of disease and the age of the subjects. He then discussed smallpox, influenza, yellow-fever and typhus. India and Pakistan are now hyper-endemic areas for smallpox, and mortalities might reach 40-50 per cent in some epidemics. Yellow-fever is endemic in West and Central Africa and heavy mortalities have occurred in recent epidemics in the Sudan and Abyssinia. Typhus, which is now endemic in the Middle East, North Africa, China and other regions, formerly caused heavy mortalities especially in persons more than fifty years of age. The influenza virus is very plastic and epidemics tend to occur when a new strain emerges. The work of McDonald and Zuckerman had shown that blood group O was more frequent among persons who had been infected with influenza A₂ virus, and unpublished work confirmed this. He thought that there was still scope for genetical investigations on populations to which certain viruses had not yet spread.

In the discussion, Prof. Barnicot asked for examples of non-immune populations which might be favourable for genetical investigations, and Prof. Stuart-Harris mentioned the measles epidemic in Greenland and polio epidemics among Eskimos. He said that Type I polio virus was absent in Tristan da Cunha before the exodus. In reply to Dr. Harrison, he said that little could be deduced about individual variations in susceptibility by measuring antibody titres. Dr. Roberts mentioned his own work on ABO blood groups in relation to *Rickettsia* infections in Nigeria, and Dr. Spicer spoke of his analysis of periodicity in deaths from respiratory diseases.

In his paper on "ABO Blood Groups and Infectious Diseases", Prof. F. Vogel said that in much of the earlier work on this topic the samples were too small, but relatively high B and AB frequencies had been found among patients with tertiary syphilis. His own work on ABO groups and smallpox had been stimulated by geographical correlations and by the claim, which was in dispute, that vaccinia virus contains A-like antigen. It was best, he thought, to seek correlations between blood group and clinical severity, and in India his team had found that scarring was more severe in subjects of groups A, B and AB or of groups A and AB than in those of group O. Clinically more severe cases among fresh smallpox cases in Pakistan were more frequent in groups A, B and also AB compared with O. Groups A and AB were in excess in a sample of post-vaccination encephalitis cases examined in Germany. Work in Austria suggested that infantile diarrhoea was more severe in group A children than in those of group O; German data for 1956 and 1960-63, which he himself had analysed, showed the same trend, but it was reversed in data for 1957-59. He felt that these various lines of work were sufficiently encouraging to deserve further attention.

In the discussion, Dr. Garhek reported a small series collected in a smallpox epidemic in Nigeria; no relation was found between ABO group and either the incidence or severity of disease. Dr. Allison expressed scepticism about the effect of ABO groups on smallpox infections. The possibility of collecting more data on tertiary syphilis, post-vaccination encephalitis and infantile diarrhoea was

discussed. Sir Graham Wilson pointed out the difficulties of diagnosis of post-vaccination encephalitis.

In a paper on the epidemiology of protozoal diseases, Mr. W. E. Ormerod said that disequilibrium between host and parasite could be adjusted by either of them becoming adapted by selection; owing to short generation times, adaptation of the parasite was likely to be more rapid. Some protozoa such as *Entamoeba coli* cause no disability in man; others, like *Plasmodium vivax*, cause illness; and some, such as *Trypanosoma rhodesiense*, are generally lethal. A benign parasite may be more harmful if other parasites are present. West Africans and American Negroes seem to have a solid immunity against *P. vivax* which is presumably genetical. This parasite may have been an important selective agent before equilibrium with man was achieved. Marked differences between certain cattle breeds in resistance to trypanosomiasis are known. This disease is enzootic in cattle but sporadic in man, and probably exerts little selective pressure on the latter. *Toxoplasma* is endemic in some human communities and may be a significant cause of abortion and still-birth. In general, if parasites can change rapidly, as can trypanosomes in contrast to plasmodia, host adaptation cannot proceed far in any direction. The highest selective pressures are likely to occur where a disease is holoendemic.

The epidemiology of toxoplasmosis and the immunity of Negroes to *P. vivax* were discussed. Dr. Allison considered that the latter phenomenon was genetical but not associated with haemoglobin variants or with G-6PD (glucose-6-phosphate dehydrogenase) deficiency.

Speaking on the epidemiology of bacterial diseases, Sir Graham Wilson said that the proportion of clinical, sub-clinical and latent infections and of uninfected people, both susceptible and immune, would depend on many factors. 98 per cent of people might catch a disease such as measles if it were introduced into a virgin population, whereas infectious anaemia of rats was widespread but latent. Probably most people are susceptible to plague and cholera, but these diseases do not occur to-day in Britain because conditions for transmission are unfavourable. Very little is known about genetical factors in disease resistance. Wide differences in the incidence of tuberculosis in troops of various nationalities in the First World War had been noted and in underdeveloped areas the disease assumes a rapidly progressive form when first introduced, but it is uncertain to what extent genetical differences are involved. Concordance work on twins is usually considered to show that genetical factors influence susceptibility to tuberculosis, but in a recent careful investigation Simonds attributed differences in twin responses mainly to environment. Certain cattle breeds and also mouse strains are known to differ widely in resistance to tuberculosis; in resistant mice there is better mobilization of mononuclear phagocytes, and strains resistant to *Salmonella typhimurium* have higher lymphocyte counts. Sir Graham Wilson thought that geneticists should direct the attention of epidemiologists to various simply inherited traits in the hope that they might be able to suggest possible connexions with disease resistance.

The role of nutrition in resistance to infections was discussed. Dr. Spicer said that both genetical and environmental factors were doubtless involved in susceptibility to tuberculosis. There had perhaps been a large epidemic of this disease reaching a peak in England in the nineteenth century. The interpretation of work on twins was further discussed and also the reasons why cholera tends to remain endemic in Bengal though occasionally breaking out elsewhere. Dr. Roberts had compared local variability in disease incidence in Nigeria with variability in blood groups and failed to find suggestive trends.

Dr. A. C. Allison spoke on "Genetical Components in Disease Resistance". He gave examples of single gene factors in bacteria conferring phage-resistance and in phages influencing infectivity. Resistance of insects to

insecticides had provided striking examples of evolutionary processes. In higher animals the picture is complicated by various immune responses. Evidence from work on bacteria, phage and on some mammals and birds indicated that genetical changes in cell surface components might affect susceptibility to attack by parasites. The susceptibility of certain mouse strains to polyoma and mouse hepatitis virus is greatly increased by thymectomy at birth. The Aleutian mink gene affects coat colour and also leucocyte morphology; the homozygotes are susceptible to a 'collagen disease' induced by a filterable organism. Dr. Allison summarized the work on sickle-cell trait and malaria and considered that the evidence for a protective effect was now quite impressive. Both in Central America and the Caribbean sickling rates tend to be low where selection due to malaria has been relaxed. Sickling rates in various regions correlate well with rates of G-6PD deficiency. He pointed out that ABO blood group heterozygotes such as AO and BO would presumably be handicapped in producing antibodies to two types of antigen and might therefore be at a selective disadvantage.

In the discussion, Prof. Vogel stressed that stable equilibrium due to heterosis might not be important in the case of ABO blood groups; one disease might push the frequency of a gene up and another push it down. Mechanisms other than heterosis for achieving equilibrium were mentioned by Prof. Penrose and Dr. Harrison. Experimental work on malarial infections in relation to sickling and G-6PD deficiency was discussed by Dr. Kirk and Dr. Allison. Dr. Edwards doubted whether protection against malaria by sickling should be regarded as an example of heterosis and whether this was the important mechanism in the case of blood group polymorphisms. Dr. Roberts mentioned his own work on both G-6PD deficiency and sickling in Nigeria.

Summarizing and commenting on the meeting, Prof. Barnicot said that many examples of disease resistance due to single genes in animals and plants had been cited, but the detailed mechanisms of gene action were generally not clear. Work on animals might suggest fruitful lines of research, but it showed only what might happen in man and not what does happen. On the whole, the meeting had emphasized the pitfalls more than the possibilities of work on genetical factors in disease resistance.

Lines of attack on this problem include (a) twin studies; (b) the study of disease incidence in families; (c) the genetical comparison of affected and control samples; (d) correlation of the geographical distributions of genes and diseases. Twin data are difficult to collect, and analysis of disease susceptibility cannot be pressed very far. Family studies are complicated by acquired immunity, and heterogeneity for age of subjects and infective dose. Comparison of affected and control populations may require very large samples to show convincing effects, though small selective pressures may be important in evolution. It may also be difficult to obtain a valid control sample. Many genes and many pathogens are known, and a blind search for associations is likely to be both fatiguing and futile. The examination of single gene traits clarifies the genetical aspect of a complex situation; in the case of multifactorial characters we may not know how much of the phenotypic variation is environmentally induced and disease resistance may depend on hidden effects of contributory genes rather than the visible phenotype. Geographical correlations may have been disturbed by relatively recent population movements and historical data on these may be meagre. In studying immigrant populations there are uncertainties about the exact origins of the migrants and the extent of intermixture in their new home. Genetical investigations of epidemics need special organization if good material is to be collected, since local conditions may not be favourable for scientific work.

N. A. BARNICOT

A SIMPLE ASSESSMENT OF PARTITION DATA FOR CORRELATING STRUCTURE AND BIOLOGICAL ACTIVITY USING THIN-LAYER CHROMATOGRAPHY

By DR. C. B. C. BOYCE and DR. B. V. MILBORROW

'Shell' Research Ltd., Milstead Laboratory of Chemical Enzymology, Broad Oak Road, Sittingbourne, Kent

SINCE the work of Overton¹ and Meyer² the conditions for the penetration of compounds into cells and the structure of cell membranes have been the subject of continuous investigation. Collander³ has shown that the rate of migration of non-electrolytes into and out of the *Nitella* cell can be closely correlated with their partition coefficients between water and a number of organic solvents. Other factors such as molecular dimensions, hydration, adsorption and hydrogen bonding undoubtedly influence the rate of penetration, but the correlations observed for a number of biologically active compounds lead one to believe that partition coefficient is of overriding importance in controlling penetration of non-electrolytes into living cells⁴.

Hansch and Fujita⁵, and Fujita, Iwasa and Hansch⁶, have recently proposed a general mathematical relationship between the penetration of a molecule into cells and a substitution constant, π , which they support with excellent structure/activity correlations obtained with eight different classes of compounds, each assayed against a specific test organism. The constant π is based on partition coefficient and is defined by the free-energy relationship:

$$\pi = \log (\alpha_X/\alpha_H)$$

α_H = partition coefficient of a parent compound

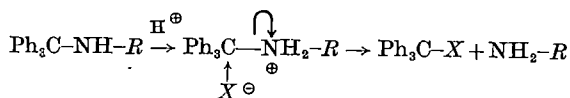
α_X = partition coefficient of a derivative

The direct determination of partition coefficient is tedious and often presents practical difficulties, particularly when the compound is highly insoluble in either of the solvent phases. We have therefore turned to a simplified assessment of partition coefficient and its derivatives by exploiting the theoretical relationship between partition coefficient (α) and R_F value deduced by Martin⁷ for liquid-liquid partition chromatography:

$$\alpha = K (1/R_F - 1), \text{ where } K = \text{constant for the system.}$$

Bate-Smith and Westall⁸ introduced the term R_M , where $R_M = \log (1/R_F - 1)$. The change in the value of R_M for a substituent (ΔR_M) is a free energy-based constant identical with π used by Hansch. It is therefore possible in principle to correlate the penetration of substances with their R_M values. Preliminary results were encouraging, and we therefore investigated the system further and found that for this purpose the chromatographic method has many advantages over the conventional method of measuring partition coefficient.

We chose a series of *N-n*-alkyltritylamines for investigation (Table 1) because their observed molluscicidal activity can be attributed primarily to different rates of penetration to the site of action. An analysis of the toxicity of a range of trityl compounds⁹ has indicated that activity is associated with nucleophilic attack at the quaternary carbon atom of the trityl group:



Molluscicidal activity depends on the rate of penetration to the site of action and the facility with which the leaving group can be displaced when at that site. In the homologous series of *N-n*-alkyltritylamines the same toxicant is formed from all compounds and the relatively small differences in the nature of the leaving group have been found to make a negligible contribution to the observed activity. This can therefore be ascribed to different rates of penetration. The compounds were prepared by heating a solution of triphenylchloromethane (0.01 mole) in dry acetone (10 ml.) with the appropriate *n*-alkylamine (0.02 mole) for 1 h under reflux. The mixture was poured into water and the solid product was collected and recrystallized several times from ethanol, or, in the case of the hexyl-, heptyl- and octyl-derivatives, which were liquids, the compounds were isolated from the aqueous mixture by extraction with ether and were purified by chromatography on neutral alumina with hexane as eluent. Melting points and other data are given in Table 1.

Table 1. THE R_M VALUES AND MOLLUSCICIDAL ACTIVITIES OF *N-n*-ALKYL-TRITYLAMINES

Compound Ph ₃ C. NH ₂ .R R =	Melting point ° C Found*	Literature	LD ₅₀ estimates and 95 per cent fiducial limits (mg/l.)			Mean RM value	Inter- plate standard error
Methyl	71-2	73†	2.10	1.94-2.31	-0.305	0.045	
Ethyl	74-6	75-7†	0.133	0.113-0.165	-0.119	0.034	
Propyl	69-71	70-2†	0.046	0.043-0.049	0.008	0.036	
Butyl	51-2	52.5-53.5‡	0.035	0.033-0.037	0.115	0.031	
Pentyl	42§	—	0.054	0.049-0.061	0.225	0.018	
Hexyl	Oil§	—	0.633	0.521-0.794	0.368	0.038	
Heptyl	Oil§	—	31.65	25.67-39.65	0.485	0.029	
Octyl	Oil§	—	> 50	—	0.620	0.030	

* Uncorrected.

† Beilstein's *Handbuch der Organischen Chemie*, 12, 1344.

‡ Ilceto, A., Fava, A., and Mazzucato, U., *J. Org. Chem.*, 25, 1445 (1960).

§ Novel compound for which satisfactory analytical data were obtained.

Chromatography was carried out on glass plates (20 × 20 cm) coated with a 250 μ thick layer of 'Silica Gel G' (Merck). After drying at room temperature for 2 h and then at 105° for 10 min the plates were impregnated by allowing a 5 per cent solution of liquid paraffin BP in hexane (v/v) to run to the top of the plate; the solvent was then evaporated at 40°. The compounds (3 μ g/spot) were applied at 1.5 cm intervals along a line 2 cm from one edge of the plate so as to be 1 cm above the level of the developing solvent. In order to avoid any systematic error, compounds were applied to positions from a set of pre-determined random allocations, none being nearer than 2 cm to either margin. In addition, *N*-tritylmorpholine was run on all plates as a standard. The plates were developed by the ascending technique in a chromatography tank under conditions of equilibrium. The mobile phase, aqueous acetone saturated with stationary phase, was allowed to run for approximately 100 min when the solvent front had advanced 14 cm from the origin. The plates were then dried and the *N-n*-alkyltritylamines

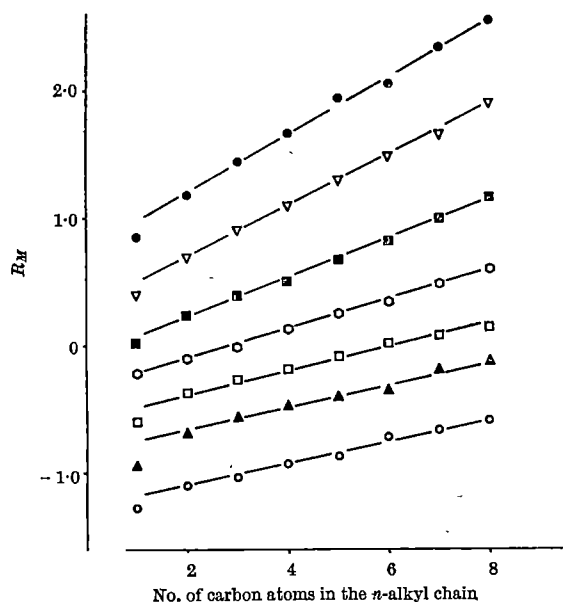


Fig. 1. Relationship between R_M values and number of methylene carbon atoms in the n -alkyl chain of N - n -alkyltritylaminines in a series of acetone/water mixtures. With the exception of the Cl compound there is a linear relationship in all systems. Proportion of acetone (v/v) in the mobile phases: ●, 0.50; ▽, 0.56; ■, 0.67; ◇, 0.75; □, 0.82; ▲, 0.83; ○, 0.91

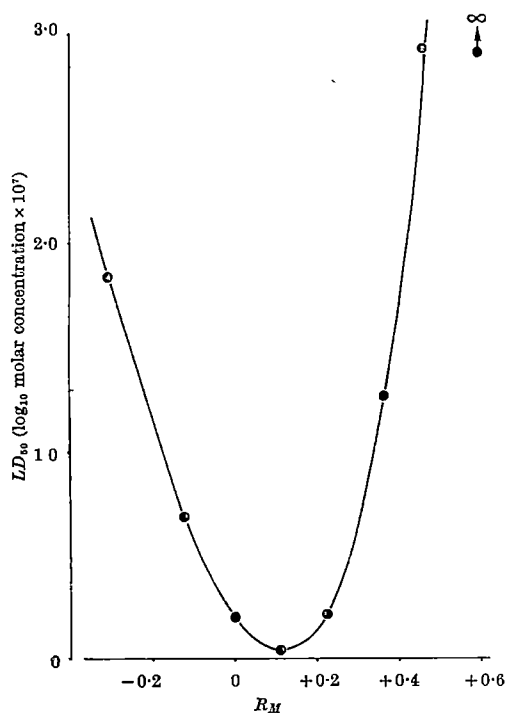


Fig. 2. Relationship between LD_{50} (\log_{10} molar concentration $\times 10^7$) and R_M when the proportion of acetone (v/v) in the immobile phase was 0.70

were detected by spraying with a solution of trichloroacetic acid (5 per cent w/v) in acetone. Yellow spots of the triphenylmethyl carbonium ion were produced after heating the plates at 100° for 5 min and, although these faded during the next 2–3 h as moisture was absorbed, they could be restored by repeating the detection procedure.

Estimates of LD_{50} (Table 1) were computed from dose-mortality data obtained with the snail *Australorbis glabratus*. Details of the procedure will be reported elsewhere⁹.

Since these compounds obey the Martin equation, that is, there is a linear relationship between R_M and the number of methylene carbon atoms in the side-chain (Fig. 1), we have related the biological activity of these compounds with their respective R_M values (Fig. 2). The quadratic relationship observed has been found in other chemical series and is attributed by Hansch and Fujita⁵ to an increase and decrease in the rate of penetration as the R_M values in a series change progressively and pass through an optimum.

The choice of chromatographic method is a matter of convenience; we have found no important differences between paper and thin-layer chromatography except that the latter is quicker and marginally more reproducible in our hands. Water is a constituent of the cell membrane and the protoplasm, so its use in one phase of the model system is obvious, but the lipoidal constitution of the cell membrane is unknown and the choice of hydrophobic phase must be somewhat arbitrary. From thermodynamic considerations, the nature of the non-aqueous phase should not affect the results qualitatively¹⁰ provided that there is no hydrogen bonding. Collander's results³ support this in that ether/water and olive oil/water partition coefficients are equally correlated with penetration into *Nilrella* cells.

Protein is absent from the aqueous and non-aqueous phases used for direct partition coefficient measurements. Therefore, the contribution of the proteinaceous material in a cell membrane is tacitly excluded from the model system. The justification for this is that the variation in penetration can be almost entirely accounted for by partition coefficient data. We were unable to detect any effect on the R_F values of the N - n -alkyltritylaminines when casein was incorporated into the silica gel support of the chromatogram (1 : 5 w/w) and we have therefore omitted protein from the chromatographic system for assessment of R_M values. We chose the reversed-phase system liquid paraffin/aqueous acetone with 'Silica Gel G' as support, although other materials, for example, alumina or Whatman No. 4 paper, impregnated with liquid paraffin were equally satisfactory and the R_F values were similar with all three types of support.

Using 'Silica Gel G' impregnated with liquid paraffin and 7 : 3 acetone/water (v/v before mixing) as mobile phase, it was possible to obtain R_F values for all members of the series of tritylaminines from methyl to octyl in the range of maximum accuracy (0.2–0.8) (ref. 11). However,

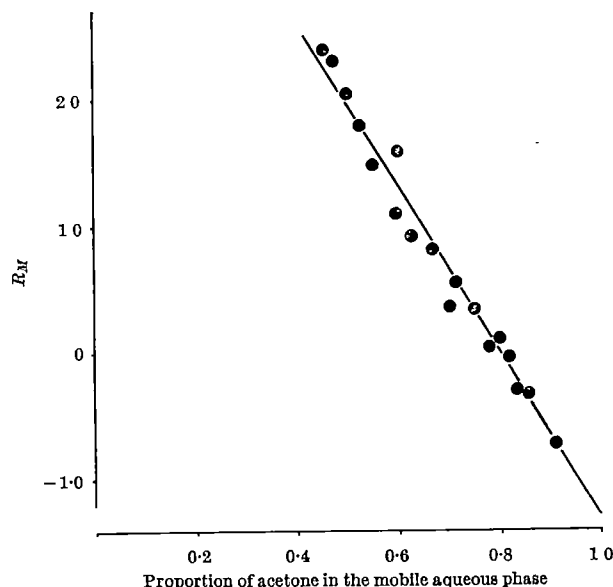


Fig. 3. The effect of solvent composition on the R_M of N - n -hexyltritylamine. Each point is the mean of at least ten determinations

in another class of biologically active compounds, the substituted benzonitriles¹², no single solvent system would give satisfactory R_F values for the most lipophilic and hydrophilic members which remained on, or ran close to, the origin and solvent front, respectively. We were able to overcome this difficulty and to extend the scope of the method by examining the R_F values of the tritylamines with mobile phases containing varying concentrations of acetone. Soczewinski and Wachtmeister¹³ have shown a linear relationship between R_M and solvent composition, provided that the solvent mixtures did not deviate markedly from ideal solutions. When the composition of the mobile phase was adjusted systematically, this relationship was confirmed for the tritylamines (Fig. 3). Given preliminary chromatographic data with a variable proportion of water in the mobile phase, it is possible to choose a solvent mixture which will give not only R_F values in the range of maximum accuracy for most of the compounds, but also maximum R_M increments (Fig. 1). The R_F values which fall outside these limits can be measured in a number of suitable mixtures and the R_M values for the primary system can then be obtained by extrapolation.

The conditions necessary for obtaining accurate and reproducible R_M values have been described in detail by Bate Smith and Westall⁸ for paper chromatography and have been reviewed by thin-layer chromatography, for example, by Stahl¹⁴ and by Truter¹⁵. The mean R_M values for the tritylamines, together with their respective inter-plate standard errors, are given in Table 1. The greatest source of error is the inter-plate variation, which is 6.5 times greater than the intra-plate variation, which was assessed by replication of a standard on each plate. Since there was no evidence that the inter-plate variation was dependent on the R_M value, the estimate of error could be pooled to give a standard error of 0.033 for a single determination and the standard error of the mean R_M value from nine determinations was 0.011. This is 1.2 per cent of the total range of R_M values examined. The mean standard error of the estimates of the log LD_{50} values expressed as a percentage of the range of these estimates is 2.6 per cent. The accuracy with which R_M can be determined is therefore adequate for structure/activity correlations because of the difficulty of assessing biological response with equal precision.

The measurement of the R_M values by reversed-phase chromatography has a number of advantages over the conventional partition measurements, the most important feature being that it is much quicker and less tedious because fewer manipulations are involved. Up to twenty

five compounds can be run simultaneously on one 40 cm × 20 cm thin-layer plate, so R_M values can be compared directly. The detection of spots by simple unspecific methods, for example, by iodine absorption or by the use of a fluorescent indicator, avoids the need for specific, quantitative analytical methods. As a corollary of this, it follows that impurities present will be detected or, alternatively, the material need not be pure provided that the salient spot can be identified. If necessary, the compounds can be extracted from thin-layer material.

The measurement of partition coefficients of sparingly water-soluble compounds demands a long period of equilibration to ensure thorough partitioning between the phases and then colloidal material has to be removed by centrifugation. After this there still remains the difficulty of measuring low concentrations of the solute. Reversed-phase paper or thin-layer chromatography in a range of solvent mixtures can give R_M values for any of these mixtures by extrapolation provided that the linear relationship between R_M and solvent composition is established. Very little material, often less than 1 µg, is required for detection. When the layer is overloaded the spots streak and such plates should be discarded, thus ensuring that the results will not be affected by the presence of colloidal material or by the degree of saturation of the phases by the substance under investigation. The chromatographic method provides a rapid reproducible means of obtaining quantitative partition data for many types of compounds that can be used to assess the roles of penetration and inherent effectiveness in the expression of biological activity. With the growing use of multiple regression techniques for the analysis of this kind of problem the importance of such data is likely to increase.

¹ Overton, E., *Studien über die Narkose* (Jena, 1901).

² Meyer, H., *Arch. Exp. Path. Pharmacol.*, **46**, 338 (1901).

³ Collander, R., *Physiol. Plant.*, **7**, 420 (1954).

⁴ Davson, H., and Danielli, J. F., *The Permeability of Natural Membranes*, second ed. (Camb. Univ. Press, 1952).

⁵ Hansch, C., and Fujita, T., *J. Amer. Chem. Soc.*, **86**, 1616 (1964).

⁶ Fujita, T., Iwasa, J., and Hansch, C., *J. Amer. Chem. Soc.*, **86**, 5175 (1964).

⁷ Martin, A. J. P., *Biochem. Soc. Symp. Camb.*, **3**, 4 (1949).

⁸ Bate-Smith, E. C., and Westall, R. G., *Biochim. Biophys. Acta*, **4**, 427 (1950).

⁹ Boyce, C. B. C. (in preparation).

¹⁰ Collander, R., *Acta Physiol. Scand.*, **13**, 363 (1947).

¹¹ Green, J., Marcinkiewicz, S., and McHale, D., *Chromatog. Revs.*, **5**, 91 (1963).

¹² Milborrow, B. V. (in preparation).

¹³ Soczewinski, E., and Wachtmeister, C. A., *J. Chromatog.*, **7**, 311 (1962).

¹⁴ Stahl, E., *Thin-layer Chromatography* (Academic Press, London, 1965).

¹⁵ Truter, E. V., *Thin Film Chromatography* (Cleaver-Hume Press, London, 1963).

SPECTRAL DENSITY FUNCTIONS OF HYDROMAGNETIC EMISSIONS AT HIGH LATITUDE

By DR. ALV EGELAND

Norwegian Institute of Cosmic Physics, Blindern, Oslo

IN this article the regular emissions in the frequency range 0.5–5 c/s are considered. Interest in them has increased considerably during the past five years, but the approach to the problem adopted here is somewhat different from that employed hitherto by other workers. The nomenclature for this type of oscillation is rather complex. In 1964 the IAGA committee (cf. Jacobs *et al.*¹) recommended that the term Pc 1 should be used for regular oscillations between 0.2 and 5 c/s, but many workers have also referred to these signals as hydromagnetic emissions, pearl pulsations, 'type A' oscillations, etc. (compare, for example, Troitskaya², Campbell³, Gendrin and Stéfant⁴, and Topley⁵). In what follows the term 'hydromagnetic

emission' (HM) will be used to describe the regular oscillations in this ultra-low-frequency range.

The principal detection systems used normally comprise iron-core loops (or air-core), low-noise amplifiers (between 0.1 and say, 10 c/s), chart and/or magnetic tape records. The use of tape makes possible careful analysis after the phenomenon has been observed. The HM emission data obtained have normally been presented in one or both of the following two types of displays: (a) amplitude against time, and (b) frequency against time (sonagrams). Both these methods of presenting data offer much valuable information concerning the micro-pulsations

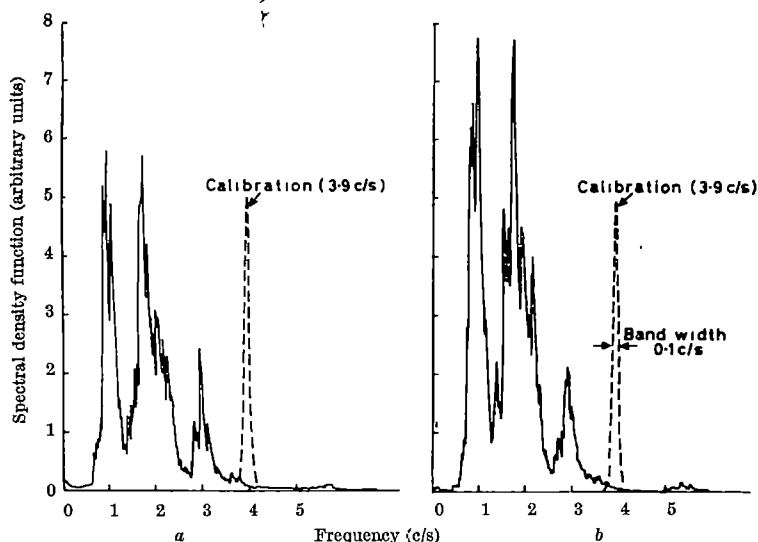


Fig. 1. Spectral density function for the Tromsø hydromagnetic emission record on December 9, 1964. *a*, Record between 2147 and 2149 M.E.T.; *b*, record between 2151 and 2153 M.E.T.

However, if we take the resolution time on the sonagrams this is normally no better than the order of 10–30 sec, and the signal amplitude of sonagrams can only be read off with an accuracy of about 20–30 per cent. This means that the bandwidths as well as the mid-band frequency of ultra-low frequency emissions cannot be determined accurately. Concerning the amplitude against time display, Pope⁶ has shown that these records must be used very cautiously when more than one emission frequency occurs, as the amplitude is strongly dependent on both the number and amplitude of beating frequencies in the emissions.

I therefore feel that if one could find a simple and accurate method of evaluating spectral density functions of HM emissions, it would be of great additional value for the two types of data presentation mentioned here; especially where bandwidths, centre-frequency and power density are concerned. Power spectra can, of course, be evaluated from amplitude-time displays, but the normal methods for reduction and computation of power spectra are time-consuming and not favourable for statistical analysis (cf. Vozoff *et al.*⁷).

This communication describes an accurate and rather simple method for power spectral analysis of micropulsations, and demonstrates two examples of HM emissions recorded in this way. The measurements were started at the Auroral Observatory at Tromsø ($\Phi = 70^\circ \text{ N.}$, $\lambda = 19^\circ \text{ E.}$) last autumn. The rapid geomagnetic fluctuations were received on an air-core loop antenna, with an effective absorbing area of approximately 3,000 m² and with the axis located in the magnetic N.–S. direction. A d.c. preamplifier, with maximum gain of approximately 100 dB for 1 μV input, was used. With a frequency-modulated adapter (cf. Mohus⁸) connected to a magnetic tape-recorder,

it is possible to record (with a tape speed of 7.5 in./sec) signals between d.c. and approximately 200 c/s. All the instruments were transistorized and battery-powered.

The tape-records are analysed on 'ISAC', a statistical analogue computer, designed and built at the Automatic Control Laboratory of the Norwegian Institute of Technology. ISAC computes correlation functions, power spectra and first-order distribution functions for signals in the frequency range 0–200 c/s. All computations, which are performed at high speed, are done automatically and the results are plotted direct by an *xy*-pen recorder. The resolution in frequency and power is high (see below). Frequency- and amplitude-calibrations are automatically carried out. ISAC weighs approximately 40 kg and may easily be carried around. (For further details concerning ISAC, compare, for example, Balchen and Blandhol⁹.)

Two samples of spectral density function of HM emissions evaluated by this method shown in Fig. 1*a* and *b*. The calculation and plotting of each power spectrum take about 8 min. No attempts at smoothing

the curve have been made. Fig. 1*a* displays the spectrum recorded on December 9, 1964, between 2147 and 2149 M.E.T., while Fig. 1*b* illustrates the spectral density of HM emission recorded 2 min later the same night: also in this case only 2 min of actual recording on the tape has been used in the analyses. The frequency accuracy is better than 0.1 c/s for both spectra.

As easily seen from the curves in Fig. 1, three dominant narrow bands are observed. Mid-band frequency as well as the bandwidth of the HM emissions can be measured very accurately direct from the curves. The centre frequency of these bands in Fig. 1*a* is 0.95, 1.7 and 2.95 c/s, while the corresponding figures for Fig. 1*b* differ by less than 0.2 c/s. The frequency widths (half-widths) for these three pronounced bands shown are 0.18, 0.32 and 0.1 c/s measured from the origin, while the corresponding bandwidths in Fig. 1*b* are 0.4, 0.13 and 0.2 c/s. The ratio between the band-width and the centre frequency ($\Delta f/f$) varies from 0.4 to about 0.03; thus the pulsations of HM emissions are normally confined to a very narrow frequency-band.

On first viewing these emission curves, it may look as if the second and third peak (measured from the origin) are the second and third harmonics of the first band, but the figures given here show that this is not the case. However, it can be objected that a rather large uncertainty exists in defining mean periods and bandwidths when such relatively short intervals of data are used. On the other hand, as these two curves are almost identical (except for the fine structure), both the mid-band frequency and the bandwidths of this HM emission should be significant.

It is difficult to devise accurate calibration for measurements of this type and, so far as I know, very little has been

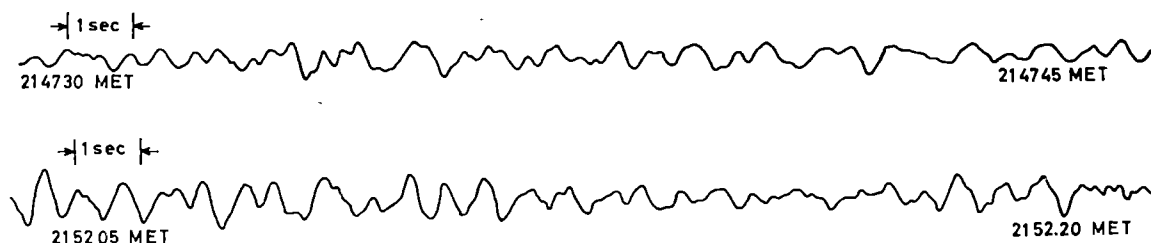


Fig. 2. Amplitude-time display. Record on December 9, 1964. upper, between 2147.30 and 2147.47 M.E.T.; lower, between 2152.05 and 2152.22 M.E.T.

written concerning signal strength of ultra-low-frequency emission. Unfortunately, no satisfactory calibration was carried out during December 9. But from later measurements it seems possible to conclude that the peaks in the spectra shown in Fig. 1 correspond to a variation in the magnetic field of the order of a few $\text{mG s}^{-1/2}$. This implies that the ultra-low-frequency emissions contain rather low energy compared with micropulsations in the frequency range between, say, 0.1 and 0.001 c/s. But, on the other hand, the amplitude resolution with this type of data presentation (cf. Figs. 1a and b) is very high.

That fine structure exists (superimposed on the regular band emission) is seen in the spectral density functions. However, detailed studies of fine structure of HM emissions can be made more directly and simply from frequency-versus-time displays (sonagrams).

With the other methods of presenting data that have been mentioned, it is very difficult to determine, with extreme accuracy, bandwidths and mean periods. This is especially true for the amplitude-time display. To illustrate this better, a small part of the amplitude-versus-time records obtained during the same time interval as the magnetic tape data used for the plotting of two power spectra discussed here, are shown in Fig. 2. The wave-form display varies more or less symmetrically about a base line but, as is easily seen, the modulation pattern is rather irregular, and they scarcely seem to exhibit a characteristic modulation frequency. Therefore, an interpretation of the spectrum based, for example, on the peak-to-peak interval can be completely misleading. It is also

impossible to arrive at the number of emission bands directly from such wave-form display.

Furthermore, it should be pointed out that the two spectral density functions of HM emissions, described here, were recorded during magnetically quiet conditions and no riometer absorption occurred during this time. Also, during the 12 h immediately before and after this time of observation, scarcely any magnetic- and/or riometer-deflexions could be seen on the diagrams.

Many other power spectra, with quite different shapes, have been obtained. These results together with theoretical considerations will be discussed in a later report.

This work was supported by the Cambridge Research Laboratories, OAR, through the European Office, Aerospace Research, U.S. Air Force, contract AF 61 (052)-811. I thank Mr. I. Mohus of the Norwegian Institute of Technology for help with the power spectra analysis.

¹ Jacobs, J. A., Kato, Y., Matsushita, S., and Troitskaya, V. A., *J. Geophys. Res.*, **69**, 180 (1964). Løkken, J. E., Shand, J. A., and Wright, C. S., *J. Geophys. Res.*, **68**, 789 (1963).

² Troitskaya, V., *J. Geophys. Res.*, **66** (1961).

³ Campbell, W. H., Paper presented at ULF Symposium, Boulder, Colorado, August 17-20, 1964.

⁴ Gendrin, R., and Stefani, R., *Magnetic Records between 0.2 and 30 c/s*, *ARGARDograph*, **74**, 371, edit. by Blackband, W. T. (Pergamon Press, 1964).

⁵ Tepley, L., Paper presented at the ULF symposium, Boulder, Colorado, August 17-20, 1964.

⁶ Pope, J. H., An explanation for the Apparent Polarization of some Geomagnetic Micropulsations ("Pearls"), *J. Geophys. Res.*, **69**, 399 (1964).

⁷ Vozoff, K., Ellis, R. M., and Garland, G. D., *Nature*, **194**, 539 (1962).

⁸ Mohus, I., *Instruction Manual for the FM-Adapter* (Norwegian Institute of Technology, 1964).

⁹ Balchen, J. G., and Blandhol, E., *Proc. First Intern. Cong. of Automatic Control* (Butterworths, London, 1960).

COINCIDENCE OF MAGNETIC DISTURBANCES WITH LOCAL EARTHQUAKES RECORDED FROM THE ETHIOPIAN RIFT SYSTEM

By PROF. PIERRE GOUIN

Geophysical Observatory, Haile Sellassie I University, Addis Ababa, Ethiopia

LABORATORY experiments¹⁻⁷ have been carried out to investigate the behaviour of rock magnetic susceptibility under conditions thought to approach those at focal depth prior to, or at the moment of, an earthquake: slow stress accumulation, sudden shear stress release, various Earth magnetic field intensities and orientations using different rock types, etc., in order to foresee what kind of magnetic anomalies might possibly be expected in the local magnetic field when an earthquake occurs.

Some authors have even expressed the opinion that some magnetic anomalies prior to a seismic event could even be used as a method for predicting earthquakes^{7,8}.

In general, however, authors do not agree^{4,6,7} on the total validity of the laboratory experiments on the grounds that they either do not reproduce the exact conditions prevailing at the seismic focal points, or simply because earthquake mechanism is not yet completely understood⁷ and varies according to local geological or tectonic conditions. I presume that the study of well-controlled field data may provide a more solid basis to the discussion of the problem. What, in fact, do geophysical stations equipped with both magnetic and seismic recorders register prior to or during a seismic event of a given magnitude (M) occurring at limited epicentral distance?

The Geophysical Observatory at Addis Ababa (09° 02' N., 38° 46' E.) is in a favourable position to answer such a question. About 175-200 km north-north-east from the station, on the western escarpment of Afar, lies the very seismically active region of Kara-Kore, from where more than 3,000 shocks were registered during the summer of 1961; two of these shocks were of $M \geq 6.2$. At a similar distance to the south-south-west on the floor of the Main

Ethiopian Rift is the dormant volcano Chubbi; this region is less active than Kara-Kore, but nevertheless experienced in 1960 an earthquake of $M = 6.3$. Thirdly, 500-600 km to the north-east of Addis Ababa is situated the seismically active region of the Gulf of Tadjura.

Since its opening, the magnetic station at Addis Ababa has been equipped with three standard Ruska variographs (H , D and Z) operating at sensitivities of about 3 γ/mm , with a time base of 20 mm/h. Prior to November 1962, the S_0 for D was 10 γ/mm .

In February 1965, two high-sensitivity rapid-run Selzer Fluxgate H and D magnetometers (supplied through the courtesy of the Institut de Physique du Globe, Paris) were put into operation with galvanometer periods of 0.5 and 4.0 sec, and with over-all sensitivities ranging from 0.02 to 0.002 γ/mm adjusted according to the level of sferics. These flux-gate magnetometers are insensitive to seismic noise and long-period magnetic variations.

In order to detect any magnetic effects related to local earthquakes, all the magnetograms since March 1959 (date of opening of the AAE seismic station) were attentively examined for unusual magnetic disturbances prior to, simultaneous with, or following a local earthquake of magnitude $M \geq 5.0$ (Eichter intensity scale). Since the Ruska H and D variometers are of the pendulum type, they are sensitive to earth tremors and, under the violence of local shocks of the aforementioned magnitude, the photographic traces disappear for periods of time often as long as 9-10 min. It should be noted, therefore, that the magnetic disturbances discussed here and indicated as coinciding with Earth tremors may have taken place either suddenly, or gradually during this 'black-out' period.

Table 1

Seismic data					Magnetic data			
No.	Date	Origin time L.M.T.	Epicentral distance	M	ΔH	ΔD (?)	ΔZ	Observations
1	1958, May 25	02:54	635 km	5.5	0	—	—	
2	1958, May 25	05:54	635	5.0	R	R	R	
3	1960, July 14	21:40	240	6.3	R	0	—	
4	1960, Aug. 8	15:28	715	5.4	0	0	0	
5	1961, Mar. 11	11:41	545	5.9	-15	0	0	ΔH may be due to normal magnetic activity
6	1961, May 29	07:60	205	5.0	-5	0	—	$\Delta H = 4\gamma$ about 36 min ahead (?)
7	1961, May 29	13:52	150	5.5	-20	0	0	Small pip at 13:30
8	1961, May 29	22:24	200	5.0	0	0	0	H-trace at night-level
9	1961, May 29	22:26	210	5.0	?	?	?	Effect covered by previous shock
10	1961, May 30	16:11	220	?	-1	0	0	
11	1961, June 2	02:29	210	6.4	-16	0	0	H-trace above night-level due to a magnetic bay
12	1961, June 2	07:51	195	6.2	-4	0	0	
13	1961, June 2	08:22	200	?	-12	0	0	
14	1961, June 2	08:45	215	5.8	0	0	0	
15	1961, June 2	10:03	175	5.5	0	0	0	Magnetic activity: $k=5$, $C_1=1.3$
16	1961, June 3	18:23	205	5.8	?	?	?	Too many shocks to detect any reliable ΔH
17	1961, June 14	23:32	210	5.7	0	0	—	$\Delta H = 2-3\gamma$ 42 min ahead (?)
18	1962, Nov. 11	18:15	915	5.6	0	—	—	H-trace at night-level
19	1963, Oct. 5	17:58	530	5.3	0	0	—	H-trace at night-level
20	1964, July 3	22:19	230	5.0	0	0	—	H-trace at night-level

R, Record missing. —, Event not recorded on that component.

Table 1 sums up the observations on Ruska magnetograms for local earthquakes of $M \geq 5.0$ at the moment of the seismic registration. On these magnetograms, no abnormal magnetic disturbances have been detected either prior or subsequent to the Earth tremors. It must be emphasized that the ΔH , ΔD and ΔZ values indicated in Table 1 do not refer to the amplitude of the pendulum oscillations, but to a sudden permanent change in the intensity of the magnetic field. The station being situated at the magnetic equator, $H \approx F$.

From Table 1, it appears that at the moment of a local earthquake:

(1) No permanent change in declination (D) or in the intensity of the magnetic vertical component (Z) has yet been observed.

(2) Magnetic disturbances prior to the seismic events listed as Nos. 6, 7 and 17 could only questionably be related to these events and are in no way outstanding.

(3) Permanent changes in the intensity of the magnetic field (restoration of the field to its normal value) are observed at the time of the registration of local earthquakes and are always negative.

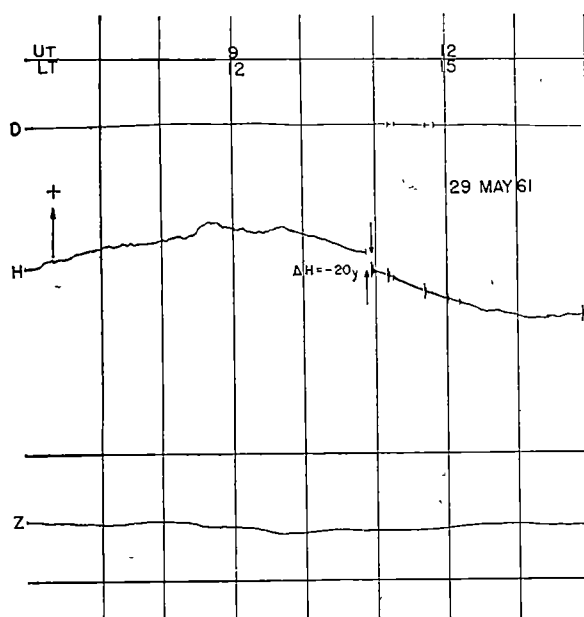


Fig. 1. Ruska standard magnetogram of May 29, 1961, showing the sudden decrease in the value of H at the moment of local earthquake No. 7 in Table 1. The tremors following that seismic event show no apparent change with intensity of the field

(4) Neglecting a possible effect of focal depth for tremors of approximately equal epicentral distances and magnitudes, the negative magnetic disturbances (ΔH) show a certain tendency to be enhanced during day-time, when the H (or F) trace is above night-time level (compare events Nos. 6 and 13 with Nos. 8, 17 and 20). There are, however, three apparent exceptions to this tendency.

(a) Earthquake No. 11 happened at night (L.M.T. 02:29) but shows a $\Delta H = -16\gamma$. The occurrence of this earthquake coincided with a magnetic bay when the H -trace was above the normal night-level. The decrease in intensity of the magnetic field by 16γ may either be due to the earthquake or may simply mark the end of the magnetic bay.

(b) Earthquakes Nos. 14 and 15 happened in the morning when the H -trace was well above night-level; the seismic magnitudes were respectively 5.8 and 5.5 and epicentral distances 215 and 175 km, but no magnetic disturbances were registered. Observations show, however, that this absence of magnetic disturbances prevails during a swarm of tremors. Earthquakes Nos. 14 and 15 were preceded by two shocks which released -4 and -12γ , respectively, within 56 min. Many other similar examples could be given. In a swarm of quakes, when tremors follow one another at short intervals, the total piezo-magnetic energy is released during the first shocks, and if a new build-up of piezo-magnetic energy does occur in such a short period of time (a few minutes) its intensity is too weak to be recorded 200 km away.

(5) The theoretical rate-of-change in the intensity of the Earth magnetic field during the hours or days preceding a major seismic event cannot be expected to be observed at magnetic stations under the influence of the equatorial electrojet; it would be hidden in the unpredictable variability of the daily Sq variation.

A question arises: Could the displacement of the H -trace on the Addis Ababa records, interpreted as a variation of the magnetic field at the moment of a local earthquake, be mistaken for seismically induced physical displacements of the working parts of the H -variometer? Such a possibility is very doubtful because:

(a) The variometers at the station are solidly cemented to their pier and their base can in no way be moved. Had they been moved under the seismic shock the baseline traces also would have been displaced, since the mirrors for these are attached to the base of the instruments.

(b) The mechanical effects would therefore be restricted to the pendulum, which is free to oscillate within the limits of its damping cup. One does not easily understand why, in a series of earthquakes of equivalent magnitudes and epicentral distances, a few shocks happening when

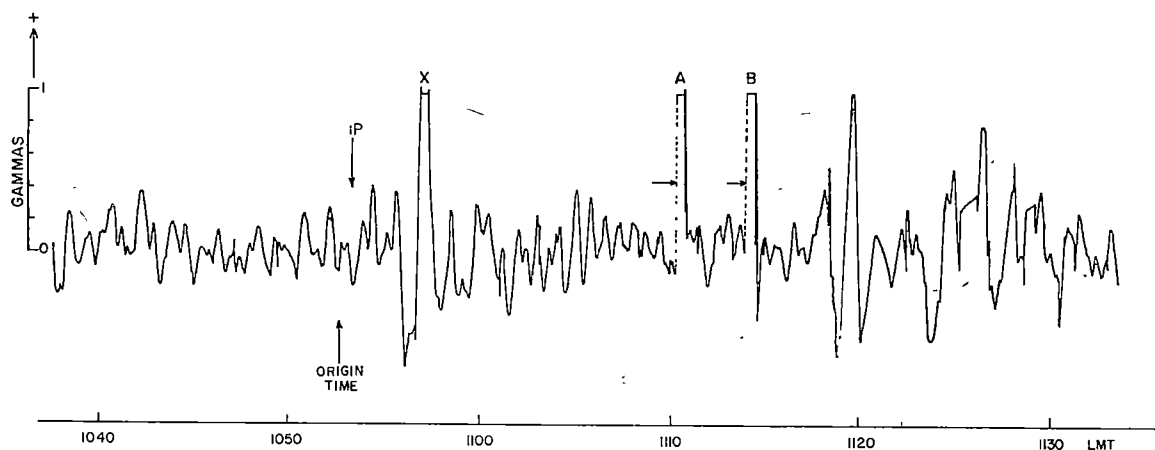


Fig. 2. Flux-gate record of April 14, 1965, showing possible magnetic effects of earthquake No. 6 in Table 2 (the record has been retraced by hand owing to the faintness of the trace)

the H -trace is above night-level would cause a physical disturbance on the H -pendulum when all the other shocks occurring at night-level hours did not produce any such physical disturbances (Table 1).

(c) The instruments have been submitted to a mechanical vibration test (because of the solidity of the pier, the seismic oscillations could not be exactly reproduced) and no permanent change in the position of the H -trace has been noticed. I think that seismic shocks as mentioned in (b) are still the best test.

Moore⁹ reported an astonishingly sudden increase of 100γ in the intensity of the total field (F) recorded on a flux-gate magnetometer at Kodiak, 66 min before the Alaska earthquake of March 27, 1964. Kodiak was 440 km away from the epicentre. Unfortunately, Moore's comments are based on a single observation.

Breiner¹⁰ mentions repeated events recorded just prior to local earthquakes, but although his records (*op. cit.* Fig. 2) show a positive disturbance 10 min before the seismic event studied, he refrains from commenting on this magnetic disturbance until more evidence becomes available.

The flux-gate magnetic records from Addis Ababa have been examined in correlation with local earthquakes, some of which are listed in Table 2. I admit that the choice of seismic events in Table 2 is not ideal, owing to generally low magnitudes and to epicentral distances higher than 300 km, but this cannot be remedied until more and bigger local earthquakes are experienced at Addis Ababa.

Table 2

No.	Date	iP (L.M.T.)	d (km)	M	Magnetic observations
1	1965, April 13	05:36:28	850	5.1	No magnetic disturbance
2	" "	23:26:58	300	2.7	" "
3	1965, April 14	04:05:08	320	2.5	" "
4	" "	06:13:05	320	3.1	" "
5	" "	07:13:08	350	3.7	Small + pips at 06:55 35 and 07:17:10
6	" "	10:53:11	350	4.3	See Fig. and comments below

Of the six events listed in Table 2, only the last one ($d = 350$ km, $M = 4.3$) showed any magnetic disturbances (Fig. 2) which could be termed unusual, but these certainly would not be differentiated from normal magnetic pulsations if it were not for the knowledge that a local earthquake had, in fact, occurred and that therefore seismo-magnetic effects might be expected.

Fig. 2 shows a part of the flux-gate magnetic H -trace recording for July 14. Both the origin time and iP -phase recorded time are indicated. The very small pips on the magnetic trace are lightning effects, well recorded on the D -trace. Prior to iP (10:53:11), no unusual magnetic

disturbance could be detected. At 10:56, a large isolated sinusoidal pulsation (x) occurred, but this is nothing unusual on the flux-gate records. Later, at 11:11 and 11:14.5, two similar very sharp and quite unusual positive pulses (A and B) appeared at the end of 30-sec abrupt increases of the magnetic field. Note that these disturbances do not appear on the D -trace.

The last two negative disturbances marked A and B are presumed to be seismo-magnetic, but further observations are necessary before a cause-to-effect relation could be affirmed.

In conclusion, from the examination of the standard magnetograms at Addis Ababa, up to now at least, it appears that:

(a) No disturbance in the local magnetic field has been observed either prior to or following a local seismic event originating in the Ethiopian Rift system.

(b) Negative stepped decreases in the intensity of the total magnetic field ($E \approx F$ at Addis Ababa) have been observed coinciding in time with local Earth tremors. (Nagata and Kinoshita⁶ would have expected $a + \Delta H$.) The amplitude of the decrease ΔF seems proportional to the magnitude of the earthquake (therefore to the stress accumulated), to its epicentral distance from the station, and most probably to the amplitude of the positive daily variation (S) of the magnetic field above the mean night level. Such a decrease in the intensity of F has also been observed by Breiner in Nevada¹⁰.

(c) The seismo-magnetic effects produced no deviation in magnetic declination such as Miyakoshi¹¹ has observed in Japan.

Unexplained phenomena have also been noted on the high-sensitivity short-period flux-gate recording either before or following local seismic events. Their polarity seems to be positive while the permanent magnetic effects on standard magnetograms have been observed to be negative. However, the time of their appearance in relation to either the origin time of the earthquake, its iP recorded time at the station or its epicentral distance, is so inconsistent that much more evidence is needed before any conclusion could be drawn.

¹ Kalashnikov, A. G., and Kapitsa, S. P., *Doklady Akad. Nauk, U.S.S.R.*, **86** (3), 521 (1952).

² Kalashnikov, A. G., *Akad. Nauk, U.S.S.R., Geophys. Inst. Trudy*, No. 25, 1962 (1954).

³ Kapitsa, S. P., *Izv. Akad. Nauk, S.S.S.R., Ser. Geophys.*, No. 6, 489 (1955).

⁴ Stacey, F. D., *Nature*, **200**, 1083 (1963).

⁵ Grabovsky, M. A., and ParEhomenko, E. I., *Izv. Akad. Nauk, S.S.S.R., Ser. Geophys.*, **5**, 405 (1953).

⁶ Nagata, T., and Kinoshita, H., *Nature*, **204**, 1183 (1964).

⁷ Stacey, F. D., *Nature*, **204**, 1184 (1964).

⁸ Kern, J. W., *J. Geophys. Res.*, **66**, 3807 (1961).

⁹ Moore, G. W., *Nature*, **203**, 5C8 (1964).

¹⁰ Breiner, S., *Nature*, **202**, 790 (1964).

¹¹ Miyakoshi, H., *Kyoto Univ. Disaster Prevention Res. Inst., Bull.*, **51** (1962).

RELATIVE SIMILARITIES IN ONE DIMENSION

By PROF. J. W. CARMICHAEL, PROF. R. S. JULIUS and DR P. M. D. MARTIN

Mold Herbarium and Culture Collection, and Departments of Computing Science and Bacteriology, University of Alberta, Edmonton

PERCEPTION, in man, is the act of deriving some meaning or message from signals transmitted to the brain from the sense organs. It consists of detection, in a set of simultaneous or sequential signals, of sets of interrelations among the signals, which we may call 'patterns'. The human brain, then, must have some mechanism for detecting patterns in signals and also for comparing recently received patterns with previously received ones. We do not know what this mechanism is, but when a pattern is recognized as a familiar one, we call the process identification. When a pattern is recognized as novel, the process is classification, or concept formation, because in the recognition of novelty we have added a new pattern to our repertory. In other words, we have added a new concept to our view of reality, or we have enlarged our perceptual sorting system for deriving meaning from signal input, or we have added a new class to our classification of all things.

This process begins at, or before, birth, and continues day by day and minute by minute. The result is a mapping, in the brain, of what we conceive of as reality. Reality for the individual can only be his map of what the world is like in relation to the signals he receives. 'Reality' in the sense of 'what the world is like in relation to man collectively' is the object of scientific investigation. We can say that whenever concepts about reality lead to reliable predictions, and hence useful responses, the concepts are valid ones. When they do not lead to reliable predictions or useful responses, the concepts are 'wrong'. Validity of concepts can only be judged on their utility, even though we may hope that the concepts of science form an undistorted mapping of the physical universe.

Of course, this process of concept formation, or development of pattern sorting systems, soon becomes complex. Without receiving new signals, we can detect patterns of patterns when we have a large store of them in our repertory (and perhaps even conceive of patterns not implicit in our repertory). This process we might call abstract conceptualization.

In the classification of bacteria, as in many problems in psychology, the attributes available for classification are largely non-visual. When the attributes in which we wish to perceive patterns are non-visual and not obviously convertible into visual form by any simple method, we are cut off from the superbly efficient, intuitive system which we have developed for sorting visual signal patterns. It is thus understandable that psychologists and bacteriologists have been leaders in attempts to develop numerical methods to substitute for visual ability in classifying. The attempts, so far, are somewhat unsatisfying, not only because they involve algorithms which may be different from our intuitive systems, but because they may yield different results. It therefore seems desirable to attempt to understand the intuitive mental procedures we follow to recognize and compare patterns, even though the mechanism by which we carry out the procedures remains unknown.

One of the many problems is to determine the procedure by which we judge one-dimensional similarities (that is, similarities among pairs of states of a single attribute). The method by which we distinguish qualitative similarity is obviously related to discriminatory ability. Things which we can discriminate between are 'different'; things which we cannot discriminate between are 'one' or 'the

same'. This has been the basis of most numerical taxonomy so far. Single attributes are considered only as having qualitative similarity (0, 1 or +, -). In some instances similarities among single attributes have been scaled by considering them as sums of qualitative similarities (+++, ++-, +--, ---). Methods for weighting the sums have also been used¹. However, so far as we can determine, no one has proposed a satisfactory definition for what is meant by quantitative or fractional 'similarity' in a single dimension².

The key to solving this problem lay in the use of a model. We assumed (hopefully without distortion) that states of any single attribute (values in a single conceptual dimension) were ordered and monotonically related to distance in a single Euclidian dimension. We then considered similarities among a number of lengths (distances in one dimension). Considering hypothetical, exact lengths, it became clear that quantitative similarity is not a function of discriminatory ability. The uncertainty in an estimate of similarity is related to discrimination, but the size of the estimate need not be. It was also obvious that quantitative similarity must be relative, since there is no similarity (other than 0 or 1) when one considers only two states in the absence of other states or a base line of some kind.

If you ask a person: "How similar are lengths of 1 and 2 units?" he may tell you they are 1/2 similar. This sounds as though similarity is simply the ratio of two lengths. Other people, given the same task, will say that the lengths have no similarity, or they will avoid answering in terms of similarity, indicating that they do not have any basis for judging this relationship. If you give a set of three lengths, $A=1$, $B=2$, and $C=12$ units, and ask how similar are A and B , most people will say that they are quite similar, and will volunteer that A and B are more similar than A and C and further that B and C are more similar than A and C . There is no doubt in most minds about the rank order of the three pairs.

Similarity is obviously a ratio or proportion of some kind. But a proportion of what? We arrived at the answer to this question, appropriately enough, by gradually recognizing that we had backed past it some time before. As one of a number of attempts to code attributes in some way so that similarities could be weighted, we had tried the relation:

$$S_{AB} = \frac{L - |A - B|}{L} \quad (1)$$

where S_{AB} is the similarity of states A and B , and L is the distance between the most dissimilar pair of states (C and D) in the set being considered. In our length model, the 'state of A ' is the value of length A in the arbitrary scale units used.

Equation (1) specifies that similarities are fractional, that they range between 0 and 1, and that they are valid only within a given range of states. If the set of lengths is enlarged so as to increase the distance between the most dissimilar pair, the original fractional similarities must be shifted towards 1. Although the fractional values change, the rank order of the similarities does not (that is, the shift is monotonic). Thus, speaking of relative similarity is redundant, since all similarities are relative to some explicit or implied range. However, we would like to retain the expression 'relative similarities' as a

term for a 'set of similarities computed according to a single procedure for a specific set of things'.

Going back to the similarity between lengths of 1 and 2, we see that those who said they were half similar assumed that the lengths were a sample from a set ranging from 0 to 2, while those who said there was no similarity assumed that they were a sample of a set (or the whole set) ranging from 1 to 2. The inclusion of a third and longer length in the set increased the range and increased the similarity of lengths one unit apart.

Verbalizing equation (1) we see that 'similarity is the fraction of the considered range not included between two states'. We now accept this as the definition of similarity in one dimension, and suggest that if you substitute 'things' for 'states' in the foregoing definition, you have a good general definition for the intuitive concept of similarity in any number of dimensions. One reason that this concept has been so elusive is that the numerator is defined by stating what is not (the considered range not included). There was no word for this quantity and we propose that it be called 'undifference' (U). Undifference is the quantity which relates the distance that does exist between a pair of states to the distance that could exist between them. Or, stated another way, undifference is the distance that a pair of states is not apart. The intuitive idea that a pair of identical states drawn from a short set is not as undifferent as a pair of identical states drawn from a long set does not apply to hypothetical lengths, because we can distinguish an infinite number of such lengths in either a short or a long set. In the hypothetical case, the following relations hold:

$$\begin{aligned} L &= |C - D| \\ U_{AA} &= L - |A - A| = L = U \text{ for this system} \\ U_{BB} &= L - |B - B| = L \\ U_{AB} &= L - |A - B| \\ U_{CD} &= L - |C - D| = 0 \end{aligned} \quad (2)$$

and hence equation (1) can be rewritten as:

$$S_{AB} = \frac{U_{AB}}{U}$$

Although the undifference is defined only to within an arbitrary, multiplicative constant (the scale used to define the states), the relative similarities are uniquely defined since the same scale is used for A , B , C and D . Undifference, as described here, was clearly anticipated by Reynolds' in his recent article on comparative elaborateness.

When we wish to use a concept, such as length, to indicate something about the nature of a real thing, uncertainty arises. We can stipulate, and hence know for certain, the state of a concept; but real things do not exist in our conceptual dimensions and states, they exist in reality (whatever that may be). Therefore, our only recourse is to stipulate tests or measures (physical sorting systems) which yield signals (measurements) which we will interpret as data (states) which we assume are applicable to our concept. A number of measurements, made by repeatedly applying the same measuring procedure, may be treated statistically to yield a mean value and a probability that the next measurement made by the same system would fall within a given distance of the mean. Unfortunately, statistics may not tell us whether our concept of what we are measuring fits what we actually are measuring, but this need not lead to error, so long as the deficiency is recognized. There are, then, two sources of uncertainty: (1) Lack of precision due to measuring error, (a) where imprecision is independent of the magnitude of the measurement over the range being measured, and (b) where imprecision is related to the magnitude over the range being measured. (2) Lack of precision due to inapplicability of the measuring system (non-unidimensionality in the quantity being measured, *vide infra*).

Let us assume first that the best estimate we can get for the state (value in arbitrary units) we are trying to determine is the mean or average of the measurements. It would seem reasonable to determine relative similarities according to these means. Thus, for equation (1) we could substitute:

$$S_{AB} = \frac{L - |\bar{A} - \bar{B}|}{L} \quad (3)$$

where

$$L = |\bar{C} - \bar{D}|$$

or perhaps

$$L = C_{\max} - D_{\min}$$

The multiplicative constant for U is now restricted by the number of states that we can reliably discriminate between. We can put any interpretation we like on 'reliably', but we suggest a confidence limit of, say, 99 per cent (where $P < 0.01$). If we wish to make our sets of relative similarities comparable to one another, then the important criterion is to have comparable P s in the different sets. By being conservative we can also minimize the likelihood of overweighting similarities where P is calculated, compared to similarities where P is judged intuitively. The confidence limit of the mean of a number of measurements is related to the unit standard deviation, so that we could calculate the undifference at $P = 0.01$ for an identical pair of means \bar{A}, \bar{A} , on a scale of length L . However, this comparatively elegant method is computationally difficult and probably unwarranted for the usual number of measurements used to compute values for states. Also, we will usually have a number of sets of similarities where U is judged intuitively. When the measuring error is approximately constant over the given range of values (case 1a), we would prefer to assume that P will be sufficiently small if we let A_{\max} be the highest measurement for A , and A_{\min} the lowest,

$$\text{and} \quad R_A = A_{\max} - A_{\min}$$

$$\text{and} \quad \bar{R} = \frac{R_A + R_B + \dots + R_t + \dots + R_n}{n} \quad (4)$$

$$\text{and} \quad U = \frac{L}{\bar{R}} \approx U_{AA} \approx U_{BB}$$

When we estimate, or judge, the states of a non-numerical attribute, and distribute them among a number (n) of ordered classes that are intuitively equally reliable, then the undifference of the system, U , is given by the number of class intervals ($n - 1$), and this may be taken as the scale length, L , for use in computing similarity. Since n and U depend on the number of equally reliable (but not necessarily equally probable) states that an attribute can exhibit, we suggest that they are measures of the relative information content for classification. If we wished to sum sets of similarities to obtain an overall similarity, then the one dimensional similarities could be appropriately weighted according to:

$$W_{SAB} = \log_2 (U + 1) = \log_2 n \quad (5)$$

For measured states, where the measuring error is not constant, but is a function of the value of the mean (case 1b), one procedure would be to compute a separate U for each pair of states by assuming that the relation:

$$U = \frac{1}{2}(U_{AA} + U_{BB}) \quad (6)$$

holds good. This would mean, in effect, that the similarities in one relative set would be based on different numbers of classes and would have different weights. To avoid this intuitively unsatisfying arrangement, and to simplify computation, it would seem better to transform the scale used, so that the error in scale units is independent of the mean of the state. We could then proceed as for case 1a. For estimated or judged states, we would intuitively compensate for value-dependent errors when we form equally reliable classes.

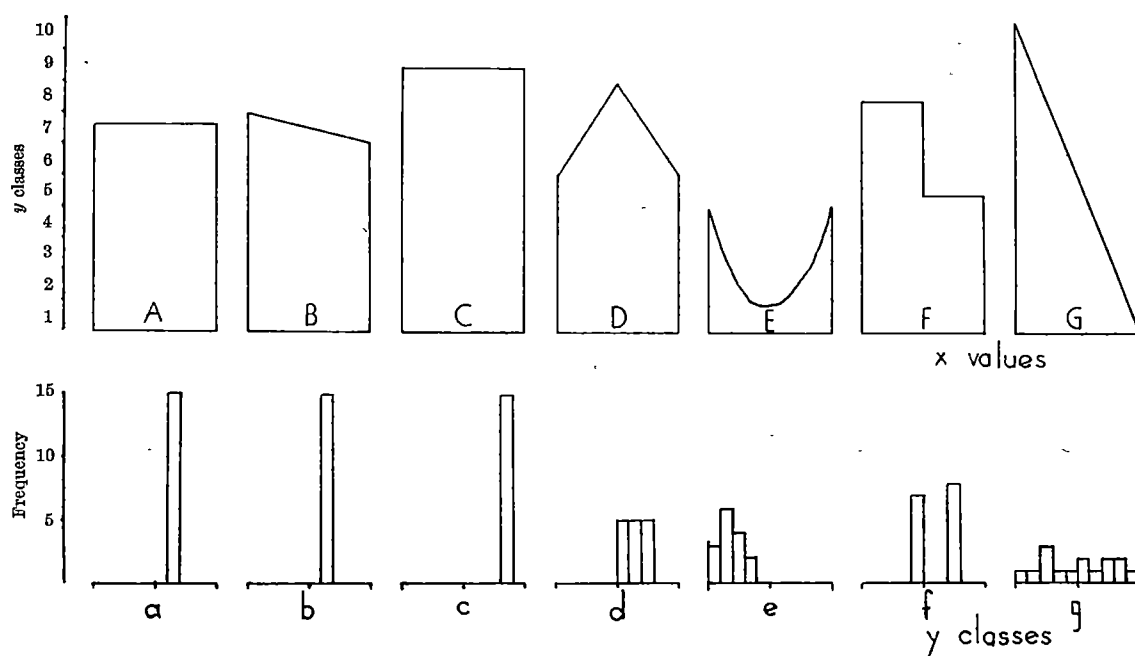


Fig. 1. A-G. Models of an attribute (y), in which the state varies over another dimension (x). a-g, Histograms derived by taking 15 measurements of y randomly distributed over x .

When the spread of values, on repeated measurement, is larger than we would expect the measuring error to be, we would assume that non-unidimensionality of the attribute is the cause. Let us assume that the error in the measuring system can be estimated. We can then make a scale of a number of classes of equal confidence and small P . If repeated measurements fall into more than one class, lack of unidimensionality is present (case 2). To illustrate this point, consider the irregular bars in Figs. 1A-G. If we assume that each of these bars has a height, in arbitrary classes on the y axis, and we repeatedly measure the height at random over the x dimension (axis), we will get the histograms shown in Figs. 1a-g. The shapes of the bars define the histograms; but if we do not know how the y measurements are distributed over the x dimension (even whether they are random or not), the histograms do not uniquely define the shapes of the bars (for example, where y is spore-length and x is the reason(s) why different spores are not the same length).

Following our previous rationale, the procedure in this case would be to compute similarities on the means for the y measurements, and the undifference as in equations (4).

Another, more presumptuous, approach would be to assume that the distribution of frequencies in the histograms had some meaning which should affect the similarities as well as the weights. With this assumption we could compute a similarity based on the undifference of each measurement in each class of A compared with each measurement in each class of B. To compute this kind of similarity we would: let the state of A be an array of frequencies, $A_1, A_2, A_3 \dots A_i \dots A_n$; and the state of B be an array of frequencies, $B_1, B_2, B_3 \dots B_j \dots B_n$; and the sum of the frequencies of A be called F_A ; and the sum of the frequencies of B be called F_B ; and the range or scale-length, $n-1$, be called L . Then the similarity of A and B is given, when $F_A = F_B$, by:

$$S_{AB} = \frac{\sum_{i,j=1}^n A_i B_j (L - |i - j|)}{\frac{1}{2} \left[\sum_{i,j=1}^n A_i A_j (L - |i - j|) + \sum_{i,j=1}^n B_i B_j (L - |i - j|) \right]} \quad (7)$$

or rewriting the above equation in simpler notation,

$$S_{AB} = \frac{U'_{AB}}{\frac{1}{2}(U'_{AA} + U'_{BB})} \quad (8)$$

When $F_A \neq F_B$, we can correct by expanding to:

$$S_{AB} = \frac{U'_{AB} \cdot F_A \cdot F_B}{\frac{1}{2}(U'_{AA} \cdot F_B^2 + U'_{BB} \cdot F_A^2)} \quad (9)$$

This equation maintains the general relation that similarity is the fraction of the range not included between the states, but both the 'range' and the 'distance' between the states are affected by the spread of the values about the means of the states. Secondly, this equation stipulates that frequencies-in-common, or close frequencies, are more important in contributing to similarity than more widely separated frequencies are important in detracting from similarity. The equation does not assume a normal distribution of frequencies, and the results with skewed distributions are intuitively satisfying. The high similarities yielded with very broad spreads or bimodal distributions (because of the second effect already noted) are somewhat disturbing intuitively. The generally higher similarities that result when we use frequencies, rather than means of single values, are reasonable enough, since the similarity is still zero when all values fall in the two most distant classes.

Not only does increasing spread result in increasing similarity, but the spread of the states results in an effective compression of the range and this should be recognized in computing the weighting of the similarity. This is easily accomplished by modifying equation (5) so that:

$$W = \log_2 \left(n \cdot \frac{\frac{1}{2}(U'_{AA} + U'_{BB})}{U'_{\max}} \right) \quad (10)$$

where $U'_{\max} = F_A \cdot F_B \cdot L$

which, if $F_A = F_B$ simplifies to:

$$U'_{\max} = F^2 \cdot L$$

When we know the way in which the y measurements are distributed over the x dimension, we would probably not want to use either of the foregoing methods. For example, if y represents growth rate and x represents different known temperatures, one could compute a similarity for the growth rate at each temperature used. How many temperatures (values of x) should we use to

compute an overall similarity for growth rate (state of y)? Obviously the range of temperatures is limited by the lowest and highest temperatures at which any of the strains will grow (that is, the only useful values of x are those for which not all the y values are identical). The number of temperature values, within this range, which would contribute new information about the distribution of y over x , is limited by the minimum temperature difference which would produce growth rates between which we could reliably discriminate. Thus, we have a finite number of test classes, as well as attribute classes, and the number in each case is based on equally reliable discrimination within the range found. If we compute a set of similarities for an attribute, in each of a number of test classes, in each of a number of test dimensions, then we would need a weighted mean similarity for the attribute under all test conditions in order to compare it with, or add it to, similarities for other attributes. The relative weight of such mean similarities for single attributes should be the log of n for the whole attribute-test system, rather than the sum of the log n 's in each test class of each test dimension (compare equation (5)).

We have promulgated a principle for determining similarities in one dimension and devised some methods for applying it to attributes to yield sets of relative

similarities and weights. An obvious way to produce, from these, sets of overall similarities (\bar{S}_{AB} 's) is to let:

$$\bar{S}_{AB} = \frac{\sum_{i=1}^n S_{AB_i} \cdot W_{AB_i}}{\sum_{i=1}^n W_{AB_i}} \quad (11)$$

The weights of the overall similarities should be the sums rather than the averages of the weights for the individual similarities.

The matrix of weighted, overall similarities then needs to be transformed into a useful classification. One promising approach appears to be through multidimensional scaling³⁻⁵. This approach might even be able to compensate for the defect that the supposed one-dimensional similarities contain varying redundancy when summed.

¹ Sokal, R. R., and Sneath, P. H. A., *Principles of Numerical Taxonomy* (Freeman and Co., San Francisco, 1963).

² *Handbook of Mathematical Psychology*, edit. by Luce, R. D., Bush, R. R., and Galanter, E., 1 (Wiley, J., New York, 1963).

³ Shepard, R. N., *Psychometrika*, 27, 125 (1962).

⁴ Shepard, R. N., *Psychometrika*, 22, 219 (1962).

⁵ Kruskal, J. B., *Psychometrika*, 29, 1 (1964).

⁶ Kruskal, J. B., *Psychometrika*, 29, 115 (1964).

⁷ Reynolds, K. A., *Nature*, 208, 166 (1965).

A.C. RESISTIVITY OF ANODIC OXIDE FILMS ON VALVE METALS

By DR. G. C. WOOD and C. PEARSON

Corrosion Science Division, Department of Chemical Engineering, Faculty of Technology, University of Manchester,

THE frequency dispersions of a.c. resistivity of anodic oxide films on niobium, tantalum, aluminium, zirconium, titanium and tungsten have been measured and also the resistivity profiles through films on niobium by an extension of the technique of Heine and Pryor¹.

Specimens in the form of sheet or as the ends of wires were generally chemically polished, followed by a 10-sec etch in 40 per cent HF in the case of tantalum and niobium. Aluminium, however, was simply etched in 0.5 N NaOH solution for 15 min at 20°C followed by a 1-sec dip in 50 per cent HNO₃ at 90°C, and zirconium was finished on 1 μ diamond suspension. The specimens were anodized, generally in 3 per cent w/v ammonium tartrate solution (pH adjusted to 7), at 10 m.amp/cm² to an appropriate cell voltage followed by current decay, or occasionally to a given overpotential. The frequency dispersions of impedance of as-formed films or of films thinned in 1 per cent HF were measured, generally in tartrate solution, using a General Radio capacitance measuring assembly type 1610-B with a 50-mV p - p signal. This permitted the calculation of resistivities, on the assumption of the validity of a simple electrical analogue for the film of a resistor in parallel with a capacitor at any one frequency. Such results are plotted as bold curves in the diagrams; dotted curves, where included, indicate the effect of correcting for double-layer resistance by the simple method of Heine and Pryor¹. Thinning was judged to be macroscopically and microscopically uniform from interference colours and transmission electron microscopy respectively. As-prepared films seemed flaw-free and the flaw density was so low in thinned films that there appeared to be negligible effect on the impedance. The film capacitance was a sound measure of film thickness when compared with interference-colour determinations.

Fig. 1 shows the frequency dispersions of average a.c. resistivity for films 230, 1150 and 2300 Å thick formed on zone-refined niobium (purity better than 99.99 per cent except for about 100 p.p.m. tantalum) at 10 m.amp/cm²,

cm², followed by appropriate current decay to a residual current of less than 20 μ amp/cm². Clearly the thicker the film the higher the average resistivity. This could suggest either that the films become more perfect the thicker they grow, or that the resistivity of the film region adjacent to the metal is lower than elsewhere. Evidence presented here favours the latter interpretation. The observation seems to be a general one for valve metals, because it holds at all frequencies for tantalum and niobium in aqueous and non-aqueous solutions and for aluminium in non-aqueous solution. It is also true for aluminium and zirconium in aqueous solution at 10 kc/s and above. The curves converge at and below 1 kc/s for aluminium in aqueous solution, probably because of effects due to gross impurity substitution of OH⁻ ions^{1,4}, and for zirconium possibly for similar reasons. The phenomenon could not be checked for tungsten because the films thinned or showed strange behaviour during and immediately after formation in many electrolytes, or for titanium because a sufficient range of film thicknesses could not be obtained. For niobium, and particularly for tantalum, the results were relatively independent of metal purity (99.8 per cent to zone-refined), anodizing electrolyte and whether the main anodizing current density of 10 m.amp/cm² was terminated abruptly or by current decay.

Figs. 2a and b give the average resistivities at 1 kc/s and 100 kc/s respectively, measured after various stages of thinning by 1 per cent HF, for a 2300 Å film formed on zone-refined niobium. Each point plotted represents the average value for the film of that thickness remaining on the specimen, not the resistivity of a small element of oxide in that location¹. Results for 230 Å films thinned in 1 per cent HF and in M.Na₂CrO₄ solution with pH adjusted to 11.2 with NaOH solution (representing a much slower thinning rate) were quite similar. The various results indicate low-resistivity oxide adjacent to the metal at both frequencies, confirming the trends reported here. According to the Heine-Pryor theory¹, this represents an n -type semi-conducting region containing oxygen ion

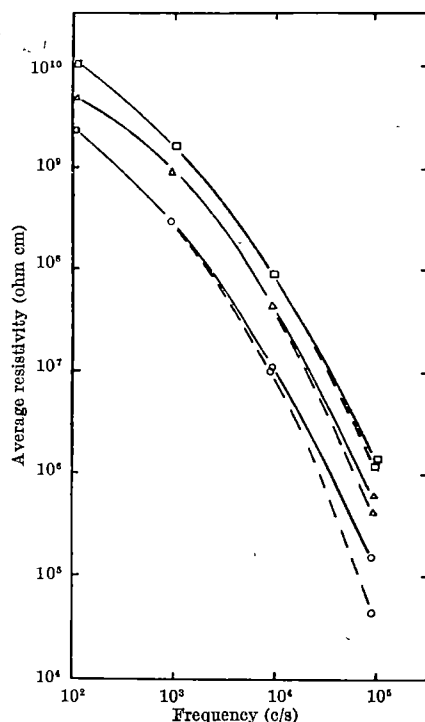


Fig. 1. Frequency dispersions of average resistivity for anodic oxide films on niobium. \circ , 230 Å; \triangle , 1150 Å; \square , 2300 Å

vacancies or possibly excess metal ions. The associated partly trapped electrons required for electrical neutrality produce principally electronic relaxation effects observable at both frequencies. Presumably this represents a steady-state situation because it is held² that about 70 per cent of Frenkel defects present during formation rapidly disappear on stopping the current. When the results at 100 kc/s, corrected for double-layer resistance, are plotted as equivalent parallel resistances, R_p (obtained by multiplying the resistivity by the thickness at each thickness), a smooth non-linear curve is obtained. If R_p is plotted against the square of the thickness, a straight line passing through the origin is obtained, indicating that the number of these n -type defects varies with the inverse distance from the metal/oxide interface for the entire film, according to the theory of Heine and Sperry³. This contrasts with films on aluminium where the metal-deficient region extends only 60–80 Å into the film for 240 Å films^{1,4}. These preliminary results require confirmation, but the difference might be due to the greater solubility of oxygen in niobium. The undulations in the outer regions at 1 kc/s may be genuine because they are semi-quantitatively reproducible, but further work is required to explain them. The initial drop was originally thought to be due to opening of flaws by the HF, but simple calculations of the contribution of conduction down the few flaws to the overall impedance do not support this argument. The average resistivities at both frequencies for films thinned to 1150 Å and 230 Å respectively agree within experimental error with the corresponding values for films formed only to these thicknesses. If films on all the valve metals could be thinned uniformly (which is doubtful), it is probable that resistivity profiles of the general form shown would always be found. Similar, but not identical, curves have been obtained for aluminium in non-aqueous solutions⁴, which should prove more amenable to quantitative interpretation than the more complex ones obtained on partially hydrated films formed in aqueous solutions^{1,4}.

Fig. 3 presents a comparison of the frequency dispersions of average a.c. resistivity of 230 Å films formed on the various metals. Table 1 lists important constants for the metal oxides obtained from many sources. The values of dielectric constants used to calculate the appropriate

Table 1. IMPORTANT CONSTANTS FOR ANODIC OXIDE FILMS ON METALS

Metal and oxide	Dielectric constant	Melting point of bulk oxide (°C)	ρ Factor (metal ion radius) (oxygen ion radius)	Refractive index	\bar{A}/V ratio
Aluminium (Al_2O_3)	8–10.5 (8)	2,050	0.38–0.39	1.52–1.67	10–14 (12)
Zirconium (ZrO_2)	22–24.7 (22)	2,700	0.60	2.13–2.2	21–25.6 (25)
Tantalum (Ta_2O_5)	27.6	1,870	0.52–0.56	2.12–2.3	17.5
Niobium (Nb_2O_5)	41.4	1,520	0.52–0.54	2.22–2.46	23
Tungsten (WO_3)	41.7	1,473	0.47	2.2	18
Titanium (TiO_2 ?)	12–114 depending on form of TiO_2 (107)	1,640	0.52	2.5–2.9	20–50 (20)

resistivities and the \bar{A}/V ratios used to estimate film thicknesses are given in brackets where a range of values is quoted. Clearly, the lower the dielectric constant of the film the higher its a.c. resistivity. In order to obtain the curves presented it was necessary to know the dielectric constants of the films and their \bar{A}/V ratios. The only case of uncertainty is titanium, where both parameters are not well defined and a variety of curve positions can be obtained. The curve for tungsten (not included) lies near that of niobium (similar dielectric constant) but rather below it because the film tends to dissolve. Now, Young⁵

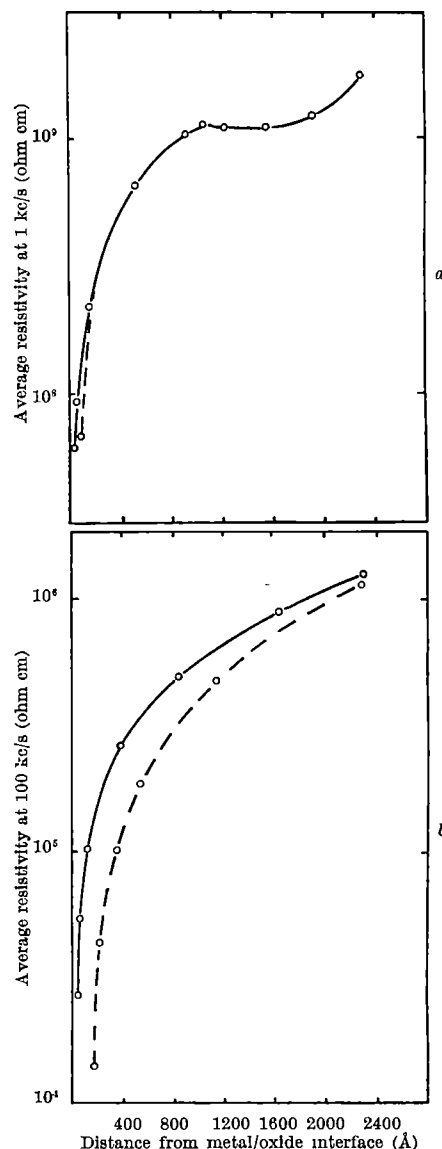


Fig. 2. Average resistivity measured during thinning of 2300 Å film formed on niobium. *a*, 1 kc/s; *b*, 100 kc/s

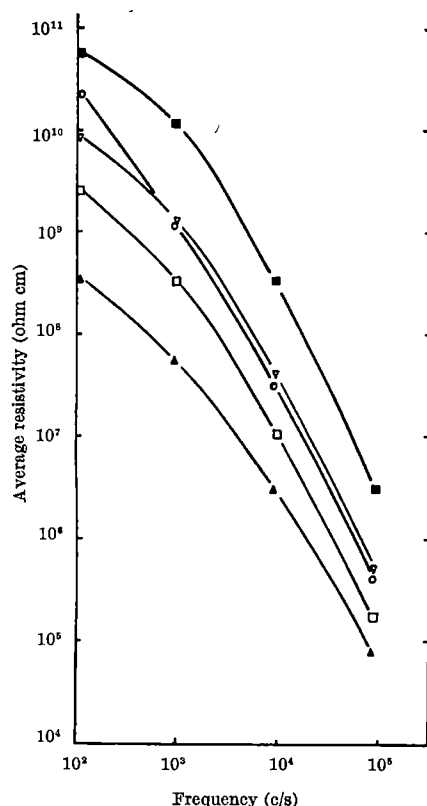


Fig. 3. Comparison of frequency dispersions of average resistivity for 230 Å films formed on various metals. ■, Al; ▴, Zr; ○, Ta; □, Nb; ▲, Ti.

has suggested that the higher the dielectric constant of the film, the lower its ionic resistivity. Although the a.c. resistivity measured with a low applied alternating voltage is complex, probably receiving contributions from several conducting processes, and is not necessarily directly related to the ease of ion movement under forming conditions at high direct voltages, the same trend seems to hold.

High a.c. resistivity (low dielectric constant) appears related to high oxide melting point (refractoriness), with titanium and zirconium as exceptions. The melting points quoted are, of course, for bulk oxides and do not necessarily apply to thin anodic films which are generally neither pure nor fully crystalline. The curve for the anodic film on zirconium may be out of position on the melting point criterion due to the film's greater tendency towards crystallinity. In any case, the thin-film, glass-forming characteristics (apparently favoured by a small ratio of metal ion radius to oxygen ion radius (ρ factor) but not well understood⁵) may be important and modify any criterion related to bulk melting point alone. Thus, Al_2O_3 would be expected to be a better glass-former than ZrO_2 on the basis of ρ factor, although it does not vitrify readily in the bulk state, and this may partly explain their relative resistivities. Apparently Ta_2O_5 and Nb_2O_5 should have good glass-forming characteristics but may be restrained from acting in this way by high ρ factors⁵. The low resistivity of TiO_2 is said to be more likely due to poor glass-forming characteristics than to impurities⁵. However, anodic films on zirconium and titanium are notoriously leaky compared with those on other metals, which may account for their behaviour. There appears to be little direct trend of a.c. resistivity with either refractive index or A/V ratio, which is not surprising.

Very recent consideration of the foregoing results, in the light of apparently more precise film and double-layer equivalent circuits, methods of determining point resistivities through the film and of applying the double-layer impedance correction, indicates that there may be a need to modify the absolute values of resistivity given here. Because of their complexity, these ideas will have to be elaborated in detail elsewhere⁶. Most of the foregoing trends still hold, but the theory based on the simple model may require some modification in due course.

We thank Prof. T. K. Ross for providing facilities and the Science Research Council for a maintenance grant to one of us (C. P.).

¹ Heine, M. A., and Pryor, M. J., *J. Electrochem. Soc.*, **110**, 1205 (1963).

² Young, L., *J. Electrochem. Soc.*, **111**, 1239 (1964).

³ Heine, M. A., and Sperry, P. R., *J. Electrochem. Soc.*, **112**, 359 (1965).

⁴ Wood, G. C., and Brock, A. J. (to be published).

⁵ Young, L., *Anodic Oxide Films* (Academic Press, London and New York, 1961).

⁶ Brock, A. J., Pearson, C., and Wood, G. C. (to be published).

COLOUR OF 'PURE' WATER*

By DR. JOHN E. TYLER

Visibility Laboratory, Scripps Institution of Oceanography, University of California, La Jolla

IN a previous article¹ a method was described for obtaining a fundamental specification of the colour of ocean or lake water, independent of the spectral variation of the natural lighting and independent of the spectral sensitivity of the photodetector.

The method consists essentially of three steps: first, the choice of suitable geometry for measuring the input flux to the body of water and the output flux from the water to the instrument; second, the measurement of a fundamental spectrophotometric property of the water body (which has been called the output/input ratio); and, third, the computation from these data of the trichromatic coefficients of the water colour. This method has recently been used to determine the colour of the exceptionally clean water of Crater Lake.

* The results used in this article were obtained during the course of research on optical methods for *in situ* detection and estimation of chlorophyll and other pigments in the ocean, a research programme which is supported by the U.S. National Science Foundation, Earth Sciences Division.

Crater Lake is the principal feature of Crater Lake National Park, a U.S. National Park in South-western Oregon. The lake is 20 miles in circumference, about 2,000 ft. deep, with its surface at an altitude of about 6,000 ft. It has no inlet other than rain or melting snow. The surrounding crater wall is sparsely vegetated and sparsely populated by wild animals, so that contamination from these sources has been small. One of the outstanding characteristics of the lake is its unusual blue colour, which is, of course, a consequence of its lack of contamination.

In 1940 the lake was examined for phytoplankton and particulate matter by Utterback and Phifer². They found that, "In the upper 20 metres of the lake, there was practically no phytoplankton . . .". They also noted that the water contained small particles of volcanic glass, but they did not undertake to determine the vertical distribution of these latter particles.

In this work, the colour of Crater Lake was measured with the same instrument used to obtain the data of the previous publication¹. The instrument was mounted underneath a small raft with the irradiance plate for measuring the input flux to the water just above the water surface, and the axis of the underwater collection system oriented horizontally. Generally clear, sunny weather prevailed during the measurements.

The average output/input ratio data for Crater Lake, shown in Fig. 1, were obtained from a location off an island near the west side of the lake. The instrument was oriented to point in a north-westerly direction in order to avoid the forward scattered flux from the Sun's direct rays.

Although it is valid to specify the colour of the water for any chosen direction, it is more difficult to obtain a satisfactory measurement in the Sun's direction because the forward scattered component of the sunlight rapidly changes in magnitude as the Sun changes its position relative to the instrument's direction of observation. With the Sun behind the instrument, repeated measurements taken an hour or more apart showed no significant changes in the output/input ratio due to the changes in the Sun's position.

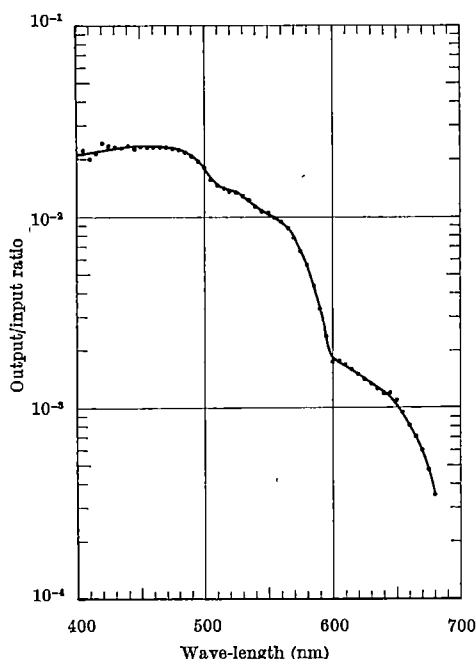


Fig. 1. Average output/input data for Crater Lake (August 23, 25, 1964)

The data of Fig. 1 have been used, together with the relative spectral distribution of the energy in sunlight above the Earth's atmosphere, to calculate the tristimulus values of the colour of Crater Lake water. This colour has been plotted in the C.I.E. co-ordinate system in Fig. 2, and from the plot the following colorimetric specification has been obtained:

Dominant wave-length: 483 mμ
Photometric brightness: 91.7 per cent
Purity: 56.0 per cent

One of the interesting features of this method for determining the colour of the ocean or a lake is the ability to make accurate comparisons of the colour of remotely separated water bodies. The cleanliness of Crater Lake can be immediately deduced from Fig. 2, and the extent of the chlorophyll contamination in the other waters illustrated can be comparatively judged. If data were available, it should also be possible to distinguish

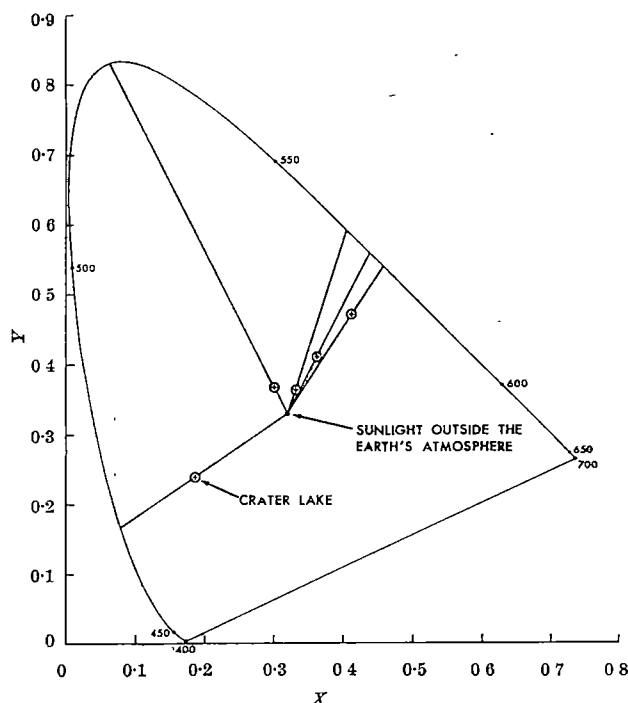


Fig. 2. C.I.E. plot to show colour of Crater Lake water relative to sunlight outside the Earth's atmosphere as a reference standard. The other points represent water containing chlorophyll (see ref. 1)

red algae from green, since these colours would occupy characteristic regions of the diagram.

One objective of this work was to obtain output/input data on the purest water that could be found. Such data could then serve as a standard, against which to estimate the contamination of other waters. There are, no doubt, many bodies of clean water which will compete for the 'honour of being the purest', and further measurements will have to be made if the ultimate standard of purity is to be found.

It is possible, however, to make one interesting comparison. In 1930, Beebe³ published a paper in which he described the spectra of the spacelight observed in a horizontal direction under water. Beebe's observations were visual, and consequently must be interpreted with care, since, for the greater depths at which he worked, his vision was scotopic and the spectral sensitivity of his eye would have been shifted considerably. On the other hand, at a depth of 800 ft., the selective absorption of light by water must have converted ordinary daylight into nearly monochromatic light. If we assume that this was the case, then we can deduce from Beebe's observations that the minimum value of the attenuation coefficient for Bermuda water occurred at 520 mμ.

From Fig. 1, the minimum value of the attenuation coefficient for Crater Lake is at about 450 mμ. It follows, therefore, that Crater Lake water in 1964 was bluer and cleaner than was the water off Nonsuch Island in 1930.

For the detection and estimation of specific pigments such as chlorophyll, it is of course more useful to work directly from the output/input data rather than from the colour of the water. References to papers on this subject are given in refs. 4 and 5.

I thank the staff of Crater Lake National Park, especially R. A. Nelson, superintendent, Donald Robinson, assistant superintendent and R. M. Brown, chief park naturalist, for their help.

¹ Tyler, J. E., *Nature*, **202**, 1262 (1964).

² Utterback, C. L., and Phifer, L. D., *Ecology*, **23**, 97 (1942).

³ Beebe, W., *Bull. N.Y. Zool. Soc.*, **33**, No. 6, 201-232 (see 226, 227) (1930).

⁴ Tyler, J. E., *Proc. U.S. Nat. Acad. Sci.*, **51**, 671 (1964).

⁵ Tyler, J. E., *J. Optic. Soc. Amer.*, **55**, 800 (1965).

RELATIONSHIP BETWEEN THE ELECTROMYOGRAM AND THE FORCE OF THE ISOMETRIC REFLEX RESPONSE OF NORMAL HUMAN SUBJECTS

By ALEX M. CLARKE*

Department of Psychology, Australian National University, Canberra

EARLY research by physiologists revealed a correlation of electrical (motor unit) activity with muscular contraction in spinal and decerebrate animals as well as involuntary contractions in man¹. Since then many reports have appeared in the literature to show that surface electromyograms (EMG) bear a linear relationship to the magnitude of voluntary muscular tension under conditions of isometric contraction²⁻⁴. However, no observations appear to have been reported of a relationship between the magnitude of the isometric response in a myotatic stretch reflex and EMG in man.

In the experiments described here, the knee-jerk was selected for examination for the following reasons: (a) the neural linkage for the α -route at the lumbar region of the spinal cord is monosynaptic, thus providing a reasonably simple pathway⁵⁻⁷; (b) the response is remarkably restricted in scope at low levels of excitation⁸: the reaction is confined to the quadriceps femoris, while synergist and antagonist muscles usually are inhibited or unaffected; (c) the subject can sit and relax in a chair specially designed to hold him in a comfortable, standard, symmetrical position since body and limb positions are known to affect reflexes^{8,9}. There is adequate evidence to establish the functional dependence of the myotatic stretch reflex on the excitation of the primary (annulo-spiral or nuclear bag) ending of the muscle spindle which is exclusively innervated in mammals by small-diameter motor nerve fibres (γ -efferents)^{6,10,11}. In this way feed-back is provided which, although being too slow to monitor reflex movements, has a strong indirect influence on the spindles, biasing them for different tensions of extrafusal muscle and rates of movement^{12,13}. The γ -efferent system itself is regulated from a number of central nervous system structures, notably the mesencephalic, diencephalic and bulbar regions of the reticular formation¹⁴. In addition, γ -discharge has been found to relate closely with change in level of cortical activity so that spindle sensitivity, generally speaking, is controlled centrally by the degree of alertness or activation of the subject^{15,16}.

Therefore, in an attempt to reduce cerebral activity in the expectation of making γ -discharge at least more consistent than it is in normal circumstances, the three young normal male adults who were used in the experiments were trained to apply Jacobson's method of mental and physical relaxation¹⁷. At least six daily sessions of 1 h each were required to train the subject individually to reach the criteria of displaying: (a) quiescent EMGs, indicating supported and relaxed muscles before and after the mechanical tap to the ligamentum patellae¹⁸; (b) discrete, highly synchronous muscle action potentials in response to the stimulus¹⁹.

Figs. 1 and 2 show comparisons between the records obtained by trained and untrained subjects.

Testing was conducted in a sound-proofed, electrically shielded and thermally insulated room in order to control auditory and visual stimuli, room temperature and relative humidity, and to suppress 50 c/s electrical interference for EMG purposes²⁰. Silver disk surface electrodes, 11 mm diameter, with a shallow central depression were applied in standard positions 3 in. apart over the belly of the muscles, rectus femoris and biceps femoris. EMG signals were amplified through push-pull circuitry and fed to a Both pen recorder. Force records were obtained by amplification of signals from silicon strain gauges mounted on the pendulum-hammer and on the ankle cuff linkage.

* Commonwealth postgraduate research scholar.

Peak forces between 1.5 kg and 3.5 kg were applied to the ligamentum patellae by varying the height from which the hammer was dropped in some sessions and, on other occasions, the hammer was dropped from a standard height for a series of trials. Under the latter conditions, it was found that subjects could maintain consistent muscle tone over short periods involving about ten taps as indicated by uniform force records.

However, irrespective of whether there were variations in the force applied to the ligament (implying variations in the extension of the muscle), slight changes in muscle-resting tension, or consistent muscle tone, between trials

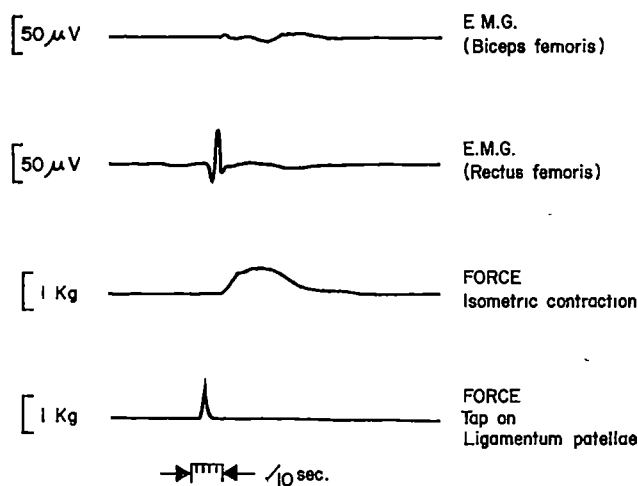


Fig. 1. Simultaneous electromyograms and force records showing electrical quiescence in the muscles of a trained and relaxed subject (G.B.). The discrete, diphasic potential in the EMG (rectus femoris) is the result of the tap on ligamentum patellae and indicates a high degree of synchrony in the discharge of muscle action potential. Negligible activity in biceps femoris.

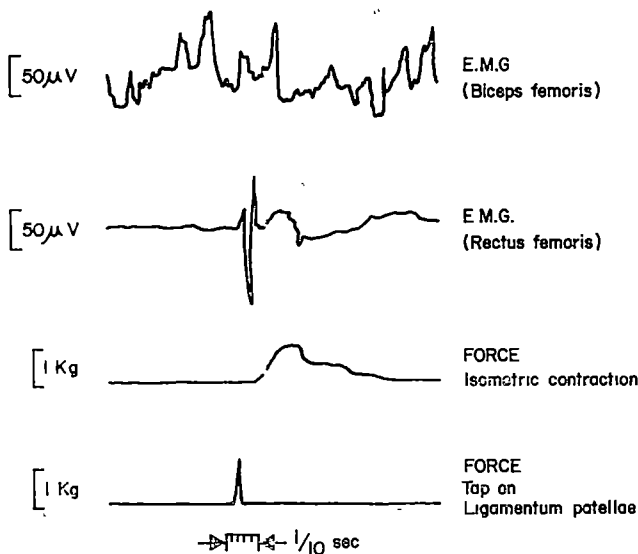


Fig. 2. Simultaneous electromyograms and force records showing random electrical activity in the muscles of an untrained and tense subject (A.M.). Hump in the force record of the isometric contraction indicates that a clonus condition has occurred. The 'interference pattern' EMG is evident in biceps femoris.

Table 1

Subject	Session	N	Isometric response impulse (g sec)		Electromyogram electrical impulse (μ V sec)		Product moment correlation*	Confidence limits of 99% for r_{pop} (Z transformation of R. A. Fisher)	
			\bar{x}	S	\bar{x}	S			
A. M.	1	15	231.1	63.9	2.83	0.27	+0.978	0.995 to 0.914	
	2	21	196.3	32.1	4.17	0.65	+0.918	0.975 0.753]
	3	21	315.1	90.6	3.25	0.98	+0.933	0.979 0.782	
	4	18	268.8	108.9	3.07	1.29	+0.943	0.984 0.790	
	5	33	276.7	105.1	2.10	1.37	+0.979	0.992 0.949	
	6	25	269.3	115.2	3.19	0.81	+0.890	0.962 0.702	
	7	25	255.9	89.9	1.84	0.95	+0.960	0.986 0.885	
	8	14	394.6	113.1	2.02	0.93	+0.977	0.995 0.885	
G. B.	1	19	137.5	31.3	3.76	0.82	+0.918	0.977 0.736	
	2	16	244.9	39.1	5.99	1.18	+0.967	0.993 0.879	
	3	21	156.9	19.7	2.12	0.57	+0.923	0.979 0.759	
	4	24	128.6	42.9	1.76	0.95	+0.934	0.978 0.811	
	5	16	243.6	23.8	4.09	0.78	+0.812	0.952 0.400	
	6	16	300.1	46.8	3.88	1.10	+0.946	0.986 0.789	
	7	26	168.6	30.6	1.89	0.59	+0.897	0.960 0.726	
	8	37	371.3	69.8	2.65	0.93	+0.935	0.975 0.861	
	9	35	324.3	69.1	1.94	0.75	+0.875	0.949 0.726	
	10	32	303.1	66.7	1.33	0.51	+0.882	0.952 0.716	
W. R.	1	19	103.8	35.8	2.28	0.60	+0.909	0.974 0.707	
	2	12	111.3	33.0	2.28	0.60	+0.923	0.985 0.623	
	3	17	227.9	45.4	1.58	0.61	+0.969	0.992 0.885	
	4	26	172.4	53.9	3.55	0.57	+0.904	0.968 0.757	
	5	25	176.4	78.6	5.07	0.87	+0.841	0.945 0.768	
	6	14	148.9	39.3	2.45	0.26	+0.887	0.977 0.592	
	7	27	79.4	64.6	2.94	0.61	+0.975	0.991 0.932	

* All correlations are significantly different from zero ($P < 0.001$).

in any one session, a high positive relationship was found between the impulse (the integral of force over time) of the isometric contraction, measured at the ankle, and the EMG impulse of the quadriceps muscle.

Table 1 provides data relevant to the product-moment correlations obtained between these two variables for the three subjects.

The explanation for this near linear relationship probably lies in the consistency of γ -innervation of the spindles under the experimental conditions imposed for these subjects and in the fact that there was no complicating activity from the flexor muscles. The γ -discharge causes the contraction of the intrafusal fibres and leads to a decreased threshold of the muscle spindle to stretch²¹. It is thought that in many instances, a reflex is augmented by an increase in γ -efferent activity²². Thus, fluctuations in the sensitivity of the spindles to stretch by variable activity of the γ -motoneurons in biasing the intrafusal muscle fibres would result in a greater or lesser number of spindle receptors firing when stimulated by the tendon tap at any given time. Assuming that the motor end-plates

of the extrafusal fibres fire proportionally to the number of spindles activated, then one may take the discrete, highly synchronous potential impulse of the EMG as the best, indirect indicator of the magnitude of γ -biasing of the spindles of the quadriceps muscle (rectus femoris). In view of the small standard deviations obtained in this variable, one might conclude that fluctuations in small fibre control of the stretch reflex response were minimal.

Some preliminary work on the correlation between the maximum force of the isometric contraction and the impulse has yielded correlations of the order of +0.992 which probably indicate that the reflex response is ballistic. In this event, the γ -innervation would be too slow to have any direct effect during the response; presumably the force-time relations of the muscle contraction under isometric conditions are programmed beforehand by at least such factors as the degree of sensitization of the spindles and the initial resting tension of the muscle. Triggering occurs when the mechanical tap imparts a sudden, brief stretch to the muscle.

Consequently, provided that there is no modification of the knee-jerk response by involvement of flexor muscles and if fluctuations in γ -control over spindle sensitivity are minimal, a high positive relationship would be expected between the EMG and the isometric contraction of the extensor muscles.

I thank Dr. J. R. Trotter for his advice.

- ¹ Adrian, E. D., and Bronk, D. W., *J. Physiol.*, **67**, 119 (1929).
- ² Inman, V. I., Ralston, H. J., Saunders, J. B. de C. M., Feinstein, B., and Wright, E. W., *E.E.G. Clin. Neurophysiol.*, **4**, 187 (1952).
- ³ Lippold, O. C. J., *Bull. Brit. Psychol. Soc.*, **17**, 57 (1964).
- ⁴ Close, J. R., Nickel, E. D., and Todd, F. N., *J. Bone and Joint Surg.*, **42**, A, 1207 (1960).
- ⁵ Lloyd, D. P. C., *J. Neurophysiol.*, **6**, 137 (1943).
- ⁶ Lloyd, D. P. C., *Res. Publ. Nerv. Ment. Dis.*, **30**, 48 (1952).
- ⁷ Magladery, J. W., Park, A. M., Porter, W. E., and Teasdale, R. D., *Res. Publ. Nerv. Ment. Dis.*, **30**, 118 (1952).
- ⁸ Cohen, L. A., *J. Neurophysiol.*, **18**, 272 (1952).
- ⁹ Sherrington, C. S., *Brain*, **29**, 467 (1906).
- ¹⁰ McIntyre, A. K., *Nature*, **168**, 168 (1951).
- ¹¹ Cooper, Sybil, *Quart. J. Exper. Physiol.*, **46**, 389 (1961).
- ¹² Kuffler, S. W., and Hunt, C. C., *Res. Publ. Nerv. Ment. Dis.*, **30**, 24 (1952).
- ¹³ Jansen, J. K. S., and Matthews, P. B. C., *J. Physiol.*, **161**, 357 (1962).
- ¹⁴ Grant, R., and Kaada, B., *Acta Physiol. Scand.*, **27**, 130 (1953).
- ¹⁵ Buchwald, J. S., and Eldred, E., *E.E.G. Clin. Neurophysiol.*, **13**, 243 (1961).
- ¹⁶ Pallard, J., *Amer. J. Phys. Med.*, **38**, 239 (1959).
- ¹⁷ Jacobson, E., *J. Nerv. Ment. Dis.*, **60**, 568 (1924).
- ¹⁸ Hayes, K. J., *J. App. Physiol.*, **15**, 749 (1960).
- ¹⁹ Denny-Brown, D., *Arch. Neurol. Psychiat.*, **61**, 99 (1949).
- ²⁰ Clarke, A. M., *Austral. J. Psychol.*, **17**, 124 (1965).
- ²¹ Mark, R. F., *Nature*, **199**, 50 (1963).
- ²² Mark, R. F., *C.R. Soc. Biol., Paris*, **156**, 705 (1962).

PURINE RICH REGULATORY SEQUENCES IN MESSENGER RIBONUCLEIC ACID

By PROF. ROY H. DOI

Biological Research Laboratories, Department of Bacteriology and Botany, Syracuse University, Syracuse, New York

THE elegant proposal for the existence of a messenger RNA by Jacob and Monod¹ has been supported by evidence obtained from phage-infected cells²⁻⁵, step-down transition cells⁶⁻¹⁰ and log phase bacterial cells^{10,11}. The results from these systems indicated that a polynucleotide fraction had most of the properties postulated for a messenger RNA (*m*-RNA), that is, a short half-life^{6,12}, a DNA-like base composition^{6,10}, a heterogeneous size^{13,14}, an affinity to ribosomes^{15,16} and a sequence complementarity to DNA^{4,6}. However, there is still a paucity of results regarding the actual template function for protein synthesis of the *in vivo m*-RNA fraction. The

most definitive results have been obtained by using synthetic polyribonucleotides^{17,18} or phage RNA^{19,20} as messages. Synthetic polymers have been used for incorporation of specific amino-acids into polypeptides. The product made in the presence of phage RNA has polypeptides ordinarily found in the phage coat protein. In binding experiments, polynucleotides²¹⁻²³ and trinucleotides²⁴ attached to ribosomes have directed the binding of specific aminoacyl-transfer RNAs. These various studies have led to the present dogma that transfer of information from DNA to protein occurs through an *m*-RNA.

In this rapid advance in our knowledge of protein synthesis, it was felt that a critical analysis of the data available would produce some useful information on the

* Present address: Department of Biochemistry and Biophysics, University of California, Davis, California.

Table 1. BASE RATIOS OF MESSENGER RNAs

Organism	DNA %GC	m-RNA							Ref.
		C	A	U	G	%AG	A/U	G/C	
<i>B. subtilis</i>	43	20.4	28.6	28.3	22.5	51.1	1.01	1.10	9
<i>B. subtilis</i>	43	20.5	31.0	26.3	22.2	53.2	1.18	1.08	9
<i>B. subtilis</i>	43	21.2	30.2	28.1	20.5	50.7	1.07	0.97	43
<i>P. vulgaris</i>	37	19.8	30.1	29.2	20.9	51.0	1.03	1.06	44
<i>P. vulgaris</i>	37	22.7	26.7	23.0	27.6	54.3	1.16	1.22	44
<i>E. coli</i>	50	19.7	26.6	28.9	23.4	50.0	0.92	1.19	45
<i>E. coli</i>	50	23.0	26.4	26.6	24.0	50.4	0.99	1.04	45
<i>E. coli</i>	50	23.5	25.9	25.4	25.2	51.1	1.02	1.07	45
<i>E. coli</i>	50	23.6	25.1	23.8	26.5	51.6	1.05	1.12	45
<i>E. coli</i>	50	24.9	25.1	23.8	26.2	51.3	1.05	1.05	45
<i>E. coli</i>	50	26.1	23.4	20.2	30.3	53.7	1.16	1.16	45
<i>E. coli</i>	50	27.3	22.1	20.2	30.4	52.5	1.09	1.11	45
<i>P. aeruginosa</i>	66	25.9	22.4	23.4	28.3	50.7	0.96	1.09	6
<i>P. aeruginosa</i>	66	27.0	21.5	21.8	29.7	51.2	0.99	1.10	6
<i>P. aeruginosa</i>	66	30.3	20.9	19.0	29.8	50.7	1.10	0.98	6
<i>S. cerevisiae</i>	36	21.7	28.6	28.7	21.2	49.8	1.00	0.98	7
<i>S. pombe</i>	—	20.6	33.8	23.6	22.0	55.8	1.43	1.07	8
<i>S. pombe</i>	—	17.8	35.0	23.8	23.4	58.4	1.47	1.31	46
<i>A. punctulata</i>	39	23.1	28.9	24.4	23.6	52.5	1.18	1.02	47
<i>A. punctulata</i>	39	22.8	28.1	27.4	21.8	49.9	1.02	0.96	47
HeLa cell	43	24.2	26.6	27.7	21.5	48.1	0.96	0.89	48
HeLa cell	43	26.9	25.4	27.3	21.0	46.4	0.93	0.78	48
β 3 phage	43	17.6	33.2	26.4	22.8	56.0	1.26	1.30	49
α phage	44	20.5	31.0	23.0	25.5	56.5	1.35	1.24	32
T2 phage	34	17.4	31.0	28.7	21.9	52.9	1.08	1.26	44
T2 phage	34	22.8	23.0	21.2	33.1	56.1	1.08	1.45	2
T2 phage	34	22.4	24.6	22.0	31.0	55.6	1.12	1.38	2
T4 phage	34	15.4	30.6	32.9	21.1	51.7	0.93	1.37	50
T7 phage	47	22.6	27.4	28.3	21.7	49.1	0.97	0.96	51
ϕ X174 phage	42	17.5	23.8	33.1	25.6	49.4	0.72	1.46	31
ϕ X174 phage	42	17.5	25.5	34.0	23.0	48.5	0.75	1.31	31

properties of *m*-RNA. A particular property of the *m*-RNA which could be analysed was the reported base compositions of the *m*-RNA fraction. Table 1 presents a list of organisms and the base compositions of the *m*-RNA fraction isolated from these organisms. Three criteria were used for inclusion of RNA base ratios in this Table. The RNAs were (a) hybridized to DNA^{4,44,50,57}, (b) synthesized after phage infection, or (c) synthesized during step-down transition. The RNAs studied were pulse-labelled or steady-state labelled with ³²PO₄ and analysed after alkali hydrolysis. Most of the RNAs were hybridized with DNA and separated from the bulk of the cellular RNA by differential sedimentation, column fractionation, or membrane filtration before analysis. Those RNAs not hybridized with DNA were obtained under conditions in which undetectable or very low levels of ribosomal and transfer RNAs were synthesized. A fact to remember is that these *m*-RNAs are heterogeneous populations.

The properties of the *m*-RNA in Table 1 can be summarized as follows:

(a) *Percentage of guanylate + cytidylate (%GC) or resemblance to DNA composition.* For most *m*-RNAs there is a resemblance of the %GC to that of the DNA. However, for many of them the %GC is significantly different from that found in the DNA. For *E. coli*, for example, the range is from 43.1 to 57.7 per cent. In *P. aeruginosa* the %GC of the *m*-RNA is much lower than that of the DNA. The deviation is less marked with the phage *m*-RNAs.

(b) *Percentage of adenylate + guanylate (%AG) or purine content of M-RNA.* All the RNAs except that from HeLa had a %AG of 50 \pm 2 per cent or higher. The *m*-RNAs have either an equivalence of purines and pyrimidines or are purine-rich in all the cases examined except one. Conversely one can state that *m*-RNAs are pyrimidine-poor. The DNA composition of the organisms analysed was from 34 to 66 per cent guanylate + cytidylate.

(c) *The adenylate-uridylylate ratio (A/U).* In most of the RNA populations the A/U ratio is equal to or greater than 1.0. In an extreme case the ratio is 1.47. If one considers a 2.0 per cent molar ratio difference between A and U as insignificant in the base composition analyses, one finds that in all cases the content of A is equal to or greater than U in *m*-RNAs.

(d) *The guanylate-cytidylate ratio (G/C).* Once again the G/C ratio is equal to or greater than 1.0 in all cases except for HeLa cells.

Before discussion of the possible significance of these facts, it is of interest to consider viruses, since viral RNA

Table 2. BASE COMPOSITION OF VIRAL RNA

Virus	Moles per cent							Ref.
	C	A	U	G	%AG	A/U	G/C	
Tobacco mosaic	18.5	29.8	26.3	25.4	55.2	1.13	1.37	52
Cucumber	18.2	25.7	30.6	25.4	51.1	0.84	1.40	52
Tomato bushy stunt	20.4	27.6	24.5	27.6	55.2	1.13	1.35	52
Turnip yellow mosaic	33.0	22.6	22.2	17.2	39.8	1.02	0.45	52
Southern bean mosaic	23.0	25.8	25.3	25.8	51.6	1.02	1.12	52
Polato X	22.8	34.5	21.4	21.4	55.9	1.61	0.94	52
Tobacco ringspot	23.2	23.9	28.2	24.7	48.6	0.85	1.06	52
Tomato bushy stunt	20.8	25.7	25.7	27.9	53.6	1.00	1.34	52
Tomato bushy stunt	20.5	25.7	25.5	28.2	53.9	1.01	1.38	52
Tomato bushy stunt	20.4	25.9	25.6	28.1	54.0	1.01	1.38	52
Aucuba mosaic	18.5	29.7	26.4	25.4	55.1	1.12	1.37	52
Rib grass	18.0	29.3	27.0	25.8	55.1	1.08	1.43	52
Reo	22.0	28.0	27.9	22.3	50.3	1.00	1.01	53
Wound tumour	19.1	31.1	31.3	18.6	49.7	0.99	0.97	53
MS2 phage	22.8	25.2	24.9	27.1	52.3	1.01	1.19	54
f2 phage	26.9	22.2	25.1	26.0	48.2	0.88	0.97	55
R17 phage	24.9	23.1	25.7	26.3	49.4	0.90	1.06	56
MS2 phage	25.5	23.3	24.8	26.4	49.7	0.94	1.04	43
Poliomyelitis	19.5	30.4	24.7	25.4	55.8	1.23	1.30	62
Influenza A PR8	24.0	23.1	32.8	20.1	43.2	0.70	0.84	52
Influenza B LEE	23.1	23.0	35.6	18.3	41.3	0.65	0.79	52

has been shown in one case to act as an *m*-RNA¹⁹. On the assumption that some viral RNAs and perhaps all are indeed *m*-RNAs, attention is now directed to their base compositions. Table 2 lists the various viruses and their RNA base compositions. The properties of viral RNAs can be summarized as follows:

(1) *Percentage of A + G or the purine content of viral RNAs.* In all cases except for turnip yellow mosaic virus and the influenza viruses, the purine content is 50 \pm 1 per cent or significantly higher than 50 per cent. Many cases exist in which the purine content is 55 per cent. In the three cases in which the purine content is less than 50 per cent, the molar content of A + G is about 40 per cent.

(2) *The A/U ratio.* About two-thirds of the analyses show that the A/U ratio is equal to or greater than 1.0. Of the one-third in which the A/U ratio is less than 1.0, some have a ratio as low as 0.65.

(3) *The G/C ratio.* In all cases except for turnip yellow mosaic virus and the influenza viruses, the G/C ratio is equal to or greater than 1.0. An extremely low G/C ratio is seen with turnip yellow mosaic virus RNA.

Before these facts are related to present knowledge of *m*-RNA compositions, a pertinent question can be asked. What type of information is present in *m*-RNA? According to present coding theories, the codon for each amino-acid and the sequence of amino-acids in the polypeptide product are determined by the sequence of nucleotides in the *m*-RNA (ref. 25). Since the *m*-RNA interacts with ribosomes during protein synthesis in an ordered manner, a recognition or starting and stopping sites for translation may be encoded into the ends of the polynucleotide²⁴. Furthermore, evidence has been presented for polycistronic *m*-RNA²⁶⁻²⁹, that is, an *m*-RNA which codes for more than one polypeptide chain. If this is indeed the case, there must exist a mechanism which allows ribosomes to recognize the end and beginnings of the cistrons. This mechanism may allow ribosomes to separate from the *m*-RNA or it may allow the finished polypeptide chain for each cistron to separate from the ribosome which continues in the translation process³⁰. This mechanism may involve specific nucleotide sequences. Therefore the total base composition of an *m*-RNA population would reflect the composition of the codons for the amino-acids in the proteins and other sequences of nucleotides which in some way 'regulate' the translation process.

Two general compositional properties of *m*-RNA are significant: (i) *m*-RNAs appear to be either purine rich, or have equal contents of purines and pyrimidines; and (ii) the A/U and G/C ratios are higher than 1.0 except in one case. For viral RNAs, (a) the purine content is higher than the pyrimidine-level in most cases examined; (b) the G/C ratio is equal to or greater than 1.0 in all cases examined except three; (c) for the A/U ratio, only two-thirds of the virus RNAs had a ratio equal to or greater than 1.0; this is significantly different from the results with *m*-RNAs.

Is there any significance or relationship of these data to other facts which are known about the information transfer

process in living systems? From recent results obtained by a variety of techniques and by many investigators, certain information has appeared which seems to have relationship to the data presented in Tables 1 and 2.

Several groups have reported that only one strand of the double-stranded DNA is transcribed. Direct evidence has been obtained with phage systems in which phage specific *m*-RNA hybridized with only one of the two strands of phage DNA³¹⁻³⁴. This information-carrying strand has been the pyrimidine-rich DNA strand in all cases reported. Thus the phage *m*-RNA are rich in purine. This suggests then that the purine-rich *m*-RNA found in non-phage systems may also be significant. It implies that information carrying genetic sites may in all cases be rich in pyrimidine.

The high purine content of the *m*-RNAs examined so far suggests that adenylate and guanylate may be encoded into sequences of nucleotides regulating translation. Recent reports appear to support this contention. In investigations with aminoacyl-transfer-RNA binding directed by trinucleotides^{35,36} and polynucleotides³⁷, the sequences AGA and UAA did not support binding of any of the aminoacyl-transfer RNAs tested. The presence of two adenylates in both cases seems significant in view of the high adenylate content of natural *m*-RNAs. From a theoretical point of view (triplet code) an *m*-RNA with a 1.2×10^6 mol. wt. can code for six polypeptides of 2.0×10^4 mol. wt. If 3 per cent of the nucleotides in this polycistronic *m*-RNA were involved in regulatory functions and two-thirds of these nucleotides were adenylate, the distribution of bases would show that adenylate is present at a molar level 2 per cent higher than that of the other bases. This calculation is based on equal distribution of the remaining 98 per cent of the bases. The percentage of nucleotides involved in regulation may be proportionately even higher, since it was demonstrated recently that a naturally occurring *m*-RNA with 36 nucleotides coded for the *in vitro* synthesis of a polypeptide containing ten amino-acids³⁸. In addition, both terminal nucleotides of this natural *m*-RNA were adenylates. The low purine content of HeLa cell *m*-RNA may be significant, since in cancerous cells one might expect some deficiency in the regulatory mechanism. This deficiency is perhaps in the information-carrying DNA strand in which pyrimidine sequences are altered or decreased.

It would be of interest to learn whether triplets with two guanylates are inactive in binding. The triplet GGU was shown to bind glycine³⁵, but the results with the other possible sequences have as yet to be reported. As the G/C ratio is usually greater than 1.0 in *m*-RNAs and the genetic code appears to be universal²⁵, it is a possibility that in DNAs with high %GC, the G containing regulating sequences may be more abundant. In this respect, *P. aeruginosa* has an A/U ratio of 1.0 but a G/C ratio of greater than 1.0. In DNAs with high %AT, the A-containing regulating sequences may be more abundant.

The presence of regulating sequences containing A or G can be masked in many ways including high frequency of amino-acid codons which are low in A and G, large *m*-RNAs with few cistrons, or large cistrons with few regulating sequences. These and other reasons may be involved in masking the regulating purine content of RNA from RNA-containing viruses. Although degeneracy in coding probably alters the composition of the *m*-RNAs in different species, the recent work from Nirenberg's laboratory^{35,36,58} suggests that all four bases are involved with equal frequency. Furthermore, the purine relationship is seen in organisms with high and low %GC in their DNAs.

Although the proposal of regulatory sequences containing purines is hypothetical, the fact remains that well-characterized *m*-RNAs do have compositional properties which appear to fit a pattern. It is of added interest that this pattern holds true for bulk RNA compositions (ribosomal and transfer (RNAs) as tabulated by Belozersky and

Spirin³⁹. Recent advances on protein-synthesizing systems^{40,41}, on the use of natural RNA templates³⁸, and on RNA sequence analysis⁴² have indicated that direct experimental evidence should emerge soon to test this hypothesis.

This work was supported by the National Science Foundation and the Atomic Energy Commission.

- ¹ Jacob, F., and Monod, J., *J. Mol. Biol.*, **3**, 318 (1961).
- ² Volkin, E., and Astrachan, L., *Virology*, **2**, 149 (1956).
- ³ Nomura, M., Hall, B. D., and Spiegelman, S., *J. Mol. Biol.*, **2**, 306 (1960).
- ⁴ Hall, B. D., and Spiegelman, S., *Proc. U.S. Nat. Acad. Sci.*, **47**, 137 (1961).
- ⁵ Brenner, S., Jacob, F., and Meselson, M., *Nature*, **190**, 576 (1961).
- ⁶ Hayashi, M., and Spiegelman, S., *Proc. U.S. Nat. Acad. Sci.*, **47**, 1564 (1961).
- ⁷ Kitazumi, Y., Ycas, M., and Vincent, W. S., *Proc. U.S. Nat. Acad. Sci.*, **48**, 265 (1962).
- ⁸ Gross, P. R., and Mitchelson, J. M., *Nature*, **195**, 305 (1962).
- ⁹ Dol, R. H., and Igarashi, R. T., *Proc. U.S. Nat. Acad. Sci.*, **52**, 755 (1964).
- ¹⁰ Ycas, M., and Vincent, W. S., *Proc. U.S. Nat. Acad. Sci.*, **48**, 265 (1962).
- ¹¹ Gros, F., Hiatt, H., Gilbert, W., Kurland, C. G., Rusebrough, R. W., and Watson, J. D., *Nature*, **190**, 581 (1961).
- ¹² Levinthal, C., Keynan, A., and Higa, A., *Proc. U.S. Nat. Acad. Sci.*, **48**, 1631 (1962).
- ¹³ Sagik, B., Green, M. H., Hayashi, M., and Spiegelman, S., *Biophys. J.*, **2**, 409 (1962).
- ¹⁴ Gros, F., Gilbert, W., Hiatt, H. H., Attardi, G., Spahr, P. F., and Watson, J. D., *Cold Spring Harbor Symp. Quant. Biol.*, **26**, 111 (1961).
- ¹⁵ Warner, J. R., Rich, A., and Hall, C. E., *Science*, **138**, 1399 (1962).
- ¹⁶ Wettstein, F. O., Staehelin, T., and Noll, H., *Nature*, **197**, 430 (1963).
- ¹⁷ Nirenberg, M. W., and Matthaei, J. H., *Proc. U.S. Nat. Acad. Sci.*, **47**, 1588 (1961).
- ¹⁸ Lengyel, P., Speyer, J. F., and Ochoa, S., *Proc. U.S. Nat. Acad. Sci.*, **47**, 1590 (1961).
- ¹⁹ Nathans, D., Notani, G., Schwartz, J. H., and Zinder, N. D., *Proc. U.S. Nat. Acad. Sci.*, **48**, 1424 (1962).
- ²⁰ Schwartz, J. H., Eisenstadt, J. M., Brawerman, G., and Zinder, N. D., *Proc. U.S. Nat. Acad. Sci.*, **53**, 195 (1965).
- ²¹ Arlinghaus, R., Favelukes, G., and Schweet, R., *Biochem. Biophys. Res. Commun.*, **11**, 92 (1963).
- ²² Nakamoto, T., Conway, T., Allende, J., Spyrides, G., and Lipmann, F., *Cold Spring Harbor Symp. Quant. Biol.*, **28**, 227 (1963).
- ²³ Kaji, A., and Kaji, H., *Biochem. Biophys. Res. Commun.*, **13**, 186 (1963).
- ²⁴ Nirenberg, M., and Leder, P., *Science*, **145**, 1399 (1964).
- ²⁵ Crick, F. H. C., *Prog. in Nucleic Acid Research*, **1**, 164 (1963).
- ²⁶ Ohtaka, Y., and Spiegelman, S., *Science*, **142**, 493 (1963).
- ²⁷ Nishi, A., and Zebin, I., *Biochem. Biophys. Res. Commun.*, **13**, 320 (1963).
- ²⁸ Martin, R. G., *Cold Spring Harbor Symp. Quant. Biol.*, **28**, 357 (1963).
- ²⁹ Gutman, B. S., and Novick, A., *Cold Spring Harbor Symp. Quant. Biol.*, **28**, 373 (1963).
- ³⁰ Ames, B. N., and Hartman, P. E., *Cold Spring Harbor Symp. Quant. Biol.*, **28**, 349 (1963).
- ³¹ Hayashi, M., Hayashi, M. N., and Spiegelman, S., *Proc. U.S. Nat. Acad. Sci.*, **50**, 664 (1963).
- ³² Tocchini-Valentini, G. P., Stodolsky, M., Aurisicchio, A., Sarnat, M., Graziosi, F., Weiss, S. B., and Gelduschek, E. P., *Proc. U.S. Nat. Acad. Sci.*, **50**, 935 (1963).
- ³³ Marmur, J., and Greenspan, C. M., *Science*, **142**, 387 (1963).
- ³⁴ Saunders, G. R., and Campbell, L. L., *Fed. Proc.*, **24**, 348 (1965).
- ³⁵ Leder, P., Rottman, F., Brimacombe, R., Trupin, J., O'Neal, C., and Nirenberg, M., *Fed. Proc.*, **24**, 408 (1965).
- ³⁶ Bernfield, M., *Fed. Proc.*, **24**, 409 (1965).
- ³⁷ Nishimura, S., Jones, D. S., Wells, R. D., Jacob, T. M., and Khorana, H. G., *Fed. Proc.*, **24**, 409 (1965).
- ³⁸ Hall, J. B., and Sedat, J. W., *Fed. Proc.*, **24**, 409 (1965).
- ³⁹ Belozersky, A. N., and Spirin, A. S., in *The Nucleic Acids*, **3**, edit by Chargoff, E., and Davidson, J. N., 147 (Academic Press, New York, 1960).
- ⁴⁰ Nishizuka, Y., and Lipmann, F., *Fed. Proc.*, **24**, 283 (1965).
- ⁴¹ Miller, R., Hausmann, E., Erickson, R., and Schweet, R., *Fed. Proc.*, **24**, 283 (1965).
- ⁴² Holley, R. W., Aggar, J., Everett, G. A., Madison, J. T., Marguise, M., Merrill, S. A., Penswick, J. R., and Zamir, A., *Science*, **147**, 1462 (1965).
- ⁴³ Dol, R. H. (unpublished results)
- ⁴⁴ Bolton, E. T., and McCarthy, B. J., *Proc. U.S. Nat. Acad. Sci.*, **48**, 1390 (1962).
- ⁴⁵ Bolton, E. T., and McCarthy, B. J., *J. Mol. Biol.*, **8**, 201 (1964).
- ⁴⁶ Mitchelson, J. M., and Gross, P. R., *Exp. Cell Res.*, **37**, 259 (1965).
- ⁴⁷ Gross, P. R., Kraemer, K., and Malkin, L. I., *Biochem. Biophys. Res. Commun.*, **18**, 569 (1965).
- ⁴⁸ Penman, S., Scherrer, K., Becker, Y., and Darnell, J. E., *Proc. U.S. Nat. Acad. Sci.*, **49**, 654 (1963).
- ⁴⁹ Yehle, C., and Dol, R. H. (unpublished results).
- ⁵⁰ Bautz, E. K. F., and Hall, B. D., *Proc. U.S. Nat. Acad. Sci.*, **48**, 400 (1962).
- ⁵¹ Volkin, E., Astrachan, L., and Countryman, J. L., *Virology*, **6**, 282 (1958).
- ⁵² Schuster, H., in *The Nucleic Acids*, **3**, edit by Chargoff, E., and Davidson, J. N., 245 (Academic Press, New York, 1960).
- ⁵³ Gomatos, P. J., and Tamm, I., *Proc. U.S. Nat. Acad. Sci.*, **50**, 878 (1963).
- ⁵⁴ Strauss, jun., J. H., and Shushelmer, R. L., *J. Mol. Biol.*, **7**, 43 (1963).
- ⁵⁵ Loeb, T., and Zinder, N. D., *Proc. U.S. Nat. Acad. Sci.*, **47**, 282 (1961).
- ⁵⁶ Mitra, S., Enger, M. D., and Kaesberg, P., *Proc. U.S. Nat. Acad. Sci.*, **50**, 68 (1963).
- ⁵⁷ Nygaard, A. P., and Hall, B. D., *Biochem. Biophys. Res. Commun.*, **12**, 98 (1963).
- ⁵⁸ Trupin, J. S., Rottman, F. M., Brimacombe, R. L. C., Leder, P., Bernfield, M. R., and Nirenberg, M. W., *Proc. U.S. Nat. Acad. Sci.*, **53**, 807 (1965).

CHANGES IN THE *t*-RNA METHYLATING ENZYMES
DURING INSECT METAMORPHOSIS

By DR. B. S. BALIGA, PROF. P. R. SRINIVASAN and PROF. ERNEST BOREK

Department of Biochemistry, College of Physicians and Surgeons, Columbia University, New York

A NUMBER of enzymes the function of which is the specific methylation of nucleic acids is now known to exist¹. While considerable information has been gathered about the mechanism of action and specificities of these enzymes, very little is known about the biological function achieved by the methylation of nucleic acids. That the methylation of DNA confers an individuality in one instance has been demonstrated by Arber², who found that host range modifications in λ -bacteriophage may be introduced by methylation.

For the enzymes which methylate *t*-RNA, the *t*-RNA methylases, no biological function has been found as yet. It has been demonstrated in our laboratory, however, that the RNA methylases can be profoundly altered in bacterial cells on phage infection or induction³.

Both the RNA and DNA methylases are species specific^{4,5}. We have speculated whether the species specificity of these enzymes may not point to their involvement in differentiation, a process requiring the greatest species specificity¹. We have now tested this hypothesis in an insect during its cycle of metamorphosis and wish to report that alterations occur in the *t*-RNA methylases not only at the transition from larva to pupa but also throughout the duration of the pupal stage right up to the emergence of the imago.

Collection of pupae and their development into adult beetles. *Tenebrio molitor* larvae were purchased from a local supply house and were maintained in open polyethylene trays on a diet of oatmeal, wheat bran and sliced carrots.

Prior to transformation into pupae, the larvae were found to have passed into an immobile phase close to the surface of the food bed. These were transferred to open glass trays and left at room temperature. Pupae were gathered from these trays daily at the same time. Pupae collected on a particular day were formed sometime during the preceding 24 h and were referred to as '1-day-old'. They were incubated at 28° in a humid atmosphere. About 50 per cent of these pupae developed into adult beetles on the seventh day. The process was nearly complete on the eighth day. Pupae ranging in age from one to seven days were picked up from these stocks and were used in the manner described below. The larvae were also collected separately and used similarly for assay of RNA methylases. (Methyl-¹⁴C)-*s*-adenosyl-L-methionine with a specific activity of 29.9 mc./mmole was purchased from New England Nuclear Corporation and diluted with non-radioactive *s*-adenosyl-L-methionine to give a concentration of 10 μ c. per 0.5 μ mole per ml.

Methyl deficient *t*-RNA was prepared by procedures previously published. Protein was estimated by the method of Lowry *et al.*⁶.

Preparation of enzyme extracts. Larvae or pupae were crushed and ground in a pre-cooled mortar with three volumes of a solution containing sucrose (0.25 M), magnesium chloride (0.001 M), and 2-mercaptoethanol (0.005 M). The homogenate was transferred into a Potter-Elvehjem type homogenizer. The mortar was washed with an equal volume of the extracting solution and the washings were added to the homogenate. The mixture was next homogenized for one minute with a motor-driven 'Teflon' pestle at 3,500 r.p.m. The homogenate was cooled for 5–10 min in an ice bath and the

homogenization was repeated for another minute. The homogenate was then filtered through cheese cloth and the filtrate centrifuged for 20 min at 27,500*g* in a refrigerated centrifuge. The faintly yellow-coloured supernatant was filtered to free it from membrane-like material floating on the surface. This filtrate, which contained 8–12 mg protein per ml. (depending on the stage of development of the pupae), was used as the source of enzymes for the methylation studies reported below. The enzyme extract was prepared fresh daily and was used immediately thereafter. All the operations connected with the preparation of the enzyme extracts were carried out in the cold (2°–5°) unless otherwise stated.

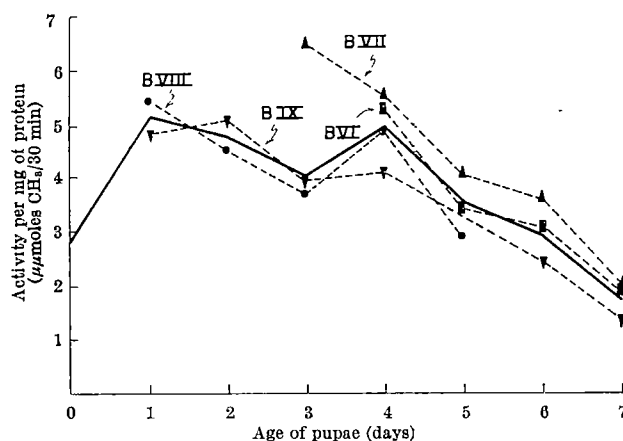
Assay of methylase activity. Methylase activity of the extracts was assayed by measuring the incorporation of ¹⁴C-methyl from (methyl-¹⁴C)-*s*-adenosyl-L-methionine into methyl-deficient *E. coli t*-RNA. The reaction mixture contained 50 μ moles *tris*-HCl buffer, pH 8.2; 5 μ moles MgCl₂; 5 μ moles 2-mercaptoethanol; 20 μ moles of (methyl-¹⁴C)-*s*-adenosyl-L-methionine (20 mc./mmole); 0.15 mg methyl-deficient *E. coli t*-RNA, water and the appropriate amount of enzyme extract in a final volume of 1.0 ml. Enzyme extract was added to the reaction mixture which had been pre-incubated at 37°. After 30 min, the reaction was terminated by the addition of 1.0 ml. of cold 10 per cent TCA followed by 3 ml. of 5 per cent TCA. The contents were cooled and centrifuged. The precipitate containing the protein and RNA was thoroughly agitated with 5 ml. of 5 per cent TCA, centrifuged as before and the supernatant discarded. The precipitate was similarly washed twice more with 5 ml. of TCA. Finally, the precipitate was dissolved in 1 ml. of 0.2 N ammonium hydroxide solution and was transferred quantitatively into stainless steel planchettes. The solution was evaporated to dryness under an infra-red lamp and the radioactivity measured in a Nuclear Chicago gas-flow low-background counter. Control experiments were simultaneously carried out in which RNA was omitted. The difference in radioactivity between the experimental and control tubes provided a measure of methyl groups incorporated into methyl-deficient *t*-RNA.

One unit of enzyme is defined as that amount which catalyses the incorporation of 1 μ mole CH₃ into *t*-RNA in 30 min under the foregoing conditions. Specific activity is defined as units of activity per mg of protein.

The amount of enzyme taken, 0.3–0.6 ml., was such that the methyl groups incorporated into *t*-RNA in 30 min did not exceed 40 μ moles. Within this range, the reaction was proportional to concentration of enzyme as well as to the time of reaction.

Extent of methylation. This was measured as in the assay of activity by incubating the reaction mixture for 4 h with 0.6 ml. of enzyme extract. No appreciable increase in methylation was noticed by incubating for a longer period.

In Fig. 1 the results of examination of the rate of incorporation of methyl groups into a methyl deficient *E. coli t*-RNA by extracts derived from larvae in their terminal stage and from pupae at various stages are presented. It is readily apparent that large changes occur in the activity of the *t*-RNA methylases almost daily within the pupae. The experiments were performed

Fig. 1. Activity of *t*-RNA methylases

on different batches of larvae and pupae over a period of several months, yet the pattern of enzyme activity is reproducible. A minimal activity on the third day is followed by a maximum on the fourth. On the seventh day, just prior to the emergence of the beetle, enzyme activity is at its lowest.

Another measurable parameter of the *t*-RNA methylases is the extent of methylation of the substrate, that is, the total number of methyl groups introduced by the enzymes in infinite time. Such determinations are presented in Fig. 2. Again large differences in total enzyme activity are apparent. For example, extracts from a 1-day-old pupa can seek out a little more than twice as many sites for methylation in *t*-RNA as extracts from a 7-day-old pupa. There is excellent concordance between the rates and total enzyme activity of the organisms in the same stage of their cycle of metamorphosis. Only in one batch of pupae (B VII) was there a 1-day discrepancy in reaching maximal activity in mid-pupa stage. The deviation from the norm by 1 day in this batch underlines the remarkable synchrony of the other batches of larvae and pupae.

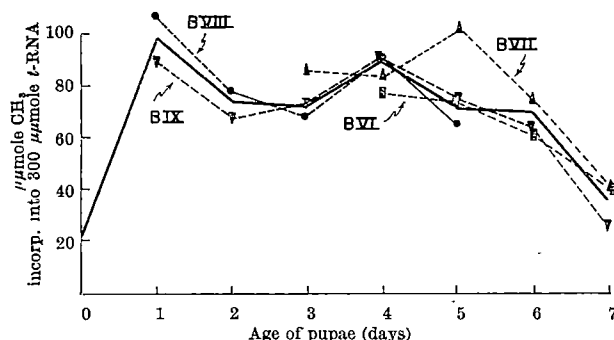


Fig. 2. Extent of methylation

It is difficult to assess the biological meaning of these changes in enzyme activity. Interpretations are hindered by our lack of knowledge of the specific biological function of the methyl groups in *t*-RNA. Some regulatory function in protein synthesis for *t*-RNA has been suggested by Ames and Hartman⁷. Methylation of the *t*-RNAs is one method of altering these molecules for changing functions. If such should eventually prove to be the case, then the fluctuations in the *t*-RNA methylases during phases of metamorphosis will assume a new meaning and a search for the new proteins the syntheses of which are directed by the changing patterns of methylating enzymes could be undertaken.

Between the first and seventh day in the life of a pupa there is approximately a 50 per cent reduction in enzyme activity, whether it is measured as a rate or extent of

methylation. The natural question posed by this finding is the cause of the reduction: it could be due to some enzyme inhibitor, or the findings could also be accounted for by the appearance of a new nuclease which essentially eliminates all or part of the substrate. In Table 1 the results of experiments designed to probe these possibilities are presented. Various amounts of enzymes from 1-day-old and 7-day-old pupae were admixed and comparisons were made with respect to both rates and extents of the methylating potencies of the extracts. The addition of small amounts of the extracts of 7-day-old pupae which themselves have low activity does not seem to diminish the levels of activity of the extracts from 1-day-old pupae. However, on the addition of larger volumes of the extract from the 7-day-old pupae, the activity, especially in terms of the extent of methylation, does become diminished. It appears as if a certain concentration of some inhibitor must be reached before it becomes effective in interfering with methylation.

Table 1. ACTIVITY OF METHYLASES AND EXTENT OF METHYLATION. MIXING EXPERIMENTS WITH EXTRACTS OF 1-DAY-OLD AND 7-DAY-OLD PUPAE

Exp. 1				Exp. 2			
1-day-old pupal extract (ml)	7-day-old pupal extract (ml)	Activity	Extent	1-day-old pupal extract (ml)	7-day-old pupal extract (ml)	Activity	Extent
0.3		14.5		0.3		11.9	
0.6		57.2	89.5	0.6		34.1	95.5
	0.3	8.5			0.3	7.1	
	0.6	15.2	55.4		0.6	16.5	65.2
0.3	0.3	26.9		0.3	0.3	24.1	
0.6	0.3	40.4	103.4	0.6	0.3	36.6	80.5
0.3	0.6	31.2		0.3	0.6	36.0	
0.6	0.6	46.6	42.0	0.6	0.6	43.6	66.7

Activity is expressed as μ moles of methyl groups incorporated into 0.15 mg of *t*-RNA in 30 min. Extent is expressed as μ moles of methyl groups incorporated in 4 h. In these experiments the total volume of the reaction mixtures was 1.5 ml

Nuclease activity in extracts of the pupae was also examined as a possible cause of the alterations observed here. Even after incubation for 4 h with these extracts, very little, if any, nuclease activity could be detected. Therefore, the reduced capacity for methylation in the extracts of the 7-day-old pupa cannot be due to the diminution of the substrate but rather to some inherent attribute of the methylases, the nature of which is obscure at present.

A rather potent nuclease activity which cleaved *t*-RNA was detected occasionally in larvae. The appearance of this nuclease activity was correlated with the conditions of the housing of the larvae. The nuclease appeared and reached a maximum within a period of two weeks in larvae which were kept in a relatively dry atmosphere. Under these conditions, the process of pupation was slowed down and ultimately ceased altogether, resulting in the death of large numbers of larvae.

The almost daily alterations in the levels of the RNA methylases during the pupal stage are highly suggestive of some basic function. This is a period of extensive differentiation aimed at the production of the new tissues and organs of the imago. The *t*-RNA methylases alter the structure of one of the cardinal components of the protein synthesizing machinery of the cell. We have also observed changes in the *t*-RNA methylases in an entirely different biological system during upheavals in its metabolic pattern. Either in bacteria infected by T2 bacteriophage or in lysogenic bacteria after induction of the latent phage by ultra-violet irradiation, major alterations in the enzyme-levels as well as in the patterns of methylation have been noticed⁸. In other words, in both instances, as a result of changes in the methylating enzymes, alterations must occur in the relative amounts of the methylated bases in the *t*-RNA of the host cell. It is difficult to imagine that bacteriophage carry information within their highly limited volume to make alterations in one of the macromolecules of the host without some purpose. We must therefore search for some regulatory mechanism at the level of *t*-RNA which is triggered by methylation.

This work was supported by grants from the U.S. National Science Foundation and from the National Institutes of Health, U.S. Public Health Service.

¹ Srinivasan, P. R., and Borek, E., *Science*, **145**, 548 (1964).

² Arber, W., *J. Mol. Biol.*, **11**, 247 (1965).

³ Wainfan, E., Srinivasan, P. R., and Borek, E., *Fed. Proc.*, **24**, 292 (1965).

⁴ Svensson, I., Boman, H. G., Eriksson, K. G., and Kjellin, K., *J. Mol. Biol.*, **7**, 254 (1963).

⁵ Gold, M., and Hurwitz, J., *Cold Spring Harbor Symp. Quant. Biol.*, **28**, 149 (1963).

⁶ Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. Biol. Chem.*, **193**, 265 (1951).

⁷ Ames, B. N., and Hartman, P. E., *Cold Spring Harbor Symp. Quant. Biol.*, **28**, 349 (1963).

INFLUENCE OF HETEROLOGOUS HISTONE ON THE SYNTHESIS OF CELLULAR AND VIRAL RIBONUCLEIC ACID AND PRODUCTION OF HAEMAGGLUTININ

By A. G. BUKRINSKAYA, A. K. GITELMAN and I. M. SHAPIRO

Institute of Virology, U.S.S.R. Academy of Medical Sciences, and Institute of Animal Morphology, U.S.S.R. Academy of Sciences

IT has been shown that histones inhibit cellular RNA synthesis both *in vivo* and *in vitro*¹⁻⁴. This effect seems to be connected with the histone-induced blockage of DNA as a template. On the other hand, a protein of a histone type was found to be produced in a cell in the first hours after its infection with a virus. This protein inhibits the cellular RNA synthesis before the onset of the viral RNA synthesis⁵. The synthesis of histone in virus-infected cells increased during the first hours after infection⁶. These data suggest that treatment of the infected cells with histone may stimulate the viral RNA synthesis.

In the present work the action of histone on the synthesis of cellular and viral RNA as well as on haemagglutinin production and infectious properties of virus has been studied.

The experiments were carried out on monolayer cultures of chicken fibroblasts. Fowl plague virus (Weybridge strain) was obtained as allantoic fluid of eleven-day-old chicken embryos. In some experiments the virus was concentrated by one cycle of adsorption and elution on chicken erythrocytes. The multiplicity of infection was about 10 and 100 ID_{50} per cell, respectively. The period of adsorption was 30 min at 4° C; the same volume of balanced salt solution was added to the control cells. After washing, the histone in balanced salt solution was added, and the cells were incubated at 37° C; this moment was considered as zero time of the experiment. ¹⁴C-cytidine was added to the medium every hour or every half-hour after infection. The monolayers were washed, cells taken off the glass, pipetted, collected on 'Millipore'

filters, treated twice with cold 5 per cent perchloric acid, twice with cold 96 per cent ethanol, and dried. After counting, the 'Millipore' filters were dissolved in acetone, RNA was hydrolysed by 1.7 M HClO₄ for 18 h at 4° C and the radioactivity was counted per μ g RNA.

The total histone was isolated from the calf thymus by the method of Hnilica and Hupka⁷ in the Institute of Medical and Biological Chemistry, U.S.S.R. Academy of Medical Sciences.

In some experiments cells were incubated in the presence of 5-fluorodeoxyuridine (FUDR), 10⁻⁶ mole/ml. being added at zero time.

As can be seen in Fig. 1, histone inhibited ¹⁴C-cytidine incorporation in the acid-insoluble fraction during the first 2-3 h. The same results were obtained in the presence of FUDR. Hence this effect is connected with the inhibition of RNA synthesis.

The inhibition of incorporation (varying from 12 to 60 per cent of the control-level) occurred in the first 2 h after infection with the allantoic virus. The incorporation was maximal 4-5 h after infection and reached about 130 per cent of the control. When concentrated virus was used

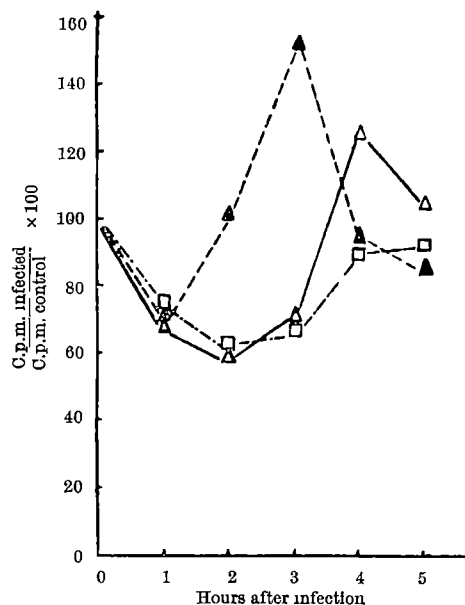


Fig. 1

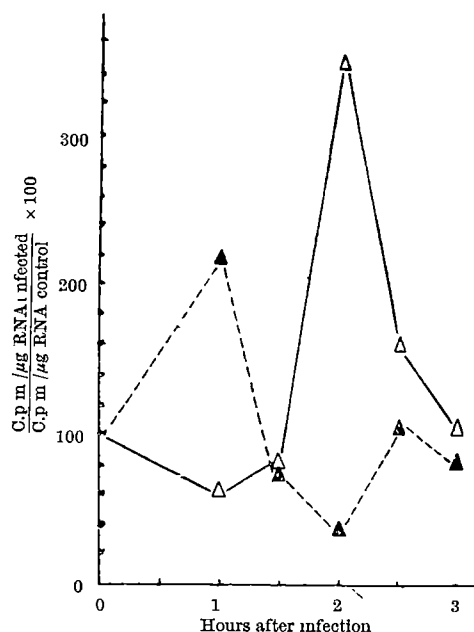


Fig. 2

Figs. 1 and 2. Rate of ¹⁴C-cytidine incorporation in acid-insoluble fraction of chicken fibroblasts. Radioactivity of histone-treated non-infected (□) and untreated infected (Δ) cells as percentage of the control, radioactivity of histone-treated infected cells (▲) as per cent of radioactivity of histone-treated non-infected cells. (1) Multiplicity of infection 10 ID_{50} per cell; each point is the average of 4 sets of experiments with 100 μ g/ml. of histone. 0.07 μ C-cytidine (specific activity 2.5 μ C per μ mole) were added in 2-3 flasks 0, 1, 2, 3 or 4 h after infection and left for 1 h. (2) Multiplicity of infection 100 ID_{50} per cell; one typical experiment with 150 μ g/ml. histone. 0.07 μ C-cytidine were added 0, 0.5, 1, 1.5, 2, 2.5 or 3 h after infection and left for 0.5 h

the minimal and maximal incorporation was found after 1 h and 2–2.5 h, respectively. Similar results with infected cells were observed in the presence of FUDR (Figs. 1 and 2). The inhibition of the incorporation seems to be caused by the formation of virus-induced repressor of cellular RNA synthesis while the stimulation of the incorporation represents the viral RNA synthesis⁸.

In the experiments with histone treatment the maximum of incorporation after infection with non-concentrated virus was higher than in the infected control, the minimum and maximum of incorporation were observed at 1.25 ± 0.3 h and 2.8 ± 0.43 h, respectively; whereas in the experiments without histone these intervals were 2.25 ± 0.3 h and 4.4 ± 0.28 h, respectively. The maximum of the incorporation in histone-treated cells infected with concentrated virus was lower than in infected controls and occurred 1 h earlier (Figs. 1 and 2). Thus, histone treatment brought about inhibition of cellular RNA synthesis and earlier synthesis of viral RNA. It may be supposed that heterologous histone acts similarly to virus-induced repressor. Their combined action inhibits cellular synthesis of RNA earlier and, probably due to this, earlier synthesis of the viral RNA occurs. There would seem to be a relationship between the time of the cellular RNA synthesis inhibition and that of the viral RNA reproduction.

The haemagglutinin titres and ID_{50} in histone-treated cells were lower as compared with infected controls (Table 1).

Table 1. HISTONE ACTION ON HAEMAGGLUTININ AND ID_{50} TITRES AT DIFFERENT TIMES AFTER INFECTION

Experimental series	Hours after infection					
	2	4	6	8	12	
Control cells	HA 0	HA 16	HA 32	ID_{50} $10^{7.6}$	HA 64	ID_{50} $10^{7.6}$
Histone-treated cells	0	2	4	10^7	8	16 $10^{7.0}$

Cells were infected with 10 ID_{50} per cell, thoroughly washed, and 100 μ g/ml. of histone were added at 0 time.

The cells were disrupted 12 h after infection by quick-freezing and thawing, haemagglutinin titres were determined in supernatant fluid with a 0.5 per cent suspension of chicken erythrocytes, ID_{50} titrated in chicken embryo pool and expressed as haemagglutinin units/ml. and ID_{50} /ml.

It is of interest to note that probably there is a comparatively narrow time-interval over which the addition of histone to the medium can influence haemagglutinin production. After the treatment of cells from 0 to 2 h, haemagglutinin titres were 8–16-fold lower than those in the control; when histone was added 3 or 5 h after infection, haemagglutinin titres were about the same as in the control. In three experiments histone was added

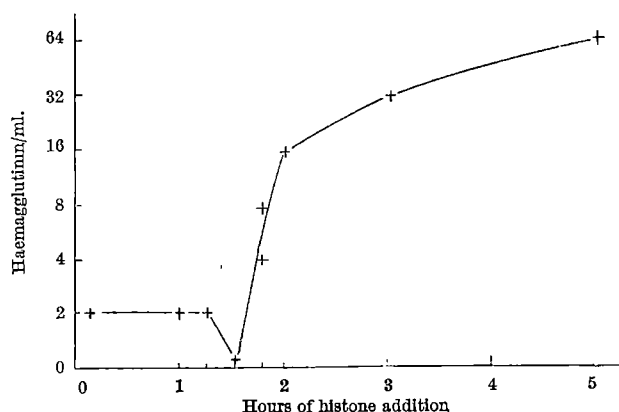


Fig. 3. Haemagglutinin production in relation to the time of histone addition. Each point is the average of 3 experiments. Cells were infected with 10 ID_{50} per cell and thoroughly washed, and 100 μ g/ml. histone were added 0, 1, 1.25, 1.5, 1.75, 2, 3 and 5 h after infection. The haemagglutinin titres were determined as described in Table 1.

to the medium every 15 min after infection. The most sensitive period to histone action was between 0 and 1.5 h after infection, the haemagglutinin titres were lowest when histone was added 1.5 h after infection (Fig. 3). Haemagglutinin production in this system starts 3 h after infection. Thus the most sensitive period was found much earlier than the process of haemagglutinin production started.

It is not yet known whether the cell genome takes part in haemagglutinin production of myxoviruses. That is why it is difficult to decide whether the effect observed is due to the binding of histone with DNA or viral RNA. Of interest is the fact that actinomycin D in low concentration (up to 2 μ g/ml.) has the same effect as histone on the synthesis of the viral RNA and haemagglutinin of PR8 and Weybridge strains of fowl plague virus^{9,10}.

¹ Huang, B. C., and Bonner, Y., *Proc. U.S. Nat. Acad. Sci.*, **48**, 1216 (1962).

² Barr, G. C., and Butler, J. A. V., *Nature*, **199**, 1170 (1963).

³ Alfrey, V. G., Litthau, V. C., and Mirsky, A. E., *Proc. U.S. Nat. Acad. Sci.*, **49**, 414 (1963).

⁴ Alfrey, V. G., Faulkner, R., and Mirsky, A. E., *Proc. U.S. Nat. Acad. Sci.*, **51**, 726 (1964).

⁵ Balandin, I. G., and Franklin, R. M., *Biochem. Biophys. Res. Comm.*, **15**, 27 (1964).

⁶ Holoubek, V., *Biochem. Biophys. Res. Comm.*, **15**, 166 (1964).

⁷ Hnilica, Z., and Hupka, S., *Biologia, Czechoslovakia*, **2**, 821 (1959).

⁸ Bukrinskaya, A. G., Gitelman, A. K., and Workunova, G. K., *Probl. of Virol.*, **5**, 569 (1964).

⁹ Bukrinskaya, A. G., *Nature*, **204**, 205 (1964).

¹⁰ Bukrinskaya, A. G., and Workunova, G. K., *Probl. of Virol.*, **8**, 657 (1964).

SUPPRESSION OF THE PUPILLARY LIGHT REFLEX IN BINOCULAR RIVALRY AND SACCADIC SUPPRESSION

By M. LORBER*, DR. B. L. ZUBER* and DR. L. STARK*

Neurology Section, Electronic Systems Laboratory and Biology Department, Massachusetts Institute of Technology, Cambridge, Massachusetts

IN 1948 Bárány and Halldén¹ reported that the pupillary response to a flash presented to the non-dominant eye of a subject experiencing binocular rivalry was smaller than the response to the same flash presented to a dominant eye. We have sought to repeat these experiments using the direct-recording infra-red pupillometer² in order to get an objective measure of this suppression.

The subject's head was firmly held in front of the pupillometer by means of a bite board and head rest.

* Present address: Information Engineering Department, University of Illinois at Chicago Circle, P.O. Box 4348, Chicago

The right eye viewed a circular field of about twenty degrees in diameter which was dimly illuminated, and in the centre of which was a small fixation cross at optical infinity. The test flash, when it appeared, was superimposed on this field and had a duration of 10 msec. The fixation target for the left eye was a small dot at optical infinity and in the centre of a uniform field illuminated with a diffuse red light. The stimulus was delivered to the right eye every 3 sec, so that during rivalry flashes were alternately presented during dominance and non-dominance (reported by the subject, who controlled a two-position switch).

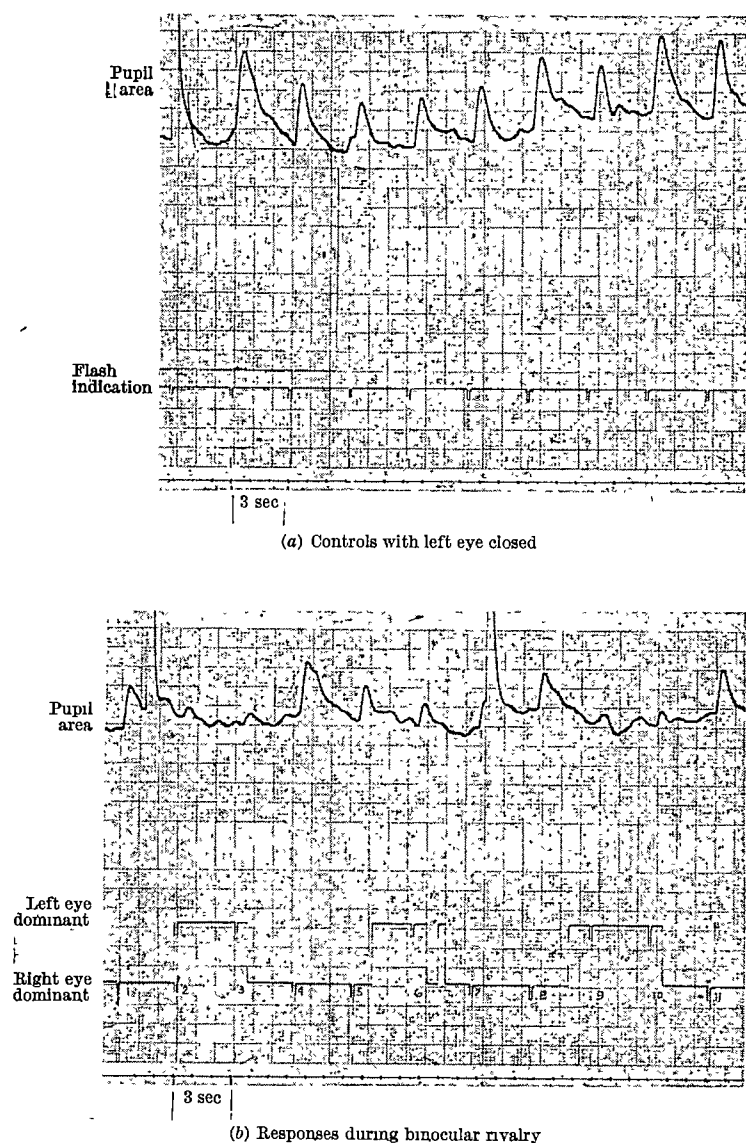


Fig. 1 Measurements of the pupillary response during binocular rivalry. *a*, Control responses with the left eye patched, *b*, responses during rivalry. Upper trace shows pupillary responses; broad shifts of base line on lower trace represent subject's state of dominance, narrow pulses indicate stimulus presentations

In Fig. 1*a* the responses to control flashes, taken with the left eye occluded, are shown. Fig. 1*b* shows responses during rivalry. Pupillary responses appear on the upper trace, while the subject's state of dominance (broad shift of the base line) and the occurrence of the stimulus (narrow pulses) are represented on the lower trace. On the whole, the responses during 'right eye dominant' are smaller than control responses, probably because the subject often experienced fusion of the two fields under this condition. There seems to be a significant decrease in the amplitude of the pupillary response when the right eye is suppressed (left eye dominant). These results have been confirmed by Richards⁹.

It has been well established that the visual threshold is significantly elevated just before and during a saccadic eye movement⁴⁻¹³. This phenomenon has been referred to as 'saccadic suppression'¹³. The experiments described here were designed to determine whether or not the pupillary light reflex is involved in this suppression.

The time required for the completion of a saccadic eye movement is short compared with the latency of the pupillary response. Thus the pupillary response to a flash presented to the moving eye may be measured by the pupillometer well after the eye has come to rest.

The stimulating apparatus (pupillometer) was basically the same as described above. Viewing conditions were monocular with the left eye occluded, and a second fixation cross was placed at the left periphery of the field for the right eye. Eye movement was always from the left peripheral to the central fixation cross, and angular distance of about eight degrees. A signal proportional to eye position was obtained by a previously described method¹². Test flashes were presented to the subject at various times before, during and after the beginning of a saccadic eye movement, and visual response (seen or unseen), pupillary response, eye movement and stimulus were recorded. A pulse with variable associated delay and constant width (10 msec) was used as the stimulus.

Pupillary responses to test flashes presented during and after the eye movement were averaged on a digital computer (GE 225) equipped with analog-to-digital converter¹⁴. For these stimuli the eye position signal served as a trigger both for the inputting sequence of the computer and for the presentation of the delayed test flash. The time of presentation of the test flash with respect to the beginning of the eye movement was determined by the delay setting. The subject indicated having seen a given test flash by depressing a push-button which caused a pulse to be registered on the recorder. Thus, visual saccadic suppression was followed on the recorder, while pupillary saccadic suppression was followed on the computer.

Responses to test flashes presented before the eye movement could not be averaged on the computer because: (1) the eye movement could not be used as an initiator; (2) the variability of the latency of eye movement was too great. In this case the subject moved his eye on receiving an auditory stimulus which also served as a trigger for the test flash. This was delayed so little that it occurred during the latency associated with the eye movement. These responses were grouped according to time of test flash presentation and hand-averaged. Saccadic suppression was followed as already described here.

Average responses to test flashes presented during and after the eye movement are shown in Fig. 2. Pupillary responses are shown in arbitrary units. Controls consisted of: curve *A*, responses to the test flash during central fixation, that is, with no eye movement; curve *B*, responses to the test flash during left peripheral fixation (no eye movement); curve *C*, responses to the eye movement with no test flash presentation. In some experiments a small pupillary response was observed in this last control, probably due to a small change in accommodation from one fixation point to the next. The average response for this control was subtracted from all computer-averaged responses (excepting controls) in order to emphasize more clearly trends in the data. Curves *D-L* represent average responses to these test flashes (control *C* subtracted out), the time of flash presentation with respect to the beginning of the eye movement appearing to the right of each response. It is clear that the average pupillary response is much diminished for short times of flash presentation. As the time of flash presentation approaches 980 msec the average response amplitude approaches that of the control responses. Fig. 3 shows psychophysical saccadic suppression and pupillary saccadic suppression results from the same experiments where test

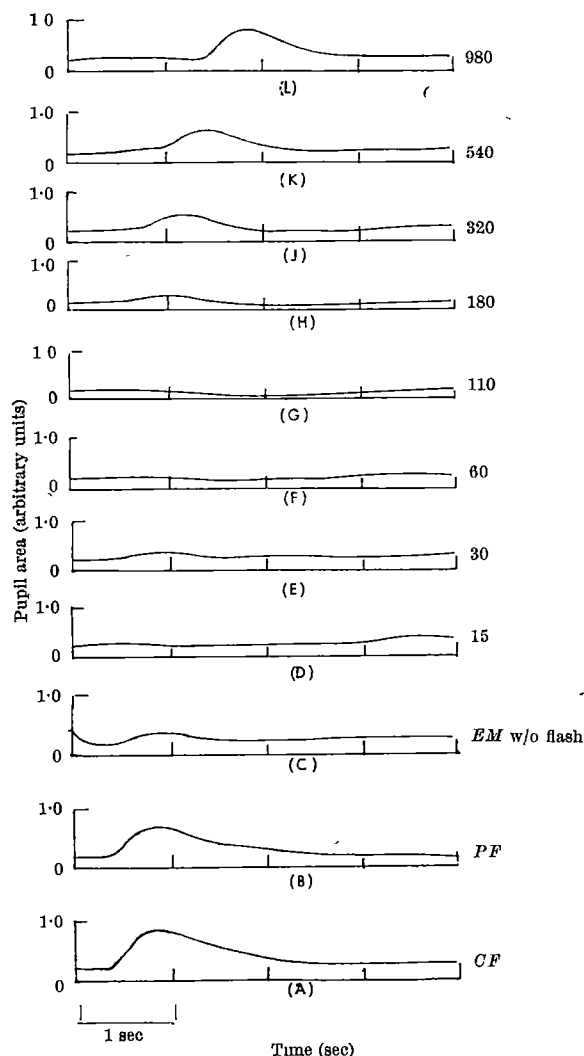


Fig. 2. Pupillary responses to test flashes presented at various times during and after eye movement. Time of presentation in msec appears at right of each average response. Curves A, B and C are controls

flashes were presented before, during and after the eye movement. The visual saccadic suppression observed in this experiment agrees in general with previously published results^{7,12}. When saccadic suppression is maximal no test flashes are seen and the average pupillary response is reduced to 10 per cent of the average response to the test flash during central fixation. While it is clear that visual and pupillary saccadic suppression occur concurrently, the time-course of pupillary saccadic suppression seems far more extensive than that of visual saccadic suppression. This experiment has been performed on

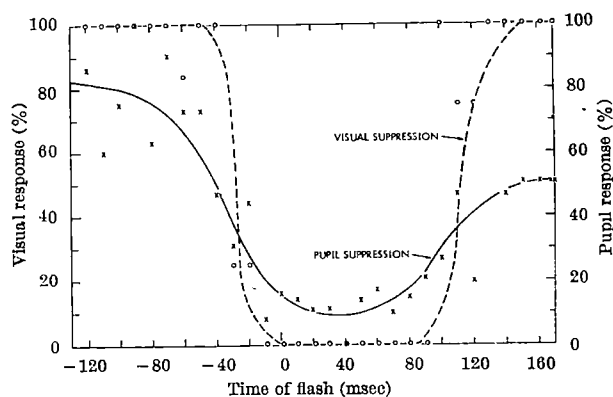


Fig. 3. Saccadic suppression and pupillary saccadic suppression as a function of time of flash presentation with respect to beginning of eye movement ($t=0$). Visual response represents per cent seen; pupil response represents average amplitude as percentage of amplitude of average response during central fixation

two subjects and the results are quite similar for both.

Much of the pupillary saccadic suppression observed in these experiments occurs before the eye movement, seemingly minimizing the probability that the suppression is caused by an artefact. Possibilities for such artefacts are non-linear addition in the pupillary system¹⁵⁻¹⁷ and illumination of new retinal areas caused by the eye movement.

Based on the results of these experiments we are not able to determine whether the suppression observed in binocular rivalry and that observed in saccadic suppression are generated by the same mechanism. It is hoped that further experiments, both neurophysiological and psychophysical, will further elucidate these as well as many other related phenomena.

Major support for this work was provided by the U.S. Public Health Service (MH-06175-02), the U.S. Navy (Office of Naval Research (Nonr-1841 (85)), and the U.S. Air Force (AF 49 (638)-1313).

¹ Bárány, E. H., and Halldén, U., *J. Neurophysiol.*, **11**, 25 (1948)

² Stark, L., *Proc. Inst. Rad. Eng.*, **47**, 1925 (1959).

³ Richards, W., *Vision Res.* (in preparation).

⁴ Dodge, R., *Psychol. Rev.*, **7**, 454 (1900).

⁵ Dodge, R., *Psychol. Bull.*, **11**, 193 (1905).

⁶ Holt, E. B., *Harvard Psychol. Studies*, **1**, 3 (1903)

⁷ Latour, P. L., *Vision Res.*, **2**, 261 (1962).

⁸ Volkman, F. C., *Optic. Soc. Amer.*, **52**, 571 (1962)

⁹ Ditchburn, R. W., *Optica Acta*, **1**, 171 (1955)

¹⁰ Holt, E. B., *Psychol. Bull.*, **3**, 68 (1906).

¹¹ Zuber, B. L., Horrocks, A., Lorber, M., and Stark, L., *QPR 73*, 221, *Res. Lab. Electronics, MIT* (1964).

¹² Zuber, B. L., Michael, J. A., and Stark, L., *QPR 73*, 217, *Res. Lab. Electronics, MIT* (1964).

¹³ Zuber, B. L., Orider, A., and Stark, L., *QPR 74*, 244, *Res. Lab. Electronics, MIT* (1964).

¹⁴ Stark, L., Sandberg, A. A., Stanten, S., Willis, P. A., and Dickson, J. F. *Ann. N.Y. Acad. Sci.*, **115**, 738 (1964).

¹⁵ Stark, L., Van der Tweel, H., and Redhead, J., *Acta Physiol. Pharmacol. Neerlandica*, **11**, 235 (1962).

¹⁶ Baker, F. H., *J. Optic. Soc. Amer.*, **53**, 1430 (1963).

¹⁷ Sandberg, A. A., *QPR 66*, 420, *Res. Lab. Electronics, MIT* (1962)

FUNCTIONAL SIGNIFICANCE OF SWEAT GLANDS AND SEBACEOUS GLANDS IN SEALS

By JOHN K. LING

Antarctic Division, Department of External Affairs, Melbourne, Australia

BIOLOGICAL investigations being carried out by Australian National Antarctic Research Expeditions at Macquarie Island (54° 30' S., 159° E.) include a study of the skin and hair cycle of the southern elephant seal, *Mirounga leonina* (L.)¹. Skin samples have been collected from five other pinnipeds also inhabiting southern latitudes but from different climatic regions.

It has been regarded as typical among mammals for the apocrine sweat glands to open into the pilary canal above the opening of the sebaceous glands, or even on to the skin surface². In the Phocidae this situation is reversed (Fig. 1), as previously noted in reports on skin from the general body surface³⁻⁵. Examination of serial sections of skin cut parallel to the skin surface of the

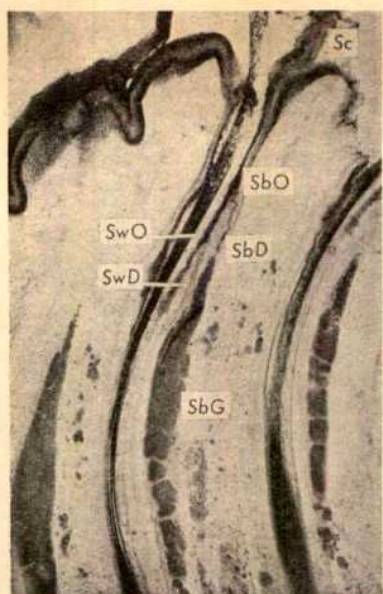


Fig. 1. Section of elephant seal skin from back, cut parallel to hair follicles to show opening, *SbO*, of sebaceous duct, *SbD*, above orifice, *SwO*, of sweat duct, *SwD*. *SbG* = sebaceous gland; *Sc* = stratum corneum. Oil red O and haemalum. $\times 27.5$

body and flippers of three phocid species confirms that the sweat ducts enter the hair canal below the sebaceous gland ducts.

Sweat glands in the Otariidae open into the pilary canal above the sebaceous gland openings over the general body surface (Fig. 2A). It is remarkable, however, that the apocrine sweat glands associated with hair groups on the flippers of the Australian fur seal, *Arctocephalus doriferus* Wood Jones, enter the pilary canal by way of a duct opening below the sebaceous gland orifice (Fig. 2B). Thus we have opposite arrangements on different parts of the same species of seal. The distally naked areas of skin on the fore and hind flippers have no cutaneous glands, although sections of 'naked' regions on hind flippers of the New Zealand fur seal, *Arctocephalus forsteri* (Lesson), have revealed minute hairs with asso-

ciated sweat and sebaceous glands. These hairs could not be seen when the outer surface of fixed material was viewed microscopically.

In the true seals (Phocidae) the sweat glands are rather insignificant, though in accurately oriented longitudinal sections their ducts can generally be traced alongside the follicle and seen to open just above the constricted region. Thus the sweat duct passes between the sebaceous gland and the follicle, and the two gland openings are visible one above the other, with the mouth of the sebaceous duct appearing in the form of a delta among the lamellae of the stratum corneum, particularly of the elephant seal. Copious lipid secretion can be demonstrated in frozen sections stained with oil red O (Fig. 1).

The sweat glands over the general body surface of the fur seals (Otariidae) are relatively enormous organs extending well below the bases of the hair follicles. Their secretory portions are much coiled and the ducts ascend outside the sebaceous glands to open into the funnel-shaped upper end of the pilary canal above the sebaceous gland opening. On the hairy proximal regions of the flippers of otariids the sweat glands are somewhat reduced and their ducts ascend between the follicle and the sebaceous gland in the same way as on all regions of phocids. In these areas the sebaceous glands are larger and the stratum corneum is thicker and more richly supplied with lipids.

The bilobed sebaceous glands of Phocidae are large compared with the size of the follicle and are situated on its ental side. These glands have unequal lobes in the Otariidae and the larger one enters the ental side of the guard hair follicle, while the smaller and more slender duct opens on the ectal side of the underfur follicles that emerge from a common pilary canal.

With the exception of the harp seal, *Pagophilus groenlandicus* (Erxleben), the relative positions of the cutaneous gland openings on the general body surface are distinct for the two families of seals. Bergersen's³ meticulous observations on *P. groenlandicus* leave no doubt that the sweat gland orifice in this species lies above the opening of the sebaceous glands. However, in addition to the guard hair, the follicle bundles of the harp seal contain 5-9 subsidiary hairs, quite a high number in comparison with other phocid species.

It has been stated that the naked areas of flippers of all Pinnipedia—including the walrus, *Odobenus rosmarus* (L.)—are devoid of sweat glands⁴. However, the appearance of liquid droplets on the glabrous palmar surface of the northern fur seal, *Calorhinus ursinus* (L.), after exposure to radiant heat was assumed to indicate that sweating had been induced⁵. Large and abundant sweat glands have been found in microscopic preparations of skin from naked regions of fore and hind flippers of the same species⁷.

I recently noticed fine hairs with associated cutaneous glands in histological sections of apparently naked skin from the fore flipper of *C. ursinus* (kindly lent by Dr. V. B. Scheffer). A similar situation obtains in the 'naked' skin of the hind flipper (but not the fore flipper) of *A. forsteri*. No cutaneous appendages have been found in naked skin of the fore or hind flippers of other southern otariids. There were, however, delicate, lipid-rich strands ascending vertically through the stratum corneum on the hairless part of the fore flipper of the Kerguelen fur seal, *Arctocephalus tropicalis gazella* (Peters), but no associated tubules were visible in the dermis. The nature of these structures remains unknown.

The phylogenetic significance of apocrine sweat glands in primates has been discussed recently in terms of the shifting position of the orifice of the sweat duct from the skin surface of the Erosimii to within the pilary canal of man². This latter situation is common for most mammals, an exception being the pig, the glands of which open on to the skin surface⁸. Thus there is no clear relationship between the amount of hair and the position of the sweat gland orifice in mammals described so far.

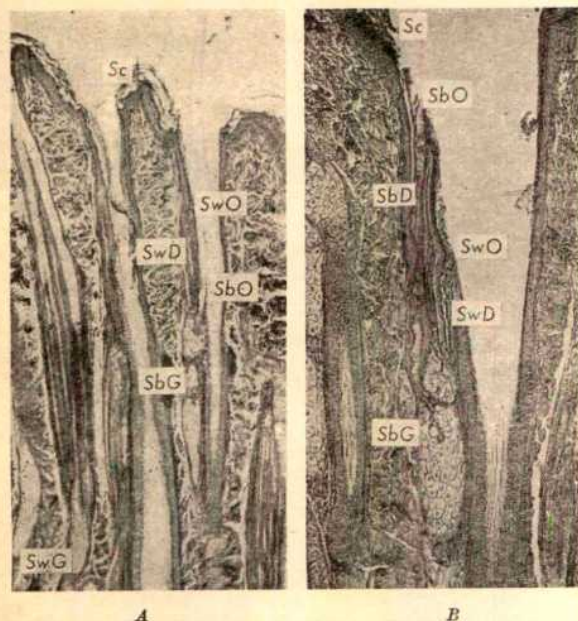


Fig. 2. Sections of Australian fur seal skin cut parallel to hair follicles showing relative positions of cutaneous gland openings into pilary canal. *SwG* = sweat gland. Other lettering as for Fig. 1. A, Dorsal body surface, $\times 54$. B, Dorsal surface of hind flipper. Mallory's triple stain $\times 64.5$

The morphology of sebaceous and sweat glands in seals may be related to the adaptation of these animals to their aquatic environment. Loss of functional hair in the thermoregulatory sense among the Phocidae and on the flippers of the Otariidae was accompanied (or followed?) by reduction in size of the sweat glands and a shifting of their excretory pore to a position below that of the sebaceous gland opening. Sebaceous glands are relatively larger in the skin of sparsely haired phocids and on less hairy parts of otariids, and these glands secrete a heavy lipid deposit within the stratum corneum. Although the sweat glands of the walrus—an almost hairless pinniped—are large, they actually atrophy during autumn⁹. The sebaceous glands are relatively small, but the nature of their secretion is not known.

Aoki and Wada¹⁰ have shown that sweat glands on the general body surface of dogs are sensitive to local stimuli such as radiant heat and drugs, but are insensitive to bodily conditions of heat stress. These authors suggested that temperature regulation is the chief function of the apocrine sweat glands of dogs. It is probable that similar local effects of sweat glands are associated with the hair bundles of heavily furred body regions of fur seals and the harp seal.

There is an apparent distinction in relative size and position of the sweat glands and sebaceous glands between the more terrestrially inclined Otariidae and the aquatically adapted Phocidae. This seems to weaken the argument for a biphyletic origin of the Pinnipedia¹¹. Sweat glands appear to be functionally important in association

with an elaborate pelage where their role is probably the regulation of local skin temperature. With the progressive loss of hair, exemplified by seals of the family Phocidae, the need for water-proofing the skin is met by enlarged lipid-secreting sebaceous glands, thermoregulation being achieved by the thick layer of subcutaneous blubber. It has been suggested that northern fur seals depend on their naked flippers for maintenance of their body temperature⁷, but this may not be a general rule in the Otariidae. The density, size and distribution of cutaneous glands, if any, and blood vessels in the naked flippers of Otariidae need to be carefully analysed before their significance can be evaluated.

Further detailed quantitative studies of the skin of pinnipeds and other aquatic mammals are required to elucidate structure and function of the integument and its associated glands. Perhaps the evolutionary path back to the water by these once terrestrial forms will then emerge.

¹ Ling, J. K., in *Biology of the Skin and Hair Growth*, 525 (Angus and Robertson, Sydney, 1965).

² Montagna, W., *Arch. Derm.* (Chicago), **88**, 1 (1963).

³ Bergersen, B., *Norske Vidensk. Akad. Skrifter*, No. 5 (1931).

⁴ Montagna, W., and Harrison, R. J., *Amer. J. Anat.*, **100**, 81 (1957).

⁵ Scheffer, V. B., *North American Fauna*, No. 64 (1962).

⁶ Sokolov, V. E., *Byull. Mosk. Obshch. Ispyt. Prirod. Otd. Biol.*, **63**, 5 (1958).

⁷ Bartholomew, G. A., and Wilke, F., *J. Mammal.*, **37**, 327 (1956).

⁸ Montagna, W., and Yun, J. S., *J. Invest. Derm.*, **43**, 11 (1964).

⁹ Sokolov, V. E., *Nauchn. Dokl. Vysshei. Shkoly. Biol. Nauch.*, No. 2, 43 (1961).

¹⁰ Aoki, T., and Wada, M., *Science*, **114**, 123 (1951).

¹¹ McLaren, I. A., *Syst. Zool.*, **9**, 18 (1960).

INTERFEROMETRIC ESTIMATION OF EFFECTS OF TESTOSTERONE AND METHANDROSTENOLONE ON THE DRY MASS OF VARIOUS RAT MUSCLES

By DR. W. STAÜBLI and DR. T. OKADA

Research Laboratories, Pharmaceutical Department, Ciba, Ltd., Basle

IN animals following castration certain target organs display marked atrophy, which is reflected in a decrease in their wet and dry weights¹. This loss of weight of hormone-dependent organs can be partly or completely reversed by giving the castrated animals substitution treatment with androgenic¹⁻³ or anabolic² steroid hormones. In the present study we investigated the myotrophic effect of testosterone and methandrostenolone ('Dianabol') on certain skeletal muscles in the castrated rat. The optical path difference of the muscle fibres, as determined with the aid of an interference microscope, was selected as a criterion of the effect exerted by the hormones on the muscles.

Male Sprague-Dawley rats weighing approximately 100 g were used. They were kept on a standardized diet, and offered drinking-water *ad lib*. The rats were divided as follows into four groups, each containing four animals: (1) untreated controls; (2) castrated controls; (3) castrated animals, given one subcutaneous injection of testosterone (10 mg/kg in sesame oil) daily for 14 days, starting on the 15th day after castration; (4) castrated animals, given one subcutaneous injection of methandrostenolone (30 mg/kg in sesame oil) daily for 14 days, starting on the 15th day after castration. The m. levator ani and the m. bulbocavernosus were selected as representative 'sexual muscles'. Since the m. levator ani displays the characteristic features of a 'white' muscle⁴, the white portion of the m. psoas was used for comparison purposes.

Immediately after removal, the muscle tissue was fixed in Carnoy's solution, mounted in paraffin, and 4 μ sections were prepared. For the purposes of interfero-

metry, the sections, after removal of the paraffin, were covered with glycerol.

The optical path difference of the muscle fibres (φ) was estimated on longitudinal sections of the fibres (Fig. 1) with the aid of a Leitz interference microscope (fluorite objective with a magnification of 50 times, A , 0.85; monochromatic light of $\lambda = 546$ m μ) by measuring the displacement of the interference bands (d/D , Fig. 1) caused by the object. The optical path difference determined in this way is in direct proportion to the dry mass per unit of area⁵ (A_1 , A_2 , A_3 , Fig. 1). As the volumes (V_1 , V_2 , V_3 , Fig. 1) of the muscle fibres fluctuated markedly in response to hormonal treatment (Fig. 1), the following volume parameters were determined on photographs of cross-sections stained with haematoxylin-eosin: (1) the number of muscle cell nuclei per unit of area (5 cm \times 5 cm); (2) the number of muscle fibres per unit of area; (3) the area of the muscle fibres as estimated by planimetry (A'_1 , A'_2 , A'_3). Since the thickness of the specimens (t , Fig. 1) was kept constant, the path difference can be regarded as proportional⁶ to the concentration of

substances ($C = \frac{M}{V}$) in the muscle fibres. The volume

V is a prism, the sides of which are marked a , l (length of the muscle fibre), and t (thickness of the section) in Fig. 1.

After the wet weight had been measured, the muscles were dried to constant weight at 110° C for the purpose of estimating their dry weight.

It is known that the areas of the fibre cross-sections in certain target muscles are considerably reduced as a result of castration⁶. Since the muscle fibres in histological

Table 1. INFLUENCE OF HORMONE TREATMENT ON VOLUME PARAMETERS OF MUSCLE FIBRES

Treatment	M. levator ani						M. bulbocavernosus						M. psoas (white)					
	Nuclei			Fibres			Nuclei			Fibres			Nuclei			Fibres		
	No. (1a)	% (1b)	No. (2a)	% (2b)	Mean (3a)	% (3b)	No. (4a)	% (4b)	No. (5a)	% (5b)	Mean (6a)	% (6b)	No. (7a)	% (7b)	No. (8a)	% (8b)	Mean (9a)	% (9b)
Untreated controls	14*	—	14*	—	13.8*	—	16	—	12	—	20.6	—	9	—	4	—	52.6	—
Castrated controls	42	+200	62	+343	3.7	-73	51	+215	69	+475	3.1	-85	16	+78	8	+100	31.5	-40
Testosterone	16	+14	11	-21	15.0†	+9	20	+25	17	+42	15.5	-25	9	0	4	0	53.4	+2
Methandrostenolone	16	+14	11	-21	14.8	+7	20	+25	16	+33	16.3	-21	8	-11	4	0	50.4	-4

No., Absolute number per unit area (5 cm × 5 cm).

%, Percentage change of untreated controls.

Mean, mean area of fibre cross-sections expressed in arbitrary units.

* Each value represents the mean of 36 to 104 individual measurements.

† This value is significantly ($P < 0.01$) higher than the corresponding value of untreated controls.

cross-sections usually have an irregular polygonal configuration, measurement of their diameter is not a suitable criterion for estimating changes in their volume. The dependence of the 3-volume parameters already mentioned on the hormonal treatment is shown in Table 1. As was to be expected⁴, castration led to a reduction in the volume of the muscle fibres, which was reflected in an increase in the number of cell nuclei and of muscle fibres per unit of area and in a decrease in the areas of the fibre cross-sections. The extent of this reduction in volume was most marked in the case of the m. bulbocavernosus, less marked in that of the m. levator ani, and least marked of all in that of the m. psoas.

The administration of testosterone and of methandrostenolone to castrated animals in the dosages indicated produced an increase in fibre volume in all the types of muscle studied (cf. Fig. 1); the reaction of these muscles to the hormonal stimulus, however, was not uniform. Thus, testosterone and methandrostenolone increased the fibre volume in the m. levator ani and in the m. psoas almost to the level of that in the untreated controls, whereas the values for the m. bulbocavernosus were lower. Both testosterone and, to a somewhat lesser extent, methandrostenolone caused the fibre volume of the m. levator ani, as estimated by measuring the area of the fibre cross-sections, to rise above the control-levels by 9 and 7 per cent respectively (Table 1).

The results of the interferometric estimation of the optical path difference are indicated in Table 2. It can be

Table 2. DEPENDENCE OF THE OPTICAL PATH DIFFERENCE (f) ON HORMONAL TREATMENT

Treatment	(Mean values \pm S.E.)		
	M. levator ani	M. bulbocavernosus	M. psoas (white)
Untreated controls	0.37 ± 0.032	0.36 ± 0.054	0.35 ± 0.039
Castrated controls	$0.49 \pm 0.088^*$	0.39 ± 0.143	$0.45 \pm 0.06^*$
Testosterone	0.39 ± 0.084	0.40 ± 0.141	0.41 ± 0.140
Methandrostenolone	0.33 ± 0.156	0.39 ± 0.079	0.36 ± 0.075

* These values differ significantly ($P < 0.01$) from those of the corresponding untreated controls.

seen that castration caused a slight increase in the optical path difference and thus in the dry weight/area of the muscle fibres (cf. Fig. 1). The administration of testosterone or methandrostenolone brings the optical path difference back again to within the vicinity of the control-levels.

The castration-induced increase in the optical path difference of muscle fibres appeared to be relatively small (Fig. 1). Since the area (A_2) of the fibres is markedly reduced following castration, the optical path difference

$\varphi (= \frac{M_2}{A_2})$ would have to show a corresponding increase

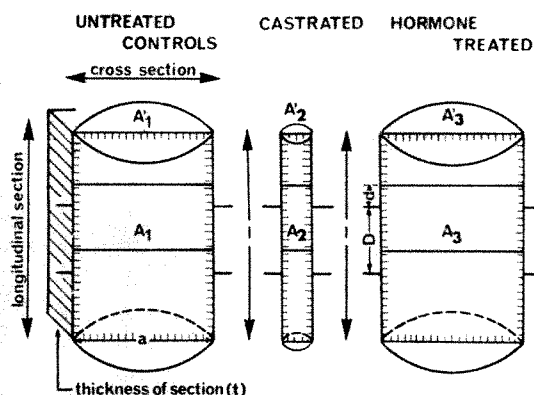
in relation to the untreated controls if the dry mass remains constant ($M_1 = M_2$). As, however, this is not the case (Table 2), it can be assumed that the muscle fibres must undergo an appreciable loss of substance as a result of castration and that the two hormones are capable of making good this loss (Fig. 1 and Table 2).

This loss of substance, demonstrated with the aid of interferometry, must also exert an effect on the concentration of dry substances in the muscle fibres. Since the considerable diminution in the volumes (Table 1), which occurs following castration, is accompanied by a loss of dry mass, the concentration in the muscle fibres of castrated animals does not undergo any substantial change in comparison with the untreated controls and the hormone-treated animals (Fig. 1). In the light of the proportional relationship between the optical path difference and the concentration⁵ in our experimental procedure, we attempted to correct the path difference with the aid of the volume parameters listed in Table 1 (Table 3). It can be seen that the following expressions yield values which—

Table 3. RELATIONSHIP BETWEEN THE CORRECTED OPTICAL PATH DIFFERENCE AND DRY WEIGHT (AS DETERMINED GRAVIMETRICALLY)

Muscle	Treatment	As percentage of untreated controls			
		$\varphi^*/(1a)$	$\varphi/(2a)$	$\varphi x(3a)$	Dry weight
M. levator ani	Castrated controls	44	31	35	38
	Testosterone	92	130	115	98
	Methandrostenolone	80	115	96	96
M. bulbocavernosus	Castrated controls	32	17	16	16
	Testosterone	86	79	84	86
	Methandrostenolone	86	83	86	88
M. psoas (white)	Castrated controls	68	69	77	—
	Testosterone	112	125	119	—
	Methandrostenolone	115	123	99	—

* φ (compare with Table 2) was corrected with the aid of the volume parameters listed in Table 1.



Dry weight W	$W_1 \gg W_2 \ll W_3 (\approx W_1)$
Area A (longitudinal section)	$A_1 \gg A_2 \ll A_3 (\approx A_1)$
Volume V (a-l-t)	$V_1 \gg V_2 \ll V_3 (\approx V_1)$ (Table 1)
Concentration $C = \frac{M}{V}$	$C_1 \approx C_2 \approx C_3$
Optical path difference φ	$\varphi_1 \approx \varphi_2 \approx \varphi_3$ (Table 2)
Dry mass $\frac{M}{A} = \varphi$	$M_1 \approx M_2 \approx M_3$
Dry mass $M = \varphi \cdot A$	$M_1 \gg M_2 \ll M_3$

Fig. 1. Diagram showing the experimental procedure

with a few exceptions to be dealt with later—tally reasonably well with the gravimetric estimation of the dry weight (Table 3): (1) ratio between optical path difference (φ) and number of cell nuclei per unit of area (1a, 4a, 7a, Table 1); (2) ratio between optical path difference (φ) and number of fibres per unit of area (2a, 5a, 8a, Table 1); (3) product of optical path difference (φ) and area of the fibre cross-sections (3a, 6a, 9a, Table 1). Among the various volume parameters, the optical path difference (expressed in terms of concentration of dry substance) appeared to be most directly related to the area of the fibre cross-sections.

Table 3 also shows that following testosterone treatment the corrected optical path difference (column 3b) of the m. levator ani and of the m. psoas is 15 and 19 per cent, respectively, above the corresponding values in the untreated controls; methandrostenolone does not display this effect.

The reason for this difference is not known. There are, however, two main possibilities:

(1) It is conceivable⁷ that the hormone-stimulated synthesis of cellular substances, that is, contractile proteins, for example, proceeds more rapidly in the region of the cell nuclei at the periphery than in those zones of the fibres further removed from the nuclei. Hence, there would have to be a concentration gradient decreasing from the periphery towards the centre of the fibre. The optical path difference, however, was determined at the periphery of the fibre. If this gradient were to be maintained for longer following testosterone than following methandrostenolone, determination of the optical path difference should yield higher values for testosterone. The figures listed in Table 2 do in fact indicate that testosterone tends to raise the optical path difference in the 'white' muscles

above the values found for methandrostenolone. Pointing in the same direction is the observation that following testosterone an inhomogeneous distribution of the myofibrils is encountered in stained histological cross-sections of the m. levator ani and m. psoas; this cytological picture could be due to the fact that hormonal stimulation of the sarcoplasm and its particulate organelles⁸ has not yet been completely arrested² at this point (14 days after castration).

(2) The second possible explanation would be that determination of the optical path difference yields higher values following testosterone than following methandrostenolone (Table 2) because the dry mass of connective tissue (*D*, Fig. 1) is affected in different ways by the hormones which nevertheless otherwise exert the same type of effect on the muscle cell.

The results of interferometric measurements of individual muscle fibres are very closely related to those of gravimetric determinations of muscle mass. Although the interferometric method is rather laborious, it makes it possible to quantitate certain cytological changes which may be encountered as a result of hormone treatment.

We thank Prof. F. Ruch of the Federal Institute of Technology, Zurich, for his advice and Dr. P. Barthe for his help in preparing the animals for the experiments.

¹ Kochakian, C. D., *Lab. Invest.*, **8**, 538 (1959).

² Desaulles, P. A., and Krähenbühl, C., in *Protein Metabolism*, edit. by Gross, F., 170 (Springer, Berlin, 1962).

³ Scow, R. O., and Roe, jun., J. H., *Amer. J. Physiol.*, **173**, 22 (1953).

⁴ Gori, Z. M., and Pellegrino, C., *Third Europ. Reg. Conf. Electron Microsc.*, Prague, B, 83 (1964).

⁵ Davies, H. G., in *General Cytochemical Methods*, edit. by Danielli, J. F., 1, 55 (Academic Press, New York, 1958).

⁶ Szirmai, J. A., in *Protein Metabolism*, edit. by Gross, F., 45 (Springer, Berlin, 1962).

⁷ Liac, S., *J. Biol. Chem.*, **240**, 1236 (1965).

A PHAGOCYTIC FUNCTION OF HASSALL'S CORPUSCLES

By Dr. J. N. BLAU

Pathology Department, Guy's Hospital Medical School, London, S.E.1

IN *The Microscopic Anatomy of the Human Body in Health and Disease*, published in 1849, Hassall¹ first described and illustrated the corpuscles which bear his name. He quotes from a 'Prize Essay' entitled *A Physiological Essay on the Thymus Gland* by John Simon, F.R.S. (Renshaw, London, 1845), who examined thymus glands from animals of varying maturity and concluded from "the application of acetic acid to the histological section", that the cells of the corpuscles had a "great affinity to embryonic cells". The origin and function of these corpuscles remain unknown or under dispute, and as recently as 1964 Christianna Smith², an authority on thymic anatomy, wrote: "The weight of evidence as to the origin of typical Hassall's corpuscles favours the theory stated by Kingsbury in 1928, that they are the expression of growth within a confined space of an epithelium which has lost its surface function not dissimilar to the epithelial pearls of keratinizing epitheliomas."

During an investigation of the entry of proteins, colloidal and particulate materials into the thymus of guinea-pigs under normal and pathological conditions, it was seen that Hassall's corpuscles were larger when the gland had involuted following either local X-irradiation or systemic cortisone. Two days after injecting indian ink into the circulation, particles of carbon were visible in macrophages inside the thymus of control animals and in greater concentration in the treated group. In addition, carbon was evident in some Hassall's corpuscles, and it is this phenomenon that is the special purpose of this article.

Young immature albino guinea-pigs (Hartley strain) of both sexes, weighing 220–370 g and 7–10 weeks old, were

grouped into animals of comparable weight and sex. Each experimental animal had its own control. The thymus was caused to involute either by local X-irradiation or by systemic injection of cortisone.

Each animal was anaesthetized with intraperitoneal Standard Veterinary Pentobarbitone Sodium B.P. (Abbotts), laid on its back and the limbs secured. A lead screen covered the animal apart from a window, 4 cm × 5 cm, through which 300 r. of X-rays were directed to the ventral aspect of the neck. The irradiation constants were 40 r./min, 220 kV and half-value layer 1.4 mm copper. Five animals were irradiated, three of which were allowed to survive for 2 days and two for 7 days when they were killed. Five control animals were anaesthetized, not irradiated and killed at similar time-intervals. In the cortisone group, five animals received subcutaneous injections of cortisone acetate B.P. (Organon) twice daily; two had 2.5 mg for 5 days and three others had 5.0 mg for 7 days after which time they were killed. Five control animals had the same number of subcutaneous injections of sterile saline in the same volume, 0.1 and 0.2 ml.

Each animal, irradiated, cortisone treated or control, had one intracardiac injection of 0.1–0.15 ml./100 g body-weight of indian ink (Günther Wagner Pelikan Werke, Hanover, Batch C11/1431a), which consisted of 10 per cent carbon black with an average particle size of 290–500 Å, stabilized with 4.3 per cent fish glue, and contained 1 per cent phenol in water. The injections were given within 1 h of irradiation or at the commencement of the series of cortisone injections, with the exception of one

radiated animal and one control animal which were injected 2 days after the initial time and killed 1 h later.

The guinea-pig's thymus, which is situated in the neck, cannot be injured by intracardiac puncture.

The animals were killed with ether anaesthesia and the tissues immediately fixed in 10 per cent formal saline. Sections, 5-7 μ thick, were cut from each lobe of the thymus, a cervical and mesenteric lymph node, spleen and from either the lung or the liver. Sections stained with haematoxylin and eosin did not provide good contrast for the small carbon particles. For this purpose light staining with methylene blue or neutral red was better.

All the animals were seen to change to a slaty grey colour during the injection of indian ink, and to return to their normal colour within minutes. At post-mortem the spleen from each animal was intensely black and the liver darker than normal. Some lymph nodes were darkened in patches. The thymus glands from animals that had been exposed to X-rays or treated with cortisone were darker than those from control animals which were pink. The treated group showed varying degrees of diminution in size of the thymus, and both groups had occasional black streaks on their surfaces.

Histologically, the spleen from each animal contained very large quantities of carbon in the sinuses of the organ. Large amounts of carbon particles were also to be seen in all the livers examined (irradiated group) and a considerable amount in the lungs (cortisone group). The lymph nodes, one cervical and one mesenteric from each animal, contained carbon particles albeit to varying extents.

After irradiation, thymic lobules showed the 'inverted' relationship of cortex to medulla, the cortex staining less deeply owing to impoverishment of thymocytes and prominence of reticulum cells. Following cortisone treatment the cortex was narrower than in control animals and showed the 'pockmarked' appearance. These cavities contained macrophages. The thymus glands from all treated animals contained a large number of Hassall's corpuscles, their size at times reaching very large dimensions.

Particles of carbon were evident in blood-vessel walls inside the gland as well as between and on the surface of lobules. The uniformly small particles in the wall of blood vessels contrasted with their size in macrophages where they ranged from small to large and in many instances had aggregated into sizable masses (Figs. 1 and 3). These heavily laden macrophages were to be found in the capsule, cortex, cortico-medullary junction and the medulla. In the cortex they frequently occupied the

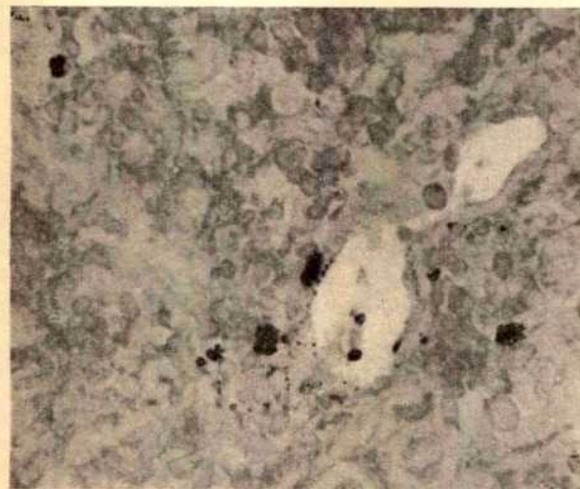


Fig. 1. Blood vessel at cortico-medullary junction from an animal treated with 2.5 mg cortisone twice daily for 5 days showing carbon in macrophages inside dilated vessel. Fine particles of carbon are seen in vessel wall and larger aggregates in macrophages adjacent to and away from the vessel (methylene blue, $\times 540$).

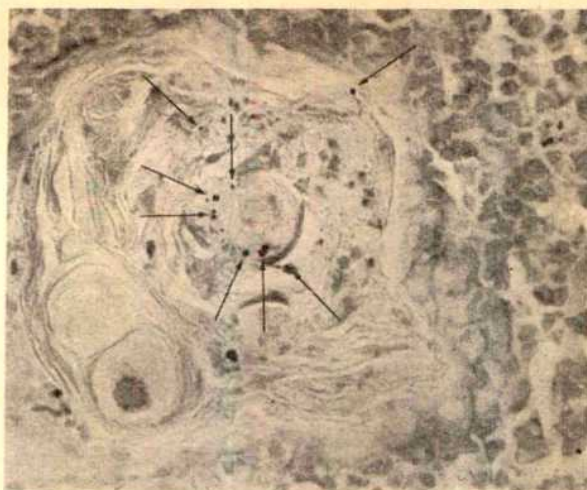


Fig. 2. Hassall's corpuscle containing carbon particles of varying sizes. Animal irradiated with 300 r locally over the thymus and indian ink injected intravascularly 7 days previously. Carbon particles, indicated by arrows, have to be distinguished from deeply staining nuclear debris (methylene blue, $\times 540$).

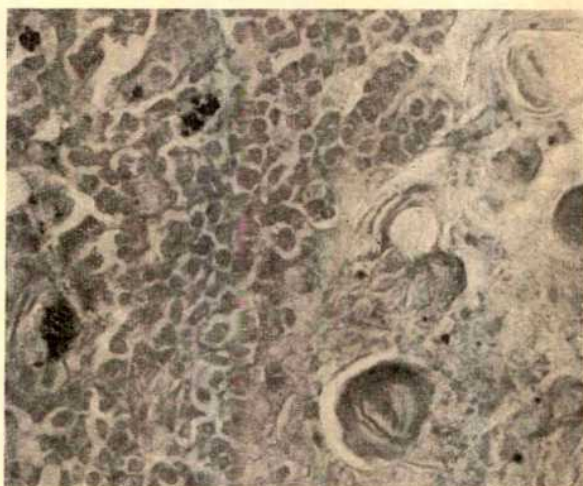


Fig. 3. From same animals as Fig. 2, showing the appearance of a 'compound' Hassall's corpuscle containing carbon. A heavily laden perivascular macrophage and 3 other macrophages not proximate to a vessel are visible (methylene blue, $\times 540$).

cavities of the 'pockmarking'. At the cortico-medullary junction where the blood vessels were dilated they were situated perivascularly. In the medulla some were close to, and others well away from, blood vessels. The concentration of carbon-bearing macrophages at the junction of the cortex and medulla was striking in some cortisone-treated animals and contrasted with the occurrence, often in patches, of solitary labelled macrophages both in the cortex and medulla in the irradiated group. In all the treated animals the quantity of carbon was much greater than in control groups, and comparison of the whole series showed that, the longer the time that had elapsed between injection of indian ink and death, the more carbon was present in the gland.

The Hassall's corpuscles consisted of a hyaline lamellated portion resembling a cut onion. Associated with this was a variable amount of deeply staining chromatin material consisting of small nuclear fragments, their differing shapes resembling polymorphonuclear leucocytes. In control animals this 'compound' corpuscle occurred far less frequently and was invariably smaller than in involuted glands. There they gave the appearance of having resulted from the fusion of two and often more corpuscles and were very large (Fig. 3).



Fig. 4. Hassall's corpuscle from an animal injected with 5 mg cortisone twice daily for 7 days. Carbon is seen outside, at the edge and inside the corpuscle (methylene blue, $\times 540$)

Carbon was rare in the lamellated portion of the corpuscle, but was evident in varying quantities and size of particle among the nuclear debris (Fig. 4). By no means did all the corpuscles in stimulated animals contain carbon, yet some control animals had a few of these 'compound' corpuscles which contained a little carbon, suggesting that involution was going on. At the periphery of a corpuscle a large cell with foamy cytoplasm was seen at times to contain one or more particles of carbon. Here as well as inside the corpuscle the variable size of the particulate matter corresponded to the variation found in macrophages (Fig. 4).

The striking morphological resemblance of Hassall's corpuscles to keratinized squamous epithelium has perhaps been one of the mainstays of the belief that they are vestigial remnants arising from epidermal invagination from the third and fourth branchial pouches. The human embryos on which Norris³ based his conclusions were examined by Ruth *et al.*⁴ in the Embryology Department of the Carnegie Institute, Washington. The latter stated that: "Norris is the only investigator who claims to have traced the development of these structures to ectoderm. His drawings are interesting but not convincing."

So far the views held on the origin of Hassall's corpuscles have obscured investigation of their potential active function. Thus, well-recognized observations of their increase in size following irradiation⁵ and systemic cortisone⁶ have been neglected. The increase in content of nuclear fragments noted in the corpuscles during involution of the thymus gland cannot be accounted for on the basis of an effete vestigial structure, neither can the presence of γ -globulin in the corpuscles of human infants, children⁷, normal adults and patients with myasthenia gravis⁸.

If Hassall's corpuscles are not vestigial structures, what then is their function? Kostowiecki⁹ argues that macrophages exist in the thymus and lists an impressive series of authors supporting his views. Green and Bloch¹⁰ showed that carbon particles appeared in macrophages in some new-born mice, Clark¹¹ confirmed the entry of ferritin in adult mice and Bailliff¹² found the thorotrast in the thymus of rats and mice. The experiments cited here, using carbon, confirm this in the guinea-pig, and other investigations with Evan's blue (unpublished) are also in agreement.

The suggestion that macrophages become incorporated and form Hassall's corpuscles has been made by Kostowiecki⁹, who found that following the intraperitoneal injection of Evan's blue the corpuscles were stained with dye. Clark¹¹, too, found this dye to be present in some cells which formed rudimentary corpuscles in mice.

The observations reported here demonstrated that particles of carbon are present in macrophages and in

Hassall's corpuscles if sufficient time is allowed to elapse after intravascular injection, and this effect was markedly accentuated if the gland was caused to involute. This function of the corpuscle has hitherto been hinted at, but not satisfactorily demonstrated for a number of possible reasons: use of the wrong animals, like rats and mice, where the corpuscles are rudimentary; use of normal animals in which the thymus gland is not involuting or involution is proceeding very slowly; failure to use a readily visible foreign particle; allowing insufficient time for the accumulation of foreign material in the gland. It is only the simultaneous presence of these four conditions which has made these observations possible. This may explain why in Marshall and White's experiments¹³ trypan blue and pneumococcal polysaccharide failed to enter the normal gland, yet gained entry when the gland was traumatized: hence their proposition of a blood-thymus barrier. Clark¹¹ supports the concept of a functional and partial barrier and the observations cited here would be in favour of such a barrier rather than an absolute one, since particulate matter, ferritin in mice and carbon in guinea-pigs, has been found in macrophages of unstimulated animals.

The mode of entry is almost certainly via the blood stream. In comparing sections from animals that had carbon injected 1 h, 2, 5 and 7 days before death, there were increasing quantities of particulate matter in macrophages situated perivascularly and well away from blood vessels. Hence a likely interpretation is that perivascular macrophages take up carbon from the walls of the blood vessels which they line, or possibly circulating macrophages migrate through these vessels, as is suggested by Fig. 1. The permeability of the small blood vessels of the thymus may well have been affected by irradiation and cortisone treatment, and certainly in the latter group vessels seemed more prominent. These perivascular macrophages might then migrate to the Hassall's corpuscles and contribute in their formation.

Whether this dynamic interpretation derived from a series of static histological sections, taken at timed intervals, is correct or not requires further evidence. However, if the definition of a reticulo-endothelial tissue is based on its ability to phagocytose foreign particulate matter, then the demonstration of carbon black inside Hassall's corpuscles must imply that they belong to this tissue system in the guinea-pig.

To what extent are these observations relevant to the behaviour of the thymus in man in health and disease? The illustrations of Hassall's corpuscles in children with involution of the thymus¹⁴ bear such close similarity to those seen in the guinea-pig after irradiation and systemic cortisone that one can see little, if any, difference. The presence of germinal centres in some patients with myasthenia gravis and in a strain of New Zealand mice¹⁵ implies that an antigenic stimulus has reached the organ. Furthermore, Marshall and White¹³ produced germinal centres in the guinea-pig thymus by direct injection of killed typhoid and paratyphoid bacilli into the exposed thymus gland. Hence it is possible that, if antigenic molecules reach the thymus via macrophages in sufficient concentration, this could provide the stimulus for the formation of germinal centres. The local conditions in the thymus which determine the formation of these centres awaits elucidation.

In conclusion, Hassall's corpuscles in the thymus glands of guinea-pigs have been shown to take up particles of carbon after the injection of indian ink into the circulation, especially when the thymus glands are undergoing involution caused either by X-irradiation or the systemic injection of cortisone. It is suggested that macrophages play an important part in the formation of Hassall's corpuscles which in certain circumstances have a phagocytic function and form a part of the reticulo-endothelial system.

This work was carried out with the aid of a personal grant from the Medical Research Council. I also thank (the

late) Prof. G. Payling Wright, Profs. S. J. DeNevasques and C. W. M. Adams for hospitality in the Pathology Department of Guy's Hospital Medical School, Prof. C. B. Allsopp and Mr. K. W. Twinn for X-irradiation, Dr. S. Leibowitz for the indian ink, Mr. Ronald Morgan for photography and Miss Lesley Kennedy for experimental assistance.

¹ Hassall, A. H., *The Microscopic Anatomy of the Human Body in Health and Disease*, 477 (Highley, London, 1849).

² Smith, C., in *The Thymus in Immunobiology*, edit. by Good, R. A., and Gabrielsen, A. E., 71 (Harper and Row, London, 1964).

³ Norris, E. H., *Contr. Embryol. Carneg. Inst.* 27, 191 (1938).

⁴ Ruth, R., Allen, C. P., and Wolfe, H. R., in *The Thymus in Immunobiology*, edit. by Good, R. A., and Gabrielsen, A. E., 183 (Harper and Row, London, 1964).

⁵ Murray, R. G., in *Histopathology of Irradiation from External and Internal Sources*, edit. by Bloom, W., 446 (McGraw-Hill, London, 1948).

⁶ Field, E. J., *J. Anat. (Lond.)* 90, 428 (1956).

⁷ Gitlin, D., Landing, B. H., and Whipple, A., *J. Exp. Med.*, 97, 163 (1953).

⁸ White, R. G., and Marshall, A. H. E., *Lancet*, ii, 120 (1962).

⁹ Kostowiecki, M., *Z. mikr.-anct. Forsch.*, 69, 585 (1963).

¹⁰ Green, I., and Bloch, K., *Nature*, 200, 1099 (1963).

¹¹ Clark, jun., S. R., in *The Thymus*, edit. by Defendi, V., and Metcalf, D., 9 (Wistar Inst. Press, Philadelphia, 1964).

¹² Bailliff, R. N., *Ann. N.Y. Acad. Sci.*, 88, 3 (1960).

¹³ Marshall, A. H. E., and White, R. G., *Brit. J. Exp. Path.*, 42, 379 (1961).

¹⁴ Siegler, R., in *The Thymus in Immunobiology*, edit. by Good, R. A., and Gabrielsen, A. E., 623 (Harper and Row, London, 1964).

¹⁵ Burnet, F. M., and Holmes, L. G., in *The Thymus in Immunobiology*, edit. by Good, R. A., and Gabrielsen, A. E., 656 (Harper and Row, London, 1964).

ANDROGEN-OESTROGEN-INDUCED TUMOURS: LACTIC DEHYDROGENASE RELATIVE TO CHAETEPITHELIOMA INDUCTION IN THE SYRIAN HAMSTER

By PROF. F. T. ALGARD

Department of Biological Sciences, Victoria University, British Columbia, Canada

AND

A. H. DODGE and PROF. H. KIRKMAN

Department of Anatomy, Stanford University School of Medicine, California

THERE is some presumptive evidence that neoplastic changes (induction) and subsequent progression from the benign to the malignant state may be accompanied by fluctuations in enzyme patterns. In view, especially, of the accumulating data suggesting a possible relationship between lactic dehydrogenase (LDH) and progression in certain tumours¹, this enzyme has been selected for an initial study on the histogenesis and growth of an androgen-oestrogen-induced, hormone-dependent epithelioma (chaetepithelioma) deriving from matrix cells of hair follicles in the flank organs (scent glands) of the Syrian hamster².

The chaetepithelioma is a particularly favourable tumour for use in such an enzyme investigation because of the essential role of sex hormones in the clear-cut succession of developmental stages³. Under endogenous androgen influence the tumour arises from epithelial cells migrating from hair bulbs into adjacent connective tissue. These cells organize the connective tissue into microscopic, laminated, epithelial-connective tissue complexes (stage 1) which, under the influence of exogenous androgen stimulation, enlarge to barely macroscopic, still preneoplastic stage 2 nodules. Stage 2 masses enlarge and fuse to form potentially malignant stage 3 neoplasms only following the addition of oestrogen to the androgen².

Initially this survey of LDH has been limited to tumour-bearing animals treated with both the sex hormones required for progression to ultimate malignancy. Tissue samples were taken at appropriate intervals during the life-history of the neoplasm for isoenzyme histochemistry using microscopic methods as well as acrylamide-gel electrophoresis.

Each histological tissue sample was sectioned serially at 20 μ on an International Harris cryostat. Both fresh and acetone-fixed sections from each sample were incubated for 10–20 min in 0.1 M DL-lactate LDH medium. Since available sulphydryl (S-H) groups are known to reduce nitro-blue tetrazolium, fresh sections were incubated in the standard LDH medium containing the S-H blocking agent, *N*-ethyl-maleimide. Each of these procedures was controlled by omitting the substrate from the incubation medium. Definitive tumour tissue (stage 3) was subjected to electrophoresis on acrylamide-gel strips according to Brody⁴. Urea (2.6 M concentration) was used to depress the slowest migrating isoenzyme⁵ in tissue sections.

LDH assessments were made on three groups of androgen-oestrogen-treated animals selected after periods

of treatment known to evoke the different stages of tumorigenesis², that is, 100–175 days for stage 1, 150–250 days for stage 2, 250 days or longer for stage 3. Histologically, the flank organ hair matrices of the short-term androgen-oestrogen-treated animals contain variable amounts of LDH ranging from slight to relatively heavy concentrations. Smaller, though less-variable, amounts of LDH occur in the outer root sheaths of the hair bulbs. Connective tissue components (dermal sheaths and papillae) always contain recognizable amounts. Laminated corpuscles (stage 1) contain recognizable quantities of LDH concentrated predominantly in the central core composed of epithelial cells. Little LDH is seen in the connective tissue laminae although scattered cells may contain as high a concentration as is present in any single cell in the central aggregate.

Stage 2 tumours, in which connective tissue components are reduced to a supporting stroma for cords of epithelial cells², contain greater concentrations of LDH than do stage 1 nodules, although considerable variation persists in different samples. With the transformation of stage 2 to stage 3 masses, the variability in amounts of LDH in different samples largely disappears and the concentration becomes distinctly greater.

Acrylamide-gel electrophoresis of stage 3 tumour tissue discloses four distinct bands of LDH. Of these, the most conspicuous is the slowest-moving isoenzyme, LDH No. 5 (ref. 4), with decreasing amounts of No. 4, No. 3 and No. 2. If the fastest-moving isoenzyme (No. 1) is present, its concentration is below the sensitivity threshold of this method. Attempts to isolate sufficient quantities of tissue from stages 1 and 2 for electrophoretic determinations have not yet been successful. These results are summarized in Figs. 1 and 2.

A 2.6 concentration of urea, which suppresses the slow-moving band (No. 5) (ref. 4) on acrylamide gel, is found in histological preparations to depress the LDH complement in the flank organ hair matrices, to completely inhibit the enzyme in stage 1 tumours, to depress the amount in stage 2 tumours to varying degrees and to diminish uniformly the reaction in stage 3 tumours. The order of magnitude of this latter inhibition is approximately 50 per cent which is roughly comparable to the percentage inhibition seen in the hair matrices.

It appears from this preliminary investigation that LDH diminishes during the initiation of stage 1 nodules, and that there is a gradual and variable synthesis of the

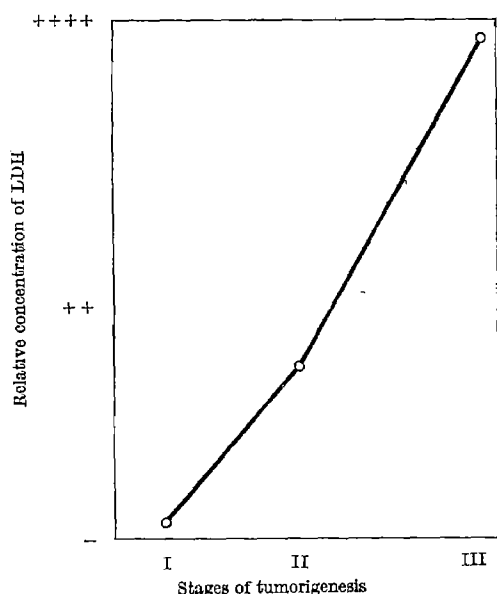


Fig. 1. Comparison of relative amount of LDH per section of the three tumour stages

enzyme, probably in more than one form, during the transformation of stage 1 to stage 2 masses and ultimately the marked elaboration of additional types and quantities of LDH during the development and growth of the definitive stage 3 tumour.

The full physiological significance of this investigation necessarily awaits the completion of similar studies of untreated males and females of varying ages, as well as animals treated independently with each of the two hormones. Of value, also, would be an investigation of the effect on types and levels of LDH in these tumours following hormone withdrawal. The correlation of hormone treatment with LDH synthesis during the life-history of this epithelioma may be physiologically significant, *in vivo*: whether there may be a direct influence on types and concentrations of LDH isoenzymes might, however, be best determined using *in vitro* techniques⁶⁻⁸. The possible

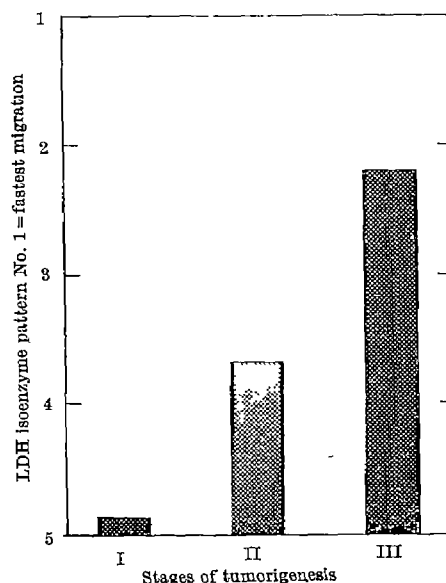


Fig. 2. Diagrammatic distribution of LDH isoenzymes in three tumour stages based on 2.6 urea inhibition investigations of sections and acrylamide-gel electrophoresis

involvement of viral LDH stimulating agents⁹⁻¹³ must await additional studies.

This work was supported by U.S. Public Health Service grants CA-04516-06, CA-02791-09 and Canadian National Research Council grant A2351. We thank Dr. R. L. Hunter for advice and for laboratory facilities.

- ¹ Goldman, R. D., Kaplan, N. O., and Hall, T. C., *Cancer Res.*, **24**, 889 (1964).
- ² Kirkman, H., and Algard, F. T., *Cancer Res.*, **24**, 1569 (1964).
- ³ Kirkman, H., and Algard, F. T., *Acta Un. Intern. Cancerum*, **20**, 743 (1964).
- ⁴ Brody, A. I., *Nature*, **201**, 685 (1964).
- ⁵ Brody, A. I., and Engel, W. K., *J. Histochem. and Cytochem.*, **12**, 687 (1964).
- ⁶ Algard, F. T., *J. Nat. Cancer Inst.*, **26**, 557 (1960).
- ⁷ Algard, F. T., *J. Nat. Cancer Inst.*, **27**, 1493 (1961).
- ⁸ Algard, F. T., *Nat. Cancer Inst. Monog.*, **11**, 215 (1963).
- ⁹ Riley, V., Lilly, F., Huerto, E., and Bardell, D., *Science*, **132**, 545 (1960).
- ¹⁰ Yaffe, D., *Cancer Res.*, **22**, 573 (1962).
- ¹¹ Crispens, C. G., *J. Nat. Cancer Inst.*, **30**, 861 (1963).
- ¹² Plagemann, P. G. W., Gregory, K. F., Swim, H. E., and Chan, K. K. W., *Canad. J. Microbiol.*, **9**, 75 (1963).
- ¹³ Gottschalk, R. G., Grantham, H. H., and Miller, P. O., *Cancer Res.*, **25**, 919 (1965).

TITRATION CURVES DEPICTING CENTRAL STIMULANT, DEPRESSANT AND DUAL STIMULANT-DEPRESSANT ACTION OF GLUTARIMIDE HOMOLOGUES IN MICE

By DR. A. SHULMAN*, GLENDA M. LAYCOCK* and J. A. HENRY
Department of Physiology, University of Melbourne

EVIDENCE has been presented from quantitative titrations in mice that certain analeptics (including bemegride) and hypnotics (including pentobarbitone sodium) act at common sites in the central nervous system^{1,2}. It has been proposed³, in terms of 'rate theory' of drug action³, that with respect to the complex formed with the responsive sites, an analeptic is an agonist with high association and dissociation rate constants (k_1 and k_2 , respectively) and thereby maintains a continued high rate of association with the sites which manifests as convulsion. On the other hand, a hypnotic has a low k_2 and prevents the action of the agonist by its prolonged association with the responsive sites. Analepsis occurs when the agonist maintains a sufficiently high rate of

association in the presence of hypnotic to overcome the depression caused by the latter. The action of these drugs has also been described¹ in terms of 'receptor occupation theory' of drug action⁴.

Independent support for these views has been obtained from structure/action investigations in mice with members of a homologous series of β, β -disubstituted glutarimides. It has been shown that β -methyl- β -ethylglutarimide (bemegride, 'Megimide'), β -methyl- β -n-propylglutarimide and β -methyl- β -n-butylglutarimide and β -methyl- β -n-amylglutarimide show the properties of agonist, partial agonist and antagonists respectively in the central nervous system; the partial agonist, which is intermediate in structure between agonist and antagonist, has dual stimulant and depressant action and, it is proposed, an intermediate k_2 . It is probable that these substances

* Full-time grantee of the National Health and Medical Research Council of Australia.

Table 1. MEDIAN CONVULSANT AND HYPNOTIC DOSES AND LEAST SQUARES REGRESSION LINES REPRESENTING TITRATION* BY PENTOBARBITONE SODIUM OF HOMOLOGOUS β,β -DIALKYLGLUTARIMIDES IN MICE

β,β -Di-alkyl glutarimide	Regression lines representing stimulant action	Regression lines representing depressant action	Median convulsant dose ($\mu\text{M/kg}$)	Median hypnotic dose ($\mu\text{M/kg}$)
Methyl, ethyl (bemegride)	$y = 0.2149x + 110.7$ (lower doses) $y = 0.0556x + 177.7$ (higher doses)	—	125.8 (123.0–137.6)†	—
Methyl, <i>n</i> -propyl	$y = 0.1027x + 112.9$ (lower doses)	$y = -0.0898x + 214.1$ (higher doses)	227.8 (227.1–228.5)†	2,232 (2,181–2,259)†
Methyl, <i>n</i> -butyl	—	$y = -0.0835x + 108.0$	—	1,840 (1,316–1,354)†
Methyl, <i>n</i> -amyl	—	$y = -0.1578x + 106.7$	—	676 (667–683)†

* The titration end-point is hypnosis in 50 per cent of a group of mice

† Estimated limits, $P = 0.05$

which differ only by the progressive addition of one methylene group to the β -alkyl substituent, and the hypnotic pentobarbitone sodium all act at common central sites which mediate both the stimulant and depressant actions of these drugs⁵.

If these views are correct, it may be anticipated that when progressively increasing doses of each of the aforementioned glutarimides are titrated against pentobarbitone sodium to an end-point of hypnosis in 50 per cent of a group of mice, the agonist will show only increasing analeptic activity, the partial agonist initial analeptic activity followed by potentiation of hypnosis, and the antagonist only increasing potentiation of hypnosis. Titration curves substantiating these views are shown in Fig. 1. In carrying out these titrations the titrants, either in aqueous solution (pentobarbitone sodium, bemegride and the *n*-propylglutarimide) or as a 1 per cent tragacanth suspension (the *n*-butyl- and *n*-amylglutarimides), were administered intraperitoneally in a temporal sequence such that their maximal effects occurred contemporaneously. The titration interval following drug administration was 10–15 min. Each titration point is the mean of at least two concordant estimations.

Theoretically, it is expected that the titration points for bemegride and its *n*-propyl homologue against pentobarbitone sodium take the form of hyperbolae⁶, but their relative analeptic potency has been estimated by fitting the points in each case by two highly significant least squares regression lines ($P < 0.001$) which intersect and show highly significant difference in slope ($P < 0.001$); each lower regression line intersects the hypnotic axis at a point non-significantly different from (0, HD_{50}) (Fig. 1 and Table 1).

It may be seen (Table 1) that bemegride is approximately twice as potent as its *n*-propyl homologue both as an analeptic to pentobarbitone sodium depression and as a convulsant. Their relative analeptic potency was calculated from the slopes of the lower regression lines, that is, where increasing doses of either glutarimide show

increasing antagonism to barbiturate depression, while their relative convulsive potency was calculated from the CD_{50} values (Table 1), estimated by plotting log. dosage of drug against probit percentage of mice which show mild clonic convulsion. By analogy with Paton's *in vitro* findings with homologous alkyltrimethylammonium cations⁷, it is likely that the k_1 values of bemegride and its *n*-propyl homologue are very similar and, hence, that the latter has a higher affinity (k_1/k_2) for the common responsive sites since, being a partial agonist, its k_2 is lower. It is also likely, judging by the close similarity in their structures and time courses of action, that a similar proportion of the peripheral dosages of the two glutarimides will reach the responsive sites during the titration interval. Consequently, it appears that k_2 of the drug-site complex is the cardinal factor determining the potency of these two drugs as stimulants in the central nervous system; this is to be expected from the assumption that k_2 limits the rate of dissociation of the drug from the responsive sites and hence its rate of association with the sites, and from Paton's definition of stimulant potency (k_2/k_1)⁸.

The inflexion in the bemegride–pentobarbitone sodium titration curve (Fig. 1) is considered to represent approaching saturation of the common sites for which the hypnotic has the greater affinity^{1,2}; a greater concentration of analeptic is required to attain the titration end-point in the presence of a hypnotic with a higher affinity when the drugs are present in excess with respect to the responsive sites than when the number of sites is adequate for maximal association of both drugs at the concentrations present. It is likely that the inflexion in the β -methyl- β -*n*-propylglutarimide–pentobarbitone sodium titration curve, in this case a change from a positive to a negative slope, also represents approaching saturation of the responsive sites since, in these circumstances, the stimulant action of the partial agonist would be expected to decline because its k_2 would be too low to permit the maintenance of an adequate rate of association when the number of free sites is limited.

The titration points of the *n*-butyl- and *n*-amylglutarimides against pentobarbitone sodium have also been fitted by least squares lines which show highly significant regression ($P < 0.001$) and which approximate very closely to the lines joining the estimated HD_{50} values of pentobarbitone sodium, 110.4 $\mu\text{M/kg}$ (109.2–111.0 $\mu\text{M/kg}$, $P = 0.05$), and β -methyl- β -*n*-butylglutarimide or β -methyl- β -*n*-amylglutarimide (Fig. 1 and Table 1). These HD_{50} values were estimated by plotting log dosage of drug against probit percentage of mice which lost righting reflex for at least 3 min.

Comparison of the slopes of the regression lines for the glutarimide hypnotics with that of the regression line for the higher doses of the partial agonist β -methyl- β -*n*-propylglutarimide shows that the *n*-amylglutarimide has approximately twice the depressant potency of its *n*-butyl homologue which is approximately equipotent to the *n*-propyl homologue (Table 1). However, the regression coefficient for the *n*-propylglutarimide gives a false

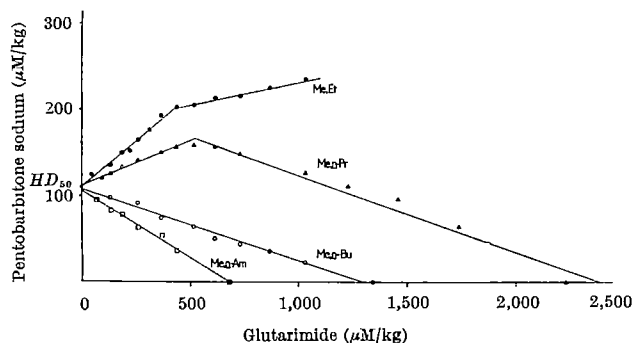


Fig. 1. Titration curves and least squares regression lines representing titration of pentobarbitone sodium by β -methyl- β -ethylglutarimide (Me,Et), β -methyl- β -*n*-propylglutarimide (Me,n-Pr), β -methyl- β -*n*-butylglutarimide (Me,n-Bu) and β -methyl- β -*n*-amylglutarimide (Me,n-Am). The titration end-point is hypnosis in 50 per cent of a group of mice. Each titration point is the mean of at least two concordant estimations

impression of its depressant potency since the regression line represents both decreasing anaesthesia, up to a dosage of 1,227 $\mu\text{M/kg}$, and potentiation of pentobarbitone-induced hypnosis at dosages beyond 1,227 $\mu\text{M/kg}$. A more accurate indication of the depressant potency of β -methyl- β -*n*-propylglutarimide is given by its HD_{50} , which was estimated by the same method already described here for the other glutarimides using a warm, near-saturated, aqueous solution (12 mg/ml.). A comparison of the HD_{50} values of the three glutarimides (Table 1) shows that β -methyl- β -*n*-amylglutarimide has approximately twice the depressant potency of its *n*-butyl homologue which is in turn approximately twice as potent as the *n*-propylglutarimide. This approximate two-fold difference in depressant potency for these drugs is similar to that shown for the stimulant potency of the ethyl and *n*-propyl homologues (Table 1) and probably reflects, primarily, a progressive fall in the k_2 of the drug-site complex representing, with each additional methylene group, the formation of a stronger bond between the glutarimide and the responsive sites.

It might be argued that potentiation of pentobarbitone sodium hypnosis by the higher doses of β -methyl- β -*n*-propylglutarimide results not from the action of the latter as a partial agonist but from its capacity to replace the barbiturate at common sites on plasma or tissue proteins or to decrease the rate of excretion, biotransformation or the extent of storage of the barbiturate, each of which would tend to increase the concentration of free hypnotic in the plasma for transfer to the responsive central sites. However, these possibilities seem unlikely since the *n*-propylglutarimide would need to be markedly more specific in these actions than its closely related ethyl, *n*-butyl and *n*-amyl homologues. The ethyl homologue, bemegride, is bound negligibly to plasma protein⁷ and shows continued anaesthetic activity against pentobarbitone sodium over a 117-fold range of anaesthetic dosage⁸, while the hypnotic *n*-butyl and *n*-amyl homologues produce no more than the expected additive depression in combination with the barbiturate.

Further, it has been demonstrated that whereas mice given β -methyl- β -*n*-propylglutarimide 500–600 $\mu\text{M/kg}$, in the absence of hypnotic, may die within 2–15 min of convulsion usually associated with maximal hindleg extensor tonus, animals receiving five times this dosage lose righting reflex within 2 min, following an initial burst of convulsion, and sleep without signs of stimulation for a period varying from 4 to 30 min. These mice wake with generalized hypertonia and excitement and show sporadic episodes of mild jumping and clonic movements the intensity of which decreases progressively until complete recovery some 3–4 h later. These observations suggest that β -methyl- β -*n*-propylglutarimide is a partial agonist which in high dosage can itself inhibit the maximal lethal convulsive action which it produces in a much lower dosage. Further, even though the drug is probably present for a considerable period at a concentration in excess of that necessary for maximal stimulation, it appears that the relationship between the k_1 and k_2 of the drug-site complex is such that the number of free responsive sites is never sufficiently high to maintain a rate of association greater than that commensurate with mild clonic convulsion.

Not only does β -methyl- β -*n*-propylglutarimide antagonize its own convulsant action but it also antagonizes that of its ethyl homologue (bemegride) and pentylenetetrazol. The *n*-propylglutarimide at a sub-hypnotic (1,800 $\mu\text{M/kg}$) or hypnotic dosage (Table 1) eliminates maximal hind-leg extensor tonus⁸ produced in mice by a 100 per cent effective intraperitoneal dosage of the ethyl homologue (290 $\mu\text{M/kg}$) or pentylenetetrazol (800 $\mu\text{M/kg}$); mice receiving 1,800 $\mu\text{M/kg}$ of the *n*-propylglutarimide show only mild stimulation and at times appear almost normal. Anticonvulsive action and potentiation of barbiturate depression by other compounds with stimulant properties

have been reported previously, for example, 5,5-diphenyl 2,4-diketooxazolidine and its hydantoin analogue ('Dilantin')⁹ and phenacyclidine and the related compound 'CI-581'¹⁰. Antagonism by high doses of stimulant action present in low doses has also been reported for the last-mentioned two compounds¹⁰.

As anticipated, β -methyl- β -*n*-propylglutarimide in low dosage (114 $\mu\text{M/kg}$, $0.5 \times CD_{50}$) potentiates the convulsive action of the ethyl homologue, decreasing its CD_{50} from 126.8 to 63.2 $\mu\text{M/kg}$ (62.7–63.7, $P = 0.05$); both drugs in a non-convulsive dosage ($0.5 \times CD_{50}$) are anaesthetic to the *n*-butyl and *n*-amyl homologues at their HD_{50} . On the other hand, a higher dosage of the *n*-propylglutarimide (1,800 $\mu\text{M/kg}$) potentiates the action of the HD_{50} of the hypnotic glutarimides.

The dual stimulant and depressant action of β -methyl- β -*n*-propylglutarimide and the form of its titration curve suggest that stimulation, manifesting as convulsion or anaesthesia, and depression, manifesting as anticonvulsive action and potentiation of hypnosis or hypnosis, are continuous phenomena. Thus, it is very likely that convulsion and hypnosis are the opposed extremes of a common physiological process occurring at the responsive central sites; the pure convulsive and hypnotic actions respectively of the ethyl and *n*-butyl homologues strongly support this contention. A hypothesis has been presented^{1,2} which suggests that convulsion and hypnosis produced by selected barbiturates, glutarimides and a variety of related drugs are the opposed extremes of physiological wakefulness occurring predominantly at excitatory synapses on reticular neurones concerned in arousal, and that anticonvulsive action is an intermediate state of depression. These views are consistent with those obtained from direct neurophysiological and neuropharmacological investigations¹¹ and with the ability of most anticonvulsive drugs to antagonize convulsion in low dosage and to produce hypnosis in higher dosage.

The relative affinities, association and dissociation rate constants and the central nervous system penetrabilities of pentobarbitone sodium, bemegride and other hypnotic and anaesthetic drugs¹ are being estimated (using the IBM 7044 computer) from titration curves of the type shown for bemegride and pentobarbitone sodium in Fig. 1 (ref. 6). These parameters, representing the action of the drugs at the common sites in the central nervous system of mice, will also be computed for the *n*-propyl, *n*-butyl, *n*-amyl and related glutarimide homologues from appropriate titration curves. It is hoped in this way to determine the progressive changes in the magnitudes of the association and dissociation rate constants which accompany the progressive addition of one methylene group to the β -alkyl substituent of the glutarimide ring. Similarly, changes in the penetrabilities of successive homologues can be examined and compared with changes in relevant physical characteristics.

It is suggested that the foregoing conclusions, concerning the central actions of β -methyl- β -*n*-propylglutarimide, and titrations of the type shown in Fig. 1 may be useful in elucidating the mode of action of other central nervous system stimulant and depressant drugs, especially those having apparently paradoxical actions. For example, these considerations have been applied to the central actions of a convulsant barbiturate and the anticonvulsant diphenylhydantoin sodium ('Dilantin'), and it seems possible that these substances, phenacyclidine and 'CI-581'¹⁰, a number of psychoactive drugs¹² and certain halogenated aliphatic ethers and paraffins¹³ also behave as partial agonists in the central nervous system and act at the same central sites common to the other drugs discussed in this article.

We thank Mr. R. Benn for his valuable assistance with the experiments and Nicholas Pty., Ltd., for their gift of the glutarimide drugs used in this investigation. This work was supported by the National Health and Medical Research Council of Australia.

- ¹ Shulman, A., *Proc. Roy. Austral. Chem. Inst.*, **31**, 41 (1964).
² Shulman, A., and Laycock, G. M. (unpublished observations).
³ Paton, W. D. M., *Proc. Roy. Soc.*, B, **154**, 21 (1961).
⁴ Symp. Drug Antagonism, *Pharm. Rev.*, **9**, 211 (1957); Stephenson, R. P., *Brit. J. Pharmacol. Chemother.*, **11**, 379 (1956). *Molecular Pharmacology*, edit. by Ariens, E. J., 1 (Academic Press, New York, 1964).
⁵ Laycock, G. M., and Shulman, A., *Nature*, **200**, 849 (1963).
⁶ Laycock, G. M., Shulman, A., and Werner, A. (unpublished observations).
⁷ Graham, J. D. P., and Nicholls, P. J., *Brit. J. Pharmacol. Chemother.*, **14**, 35 (1959).
⁸ Goodman, L. S., Grewal, M. S., Brown, W. C., and Swinyard, E. A., *J. Pharmacol. Exp. Ther.*, **108**, 168 (1953).
⁹ Butler, T. C., *Pharm. Rev.*, **2**, 121 (1950).
¹⁰ Chen, G., Ensor, C. R., Russell, D., and Bohner, B., *J. Pharmacol. Exp. Ther.*, **127**, 241 (1959). Domino, E. F., *Intern. Rev. Neurobiol.*, **6**, 303 (1964). McCarthy, D. A., Chen, G., Kaump, D. H., and Ensor, C., *J. New Drugs*, **5**, 21 (1965).
¹¹ Moruzzi, G., and Magoun, H. W., *Electroenceph. Clin. Neurophysiol.*, **1**, 455 (1949). Jouvett, M., *Arch. Int. Biol.*, **100**, 125 (1962). Brazier, M. A. B., in *Physiological Pharmacology*, 1, edit. by Root, W. S., and Hofmann, F. G., 219 (Academic Press, New York, 1963). Magoun, H. W., *The Waking Brain* (C. C. Thomas, Springfield, 1963). Moruzzi, G., *Harvey Lect.*, Ser. 58, 233 (1963). Yegishi, K., Bravo, M. C., and Verzeano, M., *Electroenceph. Clin. Neurophysiol.*, Supp. **24**, 90 (1963); Rossi, G. F., *ibid.*, Supp. **24**, 113 (1963).
¹² Chen, G., and Bohner, B., *J. Pharmacol. Exp. Ther.*, **123**, 212 (1958).
¹³ Lu, G., Ling, J. S. L., and Erantz, J. C., *Anesthesiology*, **14**, 466 (1953). Van Poznak, A., and Artusio, Jun, J. F., *Toxic. Appl. Pharmacol.*, **2**, 363, 374 (1960).

BREEDING A PELAGIC COPEPOD, *Euterpina acutifrons* (Dana), IN THE LABORATORY

By HEINZ W. NEUNES and GIAN-FRANCO PONGOLINI

Laboratorio per lo Studio della Contaminazione Radioattiva del Mare, Comitato Nazionale per l'Energia Nucleare—European Atomic Energy Community, Fiascherino (La Spezia), Italy

IN order to evaluate the uptake, distribution and turnover of radioisotopes in a marine ecosystem, it is highly desirable to evaluate results observed in the open sea in terms of experiments carried out under precisely controlled conditions in the laboratory. An essential prerequisite is therefore to have at hand a method for rearing relevant organisms in the laboratory.

Copepods form an essential element in the marine food chains. So far, however, laboratory work with pelagic copepods has always posed serious problems, for it has been impossible to rear them under culture conditions which permit them to reproduce.

Only recently, Zillioux and Wilson¹ succeeded in maintaining a culture of the coastal species *Acartia tonsa* through five generations, and Conover² had previously been able to raise eggs of *Calanus hyperboreus* to the adult stage; these, however, failed to reproduce.

The excessive growth of bacteria in the culture vessels is presumed to be one of the main factors for the difficulties; Conover² therefore added antibiotics to the water. In order to bind heavy metal ions present, Bernhard³ used EDTA and other complexing agents and was thereby able to overcome the difficulties in culturing sea urchin larvae.

Taking advantage of this experience, we have carried out breeding experiments with some species of pelagic copepods, especially with *Temora stylifera*, *Oncaea* sp. and *Euterpina acutifrons*, which are abundant in the Mediterranean. The copepods came from the laboratory's sampling zone (see Bernhard *et al.*⁴) in the Ligurian Sea, about ten nautical miles off the coast near La Spezia; the samples were taken in surface tows over a water depth of about 250 m, except for the individuals of *Euterpina acutifrons*, used to set up the first laboratory culture, which originated from the Gulf of La Spezia in the immediate vicinity of the coast.

Aboard the ship, the plankton samples were diluted with natural sea-water and kept in 5-l. polyethylene bottles to which 50 mg/l. penicillin was added. The samples were then stored at 18° C. During the 20 min required to transport the plankton from the quay to the laboratory, no further precautions were taken to keep the temperature constant. Immediately on arrival, the copepods were transferred to culture vessels and kept in a room thermostatically controlled at 18° ± 1° C.

Natural sea-water served as basis for the culture media in all cases. EDTA in a concentration of 37 mg/l. (= 10⁻⁴ mol) was added as a complexing agent. As antibiotics, penicillin and streptomycin were used alternately; two different concentrations were tested: 50 mg/l. and 6.5

mg/l. The control media contained EDTA but no antibiotics.

A suspension of a mixture of the flagellates *Tetraselmis micropapillata*, *Dicrateria* sp. and *Platymonas* sp., a dinoflagellate *Gymnodinium* sp. and the diatom *Phaeodactylum tricornutum* was added as food. The culture medium was changed at regular intervals, using a siphon fitted with nylon gauze to prevent the nauplii from escaping; the intervals ranged from 1 week to 1 month according to the experiments.

The copepods were kept either individually as 'single cultures' in Boveri capsules with a capacity of 30 ml. or in groups of more than ten individuals as 'mass cultures' in Erlenmeyer flasks ranging in capacity from 2 l. to 10 l. The flasks were filled to half capacity or less.

In preliminary survival experiments with single cultures of *Temora stylifera* and *Oncaea* sp., two concentrations of antibiotics were compared: 6.5 mg/l. penicillin, in weekly alternation with 6.5 mg/l. streptomycin, gave better results than either the controls or the higher concentration of 50 mg/l. which was recommended by Conover².

The superiority of the lower concentration of antibiotics was significant at the 95 per cent level for *Temora* ($F = 3.73$ at *d.f.* 2 and 28). For *Oncaea*, although it also survived longer in the lower concentration, the difference was not statistically significant ($F = 1.71$ at *d.f.* 2 and 34). A mixture of various zooplankton species in a mass culture lived longer in the lower concentration of antibiotics than in the higher concentration and in the controls.

The original concentration of 50 mg/l. antibiotics appears to have some detrimental effect on the vitality of the copepods, which effect is reduced or eliminated by using a lower concentration. As to the effect of the penicillin medium on the bacteria, it should be kept in mind that an aqueous solution of penicillin is very unstable in sea-water. According to Korzybski and Kurylowicz⁵, a penicillin solution at pH 5 at 0° C loses about half its activity within 8 days; in weak alkaline solutions, such as sea-water (pH 8), and also at higher temperatures, the process of inactivation requires considerably less time.

In order to evaluate under these conditions the effect of a penicillin solution on the bacterial growth in copepod cultures, an experiment was set up in which periodic counts of bacteria were made.

Three different media were tested: (a) penicillin medium (6.5 mg/l. penicillin + 37 mg/l. EDTA), (b) sterilized sea-water (autoclaved for 20 min at 110° C, + EDTA), and (c) control medium (natural sea-water + EDTA). Two Boveri capsules were filled with each medium and five

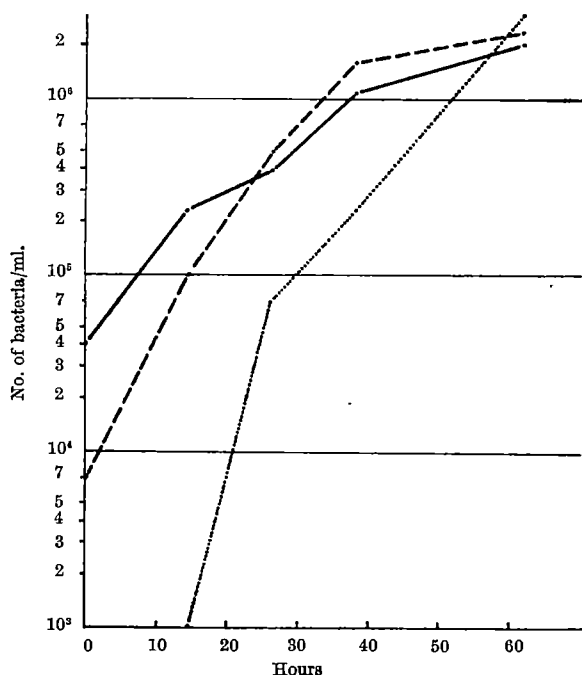


Fig. 1. Bacterial growth in copepod cultures, set up with different media: —, natural sea-water; ---, penicillin solution (6.5 mg/l.);, sterilized sea-water. EDTA was included in all media

copepodites of *Euterpina acutifrons* added per capsule. The serial dilution method was used for the bacteriological tests; the diluted samples were then poured on "peptone agar" plates (Oppenheimer and ZoBell⁶) and the developing colonies were counted in due course. Samples for the bacteriological counts were taken from the cultures at 0, 14, 26, 38 and 62 h. The results are given in Fig. 1.

The results confirm that an appreciable difference in the number of bacteria can be noted in the first 38 h only. At zero time, the number of bacteria in the penicillin medium was 18 per cent of that of the controls, at 14 h about 40 per cent; after 26 h the numbers of bacteria in the penicillin medium and in the controls were about equal. In the sterilized medium which had been contaminated with bacteria by the copepods, the bacteria needed 62 h to reach the level of the controls. A parallel experiment with mass cultures of zooplankton showed a longer survival time in the penicillin medium, but shorter in sterilized sea-water.

The fact that the penicillin medium has a bacteriostatic effect during a period of less than a day may well be of vital importance, because it is possible that the resistance of copepods against excessive bacterial growth is extremely low during the initial period in which they are probably in a weakened condition after having undergone the straining procedures of collection, the transportation to the laboratory and finally the transfer by means of a pipette into the culture vessels.

The shorter survival-time in sterilized sea-water may be due to chemical changes effected by the high autoclaving temperature.

On the basis of these findings, the lower penicillin concentration (6.5 mg/l.) was adopted for standard culture medium.

The first species of which we obtained nauplii in the laboratory was *Euterpina acutifrons* (Dana), a pelagic copepod of the sub-order Harpacticoida. The nauplii, hatched from the eggs of females caught near the coast on August 22, 1963, were transferred into a bottle with 3 l. of standard culture medium. Initially, the medium was changed weekly. The population established itself but remained at a very low density. When, however, the medium was renewed at monthly intervals, the population

density increased rapidly. The population remained in the same vessel for one year. The masses of live and dead algae and detritus collecting at the bottom were never removed and did not have any obvious detrimental effect on the culture. In August 1964, the population was divided and distributed into several new containers.

The population was examined every 4 days over a period of several months. Young naupliar stages were always predominant, which suggests a rather high larval mortality.

Generally, the density was between 1,000 and 3,000 individuals per litre, with rather marked fluctuations. But high values such as the following have been observed: 500 adults, 2,000 copepodites, 2,500 nauplii IV-VI, 9,000 nauplii I-III. These numbers were calculated for 1 l. on the basis of replicate samples of 5 ml.

In order to determine the time required for the completion of one generation, the life history of single individuals was observed. Females with eggs were transferred from the laboratory population into Boveri capsules. On the following day, the nauplii which had hatched in the meantime were transferred singly into covered salt dishes containing about 3 ml. standard culture medium. Observations were made daily and the moulted skins were counted and removed. The mortality was high: only 22 out of 50 nauplii survived to the first copepodite stage and only two of those reached the adult stage. Another experiment was therefore set up with 50 nauplii of various stages taken directly from the laboratory population. Of these, 12 developed into adults. Table 1 gives a summary of the results, combining the two experiments. At 18° C the resulting average time for the completion of larval development (egg to adult) is between 17 and 18 days. This result is confirmed by another observation: in a population started anew with ten females bearing eggs, the first F_1 -adults appeared after 14 days and the first F_2 -nauplii after 17 days.

Table 1. DURATION OF LARVAL STAGES IN SINGLE CULTURES OF *Euterpina acutifrons* AT 18° C

	N	Length of larval period (days)						Overall av. (days)
		0	1	2	3	4	5	
Nauplius	I	57	44	12	1			1.3+0.5*
	II	38	23	14	1			1.4
	III	30	1	19	9	1		1.3
	IV	26		17	7	1	1	1.5
	V	16		18	8			1.5
	VI	23		22	1			1.0
Copepodite	I	23		11	10	2		1.6
	II	22		10	9	3		1.7
	III	22	1	8	6	6	1	1.9
	IV	20	1	7	11	1		1.6
	V	14		2	8	3	1	2.2
								Total 17.5

* To the mean time length of the naupliar stage I, 0.5 days were added, because at the time 0, the nauplii were between 0 and 24 h old.

This is not very different from records of other copepod species reared under similar conditions. The non-pelagic *Tigriopus brevicornis* needs 19.5 days on the average to grow from egg to adult (own observation), and *Acartia tonsa* has a generation time (egg to egg) of 21-30 days at 17° C (Zillioux and Wilson¹).

If we assume a period of about 3 days for the time between emergence as an adult and hatching of its first eggs, a generation of *Euterpina*, under the conditions described, averages 20-21 days. On the basis of this average, we can assume that the population, which is now 18 months old, at present should contain individuals which belong to the 27th generation.

Originally it was presumed that pelagic populations of *Euterpina* might be less resistant to culture conditions than the coastal one which was used to set up our first laboratory culture. Therefore, on November 28, 1963, and January 21, 1964, two new cultures were started with *Euterpina* collected in the open sea.

The resulting laboratory population performed just as well as the original one derived from a coastal population; there appeared to be no difference in their adaptability to culture conditions. One of the populations was later transferred to a culture medium without antibiotics with no apparent detrimental effects. It seems safe to assume that antibiotics are necessary only for the initiation of a laboratory culture.

It should be noted that *Euterpina acutifrons* is closely related to the non-pelagic genus *Tigriopus* which lives in tide pools and is very easy to maintain in the laboratory. It might be interesting to carry out culture experiments with other pelagic Harpacticoida as well, in order to determine whether a relatively high adaptability to culture conditions is a general feature of this group.

We thank Dr. U. Melchiorri-Santolini from our laboratory for his valuable help and advice in the bacteriological part of the work.

This work was carried out under association contract EURATOM-CNEN 024-63-BIAI.

¹Zilliox, E. J., and Wilson, D. F., 1964-, *Twenty-seventh Ann. Meet. Amer. Soc. Limn. Oceanogr.* (in the press).

²Conover, R. J., *Proc. Intern. Council. Expl. Sea, Symp. "Zooplankton Production"*, No. 11 (1961).

³Bernhard, M., *Pubbl. Staz. Zool. Napoli*, 29, 80 (1955).

⁴Bernhard, M., Boffi, V., Lucarelli, A., Rampi, L., Melchiorri-Santolini, U., and Benedetti, A., *Com. Naz. Energia Nucleare, Roma, RT/B10* (63), 8, 11 (1963).

⁵Korzybski, T., and Kurylowicz, W., *Antibiotica*, 772 (Gustav Fischer Verlag, Jena, 1961).

⁶Oppenheimer, C. H., and ZoBell, C. E., *J. Mar. Res.*, 11, 10 (1952).

OPTICAL ROTATORY DISPERSION OF A CHLOROPLAST PREPARATION

By Dr. BACON KE

Charles F. Kettering Research Laboratory, Yellow Springs, Ohio

OPTICAL rotatory dispersion (ORD) was used more than a century ago by Biot, Pasteur and others for the study of molecular structure¹. Its use on proteins dates back to the work of Hewitt² and Jessen-Hansen³ in 1927. During the interim period, rotation measurements have been limited in the main to a few wave-lengths where convenient light sources could be found. However, in the past decade ORD has been undergoing a renaissance, largely as a result of new applications it has found in the study of molecular structure, and it is now considered one of the most powerful methods for studying conformation and conformational changes in biological macromolecules.

The most significant use of ORD in protein work is probably the estimation of helix content of a protein and the detection of helix-coil transition taking place in proteins during denaturation. Another interesting area of molecular conformation studied by ORD is that found by Blout and Stryer⁴, who showed that Cotton effect can be induced in the absorption-band region of certain symmetrical dye molecules when they are complexed with a dissymmetric helical structure of a polypeptide. They also found that the Cotton effect reverses sign when the dye is bound to a polypeptide of an opposite helical sense. When the dye is bound to a polypeptide with random conformation, no Cotton effect is observed.

As ORD is sensitive to the conformation and conformational changes in proteins and to the effect of binding small molecules, we have investigated the rotatory properties of chlorophyll⁵ and other pigments in solution as well as in the native environment of the chloroplast, to gain some insight about the nature of binding of these pigments and to correlate it with the photosynthetic activity of the chloroplasts. This article presents some preliminary observations made on a chloroplast preparation.

The problem of binding of chlorophyll to lipoprotein has been investigated by many workers in the past, mainly through such optical properties as absorption and emission. Although the state of chlorophyll *in vivo* is probably radically different from that of isolated molecules in solution, the absorption peaks of the bound pigment usually display only a slight shift as a result of binding⁶. Studies of optical anisotropy have usually led to the conclusion of a textural or form anisotropy in the chloroplasts⁷⁻⁹. More recently, polarized-absorption and -emission measurements¹⁰ with *Euglena* chloroplasts and electric-dichroism measurements¹¹ with quantasomes have

indicated an orientation of part of the chlorophyll molecules in the chloroplasts.

Chloroplasts are known to be sites of photosynthesis. As revealed by electron microscopy, chloroplasts consist of laminated systems embedded in a matrix, or stroma. The lamellae and stroma are surrounded by a double membrane. The dark layers in the laminated system are where the chlorophylls and other pigments are concentrated. Considered chemically, the laminated system is a gigantic organized complex of lipoprotein and chromophores. However, the so-called chlorophyll-lipoprotein has never been isolated from the chloroplasts in pure form. When chloroplasts are broken, the dark regions are released in particulate forms called 'grana'. The lamellae appear to be made of sub-units which were first observed by Frey-Wyssling and Steinmann¹², and have recently been given the name of 'quantasomes'¹³. The quantasomes are oblate spheroids 200 × 100 Å. It has been suggested that the quantasomes probably represent the smallest functional units capable of carrying out quantum conversion and electron transport.

The chloroplasts used in this work were prepared from spinach leaves. Quantasome aggregates were prepared according to the procedure described by Park and Pon¹⁴, except that KCl was substituted for sucrose. Further rupture of the grana was achieved by sonication, and the lamellar fragments were fractionated and freed from stroma by differential centrifugation. ORD measurements were made in the Cary 'Model 60' spectropolarimeter, with cell compartments thermostatic at 24° C. Up to 600 mμ, the bandwidth was programmed at 3 mμ. Above 600 mμ, the bandwidth was manually set at 5 mμ to avoid excessive noise because of low light intensity.

ORD spectra of isolated chlorophylls *a* and *b* in diethyl ether over the visible and near ultra-violet regions have been reported recently⁵. Negative and positive Cotton effects were found to correspond to the major absorption bands in the red and blue regions, respectively. These Cotton effects indicate that the electronic transitions corresponding to the absorption bands are optically active. The Cotton-effect inflexion points correspond closely to the absorption peaks.

The ORD spectrum of the chloroplast suspension, shown in Fig. 1, shows considerably more complexity and intensity than that of the free chlorophylls. Cotton effects corresponding to almost all the absorption peaks of chlorophylls *a* and *b* are retained in the spectrum of the lipoprotein complex. For comparison, the ORD spectrum

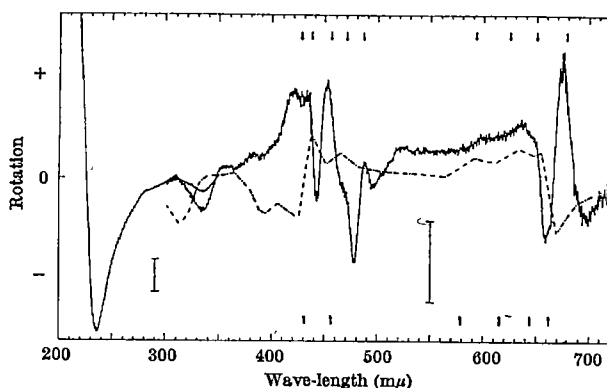


Fig. 1. ORD spectra of a suspension of the chloroplast lamellar fragments (recording trace) and an ether solution of chlorophylls *a* and *b* (dashed line). Both solutions contain the same amount of chlorophylls *a* (15 $\mu\text{g/ml.}$) and *b* (5 $\mu\text{g/ml.}$). Arrows at top indicate absorption peaks of the chloroplast suspension; arrows at bottom indicate absorption peaks of the chlorophyll solution in ether. The scale bars represent a rotation of 0.01°.

of an ether solution containing equivalent amounts of isolated chlorophylls *a* and *b* is drawn in the same figure in dashed lines. The pigment absorption peaks of the chloroplast suspension and those of the free chlorophylls in ether in the visible region are indicated by arrows at the top and bottom of the spectra, respectively.

ORD measurements of mixtures of chlorophylls *a* and *b* at various proportions showed that the magnitude of rotation is apparently additive. Consequently, the Cotton effects of the chlorophyll mixture in the red region become less sharp. On the other hand, in the chloroplast suspension, the negative Cotton effects corresponding to chlorophyll *a* (678.5 $\text{m}\mu$) and chlorophyll *b* (650 $\text{m}\mu$) are completely resolved and their magnitude greatly enhanced. The positive Cotton effects corresponding to the two subsidiary bands of chlorophyll *a* (624 and 594 $\text{m}\mu$) in the chloroplasts are also discernible.

The Cotton effects of the free chlorophyll solution in the Soret region are much better resolved, apparently because of a greater magnitude of the effects and a wider separation of the absorption peaks. However, in the chloroplasts, because of overlapping absorption by the chlorophylls and carotenoids, the assignments of the Cotton effects to the individual adsorption peaks between 400 and 500 $\text{m}\mu$ becomes difficult. This is especially true for the overlapping absorption between chlorophyll *b* (470 $\text{m}\mu$) and the carotenoid band at 455 $\text{m}\mu$ and between chlorophyll *a* (437 $\text{m}\mu$) and another carotenoid band at 428 $\text{m}\mu$. Based on the known absorption-peak position of the carotenoids, the strong negative trough near 480 $\text{m}\mu$ was tentatively designated as a Cotton effect originating from the carotenoids.

A pronounced negative trough with peak wave-length at 235 $\text{m}\mu$ was observed in the chloroplast preparation. This is similar to the 233 $\text{m}\mu$ trough first observed by Simmons and Blout¹⁵ in the protein sub-units of tobacco mosaic virus and later in various other polypeptides and proteins¹, and is believed to represent the $n-\pi^*$ transition in the peptide chromophore. The magnitude of the 233 $\text{m}\mu$ trough has been used as an approximate measure of the α -helix content¹⁶ in a polypeptide or protein. By the same procedure of calculation¹⁶, and assuming the helical protein in the chloroplast lamellae to be a right-handed α -helix, the magnitude of the 'reduced mean residue rotation' corresponded to a helical content of the order of 15 per cent in the lamellar protein.

Our present knowledge about the nature of protein and lipids in the chloroplast lamellae is rather limited. Although the amino-acid composition of whole-leaf and chloroplastic proteins has been reported¹⁷, the amino-acid sequence and protein conformation remain to be determined. Several earlier studies^{9,18,19} reported the absence of ultra-violet dichroism in chloroplasts, and conse-

quently Goedheer⁹ and Ruch¹⁸ proposed that the chlorophyll molecules were complexed with spherical protein molecules and that the pigment molecules were only slightly oriented. However, drawing analogies from the lamellar structure in the para-crystalline phase of poly- γ -benzyl-L-glutamate¹⁹, it was suspected that chloroplast lamellae might also be composed of long parallel helices, with the pigment orientation governed by the available attachment sites. The low helical content found here was indeed contrary to expectation. However, the identification of the presence and the amount of α -helices in the chloroplast lamellae protein has lent a definitive character to the structural protein in the chloroplasts and furnished a conformational basis for any orientational effect on the pigments by the protein. There is no evidence that there might be a mixture of right- and left-handed helices present in the chloroplast lamellae. However, such a possibility should not be completely ruled out without further investigation.

The enhancement of the intrinsic Cotton effects of chlorophylls and carotenoids complexed with the lipoprotein suggests a strong interaction either between the pigment molecules themselves or between the pigment molecules and the attached macromolecules. The enhancement of rotation in the carotenoid absorption region appears to be especially strong. Although the xanthophylls present in spinach (the oxygen-containing derivatives of α -carotene) are known to be optically active, examination of a hexane extract containing ten times the combined carotenoid concentration of the chloroplast sample yielded only negligible rotation, mostly buried in the noise. The strong rotation in the carotenoid absorption region suggests a possibility of an orientational effect by the lipid matrix, resulting in either an induced Cotton effect in the carotenoids including the optically inactive β -carotene or an enhancement of rotation of the intrinsic optically active xanthophylls. Presumably the lipid matrix could also exert an orientational effect on the chlorophylls through the phytol chain. A definitive clarification to these suggestions obviously requires further investigation. ORD measurements appear to offer new avenues for such studies.

After the completion of this work, I learned the results of a similar study on quantasomes by K. Sauer²⁰. It has been found that isolated chlorophyll *a* at a high concentration in carbon tetrachloride exhibits profound changes in its ORD spectrum. Sauer attributed such enhancements to chlorophyll aggregation and felt that a similar pigment-pigment interaction could also account for the enhanced ORD spectrum observed in quantasomes.

¹ Yang, J. T., in *Newer Methods of Polymer Characterization*, 103, edit by K. B. (Interscience, New York-London, 1964).

² Hewitt, L. F., *Biochem. J.*, **21**, 216 (1927).

³ Jessen-Hansen, H., *C.R. Lab. Carlsberg, Ser. Chim.*, **16**, No. 10 (1927).

⁴ Blout, E. R., and Stryer, L., *Proc. U.S. Nat. Acad. Sci.*, **45**, 1591 (1959).

⁵ Ke, B., and Miller, R. M., *Naturwiss.*, **18**, 436 (1964).

⁶ Kupke, D. W., and French, C. S., in *Encyclopedia of Plant Physiology*, by Ruhland, W., **5**, pt. 1, 298 (Springer Verlag, 1960).

⁷ Menke, W., *Kolloid-Z.*, **85**, 256 (1938).

⁸ Frey-Wyssling, A., and Steinmann, E., *Biochim. Biophys. Acta*, **2**, 259 (1948).

⁹ Goedheer, J. C., thesis, University of Utrecht (1957).

¹⁰ Olson, R. A., Butler, W. L., and Jennings, W. H., *Biochim. Biophys. Acta*, **54**, 615 (1961); **58**, 144 (1962).

¹¹ Sauer, K., and Calvin, M., *J. Mol. Biol.*, **4**, 451 (1962).

¹² Frey-Wyssling, A., and Steinmann, E., *Vjschr. Naturf. bes. Zürich*, **98**, 20 (1953).

¹³ Park, R. B., *J. Chem. Ed.*, **39**, 424 (1962).

¹⁴ Park, R. B., and Pon, N. G., *J. Mol. Biol.*, **3**, 1 (1961).

¹⁵ Simmons, N. S., and Blout, E. R., *Biophys. J.*, **1**, 55 (1960).

¹⁶ Simmons, N. S., Cohen, C., Szent-Györgyi, A. G., Wetlauffer, D. B., and Blout, E. R., *J. Amer. Chem. Soc.*, **83**, 4766 (1961).

¹⁷ Menke, W., *Ann. Rev. Plant Physiol.*, **13**, 27 (1962).

¹⁸ Ruch, F., *Exp. Cell Res.*, Supp. **4**, 58 (1957).

¹⁹ Olson, R. A., Jennings, W. H., and Butler, W. L., *Biochim. Biophys. Acta*, **88**, 319 (1964).

²⁰ Sauer, K., *Proc. U.S. Nat. Acad. Sci.*, **53**, 716 (1965).

LETTERS TO THE EDITOR

ASTROPHYSICS

Rotational Period of the Planet Mercury

In a recent communication by S. J. Peale and T. Gold¹ the rotational period of Mercury, determined from radar Doppler-spread measurements to be 59 ± 5 days², has been explained in terms of a solar tidal torque effect, taking into account the large eccentricity of Mercury's orbit, and the $1/r^6$ dependence of the tidal friction (r being the Sun-planet distance). They conclude from a very brief discussion that after slowing down from a higher direct angular velocity, the planet will have a final period of rotation between 56 and 88 days, depending on the assumed form of the dissipation function. However, from their discussion it is by no means clear why permanent deformations would imply a period of 88 days as a final rotation state after a slowing-down process. A very nearly uniform rotational motion of 58-65 sidereal-day period, that is $2/3$ of the orbital period, may indeed be a stable periodic solution. This rotational motion could have the axis of minimum moments of inertia nearly aligned with the Sun-Mercury radius vector at every perihelion passage. The orbital angular velocity at perihelion ($2\pi/56.6$ days) is close to $2\pi/58.65$ days, leading to an approximate alignment of the axis of minimum moment of inertia with the radius vector in an arc around perihelion where the interaction is strongest. The axial asymmetry of Mercury's inertia ellipsoid may result in a torque that counterbalances the tidal torque, giving a stable motion with this orientation and with a period two-thirds of the orbital period. It would therefore be possible for Mercury to have a higher permanent rigidity than that permitted by Peale and Gold.

In discussion with I. I. Shapiro³, we concluded that the actual rotational motion may have evolved via a speeding-up process from a lower angular velocity or possibly from a retrograde motion. We would point out that a 58-65-day period, precisely because it is $2/3$ of the orbital period, fits some of the old optical observations as well as the recent radar measurements.

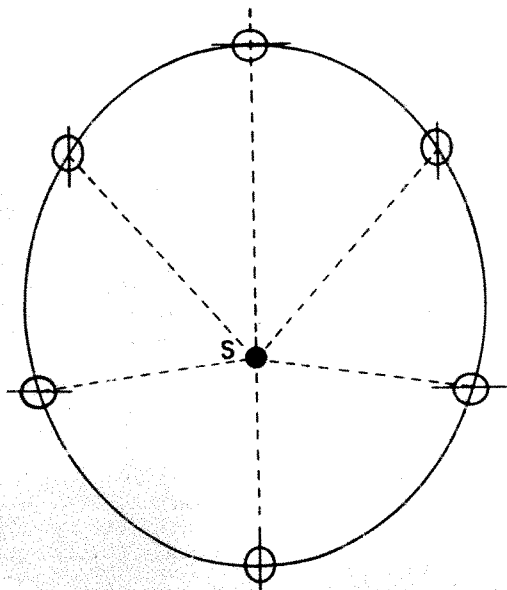


Fig. 1

In Fig. 1 a rough planar sketch is shown of the orientation of Mercury's axis of minimum moment of inertia, at different points along its orbit, given that the rotational period is two-thirds of the orbital period and that this axis is aligned with the Sun-planet vector at perihelion.

G. COLOMBO

University of Padova, Italy, and
Smithsonian Astrophysical Observatory,
Cambridge,
Massachusetts.

¹ Peale, S. J., and Gold, T., *Nature*, **206**, 1241 (1965).² Pettingill, G. H., and Dyce, E. B., *Nature*, **206**, 1240 (1965).³ Shapiro, I. I. (personal communication).

ASTRONOMY

Filamentary Nebulosity in the Vicinity of the Cetus Arc

FORMING large diameter circles across the sky are three ridges of enhanced radio emission. They are the North Polar Spur¹, 111° in diameter, the Cetus Arc², 91° in diameter, and a third, unnamed, 60° diameter loop centred on the northern celestial pole³.

The radio continuum emission from these features is identical to that from the old supernova remnants. However, the nature of the 60° diameter loop is complicated by its correlation with twelve high-velocity neutral hydrogen clouds⁴. It has therefore been suggested that two, if not the third, of these objects are the remnants of nearby supernova explosions.

The principal difficulty to the acceptance of this theory has been the lack of detection of any optical emission regions coincident with these loops^{5,6}. All the accepted supernova remnants have characteristic filamentary nebulosity bounding their radio regions.

Such nebulosity has recently been searched for, on these three radio sources, in a photographic programme carried out at the high-altitude observatories of Jungfraujoch and Pic du Midi. Photographs were taken with small, high-speed camera and filter combinations which had low detection limits for emission nebulosity.

A further effective method of detecting faint, large-scale nebulosity was also used on this problem. In this, Palomar Sky Survey plates were copied at reduced scale and very high contrast. These were then compiled to form mosaics across the radio features. Faint density changes that were continuous through adjacent plates were considered to be caused by nebulosity.

The results produced by this work are as follows: (a) No filamentary nebulosity was detected on the North Polar Spur. However, a very diffuse optical emission region was shown to be present over this area. As yet it is impossible to say whether or not this is associated with the radio feature. (b) No filamentary nebulosity has been found on the 60° diameter loop. A search for diffuse nebulosity is now being undertaken. (c) It was on the Cetus Arc that the most interesting nebulosity was discovered. This has been sketched against the 237-Mc/s radio contours and presented in Fig. 1. The most striking single feature of this optical emission zone is the huge arc of nebulosity which bounds it. This is of filamentary micro-structure and has so far been traced for a length of 28° . Within this arc, towards the Cetus Arc radio ridge, there is a zone of diffuse elongated filaments. These merge with a structureless optical emission region as the radio

ridge is crossed. This very diffuse nebulosity has not been sketched, as its extent is ill-defined.

The nebulosity are from $\alpha = 4\text{ h } \delta = +13^\circ$ to $\alpha = 3\text{ h } 40\text{ m } \delta = -15^\circ$ is comparable, in position relative to the radio feature and in structure, to the optical emission regions associated with the Cygnus Loop and IC443 (refs. 7 and 8), both of which are accepted supernova remnants. However, a further experiment is required to test conclusively the suggestions that it is associated with the Cetus Arc and that these are the optical and radio remnants of the same supernova explosion; that is, direct comparison of the Cetus Arc with the Cygnus Loop suggests that an angular movement of the Cetus Arc nebulosity can be expected of $1''$ to $3''$ per annum. This motion should be occurring away from the centre of the circle described by the Cetus Arc if this comparison is correct. In this case, during the ten years since the Palomar Plates were taken, where this nebulosity appears, the total angular displacement should amount to $10''$ – $30''$. Such a displacement could be easily detected, for the fine filaments of the nebulosity have similar angular dimensions.

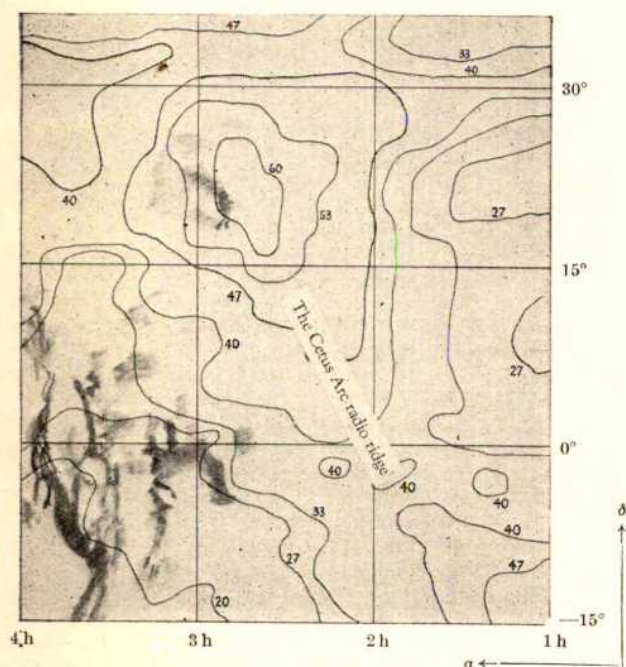


Fig. 1. The nebulosity is shown as a heavily shaded area. The continuous lines are the 237-Mc/s radio contours². Their brightnesses are given in degrees K.

This discovery of filamentary nebulosity bounding the Cetus Arc has strengthened the theory that this object and the other two similar radio features are all supernova remnants. However, the experiment to measure its rate of angular movement could, if positive results emerge, decide this issue conclusively.

J. MEABURN

Department of Astronomy,
University of Manchester.

¹ Brown, R. Hanbury, Davies, R. D., and Hazard, C., *Observatory*, **80**, 191 (1960).

² Large, M. I., Quigley, M. J. S., and Haslam, C. G. T., *Mon. Not. Roy. Astro. Soc.*, **124**, 405 (1962).

³ Quigley, M. J. S., Ph.D. thesis (1965).

⁴ Meaburn, J., *Nature*, **207**, 179 (1965).

⁵ Brown, R. Hanbury, Davies, R. D., and Meaburn, J., *Observatory*, **83**, 179 (1963).

⁶ Meaburn, J., submitted for publication in *Mon. Not. Roy. Astro. Soc.*

⁷ Minkowski, R., *Rev. Mod. Phys.*, **30**, 1048 (1958).

⁸ Hogg, D. E., *Astrophys. J.*, **140**, 992 (1964).

PHYSICS

Carathéodory's Principle and the Kelvin Statement of the Second Law

THE second law of thermodynamics has been stated in various ways. The two forms with which this communication is concerned are: (a) Kelvin's principle that it is impossible to transform an amount of heat completely into work in a cyclic process in the absence of other effects. (b) Carathéodory's principle that all points A in thermodynamic phase space are i -points¹; that is, in every neighbourhood of every point A there are points adiabatically inaccessible from A . It has already been shown that (a) implies (b)² and, in this communication, it is proposed to explain in what sense (b) implies (a).

The n -dimensional thermodynamic phase space may be thought of as having $(n-1)$ deformation co-ordinates v_1, v_2, \dots, v_{n-1} and one thermal co-ordinate, which will be taken to be the internal energy, U . By assuming the validity of Carathéodory's principle and the existence of adequate continuity conditions, it may be shown³ that all points in this space which are accessible from some given point P by quasi-static adiabatic processes lie on a single surface, and that P itself must lie on this surface. The argument may be repeated for different initial states P and the thermodynamic phase space is found to be decomposed into a family of non-intersecting surfaces. These are surfaces of constant entropy. Clearly, points lying in different level surfaces cannot be linked by quasi-static adiabatic processes.

In thermodynamic phase space, consider two points A and B ($U_B < U_A$) lying on a line of constant deformation (v_1, v_2, \dots, v_{n-1}). Since each line of constant deformation cuts each level surface in one point, the two points A and B lie in different level surfaces and, hence, cannot be linked by quasi-static adiabatic processes but, either: (i) A is accessible from B by a non-static adiabatic process, or (ii) B is accessible from A by a non-static adiabatic process, but not both. This result is a consequence of Carathéodory's principle and continuity assumptions which represent the basic premises of this communication.

Suppose (i) holds. The system may be taken from B to A by a non-static adiabatic process and the increase in internal energy is due to mechanical work W done on the system. With deformation co-ordinates fixed, the system may be returned from A to B . With the v_i constant, no work is done and the decrease in internal energy is due to heat $Q = U_A - U_B > 0$ having been removed. In this cycle, due to the conservation of energy, an amount of work is seen to be completely converted into heat.

Suppose (ii) holds. With deformation co-ordinates fixed, the system may be taken from B to A . With the v_i constant, no work is done and the increase in internal energy is due to heat $Q = U_A - U_B > 0$ having been supplied. The system may then be returned from A to B by a non-static adiabatic process and the decrease in internal energy is due to mechanical work W done by the system. Due to conservation of energy, it is seen that an amount of heat is completely converted into work in this cycle.

It follows, therefore, that for the cycles considered, either: (1) work cannot be completely converted into heat, or (2) heat cannot be completely converted into work. This multiplicity of possibilities is characteristic of conclusions based on Carathéodory's principle. Unless supplemented by additional assumptions, it leads to four possible types of thermodynamics, all similar in logical structure but distinct in detail¹.

To obtain Kelvin's principle, not only must adequate continuity assumptions be made but it must be assumed that any given cycle can be decomposed into a number of cycles of the type considered here and, in addition, that (2) rather than (1) holds. It is only in this sense that Kelvin's principle may be deduced from Carathéodory's

principle. Of these additional assumptions, the second is seen to be valid in the usual type of thermodynamics (referred to as type I in ref. 1), in which the absolute temperature is positive and heat tends to flow from high to low absolute temperatures. It is the absence of these additional assumptions which gives the converse argument², the deduction of (b) from (a), an attractive simplicity.

The form of Kelvin's principle applicable for negative temperatures states that in a cyclic process, in the absence of other effects, either heat cannot be completely converted into work or work cannot be completely converted into heat⁴. It may be noticed that, apart from the basic premises, only the assumption that any given cycle can be decomposed into cycles of the type considered here is needed to deduce this modified form of Kelvin's principle. It is only if one wants to go further and derive the conventional form (a) of the Kelvin principle that the additional assumption, that (2) rather than (1) holds, must be made.

I thank Prof. P. T. Landsberg for his advice and also for the preprint of a paper by Sears⁵, in which the converse of the argument in ref. 2 is discussed in an alternative way. This work was supported by a scholarship awarded by the Glamorgan County Council.

J. DUNNING-DAVIES

Department of Applied Mathematics,
University College,
Cardiff.

¹ Landsberg, P. T., *Thermodynamics with Quantum Statistical Illustrations*, 50, 68 (Interscience, New York, 1961).

² Landsberg, P. T., *Nature*, **201**, 485 (1964).

³ Born, M., *Natural Philosophy of Cause and Chance* (Oxford, 1949). Sears, F. W., *Amer. J. Phys.*, **31**, 747 (1963). Landsberg, P. T., *Bull. Inst. Phys. and the Phys. Soc.*, **15**, 150 (1964).

⁴ Ramsey, N. F., *Phys. Rev.*, **103**, 20 (1956).

⁵ Sears, F. W., *Carathéodory's Second Axiom and the Carnot Theorem* (to be published).

Ionizing Radiation: a Potential Lightning Hazard?

BENJAMIN FRANKLIN and Michael Faraday were until recently the only major contributors to the problem of preventing lightning damage to man-made structures. The 'Franklin Rod' and the 'Faraday Cage' systems both afford a more conductive path to earth for the lightning strike than that supplied by the structure they are intended to protect.

The electric charges generated in a thunder cloud are not distributed evenly throughout the cloud but form regions of higher intensity of electrostatic charge. It is from these regions to a region of high induced intensity of charge on the earth that the lightning discharge will tend to occur. This is because some points in this region of high intensity on the ground will liberate a stream of positive ions (known as the 'point discharge current'), resulting in a region of ionized and therefore more conductive air extending above this point. The most highly conductive path from cloud to earth is searched out by an initial low-voltage discharge; the so-called 'pilot streamer', which is followed by a second, fairly slow-moving discharge, the 'stepped leader'. This prepares a well-ionized path for the one or more main strikes which follow; these usually involve potentials of between 10^8 and 10^9 V and currents in the range of 20 to 90 thousand amperes.

The probability of any point being selected as the site of a lightning strike depends on many factors, but essentially these can be reduced to two: (1) The height of this point above other prominences in the region; (2) the magnitude of the point discharge current and thus the conductivity of the air above the point. While these factors determine the point in a region which will be struck, other factors, geographical, meteorological, etc.,

make some regions more prone to lightning strikes than others.

In the protection of man-made structures from lightning damage, the most important factor is usually considered to be the height of the building. In Great Britain it is found that churches are struck fifteen times more frequently than private dwelling-houses¹. The estimated frequency of lightning strokes to earth in England is about six per square mile per annum² of which approximately one quarter cause damage to persons or structures.

In recent years a new type of lightning protection device has been developed, and the fact that legislation governing its use has been passed under the 1960 Radioactive Substances Act would suggest that it is becoming widely used. The operation of this device is based on the ability of radioactive material to ionize the air surrounding it, the extent and degree of ionization depending on the activity and nature of the source. Suitably encapsulated radioactive sources are placed slightly higher than the roof of the building to be protected and are carefully earthed in the same way as the traditional devices. Any lightning strike entering the hemisphere of effective ionization caused by the source should be directed along the increasingly well-ionized path, strike the source itself, and be conducted harmlessly to earth. This hemisphere of effective ionization is arranged to cover the entire roof area of the building, and thus the building itself should never be struck as there will always be a more conductive path leading to the well-earthed source.

A commercially available type designed to afford a hemisphere of protection of radius 100 m contains 708- μ c. radium-226. The radiation dose rate at 100 m from a radium source of this size will not be greater than 0.00006 mr./h, and may well be less, due to absorption in the encapsulating material and also self-absorption in the source itself. This extremely small dose rate is considered to produce sufficient ionization to make this path to earth the most likely to be chosen by the lightning, and experiments carried out by the manufacturers are claimed to substantiate the effectiveness of this method. If this is so, the potential hazard due to the lightning-attractive properties of radioactive sources used for medical and industrial purposes would not appear to be widely recognized. This could result in a structure housing a source of ionizing radiation, and therefore presumably a particularly attractive site for a lightning strike, being totally unprotected against this occurrence.

Possibly persons using radioactive material for medical and industrial purposes are not aware of the claims made for the lightning-attractive powers of sources giving small radiation dose rates, or perhaps this aspect of a source used primarily for a totally different purpose has not been considered.

It is of interest to consider the case of a hospital in which kilocurie sources of γ -emitting radioisotope, for example, cobalt-60, are used for radiotherapeutic purposes. Therapy units containing these large sources are commonly installed in single-storey buildings, which are normally not fitted with any earthing system for lightning. The main radiation beam from a rotational therapy unit is frequently directed towards the roof of the therapy room for a percentage of the total treatment time. The attenuation produced by the roof is often minimal (for example, a roof light of ordinary glass) as the radiation scattered from the emergent beam is not considered to constitute a hazard to personnel at ground level.

A typical 3,000-c. cobalt-60 source gives a dose rate of approximately 125 r./min at 65 cm from the source. Allowing for absorption in the patient's body through which the beam passes, a rough calculation reveals that a dose rate of 6×10^{-5} mr./h (that is, the dose rate produced by the lightning protective device at its effective range) will still occur at a height of 62 miles. When the radiation beam is not directed upwards, only scattered radiation emerges through the roof. If one assumes that the intensity of this

scatter is only a ten-thousandth of that of the main beam, this still corresponds to a height of more than half a mile before the dose rate falls below that considered effective to attract the lightning strike to the source.

Many industries use radioactive sources giving local dose rates far in excess of 6×10^{-5} mr./h, and indeed I am aware of such a source used as a density gauge mounted on the upper section of an outdoor chemical plant synthesizing highly inflammable and explosive liquids.

In view of the foregoing, one would expect frequent reports of radiotherapy centres, industrial radiography departments, etc., being damaged by lightning; in fact, the search for reports of such occurrences has revealed only a vague rumour of this having happened once to a radiotherapy centre in the United States.

Must one therefore conclude that: (a) small sources of ionizing radiation are not effective as lightning attractors? or, (b) radiotherapy centres, etc., rarely operate during thunderstorms, and have, so far, been very fortunate?

This is a question to which some answer must be found, as at present neither of the two logical alternatives is satisfactory: either buildings 'protected' by these radioactive devices are in fact not actually protected and may well suffer lightning damage; or, sooner or later, a radiation beam will attract a lightning strike to the source of a tele-isotope unit and, unless this is as effectively earthed as a proper lightning conductor, the effects could be catastrophic. Energies of more than 5×10^{10} watts are usually involved and, apart from structural damage and the possible rupture of the sealed radioactive source, with resultant contamination, one hesitates to contemplate the fate of the unfortunate patient undergoing treatment.

P. J. GILLESPIE

Northern Ireland Radiotherapy Centre,
Montgomery House,
Purdyburn, Belfast, 8.

¹ Thunderstorm Census Organization (personal communications).

² Schonland, Sir Basil, *The Flight of Thunderbolts* (Clarendon Press, 1950).

GEOLOGY

Observations on the Mont Pelée Eruption of 1902 and their Significance to Ordovician Welded Tuffs of County Mayo, Eire

DURING recent investigation of some welded tuffs in the Ordovician rocks of eastern Murrisk, County Mayo, lenses of sillar, non-welded material were encountered at the base of the tuff-horizons. Similar layers had previously been reported by Stanton¹ and Dewey² during investigations farther west. Stanton¹ interpreted the variation in thickness of the sillar material as due to differences in the depth of sea-water present before the tuff built up to sea-level, prior to it becoming a truly welded tuff. The entire process was regarded as a 'continuous whole'.

A letter written by the British Consul in St. Pierre on May 3, 1902, some five days before the Peléan eruption which destroyed the town and its inhabitants, has been brought to my attention by Mr. R. A. Japp, of Dundee, together with samples of the early ashes produced during the eruption. With permission, an extract from this letter is relevant:

"I wrote you about the volcano smoking last week. Yesterday afternoon it began to throw out enormous clouds of steam and black smoke from a different place.

"About one this morning I was awakened by a roaring noise, something like thunder and lightning. . . . The houses and street were covered with dust . . . white, as if there had been a snow-storm.

"The dust continued to fall until there was a layer of $\frac{1}{4}$ inch, and is still falling a little. It is all very fine (I enclose sample), and as we have no glass, but only

'jalousie' windows, it drives in and penetrates everywhere . . .

"The volcano is 4 miles from here. A river running from it was swollen with dirty water and mud, and made a great stink . . . Today the smoke and dust have spread—some of the latter having gone beyond Fort de France—20 miles away. I believe there will be no danger . . ."

The sample referred to has now been examined and found to consist principally of a very fine siliceous ash, in which occur broken and angular fragments (up to 0.05 mm in diameter) of crystals of quartz and labradorite feldspar. The labradorite (An_{55-70}) shows both multiple albite twinning and complex oscillatory zoning in which outer zones have sometimes been partly torn from the cores of the crystals. Some magnetite is present, but there are no recognizable shards in this very fine-grained, yellow-green tuff.

This account is of two-fold significance. First, it directs attention to the importance of the preliminary phases of eruption which precede the extrusion and accumulation of Peléan tuffs. Secondly, it indicates that the sillar bases to welded tuffs need not be strictly contemporaneous with the overlying welded material. MacGregor³ and others have pointed out that the terms 'Peléan' and 'welded' tuffs are not necessarily synonymous.

The observation that the nearby rivers were swollen with debris from the early fall-out of ash shows that the loose ashes were rapidly transported to the sea. In areas where shallow water bordered the land, this fine ash would have been rapidly redistributed and deposited on the sea floor. Protracted activity of this type enables appreciable accumulations to develop before the extrusion of the main mass of pyroclastic material. The preliminary phases of such eruptions, therefore, may be responsible for the shallowing of the sea, so that the hot ash falling into the shallow water rapidly builds above water-level, so permitting welding to occur above the non-welded, non-contemporaneous base.

The welded tuffs of western Mayo show non-welded sillar bases in various places. In western Murrisk both Stanton¹ and Dewey² have recorded sillar layers in MT1 and MT2. In eastern Murrisk neither of these tuff layers has a continuous sillar base. Thin lenses are present on MT1, but the base of MT5 has a 4-ft.-thick layer of sillar material in the cliffs to the west of Lough Glenawough. No sillar material is present east of the lake.

The lowermost part of the sillar layer is almost devoid of shards. In places it consists of very fine dust with a few grains of quartz, which show uniform extinction. In the upper part of the sillar layer angular fragments of un-twinned plagioclase An_{25-40} are abundant in the tuff, with less frequent angular quartz grains, a few of which show the deep embayments so common in the quartz grains of the overlying, welded tuffs. Virtually all the feldspar fragments are deeply altered to sericitic material, with the result that they are distinguished with difficulty from the intratelluric material, in which minute undeformed shards are present.

With the change from non-welded to welded tuffs there is an increase in the size of the shards, from 0.01 to 0.04 mm to 0.05 to 0.20 mm. There is an accompanying change in the proportion of included lithic fragments (0.2–3.5 per cent). These two factors may result from changes in the strength of the forces active at different stages of the eruption. Large shards are to be expected from the fully frothing pyroclastic flows, whereas smaller ones result from quieter, less spectacular eruptions of the early phases.

J. McMANUS

Department of Geology,
Queen's College,
Dundee.

¹ Stanton, W. I., *Quart. J. Geol. Soc. Lond.*, **116**, 269 (1960).

² Dewey, J. F., *Quart. J. Geol. Soc. Lond.*, **119**, 313 (1963).

³ MacGregor, A. G., *Nature*, **157**, 305 (1946).

Origin of Fossil 'Zoophycos'

In the Czechoslovak Carpathians I have found imprints, described in the literature from many localities throughout the world as *Zoophycos*, *Taonurus*, *Spirophyton*, etc. The occurrence of these imprints in numerous localities in the Czechoslovak Carpathians is closely related to fine-grained sandstone strata, and sometimes to the marlstones (Cretaceous, Palaeogene). After a detailed investigation I concluded that these imprints were formed by abandoned prostomial parts (gill organs) of sedentary marine worms (Annelida, Polychaeta) from the family Sabellidae¹. The imprints are closely similar to the recent genus *Spirographis* Viviani 1805. As in that genus, the gill rays of the fossil worms are spirally wound around an asymmetrical gill lobe either to the left or to the right side, in the ratio of about 1:1. In sedimentary rocks the gill organs are either perpendicular to the bedding plane or they form flat coinciding accumulations.

The gill organs (prostomium) which are the forepart of the body are easily abandoned by the worms when they are in danger². Abandoned gill organs were formerly considered by palaeontologists as unknown, extinct plants, most probably algae or as a breeding place of marine animals, so that these fossils were regarded for a long time as problematical in origin. They are common in

Africa^{3,4}, America^{5,6}, France⁷, Germany⁸, England⁹, Switzerland⁷, Belgium^{8,9}, Luxembourg⁹, Italy¹⁰, Austria¹¹, Poland¹², Czechoslovakia¹, and the U.S.S.R.¹³, and their occurrence may be recorded anywhere in shallow-water marine sediments all over the world.

Fossil worms lived in a siphuncle as sedentary animals in colonies on the bottom of a shallow sea and, like recent forms, they fed on fine detritus and micro-organisms sinking downwards to the bottom. They received the food by means of their gill organs with variously adapted gill rays. On account of the different shape of the gill rays in fossils from the Flysch of the Czechoslovak Carpathians I proposed several genera. In that area, where these fossils occur in the rocks, a very rapid sedimentation may be supposed.

Due to their systematic classification in the animal kingdom they have become the most common fossils in the stratigraphic as well as in the topographic sense and they ought to have been referred to the zoological system long ago.

MIROSLAV PLÍČKA

Central Geological Survey,
Brno, Czechoslovakia.

¹ Plíčka, M., *Věstník Ustr. ústavu geol.*, **37**, No. 5 (Prague, 1962).

² Fauvel, P., *Faune de France* (Paris, 1927).

³ Fürst, M., and Klitzsch, E., *Revue de l'Inst. fr. du pétrole*, **18**, No. 10 (Paris, 1963).

⁴ Toit, A. L., *The Geology of South Africa* (London, 1954).

⁵ Burk, C. F., *Amer. Assoc. Petroleum Geologists Bull.*, **48**, No. 4 (1964).

⁶ Cecioni, G. O., *Amer. Assoc. Petroleum Geologists Bull.*, **41**, No. 3 (1957).

⁷ Heer, O., *Die Urwelt der Schweiz* (Zürich, 1865).

⁸ Antun, P., *Bull. Soc. Géol. Belgique*, **23**, No. 5 (1950).

⁹ Legrand, R., *Bull. Soc. Belg. Géol., Pal., Hydr.*, **57** (1948).

¹⁰ Venzo, S., *Atti Soc. Ital. Sci. Nat.*, **90** (Milano, 1951).

¹¹ Götzinger, G., *Jahrb. geol. Bundesanst.*, **94** (Vienna, 1951).

¹² Halicki, B., *Acta Geologica Polonica*, **11**, No. 4 (1961).

¹³ Gekker, R. F., *Vvedeniye v paleoekologiyu* (Moscow, 1957).

¹⁴ Lameere, A., *Précis de Zoologie* (Paris, 1931).

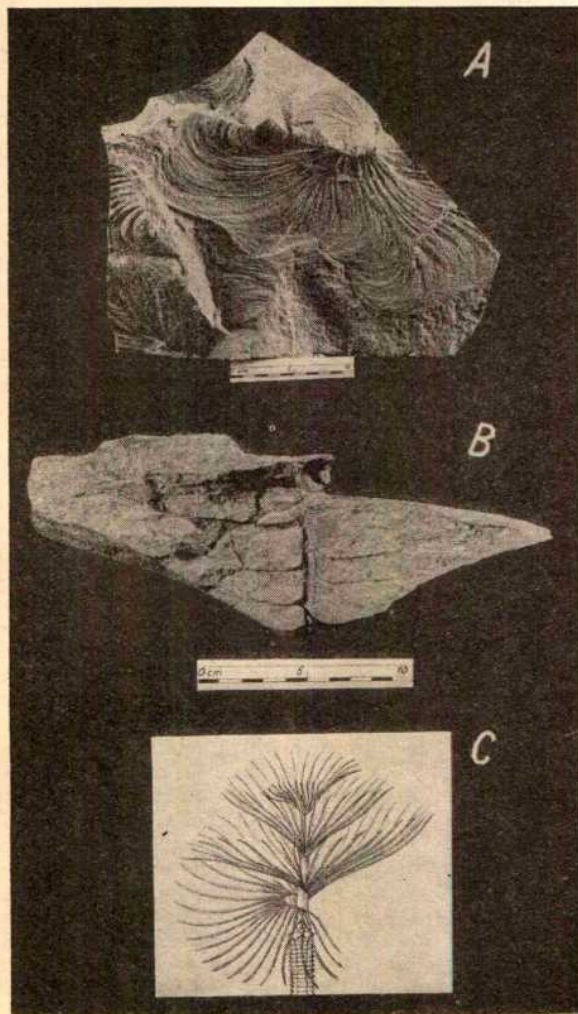


Fig. 1. A, *Zoophycos*; the imprint of the lower floor of branchial filaments when observed from above. The Carpathian flysch, Eocene, sample No. 33120B-5/1, photo Z. Šerebl. B, *Zoophycos*; the imprint of prostomial lobe with branchial filaments when observed from one side (longitudinal section). The Carpathian flysch, Palaeocene, sample No. 3497C-1/1, photo Z. Šerebl. C, *Spirographis spallanzanii* Viviani; the prostomial lobe with branchial filaments when observed from one side (A. Lameere¹⁴).

METALLURGY

Dispersion-softened Zinc Alloys

A NUMBER of zinc-based alloys containing equiaxed particles of various stable and insoluble hard second phases were prepared as part of an investigation into the high-temperature compression creep characteristics of dispersion-hardened materials¹. It was found that the dispersed particles produced a substantial strengthening effect at temperatures above 0.60 of the absolute melting temperature of zinc. Room-temperature tensile tests were made in order to measure the expected loss in ductility of zinc caused by the hard particles. It was surprising to find, therefore, that most of the dispersed particles reduced the strength and increased the ductility of zinc. These results are briefly reviewed here.

The alloys were prepared by methods of powder metallurgy in order to produce uniform dispersions of ZnO, $\alpha\text{Al}_2\text{O}_3$, carbon black, and tungsten in a void-free zinc matrix. The particle size of these spheroidal dispersoids was typically 0.1–0.6 μ . Two concentrations of second-phase particles were studied: 9 and 15 volume per cent. The composites were obtained in the form of 0.375-in.-diameter extruded rods. Specimens were annealed before testing at 385°C for 24 h. Pure zinc extrusions were also prepared from the same lot of powder as the composites and these were used to establish a basis for comparison.

Typical true stress versus true strain curves are presented in Fig. 1 for a zinc-tungsten alloy and also for a pure-zinc specimen. There are three characteristics to be noted from these curves and from the results in general: (1) The dispersed particles of tungsten actually weaken the metal. It can be seen that both the stress at the onset of plastic flow and the ultimate strength were lower for the composite than for the pure zinc. (2) The particles produced strain softening in zinc. That is, after a period of work-hardening, the effective stress required to deform

further the composite decreased with increasing strain. (3) The composites were considerably more ductile than is pure zinc. These three effects were most pronounced in the zinc-tungsten alloys, but they were also observed in each of the other composites containing spheroidal dispersed particles except for the alloys of zinc containing carbon. The influence of carbon was to embrittle as well as to weaken zinc (for example, 1-5 per cent reduction in area for Zn-C versus 14-23 per cent in pure zinc and 50-93 per cent for Zn-W). Some materials containing zinc oxide in platelet rather than in spheroidal form behaved in a more conventional manner. That is, they were stronger but slightly less ductile than pure zinc.

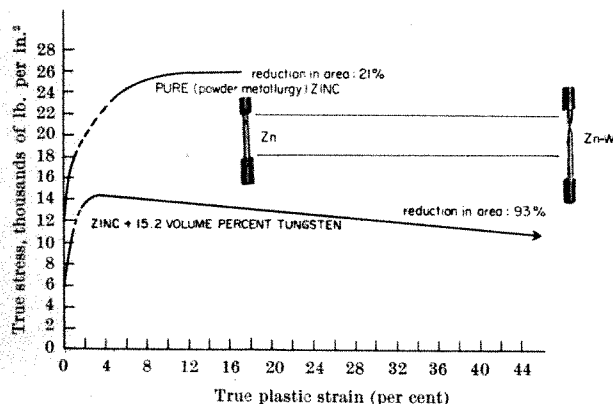


Fig. 1. Tensile behaviour of a zinc-tungsten dispersion-softened alloy compared to pure zinc. True stress-true strain curves obtained at 26° C and at a strain rate of 2 h⁻¹.

An examination of the slip bands produced in deformed specimens of pure zinc showed that the bands were coarse and widely spaced. Very fine slip lines were observed in the deformed zinc-tungsten alloy and electron microscopy was required to resolve them. Duplex slip, which manifested itself by intersecting slip lines, was common in the composites.

We believe that the fine slip and the duplex slip account for the reduced strength, work softening, and improved ductility that the alloys exhibited. Duplex slip can lead to the intersection of dislocations resulting in the formation of many jogged screw dislocations. Intersection is minimized when slip is restricted to the basal plane. The predominance of duplex slip in the composite alloys suggests that there were many more intersection-induced dislocation jogs in the alloys than in the pure zinc. The motion of jogged screw dislocations will produce an excess vacancy concentration^{2,3} which should aid the climb of dislocations over the dispersed particles. Thus the composite is strain softened and its ductility is enhanced. In addition, the ductility of the composite is high since the presence of fine and duplex slip will give a more uniform distribution of strain than the presence of coarse slip bands as was observed in zinc. It is likely that the fine slip and duplex slip observed are promoted, and indeed may be caused, by the microscopically non-uniform distribution of stresses that must exist in the alloys. This stress field heterogeneity may be caused by stress concentrations near each particle in a specimen subjected to an applied load and it may also be the result of the thermal expansion misfit between particles and matrix that develops when the alloy is cooled from the extrusion or annealing temperature. These internal stresses in the composite can lead to a low yield stress and a low ultimate strength in comparison to pure zinc.

Polycrystalline beryllium and magnesium are similar to zinc in that they exhibit limited ductility at low temperatures. This low ductility is generally attributed to the limited slip systems available in these hexagonal close-packed metals. It is possible that the addition of fine particles of selected high-melting second phases to

beryllium or magnesium may improve their ductility as was observed for zinc.

This work was supported by the Office of Naval Research, U.S. Navy. One of us (W. H. McCarthy) was the recipient of a Gordon Foundation fellowship. The assistance of Mr. D. G. Folger with some of the alloy preparation is much appreciated.

W. H. MCCARTHY
J. C. SHYNE
O. D. SHERBY

Department of Materials Science,
Stanford University,
California.

¹ McCarthy, W. H., Ph.D. dissertation, Stanford Univ., California (1965).

² Seitz, F., *Adv. Phys.*, **1**, 43 (1952).

³ Read, W. T., *Dislocations in Crystals*, 85 (McGraw-Hill Book Co., 1953).

CHEMISTRY

Structure of a Diolefin Complex of Platinum(II) $C_{10}H_{14}O_3PtCl_2$

THE nuclear magnetic resonance and infra-red spectra^{1,2} of potassium *tris* acetylacetonatoplatinum(II) suggest that the acetylacetonate anions form a square planar arrangement around the platinum with one conventional oxygen-bonded acetylacetonate chelate and two γ -carbon bonded ligands. The complex reacts with hydrochloric and hydrobromic acids to give greenish-yellow complexes of composition $C_{10}H_{14}O_3PtX_2$ ($X = Cl, Br$) which are monomeric in chloroform and non-electrolytes in nitromethane. In contrast to the 'acid' derivatives³ of the complex $K[Pt(Acac)_2X]$, these complexes are stable in solution for several days.

The nuclear magnetic resonance spectra of $C_{10}H_{14}O_3PtCl_2$ and $C_{10}H_{14}O_3PtBr_2$ indicate a symmetrical structure with two sets of pairs of methyl protons at 8.3 τ and 7.8 τ and two olefinic protons at 5.5 τ ; the methyl protons have ¹⁹⁵Pt-H coupling constants of 3 c/s (8.3 τ) and 34 c/s (7.8 τ), the protons at 5.5 τ having a coupling constant of 84 c/s. These coupling constants are consistent with the co-ordination of the platinum by olefinic groups with one pair of methyl groups directly bonded to the olefine and one set separated by a number of intermediate atoms. The infra-red spectrum is also consistent with the presence of a co-ordinated olefine grouping ($\nu_C = C = 1,495\text{ cm}^{-1}$) and the absence of a co-ordinated acetylacetonate ligand. A characteristic absorption at 1,236 cm^{-1} implies the presence of an unsaturated ether grouping, the two platinum-chlorine stretching frequencies at 325 and 335 cm^{-1} requiring a *cis*- arrangement of the chlorines in the complex. The identity of the infra-red spectra of the chloride and bromide spectra above 400 cm^{-1} shows that there are no direct carbon-halogen bonds in the complex.

β -Diketones are known to condense with elimination of water to give further unsaturated systems; the presence of only three oxygen atoms in the complex and the absence of an O-H group absorption in the infra-red and nuclear magnetic resonance spectra suggest that such a condensation has taken place in the reaction of $K[Pt(Acac)_3]$ with acids. The structure of the condensation product, which was suggested by the spectral data, has now been confirmed in detail by a single crystal X-ray diffraction analysis. The X-ray data collected, by courtesy of Dr. P. B. Braun, on a 'Paired' automatic diffractometer, have been converged to a present reliability index of 0.060, the overall stereochemistry of the complex being shown in Fig. 1.

The co-ordination of the platinum is approximately square planar with two chlorines, in a *cis* configuration, at a mean distance of 2.31 Å (σ 0.005 Å), and two donor olefinic bonds of mean length 1.45 Å (σ 0.03 Å). The bonding of each olefinic group is asymmetric, the mean Pt-C distances being 2.10 Å and 2.33 Å (σ 0.02 Å). The

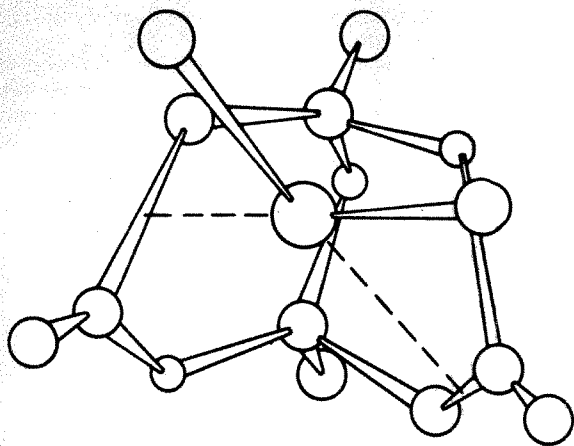


Fig. 1

intramolecular platinum-oxygen distances range upwards from 2.89 Å, more than 1 Å greater than is found in the example of the oxygen-chelated acetylacetonate anion in $K Pt(Acac)_2Cl$. The mean lengths of the bridging and remaining C—O bonds are 1.44 and 1.40 Å respectively (each with σ 0.03 Å).

D. GIBSON
C. OLDHAM
J. LEWIS

Department of Chemistry,
University of Manchester.

D. LAWTON
R. MASON
G. B. ROBERTSON

Department of Chemistry,
University of Sheffield.

¹ Figgis, B. N., Lewis, J., Long, R. F., Mason, R., Nyholm, R. S., Pauling, P. J., and Robertson, G. B., *Nature*, **195**, 1278 (1962).

² Oldham, C., and Lewis, J. (in the press).

³ Allen, G., Long, R., Lewis, J., and Oldham, C., *Nature*, **202**, 589 (1964).

Ultra-purification by Separation of Aerosol Particles

PURIFICATION methods which treat the concentration of a given impurity as a continuous quantity become ineffective when only a discontinuous distribution of that impurity is left in a material. Further purification is feasible on a dispersion principle which makes use of this discontinuity, and by which liquids have been observed free from particles at temperatures too low to cause the latter to be melted or dissolved.

A bulk sample of liquid, which after thorough pre-purification still contains a number of particles, is partitioned into a larger number of droplets which are usually suspended in a particle-free fluid. In the resulting dispersion, the particles are confined to a fraction of the droplets and thus isolated from the rest of them^{1,2}.

Liquid dispersions of metals¹⁻⁴, water⁵⁻⁷, organic compounds^{8,9}, and alkali halides¹⁰, of uniform droplet size, were subjected to under-cooling. Meanwhile, the droplets were observed either collectively or singly; their solidification was detected by inoculation of under-cooled bulk liquid⁵, by optical effects, for example, recalescence flashes³, twinkling^{8,10}, or X-ray diffraction⁴, and by other techniques. A portion of the droplets did not freeze until, far below the melting point, the critical under-cooling temperature had been reached. There is evidence that these droplets crystallized by homogeneous nucleation and therefore were free from heterogeneous impurities. Homogeneous impurities did not alter very much the critical under-cooling temperature⁹, unless, as in emulsions, they were occluded in the particles⁹.

The isolation of particle-free liquids was always incidental to the investigation of other topics and the possibility of using it to produce these liquids in appreciable quantity does not seem to have been realized. For example, a drop (2 mm diameter) of particle-free water, stored at the interface between two immiscible liquids, was instead obtained by multiple distillation without ebullition⁷.

Large samples of liquids free from particles could be produced from aerosols by separating contaminated from uncontaminated droplets. When the temperature of a monodisperse liquid aerosol is held slightly above the critical under-cooling point, all droplets containing particles are frozen. By ultra-sound of a wave-length comparable with the diameter of these droplets, the particle-free liquid droplets can then be sub-divided further. The resulting difference in particle size causes the contaminated particles to sediment before the uncontaminated ones.

Bulk samples of materials could similarly be purified from the last traces of a given homogeneous impurity. The properties of the latter determine the technique by which the aerosol particles are separated. In the case of impurities amenable to uninterrupted selective heating, it is advisable to utilize the very large negative temperature coefficient of the rate of crystallization of particle-free droplets. The noble gas, in which the monodisperse liquid aerosol is suspended, is cooled to the critical under-cooling temperature. Then only the contaminated droplets, which are subjected to induction or to high-frequency dielectric heating, remain liquid and can be dispersed ultrasonically. In other cases, for example, contaminated aerosol particles could be photo-ionized selectively by ultra-violet radiation, and electrostatically precipitated.

According to the dispersion principle, the fraction of particles free from impurities increases with the degree of dispersion. Volatile covalent compounds can be dispersed to discrete molecules within an involatile inert matrix. This makes it possible to purify small quantities of such compounds by fractional molecular distillation, in which these molecules are separately evaporated by selective infra-red radiation¹¹. The high cost of this method limits it to laboratory use.

As a guest in this Institute, I thank Prof. W. Jost for his hospitality.

STEFAN BEHRENDT*

Institut für Physikalische Chemie,
Universität Göttingen.

* Present address: Abteilung Strahlenchemie, Max-Planck-Institut für Kohlenforschung, Mülheim a.d. Ruhr, West Germany.

¹ Turnbull, D., *J. App. Phys.*, **20**, 817 (1949).

² Turnbull, D., and Cech, R. E., *J. App. Phys.*, **21**, 804 (1950).

³ Mendenhall, C. E., and Ingersoll, L. R., *Phil. Mag.*, **15**, 205 (1908).

⁴ Vonnegut, B., *J. Colloid Sci.*, **3**, 563 (1948).

⁵ Cwilong, B. M., *Nature*, **155**, 381 (1945).

⁶ Cwilong, B. M., *Proc. Roy. Soc., A*, **190**, 137 (1947).

⁷ Langham, E. J., and Mason, B. J., *Proc. Roy. Soc., A*, **247**, 493 (1958).

⁸ Thomas, D. G., and Staveley, L. A. K., *J. Chem. Soc.*, 4569 (1952).

⁹ Turnbull, D., and Cormia, E. L., *J. Chem. Phys.*, **34**, 820 (1961).

¹⁰ Buckle, E. R., and Ubbelohde, A. E., Intern. Union Pure and App. Chem. Symp. Thermodynamics (1959).

¹¹ Behrendt, S., thesis, Göttingen Univ. (1964).

Polyamide Layer Chromatography of Oestrogens

THE oestrogens were investigated by Struck¹ and Lisboa *et al.*² by thin-layer chromatography using silica-gel. The solvent systems for the silica-gel thin-layer were well-established ones which have been used for quantitative purposes³. On the other hand, Woltz and Chatteraj⁴ combined thin-layer and gas-chromatography to identify the minor oestrogenic substances in female urine.

Since oestrogens contain a phenolic group in their molecule, it is conceivable that polyamide can be used

	Table 1	
	Chloroform-butanol (95:5)	Ethanol-water (60:40)
Oestrone	0.82	0.35
Oestradiol (17- β)	0.55	0.32
Oestriol	0.21	0.48

for their separation⁵. The polyamide layer, according to Wang⁶, was applied to oestrone, oestradiol and oestriol, and both chloroform-*n*-butanol (95:5) and ethanol-water (60:40) systems showed excellent resolution (Table 1 and Fig. 1). This suggests that quantitative separation of oestrogens of complex biological fluid can be done by column chromatography with polyamide powder. It is also possible to investigate minor oestrogenic substances by means of two-dimensional polyamide layer chromatography.

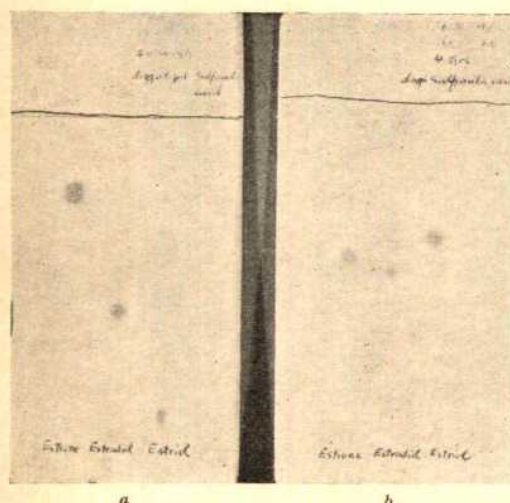
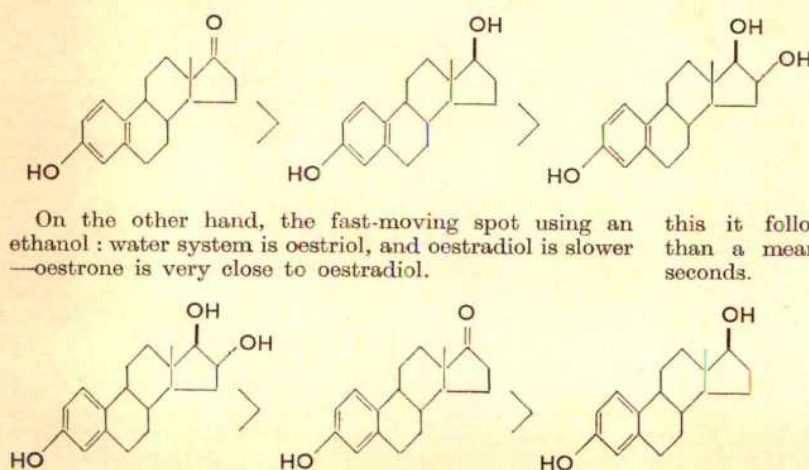


Fig. 1. Polyamide layer according to Wang (ref. 6). *a*, Solvent: chloroform-*n*-butanol (95:5); time: 1.5 h (10 cm). *b*, Solvent: ethanol-water (60:40); time: 2.5 h (11 cm). Samples, 1, oestrone; 2, oestradiol (17- β); 3, oestriol. Detection: diazotized sulphanilic acid

It was stated by Carelli *et al.*⁵ that hydrogen bonding of the phenolic group is the main force of polyamide sorption of phenols, and he has suggested a neighbour group 'screening effect'. If this is true, the oestrogens may show only a small difference on a chromatogram because they have a similar substitution pattern on the phenol ring. Differences occur, however, in the alcoholic hydroxyl groups, which are at distant sites on the 16- and 17-carbon atoms. Contrary to prediction, the chloroform-butanol system shows a marked difference in *R_F* values. It is best to assume that the alcoholic hydroxyl group in the oestrogens has some effect on their sorption to polyamide since an increase in hydroxyl groups also leads to an increase in the adhesion of substances to polyamide.



On the other hand, the fast-moving spot using an ethanol: water system is oestriol, and oestradiol is slower — oestrone is very close to oestradiol.

Although the presence of water affected the affinity of oestrogens for polyamide, it seems too early to suggest a polyamide sorption mechanism by the ethanol-water system as for the paper chromatographic-type partition process put forward by Copius-Peereboom⁷: in true partition by paper chromatography⁸, oestrone is the faster and oestriol remains at the origin, while oestradiol is intermediate between the two. There is the same tendency with chloroform-*n*-butanol systems of polyamide-layer chromatography. Our experiments on oestrogens suggest that further elaboration of the sorption mechanism is necessary.

YIH CHIH TUNG

Department of Biochemistry,
Taipei Medical College,
Taiwan.

KUNG-TSUNG WANG

Department of Chemistry,
National Taiwan University,
Taipei, Taiwan, China.

¹Struck, H., *Mikrochimica Acta*, 634 (1961).

²Lisboa, B. P., and Dizfulusy, E., *Acta Endocrinol.*, **40**, 60 (1962).

³Jacobsohn, G. M., *Anal. Chem.*, **36**, 275, 2032 (1964).

⁴Woltz, H. H., and Chattaraj, S. C., *Anal. Chem.*, **36**, 1466 (1964).

⁵Carelli, V., Liquori, A. M., and Mele, A., *Nature*, **176**, 70 (1955).

⁶Wang, K. T., *J. Chinese Chem. Soc.*, **II**, **8**, 241 (1961).

⁷Copius-Peereboom, J. W., *Nature*, **204**, 748 (1964).

⁸Boscott, R. J., *Chem. and Indust.*, 472 (1952).

Slow Inversion at the Nitrogen Atom in Certain Amides

It has long been considered that nuclear magnetic resonance (NMR) should be a suitable technique for the demonstration of slow inversion in trivalent nitrogen compounds. However, only in the case of cyclic imines, such as *N*-ethylethylenimine, has it been possible to demonstrate slow inversion by NMR¹. Recently, Saunders and Yamata² searched for evidence of slow inversion in dibenzyl methylamine, but found no evidence for non-equivalence of methylene protons in the amine itself. They were successful, however, in observing non-equivalence in the amine hydrochloride.

We wish to report evidence for slow inversion in the proton magnetic resonance spectrogram of *N*-benzyl-*N*-(orthomethyl) phenyl acetamide (I) and related compounds. The spectrogram of this compound is compared with those of *N*-benzyl acetanilide (II) and *N*-benzyl-*N*-(2,6-dimethyl) phenyl acetamide (III) in Fig. 1. The pertinent feature in the spectrogram of (I) is the multiplicity (four peaks) of the signal for the methylene protons. We have analysed these signals as an *AB* pattern with a chemical shift of 0.83 p.p.m. and a coupling constant of 14 c/s. The intensity ratio of the inner to outer lines is predicted to be 1.74 for such a pattern and was observed to be 1.67.

If a mechanism for a rapid exchange of identities of the methylene protons existed, the *AB* pattern would not be observable. Rapid inversion at the nitrogen atom is such a mechanism. From this it follows that inversion must be less rapid than a mean lifetime of something like 1–10 milliseconds.

The lack of observable non-equivalence in II and III indicates that a fairly high order of dissymmetry in the molecule is required. The requirement of a high degree of dissymmetry may be the reason that slow inversion has not been observed for acyclic trivalent nitrogen in the past. Evidently even the

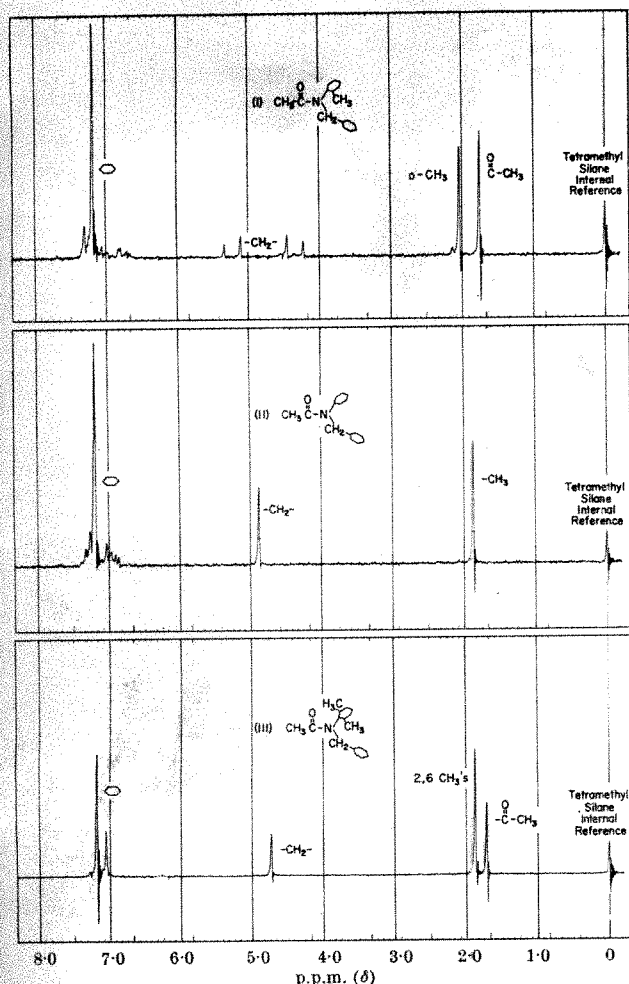


Fig. 1. Proton magnetic resonance spectrograms of three acetanilides at 60 mc/s

presence of three different substituents is not a guarantee of observable non-equivalence.

We have observed this type of non-equivalence in a number of related compounds and are continuing our study of this phenomenon.

These investigations were carried out in the course of work under contract AT(07-2)-1 with the U.S. Atomic Energy Commission.

T. H. SIDDALL
C. A. PROHASKA

E. I. Du Pont de Nemours and Co.,
Savannah River Laboratory,
Aiken, South Carolina.

¹ Bottini, A. T., and Roberts, J. D., *J. Amer. Chem. Soc.*, **78**, 5126 (1956).
² Saunders, M., and Yamata, F., *J. Amer. Chem. Soc.*, **85**, 1882 (1963).

BIOCHEMISTRY

Proteins of Wheat Flour

A NUMBER of reports have recently been published concerning investigations of the 'zwickel' (wedge) and 'haft' (adhering) protein fractions of wheat flour and their influence on the separation of flour fractions of different protein content by air-classification procedures^{1-4,11}.

These fractions were originally described by Hess, who believed that flour or endosperm proteins in the native state existed in two forms, one as a network of fibrils on the surface of the starch granules and covered by a layer of lipid ('adhering' protein), and the other as discrete particles between the starch granules ('wedge' protein). These two fractions were readily separated by differential

centrifugation in non-aqueous media and differed significantly from the equivalent fractions of the gluten isolate⁸. Hess concluded that during the conversion of native protein to gluten, mechanical treatments cause a structural chemical alteration of the protein molecules.

Jones *et al.*⁹ have shown that flour fractions separated on the basis of particle size contain different amounts of protein. Up to about 17 μ the particles include fragments of free protein with a relatively high protein-to-starch ratio. Particles of 17-35 μ contain a large proportion of free starch granules and are consequently low in protein content. Above 35 μ the particles consist of larger detached starch granules and pieces of unbroken endosperm cells and may have a similar or higher protein content than that of the parent flour¹⁰. These differences have provided the bases for the industrial air-classification of flours in the sub-sieve range (<40 μ).

Results obtained by Stevens *et al.*², based on amino-acid analyses, indicate that proteins of the 'gliadin/glutenin' type are preferentially extracted in flour fractions of small particle size (<17 μ), while the more soluble fractions appear to be associated with those fractions of larger particle size containing more starch. Protein analyses of Elton and Ewart, however, failed to indicate significant differences between these fractions⁴.

A number of workers have recently examined the protein composition of air-classified fractions using some of the newer, refined techniques of protein fractionation in an attempt to confirm Hess's original classification^{1,3,4,11}. In most cases the protein composition of these fractions differed little from that of the parent flour.

In this laboratory, the protein compositions of a number of air-classified flour fractions were examined by starch-gel electrophoresis following the methods described in previous reports^{3,12}. Acetic acid extracts (2.0 g/6 ml., 0.01 N) were indeed found to vary little from those of the parent flour.

Samples of 'wedge' and 'adhering' protein obtained from the late Prof. Hess's laboratory when similarly examined however, showed significant differences in protein composition. 'Wedge' proteins corresponded mainly to endosperm components of low electrophoretic mobility (gliadins), while 'adhering' proteins corresponded to the slowest-moving endosperm fractions together with smaller levels of components of intermediate electrophoretic mobility (albumins). Densitometric scans of these starch-gel patterns are shown in Fig. 1.



Fig. 1. Starch-gel electrophoresis of 'wedge' (solid line) and 'adhering' (broken line) proteins of wheat endosperm. Buffer: aluminium lactate-lactic acid. Voltage gradient: 8-10 V/cm⁻¹. Time, 3 h

The obvious association of 'wedge' proteins with the generally accepted gliadin fractions and those fractions adhering to the starch granules with the albumins would seem to conform with recent findings. The fact that the 'adhering' protein appears to represent mainly a distinct group of components of lowest electrophoretic mobility would seem to confirm the original observations of Hess. Protein fractionation by air-classification would therefore seem to be much less complete when carried out on the present commercial scale.

A further interesting fact, which may be of some significance in this context, emerged from a comparison of the solubility characteristics of flour and isolated gluten proteins. Aqueous extracts of flour and gluten, isolated from an equivalent amount of the same flour, were shown to contain a rather different proportion of protein components (Fig. 2). The latter contained a greater proportion of the slow-moving fractions with the exception of those of lowest electrophoretic mobility. Again the fractions of



Fig. 2. Starch-gel electrophoresis of the water-soluble proteins extracted from flour (broken line) and the equivalent amount of isolated gluten (solid line)

lowest mobility were associated with the albumin components. This could not be explained merely in terms of starch-dilution effects. Similar examination of aqueous extracts of doughs aged for various periods gave protein patterns identical with those of flour. It would therefore seem that the removal of starch during gluten isolation involves not only the removal of the bulk of the water-soluble albumins but also the fractions of lowest electrophoretic mobility, that is, those fractions associated with the starch granules.

We thank Dr. Niederaur, Institut für Mehl und Eiweissforschung, Hanover, for samples of 'wedge' and 'adhering' protein; Messrs. J. and R. Snodgrass, Ltd., Washington Mills, Glasgow, and Messrs. Spillers, Ltd., Old Change House, Cannon Street, London, for air-classified flours, and the Hercules Powder Company, Inc., for financial support.

C. B. COULSON
A. K. SIM

Arthur D. Little Research Institute,
Inveresk Gate,
Musselburgh,
Midlothian.

- ¹ Jones, R. W., and Dimler, R. J., *Cereal Chem.*, **39**, 336 (1962).
- ² Stevens, D. J., McDermott, E. E., and Pace, J., *J. Sci. Fd. Agric.*, **14**, 284 (1963).
- ³ Sim, A. K., Ph.D. thesis, Univ. Edinburgh (1963).
- ⁴ Elton, G. A. H., and Ewart, J. A. D., *J. Sci. Fd. Agric.*, **15**, 119 (1964).
- ⁵ Hess, K., *Getreide u. Mehl*, **3**, 1 (1953).
- ⁶ Hess, K., *Kolloid-Z.*, **136**, 84 (1954).
- ⁷ Hess, K., Mahl, H., Gutter, E., and Dodt, E., *Microskopie*, **10**, 6 (1955).
- ⁸ Pence, J. W., Mecham, D. K., and Olcott, H. S., *J. Agric. Fd. Chem.*, **4**, 712 (1956).
- ⁹ Jones, C. R., Halton, P., and Stevens, D. J., *J. Biochem. Microbiol. Technol. Eng.*, **1**, 77 (1959).
- ¹⁰ Jones, C. R., *Rec. Adv. Fd. Sci.*, **2**, 234 (1962).
- ¹¹ Wrigley, C. W., *J. Sci. Fd. Agric.*, **14**, 120 (1963).
- ¹² Coulson, C. B., and Sim, A. K., *Nature*, **202**, 1305 (1964).

Tumour-promoting Agents in Unburned Cigarette Tobacco

UNBURNED American cigarette tobacco is an excellent source of tumour-promoting agents¹. The aqueous extract from as little as one-half cigarette per day is sufficient to produce skin tumours in mice painted previously with 125 µg of 7,12-dimethylbenz[*a*]anthracene (DMBA). The work recorded here gives further information about the properties of the active substance.

For most of the experiments, the paper was removed from commercial cigarettes and the tobacco was extracted with aqueous barium hydroxide which was afterwards neutralized with carbon dioxide and filtered. This method provides an extract containing 0.6–0.7 g of solids per ml.¹. In one experiment, however, unprocessed commercial flue-cured tobacco was used in order to determine whether the tumour-promoting activity was due to the unburned tobacco itself, or to the several additives used in the preparation of American cigarettes. Flue-cured tobacco was less active than prepared cigarette tobacco as a source of promoting agents.

ICR Swiss female mice, 55–60 days old, were painted once with 125 µg of DMBA dissolved in 0.25 ml. of acetone. After a period of three weeks, the animals were painted five times weekly with 0.25 ml. of the various test solutions. The animals were examined weekly and the number and distribution of tumours noted. The experiments were terminated after 26 weeks of this second, tumour-promot-

ing stimulus. The biological assay system has been described more fully in other publications^{1,2}.

Each test fraction was assayed at two concentrations, the second one-half the concentration of the first. In this way a semi-quantitative comparison of various fractions was possible.

An attempt was made to fractionate the aqueous extract into acidic, neutral, and basic materials by liquid-liquid extraction techniques. However, stable emulsions of organic and aqueous phases resulted, and it was decided instead to use ion exchange resins. The neutralized aqueous extract (133 c.c. from 267 g of tobacco) was made acidic (pH 1.5) with hydrochloric acid and the solution was then passed through a column (45 × 420 mm) containing a strong-acid cation exchange resin ('Rexyn AG 50(H)'). The column was afterwards washed with distilled water until the washings were colourless. The effluents from this column were then passed sequentially through a similar column containing a strong-base anion exchange resin ('Rexyn RG1 (OH)'). The second column was also washed with water. The effluents from the second column were condensed to provide the neutral fraction. The 'AG 50' column was treated with 4 per cent barium hydroxide until Ba⁺⁺ came through the column and the effluent was colourless. The combined effluent was neutralized with carbon dioxide, filtered and condensed to provide the bases plus amphoteric compounds. The 'RG1-OH' column was washed with 1 N sulphuric acid until the effluent was colourless and the SO₄⁼ came through the column; the combined effluent was neutralized with barium hydroxide, filtered, condensed and labelled 'A' (acids). Half of each fraction was pooled to provide a reconstituted starting material that was identical to the starting material except for changes due to the experimental manipulations. The bioassays showed that much of the activity was lost (Table 1). It did not appear in any fraction or in the reconstituted crude material. The active material probably was either decomposed or not eluted from one of the columns.

A second experiment was conducted to determine whether the active material in the aqueous barium hydroxide extract would dissolve in acetone so that it might be more easily fractionated. Acetone was considered as a possible solvent because acetone-benzene extracts of cigarette tobacco have been found to be active tumour promoters¹. When the aqueous extract was condensed to remove the water, acetone did not mix with the gummy product and incomplete extraction was apparent. There-

Table 1. ACTIVITY OF 'ACIDIC', BASIC, AND NEUTRAL FRACTIONS

Fraction	Dose (tobacco equivalent per day)* (g)	No. of mice with tumours†	Maximum No. of tumours
Crude	0.5	6	12
Acids	0.5	1	1
Bases plus amphoteric compounds	0.5	0	0
Neutral	0.5	0	0
Reconstituted crude	0.5	1	1
Crude	0.25	1	1
Acids	0.25	0	0
Bases plus amphoteric compounds	0.25	0	0
Neutral	0.25	1	3
Reconstituted crude	0.25	0	1

* The amount of tobacco from which the various test solutions were obtained.

† Thirty mice per group; controls treated with DMBA alone or with the fractions alone did not develop tumours.

Table 2. ACTIVITY OF THE ACETONE-SOLUBLE FRACTION

Fraction	Dose (tobacco equivalent per day) (g)	No. of mice with tumours*	Maximum No. of tumours
Crude	0.5	6	22
Acetone soluble	0.5	3	5
Acetone insoluble	0.5	0	0
Reconstituted	0.5	2	2
Crude	0.25	0	0
Acetone soluble	0.25	0	0
Acetone insoluble	0.25	0	0
Reconstituted	0.25	2	2
DMBA alone	0	1	1

* Forty mice per group; controls treated with the fractions alone did not develop tumours.

Table 3. STABILITY OF TUMOUR-PROMOTING AGENTS

Fraction	Dose (tobacco equivalent per day) (g)	No. of mice with tumours*	Maximum No. of tumours
Fresh crude	0.5	3	3
pH 3	0.5	4	6
pH 7	0.5	5	15
pH 11	0.5	7	17
Fresh crude	0.25	0	0
pH 3	0.25	0	0
pH 7	0.25	0	0
pH 11	0.25	0	0

* Thirty mice per group; controls treated with DMBA alone or with the fractions alone did not develop tumours.

Table 4. VOLATILITY OF TUMOUR-PROMOTING AGENT

Fraction	Dose (tobacco equivalent per day) (g)	No. of mice with tumours*	Maximum No. of tumours
Crude	0.5	3	3
Distillate	0.5	0	0
Residue	0.5	10	48
Reconstituted crude	0.5	13	41
Crude	0.25	0	0
Distillate	0.25	0	0
Residue	0.25	0	0
Reconstituted crude	0.25	1	1

* Thirty mice per group; controls treated with DMBA alone or with the fractions alone did not develop tumours.

fore, the dilute aqueous extract was treated with two volumes of acetone with continuous stirring, whereon a precipitate settled out. The solution was decanted and condensed to low volume and additional acetone was added, precipitating more material. This process was repeated three more times; each time additional precipitate formed. The final solution was condensed and redissolved in a 1:1 acetone-water mixture. The precipitates were pooled and dissolved in water. As before, a reconstituted starting material was prepared by using appropriate amounts of the acetone soluble and acetone insoluble fractions. The results of the bioassays (Table 2) indicated that at least a part of the active material appeared in the acetone soluble fraction. There was no demonstrable activity in the acetone insoluble fraction. A possible loss of activity during experimental manipulation was indicated by the decreased number of tumours produced by the reconstituted crude material and by the acetone soluble fraction.

To measure the stability of the tumour promoters, additional aqueous barium hydroxide extract was adjusted to pH 3, 7 or 11 using sulphuric acid or barium hydroxide. The solutions were then allowed to stand in subdued light at room temperature. After 5 days, they were neutralized with sulphuric acid or barium hydroxide, filtered and tested on mice. The bioassays showed no evidence that the tumour promoters were unstable (Table 3). In fact, there was an increase in activity during standing, especially at the highest pH level. This increase, however, was only moderate as indicated by low activity of the diluted samples.

The volatility of the active material was tested by distilling the neutralized aqueous extract at a pressure of 18 mm to a final temperature of 27° C. One-half of the distillate and one-half of the viscous residue were pooled to produce a reconstituted starting material. The remaining distillate and residue was diluted with water to the original volume from which it was derived. Under the conditions of the distillation, the tumour promoters were non-volatile (Table 4). The residues appeared to be more active than the starting crude product, but again, this increased activity was lost by 1:1 dilution. The distillate was inactive.

The results of these various experiments suggest to us the following tentative conclusions: (1) The tumour promoter of the alkaline aqueous extract and the acetone-benzene extract of cigarette tobacco are closely related, if not identical. (2) The active material is either very highly bound to one of the ion exchange resins, or it is insoluble at extreme pH so that it is not eluted by the concentrated reagents that we used. (3) The active

material may be produced through the degradation of precursor materials in the extracts or in the leaf itself. There was a slight increase of activity when extracts were allowed to stand—particularly at elevated pH.

The biological assays of the active material indicated a definite threshold level. This is clear in every experiment. A 1:1 dilution caused almost complete loss of activity in every fraction. Thresholds are characteristic of tumour promotion and have been investigated extensively by Boutwell².

It was pointed out to us⁴ that the behaviour of the active material is similar to the behaviour of tobacco pigments which have been found in several types of tobacco⁵⁻⁸. The pigments are soluble in alkaline aqueous solution but are precipitated at very low pH. They are, in part, soluble in alcohol. They are complex compounds containing the polyphenols rutin and/or chlorogenic acid bound to proteins or polypeptides. Conceivably, these pigment materials might be degraded on standing to liberate smaller polyphenolic compounds. Furthermore, relatively low molecular weight polyphenolic compounds have been found in flue-cured tobacco⁶. It is of interest that, other than croton oil constituents, the most active known tumour-promoting agent is the polyphenol, anthralin (1,8,9-anthratril)². We are at present testing tobacco pigments isolated by a method developed by Chortyk and Stedman⁴ to determine whether these compounds or their hydrolytic products can account for the tumour-promoting activity exhibited by extracts of cigarette tobaccos.

FRED G. BOCK
RAYMOND J. SHAMBERGER
HUSTON K. MYERS

Roswell Park Memorial Institute,
New York State Department of Health,
Buffalo, New York.

¹ Bock, F. G., Moore, G. E., and Crouch, S. K., *Science*, **145**, 831 (1964).

² Bock, F. G., and Burns, R., *J. Nat. Cancer Inst.*, **30**, 393 (1963).

³ Boutwell, R. K., *Prog. Exp. Tumour Res.*, **4**, 207 (1964).

⁴ Chortyk, O. T., and Stedman, R. L. (personal communication).

⁵ Wright, jun., H. E., Burton, W. W., and Berry, jun., R. C., *Arch. Biochem. Biophys.*, **36**, 94 (1960).

⁶ Jacobson, J. S., *Arch. Biochem. Biophys.*, **93**, 580 (1961).

⁷ Runeckles, V. C., *Arch. Biochem. Biophys.*, **102**, 354 (1963).

⁸ Wright, jun., H. E., Burton, W. W., and Berry, jun., R. C., *Phytochem.*, **3**, 525 (1964).

Synthesis of Ribonucleic Acid by *Escherichia coli* during Inhibition by Azetidine-2-carboxylic Acid

In bacteria, amino-acids are needed in small amounts for the synthesis of ribonucleic acid (RNA) to occur¹ and, if an amino-acid auxotroph is starved of its required amino-acid, both protein and RNA synthesis cease². However, under certain conditions this 'stringency' is not maintained, in that protein synthesis can be sharply retarded while synthesis of RNA is less affected. In *Escherichia coli*, this differential synthesis of RNA can be brought about by incubation of cells with chloramphenicol³, puromycin⁴, or chlorotetracycline⁵, and mutants in which the control of RNA synthesis by amino-acids is 'relaxed' have also been described^{6,7}. Relaxed mutants which require an amino-acid for growth continue to make RNA when starved of their amino-acid requirement; synthesis of RNA in a relaxed strain during inhibition of protein synthesis by 5-methyl tryptophan has also been demonstrated⁸.

Differential synthesis of RNA has now been found to occur when cells of the stringent strain *Escherichia coli* K12 are inhibited by azetidine-2-carboxylic acid. This compound, while inhibiting growth, appears also to be incorporated into protein in place of proline⁹: in plants and rat liver the formation of an azetidine-2-carboxylic acid-transfer-RNA complex is apparently mediated by the

proline-activating enzyme¹⁰. Table 1 shows the results of adding azetidine-2-carboxylic acid (100 µg/ml.) to *Escherichia coli* K12 growing exponentially with a mean generation time of 70 min in a mineral-salts medium. There was an immediate slowing of protein synthesis, which, after 30 min, had been inhibited by 59 per cent; in this same period no inhibition of synthesis of RNA occurred. RNA was afterwards made at a reduced rate, but after incubation for 2 h in the presence of the analogue, the ratio of newly synthesized RNA to newly synthesized protein in the inhibited cultures was nearly twice that in the controls. Similar experiments were also carried out with the relaxed methionine-requiring mutant 58-161 of *Escherichia coli*. As shown in Table 1, addition of 100 µg/ml. of azetidine-2-carboxylic acid to cultures growing exponentially (mean generation time 75 min) produced an immediate strong inhibition of protein synthesis very similar to that observed in the stringent strain. However, RNA synthesis was initially stimulated appreciably and, although considerable inhibition developed at later times, this was less than in the stringent strain. Over a 2 h period, cells treated with the analogue synthesized about 2.5 times as much RNA per unit of newly made protein as did untreated cultures.

Table 1. SYNTHESIS OF RNA AND PROTEIN DURING INHIBITION BY AZETIDINE-2-CARBOXYLIC ACID

Organism	Time (min)	RNA (c.p.m./ml.)		Protein (c.p.m./ml.)		Inhibition (per cent)	
		Control	Inhibited	Control	Inhibited	RNA	Protein
<i>E. coli</i> K12	30	140	140	60	25	0	59
	60	430	280	145	40	35	72
	120	1,175	455	360	75	61	79
<i>E. coli</i> 58-161	30	410	515	180	95	-26	47
	60	1,075	1,055	425	165	2	61
	120	2,465	1,815	975	305	26	69

Cells were grown overnight with aeration and at 30° C in a mineral-salts medium containing DL-methionine (50 µg/ml.) and a limiting amount (66 µg/ml.) of glucose. Excess glucose (7 mg/ml.) was added to re-start growth and, after 60 min, aliquots were transferred to flasks containing uracil and cytosine (final concentration 20 and 15 µg/ml., respectively) and aeration continued. RNA and protein synthesis were followed separately by the addition of [2-¹⁴C]uracil (20 mµc./ml.) and [3-³⁵S]methionine (30 mµc./ml.) in the absence and presence of L-azetidine-2-carboxylic acid (100 µg/ml.). Measurements were made after the addition of the analogue and were of the radioactivity of cells precipitated with 5 per cent trichloroacetic acid at 0° C. Radioactivities were measured at infinite thinness with a gas-flow counter.

It has been suggested that the control of RNA synthesis by amino-acids may be mediated not by the amino-acids as such, but by their complexes with transfer-RNA. Species of transfer-RNA uncombined with amino-acids may cause repression of RNA synthesis; when molecules of transfer-RNA are joined to their specific amino-acids the repression is lifted and synthesis of RNA can continue^{7,11}. On this hypothesis differential synthesis of RNA in the presence of azetidine-2-carboxylic acid may occur in the stringent strain K12 because the analogue can undergo activation and be incorporated slowly into protein. Although protein synthesis is inhibited, uncombined transfer-RNA molecules may not accumulate immediately and synthesis of RNA is not retarded. In the relaxed mutant 58-161, differential synthesis of RNA may be made greater by the genetic defect in the control of RNA which exists in this strain.

JOAN M. MIDGLEY
D. G. WILD

Department of Biochemistry,
University of Leeds.

¹ Gros, F., *Exp. Cell Research*, **14**, 104 (1958).

² Pardee, A. B., and Prestidge, L. S., *J. Bact.*, **71**, 677 (1956).

³ Gale, E. F., *Adv. Protein Chem.*, **8**, 285 (1953).

⁴ Takeda, Y., Hayashi, S.-I., Nakagawa, H., and Suzuki, F., *J. Biochem. (Tokyo)*, **48**, 169 (1960).

⁵ Gale, E. F., and Folkes, J. P., *Biochem. J.*, **53**, 493 (1953).

⁶ Borek, E., Ryan, A., and Rokenbach, J., *J. Bact.*, **69**, 460 (1955).

⁷ Stent, G. S., and Brenner, S., *Proc. U.S. Nat. Acad. Sci.*, **47**, 2005 (1961).

⁸ Turnock, G., and Wild, D. G., *Nature*, **197**, 597 (1963).

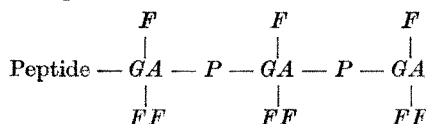
⁹ Fowden, L., and Richmond, M. H., *Biochim. Biophys. Acta*, **71**, 459 (1963).

¹⁰ Peterson, P. J., and Fowden, L., *Nature*, **200**, 148 (1963). Atherley, A. G., and Bell, F. E., *Biochim. Biophys. Acta*, **80**, 510 (1964).

¹¹ Kurland, C. G., and Maaloe, O., *J. Mol. Biol.*, **4**, 193 (1962).

'Lipid A' Component from the Cell-walls of *Pseudomonas aeruginosa*

CONSIDERABLE attention¹ has been paid to the lipopolysaccharide complexes which are found in the cell walls of Gram-negative bacteria. The extraction of whole cells or of isolated cell walls with aqueous phenol provides these lipopolysaccharides. The lipopolysaccharide component may be hydrolysed by dilute acid to give 'lipid A' and sugar. Nowotny² has suggested the following structure for the 'lipid A' derived from some strains of *Salmonella*.



F, fatty acid; GA, D-glucosamine; P, phosphoric acid.

Burton and Carter³ have recently isolated the 'lipid A' fraction from whole cells of *Escherichia coli* and, while agreeing in the main with the structure postulated by Nowotny, they point out that their findings would suggest that the D-glucosamine units are linked glycosidically and not by means of phospho-diester linkages. The precise arrangement and the identity of some of the fatty acid constituents of 'lipid A' remain undetermined.

The results obtained in our laboratories during an investigation into the structure of the cell walls of *Pseudomonas aeruginosa* agree with those of Burton and Carter and would support the suggestion⁴ that this type of structure is widely distributed in Gram-negative bacteria.

The cell walls of *Pseudomonas aeruginosa* were isolated by the method of Salton⁵, and phenol extractions of the isolated cell walls were performed as described by Westphal⁶, with the modification that the residue from the first extraction was again treated with phenol. The two phenol layers were combined and fraction PhMP was obtained by precipitation with methanol, while fraction PhMS remained in solution. The two aqueous layers were treated separately, giving rise to Aq I and Aq II as shown below.

Aq I	Aq II	Residue (R)	PhMP	PhMS
20-30%	11-13%	12-15%	20-28%	17-20%

Fraction PhMS contains 80 per cent lipid (cephalin and free fatty acids) and 20 per cent protein, while fraction PhMP is practically pure protein. The residue R is derived from the emulsion between the aqueous and phenolic layers and is a mixture consisting mainly of mucopeptide and protein. Detailed analyses of these fractions will be reported at some other time.

Although Aq I and Aq II showed some variation in quantity and elemental composition from extraction to extraction, all our information so far leads us to believe that they contain two lipopolysaccharide-mucopeptide complexes, and that Aq I and Aq II differ only in the relative proportions of these two major components.

The lipid fraction was obtained by mild hydrolysis of Aq I with boiling 1 N aqueous sulphuric acid for 30 min followed by extraction from the hydrolysate with chloroform, while the remaining aqueous layer contained D-glucose, L-rhamnose, D-glucosamine, and D-galactosamine. After removal of the acid from this aqueous layer and freeze drying, the infra-red spectrum of the residue revealed no indication of lipid material. Fractionation of the lipid on silicic acid gave two main fractions. The first fraction, presumably formed by partial degradation of 'lipid A', contained ester-bound fatty acid and free fatty acid. Thin-layer chromatography showed that the range of fatty acids was practically identical with that obtained by vigorous hydrolysis of Aq I with 6 N aqueous acid. This range included a number of acids, both saturated and unsaturated, up to about C₁₈, and also two unknown acids with high R_F values.

The second fraction was the whole 'lipid A', and the infra-red spectrum was almost identical with that obtained

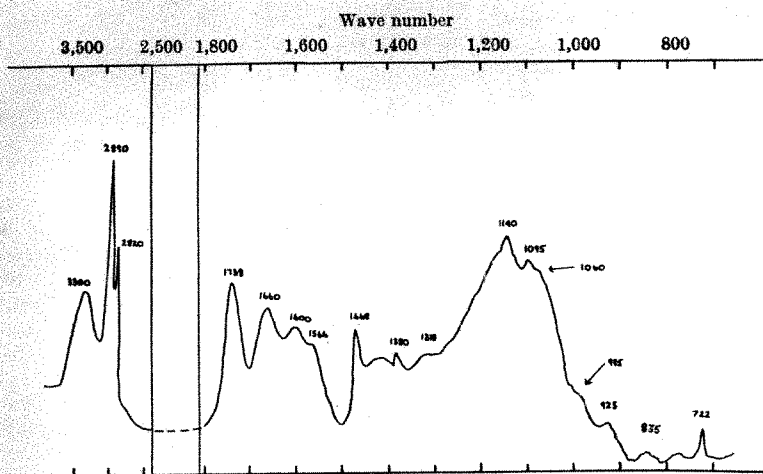


Fig. 1. The infra-red spectrum of 'lipid A' from *Pseudomonas aeruginosa*

by Burton and Carter for the 'lipid A' obtained by hydrolysis of the lipopolysaccharide from *Escherichia coli*. Examination of the infra-red spectrum of our 'lipid A' (Fig. 1), particularly in the region 750–1,000 cm^{-1} , shows the presence of moderate absorptions which Barker *et al.*⁷ associate with glycosidic linkages. Our findings thus agree closely with those of Burton and Carter and would support their suggestion that the D-glucosamine units are linked glycosidically.

K. CLARKE
G. W. GRAY
D. A. REAVELEY

Department of Chemistry,
University of Hull.

¹ Westphal, O., and Luderitz, O., *Naturwissenschaften*, **50**, 413 (1963).
Nowotny, A., Thomas, S., and Duron, O. S., *Biochemical Problems of Lipids* (B.B.A. Library, Volume 1, Elsevier, Amsterdam, 1963).
Osborn, M. J., Rosen, S. M., Rothfield, L., Zeleznick, L. D., and Horecker, B. L., *Science*, **145**, 783 (1964).

² Nowotny, A., *J. Amer. Chem. Soc.*, **83**, 501 (1961).

³ Burton, A. J., and Carter, H. E., *Biochem.*, **3**, 411 (1964).

⁴ Nowotny, A., *Fed. Proc.*, **20**, 270 (1961).

⁵ Salton, M. R. J., and Horne, R. W., *Biochim. Biophys. Acta*, **7**, 177 (1951).
Salton, M. R. J., *Biochim. Biophys. Acta*, **10**, 512 (1953).
Cummins, C. S., and Harris, H., *J. Gen. Microbiol.*, **14**, 583 (1956).

⁶ Westphal, O., Luderitz, O., and Bister, F., *Z. Naturforsch.*, **7b**, 148 (1952).

⁷ Barker, S. A., Bourne, E. J., Stacey, M., and Whiffen, D. H., *J. Chem. Soc.*, 171 (1954).

Isozymes of Arylamidase (Aminopeptidase): Source of Blood Aminoacyl Naphthylamidase in the Rat

THE qualitative approach provided by histochemical techniques makes it possible to localize enzyme activity in tissues. This, combined with the use of quantitative and other analytical methods such as electrophoresis, may help in tracing the origin and fate of an enzyme in the blood serum. This rationale has been applied in this study to certain proteolytic enzymes to which the names of aminopeptidase, arylamidase and aminoacyl naphthylamidase have been applied¹⁻³. In this report the three names are regarded as being synonymous.

In the rat, blood levels of aminopeptidase vary within a narrow range in normal conditions⁴. There is electrophoretic evidence that renal aminopeptidase, localized in cells of the proximal tubules, is the source of the urinary isozymes which are distinct from the serum aminopeptidase isozyme⁵. Certain other cells of the rat, such as columnar cells of the small intestine, hepatic parenchymal cells, prostatic epithelium, parathyroid cells, fibroblasts, macrophages, mast cells and heterophiles, contain histochemically demonstrable aminopeptidase. In order to establish a correlation between serum and tissue arylami-

dases in the rat, blood serum and supernatants of homogenates of liver, prostate, small intestine mucosa, and skin were subjected to electrophoresis in a starch-gel medium.

Prostate was homogenized at a concentration of 400 mg/ml. and liver at a concentration of 600 mg/ml. A segment of jejunum was resected. The mucosa was rinsed out gently with saline and the intestine was then cut open lengthwise. The mucosa was scraped off with a razor blade. Fragments of mucosa were homogenized at a concentration of 400 mg/ml. Tissues were disintegrated for 10 min in a Potter-Elvehjem homogenizer. Homogenates were spun at 4,000 r.p.m. in a cold centrifuge at 4° C for 45 min. Supernatants were used as enzyme sources.

An area of skin was thoroughly shaved, and the epidermis scraped off. This area, resected without including muscle or subcutaneous tissue, was minced with a razor blade. 600 mg of skin were weighed out and transferred into a homogenizing tube to which 1 ml. of distilled water was added. The sample was homogenized for 10 min while the tube was kept in dry ice. 0.5 ml. of distilled water was added and, after homogenizing for 1–2 min, the supernatant was poured into another tube and the unhomogenized plug was discarded. The supernatant was spun in a cold centrifuge at 4° C for 45 min at 4,000 r.p.m. The clear supernatant was used.

Serum and supernatant of homogenates of these organs were subjected to zone electrophoresis in a starch-gel medium⁶. A 'Heathkit' regulated power supply was used. 6.5 V/cm was kept during the time of electrophoresis, which was 6 h, at room temperature. At the end of this period, gels were sliced and incubated for the demonstration of aminopeptidase as described in a previous publication⁶. 10 experiments were performed for each enzyme source. A diagram representing the average rate of migration of proteins with aminopeptidase activity is reproduced in Fig. 1.

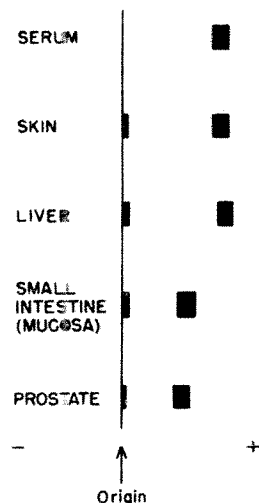


Fig. 1. Starch-gel electrophoresis of rat blood serum and tissues

Two isozymes were present in each of the organs that were studied; an isozyme remaining at the origin, and a migrating isozyme. The moving isozyme showed different but fairly similar rates of migration for each organ. The serum isozyme is most closely duplicated by the skin isozyme, suggesting that this organ might be the source of blood arylamidase.

Dermal fibroblasts showed characteristic reactivity for arylamidase⁴ (Fig 2). These cells extended all through the dermis. They were of laminar appearance with pro-



Fig. 2. Rat dermis. Aminopeptidase (L-alanyl-4-methoxy-2 naphthylamide HCl). Fibroblasts with processes intensely stained. There is an active vascular crevice in the upper left corner of this cell. ($\times 1,250$)

cesses extending along collagen bundles and abutting on small vessels in which endothelial cells were also active for aminopeptidase. This suggests that aminopeptidase of fibroblasts may be the main or only source of serum aminopeptidase.

It has been suggested on the bases of the study of similar enzymes in man that liver aminopeptidase might be the source of the serum enzyme⁷. It would seem probable that liver aminopeptidase, which by histochemical technique is localized in the peribiliary zone in dense bodies and in bile canaliculi, would be represented by the isozyme remaining at the origin. The moving hepatic isozyme resembled closely serum isozyme. Different results have been obtained for bile of man: one peak coinciding with the serum isozyme⁸ or 2 peaks with incomplete resolution, one at origin and the other migrating like the serum isozyme⁹. Bile duct obstruction in man leads to a new aminopeptidase band at origin in serum^{8,9}. This would suggest that the latter is a membrane-bound form which is also found in bile and would correspond to the bile canaliculi and dense body arylamidases.

It is concluded that, in the rat, the serum isozyme of aminopeptidase originates in the mobile isozyme of skin. Since fibroblasts are the main source of skin aminopeptidase, it is suggested that the soluble fraction of fibroblasts aminopeptidase is the main, if not the only, source of the serum isozyme, and that electrophoretically mobile liver isozyme represents cleared enzyme from serum appearing in bile. Experiments are being made to test this hypothesis.

I thank Mrs. Hannah Wasserkug for technical assistance. This investigation was supported by U.S. Public Health Service grant AMO 3219.

BENITO MONIS*

Department of Pathology,
Sinai Hospital,
Baltimore 15, Maryland.

* Present address: Instituto de Patología General, Facultad de Medicina, Corrientes, Argentina.

¹ Gomori, G., *Proc. Soc. Exp. Biol. and Med.*, **87**, 559 (1954).

² Glenner, G. G., in Patterson, E. K., Hsiao, S.-H., and Keppel, A., *J. Biol. Chem.*, **238**, 3611 (1963).

³ Rutenburg, A. M., Smith, E. E., and Kaufman, J. T., *J. Cell Biol.*, **19**, 63 A (1963).

⁴ Monis, B., *Amer. J. Path.*, **32**, 301 (1963).

⁵ Monis, B., *J. Histochem. Cytochem.*, **12**, 869 (1964).

⁶ Smithies, O., *Biochem. J.*, **61**, 629 (1955).

⁷ Smith, E. E., and Rutenburg, A. M., *Nature*, **197**, 800 (1963).

⁸ Kowlessar, O. D., Haeflner, L. J., and Riley, E. M., *Ann. N.Y. Acad. Sci.*, **94**, 836 (1961).

⁹ Smith, E. E., Pineda, E. P., and Rutenburg, A. M., *Proc. Soc. Exp. Biol. and Med.*, **110**, 683 (1962).

Evidence for the Release of Individual Amino-acids from the Resting Human Forearm

THE study of differences in concentration of substances in arterial and venous blood is well-documented, and offers a method for investigating peripheral metabolism.

In order to study this aspect of amino-acid metabolism in man, cannulation of the brachial artery and brachial vein of the same limb¹ was carried out in six subjects, four of whom were normal, one convalescing from a myocardial infarction and one suffering from benign essential hypertension. All the experiments were carried out at 10 a.m., after an overnight fast. Two simultaneous arterial and venous blood samples were withdrawn from each individual; there was an interval of 30 min between the pairs of specimens. Immediately before and again after a blood sample had been taken, the forearm blood-flow was measured by plethysmography with a mercury-in-rubber strain-gauge² and was shown to be constant. During the blood-flow measurements and while the samples were being taken, an occluding cuff around the wrist was inflated to a pressure that was 100 mm mercury above the systolic blood pressure. Soon after the blood had been withdrawn, it was centrifuged in the cold, the plasma protein precipitated with 1 per cent picric acid³ and stored at -15°C until analysed by automatic ion-exchange chromatography⁴. The means of the two arterial and the means of the two venous amino-acid measurements from each person were used for calculating the mean differences found in the group.

Table 1 shows the mean difference in venous and arterial amino-acid concentrations, expressed as a percentage of the arterial concentration, and also the differences themselves, in $\mu\text{moles}/100\text{ ml. plasma}$, in the group of six subjects. The significance of the differences, calculated by testing against the null hypothesis using both tails of the 't' distribution⁵, is also shown. During the time taken for the experiment there was no evidence of a downward drift in either arterial or venous concentrations. The venous levels were, apart from alanine, within the range delineated in a large range of normal men and women⁶. In the present series of experiments the range of alanine concentrations was 21.2–29.3 and the mean was 26.3 $\mu\text{moles}/100\text{ ml.}$

Table 1. DIFFERENCES IN CONCENTRATIONS OF PLASMA AMINO-ACIDS (VENOUS-ARTERIAL) IN THE RESTING HUMAN FOREARM

Amino-acid	Diff. (per cent)	P	Diff. ($\mu\text{moles}/100\text{ ml.}$)	P
Aspartic acid	-6	0.6	-0.24	0.3
Threonine	13	0.025	2.03	0.02
Serine ⁷ , asparagine and glutamine	7	0.05	0.89	0.025
Glutamic acid	0.4	0.6	0.34	0.8
Glycine	17	0.01	3.37	0.005
Alanine	27	< 0.001	5.42	< 0.001
α -Aminobutyric acid	1	0.8	0.01	0.8
Valine	5	0.05 < P < 0.1	0.95	0.05 < P < 0.1
Cystine	2	0.4	0.02	0.7
Methionine	16	0.01	0.30	0.01
Isoleucine	7	0.05 < P < 0.1	0.39	0.05
Leucine	11	0.01	1.14	0.005
Tyrosine	12	0.2	0.40	0.2
Phenylalanine	16	0.005	0.67	0.005
Ornithine	2	0.6	0.11	0.6
Lysine	13	0.025	2.02	0.02
Histidine	11	0.005	0.82	0.005
Arginine	16	0.025	1.28	0.02

* Estimated as serine.

The results indicate that, under the experimental conditions used, there is for some amino-acids, including many that are considered to be essential, a significantly higher concentration in venous than in arterial blood plasma; thus at rest and while fasting there is a release of individual amino-acids from the human forearm.

Furthermore, since there is a difference between arterial and venous plasma in these experiments, it may be important to measure arterial concentrations in investigations of movements of amino-acids between intra- and extra-vascular sites.

We thank Prof. F. T. G. Prunty, Drs. R. D. Lowe and I. M. Young and Mr. A. St. G. Davies for their help, and the Wellcome Trust for a grant enabling us to obtain the automatic amino-acid analyser.

D. R. LONDON
T. H. FOLEY

Departments of Chemical Pathology and Medicine,
St. Thomas's Hospital Medical School,
London, S.E.1.

C. G. WEBB

Institute of Computer Science,
University of London,
London, W.C.1.

¹ Bradley, R. D., *Lancet*, ii, 941 (1964).

² Whitney, R. J., *J. Physiol.*, 121, 1 (1953).

³ Stein, W. H., and Moore, S., *J. Biol. Chem.*, 211, 915 (1954).

⁴ Hamilton, P. B., *Ann. N.Y. Acad. Sci.*, 102, 55 (1962).

⁵ Snedecor, G. W., *Statistical Methods*, fifth ed. (Iowa State University, 1956).

⁶ Job, V., McMath, M., and Coon, W. W., *J. Surg. Res.*, 3, 85 (1963).

PHYSIOLOGY

Quantum Energy Specificity of Simple Photoreceptor Units

THE compound lateral eye of *Limulus polyphemus* has been useful in basic visual function research^{1,2}. *Limulus* ommatidia share similarities in photopigment³, scotopic luminosity function⁴, lateral neural inhibition⁵, and other functions with the scotopic mammalian retina. Light excitability of *Limulus* functional visual receptor units may be modified reversibly by doses of X-rays smaller than required for visual cell pathology⁶. X-ray excitation of *Limulus* ommatidia⁶ and vertebrate retinas⁷ has been reported.

Electrophysiological examinations of more than 100 single and multiple ommatidium preparations have demonstrated inter- and intra-preparation X-ray sensitivity variations in contrast to relatively stable visible light-excitability. Records of single optic axon spike responses to whole lateral eye X-irradiation suggest that the minimal threshold may be low relative to common human exposures. Approximately 0.025 r. is delivered in a routine dental radiographic exposure. Fig. 1 describes the stimulus-response relationship for an X-ray sensitive, dark adapted ommatidium. X-ray parameters were 50 kVp., half-value layer 0.2 mm aluminium. Abscissa, absorbed dose was measured as the incident minus the transmitted dose accumulated during the two-second stimulus. Lateral eye preparation and maintenance have been described elsewhere^{1,5,6}.

It was common to find axons of individual ommatidia which were refractory to X-irradiation doses six log units greater than Fig. 1 threshold and three log unit X-ray intensity variations. The same neural units discharged vigorously during moderate visible stimuli (~10 lux). With less frequency, whole lateral eyes have been tested the optic nerves of which responded with a normal spike output to light flashes but remained largely quiescent during X-irradiation. In these cases it was not possible to record from all optic neurones.

The optic axon potentials in Fig. 2 were recorded simultaneously from two functional unit axons. Light stimulation was provided by an R1131C (Sylvania) glow modulator tube driven by a variable square wave. The stimulus spot diameter was reduced to 75 μ at the cornea. Programmed stimulation began with a 1.7-sec light flash, followed by a 100-sec interval, and then a 4-sec X-ray exposure. A visible test flash was superimposed on the X-ray stimulus. Four records were taken at

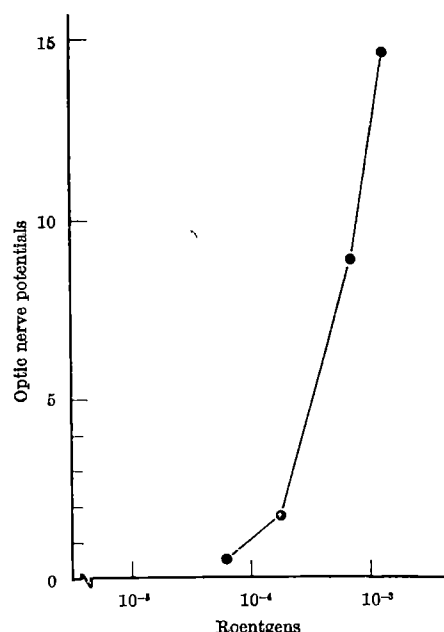


Fig. 1. Stimulus-response relationship for X-ray initiated optic nerve unit activity. Intensity of the two-second X-ray flash was varied by target-eye distance. Datum points are the means of four measures. The potentials indicated single unit activity

X-ray intensities intermediate to A and B of Fig. 2. Before A was recorded both receptors were located by the spot of light and it was determined that both axons responded similarly to the standard visible stimulus. Discharges indicative of two axons are present and may be identified by the degree of positive overshoot (downward deflexion). The unit which produced the greater overshoot emitted consistent response patterns to the standard visible test flash although the second light stimulus in each record was superimposed on and preceded by X-irradiation. Its counterpart responded during X-irradiation and more vigorously as the X-ray stimulus intensity increased.

Reports of retinograms and other retinal potentials may be interpreted to support X-ray excitation of the visual cell by a rhodopsin reaction⁷. Rhodopsin has been established as the photopigment of the *Limulus* ommatidium³, but its action spectrum has not been extended into the X-ray band. Dartnall has suggested that 'inadequate' quanta are not absorbed by the rhodopsin chromophore⁸. The X-ray quanta probably exceed the energy required for photochemical activation which becomes a matter of absorption probability in the chromophore.

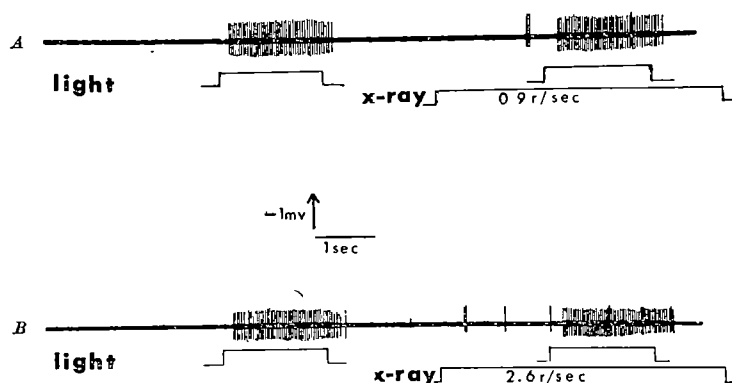


Fig. 2. Optic nerve axon potentials recorded simultaneously from two functional unit axons. Light stimulation was confined to one ommatidium. The whole eye received X-rays. In A the unit with greater positive overshoot responded to light but was not activated by X-rays which excited the unit axon identified by the smaller positive overshoot. Events in B were similar where X-ray intensity was increased. Some potentials were darkened for reproduction

However, quanta of higher energy than those of the visible band may be absorbed in photostable portions of the rhodopsin molecule¹⁰.

It is difficult to explain consistent X-ray stimulus specificity by rhodopsin bleaching. Direct high-energy quantum interaction with the neural apparatus would account for numerous unexplained reports of X-ray avoidance behaviour in animals¹¹ and sensory phenomena in human beings¹².

This work was supported by the U.S. Army Medical Research and Development Command, Department of the Army, under research contract DA-49-193-MD-2498 at the Department of Psychology, Auburn University, Auburn, Alabama.

WILLIAM W. DAWSON

Department of Ophthalmology,
College of Medicine,
University of Florida,
Gainesville.

- ¹ Ratliff, F., Hartline, H. K., and Miller, W. H., *J. Opt. Soc. Amer.*, **53**, 110 (1963).
- ² Ratliff, F., and Hartline, H. K., *J. Gen. Physiol.*, **42**, 1241 (1959).
- ³ Hubbard, R., and Wald, G., *Nature*, **186**, 212 (1960).
- ⁴ Graham, C. H., and Hartline, H. K., *J. Gen. Physiol.*, **18**, 917 (1935).
- ⁵ Lall, A. B., and Chapman, R. M., *J. Opt. Soc. Amer.*, **54**, 1167 (1964).
- ⁶ Dawson, W., and Smith, J. C., *Science*, **129**, 1670 (1959).
- ⁷ Dawson, W., in *Ninth Army Human Factors Conference*, edit. by Barber, J., 19 (Army Research Office, Washington, D.C., 1963).
- ⁸ Lipetz, L. E., *Rad. Res.*, **2**, 306 (1955). Avakyan, Ts. M., *Biofizika*, **1**, 109 (1958).
- ⁹ Bachofer, C. S., and Wittry, S. E., *Rad. Res.*, **17**, 1 (1962).
- ¹⁰ Dawson, W., and Wiederwohl, H., *Rad. Res.*, **24**, 292 (1965).
- ¹¹ Dartnall, H. J. A., *The Visual Pigments*, 90 (Wiley, New York, 1951).
- ¹² Collins, F. D., Love, R., and Morton, R. A., *Biochem. J.*, **51**, 292 (1952).
- ¹³ Garcia, J., Buchwald, N. A., Bach-Y-Rita, G., Feder, B. H., and Koelling, R. A., *Science*, **140**, 289 (1963). Furchtgott, E., *Psychol. Bull.*, **60**, 157 (1963).
- ¹⁴ See, for example, Motokawa, K., Kohata, T., Komatsu, M., Chichibu, S., Koga, Y., and Kasai, T., *Tohoku J. Exp. Med.*, **66**, 389 (1957).

Presynaptic Effects of Potassium Ion on the Mammalian Neuromuscular Junction

THE depolarization of motor nerve terminals by the application of a cathodic current increases the frequency of spontaneous miniature potentials (m.e.p.p.s), but under these conditions the number of such quantal units of transmitter released by a nerve impulse is decreased in proportion to the intensity of the presynaptic depolarization¹⁻³. Although the depolarization of the nerve terminal by elevating the potassium concentration also causes an increase in the frequency of m.e.p.p.s, the potentiation in this case continues to develop even after depolarization has reached a maximum^{3,4}. Although equivalent depolarization can be achieved both electrically and chemically, the effects of these stimuli are not entirely parallel, as the quantum content of the end-plate potential is decreased in the first case but is potentiated in the second^{1,2,5}.

Since it is evident that presynaptic depolarization alone does not account for the facilitatory effect of potassium, the question naturally arises as to whether this cation may affect some other specific process in the mechanism of transmitter release. The work recorded here was carried out in order to determine whether the action of potassium could be accounted for by an increase in the probability of stimulus-evoked transmitter release.

Investigations of the effects of potassium on neuromuscular transmission were performed *in vitro* on the rat diaphragm-phrenic nerve preparation as described by Bulbring⁶. Rats of the Wistar strain, unselected as to sex, weighing 200-300 g, were used in these experiments. The left hemidiaphragm with its phrenic nerve intact was removed under acute ether narcosis and immediately immersed in a physiological solution⁷, which was gassed with a mixture of 95 per cent oxygen and 5 per cent carbon dioxide by means of a lift pump system similar to that described by Szekeres and Vaughan Williams⁸. The temperature was maintained at $33^{\circ} \pm 0.1^{\circ} \text{C}$ throughout the experiments.

Resting membrane potentials and transients were recorded by conventional techniques^{9,10}. E.p.p.s and m.e.p.p.s were displayed at high amplification and photographed from one beam of an oscilloscope. To record the depolarization caused by a given concentration of potassium and to be able to reject fibres damaged during electrode placement, the membrane potentials of all fibres were continuously monitored on a second beam, which received signals through a direct-coupled amplifier. End-plate potentials were evoked by stimulating the phrenic nerve with repeated supramaximal stimuli delivered at frequencies of 25 or 50 c/s for 1-2 sec, and recordings were made when neuromuscular transmission was partially blocked either by the combined action of 10 mM magnesium and 1.5 mM calcium, or by curare, present in concentrations of $2-5 \times 10^{-7} \text{ g/ml}$. The addition of magnesium chloride, calcium chloride and potassium chloride was osmotically compensated for by omitting appropriate amounts of sodium chloride from the medium¹¹.

The number of transmitter quanta released by a nerve impulse was estimated in two ways according to the conditions of the experiment. When neuromuscular transmission was blocked with high concentrations of magnesium, the quantum content m of an e.p.p. was determined from the ratio of the mean e.p.p. to the mean m.e.p.p. amplitude. In curarized preparations, the estimate was made from the ratio of the variance to the mean amplitude obtained in trains of impulses¹². The concentrations of magnesium and curare used in the present work so reduced the postsynaptic depolarization by the e.p.p. that correction for non-linear summation was not necessary. The errors introduced by post-synaptic depolarization and the loss of small m.e.p.p.s into base-line 'noise' were neglected, as both factors were small.

When neuromuscular transmission was partly blocked with magnesium, an increase of the external potassium concentration from 5 to 15 mM produced a rise in the spontaneous quantal discharge frequency (m.e.p.p.s) from 5.8 to 20.8 sec⁻¹, and an increase in the mean number of quanta per e.p.p. from 4.2 to 7.2 (Table 1). To determine whether the facilitation of stimulus-linked release resulted from an increase in the size of the available pool of pre-formed transmitter quanta or whether it was due to a greater probability of release by a given impulse, additional experiments were performed with partially curarized preparations. It could be expected that the rate of initial transmitter depletion during a tetanus would increase if only the probability of release were affected, but would decline if the potassium excess had mobilized a greater number of pre-formed quanta or had speeded the reloading of vacated sites. If, on the other hand, the pool of transmitter were simply increased while the nerve impulse

Table 1. EFFECT OF POTASSIUM ON SPONTANEOUS AND STIMULATED TRANSMITTER RELEASE AT 33°C

	Normal solution 5 mM K ⁺	High potassium solution 15 mM K ⁺
m.e.p.p. freq (sec ⁻¹)	5.79 \pm 1.14	20.81 \pm 2.64
No fibres	(14)	(16)
Mean RP (mV)	79.0	54.3
Mean m *	4.15 \pm 0.75	7.19 \pm 0.47
No fibres	(15)	(28)
Mean RP (mV)	79.1	55.6

* Neuromuscular conduction partially blocked with 10 mM Mg⁺⁺, 1.5 mM Ca⁺⁺; m calculated as mean e.p.p. amplitude/mean m.e.p.p. amplitude during repetitive stimulation at 25 c.p.s.

Table 2. EFFECT OF POTASSIUM ON SUSTAINED TRANSMITTER RELEASE AT 33°C

	Normal solution 5 mM K ⁺	High potassium solution 15 mM K ⁺
Mean RP	73.4	52.2
Mean m	70.0 \pm 10.8*	132.8 \pm 14.9
No. fibres	(14)	(20)

* Neuromuscular conduction partially blocked by δ -tubocurarine $2-5 \times 10^{-7} \text{ g/ml}$.

m Calculated from the ratio of the variance to the mean e.p.p. amplitude during repetitive stimulation at 50 c/s.

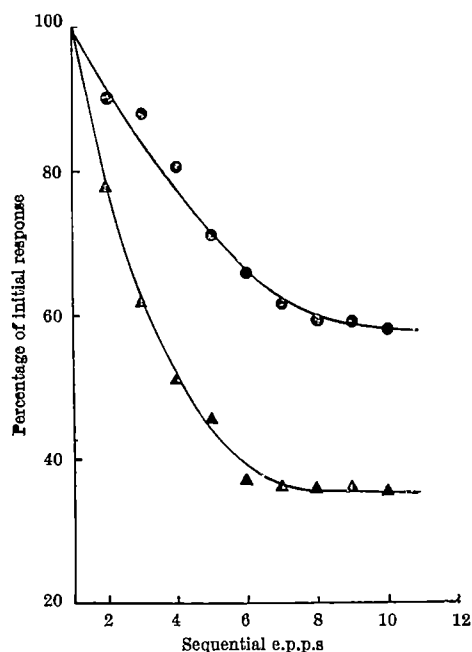


Fig. 1. The effect of potassium ions on the rate of initial transmitter depletion at 33° C. ●, Control solution containing 5 mM K⁺; ▲, high potassium solution containing 15 mM K⁺. Each point represents the average of several fibres plotted as per cent of mean initial end-plate potential amplitude. Stimulation rate 50 c/s

continued to release a constant fraction of transmitter, the depletion rate would remain stable and the absolute number of quanta released by successive impulses would rise.

It can be seen in Fig. 1 that the rate of depletion of transmitter during a tetanus was significantly increased when the potassium concentration was raised from 5 to 15 mM. The absolute number of quanta escaping per initial nerve impulse was nearly doubled by this treatment, the value of m rising from 117.3 ± 18.7 to 249.0 ± 20.2 , and the level of sustained transmitter output, estimated from trains of impulses after an initial rundown, rose from a value of 70.0 to 132.8 (Table 2).

These results indicate that the potassium has at least two direct effects on neuromuscular transmission. It clearly increases the probability of release of transmitter by a nerve impulse and it also raises the number of quanta of transmitter in the readily releasable store.

This work was supported by a grant-in-aid from the U.S. Public Health Service (HE 03693) and a contract between Stanford University and the Office of Naval Research (N0NR 225-46).

RODNEY L. PARSONS
WILLIAM W. HOFMANN
GEORGE A. FEIGEN

Department of Physiology,
Stanford University,
and
Department of Neurology,
Veterans Administration Hospital,
Palo Alto,
California.

Prolonged Administration of Atropine or Histamine in a Silicone Rubber Implant

In the use of drugs, it is often desirable to maintain a constant blood and tissue concentration of chemical for prolonged periods. Several attempts to avoid the 'saw-tooth' oscillations of drug-levels that accompany periodic dosing have been reported. Daily injections of histamine in a beeswax repository have been used to develop gastroduodenal lesions in dogs¹. Thompson *et al.*² found that a single intramuscular injection of highly insoluble pamoate salt (C1-501, cycloguamil pamoate) in mice and monkeys was successful as a repository drug against malaria. A constant level of medication, using silicone rubber as a carrier, was attempted by Folkman and Long^{3,4} for cardiac pacemaker drugs and by Powers⁵ in treating experimental malaria and schistosomiasis. We chose to investigate further the practicability of using silicone rubber to effect the slow release of drugs. Atropine and histamine were chosen for the study because they gave diverse but measurable responses, mydriasis in rats and gastroduodenal lesions in dogs.

Atropine base was placed in tubes of silicone rubber ('Medical Silastic 372', 3.140 in. intern. diam. \times 0.236 in. extern. diam.) (kindly supplied by Dow Corning Corp., Midland, Michigan), 3-6 cm in length to accommodate varying amounts of drug. Ends of the tubes were sealed with silicone medical adhesive. These tubes were washed in water, sterilized in benzethonium chloride, 1:750 ('Phemerol', Parke, Davies and Co., Detroit, Michigan) for 24 h, and placed in sterile distilled water for 24 h.

Thirteen treatments consisted of: subcutaneous tube implants of atropine base (powder) at 400, 200, 100, 50, 25, 12.5 and 6.25 mg/rat; implants of empty tubes plus subcutaneous injections of atropine base suspended in aqueous 1 per cent methyl cellulose in doses of 400, 100, 25 and 6.25 mg/rat; 2 carrier control groups: (a) empty tube implants; (b) empty tube implants plus subcutaneous injection of 1 per cent methyl cellulose. Tubes were placed in a subcutaneous channel between the scapulae, and the skin incision was closed with wound clips. The thirteen treatments were assigned to 78 Sprague-Dawley male rats according to a randomized block design with a total of six blocks. The animals were housed, one block per cage, in a room with constant temperature and humidity.

Following implant, daily observations of pupil dilatation were made with an ophthalmoscope without knowledge of individual treatment. The degree of mydriasis was recorded as one of five possibilities. The normal pupil received a rating of 0, and 25, 50, 75 and 100 per cent dilatations were rated +1 to +4 respectively. The values recorded in Fig. 1 represent the number of days each treatment group had a mean response equal to or greater than +1 mydriasis.

Atropine, enclosed in 'Silastic', produced a dose-dependent mydriasis. Duration of mydriasis was longer with the 'Silastic' tube implant than with the atropine injection. For example, at the 100 mg/kg dose-level, the 'Silastic' tube group had mydriasis for twenty-six days while the injected group had only six days of mydriasis (Fig. 1). The control groups had mydriasis for one day which was probably due to their eating animals that had had received a lethal dose of atropine.

Mortality was dependent on mode of administration. There were no deaths among the rats receiving atropine in 'Silastic' tubes. When atropine was injected subcutaneously, 400 mg was fatal to 6/6 rats and 100 mg was fatal to 3/6 rats. Animals which received injections had severe necrosis at the injection site.

Attempts were made to induce gastrointestinal lesions with histamine implants in dogs of mixed sex and breed weighing 8-10 kg. Histamine (Calbiochem, Los Angeles) was placed in silicone rubber tubes or moulded with 'RTV 5392' (Dow-Corning, Midland, Michigan). To

¹ del Castillo, J., and Katz, B., *J. Physiol.*, **124**, 586 (1954).

² Hubbard, J. I., and Willis, W. D., *Nature*, **193**, 1294 (1962).

³ Liley, A. W., *J. Physiol.*, **134**, 427 (1956).

⁴ Gage, P. W., and Quastel, D. M. J., *Nature*, **208**, 625 (1965).

⁵ Takeuchi, A., and Takeuchi, N., *J. Physiol.*, **155**, 46 (1961).

⁶ Bulbring, E., *Brit. J. Pharmacol.*, **1**, 38 (1946).

⁷ Liley, A. W., *J. Physiol.*, **132**, 650 (1956).

⁸ Szekeres, L., and Vaughan Williams, E. M., *J. Physiol.*, **160**, 470 (1962).

⁹ Ling, G., and Gerard, R. W., *J. Cell. Comp. Physiol.*, **34**, 383 (1949).

¹⁰ Nastuk, W. L., *J. Cell. Comp. Physiol.*, **35**, 39 (1953).

¹¹ Hellbrunn, L. V., *An Outline of General Physiology*, 123 (Saunders, Philadelphia).

¹² Martin, A. R., *J. Physiol.*, **130**, 114 (1955).

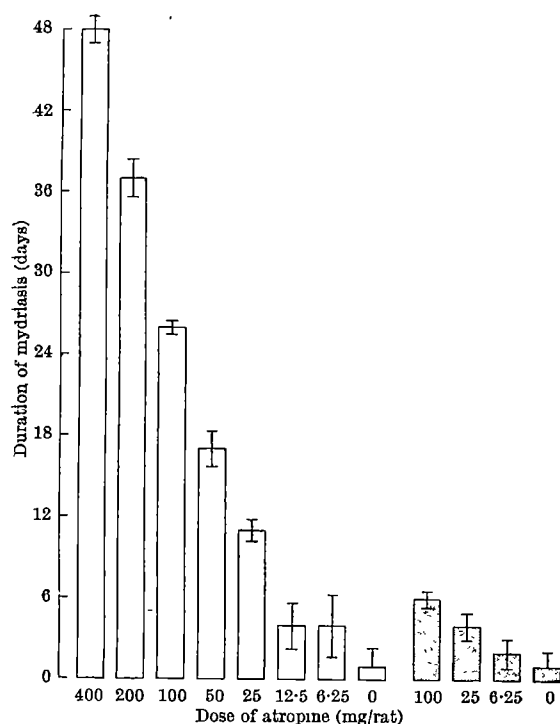


Fig. 1. Duration of mydriatic response in days \pm S.E. to graded doses of injected (stippled bars) and silicone encapsulated (plain bars) atropine in rats.

form the mould, 250 mg histamine base was triturated with 5 c.c. of the liquid silicone rubber, drawn into a plastic syringe and allowed to vulcanize. The plastic syringe was broken to remove the mould. All implants were sterilized as already described here. The tubes, a mould, or a tube plus a mould were implanted subcutaneously between the scapulae. At autopsy the stomach and duodenal areas were examined macroscopically for lesions. The results are shown in Table 1.

Table 1. ABILITY OF VARIOUS FORMS OF HISTAMINE IMPLANTS TO INDUCE LESIONS ON THE GASTRO-DUODENAL AREA OF DOGS

No. of dogs	Method of administration	Amount of histamine (g) per implant	No. of implants	No. of days between first implant and autopsy	Lesions
6	Tube (T)	1-2	1	46-68	Neg.
2	T	2†	1	63	Neg.
2	T	0.5	5*	42	Perforated duodenal
2	Mould (M)	0.5	1	42	Neg.
1	T+M	0.5-T+0.25-M	5*	42	Neg.
2	T+M	0.5-T+0.25-M	4-5*	30-42	Antral and duodenal

* The implants were placed subcutaneously at intervals of one week and autopsy performed one week after the final implant.

† Dose divided among 4 tubes, and implanted subcutaneously in a single operation.

Six dogs received implants of a single tube containing 1 or 2 g of histamine; two dogs received 2 g of histamine divided among four tubes. None of these animals showed gross changes in the appearance of the gastrointestinal mucosa when autopsied 46-68 days after implant (Table 1). Weekly implants of single tubes containing 500 mg histamine into two dogs caused perforated duodenal lesions within 42 days.

The implant of a histamine mould gave negative results. Weekly implants of a tube plus a mould produced lesions in two of three dogs. One had multiple antral and duodenal erosions, the other developed one large and one perforated duodenal lesion (Table 1).

Histamine repositories were evaluated for their effects on gastric secretions in dogs prepared with denervated fundic pouches. Single tubes of 500 mg of histamine base were subcutaneously implanted into three dogs. Secretions and acid outputs were monitored periodically during

the following eight days and compared with pre-implant rates. In a second test, a mould of 250 mg histamine base in 'RTV 5392' was implanted into each of two dogs. Secretions were monitored for 12 days. Neither the tubes nor the moulds had any prolonged marked effect on secretion. The moulds caused a slight increase in secretion on the first day only, probably due to surface-trapped histamine.

Each of four dogs was implanted subcutaneously with four 'Silastic' tubes containing 500 mg histamine (2 g total). One dog showed no change in secretion for 21 days. The second dog had a slight increase in secretion from day 7 to day 37 which returned to basal level. The third dog had an increase in secretion by the 13th day. When killed on the 19th day, numerous large craters were found in the antrum. The gastric pouch and duodenum were normal in appearance. The fourth dog had an increase in gastric secretion by the 15th day. By the 21st day, the animal was losing weight, appeared dehydrated, and had several clonic convulsions. No lesions were found in the gastrointestinal tract.

Like other drugs^{3,4}, atropine encapsulated in a 'Silastic' jacket can exert a slow and prolonged biological response. The lack of effect after a single histamine 'Silastic' tube implant on lesion production or sustained gastric secretion invalidates this type of implant for inducing experimental lesions in dogs. The level of histamine needed to induce lesions or maintain gastric secretion may be critical and cannot be attained by a single implant. The weekly histamine implants did induce gastrointestinal lesions in 4 out of 5 dogs. This type of implant regimen was not tested for its effects on gastric secretion. Chemical determinations will be required to confirm drug-levels in tissues resulting from a repository implant.

A major advantage in using silicone rubber as carrier is that the 'encapsulated' drug can be easily removed from the animal which provides constant control over the duration of exposure to the drug. Waitz *et al.*⁶ demonstrated a similar control with a dialysis bag placed in the peritoneal cavity of rats.

PAUL BASS
R. A. PURDON
J. N. WILEY

Parke, Davis and Co.,
Research Laboratories,
Ann Arbor, Michigan.

- ¹ Hay, L. J., Varco, R. L., Code, C. F., and Wangenstein, O. H., *Surg. Gynecol. and Obstetrics*, 75, 170 (1942).
- ² Thompson, P. E., Olszewski, B. J., Elsager, E. F., and Worth, D. F., *Amer. J. Trop. Med. Hyg.*, 12, 481 (1963).
- ³ Folkman, J., and Long, jun., D. M., *Ann. N.Y. Acad. Sci.*, 111, 813 (1964).
- ⁴ Folkman, J., and Long, jun., D. M., *J. Surg. Res.*, 4, 139 (1964).
- ⁵ Powers, K. G., *J. Parasitology*, 51, sec. 2, 53 (1965).
- ⁶ Waitz, J. A., Olszewski, B. J., and Thompson, P. E., *Science*, 141, 1242 (1963).

Facilitation as a Function of Temporal Spacing of Stimuli in Intracranial Self-stimulation

THE time interval between successive stimuli has been shown in a variety of situations to be crucial for the amplitude of response. For example, at certain intervals, the earlier stimulus may increase the amplitude of the response to the second stimulus. Such a phenomenon is known as facilitation, and has been demonstrated at the neural level, for example, by Lloyd^{1,2}.

In the experiment to be reported here, the time between two successive trains of electrically rewarding stimuli was varied to assess the effects of temporal spacing between them on a threshold of lever pressing.

The subjects were three male albino rats of the Sprague-Dawley strain, weighing about 250 g at the time of operation. Monopolar stainless steel electrodes, insulated except for 0.2 mm at the tip, were implanted. The co-ordinates aimed for were A 3.0, L 0.8, H 3.2 (Nos. 7 and 21) and A 2.5, L 1.0, H 3.2 (No. 38) according to DeGroot's atlas³.

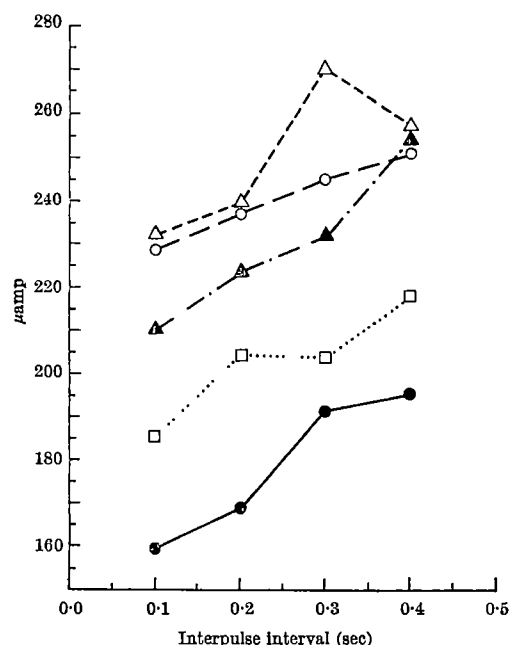


Fig. 1. The curves show how the threshold for the rewarding effect of intracranial stimulation increases as a function of separation of two 0.1-sec bursts of stimulation. The two uppermost curves for Nos. 38 and 7 show the result when the second train occurs 0.9 sec after the lever press. The other curves show the result when the first burst occurs immediately after a lever press is made. The two trains were only available once per second in both conditions. Δ - - - Δ , No. 38; \circ - - \circ , No. 38; \blacktriangle - - - \blacktriangle , No. 7; \square \square , No. 21; \bullet - - \bullet , No. 7.

The rats were trained to press a lever in a Skinner box. A press delivered a 60-c/s large-amplitude stimulus lasting for 0.5 sec. The next stimulus was not available until 0.5 sec after the termination of the previous stimulus. The intensity of the 60-c/s stimulus was so adjusted that when the stimulus was disconnected after a session lasting one minute, the animal would continue pressing for well over a minute. As the method used has already been described^{4,5}, it will be only briefly recapitulated. Short training sessions were given at the end of each of which the current was disconnected. Such training continued until the rat left the bar within a few seconds after the current was switched off. When such training was complete, a session lasting one minute, with 60-c/s stimulus at the high intensity previously used, was followed by a test period of a further minute. During the test minute, the animal could only obtain, if it pressed, a certain intensity of two 0.1-sec trains of 60 c/s. (It is found that below a certain intensity the animal will act as if the current had been disconnected and above this level will tend to keep on pressing to obtain intracranial stimuli.) The criterion taken for the cessation of pressing (behaviour on the part of the animal as if no stimulation was obtained) was the execution of no responses at all for a continuous 30-sec period during the test minute. Reward threshold was then taken to be the step in intensity of the two 0.1-sec trains just above that at which the rat met the criterion of cessation of bar pressing. The stimuli which the rat could obtain during the test periods consisted of two 0.1-sec trains of 60 c/s separated by intervals of 0.1, 0.2, 0.3 and 0.4 sec. The maximum rate at which such train couples could be obtained was once per second.

The experiment was run under two main conditions. In one condition, the first train occurred as soon as the animal pressed the lever, at the beginning of the one-second interval which had to elapse before a further stimulus couple was obtainable. In the second condition (used to control for differential average delay of reinforcement with different intervals between the two 0.1-sec trains with Nos. 7 and 38) the last pulse was always

given at the end of the second after the animal had pressed. In this way the average length of delay of reinforcement after a response was now longest for the 0.1-sec interval between trains, whereas in the first condition when the first train began at the beginning of the lever press, it was the shortest. A descending method of limits was used and the order of intervals (0.1-0.4 sec) was presented randomly from session to session.

The control of the timing of stimuli was achieved by using 'Digibit' logic panels. The 60-c/s stimulus was connected to the animal through a 'Variac' transformer and an isolation transformer. A 200-kohm resistor was placed in series with the electrodes. The stimulus to the animal was monitored on a 'Tektronix 502' oscilloscope.

The results are presented in Fig. 1. An analysis of variance was performed on the results of each individual animal in each of the two conditions separately. All the results indicate a linear trend (of current against interval between trains), the lowest level of significance being at the 0.01 level of confidence, no other effects being significant at this level.

It seems that the threshold for the rewarding effects of intracranial self-stimulation decreases the closer the temporal spacing of two identical stimulus trains. Though at present the relation of such a phenomenon to neural facilitation cannot be inferred with certainty, it seems most likely that the facilitation here observed occurs in the neural system subserving rewarding intracranial stimulation. The length of interval over which facilitation occurs is in excess of 0.4 sec. However, it is difficult to compare such a figure with others obtained under other conditions.

This work was supported by National Science Foundation grant NSF-GB-2882 and National Institute of Mental Health grant MH 10997-01.

J. A. DEUTSCH

New York University.

R. COLLINS

University of California,
Berkeley.

¹ Lloyd, D. P. C., *J. Neurophysiol.*, **9**, 421 (1946).

² Lloyd, D. P. C., *J. Gen. Physiol.*, **33**, 147 (1949).

³ DeGroot J., *Verh. Kon. Ned. Akad. V. Wet., Natuurkunde*, Ser. II, **52**, 1104 (1959).

⁴ Deutsch, J. A., *J. Comp. Physiol. Psychol.*, **58** (1964).

⁵ Deutsch, J. A., *J. Theoret. Biol.*, **4**, 193 (1963).

IMMUNOLOGY

A Specific Agglutinin in the Snail *Otala (Helix) lactea*

BLOOD group specific agglutinins (lectins) were found some years ago in various plants¹⁻³. Such substances have been found also in invertebrate animals⁴, the most recent report being that by Johnson⁵ of a weak anti-A₁ in the clam *Saxidomus gigarticus*.

We have found that the expressed body fluids of the land snail, *Otala lactea*, contain a powerful and specific anti-A agglutinin (titre for A₁ erythrocytes 8,000, for A₂ 2,000). This agglutinin, even undiluted, does not clump group O or B cells at all. It precipitates promptly and specifically with blood group A substance⁶ prepared from hog gastric mucin, and the supernatant from a mixture of these two reagents in optimal proportions contains no detectable A or anti-A activity.

We plan to investigate this interesting substance further; we already have some reason to think that this new anti-A agglutinin may prove useful in distinguishing homozygous AA from heterozygous AO erythrocytes⁷.

This work was supported by grants GM-0474, GM-06939 and HE-01076 from the U.S. National Institutes of Health, and a research grant (GB-2219) from the U.S. National Science Foundation. One of us (W. C. B.) is the

recipient of a National Institutes of Health research career award (K6-6M-14, 184-03).

WILLIAM C. BOYD
REBECCA BROWN

Boston University
School of Medicine,
Massachusetts.

- ¹ Boyd, W. C., *Fundamentals of Immunology*, third ed., 40 (Interscience Publishers, N.Y. and London, 1947).
² Renkonen, K. O., *Ann. Med. Exp. et Biol. Fenniae* (Helsinki), 26, 66 (1948).
³ Boyd, W. C., and Reguera, R. M., *J. Immunol.*, 82, 333 (1949).
⁴ Cushing, J. E., Calaprice, N. L., and Trump, G., *Biol. Bull.*, 125, 69 (1964).
⁵ Johnson, H. M., *Science*, 146, 548 (1964).
⁶ Morgan, W. T. J., and Kling, H. K., *Biochem. J.*, 37, 640 (1943).
⁷ Boyd, W. C., Batla, H. M., Diamond, M. A., and Matsubara, S., *J. Immunol.*, 89, 463 (1962).

Differentiation between Cytophilic Antibody and Opsonin by a Macrophage Phagocytic System

OPSONIC antibody by combining specifically with antigen facilitates ingestion of that antigen by leucocytes. Recently, a cytophilic antibody has been described which becomes attached to mammalian cells *in vitro* in such a way that antigen is specifically adsorbed^{1,2}. The investigation reported here was made to compare the ability of these two types of antibody to potentiate phagocytosis.

Groups of 30 to 40 mice were injected with bovine γ -globulin (BGG) or bovine plasma albumin (BPA) in Freund's complete adjuvant, or *Escherichia coli* somatic polysaccharide antigen. Antisera from each group were pooled. The γ - and β -globulins were precipitated by sodium sulphate³, purified on DEAE cellulose with a phosphate borate buffer to obtain the γ -globulin⁴, and divided into sub-fractions by electrophoresis once or twice in agar gel⁵, the extent of the separation in the gel being detected by rabbit anti-mouse globulin.

Each sub-fraction was brought to the same protein concentration of 0.25 mg/ml. They were tested for the following antibody activities:

(1) Agglutination of sheep red cells to which BGG and BPA were attached either by the tanned red cell technique⁶ or by the *bis*-diaz benzidine coupling method⁷, and to red cells treated directly by the polysaccharide⁸.

(2) Sensitization of the skin to anaphylaxis in mice⁹ (PCA).

(3) Adsorption of antigen by macrophages treated with cytophilic antibody (CT). The macrophages were in monolayers obtained by washing the peritoneal cavity of a mouse with Hanks's balanced salt solution, and diluting the suspension in Hanks's containing 10 per cent normal mouse serum from which any specific antibody had been removed by adsorption in the cold with red cells coated with the specific antigen by *bis*-diaz benzidine. Monolayers were formed by adding the suspension to glass slides for 1 h at 37° C. After washing they were treated with the antibody preparations for 0.5–1 h, washed twice for 30 min, and exposed for 1 h on a rocker at room temperature to a 0.5 per cent suspension of the antigen-coated red cells. Monolayers treated with *E. coli* antisera were also exposed to a suspension of the heat-killed organisms. After washing, the slides were examined under phase contrast. In a positive reaction large numbers of red cells or organisms became adherent to the macrophages; in a negative reaction nearly all the antigen particles remained evenly dispersed between the macrophages.

(4) Opsonization to phagocytosis. Opsonin was detected by exposing the antigen particles at 37° C to the antibody preparations for 1 h, washing them and adding the sensitized particles to the monolayers maintained at 37° C. The result was read after 0.5 and 1 h. An antibody preparation was considered to contain opsonin when the

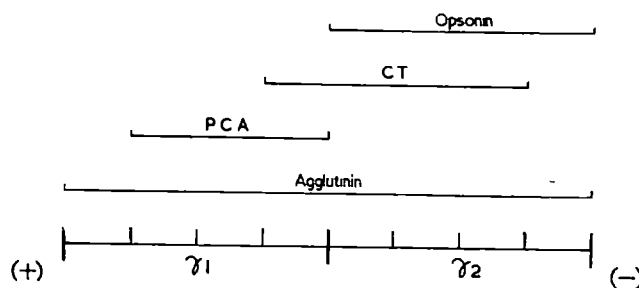


Fig. 1. Antibody activity to protein antigens in fractions of mouse γ -globulin

number of macrophages ingesting antigen was at least twice that in a test of the same antibody preparation adsorbed with the appropriate antigens.

After each test for CT antibody and opsonin, a duplicate slide of each was treated with normal mouse serum adsorbed in the cold to remove antibody to sheep cells and the specific antigen, and also adsorbed by macrophages to remove non-specific CT antibody. This provided complement-like factors which increased the number of macrophages ingesting antigen in both tests.

Two globulin fractions $\gamma 1$ and $\gamma 2$ were separated¹⁰, and each divided into 4 sub-fractions. The various antibody activities to protein antigens in all fractions were presumably those of 7S globulins since the potencies of the fractions were unchanged by treatment with 0.09 M mercaptoethanol pH 7.0.

All 8 sub-fractions of anti-BGG and anti-BPA contained agglutinins (Fig. 1). Antibody sensitizing skin to passive cutaneous anaphylaxis (PCA) was confined to 3 of the $\gamma 1$ fractions, two of which contained no cytophilic antibody (CT), thus confirming the report that cytophilic antibody does not sensitize to PCA².

Opsonin and CT antibody to BGG and BPA were both present in 3 of the $\gamma 2$ sub-fractions, but one $\gamma 1$ sub-fraction contained only CT antibody, and one $\gamma 2$ sub-fraction only opsonin. It was possible to remove selectively the CT antibody from fractions containing both by adsorption with macrophages or chopped whole mouse spleen, whereas adsorption with antigen-coated particles removed both types of antibody.

CT antibody was detectable only on macrophages. There was no adherence to lymphocytes, polymorphonuclear leucocytes or kidney epithelium, which therefore do not take up CT antibody; even 97 per cent pure suspensions of lymphocytes failed to adsorb it. The uptake of cytophilic antibody by cells separated from whole organs^{1,2,11} may be a reflexion of their content of macrophages, though uptake by mast cells has been reported and also red cells¹¹.

The proportion of macrophages ingesting protein-coated antigen particles in tests with CT antibody and opsonin is recorded in Table 1. More macrophages ingested antigen after treatment with cytophilic antibody than those treated with a similar fraction of normal serum. If serum adsorbed free of specific antigen and unrelated CT antibody was added after the antigen particles had adhered to the macrophages, ingestion was greatly increased.

In summary, the properties of cytophilic antibody specific for the proteins are that it is adsorbed by living

Table 1. PROPORTION OF MACROPHAGES, OUT OF 200, INGESTING PROTEIN ANTIGEN COATED ON RED CELLS IN TESTS WITH CYTOPHILIC (CT) ANTIBODY AND OPSONIN

	Macrophages, prior treatment	Test with	Mean % phagocytosis
(1)	Nil	Antigen	20
(2)	Normal serum protein	Antigen	17
(3)	Nil	(Antigen + CT)	28
(4)	CT antibody	Antigen	58
(5)	Opsonin	Antigen	19
(6)	Nil	(Antigen + opsonin)	92

macrophages or those gently killed by drying or freezing, but not by lymphocytes or polymorphonuclear leucocytes. Antigen treated with CT antibody, even in antibody excess, is not adsorbed to normal macrophages. Adsorption of CT antibody is blocked by previous treatment of the macrophages with CT antibody of unrelated specificity, and antigen particles adherent to treated washed macrophages may be eluted by addition of serum containing an unrelated CT antibody. Macrophages treated with CT antibody ingest more antigen particles than cells treated with similar fractions of normal serum. On the other hand, opsonin is not adsorbed to macrophages or polymorphonuclear leucocytes, but combines with antigen particles so that large numbers are ingested by both types of cell.

Analysis of *E. coli* antisera yielded a slightly different result. The CT antibody present in the intact serum was apparently inactivated during salt fractionation and separation on DEAE, and it was destroyed by mercaptoethanol. It appears, therefore, to be a 19S macroglobulin. It promoted ingestion of killed *E. coli* by the macrophages, but not ingestion of red cells coated with the somatic polysaccharide though these adhered to all the treated macrophages. It was clearly distinct from the opsonin which was stable to the fractionation procedures and unchanged by mercaptoethanol. This is similar to the finding of cell-bound 19S antibody and free 7S antibody in mice infected with attenuated *Salmonella typhimurium*¹².

The first stage of phagocytosis is contact of the particle with the surface of the phagocyte. Opsonic antibody changes the surface of the antigen particles so that they are more readily ingested by macrophages and polymorphonuclear leucocytes. Cytophilic antibody is a poor opsonin when added to free antigen, but it increases the speed and number of antigen particles becoming adherent to macrophages; as a result some of the particles are ingested. It is possible that *in vivo* cytophilic antibody sensitizes the macrophages lining sinusoids of lymph nodes, spleen, liver or other organs, so that foreign material, for example, bacteria or their toxins, are more rapidly removed from the circulation. Thus its probable function is to act as a 'cell-bound' (or 'cell-fixed') opsonin.

I thank Prof. A. A. Miles for his advice.

W. E. PARISH

Lister Institute of Preventive Medicine,
London, S.W.1.

- ¹ Boyden, S. V., and Sorkin, E., *Immunology*, **3**, 272 (1960); **4**, 244 (1961).
- ² Sorkin, E., in *The Immunologically Competent Cell*, edit. by Wolstenholme, G. E. W., 38 (Churchill Ltd., 1963).
- ³ Marrack, J. R., Hoch, H., and Johns, R. G. S., *Brit. J. Exp. Path.*, **32**, 212 (1951).
- ⁴ Tee, D. E. H., Watkins, J., Wang, M., Gammack, D. B., and Raper, J. H., *Immunology* (in the press).
- ⁵ Ovary, Z., Benacerraf, B., and Bloch, K. J., *J. Exp. Med.*, **117**, 951 (1963).
- ⁶ Boyden, S. V., *J. Exp. Med.*, **93**, 107 (1951).
- ⁷ Gordon, J., Rose, B., and Schon, A. H., *J. Exp. Med.*, **108**, 37 (1958).
- ⁸ Middlebrook, G., and Dubos, R. G., *J. Exp. Med.*, **88**, 521 (1948).
- ⁹ Ovary, Z., *J. Immunol.*, **81**, 355 (1958).
- ¹⁰ Fahey, J. L., Wunderlich, J., and Mishell, R., *J. Exp. Med.*, **120**, 223 (1964). Nussenzweig, R. S., Merryman, C., and Benacerraf, B., *J. Exp. Med.*, **120**, 315 (1964).
- ¹¹ Keller, R., and Sorkin, E., *Nature*, **199**, 709 (1963).
- ¹² Turner, K. J., Jenkin, C. R., and Rowley, D., *Austral. J. Exp. Biol. and Med. Sci.*, **42**, 229 (1964). Rowley, D., Turner, K. J., and Jenkin, C. R., *Ibid.*, **42**, 237 (1964).

BIOLOGY

Signal Value of the Genital Tassel in the Male *Tilapia macrochir* Blgr. (Pisces: Cichlidae)

In some of the mouthbrooding *Tilapia* species, during breeding season the males develop highly conspicuous appendages on their genital papillae. These are long bifid filaments which are either fringed or festooned with colourful blobs of tissue. The biological significance of

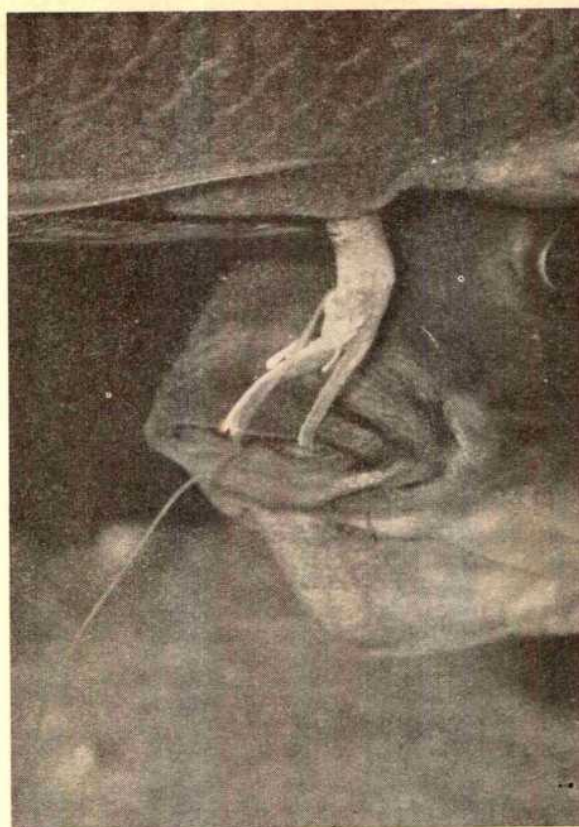


Fig. 1. Female nibbling at the male's genital appendage. The sperm thread is clearly visible at the lower left.

these 'genital tassels' is still obscure, although field-studies have indicated that they play a part during spawning activities^{1,2}. Careful observations of *T. macrochir* in our laboratory tanks have revealed some interesting details. The genital tassel of the male in this species consists of two bright white threads fringed with a few smaller threads of the same colour (Fig. 1). In a fully grown male of 30–40 cm the appendage reaches a length of 5 cm. It acts as a special signal which is presented to the 'ripe' female during courtship and spawning, and assures fertilization of the eggs in the following way:

(1) When a female is ready to spawn she enters a male's spawning pit. After some courtship movements, the male starts to drag his tassel over the bottom of the pit and up the surrounding wall. On top of the wall, he presses his genital papilla to the ground and then rises and swims tail-first back over the female across the spawning pit (Fig. 2). Simultaneously he ejects from his genital opening a long, 0.5-mm-thick whitish thread which sticks by its free end—for a very short time only—to the spot where he stopped dragging his tassel, and a few seconds later the threads drift away in the water.

(2) The female follows the male and grasps the spermatophore-like thread between her lips and inhales it. Very often she not only takes the thread but also the whole genital tassel, or parts of it (Fig. 1), into her mouth. She does this even before she has started to lay eggs and later fairly regularly following each single spawning act. She repeatedly drops some ten eggs, often from a rather peculiar position, namely, standing obliquely head-down several centimetres over the pit. She does not, however, immediately snap up the eggs into her mouth, but will usually follow the tassel-dragging male once more.

(3) The sperm-thread which the male ejects does not normally meet the eggs immediately, since it drifts away from the pit while the eggs are still laying there. The female, therefore, has to snap up the thread to bring it into contact with the eggs, which she starts to gather into

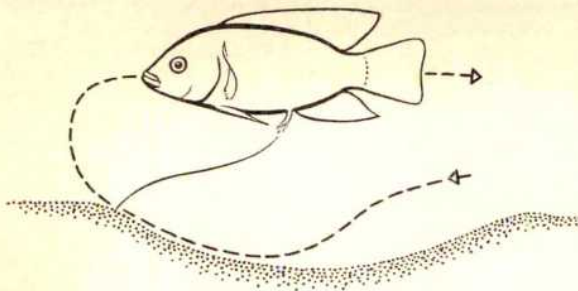


Fig. 2. Movements of the fertilizing male ejecting the sperm thread

her mouth immediately afterwards. The solid sperm mass of this species seems rather favourable in this situation since it is not blown out through the female's gill-openings as readily as would be a loose cloud of milt-fluid found in most of the other *Tilapia* and cichlid-species.

(4) The tassel directs the female's attention to the male's genital opening just before the sperm is ejected, and hence minimizes the danger of the 'spermatophore' being lost. This seems to be the main function of the tassel, which in *T. macrochir* resembles a large number of whitish sperm-threads and, indeed, is treated by the female as such. I hope to investigate this further with dummy experiments.

From the point of view of comparative zoology, it would be interesting to know whether one of the many related species has proceeded still further to true spermatophores. The whole spawning procedure of *T. macrochir* offers phylogenetically important parallels to that of the *Haplochromis*-species in which fertilization of the eggs also takes place within the mouth of the female, and is ensured by egg-dummies painted on the male's anal fin, which are treated by the female as real eggs³⁻⁵.

W. WICKLER

Max-Planck-Institut für Verhaltensphysiologie,
Seewiesen ü. Starnberg, Germany.

¹ Lowe, R. H., *Behaviour*, **9**, 140 (1956).

² Ruwet, J.-Cl., *Behaviour*, **20**, 242 (1963).

³ Wickler, W., *Nature*, **194**, 1092 (1962).

⁴ Wickler, W., *Z. Tierpsychol.*, **19**, 129 (1962).

⁵ Wickler, W., *Encyclopaedia Cinematographica* (Göttingen), Films No. E 470, E 523.

Emission of Feulgen-positive Particles during the *in vitro* Maturation of Toad Ovocytes

THERE has recently been a growing interest in the possible role and localization of cytoplasmic deoxyribonucleic acids (DNA) in amphibian eggs. Our own observations¹⁻³ suggest that large amphibian ovocytes contain at least two different kinds of DNA: a major constituent, which is bound to the yolk platelets and is probably a reserve material of low molecular weight, and a minor component which surrounds the germinal vesicle of the larger ovocytes: the latter is relatively resistant to acid hydrolysis and might originate from the nuclear sap which, as shown by Izawa *et al.*⁴, contains appreciable amounts of DNA. It is of interest to examine the distribution of DNA in ovocytes which, as a result of a treatment with pituitary hormones, undergo maturation (breakdown of the germinal vesicle which results in the mixing of the nuclear sap and the cytoplasm). In fact, some 25 years ago, I examined⁵ DNA distribution during the spontaneous maturation of frog (*Rana temporaria*) ovocytes: it was observed that the breakdown of the germinal vesicle is followed by the appearance of small Feulgen-positive

granules which lie close to the chromosomes. Their origin and fate could not be established.

More precise observations have now been made on ovocytes the maturation of which had been obtained *in vitro*, following the technique of Dettlaff *et al.*⁶. Large ovocytes of *Bufo bufo* or *Xenopus laevis* were placed in 15 ml. of Ringer's solution containing 4 crushed pituitaries of the same species. They were fixed in Zenker every 15 min; alternate sections were stained with methyl green-pyronine and with the Feulgen reaction. Four experiments were made with *Bufo* and one with *Xenopus*. In all of them but one (*Bufo*), *in vitro* maturation was successful. Two experiments with *Triturus* and one with *Pleurodeles* failed: the breakdown of the germinal vesicle was still incomplete after a treatment with 9 pituitaries for more than one day. Our observations are thus, for the time being, restricted to the Anurans.

In both *Bufo* and *Xenopus*, after a 3-h hormonal treatment, the nuclear membrane shows numerous invaginations. They stain strongly with pyronine and appear on the vegetal side. At the same time, the nuclear sap becomes more hydrated and basophilic. The chromosomes are condensed, while the nucleoli, which have moved towards the animal side of the germinal vesicle, begin to shrink and to lose their basophilia. These changes in ribonucleic acid (RNA) distribution might correspond to the synthesis of small amounts of heterogeneous RNA observed by Brown and Littna⁷.

A couple of hours later, the vegetal side of the nuclear membrane is completely broken down; the nuclear sap is now fibrous in appearance and looks like a giant spindle; it carries the strongly Feulgen positive chromosomes. At the limit of the 'spindle', where remnants of the nuclear membrane can still be seen, very small Feulgen-positive particles can be observed. They are never in visible contact with the chromosomes and are very numerous (about 100).

Later on, these fine particles coalesce to form larger spherical Feulgen-positive bodies, which surround the spindle and the chromosomes. Both bodies and spindle move together towards the animal pole. Finally, after a 7-8-h treatment with the pituitary hormones, one or two large bodies, which stain intensely with Feulgen, can be observed in the cortex of the ovocyte, at a short distance from the maturation figure (Fig. 1). Judging from the intensity of the Feulgen reaction (which is negative after treatment of the sections with deoxyribonuclease), these cortical bodies must contain much more DNA than the chromosomes.

The origin and fate of these cortical Feulgen-positive spheres (which can no longer be seen in ripe unfertilized eggs) will remain enigmatic so long as combined autoradiographic experiments (incorporation of thymidine) and electron microscopy observations have not been performed. Although it is impossible for the time being to exclude a chromosomal or nucleolar origin, the most likely interpretation of our observations is that the

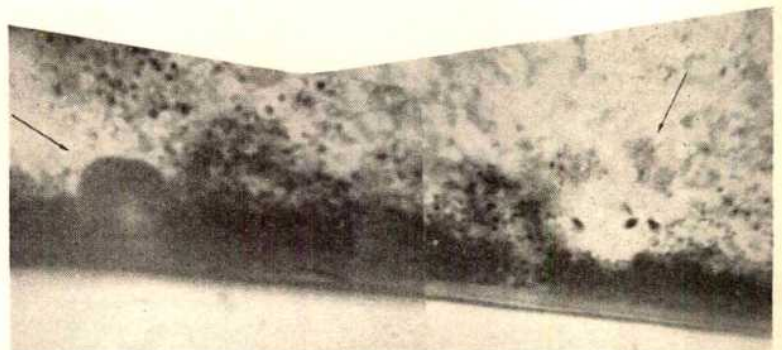


Fig. 1. Cortex of a *Bufo bufo* ovocyte undergoing *in vitro* maturation after 7 h. Arrow on the left, maturation spindle and chromosomes; arrow on the right, Feulgen-positive sphere

Feulgen-positive granules and spheres arise from the nuclear sap, when it mixes with the cytoplasm. If so, they would have the same origin as the DNA found around the germinal vesicle in very large *Triturus* oocytes².

It can be concluded from these observations that, in all anuran species examined (*Rana*, *Bufo*, *Xenopus*), mixing of the nuclear sap with the cytoplasm, as a result of an *in vivo* or *in vitro* hormonal treatment, leads to the production of extra-nuclear DNA, which ultimately moves into the cortex of the egg. This cortical localization of the Feulgen-positive spheres might be very significant, since we know the extreme importance of the dorsal cortex (grey crescent) for morphogenesis (gastrulation, neural induction)⁸. As I have pointed out recently⁹, the dorsal cortex of the fertilized egg must, ultimately, control the activation (or de-repression) of certain genes in the dorsal lip of the blastoporus (organizer). It is conceivable that this de-repression might be mediated, as suggested recently by Frenster¹⁰, by a DNA-like RNA, which should be, in the present case, synthesized on this cytoplasmic DNA template. Very recent experiments by Curtis¹¹ suggest that the dorsal cortex of *Xenopus* eggs possesses its own heredity: his results can only be understood if one assumes that it contains particles capable of independent replication. The presence of DNA, originating from nucleocytoplasmic interactions during maturation, in the cortex might perhaps account for these curious observations. Another attractive possibility is that the cortical DNA might play a part in the determination of the germ-cells, since it is known, from the work of Blackler¹², that ultra-violet irradiation of the vegetal pole in fertilized eggs results in sterility. Much more work is obviously needed before the function of the 'cortical DNA' becomes clear.

This work was carried out under contract Euratom-U.L.B. 016-61-10 ABIB.

J. BRACHET

Laboratoire de Morphologie animale,
Faculté des Sciences,
Université libre de Bruxelles,
Belgium.

¹ Baltus, E., and Brachet, J., *Biochim. Biophys. Acta*, **61**, 157 (1962).

² Brachet, J., and Quertier, J., *Exp. Cell Res.*, **32**, 410 (1963).

³ Brachet, J., and Fleq, A., *Exp. Cell Res.*, **38**, 153 (1965).

⁴ Izawa, M., Alfrey, V. G., and Mirsky, A. E., *Proc. U.S. Nat. Acad. Sci.*, **50**, 811 (1963).

⁵ Brachet, J., *Arch. Biol.*, **51**, 151 (1940).

⁶ Dettlaff, T. A., Nikitina, L. A., and Sroeva, O. G., *J. Embryol. Exp. Morph.*, **12**, 851 (1964).

⁷ Brown, D. D., and Littna, E., *J. Mol. Biol.*, **8**, 669 (1964).

⁸ Curtis, A. S. G., *J. Embryol. Exp. Morph.*, **8**, 163 (1960).

⁹ Brachet, J., *Prog. Biophys. and Mol. Biol.*, **15**, 99 (1965).

¹⁰ Frenster, J., *Nature*, **206**, 1269 (1965).

¹¹ Curtis, A. S. G., *Arch. Biol.* (in press).

¹² Blackler, A. W., *J. Embryol. Exp. Morph.*, **6**, 491 (1958).

Wheel-running Preferences of *Peromyscus*

THE bases for the strong predilection of captive and domestic rodents for wheel-running are not fully understood. It is clear that wheel-running provides a relatively strenuous form of muscular exercise which is not otherwise available to confined animals. In addition, the acts of manipulation and control of the movements of an activity wheel may be rewarding to rodents, quite independently of exercise¹. Rodents also find it rewarding to run motor-driven activity wheels which they start and stop by pressing levers¹.

That the attractiveness of wheel-running cannot be attributed solely to the fact that it is an outlet for vigorous muscular activity is shown by the finding that rodents in extensive enclosures which contain complex burrow-simulating mazes and hundreds of feet of linear runways frequently spend much of their time running an activity wheel². The present study grew out of the observation that deer mice also learn to run activity wheels shaped

into squares, triangles, ellipses, etc., even when round wheels are also available³.

A 15×15×14 in. enclosure was constructed of 'Perspex' and hardware-cloth, containing four adjacent coaxial activity wheels made of 3-in. strips of 1/8-in. mesh hardware-cloth. Dual ball-bearing mountings gave all wheels a very low frictional torque. The 'round smooth' and 'large round' wheels were 10 in. in diameter, the former containing an inner lining of acetate film. The 'small round' wheel was 6 in. in diameter, and the 'square' wheel was 7 in. square. The highest rates at which these wheels were run were: smooth round, 84; large round, 90; small round, 138; and square, 114 r.p.m. Wheel revolutions were sensed by a microswitch actuated by 1/4-in. ball bearings on the end of a half-inch metal strip forming a spoke of the wheel. The spokes extended through slots in the 'Perspex' roof of the enclosure, on which the microswitches were mounted.

Two litters of young captive-born canyon mice, *Peromyscus crinitus* (litter 1, 8 weeks old, 2♀, 1♂; litter 2, 7 weeks old, 3♂), and 2 old males, captured 3 years previously, were selected for study. The old animals had had experience running motor-driven and freely turning wheels 2½ years earlier (see ref. 1). The old males were studied individually, whereas the 3 animals in each litter were studied in consort.

Simply making activity wheels available to inexperienced *Peromyscus*, even for several weeks, does not necessarily give information concerning the preferences of experienced animals for the different types of wheels, because *Peromyscus* generally tend to be conservative in their behaviour⁴. Thus, whichever wheel an animal learns to run first often monopolizes its subsequent activity—the other wheels virtually being ignored. The following programmes were adopted to ensure that the animals would become experienced in running all the wheels. Using individual males, all 4 wheels were made available at first (phase 1) until a constant pattern of wheel running was established and maintained for 2–4 days. The wheel in which the most revolutions were run (which generally was the one in which the most time was spent running) was then locked, and the experiment was continued with 3 wheels unlocked (phase 2) until a new constant pattern was established and maintained for 2–4 days. The favoured wheel of the 3 was then locked (phase 3) and, afterwards, the favoured wheel of the remaining 2 (phase 4). After the animal had learned to run the last unlocked wheel, the wheel of third choice was unlocked until a constant pattern of running was established and maintained for 2–4 days (phase 5). The wheel of second choice was then unlocked (phase 6) and, finally, the wheel originally of first choice was unlocked (phase 7). Phase 7, in which all 4 wheels were available to the experienced animal, lasted at least 6 days. The programme with groups of 3 young animals in consort differed solely in that only 2 wheels were locked (that is, phases 4 and 5, being unnecessary, were omitted).

Running comparisons were made on the basis of average numbers of revolutions run in the different wheels per night. Time spent running, although not measured, is roughly proportional to the product of the number of revolutions run and the circumference of the wheel (7π for the square wheel). The values plotted in Fig. 1 are the averages for the adapted running of the last 2 or more days of each phase. A bright-dim light cycle (L:D 14:10) was used. Recording methods and other experimental conditions have been described elsewhere^{1,2}.

In phase 1, animal 20 ran the large round wheel almost exclusively (13,202 revolutions). When this wheel was locked it preferred the round smooth wheel (21,284 revolutions). In phase 3 it ran 28,679 revolutions in the square wheel and only 5,610 in the small round wheel (Fig. 1). With only the small round wheel unlocked (phase 4) it ran 43,051 revolutions. When the square wheel was unlocked in phase 5 the animal resumed its preference for it (38,905 compared with 7,167 revolutions) and maintained this

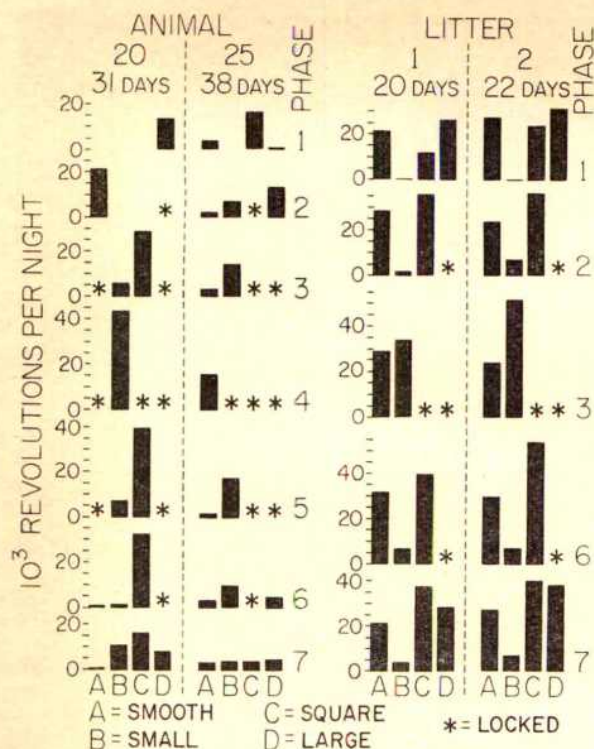


Fig. 1. Wheel running by canyon mice during the programmes described in the text. The figures for days at the top of each data plot refer to the total durations of the programmes

preference in phases 6 and 7. Thus, although the square and small round wheels were not run in phases 1 and 2, they became the wheels of preference in subsequent phases, with the square wheel very much favoured in phases 5 and 6 (Fig. 1). Animal 25 ran much less than animal 20. It preferred the square wheel in phase 1, the large round wheel in phase 2, and the small round wheel in phase 3. It returned to the small round wheel in phase 5, maintained this preference in phase 6 and showed a slight preference for the large round wheel in phase 7.

The 2 groups of young litter mates showed identical sequences of wheel-running preferences (Fig. 1). In phase 1, the order of preference was: large round, smooth round, and square; the small round wheel was more or less ignored. In phase 2 the square wheel was preferred, with some running of the small round one. In phase 3, the small round wheel was preferred to the smooth round one. The preference for the square wheel persisted when it was unlocked (phase 6), but the smooth round wheel now was run much more than the small round wheel. Finally, phase 7 saw a return to running the large round wheel, which had been preferred in phase 1, but the square wheel remained the preferred wheel.

The results of this exploratory study suggest that: (1) although canyon mice may not run certain activity wheels at all when several types are available, none the less they can learn to run these wheels with great facility; (2) the preferences that are established by giving inexperienced animals a choice of several different types of activity wheels usually differ from those of the same animals after they have had experience in running all of the wheels; (3) a wheel with a hardware-cloth surface is preferred to one with a smooth surface; and (4) experienced animals may prefer a square wheel to a round one.

The implication that can be drawn tentatively from the generally slight to marked preference of experienced animals for a square wheel is that confined wild rodents tend to select forms of exercise that require quick reflex actions and split-second timing and co-ordination of movements (running the square wheel required jumping at the

corners at rates up to 9 times per second) in preference to forms in which vigorous muscular activity is the primary requirement. In view of these findings, it appears that round activity wheels may not be the most suitable type for use in studies of confined wild rodents. Experiments designed to explore these implications further with larger numbers of animals of several species are in progress.

This work was supported by grant MH-05001 from the National Institute of Mental Health, U.S. Public Health Service, and grant G-14533 from the National Science Foundation.

J. LEE KAVANAU

Department of Zoology,
University of California,
Los Angeles.

D. H. BRANT

Department of Biology,
Humboldt State College,
Arcata, California.

¹ Kavanau, J. L., *Behaviour*, **20**, 251 (1963).

² Brant, D. H., and Kavanau, J. L., *Nature*, **204**, 267 (1964). *Ecology*, **46**, 452 (1965).

³ Kavanau, J. L. (unpublished).

Methylcholanthrene Carcinogenesis influenced by Vegetable Extracts

A GROUP of 700 three-month-old male mice received subcutaneous injections of 0.1 mg methylcholanthrene in benzene and were then observed for the appearance of tumours for 120 days. The injected mice were divided into four groups which were fed differently.

Group I, the controls, were fed on our standard ration of oats added to maize groats boiled in milk, to which cod liver oil, linseed oil and a polyvitamin preparation were added.

Group II received no raw vegetable food from the day of birth. In place of oats they received bread boiled in milk; their litter of beechwood shavings was sterilized in the autoclave and the complete destruction of enzyme activity in food and litter was confirmed by testing for catalase, which was always absent.

Group III were treated similarly except that they were deprived of vegetable food only from the day of injection.



Fig. 1. Symptom of virosis on leaves of *Nicotiana glauca* (light green mosaic) after treatment with aqueous-extract of oats and the seeds of its usual weeds (*Vicia* sp., *Brassica rapa*, *Agrostemma githago*, *Cirsium* sp.).



Fig. 2. Symptom of virosis (malformation) on the leaves of hybrid tomato provoked with aqueous extract of oats and the seeds of its usual weeds (*Vicia* sp., *Brassica rapa*, *Agrostemma githago*, *Cirsium* sp.)

Group IV were fed like Group I, but given subcutaneous injections of 0.1 ml. 'vegetable extract' twice weekly for six weeks.

The 'vegetable extract' was prepared as follows: 10 g oats was ground to powder, and 100 ml. distilled water added. After standing 1-2 h it was centrifuged for 1 h at 4,000 r.p.m., and the supernatant filtered through a Seitz EK filter. This extract contained a number of plant viruses, since on testing in tobacco, tomatoes and beans characteristic changes occurred (Figs. 1 and 2).

Table 1. EFFECT OF FRESH AND AUTOCLAVED VEGETABLES ON FREQUENCY OF METHYLCHOLANTHRENE-INDUCED TUMOURS IN MICE

Group	No. of animals at start of experiment	No. of animals developing tumours	No. of animals surviving to 120 days without tumours	Total No. of animals completing experiment	χ^2
I	300	(95-83)	108	192	300
II	100	(31-62)	11	88	99
III	150	(46-95)	27	120	147
IV	150	(47-60)	76	73	149
Total	700	222	473	695	86-57

Total $\chi^2 = 86.57$. $P \leq 0.001$.

The results are given in Table 1. It is clear that this vegetable extract enhances, whereas a diet free from raw vegetable materials depresses, the carcinogenic activity of methylcholanthrene. Is it the plant viruses which are the cause of this difference?

V. VENDEG

Virus Research Laboratory,
University of Tirgu-Mures, Romania.

ANATOMY

Sex Differences in the Rate of Tissue Regeneration in the Rabbit's Ear

It has been found that when wounds are made through the full-thickness of the pinna of the rabbit's ear, and tissue of area 1 cm² is excised (Fig. 1), the tissue deficit is almost completely made good within 98 days, though a small hole always remains (Fig. 2). This entails the regeneration of relatively mobile dorsal skin, elastic cartilage, connective tissue, nerve fibres and immobile

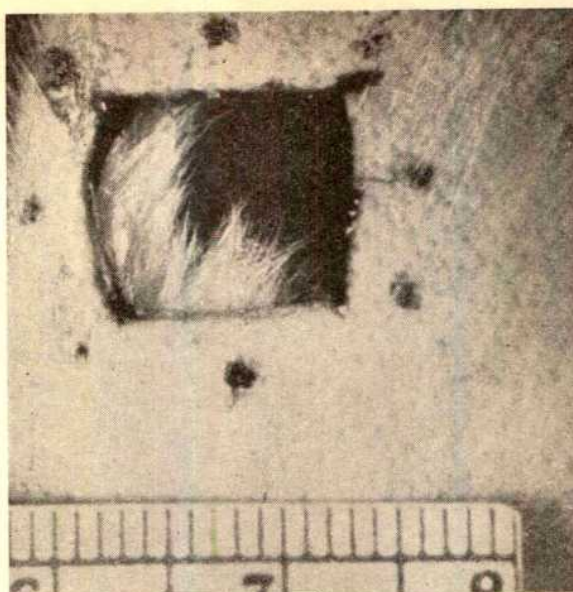


Fig. 1. Appearance of the inside of the ear following removal of a full-thickness portion 1 cm x 1 cm in area

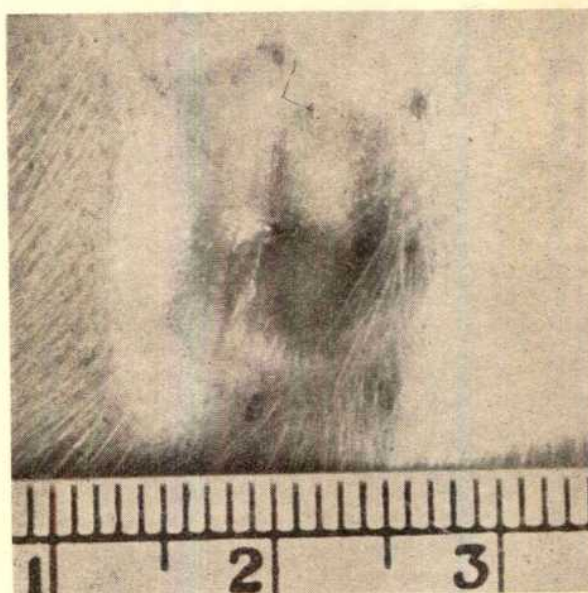


Fig. 2. Appearance of the ear after 98 days. A small central hole remains, but the deficit has otherwise been made good

ventral skin. Furthermore, the process takes place significantly faster in males than in females. It has been reported elsewhere that if either skin¹ or cartilage² is removed from the rabbit's ear it regenerates, the skin completely and the cartilage partially. The replacement of full-thickness deficits has not, however, been reported.

Tissue of area 1 cm² was removed with a specially designed punch from the ears of 30 rabbits (18 male and 12 female) under 'Nambutal' anaesthesia. Care was taken to avoid major blood vessels. Photographic records were kept of the progress of regeneration, the ears being photographed every 7 days. The areas of (a) the central hole, and (b) the zone bordered by the old ventral skin, were calculated from projections of the transparencies on to graph paper by outlining the areas and counting the enclosed squares. The area of the new tissue (a measure of the amount of growth) was determined by subtracting (a) from (b). The amount of contraction (the process whereby the size of the initial deficit is altered by movements of the old adjacent skin) was obtained by subtracting (b) from the initial deficit area. All measure-

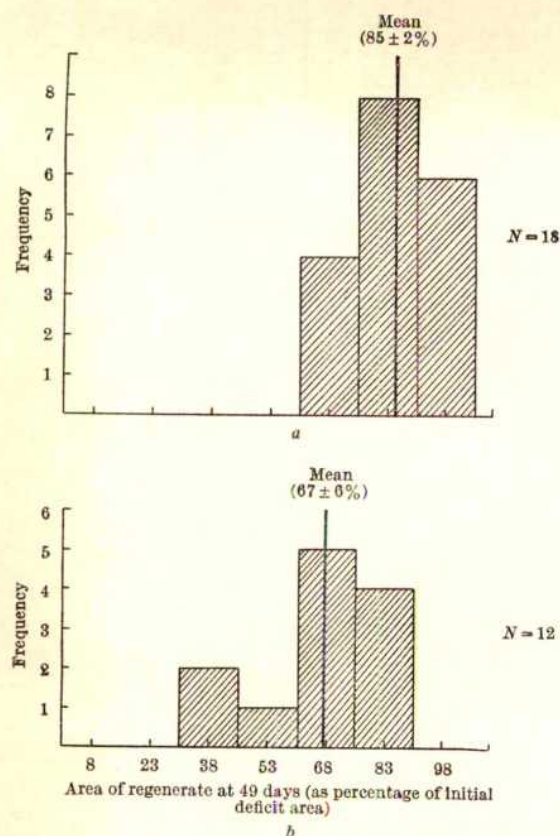


Fig. 3. Histograms showing variation in growth of the regenerate in male (a) and female (b) animals

ments of growth and contraction were expressed as percentages of the initial deficit area.

By 49 days there was a difference of 18 per cent between the areas of the regenerate in males (85 ± 2 per cent) and in females (67 ± 6 per cent); this difference was found to be highly significant ($P < 0.005$). The data on which this was based are given in Table 1 and illustrated in Fig. 3. The difference between contraction in males (3 ± 3 per cent) and in females (1 ± 5 per cent) was not found to be significant ($P > 0.3$). It is possible that the regeneration

Table 1. VARIATION IN GROWTH OF THE REGENERATE IN MALES AND FEMALES

	Area of initial deficit (cm ²)	Area bordered by old skin at 49 days (cm ²)	Area of hole at 49 days (cm ²)	Area of regenerate as % of initial deficit
♂	0.95	0.87	0.02	90
	1.03	1.06	0	103
	1.00	0.94	0.01	93
	0.99	1.08	0.29	80
	1.12	1.12	0.18	84
	1.01	0.88	0.05	82
	1.05	0.99	0.28	68
	1.10	0.83	0	75
	0.98	0.76	0.10	67
	0.99	0.95	0.02	94
	0.95	1.09	0.30	83
	0.98	1.17	0.29	90
	1.02	1.08	0.27	79
	0.98	1.00	0.06	96
	1.02	1.07	0.02	103
	1.05	0.94	0.07	83
	1.02	0.99	0.04	93
	1.10	0.84	0.07	70
			(N = 18, $\bar{x} = 85 \pm 2\%$)	
♀	1.11	1.22	0.43	71
	1.08	1.18	0.38	74
	1.07	1.05	0.60	42
	1.02	0.92	0.57	34
	0.97	0.92	0.13	81
	1.00	1.03	0.18	85
	1.01	1.10	0.45	64
	0.99	1.01	0.15	87
	1.09	1.16	0.43	48
	1.05	1.30	0.44	82
	1.15	0.78	0.02	66
	1.03	0.76	0	73
			(N = 12, $\bar{x} = 67 \pm 6\%$)	

rate is influenced by androgens. Although in large doses they inhibit the production of granulation tissue^{3,4}, most indications are that they promote reparative processes⁵⁻⁷. This may explain the faster rate found in males. Investigations into this possibility are now in progress.

The rabbit's ear provides a useful material for the investigation of mammalian tissue regeneration. Its flatness and position facilitate the keeping of photographic records of the progress of regeneration from which one parameter of this process, surface area, can be measured accurately. It is thus possible to make quantitative observations of mammalian tissue regeneration over a period of time without causing further trauma which would naturally interfere with the process. It also provides an opportunity to study quantitatively the effects of mechanical and metabolic interference on mammalian regeneration at the tissue level.

We thank the Endowments Committee of Guy's Hospital for a grant towards the expenses of this investigation and the Department of Medical Illustration, Guy's Hospital, for the preparation of the illustrations.

J. JOSEPH
MARY DYSON

Department of Anatomy,
Guy's Hospital Medical School,
London, S.E.1.

¹ Joseph, J., and Townsend, F. J., *Brit. J. Surg.*, **48**, 557 (1961).

² Joseph, J., Thomas, G. A., and Tynen, J., *J. Anat.*, **95**, 564 (1961).

³ Taubenhaus, M., and Amromin, G. D., *Endocrinology*, **44**, 359 (1949).

⁴ Taubenhaus, M., and Amromin, G. D., *J. Lab. Clin. Med.*, **36**, 7 (1950).

⁵ Barbera, V., Pollice, L., and Mazzarella, L., *Experientia*, **18**, 424 (1962).

⁶ Needham, A. E., in *Fundamental Aspects of Normal and Malignant Growth*, edit. by Nowinski, W. W., 370 (Elsevier, Amsterdam, 1960).

⁷ Justin-Besancon, L., Rubens-Duval, A., and Villamey, J., *Sem. Hôp. Paris*, **29**, 1998 (1953).

Possible Role of Neuroglia

In a recent communication¹, Hertz has postulated an impulse transmission system in which "potassium ions, which have been lost from one nerve cell during its activity, are transported through neuroglia cells to the outer surface of another nerve cell, which is then depolarized and stimulated; that is, a neuronal-neuroglial-neuronal impulse system". For such a system to operate, the neuroglia cells should bear an appropriate topographical relationship to the nerve cells and should have a capacity for active ion transport; for the pathways to be selective there should be no significant diffusion of potassium ions between cells. Hertz has given good supporting evidence for the second premise, the capacity of glia cells for active ion transport; but the first and third require examination.

The direct synaptic connexion between nerve cells and glia cells which Hertz mentions was described by Scheibel and Scheibel on the basis of Golgi preparations². While these techniques retain an important role in the mapping of neuronal processes, they are no longer acceptable for the demonstration of intimate neural contacts and their interpretation is questionable. Moreover, the synapse-like contacts the Scheibels described were limited to "the application of axonal boutons en passage and boutons terminaux to the cytoplasm and/or nucleus of the oligodendrocyte". Such limited contacts, if they exist, are not highly relevant to a general theory of impulse transmission. More relevant are certain observations by electron microscopists. When adjacent cells come together the outer limits of their osmophilic plasma membranes usually remain separated by an interval of 100–150 Å. In certain situations this interval closes with fusion between the surface dark layers of the adjoining cells. Closed contacts in the central nervous system are formed between glia cells and processes and between endothelial cells³ but have not been observed between neurones and neuroglia. Furthermore, astrocytic processes are arranged in such a way that they isolate the receptive surfaces of

neurones from all axon terminals except those that synapse specifically on them⁴. Since neuroglia-neuronal contacts are open, there exists a perineuronal space of 100–150 Å; this may be contained by closed contacts between glia cells and, as will be seen, is available for diffusion. While neuroglia-neuronal contacts are open and apposed plasma membranes are not thickened, the contacts are not unspecialized. Where certain glial processes abut against neuronal perikarya or dendrites, there is often a sub-surface cistern beneath the neuronal plasma membrane⁵. The sub-surface cistern may have a mitochondrion closely applied to the inner surface⁶. Taken with the presence of an $\text{Na}^+ - \text{K}^+$ stimulated ATPase at the outside of the glia cell membrane⁷ and the observation that cortical neuroglia reacts to an increase in potassium concentration by an increase in oxygen uptake⁸, these observations suggest that certain glia cells may be concerned with the control of the ionic content of the perineuronal intercellular space. Such a control system is necessary to prevent continuous depolarization and spread between neighbouring synaptic areas and along the axonal membrane; the possibility of interference between excitatory and inhibitory synapses here is obvious. Potassium ions are lost to the cell not only during the falling phase of the spike potential during impulse transmission but also during the production of the excitatory post-synaptic potential and particularly during the production of the inhibitory post-synaptic potential⁹. It is suggested that, particularly in the cerebral cortex, excess potassium ions from these sources may be mopped up by active transport into glia cells. It is further suggested that under physiological conditions the potassium ions would be restored to the neurones, perhaps by active transfer via the sub-surface cisterns with an apposed mitochondrion providing the energy to drive the ionic pump. Clearly such a system could be overloaded but, given a low-resistance bypass, short-term overloading is likely to result in a damming up of potassium ions⁹ and diversion at the input stage rather than in overflow at the output stage.

Such a low resistance pathway is provided by the intercellular spaces, which are not, however, restricted to those parts of the central nervous system where potassium-induced stimulation of respiration has been demonstrated. In this context the volume of the extracellular space is an irrelevancy. What matters is not the volume (only 5–7 per cent of the total tissue volume¹⁰) but the arrangement of the intercellular spaces and their effective width. It has been argued that the osmiophilic lines on electron-micrographs do not represent the full thickness of the plasma membrane and that unstained outer layers keep them a uniform distance apart. However, as has been mentioned, the osmiophilic lines can fuse and the bulk of evidence favours the view that the lines represent essentially the full thickness of the membranes¹¹. Diffusion through narrow clefts is not free and a theoretical formula has been used to estimate the restriction they impose¹²:

$$\frac{D'}{D} = \frac{(1 - a/r)^2}{1 + 2.4 a/r}$$

where D'/D is the ratio of restricted to free coefficients of diffusion, a is the radius of the particle and r is the radius of the channel. On this basis, the restriction to potassium ions (hydrated radius 1.25 Å) diffusing through a channel of 125 Å diameter would not be great ($D'/D = 0.92$). However, this formula probably overestimates the restriction since it includes a correction for steric effects at the entrance to the channel which does not apply and presumes a cylindrical channel of radius r instead of a linear slit of half-width r . Manegold has demonstrated experimentally that, on the average, the slit half-width needs to be reduced to 15 times the particle radius if diffusion is to be restricted by 50 per cent¹³. On this basis, again, the restriction to the diffusion of potassium ions through 100–150 Å intercellular spaces should be slight.

More significant than either of these approaches is the demonstration that spaces of these electronmicroscopic dimensions in the central nervous system of the leech provide a rapid and effective pathway for the movement of ions and small molecules¹⁴.

Since this primarily perineuronal pathway has been shown to exist and to be available for diffusion, it is unnecessary to involve the neuroglia as an active intermediary in Leão's spreading depression¹. According to the neuronal-contiguous theory of its transmission, potassium or a special substance which may be glutamic acid is released from depressed neurones to excite nearby neurones¹⁴. One need not look beyond the intercellular space to route this spread or to explain the source of the water and ions which enter the apical dendrites¹⁵. Certainly the advance of spreading depression (2–6 mm/min) is too fast to be accounted for solely by diffusion and too slow for orthodox nervous conduction alone, but this is no argument against a combination of these processes in a neurone-space-neurone sequence of spread. This perhaps operates when the potassium mopping-up mechanism of the neuroglia is overloaded. An important objection to a theory involving neuroglia in the sequence is the limitation of spread from one type of cortex to another. Thus, depression does not spread from neocortex to cingulate mesocortex or to the hippocampus or vice versa¹⁴. In these situations the neurones are discontinuous and spread fails to occur although the neuroglia cells are contiguous.

Finally, the possibility and time-scale of diffusion of potassium ions through intercellular spaces militates against the suggestion¹ "that the early stages of memory should be related to changes in ionic distribution". Although the long-lasting type of memory trace seems to be characterized by RNA synthesis, with changes in the base composition, in neurones¹⁶ and neuroglia¹⁷, the early stages, which occur within a few hours of training, may be unaffected by RNA inhibitors¹⁸. Some intermediary process of longer duration must follow the short-term ion changes. Perhaps this includes the induction of ion-stimulated enzymes in response to overloading of a potassium mopping-up and restoration mechanism centred on the neuroglia.

C. P. WENDELL-SMITH
M. J. BLUNT

School of Anatomy,
University of New South Wales,
Kensington, Australia.

- ¹ Hertz, L., *Nature*, **206**, 1091 (1965).
- ² Scheibel, M. E., and Scheibel, A. B., in *Biology of Neuroglia*, edit. by Windle, W. F., 5 (Charles C. Thomas, Springfield, Ill., 1958).
- ³ Peters, A., *J. Anat. (Lond.)*, **96**, 237 (1962).
- ⁴ Peters, A., and Palay, S. L., *J. Anat. (Lond.)*, **99**, 419 (1965).
- ⁵ Gray, E. G., in *Electron Microscopy in Anatomy*, edit. by Boyd, J. D., Johnson, F. R., and Levez, J. D., 54 (Edward Arnold, London, 1961).
- ⁶ Herndon, R. M., *J. Cell Biol.*, **18**, 167 (1963).
- ⁷ Cummins, J., and Hyden, H., *Biochim. Biophys. Acta*, **60**, 271 (1962).
- ⁸ Eccles, J. C., *The Physiology of Synapses* (Academic Press, New York, 1964).
- ⁹ Frankenhaeuser, B., and Hodgkin, A. L., *J. Physiol.*, **131**, 141 (1956).
- ¹⁰ Horstmann, E., and Meves, H., *Z. Zellforsch. mikroskop. Anat.*, **49**, 569 (1959).
- ¹¹ Coggeshall, R. E., and Fawcett, D. W., *J. Neurophysiol.*, **27**, 229 (1964).
- ¹² Nicholls, J. G., and Kuffler, S. W., *J. Neurophysiol.*, **27**, 645 (1964).
- ¹³ Cited by Pappenheimer, J. R., *Physiol. Rev.*, **33**, 387 (1953).
- ¹⁴ Ochs, S., in *International Review of Neurobiology*, edit. by Pfeiffer, C. C., and Smythies, J. R., **4**, 1 (Academic Press, New York, 1962).
- ¹⁵ van Harreveld, A., and Schaeff, J. P., *J. Cell. Comp. Physiol.*, **54**, 65 (1959).
- ¹⁶ Hyden, H., and Egyházi, E., *Proc. U.S. Nat. Acad. Sci.*, **48**, 1366 (1962).
- ¹⁷ Hyden, H., and Egyházi, E., *Proc. U.S. Nat. Acad. Sci.*, **49**, 618 (1963).
- ¹⁸ Barondes, S. H., and Jarvik, M. E., *J. Neurochem.*, **11**, 187 (1964).

I AGREE with Drs. Wendell-Smith and Blunt that three pre-requisites of the suggested neuronal-neuroglial-neuronal impulse transmission system are: (1) an appropriate topographical relationship between nerve cells and neuroglia; (2) a high capacity for ion transport in glia cells; (3) no significant intercellular diffusion. Since

Wendell-Smith and Blunt regard my supporting evidence for the second of these premises as good, only the two others will be discussed here.

As regards the topographical relationships, no direct synaptic connexion between nerve cells and glia cells is envisaged in the postulated impulse transfer system. The reference to Scheibel and Scheibel's findings was made to emphasize that the idea of a co-operation between neurones and neuroglia in brain function is not a new one, but it was clearly stated that "participation of neuroglia in the transfer of impulses does not . . . necessarily require any direct synaptic contact between nerve cells and glia cells". The mechanism suggested by me represents another, totally independent, way of transferring impulses between the two cell types; a direct synaptic contact is meaningless in this system and the evidence against the findings by the Scheibels accordingly irrelevant in this context.

In the initial phase of the suggested potassium-mediated transfer of impulses "potassium ions lost from a neurone during excitation are transported actively from outside it into glia cells". This is consistent with the widely accepted presence of narrow (100–150 Å) intercellular channels surrounding the cells, and it is important that the volume available for diffusion is small and the routes of diffusion long and complex. The release of potassium ions may therefore cause a temporary increase in the local concentration of potassium, which in turn stimulates the active transport of potassium ions into glia cells.

Admittedly, some diffusion may occur along the intercellular clefts. The extent of this diffusion in mammalian brain cannot possibly be estimated from measurements on the leech central nervous system, which histologically differs grossly from the vertebrate brain^{1,2}; and the findings by Nicholls and Kuffler³ that, for example, sodium ions do not diffuse into certain types of leech glia cells cannot be universally valid, since the sodium space of brain is much larger than the true extracellular space⁴. It is furthermore of no importance that some potassium ions may become passively distributed in the extracellular space—if only the potassium concentration here does not increase so much that a general excitation of nerve cells occurs. What matters is, whether the active transport is large enough to secure that a sufficient fraction of the released ions are transported via predetermined, regulated pathways to exert a selective action on certain nerve cells. To which degree this requirement is fulfilled cannot be estimated by regarding only the possibilities for diffusion but requires knowledge of the efficiency and rate of active transport. No such information is available. It seems, however, thought-provoking that the extracellular fluid in brain cortex is restricted to narrow clefts, and that the extracellular space is small. In this way the active, regulated transport is probably given a possibility to compete effectively with free diffusion. Wendell-Smith and Blunt seem to agree on this point of view, since they suggest that "excess potassium ions may be mopped up by active transport into glia cells".

The fate of the 'mopped-up' potassium ions will depend on the anatomical inter-relationship between adjacent glia cells and thus the possibilities of active transport from one cell directly into another as well as from the 'terminal' glia cell to the perineuronal cleft. The fusion between surface layers of adjoining glia cells referred to by Wendell-Smith and Blunt may constitute the anatomical basis for such a transport.

The hypothesis of a potassium transport through glia cells was primarily provoked by the biochemical, topographical, developmental, and pharmacological analogies between the potassium-induced stimulation of oxygen uptake and spreading depression. It is a result of an effort to combine neurochemical, neuroanatomical and neurophysiological evidence and experimentally less well founded than the initial uptake of potassium ions by glia cells.

I admit it is "unnecessary to involve the neuroglia as an active intermediary in Leão's spreading depression", but the concept that neurones behave like pools of potassium ions which are released by potassium and in turn transported actively by glia cells to propagate the response fits with the evidence mentioned and gives a simple explanation of the rate of the spread. The massive, self-propagating character of the response is probably due to the initial excitation of a very large number of neurones. The subsequent release of potassium ions must accordingly also be large, and they are transported in all directions; the absence of either cell type must, of course, inhibit the propagation. By the term 'overloading' is understood that the potassium release is "so intense that a certain time is required for its uptake in neuroglia"; this is the reason that neurophysiologically a depression is observed instead of an activation and consistent with the concept by Wendell-Smith and Blunt: "overloading is likely to result in a damming up and diversion at the input stage". Altogether, I therefore find that their concepts differ only slightly from mine, and this may perhaps be taken as an indication that the proposed mechanism is at no variance with present neuroanatomical thinking.

L. HERTZ

Biochemical Institute A,
University of Copenhagen,
30 Juliane Mariesvej, Copenhagen.

¹ Coggeshall, R. E., and Fawcett, D. W., *J. Neurophysiol.*, **27**, 229 (1964).

² Kuffler, S. W., and Potter, D. D., *J. Neurophysiol.*, **27**, 290 (1964).

³ Nicholls, J. G., and Kuffler, S. W., *J. Neurophysiol.*, **27**, 645 (1964).

⁴ Zadunaisky, J. A., and Curran, P. F., *Amer. J. Physiol.*, **205**, 949 (1963).

ENTOMOLOGY

An Improved Method for obtaining, *in vitro*, Uniform Cell Monolayer Sheets from Tissues of the Tick, *Hyalomma dromedarii* (Ixodidae)

THE first report on the cultivation of tick (Ixodidae) tissues *in vitro* was that of Řeháček¹ in Czechoslovakia, who obtained proliferation of fibroblast type cells in explants from developing adults of *Dermacentor marginatus*. Řeháček^{2,3} afterwards improved his techniques and was able to obtain cell monolayers by trypsinizing tissues of developing adults of *D. marginatus*, *D. pictus* and *Hyalomma dromedarii* and growing them in test-tubes and Carrol flasks. His methods for preparing tick tissues and of culturing them are fully described elsewhere⁴.

In culturing tissues from developing adults of *H. dromedarii*, we found that certain modifications in Řeháček's methods and of his medium produced better results. There was improved dissociation of the tissues and hence a higher cell yield when the tissues were agitated by a magnetic stirrer in 0.25 per cent trypsin which had been prewarmed in a 37° C water bath. The resulting cell suspension was filtered through sterile gauze to remove large pieces of undissociated tissue.

The pH of the medium was adjusted to 6.9, 0.2–0.4 higher than in Řeháček's experiments, and this appeared to produce more rapid attachment of the cells to the glass. At 27°–29° C, adherence of cells to the glass surface started in less than 20 min and, by 18–20 h, cell attachment was nearly complete. In our earlier trials, we used Leighton tubes with and without cover-glasses and incubated them horizontal. In these tubes, there was a tendency for the cells, over the major part of the glass surface, to aggregate in clumps from which fibroblast type cells grew out, but they never formed a continuous sheet. Although towards the bottom part of the tubes there was a narrow strip where the growing cells had formed a more or less continuous sheet, growth of the culture as a whole was by no means as satisfactory as the uniform cell monolayers obtained in vertebrate cell cultures. Mitoses in the Leighton tube cultures were seen up to 10 days, the same period as in Řeháček's cultures⁴.

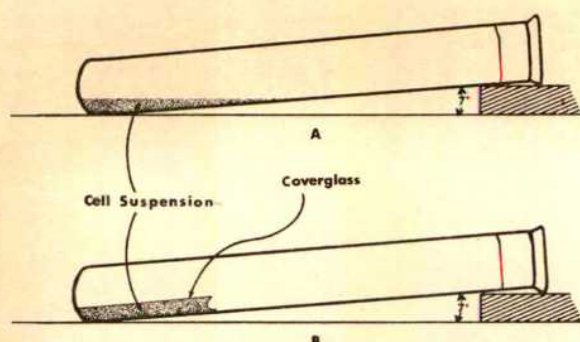


Fig. 1. Cultivation of tick cells in test-tubes, without cover-glass (A) and with raised cover-glass (B)

In subsequent investigations to improve the cultures, we used 'Pyrex' glass test-tubes 125 mm x 16 mm, incubated at an angle of about 7°, and found that, as in the Leighton tubes, by about the 4th day, there was a patch of cells growing into a sheet towards the bottom of the tubes, but over the rest of the glass surface the cells formed scattered aggregates (Fig. 2, B, D). We next introduced into the test-tubes, before seeding of cells, rectangular cover-glasses (40 mm x 11 mm) primarily with the object of obtaining growing cells on the cover-glasses for microscopical examination. However, the width of the cover-glass was such that it remained raised above the curved side of the tube (Fig. 1, B) with the cell suspension underneath it. In such tubes the cells always grew out on the glass surface of the tube as an excellent, uniform monolayer sheet, consisting mostly of epithelial type cells. There were no cells, or only a few, attached to the cover-glass. In appearance, these cell sheets (Fig. 2, A, C) were similar to those obtained from vertebrate cells. Furthermore, the metabolism in the tubes appeared to be higher with cover-glasses than without as evident from the more rapid change in the pH of the covered medium. The cell sheets in the tubes with cover-glasses have, with periodic changes of medium, been kept for up to 35 days without any obvious signs of degeneration. There was recurring mitotic activity and mitoses were common even 30 days after seeding, the longest period recorded so far for tick tissues *in vitro*.

What caused and maintained the better growth in the tubes with cover-glasses in these preliminary investigations is not clear. Cell aggregation which occurred by about the 4th day in tubes without cover-glasses would appear to slow down metabolism. One explanation may be that the partially closed system of the cell suspension

in medium trapped between the overlying cover-glass and the curvature of the tube produces physical forces which prevent the cells from aggregating. Towards the free surfaces of the medium, that is, at the end and less so at the edges of the cover-glass where these forces may be less, the cells do tend to form clumps, particularly at the open end (Fig. 2, A, C). It is also possible that this confined system produces, except towards the limits, partially anaerobic conditions which may be more suitable for the continued growth of the cells, or that the carbon dioxide evolved during the metabolic processes is prevented from diffusing out to any great extent, thus increasing the carbon dioxide tension of the medium. These aspects are now under investigation. But, whatever the explanation may prove to be, inclusion of an overlying cover-glass appears to maintain consistently better cell growth and a more uniform cell monolayer sheet than if it is omitted. This modification of technique may also enhance the utility of the cell cultures for the cultivation of viruses and other pathogenic organisms, the purpose for which the technique is primarily intended.

We thank Dr. J. Řeháček of the Institute of Virology, Bratislava, Czechoslovakia, for his help at the beginning of these experiments, also Prof. D. S. Bertram, director of this Department, and Prof. Forrest Fulton of the Department of Bacteriology and Immunology for their advice, Mr. D. F. King for staining the tubes, Mr. C. J. Webb for taking the photograph and Miss Anne Caisley for preparing Fig. 1.

M. G. R. VARMA
WENDY WALLERS

Department of Entomology,
London School of Hygiene and Tropical Medicine,
London, W.C.1.

¹ Řeháček, J., *Acta Virol.*, 2, 253 (1958).

² Řeháček, J., *Acta Virol.*, 6, 183 (1962).

³ Řeháček, J., *Ann. Epiphyties*, 14, 199 (1963).

⁴ Řeháček, J., *J. Med. Entomol.* (in the press).

MICROBIOLOGY

Occurrence of Dimorphic Forms of *Mycobacterium phlei*

DURING the course of an investigation concerning the lipid fractions of *Mycobacterium phlei* it was found that liquid cultures which had been incubated for a period of 1 week at 37° showed the formation of a dry, rough pellicle. However, after incubation for 5 weeks only a sparse pellicle remained and the rough pellicle observed at 1 and 2 weeks had fallen to the bottom of the pan, forming a thick, mucoid deposit. This deposit was unlike the granular deposit obtained in young cultures of mycobacteria and it was therefore decided to investigate the morphology of the organisms in these cultures. Using the method described in this communication, three morphological phases of growth from *M. phlei* were consistently obtained, which formed part of a definite cycle of growth.

Non-acid-fast stages in the life-cycle of different members of the Actinomycetaceae and Mycobacteriaceae have frequently been observed¹. Rich² noted that considerable differences in the morphology of individual organisms could be found and this depended on the age of the culture, the character of the medium and the conditions of growth. Csillag³ has obtained various non-acid-fast organisms from *Mycobacterium tuberculosis* var. *hominis* and atypical mycobacteria. The typical acid-fast bacilli were termed 'Form I' mycobacteria and five different non-acid-fast types of organ-

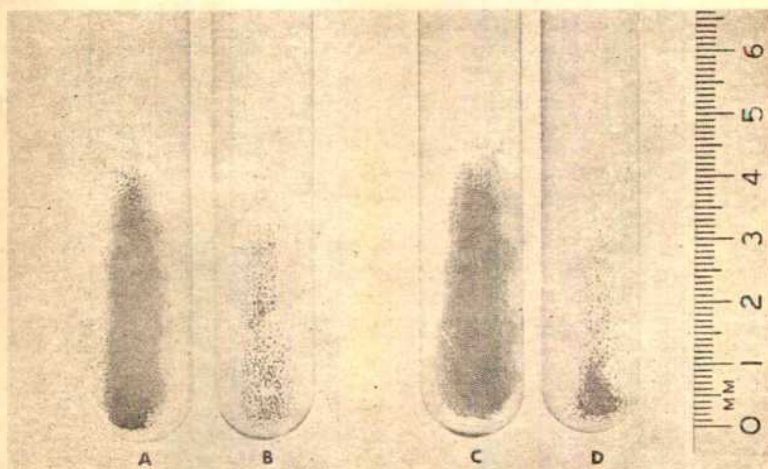


Fig. 2. A, B, Culture 7 days after seeding, stained with Mallory's stain; A, with raised cover-glass; B, without cover-glass. C, D, Culture 10 days after seeding, stained with haematoxylin and eosin; C, with raised cover-glass; D, without cover-glass

ism were grouped together under the heading 'Form 2' mycobacteria. Csillag⁴ found that a coccoid form could be obtained from the 'Form 2' mycobacteria and regarded these as conforming with the definition of mycococci, which were originally described by Krassilnikov⁵. The genus *Mycococcus* is defined in *Bergey's Manual*⁶ as consisting of cells of variable size and shape, generally spherical, occurring singly, in short chains or in clumps. Multiplication is by fission, constriction or bud formation. The cells are non-acid-fast, Gram-positive, aerobic and grow well on ordinary culture media.

The media used were: (a) 1 per cent glucose meat extract broth; (b) meat extract agar; this was prepared by mixing 1 lb. fat-free minced horse heart, 10 g peptone, 5 g sodium chloride and 1 l. tap-water, and heating for 30 min at 65°. The following day the mixture was heated for 2 h at 100°, filtered through coarse paper and the pH adjusted to 8.5. The broth was steamed for 45 min and the phosphate deposits filtered off on fine filter paper. Difco agar (14 g) was added and the mixture steamed for 30 min, filtered and sterilized at 15 lb. for 20 min; (c) a modified Sauton medium^{7,8}: 5 lb. potatoes were left overnight in 4 per cent KMnO_4 solution, washed in distilled water, peeled and chipped. Distilled water (5 l.) and glycerine (1 l.) were added to the chipped potatoes and the mixture was left to infuse at 60° overnight. The potato extract was filtered off through gauze. The following ingredients were added to the extract: 48 g sodium glutamate, 3 g K_2HPO_4 , 0.3 g brown ammonium iron citrate, 12 g citric acid, 3 g magnesium sulphate. The total volume was adjusted to 12 l. and the medium was steamed for 30 min. After filtering off any deposits the medium was distributed in 500-ml. amounts in 2-l. Caroll flasks (J. Jobling and Co., Ltd.) and autoclaved at 10 lb. for 10 min.

Cultivation of the organisms was as follows: A starter culture was prepared by inoculating a flask of 1 per cent glucose broth with *M. phlei* and this was incubated for 3–4 days at 37°. Part of the pellicle from this starter culture was transferred on a large loop to the surface of the Sauton medium. The cultures were incubated for six weeks at 37°.

Smears of the deposit in the Caroll flask were stained by the method of Gram and the Ziehl Neelsen method⁹. Examination of the stained preparations showed that 25 per cent of the cells were acid-fast and the remaining 75 per cent were non-acid-fast (Fig. 1), and these showed two different morphological types. The first of these was a Gram-positive rod with square ends; the rods sometimes appeared curved or bent double. The second type was a poorly stained Gram-negative organism containing a large clear ovoid inclusion.

Meat extract agar plates, previously incubated for 48 h to check sterility, were inoculated with the deposit from the 6-week-old cultures and incubated at 37°.

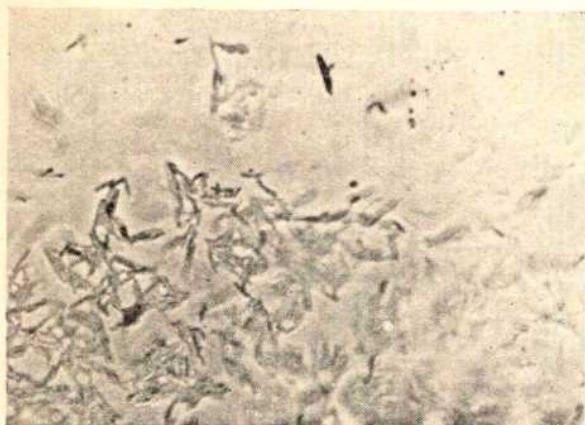


Fig. 1. Cellular morphology of a six-week-old culture of *M. phlei* ($\times c. 750$)

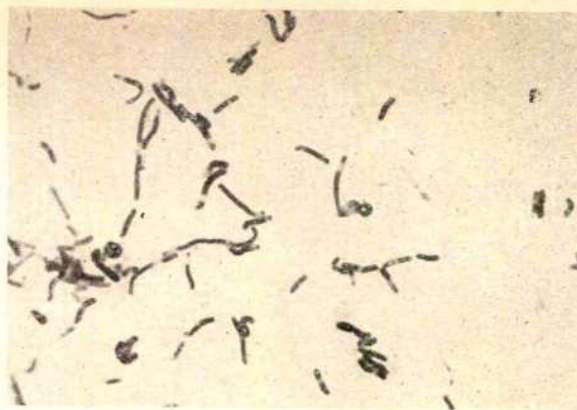


Fig. 2. Gram stain of Phase 1 of *M. phlei* ($\times c. 750$)

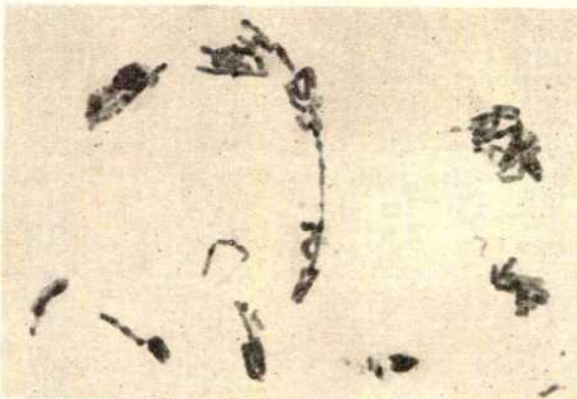


Fig. 3. Sudan black stain of Phase 1 of *M. phlei* ($\times c. 750$)

After 36 h large flat colonies of acid-fast *M. phlei* were present; but after longer incubation small regular colonies, 0.4–0.6 mm in diameter, and larger irregular colonies, 2–3 mm in diameter, were found. From both types of colony sub-cultures were grown on meat extract agar. However, it was found that when these organisms had been sub-cultured several times the morphology, which had been checked at each stage, was obviously changing. Consequently, a further set of meat extract agar plates was inoculated with the rod-form, incubated for three days at 37° and then left at room temperature for one month. It was found that the colonies on these plates, which were 3–6 mm in diameter, contained coccoid forms of variable size, arranged singly, in pairs or in clusters.

The morphology and staining reactions of the organisms isolated may be summarized as follows:

Phase 1. Large, non-acid-fast, motile, Gram-variable rods with a thick cell wall and a thin capsule. Each cell contained two or more large inclusions which were quite distinct and stained positively with Sudan black⁹ (Figs. 2 and 3).

Phase 2. Small, Gram-negative, motile, pleomorphic organisms containing a large inclusion which almost filled the cell. These inclusions were sometimes acid-fast but only gave a very weak spore-stain reaction. After delipidation of the smear with alcohol-ether (1:1) for 2 min these ovoid inclusions were strongly stained by a spore-stain method⁹. It is possible that these reactions may be related to the acid-fastness (Figs. 4 and 5).

Phase 3. Gram-positive, capsulated, non-motile cocci of variable size, lying singly, in short chains or small clusters. These organisms contained no large inclusions and were non-flagellated (Fig. 6).

These phases 1 and 2 of growth from *M. phlei* are identical to two phases of growth included under the term 'Form 2' mycobacteria by Csillag⁴. Phase 3 from *M. phlei*



Fig. 4. Spore stain of Phase 2 of *M. phlei* after delipidation of the smear ($\times c. 750$)



Fig. 5. Gram stain of Phase 2 of *M. phlei* showing the unstained ovoid inclusions ($\times c. 750$)

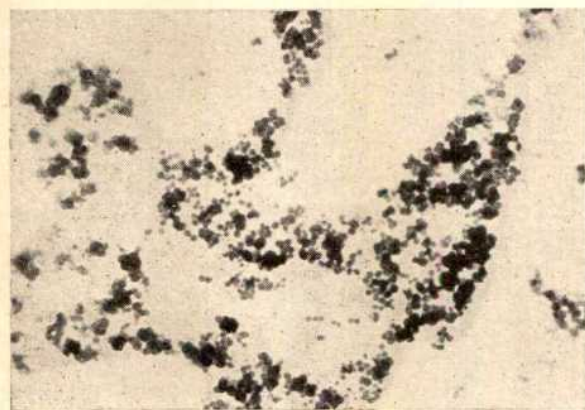


Fig. 6. Gram stain of Phase 3 of *M. phlei* ($\times c. 750$)

corresponds to the 'mycocoecal stage' isolated by Csillag⁴. Since it is possible to obtain the same phases of growth, the method described here has some advantages over previously reported procedures. The phases 1, 2 and 3 from *M. phlei* were obtained within 7 weeks without aeration of the cultures, whereas the 'Form 2' and 'mycocoecal' stages were obtained after 14–52 weeks incubation with intermittent aeration^{3,4}.

The existence of a growth-cycle in mycobacteria has been suggested by various workers¹⁰ although the precise nature of this cycle has never been determined. McClung¹¹ has described the cyclic nature of growth and the morphological changes which occur in the different species of the genus *Nocardia*. It is well known that a close relationship exists between the two genera *Nocardia* and *Mycobacterium*^{12,13}. Consequently, if such a growth-cycle could be found in all species of mycobacteria there would be additional evidence to suggest that *Nocardia* and *Mycobacterium* should be included in the same family.

Further biochemical and serological experiments are being carried out with these phases of growth isolated from *M. phlei* in order to determine the nature of this growth cycle.

I thank Miss Kate McLean for her technical assistance in this work.

D. E. S. STEWART-TULL

Department of Bacteriology,
Anderson College,
University of Glasgow,
Glasgow, W.1.

¹ Waksman, S. A., *The Actinomycetes*, 2 (Baillière, Tindall and Cox, London, 1961).

² Rich, E., *The Pathogenesis of Tuberculosis* (Springfield, Thomas, 1944).

³ Csillag, A., *J. Gen. Microbiol.*, **26**, 97 (1961).

⁴ Csillag, A., *J. Gen. Microbiol.*, **34**, 341 (1964).

⁵ Krassilnikov, N. A., *Diagnostik der Bakterien und Actinomyceten* (Gustav Fischer, Jena, 1959).

⁶ Breed, R. S., Murray, E. G. D., and Smith, N. R., *Bergey's Manual of Determinative Bacteriology*, seventh ed. (1957).

⁷ Caneela, F. J., and De Bravo, U., *Bull. Hyg.*, **33**, 68 (1958).

⁸ Takeya, K., and Hisatsune, K., *J. Bact.*, **85**, 16 (1963).

⁹ Mackie, T. J., and McCartney, J. E., in *Handbook of Bacteriology*, edit. by Cruickshank, R., tenth ed. (Livingstone, London, 1960).

¹⁰ Wilson, G. S., and Miles, A. A., *Topley and Wilson's Principles of Bacteriology and Immunity*, fifth ed., 541 (Arnold, London, 1964).

¹¹ McClung, N. M., *Kansas Acad. Sci.*, **58**, 50 (1955).

¹² Cummins, C. S., *J. Gen. Microbiol.*, **28**, 35 (1962).

¹³ Cummins, C. S., and Harris, H., *J. Gen. Microbiol.*, **18**, 173 (1958).

VIROLOGY

Propagation and Properties of Hepatitis Virus

TESTING a newly developed chemical reaction, we found the virus of hepatitis to consist of pure deoxyribonucleic acid without proteins, contrary to other known forms. This was investigated and confirmed as follows: by treatment with alcohol the virus can be precipitated like the nucleic acids and can be dissolved afterwards without loss of activity. With other virus such treatment led to total inactivation^{1,2}. This property of the hepatitis virus enables one to prepare highly concentrated and pure samples, which do not contain proteins from tissues or other virus.

The hepatitis virus itself cannot permeate into the cell, the protein hull of virus being, as is well known, responsible for the affinity to the host cells. However, if the virus is brought into the cell by special treatment, then propagation and formation of plaques occur as with other viruses. The fact that, in such cases, the virus can be propagated in many tissue cultures confirms its character as a pure nucleic acid³.

Enzymes that cleave nucleic acids degrade the virus, but trypsin, papain, Na-deoxycholate and ether are without any effect.

In vitro, the virus can be fixed by actinomycin like other deoxyribonucleic acids.

The following factors also indicate that the nucleic acid of the hepatitis virus is deoxyribonucleic acid (DNA): (1) Total inactivation by ENase, but not RNase; (2) 5-iodo-2'-deoxyuridine yielding an inhibition of propagation *in vivo*, while substances influencing the metabolism of RNA, such as 8-azaguanine, 2-thiouracil and 5-fluorouracil, have no influence; (3) actinomycin inactivates the virus *in vivo* as well as *in vitro*; it is known that actinomycin blocks DNA, but not RNA, *in vitro*; (4) estimation by the method of Schmidt and Thannhauser⁵ confirms the DNA character of the virus.

The hepatitis virus can be propagated on the chicken embryo by the following technique: (1) Treating the chorio-allantoic membrane with hyaluronidase just before inoculation; this loosens the tissue and thereafter the virus can passively permeate into the tissue; (2) the

cytotoxic effect, especially from sera, can be removed by dilution, otherwise it prohibits multiplication of the virus; (3) the effect of DNA in cleaving enzymes such as DNase I and DNase of tissue origin will be greatly reduced by adding 0.01 moles Na-citrate and shifting the pH up to 8.5.

In tissue cultures the cells were washed before inoculation with 1 M NaCl, pH 8.2. The inoculation fluid contained hyaluronidase. Further propagation for many passages in tissue cultures is possible only if the pH of the medium does not go below 7, since pH 6.5 strongly decreases the active virus particles. The optimum pH for preservation of the hepatitis virus is between 8.5 and 9.5.

Plaque formation on the chorio-allantoic membrane appeared on the third day, and in liver and spleen on the fourth day after inoculation, the foci being similar to those of other virus, both morphologically and histologically⁶. In tissue cultures the beginning of the cytopathogenic effect was seen on days 3-4 after inoculation.

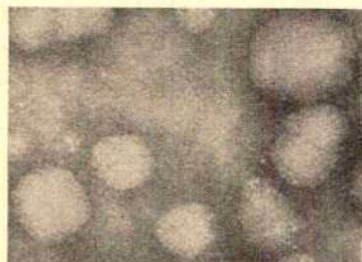


Fig. 1. Human hepatitis virus stained with uranyl acetate ($\times 100,000$)

Electron microscopy shows the virus to have a polyhedral shape (Fig. 1). Negative staining with 1 per cent uranyl acetate makes it possible to measure the diameter, which is between 40 and 150 m μ . To determine whether the smaller particles are parts of larger ones, or are the virus itself, we separated the particles by ultracentrifugation. These were then tested for infectivity and diameters measured. The results showed that the smallest particles represent the virus itself.

The virus was propagated from 144 cases of acute and chronic hepatitis epidemica, from serum hepatitis and from a series of normal blood donors. The virus was found in sewage as well as in blood and in faeces. All 144 virus stems tested were identical in electron micrographs and by immunological tests.

For preparation of immune serum from rabbits, the injection of the pure virus suspension with Freund's adjuvant is recommended together with simultaneous application of anabolic steroids. With immune sera prepared in this way 144 stems were tested by neutralization and haemagglutination with erythrocytes of guinea-pig, and a complement fixation test. As has been stated, they all gave the same reaction.

Neutralizing and haemagglutination-inhibiting antibodies were found in patients after complete healing, while complement-fixing antibody was present as well as the virus only in chronically active cases. An efficient disinfectant is 'Havisol' (Schulke and Mayr G.m.b.H., Hamburg), based on phenol. The 6 per cent solution inactivates massive, protein-poor virus suspensions in 2 min. With more proteins present the virus was inactivated by 'Havisol' and 'Parmetol' in 2 per cent solution after 15 min.

As expected, the virus was much more resistant to heat than other viruses, and heating to 75° C for 1 h had no effect at all. Heating above 170° C was necessary to inactivate pure virus suspension with 10 p.f.u., within 1 h. The same suspension was inactivated in the autoclave after 30 min at 2 atmospheres at 134° C. With protein and cations, present heating for 1 h at 195° C, or at two atmospheres, was required to kill the virus. The addition of 2 per cent sodium carbonate led to the total loss of activity within 20 min at 100° C.

The hepatitis virus was more resistant to ultra-violet radiation. Irradiation of 0.5 ml. solutions in Petri dishes at a distance of 10 cm, using a mercury vapour lamp giving 2540 Å emission, produced an area of inactivation of 8.5 cm diameter after 6 min.

Since it was not possible to inoculate man with the virus, we performed the classical experiment of Neefe *et al.*⁷ on human volunteers, as with the propagated virus in chicken embryo. The results were exactly as described by these authors.

Z. F. CH. KACHANI

Department of Colloid Chemistry,
University of Kiel.

¹ Chargaff, E., and Davidson, J. N., in *The Nucleic Acids*, 1, 382 (Academic Press, New York, 1955).

² Maassab, H. F., *J. Immunol.*, **90**, 265 (1963).

³ Herriot, R. M., *Science*, **134**, 256 (1961).

⁴ Tatum, E. L., *Proc. U.S. Nat. Acad. Sci.*, **48**, 1238 (1962).

⁵ Schmidt, G., and Thannhauser, S. J., *J. Biol. Chem.*, **161**, 83 (1945).

⁶ Kachani, Z. F. Ch., *Arch. Hyg. Bakt.*, **147**, 546 (1963).

⁷ Neefe, J. R., Stokes, J., Baty, J. B., and Reinhold, J. G., *J. Amer. Med. Assoc.*, **128**, 1076 (1945).

'Ring-forms' of Reovirus Particles

SEVERAL investigations utilizing electron microscopy have shown that during reovirus synthesis, progeny virus consisted of particles containing nucleic acid surrounded by protein coats (capsids), and others which were devoid of a central core¹⁻⁴. Negative staining techniques⁵ have afforded a means of following the course of events in infected cells.

The L-48 line of mouse fibroblasts was received from Dr. C. P. Stanners⁶ and maintained in this laboratory in monolayer bottles in Eagle's medium with twice the usual concentration of amino-acids and 10 per cent calf serum. Cells were transferred every third day.

A type 2 strain of reovirus (988) (ref. 7) was prepared and concentrated according to methods previously described⁸.

Sixteen-ounce monolayers of L-48 cells containing 1×10^7 cells were changed with 50 c.c. of pre-warmed medium (35° C) containing Eagle's as before, and 5 per cent foetal bovine serum which had been tested and found to be free of reovirus haemagglutinating inhibiting antibodies⁷. Each 10-oz. bottle was infected with 1×10^6 haemagglutinating units⁹ of reovirus 988 in a 1-ml. volume. This ensured a multiplicity of virus to cell of 100-300 (ref. 10) allowing every cell in the culture to be infected at the time of virus inoculation.

The cultures were then incubated at 35° C for 1, 4, 12, 16, and 36 h. At each of these times supernatant medium was poured away, and the cells washed 10 times with 100 ml. of Earle's balanced salt solution (4° C). The remaining cells were then scraped from the glass with a rubber policeman, and allowed to swell in 3 ml. of a hypotonic medium (10^{-2} M tris, 10^{-3} KCl, 10^{-3} MgCl₂, pH 7.4) for 15 min (4° C). Cell membranes were then disrupted by 10 gentle strokes in a glass Tenbroeck homogenizer (4° C).

These lysates of virus-infected cells were examined on thinly spread films by electron microscopy. Crystalline bovine serum albumen was added to a final concentration of 0.286 mg/ml. to facilitate spreading. Five per cent phosphotungstic acid (PTA) neutralized (pH 7.0) with 1 M KOH was coated with a light film of talcum. One drop of cell lysate was then spread on to the surface. The films remained on the surface of the PTA solution for 5 min. They were lifted on to 'Formvar'-coated grids, and dried on filter paper. The grids were examined in an RCA EMU 3E electron microscope. Observations were made at initial magnifications of 5,000-18,000 diameters.

Virus particles were seen in preparations from infected cells as early as 1 h and as late as 36 h after infection. Complete virus was 69 m μ in diameter and contained a dense core of nucleic acid measuring 48 m μ . Enucleate

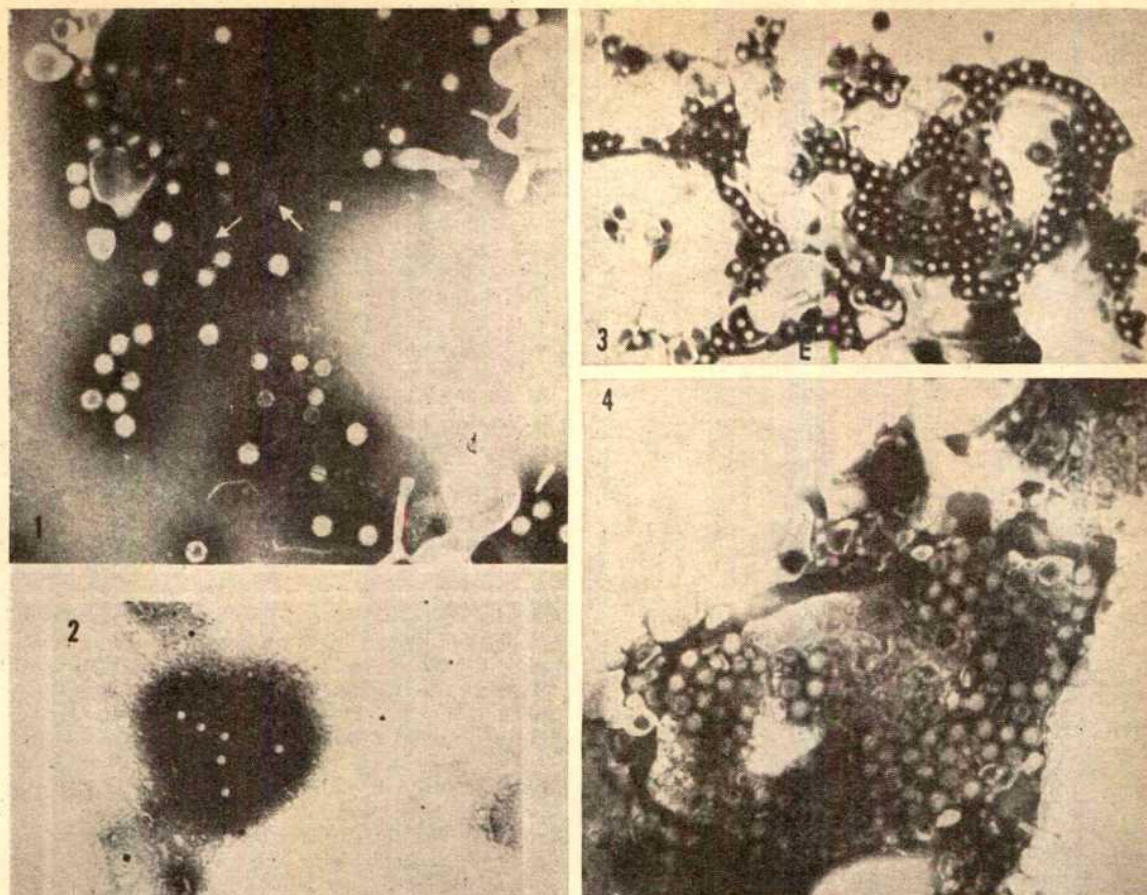


Fig. 1. Complete virus, enucleate capsids and a third type of particle, 'ring forms', are seen 12 h after infection. Complete virus and enucleate capsids are 69 m μ in diameter. The 'ring forms' (arrows) are enucleate and 48 m μ in diameter. (\times c. 55,380)

Fig. 2. A group of nucleic acid cores in a preparation made 4 h after infection. (\times c. 35,840)

Fig. 3. Free virus, cytoplasmic vesicles and canaliculi are seen in a preparation 16 h after infection. Capsomeres of the protein coat are discernible. There are enucleate particles and whole virus. (\times c. 35,800)

Fig. 4. Sixteen h after infection, clusters of virus enclosed by a limiting membrane or sac are seen (lower right). (\times 61,800)

particles with a protein coat of capsomeres (capsid) but without central cores were present in all preparations.

At 12 h or more following infection of *L*-cells by reovirus, several changes in virus progeny were observed. In addition to complete and coreless forms, a third type of particle was noted in some fields. These were empty particles with an outer limiting membrane. Capsomeres were not seen (Fig. 1). These 'ring forms' had a diameter of 48 m μ , equal to that of the central core of the complete particle. A fourth type of virus material of similar dimension was also observed. These were solid electron-dense polygonal particles (Fig. 2) which appear to be the nucleic acid or core material.

At 16 h after infection aggregations of virus particles enclosed within a limiting membrane or sac were seen (Figs. 3 and 4). Several points concerning reovirus became evident. The appearance of ring forms at 12 h after infection suggests new virus synthesis. The ring could well be protein. This suggests that reovirus protein consists of 2 morphological types, capsomere and 'ring'. The inner layer of the ring was noted by Vasquez and Tournier². It had never been observed free of capsomere protein, and the time of appearance was not described. 'Ring forms', which have a diameter equal to that of the nucleic acid core, could serve as an outer covering of the nucleic acid or as an inner lining for capsomeres.

The observation of virus aggregates in a sac is reminiscent of an earlier observation by Horne and Nagington for poliovirus¹¹. Such a cytoplasmic structure has recently been shown to be involved in the synthesis and assembly of poliovirus components¹².

This work was supported by grants 1 T1 A1 261-01 and A1-05721-01A1 from the U.S. National Institutes of Health, and G-24281 from the U.S. National Science Foundation.

Note added in proof. Findings similar to those reported here have appeared in two recent publications from another laboratory: Mayor, H. D., and Jordan, L. E., *Exp. Mol. Path.*, 4, 40 (1965). Mayor, H. D., Jamison, R. M., Jordan, L. E., and Mitchell, M. Van, *J. Bact.*, 89, 1548 (1965).

FREDERICK J. GROSE
MAURICE H. BERNSTEIN
A. MARTIN LERNER

Departments of Anatomy, Microbiology and Medicine,
Wayne State University School of Medicine,
and Detroit Receiving Hospital,
Detroit, Michigan.

¹ Caspar, D. L. D., Dulbecco, R., Klug, A., Lwoff, A., Stoker, M. G. P., Tournier, P., and Wildy, S., *Proposals: Cold Spring Harbor Symp. Quant. Biol.*, 27, 49 (1962).

² Harford, C. G., Hamlin, A. and Middelkamp, J. N., *J. Lab. Clin. Med.*, 60, 179 (1962).

³ Vasquez, C., and Tournier, P., *Virology*, 17, 503 (1962).

⁴ Jordan, L. E., and Mayor, H. D., *Virology*, 17, 597 (1962).

⁵ Horne, R. W., in *Techniques for Electron Microscopy*, edit. by Kay, D., 150 (Blackwell Scientific Publications, Oxford, 1961).

⁶ Stanners, C. P., Till, J. E., and Siminovitch, L., *Virology*, 21, 448 (1963).

⁷ Lerner, A. M., Cherry, J. D., Klein, J. O., and Finland, M., *New Engl. J. Med.*, 267, 947 (1962).

⁸ Lerner, A. M., *Bact. Rev.*, 28, 391 (1964).

⁹ Lerner, A. M., Cherry, J. D., and Finland, M., *Virology*, 19, 58 (1963).

¹⁰ Lerner, A. M., Cherry, J. D., and Finland, M., *Proc. Soc. Exp. Biol.*, 110, 727 (1962).

¹¹ Horne, R. W., and Nagington, J., *J. Mol. Biol.*, 1, 333 (1959).

¹² Penman, S., Becker, Y., and Darnell, J., *J. Mol. Biol.*, 8, 541 (1964).

GENETICS

'Complement Fractionation' in a Natural Hybrid between *Rubus procerus* Muell. and *R. laciniatus* Willd.

Rubus procerus Muell. and *R. laciniatus* Willd. occur sympatrically over a wide range of area in the coastal Pacific north-west of the United States. Natural hybridization between these two pseudogamous apomictic species has been shown to take place by Bammi and Olmo¹. One such partially sterile natural hybrid (6412A), collected from Riverton, Oregon, proved very interesting cytologically. The present report deals with the peculiar cytological behaviour of this hybrid clone.

The chromosome analyses in this study were made from temporary squash preparations according to the alcoholic-carminic technique described by Snow².

All clones of *R. procerus*, *R. laciniatus* and the hybrid clone were tetraploids with 28 chromosomes in their somatic cells. Stages prior to diakinesis or metaphase I were not suitable for analyses.

Both *R. procerus* and *R. laciniatus* behave as segmental allopolyploids. Usually not more than 1-2 quadrivalents are formed at microsporogenesis. The hybrid clone (6412A) showed a peculiar kind of abnormality. In about 60 per cent of the microsporocytes two chromosomal plates were present, instead of the expected one, at metaphase I. These two plates behaved as independent units during the rest of the meiosis. Various other abnormalities like non-disjunction, lagging of certain chromosomes at first and second division of meiosis, separate or in conjunction with the above described abnormality, were also observed. About 1.5 per cent of the microsporocytes in *R. laciniatus* also showed more than one group of chromosome at metaphase I. The net result of the phenomenon of multiple plates at metaphase I and the behaviour of these groups of chromosomes to operate independently of each other in subsequent stages results in the production of an excess of sporads at telophase II. Table 1 shows that at telophase II, this plant had only 29-30 per cent of the cells with four sporads. This is in contrast to the clones of the parents and the hybrids, where cells with four sporads were about 97-99 per cent of the total. Almost all the rest of the cells had excess sporads.

Table 1. FREQUENCY DISTRIBUTION OF CELLS AT TELOPHASE II WITH REGARD TO NUMBER OF SPORADS PER CELL IN THE SUSPECTED HYBRID CLONE 6412A

Total cells studied	Cells with sporads numbering												
	4+	1	2	3	4	5	6	7	8	9+			
273	Micronuclei No.	18	1	3	8	4	80	42	65	12	42	9+	2
	Per-centage	6.59	0.37	1.10	2.93	29.30	15.38	23.81	4.40	15.38	0.74		

Thompson³ introduced the term 'complement fractionation' for the general phenomenon wherein "the chromosome complement is sub-divided into independently operating groups within a cell". Thompson³, in working with the cultivated variety 'Boysen' of the genus *Rubus*, found a similar situation of sub-division of the chromosome complement into independently operating groups within meiotic cells. In her interpretation there were no definite implications as to the genetic constitution of the end-product. However, the present study goes a step further in proposing that the two sub-groups of chromosomes at metaphase I in fact belong to different genomes, namely *R. procerus* and *R. laciniatus*. This interpretation is based on the following observations.

(1) Sub-division does not seem to be a completely random process. The number of bivalents in each group at metaphase I tends to be 7 (14 being the gametic number for both *R. procerus* and *R. laciniatus*).

(2) Rarely, more than 2 sub-groups were observed at metaphase I.

(3) Each sub-group seems to have a separate spindle. This is supported by the following observations:

(a) Lack of synchronization of 2 groups in some cells, and
(b) their separation in different planes in a 3-dimensional cell.

(4) Counts made at anaphase II show that chromosomes number in each group tends to be 7.

Since the frequency of this phenomenon is different in different clones, it is assumed to be genotypically controlled.

No definite conclusion as to the time or method of origin can be drawn from this work. Some indication of sub-grouping of chromosomes in somatic cells was also seen. It is probable that actual separation of genomes had taken place in the premeiotic cells.

The presence of 'complement fractionation' in the cells of an organism has many implications. It may be an indication of its obvious or concealed hybridity. It is quite conceivable that one of the resulting gametes may be functional and may take part in fertilization. This could result in the formation of unique segregants which would be expected to be rare or impossible in the normal reproductive pattern. As pointed out by Thompson³, the separation of single genomes which form functional gametes can have value in the discovery of ancestral species of polyploids. There, of course, then exists the fascinating possibility that the genome of an ancient diploid trapped inside the constitution of a polyploid (in this case, an apomictic one) might also be released. This phenomenon might also provide a method by which the polyploid level of a species may go down.

Examples of 'complement fractionation' have so far been reported in cultivated plants of hybrid origin, for example by Thompson³ and in tomato by Gottschalk⁴, and in *Rubus* by Hull and Britton⁵, that were previously subjected to colchicine treatment. This is probably the first instance where a naturally occurring plant shows this phenomenon.

I thank Prof. H. P. Olmo for advice.

R. K. BAMMI

Department of Viticulture and Enology,
University of California,
Davis, California.

¹ Bammi, R. K., and Olmo, H. P. (in the press).

² Snow, R. S., *Stain Tech.*, **38**, 9 (1963).

³ Thompson, M. M., *Amer. J. Bot.*, **49**, 575 (1962).

⁴ Gottschalk, W., *Z. Indukt. Abstam. und Vererbungslehre*, **89**, 52 (1958).

⁵ Hull, J. W., and Britton, D. M., *Univ. Md. Agric. Exp. Sta. Bull.*, **A-91** (1958).

SOIL SCIENCE

Thermistor Hygrometer for determining the Free Energy of Moisture in Unsaturated Soils

IN any investigation involving the movement of moisture through soils, the measurement of the free energy or total potential of the soil moisture still remains one of the chief problems. The basic requirement of any satisfactory technique for such measurement is that it should depend on some property which is uniquely related to the free energy of the soil moisture and is in no way affected by the soil type. One such property is the relative vapour pressure or humidity in equilibrium with the soil moisture as given by equation (1).

$$h = \frac{RT}{Mg} \log_e \frac{p}{p_0} \quad (1)$$

where h = free energy (cm water); R = universal gas constant; T = abs. temp.; M = molecular wt. of water;

$g = 981 \text{ cm/sec}^2$; $\frac{p}{p_0}$ = relative humidity.

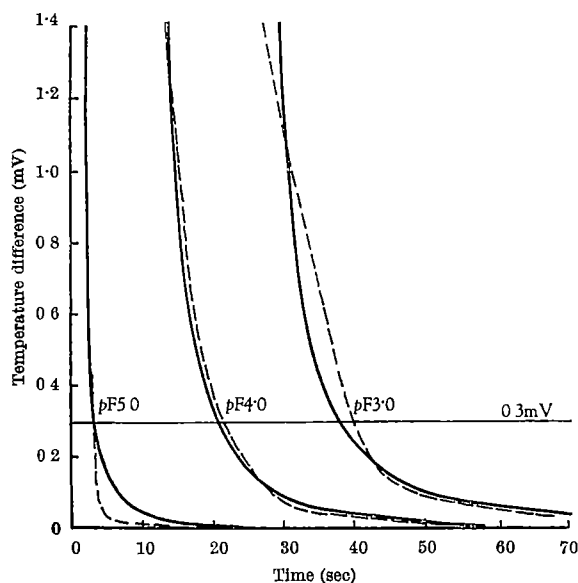


Fig. 1. Comparison of theoretical (solid lines) and measured curves (broken lines)

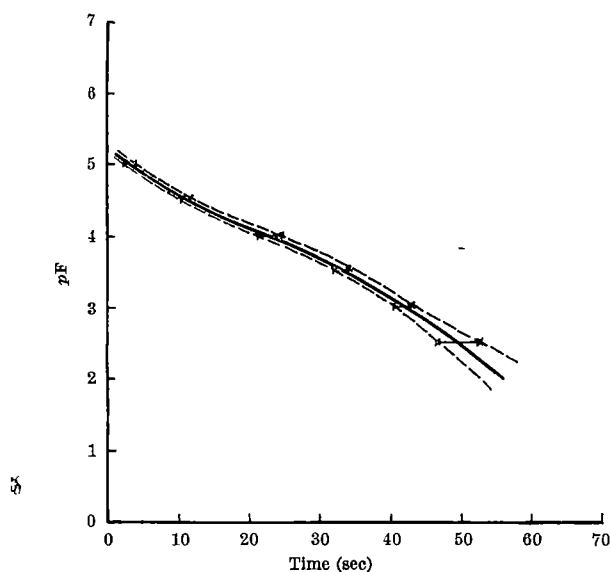


Fig. 2. Probe P; time to reach 0.3 mV for 16-h reading

The amount of capillary condensation in a porous medium from some fixed initial condition is closely related to the ambient humidity. The porous medium chosen for study was the micro-porous glass coating on a thermistor bead. It has been shown^{1,2} that glass surfaces may have effective surface areas many hundred times greater than the nominal area of the surface. The initial condition was established by heating the thermistors with their normal operating current in the room atmosphere at approximately 60 per cent humidity for 1 h. The capillary moisture which afterwards condensed on the glass surface could then be measured by the heat energy required to vaporize this moisture or more simply the time delay in the temperature rise as current was passed through the thermistor.

The original experimental work was carried out using a two-thermistor probe sealed into a sample tube. One thermistor, enclosed in an air-tight compartment brought to approximately 0 per cent humidity with silica gel, was used as the standard in a Wheatstone bridge circuit with a 1.35-V d.c. supply from a mercury cell, while the second thermistor was open to the sample atmosphere. The thermistors were 'zeroed' to their 'final' condition by

passing the current for 1 h. After allowing a period of 16 h for capillary condensation to take place, the current was again switched on. Typical readings are shown in Fig. 1 for Stantel 'P23' thermistors and for standard sodium chloride solutions supported on filter paper.

Although the standard thermistor compensated for small changes in mean temperature, differences in temperature between the thermistors and the sample could cause serious errors. These errors were minimized by carrying out the experimental work in a constant temperature room ($\pm 0.1^\circ\text{C}$) with the sample tube and probe placed in an 18-in. cube of polystyrene insulating foam.

Fig. 1 also shows theoretical curves of the temperature difference between a 'F23' thermistor in dry air (that is, the standard) and the second 'P23' thermistor also in dry air, but after time delays corresponding to the experimental curves. These curves were calculated³ assuming the heat is produced in a sphere of perfect conductor surrounded by an infinite region.

In most of the later work, the whole temperature curve was not recorded, and only the time to reach a temperature difference corresponding to a bridge output of 300 μV was measured on a stop-watch. This gave a satisfactory curve between Schofield's pF and the reading in seconds between pF 2.5 and 5.0 as shown in Fig. 2. During the duration of the test, a period of about 2 months, no surface contamination of the porous surface was apparent. Similar curves were also obtained for other types of thermistors and for other capillary condensation times. Times less than 1 h still gave satisfactory calibration curves for more rapid measurements in the range pF 2.5 to 5.0, but with a lower sensitivity.

In the present work a new simple two-thermistor probe is being used with the standard coated with water repellant. Although this work is not complete, similar curves are being obtained to that shown in Fig. 2, using equipment that can be purchased for a few pounds. This technique shows considerable promise in the measurement of free energy of soil moisture in the range pF 2.5–5.0.

B. G. RICHARDS

C.S.I.R.O. Soil Mechanics Section,
P.O. Box 54, Mt. Waverley,
Victoria, Australia.

¹ Adam, N. K., *The Physics and Chemistry of Surfaces* (Oxford, London, 1941)

² Imelik, B., *Proc. Tenth Symp. Colston Res. Soc.*, Bristol (1958).

³ Carslaw, H. S., and Jaeger, J. C., *Conduction of Heat in Solids* (Oxford, London, 1959).

Photographic Reproduction of Soil Fabric Patterns

An important prerequisite for a satisfactory fabric analysis of any complex, multi-component fabric pattern is the requirement that the component individuals can be relatively easily recognized as to their nature and their geometrical relationships towards adjacent individuals. In rocks, for example, the various mineral individuals can, under average conditions, be recognized readily and clearly distinguished from adjacent crystal individuals. As a result, rock fabric analysis can usually be carried out directly on the image provided by the microscope.

The microscopical image of soil fabric patterns is, as a rule, very complex. Thus, particularly in spatial distribution analysis¹, the nature of the individuals of the various components may be rather difficult to establish, or boundaries between components may appear hard to locate. For this reason, even elementary soil fabric analysis tends to develop into an extremely slow, tiresome and costly process, particularly if areas larger than the field of the microscope or even whole thin sections are involved.

The obvious solution to this problem is to replace the original microscope image of the pattern by a simplified

Table 1. PHOTOGRAPHIC EFFECTS OF THE VARIOUS STEPS IN THE PROCEDURE

Step No.	Photographic product	Type of light	Appearance of 'primary' fabric elements		
			Skeleton grains	Clay matrix	Pores
Step 1 A B	Negative Positive transparency	Ordinary parallel light Ordinary light, parallel beam or diffuse	Black White	White* Black	Black White
Step 2	Negative	Plane polarized light, rotating crossed Nicols	Black	White	White
Step 3	Combination of positive transparency (1B) and negative (2)	Ordinary parallel light	See step 4	Black	White
Step 4	Negative	Ordinary parallel light under an angle of approx. 70°-80°	White areas surrounded by fine, black lines	White	Black
Step 5	Print or enlargement of negative (4)	Ordinary light, parallel beam or diffuse	Black areas surrounded by fine, white lines	Black	White

* Depending on the colour of the clay material in the thin-section. If sufficiently reddish, the result can be obtained by using the appropriate type of photographic emulsion. If the colour of the clay material is insufficiently reddish, a suitable preferential staining of the clay can be considered.

graphical model which contains all the required information but no unnecessary details. The ideal graphical model therefore should possess the following major properties: it should show all relevant constituents in their true size and shape ratios, and in their mutual spatial relationships; it should also show all the individuals of any one constituent in the same way (for example, skeleton grains in black, clay matrix in grey, pores in white, etc.). Simple drawing or tracing of the microscope image seems attractive, but is, particularly with the usually complex soil fabric patterns, extraordinarily time-consuming and often insufficiently accurate. Ordinary photomicrography, also in polarized light between crossed Nicol prisms, is accurate indeed, but still has the main disadvantage of complexity of the original microscope image, and in addition shows the loss of a number of diagnostic characteristics (for example, extinction angle between crossed Nicol prisms, birefringence, etc.). In many instances these characteristics must be determined later in the original microscope image, leading again to an excessive loss of time.

Over some period of time we have tested several combinations of photomicrographic and drawing techniques. Some of these have been applied for a time, but have been rejected, mainly because they were too time-consuming. We have finally adopted a method which virtually satisfies the two conditions of the ideal model. It is a purely photographic technique which does not demand an excessive amount of time spent on routine work, but rather calls for appreciable photographic experience and skill, as well as for very accurate working methods.

The steps in the actual operation can briefly be described as follows:

1. (A) The area of the soil thin section to be investigated is photographed at the desired magnification and in ordinary transmitted light. (B) From the negative

obtained a positive transparency is made by contact printing.

2. The same area of the thin section is then photographed again without altering the position of camera, microscope or thin section. The exposure is now made, however, in plane polarized light between crossed polarizing filters or Nicol prisms. During exposure the crossed polarizing filters or prisms are simultaneously rotated through an angle of at least 180°. The result of this exposure is a negative, which is directly used in the next step.

3. The positive transparency of step 1B and the negative of step 2 are now put together with the sensitized sides of both film-sheets on the outside and the corresponding parts of the images fitting very accurately. The fitting together of the corresponding parts of the images is the most critical part of the whole procedure, and must be carried out with the utmost care to ensure success of the whole operation. Once the fit of the two film-sheets is perfect, they should be joined semi-permanently or permanently.

4. From the joined set of the positive transparency and the negative, a new negative is now produced in the following manner. The combined two film-sheets, with the third, new film-sheet directly underneath, are exposed to a beam of parallel light (for example, from a photographic enlarger or a microscope lamp) in such a way that the plane of the three film-sheets makes an angle of about 70°-80° with the axis of the light beam. While exposing, the stack of three film-sheets, maintaining the previously mentioned angle of 70°-80°, is rotated through an angle of at least 360° around the axis of the light beam.

5. The new negative produced in step 4 can now be printed or enlarged in the ordinary way.

The final result of the whole operation is demonstrated in Fig. 1, which shows an area of a thin section of an arid red earth from Alice Springs, N.T., Australia. Nearly all skeleton (quartz) grains are shown as black areas surrounded by a fine white line. The very few quartz grains which are shown as white or whitish areas in the figure are those which in the original thin section were cut perpendicular or nearly perpendicular to their optical axis. The majority of these reveal themselves in the photograph immediately by their size and shape. In only very few instances will it be necessary to return to the original thin section for additional information. In the same figure the clay matrix is shown in solid black, and the pores in solid white.

The results and effects of the various steps are described in Table 1.

D. LAFEVER
M. KURBANOVIC

C.S.I.R.O.

Soil Mechanics Section,
P.O. Box 54, Mount Waverley,
Victoria, Australia.

Lafever, D., *Proc. Fourth Austral. N.Z. Conf. Soil Mech. Found. Eng.*, 1963
185 (1964).

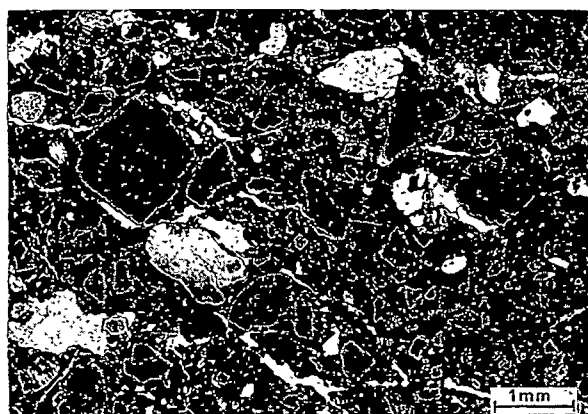


Fig. 1. Photographic reproduction of the fabric pattern of an arid red earth, Alice Springs, N.T., Australia. (Depth of the sample below the surface: 5 ft. 6 in.)

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, November 8

PLASTICS INSTITUTE, PLASTICS PROPERTIES DISCUSSION CIRCLE (at the Mandeville Hotel, Mandeville Place, London, W.1), at 3 p.m.—Mr. R. A. Horsley: "Creep Properties of Thermoplastics".

SOCIETY OF CHEMICAL INDUSTRY, COLLOID AND SURFACE CHEMISTRY GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Mr. G. P. C. Chambers: "The Colloidal and Surface Properties of Clay Minerals".

INSTITUTION OF ELECTRICAL ENGINEERS, CONTROL AND AUTOMATION DIVISION (at Hatfield College of Technology, Hatfield), at 6 p.m.—Discussion on "Is Laboratory Work Really Necessary?" opened by Dr. K. R. Sturley.

INSTITUTION OF MECHANICAL ENGINEERS, NUCLEAR ENERGY GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Fuel Element Behaviour in Gas-Cooled Reactors".

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, W.C.2), at 6.30 p.m.—Mr. A. G. Page: "Some Manufacturing and Processing Techniques in the Electronics Industry".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Lord Rennell of Rodd: "Heinrich Barth and the Opening Up of Central Africa".

Tuesday, November 9

UNIVERSITY OF LONDON (at Imperial College of Science and Technology, London, S.W.7), at 1.30 p.m.—Mr. E. F. Schumacher: "Homo Viator and Homo Sapiens".*

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, Westminster, London, S.W.1), at 2.30 p.m.—Mr. P. R. Barnard and Mr. R. Paul Johnson: "The Ultimate Strength of Composite Beams"; Mr. R. Paul Johnson, Mr. C. H. Finlison and Mr. J. Heyman: "The Plastic Behaviour of Continuous Beams" and "A Plastic Composite Design".

ZOOLOGICAL SOCIETY OF LONDON (at the Zoological Gardens, Regent's Park, London, N.W.1), at 5 p.m.—Scientific Papers.

INSTITUTION OF ELECTRICAL ENGINEERS, CONTROL AND AUTOMATION DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. D. E. Hesmondhalgh: "The Design of the A.C. Servomotor".

INSTITUTION OF THE RUBBER INDUSTRY, LONDON SECTION (at the Royal Society of Tropical Medicine and Hygiene, 26 Portland Place, London, W.1), at 5.30 p.m.—Mr. L. R. Mernagh: "Tyres 1965". 7 p.m.—Dr. A. Schallamach: "Tyre Wear is Complex".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 5.30 p.m.—Sir John Baker, O.B.E., F.R.S.: "The Excitement of Engineering Design" (Afternoon lecture for Sixth Form Boys and Girls from Schools in London and the Home Counties. To be repeated on November 10, 16 and 17).

ROYAL INSTITUTION OF NAVAL ARCHITECTS (Joint meeting with the Institute of Marine Engineers, in the Memorial Building, 76 Mark Lane, London, E.C.3), at 5.30 p.m.—Mr. A. F. Willens and Mr. D. R. Murray Smith: "Fire Fighting and Fire Protection in Ships".

UNIVERSITY OF LONDON (at Imperial College of Science and Technology, London, S.W.7), at 5.30 p.m.—Prof. D. Rogers: "The Portraiture of Molecules" (Inaugural Lecture).*

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. J. Ralph Audy (U.S.A.): "Red Mites and Typhus. III. Emergence of the Typhus Group of Fevers".*

INSTITUTION OF MECHANICAL ENGINEERS, AUTOMOBILE DIVISION (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Mr. A. R. Reece: "Principles of Soil-Vehicle Mechanics".

UNIVERSITY OF LONDON (in the Mechanical Engineering Building, Imperial College of Science and Technology, London, S.W.7), at 6 p.m.—Prof. W. R. Sears (University of Cornell): "Waves". (Third of four lectures on "Magneto-Fluid Dynamics").*

INSTITUTE OF SCIENCE TECHNOLOGY, LONDON BRANCH (in the Anatomy Lecture Theatre, University College, Gower Street, London, W.C.1), at 6.30 p.m.—Prof. J. Z. Young, F.R.S.: "The Memory in the Brain" (Distinguished Visitors' Address).

Tuesday, November 9—Thursday, November 11

INSTITUTION OF MECHANICAL ENGINEERS, RAILWAY ENGINEERING GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1)—Convention on "Interaction Between Vehicle and Track".

Wednesday, November 10

POLAROGRAPHIC SOCIETY (at the National Physical Laboratory, Teddington, Middlesex), at 2.30 p.m.—Meeting on "E.S.R. and Polarography".

SOCIETY FOR ANALYTICAL CHEMISTRY (at the Wellcome Building, Euston Road, London, N.W.1), at 3 p.m.—Meeting on "Automatic Analysis".

ENVIRONMENTAL GROUP (in the Department of Electrical Engineering, Imperial College of Science and Technology, London, S.W.7), at 6 p.m.—Discussion on "The Adequacy of our Current Measures of the Environment".

INSTITUTE OF METALS (at 17 Belgrave Square, London, S.W.1), at 6 p.m.—Prof. C. S. Barrett (University of Chicago): "X-ray Diffraction at Low Temperatures".

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, JOINT I.E.R.E./I.E.E. MEDICAL ELECTRONICS GROUP (at 9 Bedford Square, London, W.C.1), at 6 p.m.—Dr. D. W. Hill: "Medical Electronics Round the World".

SOCIETY OF INSTRUMENT TECHNOLOGY (at Manson House, 26 Portland Place, London, W.1), at 6 p.m.—Mr. G. M. E. Williams: "Control in Automatic Mechanical Production".

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP—FOOD ENGINEERING PANEL (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Mr. A. Hudson: "Diversification in Packaging".

Wednesday, November 10—Thursday, November 11

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2)—Conference on "Power Applications of Controllable Semiconductor Devices".

Thursday, November 11

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. S. W. Kuffler (Harvard Medical School): "Physiological Properties of Vertebrate and Invertebrate Neuroglial Cells and the Movement of Substances Through the Nervous System" (Ferrier Lecture).

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, Westminster, London, S.W.1), at 5.30 p.m.—Discussion on "Access to Airports" introduced by Mr. C. A. Harvey.

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. J. Ralph Audy (U.S.A.): "Red Mites and Typhus. IV. The Impalpable Circus".*

CHEMICAL SOCIETY (in the Chemistry Lecture Theatre, King's College, Strand, London, W.C.2), at 6 p.m.—Dr. B. A. Thrush: "The Formation of Electronically Excited Molecules in Simple Gas Reactions" (Tilden Lecture).

UNIVERSITY OF LONDON (in the Mechanical Engineering Building, Imperial College of Science and Technology, London, S.W.7), at 6 p.m.—Prof. W. R. Sears (University of Cornell): "Two Weird Boundary Layers". (Last of four lectures on "Magneto-Fluid Dynamics").*

OIL AND COLOUR CHEMISTS' ASSOCIATION (in the Small Physics Lecture Theatre, Imperial College of Science and Technology, London, S.W.7), at 7 p.m.—Mr. F. G. Dunkley: "Airless Spray Painting".

PHARMACEUTICAL SOCIETY OF GREAT BRITAIN AND THE OSLER CLUB (at 17 Bloomsbury Square, London, W.C.1), at 8 p.m.—Dr. F. Guerra and Prof. F. E. Camps: "Phantasica (Drugs, Dreams and Addictions)".

Friday, November 12

ASSOCIATION OF APPLIED BIOLOGISTS (in the Lecture Hall of the British Museum (Natural History), Cromwell Road, London, S.W.7), at 10.50 a.m.—Symposium on "Yield and Quality in Potatoes".

LINNEAN SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 11 a.m.—Meeting on "The Flora Europaea Project". Speakers to include Prof. H. Merxmüller (Munich), Prof. D. A. Webb, Prof. D. H. Valentine, Prof. J. Dostál (Czechoslovakia) and Dr. S. M. Walters.

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 4 p.m.—Dr. S. Callender: "Iron Absorption".*

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. B. M. Weedy and Mr. A. M. Parker: "Method of Predicting the Thermal Loading of an Oil Circuit Breaker".

UNIVERSITY OF LONDON (at King's College, Strand, London, W.C.2), at 5.30 p.m.—Prof. M. J. Langeveld (Utrecht): "Educating a Whole World".*

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Dr. J. R. Napier: "Evolution of the Human Hand".

Saturday, November 13

INSTITUTE OF LINGUISTS (at the British Academy, Burlington Gardens, London, W.1), at 3 p.m.—Mr. Peter F. D. Tennant, C.M.G., O.B.E.: "Language, Communication and Misunderstanding" (Sixth Threlford Memorial Lecture).

INNER LONDON EDUCATION AUTHORITY (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Mr. B. A. L. Cranstone: "The British Museum Expedition to New Guinea".*

Monday, November 15

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 10.30 a.m. and 2.30 p.m.—Colloquium on "Phase Measurement Throughout the Spectrum".

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Dr. D. E. Stevenson: "The Assessment of Possible Health Hazards Associated with the Use of Pesticides".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. J. Ralph Audy (U.S.A.): "Red Mites and Typhus. V. Old and New Horizons".*

INSTITUTION OF MECHANICAL ENGINEERS, MANUFACTURE AND MANAGEMENT GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "The Engineer and the Law".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Prof. Colin Cherry: "The Nature of Human Communication". (First of three Cantor Lectures on "World Communication").

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (at the College of Further Education, Hatfield Road, St. Albans), at 7.30 p.m.—Dr. J. S. Goutlay and Dr. C. K. Warren: "The Chemist's Contribution to the Building Industry of the Future".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned.

ASSISTANT IN VETERINARY CLINICAL BIOCHEMISTRY—The Secretary of the University Court, The University, Glasgow (November 10).

DEMONSTRATOR/SENIOR DEMONSTRATOR IN THE DEPARTMENT OF GEOGRAPHY—The Registrar, The University, Newcastle upon Tyne, 2 (November 12).

LECTURER OR ASSISTANT LECTURER (with considerable field experience of applied geology, preferably on the side of engineering) in GEOLOGY AND MINERALOGY—The Secretary, The University, Aberdeen (November 13).

LECTURER (COMPUTER MANAGER) (graduate with experience of computer systems and administration); and a SENIOR LECTURER (with suitable experience in some aspect of computing science such as system programming) IN THE COMPUTING DEPARTMENT—The Secretary of University Court, The University, Glasgow (November 15).

LECTURERS or ASSISTANT LECTURERS (2) IN THE DEPARTMENT OF PHILOSOPHY—The Registrar, The University, Leeds, 2 (November 15).

PROFESSOR IN MATHEMATICAL STATISTICS AT THE UNIVERSITY OF CAPE TOWN—The Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1; and The Registrar, University of Cape Town, Private Bag, Rondebosch, Cape Town, South Africa (November 15).

LECTURER IN CONTROL or DYNAMICS IN THE DEPARTMENT OF ENGINEERING—The Registrar, The University, Leicester (November 17).

SENIOR LECTURER and a LECTURER IN THE NEW LABORATORY OF GENERAL PHYSIOLOGY—The Registrar, The University, Leicester (November 17).

HEAD OF THE DEPARTMENT OF BIOLOGY—The Clerk to the Governors, Woolwich Polytechnic, London, S.E.18 (November 19).

LECTURER or ASSISTANT LECTURER IN MEDICINE—The Registrar, The University, Sheffield (November 20).

LECTURER or ASSISTANT LECTURER (preferably specialized in modern analysis or topology) IN PURE MATHEMATICS—The Registrar, The University Sheffield (November 20).

UNIVERSITY DEMONSTRATOR (with an honours degree and postgraduate teaching and/or research experience) IN AGRICULTURE—The Secretary, School of Agriculture, University of Cambridge, Downing Street, Cambridge (November 20).

LECTURER (preferably with an interest and competence in physiological and animal psychology) IN PSYCHOLOGY at the University of New England, Armidale, New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 22).

LECTURER (qualified in chemistry, physics, or a branch of technology, and with industrial or postgraduate experience in a branch of materials science) IN MATERIALS and MOLECULAR SCIENCE—The Clerk to the Governors, Woolwich Polytechnic, London, S.E.18 (November 22).

LECTURER/SENIOR LECTURER IN BIOCHEMISTRY at the University of New England, Armidale, New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 26).

TEACHING FELLOWS/DEMONSTRATORS (2) (preferably with an honours degree in rural science, agriculture or science with specialization in plant sciences) IN AGRONOMY at the University of New England, Armidale, New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 26).

CHAIR OF ZOOLOGY at the University of Malaya—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Kuala Lumpur, November 30).

HEAD (senior research worker, preferably holding veterinary or medical qualifications) OF THE PHYSIOLOGY and BIOCHEMISTRY DEPARTMENT—The Secretary, Houghton Poultry Research Station, Houghton, Huntingdon (November 30).

LECTURER (preferably with a veterinary qualification and interested in chemical pathology) IN THE DEPARTMENT OF PATHOLOGY—The Secretary, The Royal Veterinary College (University of London), Royal College Street, London, N.W.1 (November 30).

PROFESSOR OF PHYSICAL CHEMISTRY at Rhodes University, Grahamstown, South Africa—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, November 30).

SENIOR LECTURER and a LECTURER IN SOCIAL ANTHROPOLOGY IN THE DEPARTMENT OF AFRICAN STUDIES, University of Natal, Durban—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, November 30).

SUPERVISING GEOLOGIST (with a university degree with geology as a major subject, extensive experience in the examination and assessment of mineral deposits, together with administrative ability) IN THE GEOLOGICAL SURVEY BRANCH OF THE DEPARTMENT OF MINES—The Public Service Commissioner, 184 St George's Terrace, Perth, Western Australia (December 3).

LECTURER or SENIOR LECTURER IN CHEMICAL PATHOLOGY at the University of Otago Medical School, Dunedin, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1, or the Registrar of the University (New Zealand and London, December 15).

ANIMAL NUTRITIONIST (national of the United Kingdom or the Republic of Ireland, with a degree in agriculture with post-graduate training or experience in biochemistry or agricultural biochemistry) with the East African Agriculture and Forestry Research Organisation, to investigate metabolism of tropical and temperate type cattle with a tropical environment—The Appointments Officer, Room 301, Ministry of Overseas Development, Eland House, Stag Place, London, S.W.1, quoting Ref. PC213/214/016.

CHAIR OF PHILOSOPHY—The Dean, Faculty of Arts and Science, McGill University, Montreal, Canada.

CHIEF TECHNICIAN (preferably with some knowledge of glassblowing and experience with instrumental analysis, for example, gas chromatography) to take charge of chemical laboratories and stores in the Chemical Engineering Department—The Establishment Officer, University College, Gower Street, London, W.C.1, quoting Ref. Chem. Eng. 15.

LECTURER or ASSISTANT LECTURER (with a degree in physics or general science and/or an ophthalmic optical qualification) IN THE DEPARTMENT OF OPHTHALMIC OPTICS—The Academic Registrar, Northampton College of Advanced Technology, St. John Street, London, E.C.1.

MASTER (preferably graduate in mechanical sciences or engineering) to teach MATHEMATICS and some PHYSICS—The Headmaster, The School, Oundle, Peterborough.

RESEARCH ASSISTANT IN THE DEPARTMENT OF METALLURGY to study the behaviour of point defects in metals using electron microscopy and electrical resistivity techniques—The Registrar, University College of Swansea, Singleton Park, Swansea.

RESEARCH TECHNICIAN (preferably with some experience in growing plants under controlled conditions and of chemical analysis) to assist Prof. J. F. Sutcliffe in an investigation of the absorption and movement of mineral salts in intact plants—The Assistant Registrar (Establishment), University of Sussex, Stanmer House, Brighton, Sussex.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

The National Plan. (Cmdnd. 2764.) Pp. xviii + 474. (London: H.M. Stationery Office, 1965.) 30s net.
Bacteria and the Biologist. By Prof. S. Dagle. (An Inaugural Lecture delivered before the University of Leeds on 23 November, 1964.) Pp. ii + 23. (Leeds: The University, 1965.) 2s. 6d.

Ministry of Agriculture, Fisheries and Food: National Agricultural Advisory Service. Experimental Husbandry Farms and Experimental Horticulture Stations—Sixth Progress Report. Pp. v + 70 + 4 plates. (London: H.M. Stationery Office, 1965.) 6s. 6d. net.

Dislocations in Lithium Fluoride: a Microscopical Exercise in Plastic Deformation Using Dislocation Etch Pit and Birefringence Techniques, with Appropriate Crystallographic Background. By Dr. C. W. A. Newey and Dr. R. W. Davidge. (Annotated Metallographic Specimens. Special Series—Exercises in Materials Science) Pp. iv + 41. (Betchworth, Surrey: Metallurgical Services, in association with Scientific Techniques, Ltd., 1965.)

Medical Research Council. Memorandum No. 43: Spectral Requirements of Light Sources for Clinical Purposes. (Joint Committee on Lighting and Vision.) Pp. vii + 56 + 2 plates. (London: H.M. Stationery Office, 1965.) 6s. 6d. net.

Memoirs of the Royal Astronomical Society, Vol. 69, Part 4: Atomic Wave Functions, Collision Cross-Sections, and Transition Probabilities of Fe Ions. By T. K. Krueger and S. J. Czyzak. Pp. 145–182. (London: The Royal Astronomical Society, 1965.)

University of London. University College Calendar, 1965–66. Pp. xci + 478. (London University College, 1965.)

The Nuffield Foundation. Report for the year ended 31 March 1965. Pp. xiv + 161. (London: The Nuffield Foundation, 1965.)

Other Countries

Organization or Economic Co-operation and Development. Office Automation: Administrative and Human Problems. By W. H. Scott. (Industrial Relations Aspects of Manpower Policy—1) Pp. 103. (Paris: Organization for Economic Co-operation and Development; London: H.M. Stationery Office, 1965.) 10 francs; 15s.; 2.50 dollars.

World Health Organization. World Directory of Post-Basic and Post-Graduate Schools of Nursing. Pp. 223. 16 Sw. francs; 26s. 8d.; 5.25 dollars. Technical Report Series, No. 311: Special Courses for National Staff with Higher Administrative Responsibilities in the Health Services—Report of a WHO Study Group. Pp. 31. 2 Sw. francs, 3s. 6d.; 0.60 dollars. (Geneva: World Health Organization; London: H.M. Stationery Office, 1965.)

Alfa Inorganics, Inc., Beverly, Massachusetts. 1965 Catalog of Inorganic Research Chemicals. Pp. 128. (Beverly, Massachusetts: Alfa Inorganics, Inc.; Reading: Ralph N. Emanuel, Ltd., 4 Gasworks Road, 1965.)

Exams—Where Next? By J. Lloyd Breerton. Pp. viii + 145. (Victoria, B.C.: Pacific Northwest Humanist Publications, 1965.)

Metropolitan Life Insurance Company. Statistical Bulletin, Vol. 46 (July, 1965): Geographic Differences in Longevity Diminishing. Recent Immigration to the United States. Reported Frequency of Chronic Respiratory Diseases as Causes of Death: General Findings. Mortality Among Policyholders in First Half of 1965. Pp. 12. (New York: Metropolitan Life Insurance Company, 1965.)

Iceland. The University Research Institute—Department of Fisheries. Rít Fiskeldildar, Vol. 4, No. 2: The 'Aegir' Redfish Larvae Expedition to the Irminger Sea in May 1961—Cruise Report and Biological Observations. Pp. 86. (Reykjavik: Hafnarskólastofnunin, Marine Research Institute, 1965.)

The United Republic of Tanzania: Ministry of Industries, Mineral Resources and Power. Annual Report of the Geological Survey Division, 1964. Pp. 23. (Dodoma: Department of Geological Survey, 1965.) Shs. 5/50.

Rubber Research Institute of Malaya. Annual Report 1964. Pp. 108. (Kuala Lumpur: Rubber Research Institute of Malaya, 1965.)

Publications de l'Institut National pour l'Étude Agronomique du Congo. Série Scientifique, No. 107: Contribution à l'Étude des Effets du Gamétocide FW 450 sur la Fertilité Mâle et Femelle de *Gossypium hirsutum* L. Par P. Bouharmont et P. Pochet. Pp. 45 + 14 photographes. (Bruxelles: Institut National pour l'Étude Agronomique du Congo, 1965.) 90 francs.

Modern Concepts and Practices in Seed Testing and Their Historical Development. (Proceedings of the International Seed Testing Association, Vol. 3, No. 1.) Pp. 118. (Wageningen: International Seed Testing Association, 1965.) 12s. 6d.; 1.20 dollars.

Council of Europe. Organization for Economic Co-operation and Development. Science and Parliament: Second Parliamentary and Scientific Conference organized jointly by the Council of Europe and the Organization for Economic Co-operation and Development, Vienna, May 23–27, 1965—Final Report. Pp. 177. (Paris: Organization for Economic Co-operation and Development; London: H.M. Stationery Office, 1965.) 8 francs; 12s. 6d.; 2 dollars.

Cuba. Instituto Nacional de la Pesca: Centro de Investigaciones Pesqueras. Boletín de Divulgación Técnica No. 2: Instrucciones para la Instalación Operación de Autoclaves. Por J. J. Franco. Pp. 32. Nota Sobre Investigaciones No. 6: El Ostion Antillano: *Crassostrea rhizophora* Guilding y su Cultivo Experimental en Cuba. Por B.A. Saenz. Pp. 34. Contribución No. 21: Las Pesquerías de Cuba y Algunas Recomendaciones para su Intensificación. Por Hermann Ritzhaupt. Pp. 110. (Playa Habana, Bauta, Habana: Centro de Investigaciones Pesqueras, 1964 and 1965.)

Annals of the New York Academy of Sciences. Vol. 128, Article 1: The Staphylococci: Ecologic Perspectives. By A. C. Baird-Parker and 42 other authors. Pp. 1–456. 8 dollars. Vol. 130, Article 1. Antiviral Substances. By L. W. Akers and 101 other authors. Pp. 1–482. 8 dollars. (New York: New York Academy of Sciences, 1965.)

International Pacific Salmon Fisheries Commission. Bulletin No. 17: The Migration, Composition, Exploitation and Abundance of Odd-Year Pink Salmon Runs in and Adjacent to the Fraser River Convention Area. By A. S. Hourston, E. H. Vernon and G. A. Holland. Pp. vi + 151. (New Westminster, B.C.: International Pacific Salmon Fisheries Commission, 1965.)

Editorial and Publishing Offices of "NATURE"

MACMILLAN (JOURNALS) LIMITED

4 LITTLE ESSEX STREET, LONDON, W.C.2

Telephone Number: Temple Bar 6633. Telegrams: Phisus London W.C.2

Annual subscription: Inland £14, Overseas £13 15s., payable in advance, postage paid to any part of the world.

Advertisements only should be addressed to

T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2.

Telephone Number: Holborn 4743

Registered as a newspaper at the General Post Office

Copyright © Macmillan (Journals) Limited, November 6, 1965

NATURE

No. 5011

SATURDAY, NOVEMBER 13, 1965

Vol. 208

Published by
MACMILLAN (JOURNALS) LTD.

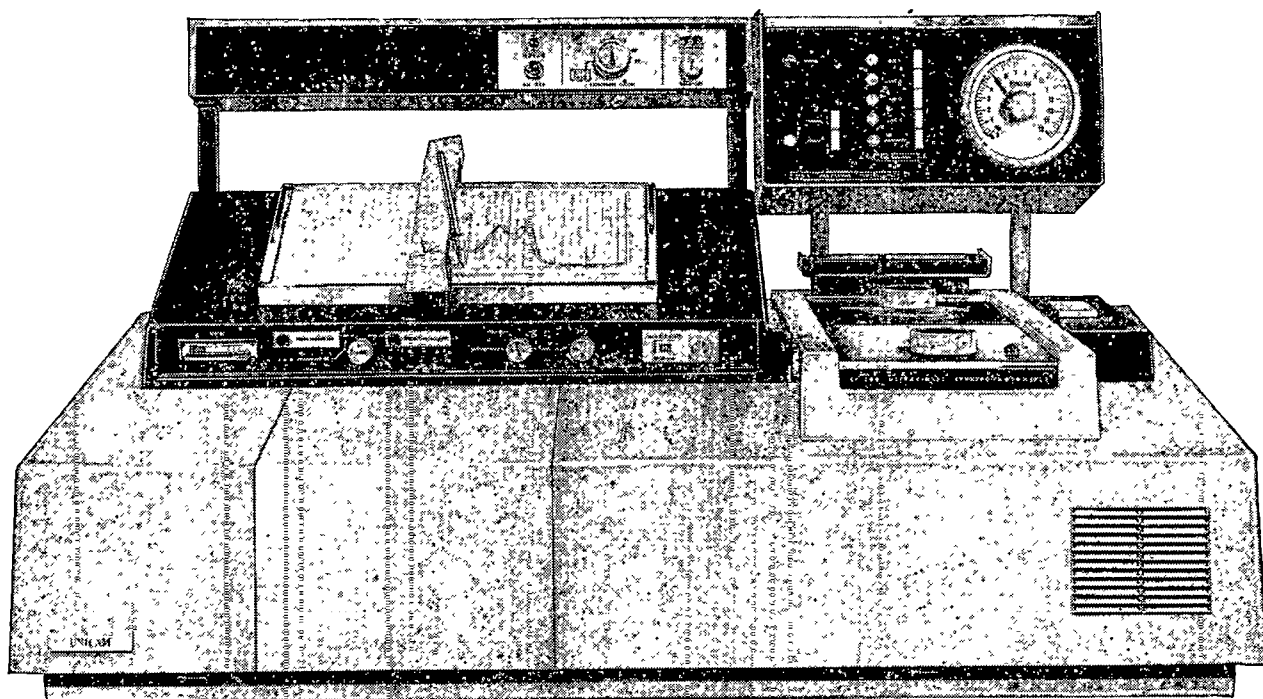
CONTENTS

	Page
TRAINING FOR MANAGEMENT IN INDUSTRY	613
LOWER PALAEOZOIC STRATIGRAPHY. By Prof. A. Williams	614
GEOLOGICAL THESIS ON HADHRAMUT. By F. E. Wellings	615
A USE FOR MATRICES. By Prof. W. A. Tuplin	616
PROGRESS IN MAGNETISM. By Dr. C. A. Bates	616
ANALYSIS OF SURFACE-ACTIVE AGENTS. By Dr. P. Robson	617
LEATHER ANALYSIS AND TESTING. By Prof. A. G. Ward	617
APPLIED ECOLOGY. By Dr. A. Macfadyen	618
'STRASBURGER' REVISED. By Dr. A. Allsopp	618
PHYSIOLOGY OF PLANTS. By Dr. C. R. Metcalfe	619
UNIVERSITY OF KHARTOUM, SUDAN. By Prof. M. A. Nour	620
SOCIAL AND INDUSTRIAL GERONTOLOGY IN GREAT BRITAIN. By C. E. Fleming	623
OBITUARIES:	
PROF. R. SPÄRCK. By Dr. Torben Wolff	625
MR. K. E. B. JAY, M.B.E. By R. M. Fishenden	625
DR. DUNCAN A. MACINNES	626
PROF. HERMANN STAUDINGER. By Prof. C. E. H. Bawn, C.B.E., F.R.S.	626
NEWS AND VIEWS	627
MEETING OF BIOCHEMISTS IN SPAIN. By Dr. J. R. Villanueva	632
LUNG TUMOURS IN ANIMALS. By Prof. L. Severi	632
PLANNING A NEW MEDICAL SCHOOL	634
NATURE CONSERVANCY IN BRITAIN	635
ORIGIN OF ATYPICAL METEORITES FROM THE ARIZONA METEORITE CRATER. By Prof. Michael E. Lipschutz	636
MECHANISM OF STORED-ENERGY RELEASE AT 200° C IN ELECTRON-IRRADIATED GRAPHITE. By Prof. E. W. J. Mitchell and M. R. Taylor	638
SOME CHARACTERISTICS OF TROPICAL THUNDERSTORMS. By Prof. S. V. C. Aiya	641
EFFECTS OF PRONOUNCED ELECTRON-CONTENT VARIATIONS ON DOPPLER SHIFT AT LOW ELEVATIONS. By Haim Soicher	644
FRACTIONATION OF RIBONUCLEIC ACIDS IN THE ZONAL ULTRACENTRIFUGE. By Dr. J. R. B. Hastings, Dr. J. H. Parish, Dr. K. S. Kirby and E. S. Klucis	645
EFFECT OF STEROIDS AND 'TRITON X-100' ON GLUCOSE-FILLED PHOSPHOLIPID/CHOLESTEROL STRUCTURES. By Dr. Gerald Weissmann, Dr. Grazia Sessa and Dr. Sigmund Weissmann	649
DISTINCTION BETWEEN THE EFFECTS OF ANTIVIRAL AND ANTICELLULAR POLYOMA ANTIBODIES ON POLYOMA TUMOUR CELLS. By Ingegerd Hellström	652
INTERSPECIES DIFFERENCES IN RESPONSE TO POLYPEPTIDES AS PERMEABILITY FACTORS. By Dr. Jean Carr and Prof. D. L. Wilhelm	653
A SIMPLE BIOASSAY FOR ANTIOXIDANTS BASED ON PROTECTION OF <i>Tetrahymena pyriformis</i> FROM THE PHOTODYNAMIC TOXICITY OF BENZO(a)PYRENE. By Dr. Samuel S. Epstein, Inna B. Saporoschetz, Myra Small, Weldon Park and Nathan Mantel	655
THREE HAEMOGLOBINS K: WOOLWICH, AN ABNORMAL, CAMEROON AND IBADAN, TWO UNUSUAL VARIANTS OF HUMAN HAEMOGLOBIN A. By Dr. N. Allan, D. Beale, D. Irvine and Dr. H. Lehmann	658
APOFERITIN SYNTHESIS IN HUMAN ERYTHROID CELLS IN THALASSAEMIA. By Dr. Edwin H. Eylar and Dr. Gastone Matoli	661
FORMATION OF INTRA-MITOCHONDRIAL GLUCOSYL-OLIGOSACCHARIDES FROM GLUCOSE BY INTACT NOVIKOFF ASCITES HEPATOMA CELLS. By Dr. V. N. Nigam and Dr. G. de Lamirande	664
GUANASE ACTIVITY OF THE SYMBIONTS AND FAT BODIES OF THE COCKROACH, <i>Leucophaea maderae</i> . By Dr. Leon L. Pierre	666
DIFFERENT MOLECULAR SPECIES OF MOUSE INTERFERON INDUCED BY STATOLON. By Prof. T. C. Merigan and Dr. W. J. Kleinschmidt	667
AUTORADIOGRAPHIC IDENTIFICATION OF THE D (13-15) CHROMOSOME RESPONSIBLE FOR D ₁ TRISOMIC PATAU'S SYNDROME. By Dr. F. Giannelli	669

(Continued overleaf)

UNICAM**SP.800**

spectrophotometer
for routine U.V. analysis
with automation accessories



Save operator time, increase speed and efficiency with Unicam automation for:

ENZYME REACTION RATES

LIQUID COLUMN CHROMATOGRAPHY

**ALL ROUTINE ULTRAVIOLET AND
VISIBLE SPECTROSCOPY**

Please send for descriptive brochure

The brilliant new Unicam SP.800 Spectrophotometer is setting new standards in ultraviolet and visible spectroscopy. The widely acclaimed flat bed presentation, the beam balance smoothing cam and the unique second sample position are among the features which establish it as *the* outstanding U.V. spectrophotometer. Additionally Unicam now offers automation equipment which provides:

AUTOMATIC interchange of up to 4 sample and 4 reference cells

AUTOMATIC re-cycling over any preselected wavelength range

AUTOMATIC recordings at predetermined time intervals

Unicam Precision Spectrophotometers

Unicam Instruments Ltd, York Street • Cambridge • Telephone: Cambridge 61631 • Telegrams: Unicam Cambridge • Telex 81215

LETTERS TO THE EDITOR

ASTROPHYSICS		Page	HAEMATOLOGY		Page
Measured Velocities of Interplanetary Dust Particles.— C. S. Nilsson, W. M. Alexander, C. W. McCracken, O. E. Berg and L. Secretan		673	Haptoglobin Johnson in Australian Aborigines.— D. W. Cooper, H. B. M. Lewis and Dr. E. M. Nicholls		694
PHYSICS			Low Concentrations of Serum Haptoglobin in Mothers of Children with Haemolytic Disease of the Newborn. —Jan Pintera		694
A Dense Packing of Hard Spheres with Five-fold Symmetry.—B. G. Bagley		674	HISTOCHEMISTRY		
GEOPHYSICS			Cholinergic Excitation and Inhibition in the Cerebellar Cortex.—P. Kása and Dr. B. Csillik		695
Damping of <i>S</i> Waves.—Sir Harold Jeffreys, F.R.S.		675	PATHOLOGY		
Cassiterite as a Carrier of Palaeomagnetism.—V. Hanuš and M. Krs		675	Immunological Tests in Helminthic Diseases.—Dr. R. Elsdon-Dew		696
GEOLOGY			Lymphatic Neoplastic Transformation of Thymus in Mice with Myeloid Virus-induced Leukaemia.— L. Chieco-Bianchi, Dr. L. Fiore-Donati, G. Tridente and N. Pennelli		697
Isotopic Ages and Andean Uplift.—Dr. R. W. R. Rutland, Dr. J. E. Guest and Dr. R. L. Grasty		677	IMMUNOLOGY		
CHEMISTRY			Histocompatibility Antigens in Transplanted Mouse Eggs.—Dr. Richard L. Simmons and Paul S. Russell		698
Nomadic Behaviour of Yttrium Ions and their Struc- ture in Solution.—Dr. R. C. Vickery		678	RADIOBIOLOGY		
Use of Infra-red Spectroscopy to determine the Orientation of Pyridine sorbed on Montmorillonite. —J. M. Serratosa		679	Tests on <i>Drosophila</i> for the Production of Mutations by Irradiated Medium or Irradiated DNA.—V. L. Chopra		699
Relaxation Effects in the Electrophoresis of Disperse Systems.—J. N. Shaw and Dr. R. H. Ottewill		681	Mutagenic Effect of Irradiated and Unirradiated DNA in <i>Drosophila</i> .—A. E. Khan and Dr. T. Alderson		700
Ultra-violet Absorption Spectra of Aromatic Nitrenes and Dinitrenes.—A. Reiser and V. Frazer		682	Lack of Mutagenic Effect of Irradiated <i>Drosophila</i> Medium.—Dr. O. S. Reddi, G. M. Reddy, J. J. Rao, D. N. Ebenezer and M. S. Rao		702
BIOCHEMISTRY			BIOLOGY		
Transphosphorylation of Adenosine Di- and Tri-phos- phates in the Presence of Calcium Phosphate Precipitates.—Dr. R. W. Burley		683	Sexual Dimorphism in <i>Tilapia</i> .—J. Chervinski		703
An <i>in vitro</i> Action of Human Growth Hormone in the Presence of Actinomycin D.—T. E. Martin and Prof. F. G. Young, F.R.S.		684	Presumed Sensory Cells in Fish Epidermis.—Dr. Mary Whitear		703
Long-spacing Segments from Renatured $\alpha 1$ Sub-units of Collagen.—Klaus Kuhn, Christian Tkocz, Bodo Zimmermann and Gundolf Beier		685	Taxonomic Investigation of Several <i>Brassica</i> Species using Serology and the Separation of Proteins by Electrophoresis on Acrylamide Gels.—Dr. J. G. Vaughan, A. Waite, Dr. D. Boulter and S. Waiters		704
Glycogen in Yeast Form of <i>Paracoccidioides brasiliensis</i> . —Dr. Fuminori Kanetsuna and Dr. Luis M. Carbonell		686	Seasonal Changes in the Histological Structure of the Spinal Column of <i>Sorex araneus</i> (L.).—Lauri Saure and Heikki Hyvärinen		705
PHYSIOLOGY			Effects of Starvation and Protein Depletion on Mercury Retention in Two Strains of Chickens.—Donna V. Larkin, V. L. Miller, G. E. Bearn and Dr. C. M. Hamilton		706
Plasma Growth-hormone Levels in Chronic Starvation in Man.—Dr. Vincent Marks, N. Howorth and F. C. Greenwood		686	ANATOMY		
Fasting Levels of Growth Hormone in Men and Women.—Dr. C. Faiman		687	Ultrastructure of the Phrenic Nerve.—Dr. Andrew S. Wilson and Douglas G. Silva		707
Antidiuretic Hormone and Bile Flow.—Earl Homsher and Dr. George C. Cotzias		687	Electron Microscopic Observations on the Carotid Body.—Dr. T. J. Biscoe and Dr. W. E. Stehbins		708
Alcohol and Evoked Potentials in the Cat.—Arlene K. Schweigerdt, Dr. Anant R. Dravid, Ann H. Stewart and Dr. Harold E. Himwich		688	MICROBIOLOGY		
Proposed Physiological Function for Plasma Cholin- esterase.—H. S. Funnell and Prof. W. T. Oliver		689	Timing of Protein Synthesis during Germination and Outgrowth of Spores of <i>Bacillus cereus</i> Strain T.— W. Steinberg, Prof. H. O. Halvorson, A. Keynan and E. Weinberg		710
Introduction of a Permanent Electrode into the Pituitary Gland of the Cow: Influence of Electrical Stimulation on Milk Ejection.—B. Hynek, Dr. J. Bilek and M. Lis		690	VIROLOGY		
PHARMACOLOGY			Nucleic Acid of Infectious Laryngotracheitis Virus.— G. A. Tannock		711
Chloroquine Resistance and the Nature of Malarial Pigment.—Prof. Irwin W. Sherman, J. Brian Mudd and Prof. William Trager		691	SOIL SCIENCE		
Mepacrine- and Primaquine-resistant Strains of <i>Plas- modium berghei</i> , Vincke and Lips, 1948.—Dr. W. Peters		693	Measurement of Exchangeable Aluminium in Acid Soils.—J. B. Skeen and Dr. M. E. Sumner		712

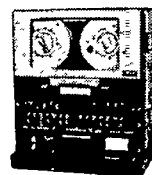
What do you do with data?



As makers of a range of rugged, reliable, economical and flexible Data and Instrumentation Recording equipment, we know a thousand and one ways of using magnetic recording for data acquisition, storage and analysis—in aeronautics, medicine, industry and university research work. Magnetic recording provides a wide range of band widths and high information packing density. Data expansion and contraction facilities are available which are not possessed by other forms of data acquisition. Thermionic equipment is the lowest priced range of digital and analogue recorders for research—we designed it on the unit principle for complete flexibility of use and we're sure it will answer your data handling problems.

You tell us!

Tell us what your particular research requirements are and we'll show you how THERMIONIC Data Recording Systems can meet them quickly, simply, cheaply. Drop a line to us today.



THERMIONICS



DATA & INSTRUMENTATION RECORDING EQUIPMENT

THERMIONIC PRODUCTS (Electronics) LTD., Hythe, Southampton. Tel: Hythe 3265-9. Cables: Technico Hythe Southampton
A MEMBER OF THE CONTROLS & COMMUNICATIONS GROUP

Lock

Gas Chromatograph Test Set & Reference Signal Source



enables the Chemist or Instrument Engineer to check quickly the operation and performance of his complete Gas Chromatograph equipment. If the equipment is not operating correctly, it will quickly localise the faulty section. No electrical knowledge is necessary to use this Test Set, which is suitable for Flame Ionisation, Thermistor type and Katharometer Gas Chromatographs.

Please write for details now:-



A. M. Lock & Co. Ltd.
Prudential Buildings,
79, Union Street, Oldham, Lancs.
Telephone: MAIn 0333

TRAINING FOR MANAGEMENT IN INDUSTRY

IN one of the most recent of a series of articles on "Research Establishments in Europe" which has been appearing in *Chemistry and Industry*, Dr. Max Stoll, in describing the research laboratories of Firmenich et Cie, Geneva, has a passage on the organization of the laboratories which is highly pertinent to the present discussions on education for management, recruitment and training for the Civil Service and the like. Dr. Stoll observes that throughout his career of forty years with Firmenich the management has always been 'research-minded' and this has ensured the greatest possible efficiency in research. For this his basic principle is to ensure the deepest interest of his research chemists in their work and accordingly they have the utmost freedom.

Dr. Stoll develops his point with the significant comment that three of the directors studied chemistry at the Swiss Federal Institute of Technology, two presenting research theses for a Ph.D. It was not surprising that with a broad-minded and scientifically trained management, Dr. Stoll's rejection of the idea of progress reports met with complete understanding. He is convinced that chemists will write reports spontaneously as soon as they have anything to report, and that insistence on an automatic progress report is a senseless and unnecessary burden. Freed from this, properly selected staff will automatically become interested in the problems of the company, and Dr. Stoll finds that where the choice is wide they tend to choose the most challenging, which he usually encourages them to do, and having made their choice the problem is attacked with much enthusiasm and ingenuity.

The freedom that is thus progressively developing is one thing that makes all the difference between efficient and non-efficient research. Dr. Stoll recognizes, of course, that sometimes this freedom may involve inconvenience: for example, if the company wishes to exploit very rapidly an interesting product which a research chemist has discovered it may be necessary to open the problem to all members of the fundamental research group if the original discoverer does not arrive at an economically advantageous synthesis sufficiently swiftly. Even so, this internal competition can be handled so as to prove a stimulant rather than a frustration.

Here, too, the ultimate responsibility is that of management, which must equally be on the alert to avoid any deterioration in morale such as could, for example, result from continued lack of success in attacking a particularly difficult problem. Publications policy presents its own problems, and Dr. Stoll also suggests that the freedom on which he places such emphasis can minimize these difficulties too as the research worker finds himself a part of the company as well as of his research group. Here again he emphasizes that the corollary is a highly efficient laboratory for developing manufacturing processes.

Dr. Stoll's two main points—the need for a management which has been scientifically trained and which is capable of undertaking the character of scientific work and the essential conditions in which such work is effective, and the provision of the maximum freedom for the investigator—are not always sufficiently to the front even in discussions on the management of research in Britain. Moreover, it should be noted that there is a corollary or implication that is also often overlooked: the freedom he advocates is unlikely to be abused unless the selection of

staff has initially been at fault. This is another responsibility of management which has recently come to the front very much so far as the Scientific Civil Service is concerned, but it is equally important in business and industrial management generally, and was, in fact, one count in an indictment of the training of young people for industry in a forthright report this summer from the Central Committee for Study Groups.

This report maintained that management is too often ignorant of recent advances in education and does not know what qualities are required for different jobs. Generally, the seventeen independent study groups who contributed to the report thought that Britain was suffering from an incalculable loss of potential. While the report itself is concerned more with the training of those entering industry direct from school, advocating replacement of the present apprenticeship system with a single channel of entry into industry for all school-leavers and the termination of set periods of apprenticeship, its conclusions imply management of the same calibre and qualities as Dr. Stoll emphasizes. This amounts, in effect, to something like the managerial revolution which Mr. John Marsh, director of the British Institute of Management, told Section J (Psychology) of the British Association for the Advancement of Science, at its meeting in Cambridge on September 7, had already started in Britain, involving the Government, business, academic institutions and managers themselves.

Mr. Marsh emphasized that our standard of living would depend increasingly on the calibre of management, but considered that the prime responsibility of the Government was to provide the economic conditions which encouraged good management. It was in this context that he asserted that the problem of managerial obsolescence was perhaps the greatest challenge in what he had described as a managerial revolution. In essence, it involved an attitude of mind which it should be the purpose of the business schools and other academic institutions concerned to provide. Mr. D. Pym, who discussed more particularly the identification of effective managerial performance, asserted that at present too many mediocre men were to be found in the highest management of British industry. This would seem to point to indifferent selection as well as to weaknesses in management in an earlier generation, but once again it substantiates in the general field Dr. Stoll's contention in regard to research.

The third paper in this discussion on management training on September 7 came from Mr. W. G. McClelland, the first director of the new Business School which opened in Manchester on September 27. The London school is expected to open next year, and meanwhile the Manchester school has a staff of seven, but it hopes to increase this eventually to about fifty. The opening course, which will last for twelve weeks, is attended by executives between thirty-five and forty from some of the largest companies in Britain, and is intended for those who have shown unusual talents for management. The task of the school is, in fact, seen not as to train managers but to educate them, and Mr. McClelland's paper at Cambridge was concerned with the practicability of this task.

He thought, first, that the tasks of the highest levels of management could be specified broadly enough for this purpose. The manager could, for example, be taught

techniques, such as those of forecasting and the interpretation of accounts, which would help him to make good decisions on courses of action. Through these his understanding of the social, economic and political environment, in which his firm operated and on which his decisions often depend, could be developed. Mr. McClelland agreed that it was very difficult to assess the results of any such training or education, particularly as those selected for courses were probably already earmarked for promotion. Moreover, training which was effective for one man in one situation could be disadvantageous for another in some other situation.

Thirdly, Mr. McClelland, considering the problems of personal development to which this formal education might contribute some solution, emphasized the wider perspectives which specialists taking up senior positions needed. External management courses could enable these needs to be met as well as put technical expertise in a context of wider considerations, and encourage a higher quality of decision. Finally, he insisted that training for business management was essentially an investment, carrying indeed a certain element of risk, as the manager might leave the firm (although this need not be a national loss) but it also offered the probability of a high return.

In so far as poor management may be a contributory factor in the disputes which have led to unofficial strikes and working-to-rule, Mr. McClelland's argument could easily be substantiated. Even more important where good industrial relations are concerned, efficient management could promote the mutual confidence and good understanding which could help the Trade Unions to get on with the long-overdue overhaul and reform of the whole structure of the movement and its adaptation to the conditions of the technological age in which we live. For this reason the wide support which the new business schools have received from industry is encouraging. A report from the Confederation of British Industries, issued in August, appealed for the fullest co-operation from industry in establishing the new Business Schools in Manchester and London, urging that the Schools would not meet the needs of industry without such co-operation.

The Confederation considered that business education has two main tasks. One is the development and teaching of business studies based on such subjects as economics, accountancy, law and sociology, in the same way that education for the medical or legal profession is based on appropriate subject groups, and the application of this knowledge to business. The other task is to develop the personal qualities needed for successful management, and the report suggests that these must include a critical awareness of environment, strong powers of perception, and ability to assess achievement, to take correct decisions, to interpret human behaviour, and to use labour and materials to achieve a specified target. The Confederation also believes that the Schools should serve two groups: besides courses for managers with some years of experience in industry on the threshold of more senior posts, there should be courses for those who have recently graduated.

The Confederation's views are thus closely in line with the views expressed by Mr. McClelland, and the first task of business education appears to be very much what is already being attempted inside the Civil Service at the Centre for Administrative Studies. On the other hand, in some of the evidence to the Estimates Committee in its recent enquiry into recruitment to the Civil Service, some uneasiness was apparent as to the possible effect of proposed methods of recruitment to the Department of Economic

Affairs building up a corps of specialists with too narrow an outlook and range of experience. Government economists of all people will need constant refreshment from outside, and use of the new Business Schools might be one answer. It should not be imagined, however, that the new Business Schools by themselves can meet more than a fraction of the need, important as that fraction may be. Their work will need to be supplemented by the fullest possible use of other facilities for education in management and by a considerable programme of well-planned research, in which field the new Social Science Research Council should in due course make felt its own influence. Beyond this, however, the establishment of the new Business Schools and the wider recognition that the question of education for management is being faced realistically by industry and tackled with determination should have a beneficial effect on recruitment to industry. At the Cambridge meeting of the British Association, Mr. D. W. Hutchings described an enquiry undertaken at the request of the Department of Scientific and Industrial Research in view of the relatively small proportion of engineering graduates who accepted awards to stay on for research. He expressed the view to the Engineering Section that an alarming proportion of our ablest graduates in science were intent on university careers, although in technology, on the other hand, far too few graduates were ready to undertake academic research. Even in arts it appears, however, that industry now has increasing difficulty in obtaining recruits: an increasing proportion of the ablest graduates remain at their university, and those who do leave tend to prefer teaching, the professions or the Civil Service to industry. Clearly there is a problem of priorities which is outside the scope of a discussion of the methods of selection or training for management, but its existence emphasizes the far-reaching importance of a determined and urgent effort to ensure that management in industry and elsewhere is of the highest quality. Its importance for our economic, scientific and industrial effort is decisive, and as management is seen to be efficient its effect on the recruitment of first-class talent could also be decisive. Nor should it be forgotten that in the long run it is on the quality of management in a wide range of spheres that the success of our efforts at national and regional level depends.

LOWER PALAEOZOIC STRATIGRAPHY

Les Temps Fossilifères

Par Prof. Henri Termier et Geneviève Termier. 1: Palaeozoïque Inférieur. Pp. v+690. (Paris: Masson et Cie., 1964.) 270 francs.

BOOKS by individuals about the history of the Earth fall into two categories. The majority are written with the undisguised intention of capturing part of the growing undergraduate market. They are, in the main, unpretentious accounts with a strong regional bias and, despite the advance notices of their publishers, are usually sufficiently restrained in their treatment of the elements of stratigraphy to be freely prescribed to students enmeshed in one's own impressions of the subject. The remainder are much more ambitious theses which, by deployment of detail, contrive to win recognition as definitive works of reference. They appear with increasing rarity, because for several decades now it has been beyond the capacity of any one man to maintain a high standard of scholarship and first-hand experience over so wide a field as world stratigraphy, which explains the modern preference for collaborative works compiled

by several experts. It is therefore with mixed feelings that one opens a volume on Lower Palaeozoic stratigraphy and palaeogeography, even though it has been prepared by H. and G. Termier, surely the most prolific partnership ever known to geology.

On the whole, Volume 1 of *Les Temps Fossilières* well survives a scrutiny, sharpened by a familiarity with the geology of certain regions that is necessarily greater than that of the authors. The Termiers have evidently missed very little background information and the data selected for publication have been marshalled and interpreted in an interesting way. Sixteen time-stratigraphic units have been recognized, and in the sections devoted to each there are chapters on the absolute time range of the epoch, regional stratigraphy, earth movements, igneous activity, sedimentary deposition, palaeoclimates, palaeogeography, palaeontology and palaeoecology and also an extensive bibliography. The text is supported by 124 stratigraphical tables and 441 figures, including well-designed regional maps showing rock distributions and palaeogeographic reconstructions and excellent illustrations of successions, sedimentary features and fossils.

There are, of course, some disadvantages to sorting inter-related observations into such neat pigeon-holes. The interpretations offered within each chapter of a section are concerned with different aspects of the Earth's history during a given segment of time. But since they are all ultimately dependent on the distribution, disposition and characteristics of the same rocks, reiteration can become noticeable and the maintenance of separate chapters (especially on climate, sedimentation and palaeogeography) contrived in some sections. Yet the segregation of information can also be effective. The Caledonian orogeny, for example, does not burst unheralded on the scene as in so many other text-books. The precursory phases as well as the climactic movements are described in their stratigraphic context from one section to the next and their effects are well brought out in the palaeographic interpretations.

In a study as comprehensive as this, some information is bound to show signs of only partial digestion. The tell-tale traps of recording "*Orthis testudinaria*" in the basal Ordovician (p. 308), of using *Dicoelosia* (p. 543) as well as its synonym *Bilobites* (p. 521), of assigning—here and there—genera to the wrong superfamilies, of identifying the Manx Slate as Pre-Cambrian (p. 82) and of finding only "Tethyan" and "Balto-Scandinavian" elements in the Caradocian faunas of Britain (p. 396), suggest some unevenness in textual accuracy. But the most serious criticisms of the study arise from the arrangement and content of the stratigraphical tables and the status assigned by the authors to some of the major time-stratigraphical units.

The stratigraphical tables are so arranged that the successions selected to illustrate the geology of a region are grouped together to show the ranges of named units but not necessarily an accurate correlation of them. Moreover, no standard succession is used to give correlative continuity from one table to the next, so that, in the absence of diagnostic fossils, the tables can become unconnected columns of rock units. This lack of stratigraphical discipline has led to obvious errors. Thus, the Ashby State of North America is, in one table (p. 377), restricted to the zone of *Nemagraptus gracilis*, while members assigned to it by American geologists are, in another table (p. 345), included in the zone of *Glyptograptus dentatus*; the Kosov Beds of Czechoslovakia with *Akidograptus acuminatus* are included in the Ordovician (p. 419); and the "Hirnantian" of Shropshire is equated with the Drummuck Mudstones of Scotland (p. 465). In a review of this scope one would also expect the standard section on which the correlation of a region is based to be invariably given. But this is not so. The Llandovery shelly facies of Britain, for example, are not represented

by successions in the type area but by an imperfect version of the less well-known outcrops in south Scotland.

Finally, one must join issue with the authors for the way they use 'Tremadoc' and 'Silurian' as systemic names. This procedure is unwarranted and creates many more difficulties than it can ever overcome. Their recognition of the discredited Ozarkian as a synonym of the Tremadoc should have arrested any temptation to resolve controversy over the systemic affiliations of the series in this manner. Moreover, to resuscitate Grabau's 'Silurian' as an answer to facies shifts across the generally accepted Siluro-Devonian boundary is to ignore the fact that all stratigraphic measurements of time are man-made and liable to be inappropriate in some succession or other.

A. WILLIAMS

GEOLOGICAL THESIS ON HADHRAMUT

The Stratigraphy and Structure of the Eastern Aden Protectorate

By Z. R. Beydoun. (Overseas Geology and Mineral Resources Supplement Series No. 5.) Pp. viii+107+illustrations and maps. (London: H.M.S.O., 1964.) 27s. 6d.

THE first thing to note about this geological survey is its size, 92,000 square miles (about as big as the United Kingdom); and the second is that such a remote rugged mountain and desert plateau region—virtually unknown before the Second World War—was mapped in fifteen years after 1945. Previously, the only geological exploration had been around the fringes in Yemen, Somaliland, Socotra and the Oman mountains, while the shield and southern half of Arabia remained geologically another 'empty quarter'. The barren limestone plateau north of the Wadi Hadhramut was first crossed by Philby in 1936 and the Mahra territory in 1946 by Thesiger.

In the 1920s and 1930s there were sporadic geological reconnaissances along the coast around Mukalla and into the Wadi Hadhramut, but the first detailed stratigraphic work was done by Wetzel and Morton over 20,000 square miles of Mahra country in 1947–48, close on Thesiger's heels, and also by camel, a very arduous expedition. Dr. Z. R. Beydoun, who spent six consecutive field seasons, 1953–59, in the Eastern Aden Protectorate, was able to use 'Land-Rovers', astrofixes and aerial surveys; but most of the 'main motorable tracks' shown on his locality map did not exist at the beginning, nor were there any drilled wells of potable water.

Having worked on similar stratigraphic sections in southern Jordan and visited the neighbouring countries of Aden, Socotra, Somaliland, Dhofar and Oman, he is particularly well qualified to present this thesis, which is a good example of the high standard of stratigraphical, palaeontological and petrographical work now undertaken by oil companies, even on formations that are only of background interest to oil-finding.

The quality of this publication is also extremely high, comprising 3 loose geological maps in colour and 16 plates (including 3 regional stratigraphic correlations) in 100 pages of text.

For the sake of readers who are not familiar with the many references from surrounding countries, it is a pity that an outline map of the Arabian peninsula was not included. The salt comes should also have been marked on the tectonic map (Plate XV).

The Eastern Aden Protectorate belongs to the foreland or shelf belt surrounding the Arabo-Somali granite nucleus. Pre-Cambrian and probable Cambrian basement rocks—folded and faulted on north-south lines and then eroded—are unconformably overlain by basal sands, limestones and marls with local evaporites of Middle-Upper Jurassic age.

This big unconformity is regional for western and southern Arabia from Yemen and Aden to Dhofar. Fossiliferous Cambrian is known only from the Dead Sea, where it is followed by marine Triassic. Oman and eastern Saudi Arabia have marine Palaeozoic and Triassic below the transgressive Jurassic. The Eastern Aden Protectorate Jurassic is highly fossiliferous and its evaporites have given rise to salt domes on the western frontier with Yemen. Dr. Beydoun considers the salt to be of Jurassic age and diapiric in action, contrasting with the accepted Cambrian age of the Oman piercement plugs and the Miocene age of the Red Sea ones.

I was struck by the anomalous trend of the evaporite facies line in this area, west-north-west-east-south-east, the same as the faults among which the domes are located, and wondered if there was a genetic connexion.

The facies lines of the rest of the Jurassic and of the Cretaceous are north-south from Saudi Arabia to Somaliland. The Jurassic of the Eastern Aden Protectorate and Yemen correlate well, including the evaporite facies.

The Cretaceous consists of sand in the west and limestones and marls in the east, and this period and the succeeding Tertiary one of limestones, shales and evaporites are subdivided into litho-stratigraphic units which are described in detail with faunal lists and regional correlations.

The dominant structure of the Eastern Aden Protectorate is the gentle east-west geo-anticlinal Hadhramut arch in Palaeocene limestone forming a plateau between the sands of the Rub-al-Khali and the Gulf of Aden.

There is no space in this review for the controversial rift-fault tectonics which dominates the last twenty pages on structure, especially as Dr. Beydoun quotes fully many publications on neighbouring areas. In conclusion, he and his sponsors (Iraq Petroleum) are to be congratulated on a very valuable contribution to the geology of this remote region. He and his assistants and colleagues deserve high praise for their strenuous and painstaking field mapping under adverse topographic conditions—well illustrated in the photographs. F. E. WELLINGS

A USE FOR MATRICES

The Matrix Analysis of Vibration

By Prof. R. E. D. Bishop, G. M. L. Gladwell and S. Michaelson. Pp. x+404. (London: Cambridge University Press, 1965.) 100s. net.

THE Matrix Analysis of Vibration is basically mathematical and it treats the subject of matrices in a much more general manner than might be associated with the title's particular association of it with vibration. The nature of the answers to many of the numerous examples makes this clear, but others show that the reader is recommended to do a fair amount of numerical computation in order to ascertain the tenacity of his grip on the process of practical application of matrix methods and also no doubt to give him some idea of the tedium and irritation that are liable to be found in long calculations that are unavoidable in solving certain types of vibration problem.

As the first step in making calculations in connexion with vibration of any actual system, simplifying assumptions are used in representing the physical attributes of the system by symbols or numbers. Out of these it is usually possible to build an equation or equations. The next stage is the purely mathematical one of solving the equations, and it is with this stage that this book is almost wholly concerned.

As the matrix method is not applicable to a system with an infinite number of degrees of freedom (and strictly, this means every system) the book considers in some detail the problem of determining the dimensions of a "lumped-mass" system closely equivalent to the actual system. The

labour of solving the equations is then left to a digital computer. It is odd to recollect that before such aid was readily available, lumped mass systems were sometimes replaced by distributed mass systems in order to ease calculation by the conventional methods of the time.

Mention is made of the fact that where the equation representing a vibration problem is found to be highly sensitive to small changes in the arguments, the physical system is likely to be correspondingly temperamental. If it is, close prediction of its behaviour is impossible and so meticulous calculation may be purposeless.

The note on p. 227 that two methods of calculation give results differing by only 0.645 per cent inspires the remark that a discrepancy of ten times that amount is gratifyingly small in quantitative predictions of torsional vibration in some practical cases. This is, perhaps, not a valid comment in reviewing a book that is avowedly mathematical and that deals very clearly and very thoroughly with the principles and practice of using matrix methods for solving linear equations with large numbers of unknowns.

The question as to whether any particular vibration problem can be solved with adequate accuracy and speed without resorting to matrix analysis is usually one that demands attention from both the engineer and the mathematician, if it is asked at all. Whatever the answer may be, calculation in practice is preferably carried out in at least two different ways, even if none of them is rigorous, in order to obtain some safeguard against gross errors.

W. A. TUPLIN

PROGRESS IN MAGNETISM

The Theory of Magnetism

An Introduction to the Study of Cooperative Phenomena. By D. C. Mattis. Pp. xvi+303. (New York and London: Harper and Row, 1965.) 86s.

Physics of Magnetism

By Prof. S. Chikazumi. English edition prepared with the assistance of Stanley H. Charap. (Wiley Series on the Science and Technology of Materials.) Pp. xii+554. (New York and London: John Wiley and Sons, Inc., 1964.) 120s.

HISTORIES of physics and magnetism have much in common, beginning with the discovery of the lodestone by the Greeks. Modern texts on magnetism tend to overlook the work of past philosophers and it is very refreshing to find such an excellent account of the past introducing the main text of the first book.

A student fresh to the theory of magnetism is confronted with literature of alarming proportions. *The Theory of Magnetism* attempts to organize this material by treating magnetism essentially as one of the most important examples of co-operative phenomena. The emphasis throughout is on strong interactions between two or more particles and especially those between electrons and spins. The book itself begins with a review of the quantum mechanical idea of exchange and discusses various concepts derived from it. Magnetic moments are measures of angular momentum, and thus a detailed quantum mechanical discussion of angular momentum is given. Important relationships, formulae and tables are presented in an unorthodox but very attractive way. Many electron wave-functions complete the mathematical basis of the theory and most of the more important theorems and rules are clearly presented.

Spin waves form the principal topic of the book, and thus a study of the statics and dynamics of magnetism follows. It commences with a semi-classical treatment and leads to the introduction of magnons and concludes with spin waves in insulators. Magnons in metals are treated separately and are derived from detailed discussions of the properties of electrons in metals. The third and final

part of the book deals with the effects of temperatures on spin systems, and consequently the thermodynamics and statistical mechanics of magnetism are considered. Phase transitions and the Ising model are discussed at length. Tables for indirect exchange theory form a very useful appendix and the book concludes with a complete and up-to-date bibliography of collateral reading.

Magnetism is far from all theory, and this is clearly emphasized in the *Physics of Magnetism*. A general and fully comprehensive review of magnetism is presented with an emphasis on physical concepts. One departure from common practice is the use throughout of the MKSA system of units, although the treatment is as non-mathematical as possible. The book begins with a classification of magnetic substances and then describes the properties of a ferromagnetic body. Theoretical background for the origin of magnetism and the theories for ferromagnetism and ferrimagnetism form the second part of the book. The discussion tends to be pictorial, and the reader needs little in the way of quantum mechanics. The book is centred around magnetic domains, and a fully comprehensive discussion of this concept is given with numerous figures and photographs. The domain concept is extended to account for the various observed magnetization processes, and many detailed results and conclusions are presented. More specialized topics form the last part of the book—some of the more important topics discussed are induced magnetic anisotropy, the ferromagnetism of thin films, thin wires and fine particles, various magnetization phenomena, the rare earth metals and also techniques for investigating internal magnetic structure. Probably the most important aspect of magnetism concerns its technological application, and this is not forgotten with a brief discussion of the types of magnetic materials and special applications of them. Data relating to magnetic substances are collected in an appendix.

In my own opinion, both books are most successful in their intending aims. *The Theory of Magnetism* is intended for the research worker, and caters particularly for the theoretician. The major achievement is to step from most complicated situations to accurate arguments with simple explanations only. The style of the book is unconventional and many topics are presented in a textbook for the first time. *Physics of Magnetism* is pitched on a somewhat less-advanced level as it caters for undergraduates as well as research workers. For an experimentalist, it contains most of the important facts appertaining to magnetism and contains lucid explanations for them. Both contain numerous problems for the reader to test his knowledge and its applications. There is little material common to both books and together they cover most of the field of magnetism. They are both attractively presented and may be recommended with confidence.

C. A. BATES

ANALYSIS OF SURFACE-ACTIVE AGENTS

Identification and Analysis of Surface-active Agents by Infrared and Chemical Methods

By Prof. Dieter Hummel. Translation by Dr. E. A. Wulkow. Text Volume. Pp. xiv + 386. Spectra Volume. Pp. 166 (466 spectra). (New York and London: Interscience Publishers, a Division of John Wiley and Sons, Inc., 1964.) 150s. the two volumes.

PROF. HUMMEL'S work on the systematic identification of surface-active agents from their infra-red spectra was reported at the third International Congress of Surface Activity in 1960. His book, first published in German in 1962, contains a much expanded version of his own work as well as chemical methods for the identification of surfactants, which were added in order to broaden the analytical character of the book.

Identification and Analysis of Surface-active Agents by Infrared and Chemical Methods is the translation, without modification, of the earlier book and hence the literature is covered only to 1960. In the area of detergent analysis, as in most other fields of research, progress has been notable and rapid in the past 5 years, and thus much of the content of the book is out of date. For example, gas liquid chromatography is mentioned only once and thin-layer chromatography not at all. For this reason, the book fails in the author's expressed intention of providing a general book on detergent analysis. It remains, however, an extremely important reference volume for the detergent analyst and will be particularly useful in the identification of little-known actives.

A comprehensive list of surfactants (or 'tensides' as the author proposes all interfacially active materials should be called) is reviewed systematically with copious notes on methods of manufacture, physical properties and infra-red spectra. This chapter (141 pages) and the volume of spectra form the useful content of the book. The introductory chapters on structural characteristics of surface-active agents (13 pages) and the determination of the electrochemical behaviour of surface-active agents (12 pages) are oversimplified and contain a number of errors. For example, the cationic character of amine oxides in aqueous solution is ascribed to the formation of free amine by hydrolysis. In fact the cationic species present is the conjugate acid of the amine oxide.

The later chapters on chemical methods for determining the structural characteristics of tensides (24 pages) and methods for the quantitative analysis of tensides (37 pages) suffer most from the omission of recent research. This is unfortunate since there is no good modern review of the analysis of mixtures of surfactants which can aid the detergent analyst.

The tables at the end of the book (114 pages) summarize much of the information given in the text. Most of them are useful but one should question the value of recording in tables the results of empirical tests (for example, the Wurzschnitt reaction with iodine) which cannot be used with confidence for the identification of specific materials.

The volume of spectra is well produced and peak positions and intensities are easily read direct from the figures. The text volume is printed in Times Roman type on good quality paper and both volumes are well bound. The translation is adequate but suffers from literal translation in parts. The lack of typographical errors in the whole work is notable. The book costs £7 10s. which, combined with the lack of new information it contains, will prevent its use in all except specialist laboratories.

P. ROBSON

LEATHER ANALYSIS AND TESTING

The Chemistry and Technology of Leather

Edited by Fred O'Flaherty, William T. Roddy and Robert M. Lollar. Vol. 4: Evaluation of Leather. (American Chemical Society Monograph Series, No. 134.) Pp. viii + 440. (New York: Reinhold Publishing Corporation; London: Chapman and Hall, Ltd., 1965.) 17.50 dollars; 144s.

THE publication of Volume 4 of the American Chemical Society Monograph Series No. 134, *The Chemistry and Technology of Leather*, completes the Series begun with the appearance of Volume 1 in 1956. As in the previous volumes, each chapter is contributed by one or more authorities in the particular field, but in this volume all the authors have been drawn from the United States. Included in the volume are the analysis and assessment of raw materials and finished leather, including physical test methods. A valuable chapter on fungal resistance of leather has been contributed by T. C. Cordon of the Eastern Regional Laboratory of the U.S. Department of

Agriculture. The chapter on leather finishing by R. Shaw, drawing on his experience with the Rohm and Haas Co., is out of place in the present volume. It is also more discursive and qualitative than is required in a monograph.

It is unfortunate that analytical and physical testing methods for leather are still not co-ordinated by a single body. The commissions of the International Union of Leather Chemists Societies have been particularly successful in securing agreed physical test methods which many countries are adopting and have also made progress with chemical analytical methods. The American Leather Chemists Association (A.L.C.A.) has, however, continued to develop methods independently. This division is reflected in the various chapters of this book, with often a disproportionate emphasis on the A.L.C.A. methods.

The discussion of analytical methods, particularly when their basis is largely empirical as is often the case with leather and its raw materials, does not make easy reading. Nor is the quotation in full of an A.L.C.A. method justified. A fuller discussion of the accuracy needed in the various determinations would have been valuable, compared with that provided by the methods given.

The chapters on physical testing have greater general interest, especially that by Mieth Maeser on dynamic and non-destructive testing, which refers especially to shoe leathers and their performance.

The American Chemical Society's leather monograph first appeared under the authorship of J. A. Wilson, who presented a coherent and forceful point of view, in some instances outlining approaches only recently being taken up again. The change to the present multi-volume form, with a wide array of authors, has necessarily resulted in the loss of unified consideration. The chapters have varied in merit and value more than is usual in a book of this character, but this reflects the limited number of persons making significant contributions to leather science and technology. Despite the faults, to which reference has been made, access to the final volume and to the whole work will be a necessity for all seriously concerned with leather, although their use of them will require that they be discriminating.

A. G. WARD

APPLIED ECOLOGY

Ecology and the Industrial Society

Edited by Gordon T. Goodman, R. W. Edwards and Dr. J. M. Lambert. (A Symposium of The British Ecological Society, Swansea, 13-16 April 1964.) Pp. viii+395. (Oxford: Blackwell Scientific Publications, 1965.) 70s. net.

AMONG the outstanding questions raised by this, the British Ecological Society's fifth symposium volume, is the future of ecology as a source of practical guidance for the management of our man-dominated environment. Although the Society has, by the publication last year of the *Journal of Applied Ecology*, asserted that ecology has such a role, the sixteen articles in this volume are not unanimous on this point. In his opening address Prof. Clapham advocates the direction of university research towards increasing biological productivity and providing a scientific basis for conservation policy, a view that is supported by Britain's full backing of the International Biological Programme. This view is also implicit in suggestions by several authors that reclamation policies are far too empirical and lack a basis in theory. The opposite view is ably put, however, by Hynes, who claims that, in the field of water pollution, the scientific knowledge already exists and that current problems are nearly all in the fields of administration and politics. That such differences are not solely due to differing progress in different fields is indicated by Hawkes's views that, in the closely related field of sewage treatment, many fundamental ecological problems remain.

In fact a striking characteristic of many papers in this collection is the revelation of interesting fundamental problems at the root of purely practical investigations. A few examples are: (a) Bradshaw's demonstration that extreme conditions in contaminated environments can facilitate the study of high-speed population evolution and that conspecific plant populations have rigid spatial boundaries in contaminated ground; (b) Hawkes's views on the effect of temperature on turnover and biomass of sewage decomposers and the stimulation of bacterial activity by grazing animals; (c) Templeton's stress on the need for food chain studies of marine pollution by atomic wastes (consumption of *Porphyra* (laverbread) in Swansea limits waste disposal from the Windscale plant in Cumberland), and his demonstration that radionuclides in the sea sink or float according to their role in phytoplankton physiology.

There are, however, many points of more immediate practical importance: Hynes contends that much routine toxicity testing is a waste of money because the laboratory tests used are not related to survival in Nature; Moore concludes that we have now reached the time when fish for human consumption should be monitored for organic chlorine residues; Hawkes believes that sewage treatment in many places to-day is inferior to the best practices of the Assyrians and the Babylonians, and that diseases such as bovine cysticercosis are on the increase as a result; Gilbert's demonstration that the species range of lichens provides a practical method for detecting air pollution.

This is a well-edited book, well provided with indexes and well illustrated; the only error which seems important enough to mention is the maximal figure (p. 249) of "900,000 invertebrate animals per m² of soil"; this must surely apply to arthropods only, for nematodes occur in their tens of millions and Protozoa in far greater numbers. Inevitably the volume contains highly specialized papers in which little attempt is made to relate the material to the rest of the symposium or explain its significance, although these are far fewer than in many such volumes. The custom of publishing all papers read at meetings (and presumably chosen by the editors in the first place) is well established, but it costs editors' time and readers' money; perhaps future organizers might reserve the right at the outset to publish only a proportion of the more successful papers.

A. MACFADYEN

'STRASBURGER' REVISED

Strasburger's Textbook of Botany

Rewritten by R. Harder, W. Schumacher, F. Firbas and D. Von Denffer. New English Edition from the Twenty-eighth German edition. Translated by Peter Bell and David Coombe. Pp. xvi+846. (London: Longmans, Green and Co., Ltd., 1965.) 84s.

THE publication of a new English edition of *Strasburger's Textbook of Botany* is an important event for many English-speaking students of the subject. Most pre-war students of botany were well acquainted with the previous English edition, published in 1930, but in the absence of more recent translations of the later German editions, it is scarcely surprising that 'Strasburger' is not so well known to modern students. Eleven German editions have appeared since the last English edition and the present translation is from the twenty-eighth German edition, published in 1962.

As in previous editions, four well-known authors deal with the principal aspects of botany: D. Von Denffer with morphology, W. Schumacher with physiology, R. Harder with lower plants, and F. Firbas with seed plants and plant geography. The whole is a remarkably comprehensive account which provides a valuable introduction to almost all aspects of botanical science.

Some botanists have questioned the value of extensive compilations of this kind and prefer shorter accounts of

selected aspects of the subject. This approach, however, carries the danger, which is undoubtedly a feature of certain modern botany courses, that the student may be well informed on recent spectacular advances in such topics as plant physiology, biochemistry, fine structure and especially those aspects collectively described as "molecular biology", but may have virtually no knowledge of other aspects of botany, which are less fashionable, but equally important to a thorough understanding of the plant. The new edition of *Strasburger's Textbook* is a timely corrective to such a restricted outlook. It deals in detail with various topics, such as morphology and the physiology of growth and movement, which are rarely adequately treated in general text-books. It is true that it is difficult for so broadly based a work to keep abreast of present developments in rapidly advancing branches of botany. But with the present spate of specialist volumes and semi-popular journal articles there is little risk that the more recent advances will be overlooked by the enquiring student. The merit of a work such as 'Strasburger' is that it provides a reasonably up-to-date account of the established facts and principles of botany, which, in the words of the translators, will equip the botanist "not only to deal with the theoretical and practical problems of to-day but also to formulate and solve those of to-morrow".

The individual parts of the present edition are all of considerable merit. The section on morphology describes some of the principal discoveries in the fine structure of the cell, but is above all a thorough account of the form and structure of plants. The critic may object that there is little reference to modern experimental studies of morphology, but the results of such work are perhaps still somewhat controversial and require more detailed discussion than can be accommodated in a general text-book. In the physiology section, the parts dealing with metabolism appear to be up to date, at least so far as respiration and photosynthesis are concerned, although our knowledge of the role of nucleic acids in protein synthesis has advanced considerably since this 1962 edition. The physiology of growth and movement is well covered. This section was published late enough to include a treatment of gibberellins, but too early to include an account of phytochrome.

In the systematic section, only seven major divisions are recognized although the authors point out that bearing in mind the purposes of a text-book certain simplifications have been made intentionally. Apart from the Cyanophyta, which are rightly placed in a separate division, all the algae are included in a single division Phycophyta, but this false impression of unity is corrected later by a diagram emphasizing the independent origin of the principal algal groups. With so extensive a field to cover it is inevitable that much should be omitted, but many interesting details are described, and in the part devoted to diatoms there is an up-to-date account of the sexual reproduction of both pennate and centric diatoms. The section on seed plants is very condensed but gives a broad survey of the principal orders. The concise account of plant geography includes a short review of the plant communities of central and north-west Europe.

A useful set of references covers each major section of the work and has been added to since the 1962 German edition. Even certain useful books published in 1964 are included, for example, *The Structure and Life of Bryophytes*, by E. V. Watson.

For a comprehensive text-book, 'Strasburger' is remarkably readable and both authors and translators have done their work well. Although described on the verso as covering the work of students in their final year at school, at technical colleges and in the first years of universities, it is far more than an introductory text, and most botanists would benefit from having 'Strasburger' readily available on their shelves. It is well illustrated and produced and at 84s. is very good value indeed.

A. ALLSOPP

PHYSIOLOGY OF PLANTS

Encyclopedia of Plant Physiology

Edited by W. Ruhland. Vol. 15: Differentiation and Development, Part 1: Pp. lxiv+1647; Part 2: Pp. xliii+1362. (Berlin, Heidelberg and New York: Springer-Verlag, 1965.) Ganzleinen DM 748; Subskriptionspreis Ganzleinen DM 598.40.

THE publication of a scientific treatise comprising 70 chapters written by 53 authors and consisting of more than 3,000 pages is a notable achievement, even in times when the breaking of records is an almost everyday occurrence. When, furthermore, we realize that the two books form only the latest two-part volume of the famous *Encyclopedia of Plant Physiology*, which has been appearing in parts in recent years, we must be still more impressed by the sheer mass of data concerning the life-processes of plants that have been accumulated.

The new volume covers the all-important branch of plant physiology that passes under the general title of "Differentiation and Development". The title itself, however, scarcely indicates the range of topics that are covered, for the authors have set out not only to summarize what is known about the structural and physiological changes that are involved in the life of the plant, but they also enter into the field of cytology and genetics sufficiently to indicate how growth and differentiation are basically controlled by genes.

The editor, Dr. Anton Lang, says in his introductory essay that: "he has misgivings that the volume, in times to come, may be considered as the last major monument—not to say epitaph—of an era to which future generations of biologists will refer, with a mixture of condescension and (at least we hope) nostalgia, as the dark ages of developmental physiology". He takes this dim view because the recent outstanding advances in genetics seem to him to keep other branches of biology somewhat in the shade. He continues, however, in a more hopeful vein when he says: "Genetics and developmental physiology are theme and countertheme. The objective of genetics is to understand how the hereditary information of organisms is written, that of developmental physiology, how it is read". He also expresses a hope that the unveiling of the 'genetic code' will help to throw further light on the connexion between the two disciplines.

It is impossible to summarize the contents of the volume very adequately in a few words. It must suffice to say that the treatment is many-sided and very comprehensive. We can, for example, read about the physiology of abscission, seed dormancy and germination, graft hybrids, photoperiodism, auxins, fasciation, as well as about abnormal differentiation caused by fungal invasion and attack by insects or viruses. We can learn such practical details as that seeds can often be more effectively stored in sealed tin cans than in the time-honoured paper packet. On the other hand, we can pass on to the complexities of correlation in plant development. The volume ends with German/English and English/German indexes to the subject-matter and there is a third index to authors. Literature is cited at the end of each chapter.

The printing and lay-out of the book are very good. There is some overlapping of subject-matter as, for example, on pp. 175 and 348 of Part 2 respectively, but this is almost unavoidable in such a large volume, written by so many authors. All readers will be indebted to the authors and editor for bringing together and summarizing such a wealth of information in a readable form. It is to be feared, however, that the very high price of the volume will make it less generally available than it deserves to be. This is a fault that should be avoided especially as it is most desirable for the existing knowledge of this rapidly advancing subject to be disseminated widely before it is out of date.

C. R. METCALFE

UNIVERSITY OF KHARTOUM, SUDAN

SILVER JUBILEE OF THE FACULTY OF AGRICULTURE, SHAMBAT

By PROF. M. A. NOUR

Dean of the Faculty

IN early December 1965 the campus of the Faculty of Agriculture of the University of Khartoum will welcome distinguished guests from most African countries, and from many institutions elsewhere, all of whom are intimately concerned with agricultural education at university-level, and each eminent in the field of his own interest. The Food and Agriculture Organization of the United Nations has kindly agreed, at our invitation, to hold concurrently an Inter-African seminar to discuss "The Role of Faculties of Agriculture in Developing Societies". Most of the participants, invited from some twenty-four African countries, will be from among those who are playing a leading part in policy and decision-making in agricultural higher education and planning in their respective countries. Thus the Faculty will open its doors, to share a happy historical occasion during the celebration of its twenty-fifth anniversary with men of eminence in the field of agriculture, and will participate with them in the discussions at the seminar.

On such occasions it is pertinent to indulge in some introspection, on one hand, and speculation on the other. In 1957 my predecessor, Prof. R. J. McIlroy, gave an account of the Faculty at that stage of development¹, but since then nothing has been written on this score for readers outside the Sudan. What follows, therefore, is an endeavour to bring the information on the structure and function of the Faculty up to date.

The Faculty in 1957 consisted of three departments, with a total staff complement of eleven; now there are eight departments and thirty academically qualified personnel. The Faculty is now composed of the Departments of Agricultural Botany, Agricultural Engineering, Agronomy, Animal Production, Biochemistry and Soil Science, Crop Protection, Horticulture and Rural Economy. Eight years ago there were three Sudanese

and eight expatriate lecturing staff; now the number of Sudanese is twenty-four, all of whom have received a Ph.D. degree from universities in the United Kingdom or the United States. Six more are abroad at present and will join the staff on their return within the coming three years. The number of undergraduate students in 1956-57 was 39; now it is 167. And in 1957 no post-graduate training was available, although the Faculty was becoming keenly conscious of its role in this field, and a comparatively small but sound research development was in progress. Since then, 10 candidates have obtained their M.Sc. degrees here, 10 more are working towards it, and two are registered for the Ph.D. course. From 1938 (when the Faculty started) to 1957 some 20 publications appeared in international journals or as books, whereas more than 90 publications of comparable scientific standing have appeared since then. Because the Faculty in 1957 was just emerging, the scattered nuclei of research activities were then novel developments, whereas research is now in progress in almost all the disciplines of agriculture with perhaps the greatest momentum on the production and protection of cotton, sorghum, millet, broad bean, winter vegetables, forage crops and citrus. Nutrition of poultry and large animals and their breeding and adaptability are being investigated, while problems of salinity, irrigation and fertility of heavy clays are receiving attention by members of the Soil Science and Engineering Departments. The Faculty has become increasingly conscious of its geographical location, in relation to the Sudan and Africa, and this has led to a concerted effort in the past few years to co-ordinate team research on short-term, as well as more far-reaching, investigations of dry-land problems. To go further would be to submit a research report, but a *Jubilee Bulletin* of the Faculty will be published, available on request, containing details of present trends in research and a list of all published work.

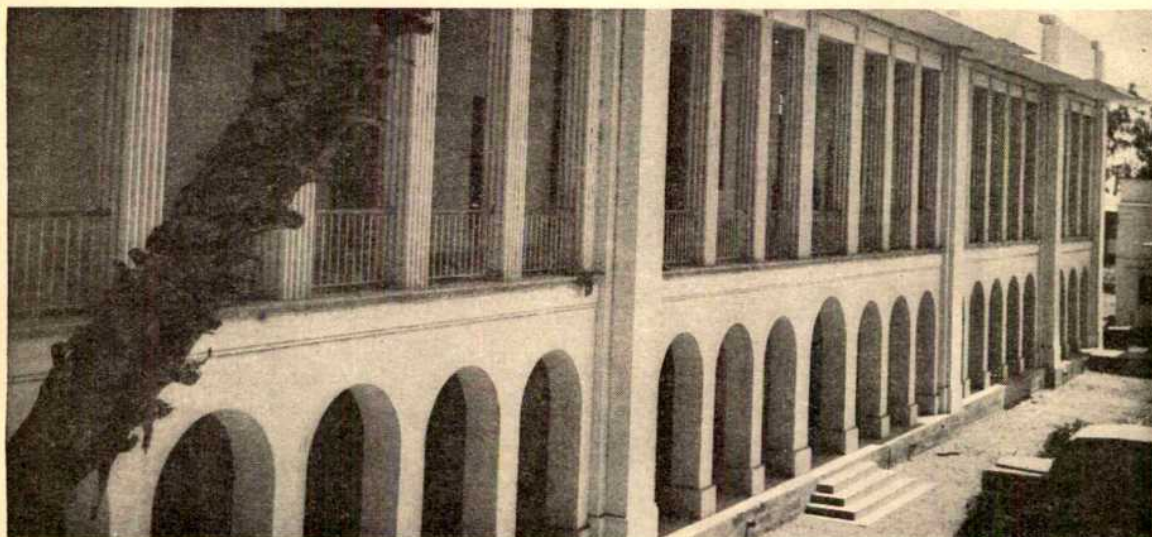


Fig. 1. Façade of the main building of the Faculty of Agriculture, University of Khartoum

The physical capacity of the Faculty has been greatly increased by a building programme which cost about £S.150,000, excluding a corresponding expansion of hostels, which house all the students, and the rapidly growing annual budget. A generous grant from Barclays Bank of £S.30,000 is allocated for building a new Department for women students to study home science and community development, the intake for which is planned to start in 1966-67. In addition the Rockefeller and Ford Foundations have donated 107,000 dollars and 75,000 dollars, respectively, towards the cost of research and improved teaching facilities in the Faculty.

The reference library at Shambat (which also serves the Faculty of Veterinary Science) has increased almost tenfold in book and journal accessions, and now houses some 25,000 volumes and more than 500 journals, apart from considerable stocks of standard text-books available in departments for borrowing by the students. Other faculty service facilities have been added since 1957, such as a plant environment control unit and a tropical shade house costing some £S.30,000 to erect, an agro-meteorological station which contains examples of virtually all the instruments available for recording soil and atmospheric climatological data, and a photographic unit. The recently established isotope research unit is bound to initiate new approaches towards agricultural problems under dry-land and irrigated conditions. There are two farms, which are in increasing demand for demonstration and experimental purposes, although the controversy over the functions of a university farm is as lively as ever.

The structure of the undergraduate courses has also undergone far-reaching changes. The old system, based on two years in the Faculty of Science and three years in the Faculty of Agriculture, has been re-modelled, and now involves one year in the Faculty of Science and four in the Faculty of Agriculture, the latter being devoted to basic and general topics for the first three years, leaving the final year for some degree of specialization. While we have been primarily concerned with the design and detail of a new type of final year, course structures in the preceding three years have also been reviewed to provide a logical yet attractive sequence in the framework, content and functional inter-relationship of these courses. This has been done and has, with remarkably few modifications, withstood the test for the past three years.

The final-year course is of an honours nature (implying a greater degree of specialization in one discipline rather than a particular level of excellence in the examination). In order to produce graduates with a measure of competence in one of the many branches in agricultural science, there was obviously the possibility of recommending a single-discipline specialization in the final year, thus offering a 'special' degree following the pattern of the University of London. This avenue was explored, but the academic hazards involved (not to mention the financial implications) became abundantly clear and single-subject specialization has been left, as in the past, to the postgraduate level.

The design of degree that has been found most appropriate for the requirements of a developing society is based on a combination of selected courses in the final

year, with the choice of discipline as the main option but with four additional supporting (ancillary) courses from other departments. The commitment of these supporting courses in formal time-table hours is the same as for the main option, namely, eight hours in total; but a student is expected, in addition, to submit a short dissertation in a subject of his choosing.

In recommending this structure, and now for the first year testing it, the Faculty has been keen to achieve a higher plateau in intellectual and academic activities than at the general degree level. The 'group specialization' design of the courses not only brings the teaching staff of each department closer together for purposes of co-ordination (such as in seminars and study groups), but will also strengthen the links within and between departments to a much greater extent than has hitherto been the case.

The main objective is to attempt to produce young men and women: (1) adequately aware of the underlying scientific and social principles governing agriculture; (2) with a good measure of competence in their grasp of the principles and practices of crop and animal production, together with (3) developing a special interest in one agricultural science, though by no means exclusively specializing in it. Thus we have avoided exclusive specialization even in the final year, unlike many faculties of agriculture in other countries, where the last one or even two years are devoted to one discipline only. Presumably this reflects the needs of those countries for specialist graduates from the first degree; but so far as can be assessed, the Sudan and other developing countries will continue to need graduates in agriculture with a fairly broad background.

The underlying major fault in the general degree in agriculture was that it tried to cover a wide range throughout and in a continuously expanding array of disciplines. Two cracks are bound to develop in any such structure. Members of an expanding staff, each with his own speciality, claim a *de facto* recognition by demanding their individual shares of the teaching time-table, which may well lead to interdepartmental stresses and jealousies. The second crack is that, as a result, the students become bored and bogged down, and often lose any interest that may originally have sparked their imagination towards agriculture. Some years ago a student described our courses as the "stuff that makes the head", consisting of approximately equal portions of skin, bone, cartilage, fat, grey matter and nondescript tissue thrown together in the stew. He concluded: "it tastes queer".

After lengthy and intense discussions, and tense moments of conflict, the staff of this Faculty became convinced that we must follow this new 'group specialization' route, which gives a wide coverage in the first three years, perhaps with a "queer taste". But the final year, handled with imagination and confidence, may well achieve the objectives it is designed to serve. It provides more time for students to think, read and enjoy their respective subjects. The educational commandments quoted by Whitehead in order to guard against mental dry rot, "Do not teach too many subjects" and "what you teach, teach thoroughly", are, we hope, followed in this final

Table 1. YEARLY INTAKE OF STUDENTS FROM THE FACULTY OF SCIENCE INTO THE FACULTY OF AGRICULTURE AND NUMBER OF GRADUATES

	Years													
	1938/39	1939/40	1940/41	1941/42	1942/43	1943/44	1944/45	1945/46	1946/47	1947/48	1948/49	1949/50	1950/51	1951/52
Intake	6	—	—	6	—	5	—	5	—	7	—	—	—	11
Output	—	—	—	—	6	—	—	11	—	5	—	6	—	—
	Years													
	1952/53	1953/54	1954/55	1955/56	1956/57	1957/58	1958/59	1959/60	1960/61	1961/62	1962/63	1963/64	1964/65	1965/66
Intake	—	10	16	10	13	18	17	23	8	5	19	48	44	50
Output	—	8	8	13	10	11	16	15	27	16	10	5	5	—

year. Judging by the appearance of the present final year students, one certainly sees more eyes with sparks in them than previously; but it is still too early to form an objective opinion on whether we have satisfied both the tastes of the student and the needs of the country.

On the occasion of our twenty-fifth anniversary one must ask whether this Faculty is serving the needs of the country efficiently and purposefully. Are we going at the right pace in the right direction, usefully employing all the available manpower resources? Other things being as they are, the Sudan (and indeed Africa) will remain agrarian for some time to come, and graduates in agriculture will continue to hold posts of leadership in the agricultural sector. A Faculty of Agriculture in Africa must serve as a catalyst to speed up the reaction between new ideas and old ones. 'Tradition and Change' has been the theme of many a formal and informal gathering, but the role of the Faculty of Agriculture in this debate has never been brought fully into focus. We are on the march, but one wonders at times of introspection whether, like the march of dons in medieval ceremonial robes at graduation day, our march forward is colourfully academic but of little benefit to the agrarian community. On the whole, universities in Africa have much less contact with their own people than with the West, that has offered to graft a university system of its own pattern on to our African stock. Even if this state of affairs can be relaxedly tolerated in the sphere of chemistry and medicine, it has to be critically scrutinized every now and then—say, at least every twenty-five years—in the domain of agriculture, where science is drawn most closely into contact with the everyday needs of man. In Africa the needs of man are elemental, and so the value of scientific findings for stimulating rural development must logically be scrutinized. Earlier it was said that in the past decade some ninety publications by the Faculty staff have appeared in the international journals. But let us be frank about these publications—they have been effectively transfixed in print, they helped some of us admirably (including myself) to obtain a higher status and income, but on the whole they have remained inert entities rather than activating catalysts. Perhaps here lies one of the main weaknesses of the Faculty *vis-à-vis* the community. The system of the land grant college of the United States has often been suggested as more suited to our needs, involving closer co-ordination between the researcher, the teacher, the planner and the farmer.

Agricultural publications in Africa tend to meet many obstacles before they can even reach the farmer. The concept of the 'agricultural inspector' has prevailed for too long to allow the 'agricultural adviser' elbow-space. Graduates of agriculture have believed that their function was to wield authority (they even until recently dressed in khaki) rather than to teach and extend, and it is significant that courses in extension and management have not yet been introduced in most African Faculties. Agricultural extension, as a systematic and studied plan of campaign, is a new service in the Sudan, and the Faculty has certainly not yet played its full part in leading the minds of the graduate, and hence the policy and decision makers of the future, towards the concept of extension rather than inspection.

Schultz states that: "To produce an abundance of farm products requires that the farmer has access to and has the skill and knowledge to use what science knows about soils, plants, animals and machines. To command farmers to increase production is doomed to failure"³. The role of the Faculty in extension has not yet received sufficient attention, although this is a vital question because Africa now, more than ever before, needs effective extension services.

Africa is an agricultural continent, under-populated except around the main rivers; its peoples depend mainly on subsistence production; and its rains are mainly seasonal and confined to a small portion of the year.

Moigs estimated that 42 per cent of Africa is arid and 22 per cent semi-arid, the remaining 36 per cent receiving adequate precipitation. Twenty-six countries in Africa have large tracts of land classified as dry⁴. Dekker states that only 16 per cent of the African population lives in areas of adequate (over 1,500 mm) rainfall⁵. Of the whole population in Africa (some 120 million people), it is estimated that only about 7.5 per cent live in cities of 200,000 inhabitants, and most of the remainder are agriculturists or nomads depending on a dry-land pattern of subsistence. In the hot arid regions, evapo-transpiration losses are much higher than in cooler climates, and irrigation is essential if the subsistence-level is to be raised as a result of cash cropping and profitable animal production. Modern concepts and technologies in agriculture must be made available to the farmer in order to obviate the elemental hazards and make progressively higher targets possible in production and marketing. Sociology and economics must be harmoniously utilized, as well as the physical and biological sciences, to improve agriculture in Africa.

The role of the graduate in agriculture is paramount, in both planning and execution, yet the Faculties of Agriculture in Africa have not, I think, fully exercised their capabilities and accepted their responsibilities in this context. Let me hasten to add that I do not wish to see the Faculty encroaching on the equally important role of farmer training schools and institutes of agriculture of sub-university level. That would obviously be disastrous and undesirable. Let me further add that a relatively young institution cannot assume greater tasks than it can effectively shoulder without frustration and confusion. But the Faculty, in its natural process of growth, needs to be fully aware of what the community expects from it. What I have said here is neither profound nor new, but is it not often true that the obvious is not said and gradually forgotten? What does the community want us to do? It wants more and better graduates, and it wants more and better planned research, the results of which are more easily taken into the service of the rural community.

The number of Faculties of Agriculture and their graduates in Africa, excluding the United Arab Republic, is pitifully meagre, a total of some 20 or 25, each producing an average of 20 or 25 students a year. The Ten Year Plan of Economic and Social Development of the Republic of the Sudan foresees, on the conservative side, a need for about 1,000 trained administrators, research workers and professional agriculturists by 1972. At the present rate of development this Faculty can only hope, at best, to produce an additional 500 by that date. Thus in terms of numbers we shall be unable (despite our modest achievements) to satisfy the needs of the community, because development is necessarily limited by socio-economical, historical and geographical factors. In terms of quality, we aspire to give the best we can afford. We hope to graduate men and women with a spark of original interest in their profession; but the Sudan and the rest of Africa, not to mention the world, still tends to regard the agricultural 'profession' with guarded zeal in years of economic distress, and with an indifferent yawn when the rains are good. Disastrous fluctuations in yield of crops and pastures in this vast continent are so common that there is little recognition of the value of our service by the peasant and the nomad, a tiresome, vicious circle which will have to be broken in time by education at all levels. The Faculty has a role by developing a sense of pride, enthusiasm and dedication in the profession, both in staff and graduates.

The Faculty of Agriculture has, therefore, plenty of problems ahead, and yet it has not been unsuccessful in its first twenty-five years. It is the oldest of the agricultural Faculties in Africa, excluding one or two in the United Arab Republic and South Africa, and it is keenly aware of its geographical position, in the centre of an arid

zone and at the northern tip of the Gezira Scheme, one of the largest and oldest irrigated schemes in Africa. This Faculty has planned to develop its courses and its researches along lines which are compatible with the typically arid African environment in which it is so aptly situated.

¹ McIlroy, R. J., *Nature*, **179**, 394 (1957).

² Whitehead, A. N., *The Aims of Education and Other Essays* (Benn, London, 1962).

³ Schultz, T. W., *Transforming Traditional Agriculture* (Yale Univ., New Haven, 1964).

⁴ Meigs, P., in *Proc. Seventeenth Intern. Geog. Cong.*, Washington, D.C. (1952).

⁵ Dekker, G., in *Man and Africa*, edit. by Wolstenholme, G., and O'Connor, M. 30 (Churchill, London, 1965).

SOCIAL AND INDUSTRIAL GERONTOLOGY IN GREAT BRITAIN

By C. E. FLEMING

Lecturer in Social and Industrial Medicine, University of Sheffield

Community Reactions to Population Ageing in Great Britain

THE 1949 *Report of the Royal Commission on Population*¹ outlined the demographic situation in Great Britain and presented a challenge. "... the ageing of the population of Great Britain ... has been steadily in progress since about 1891 ... it must be expected to continue in the future ... it represents the delayed effect of changes which we have neither the power nor the desire to reverse, and to this extent the appropriate attitude must be not 'must we try to prevent this?' but 'how can we best adjust ourselves to it, so as to minimize the disadvantages and make the best use of any opportunities which it presents?'" (p. 111). One of the most outstanding features of a Western society is the inadequacy of its investigations of later ageing (especially for the sociological, psychological and related sciences), of applying research findings in social and industrial life and in basic organizations and institutions which would be the foundation for such thought and action. This is particularly true of Great Britain.

As well as carrying out industrial gerontological research (the science of ageing, *not* old age) in the British iron and steel industry and the Sheffield cutlery industry and social gerontological research in the homes of retired cutlery workers, I have been working on an investigation into community and individual reactions to population 'ageing'. The expectation of life at birth has almost doubled here in the past hundred years. This opens up great possibilities for individual and societal development. But, by and large, the complex problems involved have been handled along the lines of the past—with some quantitative improvements. Ideologically and organizationally, we are not equipped to cope with the present and impending situation.

Provision for Old People in Britain

Great Britain is well in advance of most countries in the provision of an almost universal national retirement pension, but this is comparatively low. In Sweden, the retirement pension for a single man is equivalent to some 25–30 per cent of an average working income and about 40 per cent for a married couple. This will shortly be based on the average of the fifteen best-paid years. In Federal Germany, the national retirement pension is linked with the cost of living². In Britain there is a universal National Health Scheme, which is of major benefit for older people, but there is still room for improvement in it; and, unlike France, Britain has no University Department of Geriatric Medicine. Again, in Britain the system of statutory and voluntary services for the old compares very favourably with that of most other countries; but it is still in its infancy and such services have a very limited coverage³.

Psycho-social and Industrial Gerontology in the United States

The three main divisions of research on ageing are: (a) biological; (b) clinical medical; (c) psycho-social and

industrial. The last-mentioned is a very recent development, but it is now widely agreed that it is of major importance to society.

One of the outstanding papers given at the 1963 Copenhagen Congress of the International Gerontological Association was that of Dr. Wilma Donahue, of the University of Michigan. She gave a factual report on "Organization of University Training for Teaching and Research in Psycho-Social Gerontology". It may be mentioned that the United States is demographically 'younger' than Great Britain. Dr. Donahue outlined the American academic situation. University departments of social and industrial gerontology and the like have been established in at least six American universities and fifteen special university units; there are three multi-university organizations for promoting such teaching and research and applied research; there are twenty-six departments of psychology and twenty-two departments of sociology paying special attention to social and industrial gerontology. Yet Dr. Donahue states that in this connexion: "Effort has been too limited and progress too slow".

In the United States there is a national Gerontological Society with a professional and lay membership of more than a thousand. The section concerned with social, psychological and industrial gerontology is one of the largest and most important of the three. In addition to this, a considerable number of the member States have their own gerontological societies. There is a great deal of publicity given to the subject of ageing in all its aspects in American newspapers, journals, books and sound radio and on television programmes. The Federal and State Governments and the private research foundations have made available quite large sums of money for research in all aspects of ageing.

Psycho-social and Industrial Gerontology in Great Britain

What are the comparative university and organizational provisions in Great Britain? I take my statistics from *Scientific Research in British Universities*⁴ and the *Register of Social Research on Old Age*⁵. No British university has a department for social, let alone industrial, gerontology. In all, in seven British universities, research workers are listed as working in some aspects of ageing; but this is chiefly with socio-medical surveys and the like. Less than one university research worker in a thousand in Great Britain is listed as engaged on any research project however remotely connected with ageing or old age. It would seem that all research workers in this field in Britain are self-trained; in the United States the university departments already mentioned have trained several hundreds. Within recent years more publicity has been given in Great Britain to the subject of ageing, but it has been chiefly along the lines of the 'care and comfort of the aged poor'. At long last a Social Science Research Council is to be formed in Great Britain; but no mention is made of its plans in connexion with research in social and industrial gerontology. Governmental and other

funds for research in this important field are extremely small in Great Britain.

Social and Industrial Gerontological Research Publications in the United States

One can refer to the *Classified Bibliography of Gerontology and Geriatrics*⁶. This lists the enormous amount of publication in all fields of research on ageing in the United States, and though this varies in quality, some of it is very good. A large bulk of it has to do with social and industrial gerontology. There are three major publications by the University of Chicago Press⁷⁻⁹. One could go so far as to say that the vast bulk of publication in this sphere comes from the American press, but unfortunately most of this is oriented to the American scene.

How Old is Old?

The population of England and Wales, as shown by the Census of 1961, gives us some statistics on those who could be classified as old in the community. If we take chronological age of sixty as 'old', this gives us 16.3 per cent. One person in six among us is old. But if we take it as fifty years and over, this gives us 31.8 per cent. Almost one person in three in the population of Britain can be described as 'old' by the age-typing commonly used on the factory floor. Again, let us take forty years and over. This gives us 45.4 per cent. The biologists tell us that physical later ageing commences as early as age thirty, and the psychologists that certain aspects of mental ageing are well established at age twenty. We do not know exactly what 'ageing' is; but for most people some pathological, physical or psychological concomitants of later ageing are present by age forty. On this reckoning, about half the population of Britain is old. This is based on the 1961 figures. In 1961 the largest five-year age-group was 10-14 years; the second largest 0-4, but the third largest was for people aged 35-39. In 1961, people between the ages of 35 and 54 could be considered 'middle-aged' (or shall we say in the 'prime of life'?). This twenty-year age grouping made up 27 per cent of the population of the period. It is a 'bulge' moving up the population age structure.

Gerophobia in contemporary Great Britain. There is considerable evidence that in Great Britain, indeed throughout the Western world, we suffer badly from gerophobia—which I have defined as a "morbid or pathological fear of late ageing". I have investigated this in the national Press as well as in my contacts in industry and society.

There is a connexion here with what the American social gerontologists call the 'role-less role' of many retired people in modern Western societies.

Emotional health from middle age; the quality of life at later years. The motto of the American Gerontological Society is: "To add life to years not years to life". A central problem is that of the psychological (mental emotional) well-being of not only older people, but also of people from middle years onwards. This relates with having some role and function in society and, indeed, in industry—where that is desired and possible. Recent publications in psychiatry and social psychiatry have emphasized this (ref. example, ref. 10).

Male Work-force 'Ageing' in the British Iron and Steel Industry and the Cutlery Industry

In Great Britain there is a middle-aged male work-force; more than 50 per cent of the men are aged forty years and more. But these average figures hide the considerable proportion of companies and departments where the male work-force is very much 'older'. I have investigated the age composition of the male work-force of the British iron and steel industry. A half-page report of this, entitled "Survey of the Age Structure of the British

Iron and Steel Industry", was published by the *Manchester Guardian* of November 5, 1957¹². It covered more than 145,000 workers in 96 companies of all sizes in six national areas with large sub-samples for the skilled divisions and for twelve major occupational divisions. A salient finding was the large proportion of departments and companies with considerable numbers of older men—those having from one in five to one in three of their men age fifty-five and over, and with relatively high proportions of other later age groupings. More recently, I carried out an age composition survey of the Sheffield cutlery industry. Some of the male work-forces there have extraordinarily high proportion of men at later years. A 1952 (ref. 11) sampling of the age composition of the Sheffield cutlery and associated products industries gives an indication of this (Table 1).

Table 1

Proportion of male workers in given age grouping (years)	Sample Sheffield cutlery and associated products 1952 (% of total)	Estimate for national male work-force (Ministry of Labour mid-1952) (% of total)
(i) 45 and over	56	38
(ii) 55 " "	34	17
(iii) 65 " "	16	4

Organizational and Institutional Necessities for Contemporary Great Britain

There appear to be four prime necessities if Britain is to take up this challenge of population 'ageing' (or is it 'maturing'?):

(1) A widely membered professional and lay British Gerontological Society, with a section for sociological, psychological and industrial gerontology as well as sections for biology and clinical medicine. In Great Britain there are a British Society for Research on Ageing (chiefly for biologists) and a British Geriatric Society (for the medical profession); but there is no comparable organization for the social sciences.

(2) At least one British University Department of Social and Industrial Gerontology—for pure research, applied research and university and extra-mural teaching. It will take some time to bring into being such a university department. But possibly Her Majesty's Government will help in this respect.

It is suggested that a number of Regius chairs of social and industrial gerontology be established in Britain in the coming quinquennium.

(3) A British Institute of Applied Gerontology, which would bring together concerned academics and leaders from both sides of industry and from the social services, etc.

(4) An Inter-Ministry Committee on Sociological, Psychological and Industrial Gerontology, to help, advise and guide the Government, the public and concerned bodies and to facilitate the acquisition of necessary funds for the furtherance of gerontological research and its application in all relevant aspects of this multi-disciplinary science and, particularly, in its sociological and industrial aspects.

In recent decades new faculties have been established in different universities in Britain, yet faculties of social science are still comparatively few in relation to the numbers of such in the United States; but even newer faculties are required for the universities of the twentieth century. The University of Chicago has established a Faculty (or is it only a Department?) of Human Development. This is a very good title because it enables us to see ageing in life perspective. It is to be hoped that in the coming decades a number of new departments, if not faculties, of this nature will be established in Great Britain.

The sixth congress of the International Gerontological Association is to be held in Vienna during June 26–July

2, 1966. It would be highly desirable if Great Britain could form a nucleus of a National Gerontological Society with this missing third section for a sociological, psychological and industrial gerontology. Great Britain seems to be one of the few modern States which has not brought about this organizational development. I should like to contact all those interested in the proposals. Research is important, applied research is even more important; but for us now yet more important is an appropriate national organization. My address is: 26 Dransfield Road, Sheffield 10.

It is hoped to convene a meeting shortly in Birmingham, as a central city, to discuss this more thoroughly and to bring such an organization into being. I should appreciate comments on this article, offers of support and, indeed, offers of funds; a start could be made with a few thousand pounds, and this should yield a considerable return to the nation. This has been a very brief sketch of the situation, but I appreciate that the whole matter must be discussed by people from different university disci-

plines, from industry and the Trade Unions, and from Government departments, etc.

¹ *Report of the Royal Commission on Population* (Cmd. 7695, H.M.S.O., London, 1949).

² *Age and Employment* (O.E.C.D., Paris, 1962).

³ Townsend, P., and Wedderburn, Dorothy, *The Aged in the Welfare State* (G. Bell and Sons, Ltd., 1965).

⁴ *Scientific Research in British Universities, 1963-64* (D.S.I.R. and British Council, H.M.S.O., 1964).

⁵ *Register of Social Research on Old Age, 1960-63* (National Corporation for the Care of Old People).

⁶ *Classified Bibliography of Gerontology and Geriatrics*, Suppl. I, 1945-55; Suppl. II, 1956-61; edit. by Schock, N. (Stanford Univ. Press, 1951).

⁷ *Handbook of Social Gerontology: Societal Aspects of Ageing*, edit. by Tibbitt, E. (Univ. Chicago Press, 1960).

⁸ *Ageing in Western Societies - A Comparative Survey*, edit. by Burgess, E. W. (Univ. Chicago Press, 1960).

⁹ *Handbook of Ageing and the Individual: Psychological and Biological Aspects*, edit. by Birren, J. E. (Univ. Chicago Press, 1959).

¹⁰ Post, F., *The Clinical Psychiatry of Later Life* (Pergamon Press, 1965).

¹¹ Fleming, C. E., *Vita Humana*, 694, 177 (1963).

¹² Fleming, C. E., "The Middle-aged Bulge. A Survey of Age Structure in the Iron and Steel Industry", in *The Manchester Guardian* (now *The Guardian*), November 5, p. 9 (1957).

OBITUARIES

Prof. R. Spärck

WHEN Ragnar Spärck died on June 20 at the age of sixty-eight his passing left a gap in many circles, created by his central position in Danish zoology, his wide intellectual interests and his extraordinary personality. Few men in his profession have filled so many posts and performed such various duties.

For a period of almost forty years Spärck taught zoology in the University of Copenhagen, from 1937 as professor, occupying the old tradition-rich chair of systematic zoology in the University. The general acknowledgment in Denmark of taxonomy as a necessary background for more recent trends in zoology is largely due to his education of a whole generation of zoologists. Among the students he was equally popular as an inspiring teacher and a cheerful mid-point during social events and on excursions. His elegant appearance as University opponent at public defences of more than fifty theses was widely appreciated, even by the candidates.

Already as a student, Spärck had acquired a position at the University Zoological Museum. In the early 'twenties he put forward a bold plan for a new museum building; but it was not until he became professor and head of the Museum Council that he could put authority behind his plans. The Second World War postponed the erection of new and badly needed quarters, but in 1960 the Students' Laboratory and Study Collection was finished and three years later the large staff and rich collections of the Museum moved into an adjacent, well-planned building. He also played an active part in the consequent erection of a third, now almost finished, spacious building for comparative anatomy and experimental zoology.

Spärck's contribution to science falls mainly within two fields, marine biology and history. As a pupil of C. G. Johs. Petersen, he carried on his investigations of bottom communities, mainly in areas outside of Denmark. He wrote his doctoral thesis on the biology of the European oyster and served for the rest of his life as an adviser for the oyster cultivation in the Limfjord. Only to a lesser extent did he join marine expeditions (for example, in the Mediterranean during the *Dana* Expedition, in East Greenland, and in South Africa during the *Galathea* Expedition). However, both before and after the War he was very active behind a long series of Danish expeditions to East Greenland, the Iranian Gulf, West Africa, and not least the *Galathea* Deep-Sea Expedition. He was vice-president of the *Galathea* Committee and acted as the co-ordinator on the 'home-front', an extremely

important but not widely recognized position during accomplishment of large expeditions.

During his later years Spärck devoted much of his energy to the history of natural sciences in Denmark. A profound interest in the work and conditions of the predecessors, an extraordinary memory for details and a superior view for general lines was the basis for his important authorship of books and papers on historical subjects, for example, on the Zoological Museum and zoology teaching during 300 years, on early Danish expeditions, on the Natural History Society, and on Japetus Steenstrup, the leading Danish zoologist of the last century, with whom Spärck had so much in common.

Spärck's work for nature conservancy was legendary. He was a member of the Conservancy Council for almost forty years, the last sixteen years serving as a chairman. He knew his native country as few others and had an ability to make odds and ends meet in the not always easy negotiations with authorities and proprietors. He was also a leading force behind the Danish game sanctuaries, and, thanks to his initiative, a long series of papers on game biology have been published.

Spärck was well known abroad. He liked travelling and as a result of his broad interests and attainments he attended congresses and meetings within many fields of zoology and marine biology, museum work, nature conservancy and zoological gardens. He was a member of the International Committee of Zoological Congresses and the president of the Copenhagen Congress in 1953. He was also for six years president of the zoological section of the International Union of Biological Sciences and was a member of several other international committees (for example, on Zoological Nomenclature, the Zoological Record and Nature Conservancy).

TORBEN WOLFF

Mr. K. E. B. Jay, M.B.E.

MR. K. E. B. JAY, who was well known for his popular books on atomic energy, died at his home in East Hendred, Berkshire, on August 3, at the age of fifty-five. Kenneth Edmund Brian Jay was the son of Joseph Jay, a company secretary, of Amersham. From 1923 until 1929 he was educated at University College School, London, and then went on to University College, where he read physics. After only a year, his studies were interrupted by a long and painful illness, leaving him with a serious physical disability, which remained for the rest of his life. Jay resumed his studies in 1935, and in 1938 he graduated

with first-class honours in physics, and was awarded the Granville scholarship. After taking his degree, he began research on electron diffraction under Prof. E. N. da C. Andrade, who was much impressed by his character and ability.

At the beginning of the Second World War, Jay joined the Air Ministry Telecommunications Research Establishment (later the Royal Radar Establishment). It was here that his abilities as a writer first came to notice, and after a short spell of experimental work he was attached to the chief superintendent, Mr. A. P. Rowe, to prepare the establishment progress report and other documents. Rowe attached great importance to presenting technical information to the layman in an intelligible form, and he would not tolerate jargon; his views undoubtedly left their mark on Jay's future career. When the Telecommunications Research Establishment moved to Malvern, Jay set up an information room, where all forms of graphical presentation were used to bring out the contribution of radar to the successful conduct of the War. During this period Jay did much to strengthen the chain of communication which was so successfully established between the scientists in the Telecommunications Research Establishment and the serving officers in the Royal Air Force.

In 1945 Jay went to the Cabinet Office to work on the *Official History of the War*. There he conducted the researches and composed the narrative relating to the history of radar, which was incorporated in the volume on the *Design and Development of Weapons*.

Three years later, Jay joined the Atomic Energy Research Establishment at Harwell as information officer, and in this post he laid the foundation of the present information service. However, he retained his interest in the more popular presentation of scientific information, and when the declassification of atomic information began to gather momentum in 1951, he put forward a proposal for a book on the work of the Atomic Energy Research Establishment. This was published in 1952 under the title *Harwell, the British Atomic Energy Research Establishment*; it was the first serious description of post-war nuclear research in the United Kingdom, and was an immediate success. This success led to Jay's transfer to a full-time, scientific-writing post, and in the next few years there followed four more books: *Britain's Atomic Factories* (1954), *Atomic Energy at Harwell* (1955), *Calder Hall* (1956) and *Nuclear Power Today and Tomorrow* (1961). Although the visits to distant establishments were a serious physical burden, he perhaps most enjoyed writing the books on the factories and Calder Hall, where an account of the scientific principles involved was combined with a fascinating story of engineering initiative and enterprise.

In addition to these major works, Jay was responsible for many years for writing the chapters dealing with research in the Atomic Energy Authority's annual report, and for a series of progress reports on activities at the Atomic Energy Research Establishment prepared for internal use in the Authority. He also wrote an introductory chapter to the first volume of Mrs. M. Gowing's official history of the United Kingdom atomic energy project, *Britain and Atomic Energy, 1935-45*.

In all his writing Jay took immense pains both to keep in mind the needs of his readers for a clear and simple exposition, and also to preserve scientific accuracy. In this way he was able to achieve the objectives he set himself, and at the same time to retain fully the confidence of the scientists about whose work he wrote: nearly all his writing was based on first-hand discussion with them.

A catalogue of his written work, however, cannot adequately represent the impact which Jay made on Harwell, and the Atomic Energy Authority. He was an excellent lecturer both in the history of atomic energy and on the presentation of scientific information; he was an active worker for standardization of nomenclature, and

was chairman of a British Standard Institution sub-committee which produced their *Glossary of Terms used in Nuclear Science*. But perhaps most important, in the face of physical adversity he showed a cheerfulness and determination which were an example to all, and with his warm personality, he inspired the affection as well as the professional admiration of all who knew him. His award of the M.B.E. in 1956 was universally acclaimed.

Outside his office, as at work, Jay did not allow his disability to limit his activities. He was chairman of the Atomic Energy Research Establishment's Amateur Radio Club, an active worker for his parish church, and a member of the Science Writers' Guild. He was also author of some illustrated children's books on science, including *British Nuclear Reactors* (1960).

Science is becoming more complex, more expensive and more difficult to understand; at the same time, its understanding by laymen in industry and Government must increase if science is to be efficiently applied. Kenneth Jay had an outstanding ability to bridge this gap in communication between scientists and laymen; there is a great need for many more like him.

R. M. FISHENDEN

Dr. Duncan A. MacInnes

DR. DUNCAN A. MACINNES, member emeritus of the Rockefeller University, died on September 23, in Hanover, New Hampshire. He was eighty years of age and had been active in scientific research until this past summer.

Dr. MacInnes had been affiliated with the Rockefeller University since 1926 and had been a member emeritus since 1950. During his career, he distinguished himself in teaching and research in several universities and as a civilian with the Office of Scientific Research and Development during the Second World War.

Many honours were conferred on Dr. MacInnes. He received the Nichols Medal in 1942, awarded by the American Chemical Society to stimulate original research. In 1948 he received the Acheson Medal, awarded every two years by the Electrochemical Society. He was also honoured with the Presidential Certificate of Merit in 1948.

Dr. MacInnes's field of research had been largely directed towards the study of electrolytes in aqueous solution. As emeritus member, he had continued to conduct laboratory research.

Born in Salt Lake City, Utah, March 31, 1885, he received his B.S. degree from the University of Utah in 1907. In 1909 he received his M.S. degree from the University of Illinois and his Ph.D. degree in physical chemistry in 1911. During the next six years he was successively an instructor and an associate in chemistry at the University of Illinois. During 1917-26 Dr. MacInnes carried out physical chemistry research at the Massachusetts Institute of Technology, first as an assistant and then as an associate professor. In 1926 he became an associate member of the Rockefeller Institute (now the Rockefeller University), in 1940 a member, and member emeritus in 1950.

He was a member of the National Academy of Sciences, the American Association for the Advancement of Science, the American Chemical Society, the Electrochemical Society (for which he served as president during 1935-37), the American Philosophical Society, and the Harvey Society.

✓ Prof. Hermann Staudinger

HERMANN STAUDINGER, whose death occurred on September 8, at the age of eighty-four, was a pioneer in the study of macromolecules and one of the founders of the subject of polymer chemistry. Born at Worms (Rhein) on March 23, 1881, he was educated at Halle, Darmstadt and Munich. In 1907 he became a lecturer in

chemistry at the University of Strasbourg, and after one year there he was appointed as an extra professor at Karlsruhe Technical High School (1908-12). After holding an ordinary professorship at the Federal Technical School, Zurich (1912-26), he was appointed to a professorship at Freiburg im Breisgau in 1926—a post which he held until he retired on reaching the age of seventy.

In his early years, Staudinger worked in the field of classical organic chemistry and with its well-defined substances which could be fully characterized by standard methods. The outcome of this work was the well-known monograph on *The Chemistry of the Ketenes*. It was not until the early 1920's that he selected as his field—at that time scarcely thought to be worth the consideration of an organic chemist of his reputation—the study of natural organic substances of high molecular weight. In this area he performed, with numerous collaborators, an enormous amount of experimental work on materials varying from natural materials, such as starch and cellulose, to synthetic substances, such as polystyrene and polyaldehydes, applying purely organic methods. He demonstrated that large linear molecules could be formed from small molecules by ordinary chemical reactions and, what is more, maintain their individuality even when they were subjected to chemical modification. Through this work he built up information for the foundation of the new branch of macromolecular chemistry. In one of his first publications he deplored the prevailing tendency to formulate polymeric substances as association compounds held together by "partial valencies" and he proposed the chain formulations of polystyrene and polyformaldehyde which are the ones accepted at the present time. He also advocated the long-chain structure of rubber and attributed the colloidal properties of these substances entirely to the size of their primary valence molecules. His chemical studies left no doubt as to the linear chain-like structures of these substances which he called 'macromolecules'.

In a paper published in 1926, Staudinger remarked that "many of the properties of synthetic high polymers are so similar to those of natural origin that they may be used as substitutes for them. It is not improbable that sooner or later a way will be discovered to prepare artificial fibres from synthetic high molecular-weight products because the strength and elasticity of natural fibres depend exclusively on their macromolecular structure". How brilliantly this prediction was fulfilled in his lifetime is commonplace knowledge. Staudinger's views were not

widely accepted at once. Many of his colleagues in high academic positions remained for a long time unconvinced. They did not approve either of his methods and results or the manner in which he elevated his own working field to a "new branch of organic chemistry". Many will recall the numerous occasions in the 1920's and early 1930's when the history of polymer science was made in the eloquent clashes between Staudinger—who relentlessly championed the macromolecular hypothesis—and those who believed that polymers were aggregates of small molecules. One of his best-known contributions was the establishment of a relationship between the viscosity of a solution of a high polymer and its molecular weight. As with other aspects of his work, much doubt and controversy centred on the so-called viscosity law which Staudinger proposed in 1930. The difficulties, however, were soon resolved, and the utility of the later modified relationship between viscosity and molecular weight played an important part in the determination of molecular weights and in the development of the physical chemistry of high polymers.

Staudinger's importance in the development of polymer chemistry is now universally accepted. His prodigious output of research and publications and his never-failing enthusiasm in a field in which he had to fight at every step to establish new concepts or justify new methods places him first when it comes to the question of who contributed the greatest number of facts and figures to the new macromolecular chemistry.

In addition to his outstanding scientific achievements, Staudinger was also a great teacher, who succeeded in transmitting his new ideas and creative thinking to the very large number of students always working in his laboratory. These students carried with them into academic life and industry the convictions of their master, and a generation of leaders in polymer science emerged.

Staudinger's many honours included the Nobel Prize for Chemistry in 1953; honorary degrees of Karlsruhe (1950), Mainz (1951), Salamanca (1954), Zurich (1955) and Strasbourg (1959); and honorary membership of numerous academies of science and scientific societies. He was awarded the Leblanc Medal of the French Chemical Society (1931), Cannizzaro Prize, Rome (1933), and Goldene Ehren-Medaille des Vereens der Textil-chemiker (1962). He was made an honorary citizen of Freiburg in 1955.

C. E. H. BAWN

NEWS and VIEWS

Royal Society of Edinburgh: Elections

At the annual statutory meeting of the Royal Society of Edinburgh, held on October 25, the following were elected to the Council of the Society for the 183rd session: *President*, Prof. J. N. Davidson; *Vice-Presidents*, Prof. C. F. Davidson, Dr. M. Ritchie, Prof. J. Allen, Dr. G. H. Mitchell, Prof. A. E. Ritchie, Prof. C. H. Waddington; *General Secretary*, Prof. N. Feather; *Secretaries to Ordinary Meetings*, Dr. A. F. Brown, Prof. W. L. Weipers; *Treasurer*, Dr. J. R. Peddie; *Curator of Library and Museum*, Dr. R. Schlapp; *Councillors*, The Right Hon. Lord Balerno of Currie, Dr. J. M. Jackson, Prof. I. N. Sneddon, Prof. P. E. Weatherley, Dr. Neil Campbell, Prof. P. L. Pauson, Prof. J. R. Raeburn, Prof. J. D. Robertson, Prof. P. W. Brian, Dr. H. E. Butler, Sir David Lowe, Dr. D. G. Sopwith.

Metallurgy in the University of Oxford:

Prof. W. Hume-Rothery, O.B.E., F.R.S.

PROF. W. HUME-ROTHERY will retire from the Isaac Wolfson chair of metallurgy in the University of Oxford

on September 30, 1966. His long and distinguished scientific career has been spent almost entirely in Oxford, and the date will be a sad occasion for his friends and colleagues. Prof. Hume-Rothery intended to make his career in the Army (and would surely have become a general!), but a serious illness as a young man left him totally deaf, and he turned to science instead. After taking an Oxford first in chemistry, he worked for his Ph.D. at the Royal School of Mines, and then returned to work in Oxford in 1926. Using his chemical background to great advantage, he began there the work on the theory of alloy formation for which he is so renowned, and the Oxford school of alloy chemistry eventually grew into the present Department of Metallurgy. In 1957 he became the first professor of metallurgy in Oxford, and the choice was so unquestionable that the University dispensed with the formality of an election. Throughout his career, "H. R." has played a leading part in associating metallurgy with solid-state physics and chemistry. He pioneered the scientific approach to metallurgy, not only by his own research, but also by his brilliant expositions of basic theory in books which

have been used by generations of students and which have been translated into many languages. His early work was accomplished with slender resources in cramped accommodation, but led to widely acclaimed generalizations such as the size factor rule and the existence of electron compounds. He was elected a Fellow of the Royal Society in 1937, and has received awards and honours from scientific societies in Britain, the United States, The Netherlands and Italy, including the Platinum Medal of the Institute of Metals in 1949.

Metallurgy at Oxford has always been associated with the name of Hume-Rothery, and without his enthusiasm it would never have attained the status of an independent Final Honour School. His interests in teaching no less than in research have been fully engaged since the Second World War, and he hands on to his successor a Department which already enjoys a high reputation. All will wish "H. R." a long and happy retirement, but it will be surprising if he does not immediately begin work on another book.

Prof. P. B. Hirsch, F.R.S.

DR. P. B. HIRSCH, reader in physics at the Cavendish Laboratory, Cambridge, has been elected to the Isaac Wolfson professorship of metallurgy in the University of Oxford, as from October 1, 1966, in succession to Prof. W. Hume-Rothery. He was born in 1925, and graduated at Cambridge in 1946, with a first-class honours degree in physics. He then joined the late J. N. Kellar as a research student in the Cavendish Laboratory, and together they developed an X-ray microbeam technique for the study of the microstructure of deformed metals, under the direction of Dr. W. H. Taylor. In 1950, Dr. Hirsch initiated at the Cavendish Laboratory, on behalf of the National Coal Board, an X-ray diffraction study of the structure of coals and of the carbonization process. From about 1955 onwards he turned his attention again to problems in plasticity, work-hardening and defects in metals. He was responsible for developing, with Dr. M. J. Whelan, the electron microscope transmission technique for the study of defects in crystals. This work led in 1956 to the first direct observations of the movement of dislocations in metal foils. During the next few years Dr. Hirsch and his colleagues developed the experimental techniques and theories of image contrast which make it possible to determine from electron micrographs the detailed nature of defects. During this period the collaboration of Hirsch, Howie and Whelan was particularly successful. The technique was also applied to a number of problems, including dislocation interactions, defects in quenched and irradiated metals, dislocation distributions in metal crystals deformed in tension and fatigue, and recrystallization. This work was a major and fruitful advance in metal physics and it established the electron transmission microscope technique as a powerful tool in metallurgy. Dr. Hirsch also made outstanding contributions to the theory of dislocations and work hardening. It is recognized internationally that the group which has grown around him in the Cavendish is a leading one in the field of metal physics. Dr. Hirsch was elected a Fellow of Christ's College, Cambridge, in 1960, a Fellow of the Royal Society in 1963, and was awarded the Rosenhain Medal of the Institute of Metals in 1961, and the C. V. Boys Prize of the Institute of Physics and the Physical Society in 1962. When he takes up his new appointment he will be responsible for the proposed expansion of metallurgy at Oxford and for broadening the interests of the Department towards materials science.

Geography in the University of Reading :

Prof. A. Austin Miller

PROF. A. AUSTIN MILLER retired from the chair of geography in the University of Reading on September 30 after nearly forty years in the Department of Geography there. A graduate in geology of University College,

London (B.Sc. first-class honours 1922, M.Sc. 1925, D.Sc. 1940), he was appointed lecturer in geography at Reading in 1926 and professor of geography in 1943. His work has dealt mainly with the physical aspects of geography and in 1931 he established an international reputation for himself with the publication of *Climatology* which, with due revision through nine editions and several translations, remains a standard text-book in 1965. In addition to many papers, his publications also include *The Skin of the Earth* (1953) and (with M. Parry) *Everyday Meteorology* (1958). For his work in geomorphology and climatology he received the Murchison Award of the Royal Geographical Society in 1963. A founder-member of the Institute of British Geographers, he became in due course its youngest president in 1946-48, and later was president of Section E of the British Association for the Advancement of Science (1956) and of the Geographical Association (1963). He has served on the Council of the Royal Geographical Society and was advisory editor on geography to the new *Chambers' Encyclopaedia*, 1945-49. In 1958 he was elected Fellow of University College, London, the same year in which he was visiting professor at the Universities of Indiana and British Columbia. He also held offices at meetings of the International Geographical Union at Lisbon 1949, Washington 1952, Rio de Janeiro 1956, and Stockholm 1960.

Prof. T. G. Miller

MR. T. G. MILLER, who has just taken over the chair of geography at the University of Reading from Prof. A. Austin Miller, is a geologist by training. Demobilized after the Second World War with the rank of major, he then graduated in geology at Cambridge and was appointed University demonstrator in geology and Fellow of Jesus College, a post that he occupied until 1953, when he transferred to the University College of North Staffordshire (now University of Keele). Prof. Miller's interests have been in the realms of physical geography—exemplified in his *Geology and Scenery of Great Britain*—in Carboniferous palaeontology and in stratigraphy; while in the Territorial Army he has been concerned with terrain studies from a military point of view. He thus continues at Reading the wide interest in the physical aspects of geography that Prof. Austin Miller has cultivated for almost forty years.

Assistant Chief of the National Bureau of Standards Textile and Apparel Technology Center :

Dr. F. C. Brenner

DR. F. CECIL BRENNER has been appointed assistant chief of the Textile and Apparel Technology Center of the National Bureau of Standards Institute for Applied Technology. He will be responsible for developing new research projects and managing existing ones. The programme of research of the Textile and Apparel Center is designed specifically to meet the needs of industry. Dr. Brenner will maintain close contact with technical and trade associations and educational institutions as well as with industry, enabling him to carry on research useful to the industry. He is particularly well qualified for his new position as he has conducted research on fabric properties for end-use performance. He has also investigated the effect of textile finishing processes on the mechanical behaviour of fabrics which determines their stiffness, drape and wrinkling performance. Recently, Dr. Brenner was a member of the Chemstrand Research Center in Durham, North Carolina, and earlier of Johnson and Johnson Co. He gained his Ph.D. degree in polymer chemistry at the Polytechnic Institute of Brooklyn, and was appointed an instructor at the Institute, and later an assistant professor of physical chemistry at Vanderbilt University. Dr. Brenner is a member of the American Chemical Society, Fiber Society, Inc., and the American Association of Textile Chemists and Colorists.

Social Science Research Council

IN a written answer in the House of Commons on November 2, Mr. A. Crosland, Secretary of State for Education and Science, stated that further to the appointment of Dr. M. Young as chairman of the Social Science Research Council, the following members of the Council had also been appointed: W. O. C. Adamson, A. L. C. Bullock, Prof. J. Drever, Prof. R. W. Firth, Sir William O. Hart, Prof. Marie Jahoda, Lord James of Rusholme, Prof. R. G. Lipsey, Prof. W. J. M. Mackenzie, Prof. T. H. Marshall, Prof. C. A. Moser, L. Murray, Prof. R. M. Titmuss and G. D. N. Worswick. A draft of the Charter to be granted to the Council has been approved by Her Majesty in Council, and a draft Order specifying the objects of the new body and declaring it to be a Research Council for purposes of the Science and Technology Act, 1965, has been laid before Parliament.

Desalination of Sea-water

IN a written answer in the House of Commons on October 27, Mr. F. Cousins, the Minister of Technology, stated that the Atomic Energy Authority and industry had completed a design for a plant to produce 30 million gallons of fresh water a day from sea-water. Further design work had been done, particularly in respect of dual-purpose plants for producing electric power and fresh water from nuclear reactors, and economic studies on these plants had been made in some detail. The great contribution already made by Britain had been recognized at the recent international conference on desalination in Washington. More than two-thirds of all land-based desalination capacity in the world, including the biggest sea-water distillation plant at Kuwait, with a capacity of 1.4 million gallons a day, was built by British industry. Much interest was also shown at the Washington conference in Britain's design studies for combined power and distillation plants. In conjunction with Weir Westgarth and the nuclear engineering industry, the Authority had done much to improve the steam cycle of these dual-purpose plants. The flash-distillation process remained the most promising process so far available, and every effort was being made to improve its technical and economic efficiency. Work had also been started on alternative processes, such as reverse osmosis and electrodialysis, which might become increasingly important.

Research and Development in the Building and Civil Engineering Industries

IN a written answer in the House of Commons on November 1, Mr. C. Pannell, the Minister of Public Building and Works, stated that during the past 9 months the Directorate General of Research and Development had made a comprehensive review of research and development related to the building and civil engineering industries. It had commissioned from universities some research projects relevant to the construction industry and was studying proposals for increasing the resources available for building research and the dissemination of technical information in the industry. Design work had started on a £1 million pre-production programme for the Department's steel-framed system, Nenk. Further progress had been made with dimensional co-ordination and a start had been made on the change to the metric system. The Department had continued its work on winter building and had issued a film on techniques suitable for smaller builders. It continued to collect, analyse and disseminate statistical information, and to advise on the construction industries in connexion with the Government's economic planning. The Ministry was represented on the Economic Development Committees for the civil engineering and building industries by members of the Directorate.

Solway Firth Barrage

IN answer to a question in the House of Commons on November 3, Mr. W. Ross, the Secretary of State for Scotland, said that, as a result of the moratorium on capital expenditure announced in July, it was not possible to proceed immediately with the full-scale feasibility study of the Solway Firth Barrage. However, arrangements had been made for Messrs. Babbie, Shaw and Morton to start a preliminary study, costing some £10,000-£15,000, on the quantity and quality of water that would be made available by a barrage.

Second Conference on the Countryside in 1970

WHEN the Duke of Edinburgh inspected National Nature Week in May 1963, what impressed him most was that so much goodwill and so many bodies interested in the care of the countryside were meeting with so little success in saving it. This he attributed to a lack of thorough enquiry and clear thinking which might enable all concerned to work in harmony and produce early and worth-while results. At his initiation, a study conference with that object was convened. It was felt that the countryside was in grave danger of irreparable damage by 1970. The first study conference in November 1963 brought the parties together, obtained fairly widespread agreement and helped towards resolving certain special problems such as the misuse of toxic chemicals on the land (*Nature*, 201, 958; 1964). It could not, however, tackle many deeper and more complex problems which needed more time and manpower.

This task has now been undertaken by twelve study groups and a number of other combined operations, and the results came before the second study conference opened by the Duke of Edinburgh on November 10 at the Royal Society of Arts. That day and the next were devoted to intensive examination by those responsible for preparing the conference documents with the view of bringing them together as a whole and defining ways and means of obtaining positive action. At this stage, Lord Strang, chairman of the National Parks Commission, and Lord Robens, chairman of the National Coal Board, took the chair in turn. On November 12 the final reports went to a larger plenary meeting at Fishmongers' Hall, at which Mr. F. Willey, the Minister of Land and Natural Resources, made a policy statement on behalf of the Government. Spokesmen of other national interests included Mr. John Davies for the Confederation of British Industries, Prof. C. Buchanan on "Road Use in the Countryside", Mr. Jack Longland on education, Mr. W. Deedes on "Information", and Mrs. G. L. S. Pike of the National Federation of Women's Institutes, speaking from the point of view of the country dweller.

The conference was sponsored by the Royal Society of Arts, the Nature Conservancy and the Council for Nature.

Central Building Research Institute, Roorkee, India

IT is always a matter of interest to students of building technology to learn, if only for comparative purposes, of the progress of research and of various lines of enquiry pursued in this field by organizations similar to the British Building Research Station, which have been established in other countries. India is no exception to this, as is clear from the annual report of the Central Building Research Institute, 1964-65 (India: Council of Scientific and Industrial Research. Pp. ix + 83. Roorkee (U.P.): Central Building Research Institute, 1965). In his foreword, G. Pande, chairman of the Executive Council of the Central Building Research Institute, makes these points: "... the trend of research in the Institute has been project oriented and greater emphasis has been given during the year on projects which have immediate impact on the building industry by way of increasing

productivity and reducing costs, with the maximum utilization of indigenous materials and improved techniques. . . . The Institute has also made good progress in the field of international co-operation, particularly in the exchange of research programmes and research results which will help in improving the quality of research work here". Some idea of the scope of the work of the Institute is conveyed by the titles of the sections into which this report is divided: building materials; soil engineering; structural engineering; efficiency of buildings; building processes, plant and productivity; architecture; information and survey; extension; and administration. In the introduction to the report, the director of the Institute, Dinesh Mohan, mentions several items of importance as affecting the progress of its work during the year under review; among these are: the installation of an IBM digital computer and the starting of a Computing Centre; an extension service, the opening of small liaison cells in other parts of the country, a start being made at Calcutta and Bhopal; the new laboratories of the Efficiency of Buildings Division; the difficulty of recruiting staff for vacant scientific posts, especially in the engineering disciplines, which has hampered progress in a number of research projects; and the popularity of the *Building Digests* issued by the Institute at regular monthly intervals. The Central Building Research Institute has now completed twelve years of its existence in the service of the building industry, and its close ties with international organizations, through the International Council of Building Research and Documentation, will ensure that its work in future will proceed, as in the past, on most progressive lines.

Chemical Industries Association, Ltd.

In 1964, the Association of British Chemical Manufacturers and the Association of Chemical and Allied Employers decided that it was vital, in view of the increased involvement of Government, irrespective of party, in industrial affairs, that their two organizations should merge to form a new and more powerful body. This merger has now been successfully concluded with the formation of the Chemical Industries Association, Ltd., which formally comes into being on January 1, 1966. The officers of the new Association will be: *President*, P. C. Allen; *Vice-Presidents*, Lord Netherthorpe and F. L. Waring; *Director General*, J. C. McEntee; *Director of Trade Affairs*, H. W. Vallender; *Director of Industrial Relations*, J. T. Collins; and *General Secretary*, A. J. Chant. Further information can be obtained from the Association of British Chemical Manufacturers, Cecil Chambers, 86 Strand, London, W.C.2.

Food Poisoning

Food poisoning, the subject of a symposium in *The Practitioner*, is a problem of unknown extent (195, No. 1165; July 1965). As Dr. G. E. Breen points out in the opening article, "it is probable that the condition is very much under-reported", and, in his opinion, unreported cases may amount to nearly 50 per cent of the overall incidence. In noting that there were 3,919 cases of human salmonellosis proved in England and Wales in 1963, Dr. J. Taylor comments that "this figure is probably about one-third of the actual number, as so many cases are not notified". In the United States it has been estimated that there are 2 million cases of salmonellosis every year, but whatever the precise figures, the incidence is much too large and leads to a great deal of personal inconvenience and loss of working time. Much of it could be prevented if only housewives, shopkeepers and restaurant keepers would observe certain simple rules of hygiene; however, while prevention is the ideal way of tackling the problem, there will always be a modicum of cases, no matter how efficient preventive measures may

be. It is in the detection of these that the general practitioner occupies the key position. The ultimate responsibility for coping with any 'incident' rests on the medical officer of health, but he is obviously at a disadvantage unless he is notified of a case at the earliest possible moment by the family doctor. The symposium has been prepared in order to provide an up-to-date, authoritative account of what can be done to control this unnecessary incidence of disease.

Care of Laboratory Animals

PUBLICATIONS Nos. 1284 and 1285, issued by the National Research Council of the National Academy of Sciences, 2101 Constitution Avenue, Washington, D.C., will interest all who are concerned with the maintenance of laboratory animals and their use in experimental work (No. 1284: *Laboratory Animals: Animal Medicine*. Pp. vii + 35. 1.25 dollars. No. 1285: *Laboratory Animals: Training*. Pp. v + 57. 1.50 dollars. Washington, D.C.: National Academy of Sciences-National Research Council, 1965). They outline the plans put forward by the Institute of Laboratory Animal Resources for graduate training in laboratory animal medicine. College programmes now providing this training are examined. For this purpose, eighteen veterinary schools have been visited. Other publications in the series on *Laboratory Animals* are entitled: *The Shipment of Laboratory Animals* (1.50 dollars); *A Directory of Sources of Laboratory Animals, Equipment and Service* (2.00 dollars); *Recommended Minimum Standards for the Shipment of Laboratory Primates* (1.50 dollars). These publications express the welcome recognition in the United States of the fact, long recognized in Britain, that animal care is a profession and a discipline, and that those who look after laboratory animals should be fully trained for their work. They need to understand the biology of the animals under their care, their sanitation, hygiene, housing, shipment and the equipment needed for this. Furthermore, they should be able to ensure their safety and deal with such matters as administration, management and the keeping of records.

Veterinary Non-proprietary Names

THE British Veterinary Codex Revision Committee has adopted the following non-proprietary names for the veterinary substances indicated:

Non-proprietary name	Other names
Oxyclozanide	3,5,6,3',5'-pentachloro-2,2'-dihydroxybenzanilide; I.C.I. 46,638.
Pyrimithate	2-dimethylamino-6-methylpyrimid-4-yl diethyl phosphorothionate; I.C.I. 29,661.
Tetramisole	2,3,5,6-tetrahydro-6-phenylimidazo-[2,1-b]thiazole; I.C.I. 50,627

The non-proprietary names are reported to be free from conflict with trade marks registered in Great Britain and Northern Ireland, and these names, or names resembling these names, will not be registered as trade marks for pharmaceutical products or drugs in those countries. Some of the names, other than the chemical names, appearing in the second column here are registered trade marks. The adoption of a non-proprietary name does not necessarily imply that the British Veterinary Codex Revision Committee recommends the use of the substance in veterinary medicine or that the substance will be included in the *British Veterinary Codex*, although if a substance is included, it is intended that the non-proprietary name shall be the title of the monograph. The British Veterinary Codex Revision Committee has undertaken, at the request of the Association of the British Pharmaceutical Industry, to provide non-proprietary names for veterinary products, and all requests from manufacturers and other interested persons for the provision of such names should be addressed to the Secretary, British Veterinary Codex Revision Committee, Pharmaceutical Society of Great Britain, 17 Bloomsbury Square, London, W.C.1.

Mitral Valve Disease

A SYMPOSIUM on advances in diagnosis and treatment of mitral valve disease was held at a regular meeting of the Section of Biological and Medical Sciences at the New York Academy of Sciences on October 14, 1963 (*Ann. N.Y. Acad. Sci.*, 118, Article 10: *Mitral Valve Disease*, by J. D. Baker and 11 other authors. Pp. 469-536. New York: New York Academy of Sciences, 1965). The moderator was G. H. Humphreys, who summed up the symposium as follows: "Disease of the mitral valve, usually of rheumatic but occasionally congenital origin, continues to be a major cause of disability in human beings in most countries of the world. It is interesting that the same valve is a frequent cause of heart failure in dogs, though the etiology appears to be different. It is of importance, therefore, that the search for better understanding of the pathological physiology, and improved diagnostic methods often based on, and sometimes adding to, this understanding be continuously pursued. At the same time, methods of more effective surgical correction of the valve are now available, so the criteria for selection of patients and choice of procedure must constantly be refined. It is evident from the papers presented in the symposium that much remains to be done in this field. If the experience in animal hospitals can be correlated with that in human, it is possible that new avenues of investigation may be opened".

The Association of Clinical Biochemists

THE following have been elected officers of the Association of Clinical Biochemists for 1965-66: *President*, Prof. R. H. S. Thompson (Courtauld Institute of Biochemistry, London); *Chairman*, Dr. R. Gaddie (General Hospital, Birmingham); *Secretary*, Dr. A. H. Gowenlock (Chemical Pathology Department, University of Manchester); *Treasurer*, Dr. D. B. Horn (Royal Victoria Infirmary, Newcastle upon Tyne).

Bullard Forest Research Fellowships

APPLICATIONS are invited for research fellowships in forest resources which are awarded annually by Harvard University from the Charles Bullard Fund. Such fellowships are tenable for one year and carry stipends up to 15,000 dollars. They are open to men in public service, in academic careers and in private forestry, with or without advanced degrees, who show promise of important contributions to forestry, which study and research at Harvard University will help to fulfil. Further information can be obtained from the Committee on the Charles Bullard Fund for Forest Research, Littauer Center 119, Harvard University, Cambridge, Massachusetts.

Guinness Awards for Science and Mathematics Teachers

THE Guinness Awards for Science and Mathematics Teachers are now to be extended so as to include Africa. This extension follows experience of the first results in the Guinness Awards programme in Ghana during 1964. The Awards scheme will now include the whole of English-speaking Africa. It is to be carried out in association with the Afro-Anglo-American conference financed by the Carnegie Corporation. Money prizes totalling £350 and certificates will be awarded. The purpose is to encourage teachers in schools and colleges in Africa to develop and report on the teaching of science in Africa with special reference to adaptations related to the African environment. The scheme for Africa will go into operation immediately and the first Awards are expected to be announced in April 1967.

The Guinness Awards for Science and Mathematics Teachers are also to be extended so as to include all schools run overseas by the Armed Forces. The three Services have agreed to collaborate, and the Royal Air Force

Educational Organization is to act as the co-ordinating centre. The first Awards will be announced in April 1967. Also to encourage mathematics, it has been agreed that financial and administrative aid shall be provided for the following two schemes: first, a National Mathematics Contest, now in its third year, in which the entrants are students in secondary schools, mainly grammar and public schools (in 1965 there were 5,000 entrants from 109 schools in the United Kingdom for this contest); secondly, the Mathematics Olympiad, organized by Prof. W. K. Hayman, of the Department of Mathematics, Imperial College of Science and Technology, and Mrs. Hayman, a mathematics teacher at Mayfield School, London. This scheme began in 1966. The first Awards under the auspices of the Guinness Awards for the National Mathematics Contest and for the Mathematics Olympiad are to be made in April 1966. As a result of the extensions to the Guinness Awards outlined here, the total value of prize money to be awarded annually will now be £1,500. Further information can be obtained from Mr. Maurice Goldsmith, c/o *The Science Teacher*, 60 Paddington Street, London, W.1.

Announcements

MR. W. T. COOPER, research editor of the Division of Coal Research, Commonwealth Scientific and Industrial Research Organization, has been appointed to succeed Prof. N. Y. Kirov as chairman of the Institute of Fuel (Australian Membership).

A MEETING of the Welsh Soils Discussion Group on "The Fauna and Flora of the Soil" will be held in the University College of North Wales, Bangor, on November 24. Further information can be obtained from C. C. Rudeforth, Soil Survey, Trawscoed, near Aberystwyth, Cardiganshire.

AN ordinary meeting of the Scottish Section of the Society for Analytical Chemistry will be held in the University of Strathclyde on November 26. A paper on "Separate or Calculate" will be presented by Dr. A. L. Glenn. Further information can be obtained from the Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1.

A LECTURE on "The Reliability of Electronic Systems" will be delivered by Dr. Irving Bosinoff (Mitre Corporation, Bedford, Massachusetts) at the Institution of Electrical Engineers on December 3. Further information can be obtained from Dr. G. S. Brayshaw, Northampton College of Advanced Technology, St. John Street, London, E.C.1.

A JOINT meeting of the Scottish Section of the Society for Analytical Chemistry and the local sections of the Chemical Society, the Royal Institute of Chemistry and the Society of Chemical Industry will be held in the University of Strathclyde on December 3. A paper on "Chemistry for Profit" will be presented by Dr. J. W. Barrett. Further information can be obtained from the Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1.

THE 1966 Gordon Research Conferences will be held in Santa Barbara, California, during January 24-February 4. The purpose of the Conferences is to stimulate research in universities, research foundations and industrial laboratories and is achieved by an informal type of meeting consisting of scheduled lectures and discussion groups. The programme will include conferences on: polymers (January 24-28); chemistry of ageing (January 24-28); electrochemistry (January 31-February 4); formulation of research policies (January 31-February 4). Further information can be obtained from W. George Parks, Department of Chemistry, University of Rhode Island, Kingston, Rhode Island.

MEETING OF BIOCHEMISTS IN SPAIN

DURING July 28–31 the third general meeting of Spanish biochemists was held at the University of Oviedo, organized by the Spanish Society of Biochemistry and sponsored by the Ministry of Education and the University of Oviedo. The Minister of Education, Prof. M. Lora Tamayo, himself a biochemist, was the honorary president of the meeting.

At the opening session, Dr. A. Sols and Dr. J. R. Villanueva, president and secretary, respectively, of the Spanish Society of Biochemistry, gave a short historical review of the Society including its progress as shown through the programme of these meetings and other activities. Prof. F. Grande Covian, of the University of Minnesota, spoke in representation of the scientists coming from abroad, and finally the acting rector of the University expressed his pleasure at being with the Spanish biochemists.

The meeting centred about three symposia: (a) "Enzymatic Regulation"; (b) "Microbial Metabolism"; (c) "Hormonal Metabolism". The first symposium held was under the chairmanship of Prof. S. Grisolia, of the University of Kansas, and Dr. Gertrudis de la Fuente (Madrid) described the work on the allosteric effects in hexokinases, followed by M. L. Salas, who discussed the allosteric regulation of the yeast phosphofructokinase. C. Gancedo (Madrid) described some results on the allosteric yeast fructose-1,6-diphosphatase and the regulation of gluconeogenesis. Dr. Gertrudis de Torrontegui (Madrid) described the level of pyruvate carboxylase and related enzymes in micro-organisms grown on different carbon sources. Prof. S. Grisolia presented the work carried out in collaboration with Dr. Torralba and J. Tecson at the University of Kansas on the reversible transformation of phosphoglyceromutase in diphosphoglycerate phosphatase. J. Salas (Madrid) described results of the stability and synthesis of the liver glucokinase *in vitro* and *in vivo*. Finally, A. Sillero described the induction and turnover of the glucokinase in liver. The session was followed by a general discussion, and at the end Drs. A. Sols and S. Grisolia summed up the more important points of the symposium. This session was followed by a lecture by Prof. J. Oró, University of Houston, on recent researches on hydrocarbons of biological origin found in Pre-Cambrian sediments and meteorites.

In the second symposium, under the chairmanship of Dr. C. Asensio (Madrid), Dr. D. Vázquez (Cambridge) described investigations of the effect of some antibiotics inhibiting the synthesis of proteins at the ribosomal level. Prof. R. Parés (Barcelona) described work on amino-acid excretion by a coliform. Dr. E. Muñoz (Liège) described recent work carried out in collaboration with Drs. Ghuyssen and Petit on the structure of glucopeptides of the bacterial cell wall. Dr. Concepción García Mendoza (Madrid) described the purification and chemical analysis of the cytoplasmic membrane of yeast, and finally Dr. J. R. Villanueva (Madrid) reviewed the ways in which information about yeast cell wall structure

has been obtained and described some results obtained by his group at the Institute for Cell Biology. At the end of this symposium a discussion on "Unity and Variety in Microbial Biochemistry" was held, the main discussants being Drs. Losada, Mayor, Montoya, Parés, Regueiro, Sols and Villanueva. The session was followed by an address by Dr. M. Losada, director of the Institute for Cell Biology, CSIC, Madrid, who described the mechanism of the photosynthetic reduction of nitrate to ammonium.

In introducing the third symposium, Prof. F. G. Valdecasas, rector of the University of Barcelona, made some comments on the importance of the subject of hormonal metabolism. Dr. J. Gómez Acebo (Madrid) showed a collection of electron micrographs of pancreas cells during the process of insulin secretion *in vitro*. Dr. C. López Quijada (Madrid) reported the effects of iodoacetate and cysteine on the antigenic power of insulin. Prof. C. Osorio (Granada) described the process of secretion of thyroxine in bile of monkeys. Dr. J. A. Sanchez-Martin (Madrid) discussed the action of thyrotrophic hormone on the intra-thyroid metabolism of the propylthiouracil. Dr. E. Herrera (Madrid) discussed the problem of experimental goitrogenesis, Dr. A. Colás (University of Oregon) described the biosynthesis of the oestriol during pregnancy, and finally Dr. A. Oriol-Bosch (Madrid) described the metabolism of tritiated progesterone by the adrenal cortex of the guinea-pig. The session ended with an address by Prof. J. L. Rodríguez Candela, director of the Marañón Institute, CSIC, Madrid, who described investigations of his group and discussed the regulation of the insulin secretion. The symposia were followed by sessions where short communications were read. In all, about forty papers were presented.

During the closing session of the meeting, Prof. F. Grande Covian gave a lecture on the role of the dietetic factors in the regulation of the plasma lipids.

Several social events were also organized, including receptions in the University and the Town Hall, and an excursion to the beautiful resorts of the Asturias coast. Nearly two hundred people attended this meeting, including a notable group of Spanish research workers from abroad and many young research students. The meeting was very successful, especially in encouraging the strong development of biochemistry in Spain, which has been proceeding during the past five years. The next general meeting of the Society will be held in the University of Granada in the autumn of 1966.

The whole of the Oviedo University meeting made an important contribution to the way in which science, and in particular biochemistry, is growing in Spain. The progress has been strongly stimulated by the marked interest taken in biochemistry by some distinguished professors and research workers from the Consejo Superior de Investigaciones Científicas and the University. Recently, increasing attention has been directed in Spain to the means of developing scientific policy and the mechanisms of scientific advice.

J. R. VILLANUEVA

LUNG TUMOURS IN ANIMALS

AN international conference on "Lung Tumours in Animals" was held at the Division of Cancer Research, University of Perugia Medical School, during June 24–29. The conference brought together many geneticists, physiologists, pathologists, virologists and biochemists, whose main interests lay in basic cancer research.

Following the opening of the conference by Prof. G. Ermini (Rettore Magnifico of Perugia University), L. C. Strong (San Diego, U.S.A.), addressing the audience as the representative of the doctors 'honoris causa' of the

University of Perugia Medical School, said that the contributions of that small select group of scientists to the human knowledge of cancer had indeed been great—and that no small part of these contributions had been made, and would presently be made, at the Conferences of the Division of Cancer Research, Perugia.

H. L. Stewart (Bethesda, U.S.A.), speaking as the representative of the National Cancer Institute, Bethesda, commented that the conference should contribute much to our knowledge of the comparative pathology of pulmon-

ary tumours and help to decipher some of the puzzles that remain obscure about cancer of the lung in human beings.

Prof. L. Severi, in an introductory talk, commented that, because of the apparent increase in human lung cancer attributed to atmospheric pollutants and, on the basis of statistical investigations, especially to smoke, the conference was most timely. He pointed out, however, that lung cancer occurs in animals, which do not smoke, and that no conclusive evidence was available to show that tumours could be induced in them either with smoke or with the products of tobacco.

Lectures were given at the conference to emphasize important aspects of pulmonary carcinogenesis in animals as a whole, and a number of papers were presented. Of special interest was the brief address of Prof. W. I. B. Beveridge on the World Health Organization's programme in comparative oncology.

From Heston we learnt that lung tumours in the mouse are the most suitable for genetic study. There are sub-strains with high- and with low-pulmonary-tumour incidence. The genes which influence the development of induced tumours have the same effect on the development of the so-called spontaneous tumours. These are tumours with a high degree of heritability, and at least 10 known genes are connected with the increase or decrease in pulmonary tumours. After discussing the anatomy, histology and electron microscopy of the lungs of the animals most used in experimental research on lung tumours, Peacock directed attention to the double exposure of alveolar epithelium to carcinogens, on one hand, through the air breathed, and on the other through blood, which is responsible for conveying fat-soluble substances directly from the alimentary canal.

As the environment (broadly speaking) would seem to be important in pulmonary carcinogenesis, which may be supposed to overcome the body's defences, Bell examined the protective physiological mechanisms of the lung in small laboratory animals and birds, the respiratory physiology of which is little known or studied. Kinoshita reviewed recent progress in research on pulmonary carcinogenesis, and pointed out that many newly discovered causative agents for various species of animals have been introduced. The neoplasms are apparently alveolar and bronchiolar in origin, often undergoing squamous metaplasia. Even minute doses of the agents, given to pregnant or nursing mothers or to the progeny at birth, can be shown to produce lung cancer in mature age.

According to Roe, mice are not ideal for research on lung cancer in man, especially because of differences in airway size and mucus production; they represent, however, a valuable tool in that they make possible the study of a large number of animals and the rapid induction of pulmonary tumours. Such tumours are induced more easily with techniques which prevent the rapid elimination of the carcinogen from the pulmonary tissue. From this fact Shabad developed the theory that, in man, disturbances in the defence mechanisms of the lung could promote the deposition and the retention of carcinogens and the induction of tumoral proliferation. By histological analysis of pulmonary tumours of a number of different animals, by comparison between the lung tumours of the various species and comparison of these with human lung tumours, and by considerations of tumour classification, H. L. Stewart has been led to the conclusion that these are for the most part tumours that have not been studied in depth.

Spontaneous and So-called 'Spontaneous' Lung Tumours

Evidence points to the belief that spontaneous pulmonary tumours in animals are rare. In cattle they are exceptional (Migaki and Brandy; Montroni and Barboni), about 50 cases having been described (Owen); in the cat, according to Stünzi, they represent 12 per cent of all carcinomata; in the dog, where they do not seem to be on

the increase (Cohen), they occur in between 0.2 and 0.6 per cent of the autopsy material, respectively, at Richland (Washington) (Clarke *et al.*) and Storrs (Connecticut) (S. W. Nielsen). In both domestic animals (Dahme) and in captive wild mammals and birds (H. L. Stewart), the cases are usually of adenocarcinomata and squamous-cell carcinomata; alveolar-cell carcinomata are infrequent and are divided into unicentric and multicentric—the latter corresponding (Dahme) to pulmonary adenomatosis in sheep (jaagsiekte).

Because lung adenomatosis or foetalization frequently occurs in many circumstances and due to many causes, care (Schiefer) must be taken in formulating the diagnosis of adenomatosis, strictly defined, in sheep; its real nature is still doubtful, since the possibility of its being inflammatory cannot be excluded (Cohrs). Perhaps, in this regard, it is appropriate to consider the 'jaagsiekte complex' (Marsh) which consists of two morbid entities: (a) pulmonary adenomatosis in sheep (jaagsiekte), which may be a tumour capable of metastasising; (b) progressive chronic pneumonia, to which 'maedi' belongs. This latter disease (Gudnadottir) has appeared in sheep in Iceland in the past twenty-six years. It is a generalized infection which produces pulmonary lesions, lesions in the lymphatic system and probably in the central nervous system; it would seem that it may be transmitted to healthy animals by the coughing of affected animals. It is a virus disease, the fundamental characteristics of which we have now learned (Thormar).

Pleuropneumonia-like organisms have been shown in a series of sheep with adenomatosis, both serologically and culturally (Mackay). A lesion similar to adenomatosis in sheep (jaagsiekte) is found in man, and this is an alveolar carcinoma of the lung, which has a peculiar clinical progression (Cunningham). The so-called spontaneous lung tumours in mice (the lung tumours of inbred strains) are dependent on many factors, genetic and extragenetic. Johnson and Strong showed that, in all probability, it is possible to control the incidence of lung tumours, a genetically controlled biological characteristic, with selection on a maternal age basis. Rabotti reported on the biology of lung tumours in *BALB/cN* mice, a sub-strain of intermediate lung tumour incidence, and presented data on incidence (which can vary by extragenetic influence), progression and the findings by electron microscopy and tissue culture.

Induced Lung Tumours

It is possible to induce hyperplastic, metaplastic and/or tumoral lesions in the respiratory apparatus of animals in many ways.

(1) *Ionizing radiation.* After a review of lung carcinogenesis by ionizing radiation (Cember), papers were read on the possibility of obtaining tumours: (i) in the dog, with plutonium particles (Park *et al.*; Clarke *et al.*) and with X-rays (Andersen and Guttman); (ii) in the rat, with β -rays (Kuschner *et al.*); (iii) in the mouse, with X-rays at high altitude (Mori-Chavez).

(2) *Viruses.* Squartini *et al.* found indications that viruses and hormones might be involved in lung carcinogenesis in mice (*BALB/c/Cb/Se* sub-strain). Rabotti found viral particles in spontaneous lung tumours in mice of the *BALB/cN* sub-strain. The virus involved might be regarded as a possible environmental extragenetic factor in the development of lung tumours in mice of this sub-strain. Smith and Miller carried out research to show a virus in a transplantable tumour of mouse lung origin. In the hamster, with cultures of a human lymphoma containing a virus, S. E. Stewart obtained pulmonary lesions. Leuchtenberger and Leuchtenberger concluded from experiments on mice with influenza virus, and with influenza virus in combination with cigarette smoke, that influenza virus would appear to be an 'infectious' RNA virus, which under certain conditions may be implicated in malignant transformation

of cells. Such a conclusion supports the hypothesis that influenza virus may be a co-factor in the development of bronchogenic carcinoma. Harris and Negroni, in a series of experiments, similarly exposed mice to influenza virus and also to influenza virus in combination with cigarette smoke. The results with influenza virus alone gave a low incidence of malignant lung tumours developing rather late in the life of the mice; preliminary results with the combination of influenza virus and cigarette smoke indicate that tumours appear earlier. Of particular interest in the latter series of experiments is the appearance of a squamous cell carcinoma not unlike the lung tumours observed in human smokers. The authors do not regard their findings as conclusive.

(3) *Atmospheric pollutants*. Tumours: (i) in the rat, with nickel carbonyl (Sunderman), and with asbestos (in the pleura, mesothelioma) (Wagner); (ii) in fowls, with asbestos (axillary air-sacs) (Peacock); (iii) in the hamster, with asbestos (in the pleura, mesothelioma) (Smith), with benzo(a)pyrene by means of dust particles (Saffiotti *et al.*), and with cigarette smoke (metaplastic and papillary processes in the trachea and bronchi) (Dontenwill and Wiebecke).

Experiments in mice have yielded the following results: (i) with denicotinized tobacco smoke condensate (decrease in the development of pulmonary tumours by 3,4,9,10-dibenzpyrene) (Homburger and Treger); (ii) by the inhalation of tobacco smoke (after a year a single pulmonary tumour) (Harris and Negroni); (iii) with tobacco tar fractions (no increased incidence of pulmonary tumours) (Orr and Woodhouse). From the point of view of human pathology, Carnes and Moses reported that epithelial lesions have been studied, in autopsies, in relation to three forms of atmospheric pollution: (a) smoking history; (b) residence, classified by size; (c) occupation. Epithelial lesions of the trachea and bronchi proved to be more severe in those cases with a smoking history. According to Kreyberg, cigarette smoking, and to a lesser extent pipe smoking, may be considered to be particularly responsible for lung cancer.

(4) *Chemicals*. Tumours: (i) in the rat, with *N,N'*-2,7-fluorenylenebisacetamide and related aromatic amines (Morris), 9,10-dimethyl-1,2-benzanthracene, 3,4-benzpyrene and 3-methylcholanthrene (Crocker and B. I. Nielsen), 20-methylcholanthrene and 3,4-benzpyrene (Laskin *et al.*), *N*-methyl-*N*-nitrosourethane (Schoental); (ii) in the mouse *N*-nitrosodiethylamine and *N*-nitrosodimethylamine (Takayama and Oota), 4-nitroquinoline-*N*-oxide (Kinosita), 3-methylcholanthrene and 4-nitroquinoline-*N*-oxide, new-born and adult (Tsubura and Kimura), urethane (Tannenbaum; Ribacchi and Giraldo; at high altitude, Mori-Chavez), 'Imferon' (Langvad), hydrazine (Bianciffiori *et al.*) and its derivatives (Clayson *et al.*); (iii) in the hamster, with diethylnitrosamine (Dontenwill and Wiebecke); (iv) in the white Pekin duck, with methylcholanthrene (Rigdon).

In the rabbit, Griecute produced adenomatosis with dimethylbenzanthracene, and Moran showed that cortisone, administered after pulmonary lesions had been produced by chemicals, resulted in temporary hyperplasias of the epithelial type. In the pulmonary tumours of the rat by dimethylnitrosamine and diethylnitrosamine,

changes occur in the protein-levels which seem to reflect systemic alterations in the whole organism (Hoch-Ligeti). The various degrees of alkylation of nucleic acids in the lungs and in other organs of the mouse, hamster and rat after treatment with carcinogenic nitroso compounds were studied in relation to the susceptibility of the organ to the carcinogen (Magee).

Briand and Kieler investigated the carcinogenic and co-carcinogenic effect of various oxygen tensions on murine lung cells grown *in vitro*. Nitrosamines may be contained in cigarette smoke even though they have not been demonstrated; it is possible, however, that they disappear rapidly (Boyland and Roe). Urethane may act as an accelerator only (Bentvelzen and Szalay) and, according to Boyland, by means of *N*-hydroxyurethane. It has been shown in the rat that even a serious lesion in the pulmonary tissue is not sufficient for the development of a cancerous growth in the presence of a weak carcinogen (Stanton and Blackwell). Boeryd and Mellgren reported on the influence of heparin and epsilon-amino-caproic acid on pulmonary metastases of malignant tumours in mice.

Summary

In the final session of the conference, under the chairmanship of Lord Florey, Prof. Severi gave a summary of the contributions to the scientific programme. Little is known of the epidemiology, the biological behaviour and the pathology of spontaneous lung tumours in animals. The World Health Organization's programme in comparative oncology certainly is welcome. Attempts to induce tumours in the respiratory system by means of physical, viral and chemical agents must be developed extensively, so that research in the field of pulmonary carcinogenesis can be widened. On this point, Balò considered drugs capable of producing lung tumours in animals while Sprunt reported that, by irritation of the pulmonary epithelium with non-carcinogenic substances, lung tumours can be produced in the rabbit. The last mentioned is perhaps an important step for the understanding of pulmonary carcinogenesis. The results reported on experimental carcinogenesis by tobacco did not extend our knowledge beyond the known facts: that smoke condensate is a complete carcinogen, if a weak one, for the skin of the mouse and the rabbit, for the mouse cervix, for the subcutaneous tissue of the rat and also for the trachea of the dog. Nothing is known for sure of its carcinogenic action on the bronchial epithelium, perhaps because of the technical difficulties. A great deal is known about lung cancer in man, and a very great deal about so-called 'spontaneous' and induced lung cancer in animals, but the cause of lung cancer in man or of spontaneous lung cancer in animals remains obscure.

Prof. Severi agreed with Hockett that much systematic, methodical, step-by-step research with animal models must be done. So far as man is concerned, what is not understood cannot be prevented. For this reason there is little to expect from preventive medicine, but much to hope for from basic research.

The *Proceedings* of the conference will be published, as usual, by the Division of Cancer Research, P.O.B. 167, Perugia, Italy.

L. SEVERI

PLANNING A NEW MEDICAL SCHOOL

IN July 1964 the Minister of Health informed Parliament that a new medical school was to be established in the University of Nottingham, with an intake of 100 students a year, in conjunction with a new teaching hospital of 1,200 beds. For three years before this announcement, the University had been engaged in negotiations, during which much thought was given to the problems involved.

In October 1964 the University Council, at the request of the Senate, decided to set up a Medical School Advisory Committee to offer advice and recommendations to the University on medical education, teaching and research, on the best arrangements for the nature and lay-out of the buildings required, and on the University's administrative relations with other bodies concerned. The Com-

mittee's report has now been published*, and as the proposed new medical school is the first to be established in Britain since the Welsh National School of Medicine, set up in Cardiff in 1893, the report is of considerable interest.

The report begins with a brief review of medical education in the past and then discusses the purpose of a university medical school, which should, it considers, like the university itself, have two chief functions: the pursuit of knowledge and the education of the young—that of training for a profession, although a vocational objective, is added. It is accepted, as a fundamental tenet, that the new medical school will encourage research, particularly in the growing points of medical science, and also that teaching and research are complementary. The Committee adopts three guiding principles and attempts (1) to shape the pre-clinical part of the course to conform in pattern and objective with that of other science departments in the University; (2) to integrate and co-ordinate so far as possible pre-clinical and clinical departments and studies; (3) to plan the government, staffing and services of the hospital, to meet the needs of both the University and the community.

The curriculum and buildings of the new medical school should be designed in such a way as to develop and strengthen the links between: (1) the medical school and the rest of the University; (2) the pre-clinical and clinical parts of the school; (3) the hospital and the community it serves. The Committee suggests that the medical school and hospital should be known as the "University of Nottingham Medical Centre". Because teaching and research are so closely associated it is imperative that the new medical school should offer facilities for research in terms of time, space, staff, equipment and money which will attract only the best.

The total of 100 medical students per annum is accepted as the number at which the new school should aim, but the building should be flexible enough to enable this number to be increased. No dearth of good candidates is expected, but over-specialization at school has serious consequences for medicine. Boys and girls wishing to study medicine, who have passes in Advanced-level Examinations in both arts and sciences or arts subjects alone, should be encouraged; suitable arrangements should then be made to teach them the chemistry, physics and biology required for the study of medicine. Students should be selected for their intelligence (as revealed by Ordinary- and Advanced-level Examination passes) and for their personal qualities and capacity for citizenship, as shown by their school records and by interview. Every effort should be made to reduce the segregation from other students, inevitable to some extent in a medical student's studies, by sharing in common residence, meals, sports and social facilities. Every inducement should be given to students to incorporate themselves into the general life of the university. Certainly, the present science library should be extended to cater for medicine.

As regards the curriculum, the report suggests that this should be designed to cultivate a student whose curiosity is enhanced and not diminished, who is familiar with the broad field of medical science and who has

acquired the habit of learning. He should also have assimilated the ethos of medicine. The curriculum should be planned with the view of avoiding the chief defects of contemporary medical education, such as overcrowding, lack of integration between subjects taught at the same and different times, and too many examinations. Accordingly, it is recommended that lectures should not be excessive in number and attendance should, so far as possible, be voluntary. The student should have sufficient free time to read and work on his own initiative, both in the library and in the laboratory or wards. Details of the curriculum should be planned by an inter-departmental committee of teachers, appointed by the Faculty of Medicine with an independent chairman. This committee should include junior staff, and should seek opinion from students, and keep the curriculum continuously under review. To synthesize the contributions of different disciplines, lectures on different aspects of a single problem should be delivered consecutively and followed by group discussions. Clinical demonstrations should be given throughout the pre-clinical period so as to emphasize the unity of knowledge and alleged vocational aspects of the curriculum, but it should not be allowed to dominate the educational aspects. Medical examinations should be reduced to the minimum compatible with the regulations of the General Medical Council.

It is suggested that the curriculum should consist of a period of three years' training for the biological scientist, whose interest is centred on man, followed by two years of clinical training and two pre-registration years. For those students who have not studied science at school, the University should provide an introductory year of science. The first-degree course in medical biological sciences is visualized as forming the third limb of a tripod, of which the other two would be a school of general biological sciences, within the faculty of pure science, and a school of agricultural biological sciences within the faculty of agricultural sciences. The teaching hospital should provide for the needs of the community and of the University and its services, and include geriatrics, psychiatry and infectious diseases in a certain area. The size of the units may require modification to meet the teaching and research needs of the University. The teaching hospital and medical school should be designed and constructed as a unit, to permit as free as possible an interchange of people and a maximum flexibility. It is suggested that the governing body of the new teaching hospital should be constituted by the Regional Board and the University (as equal partners), and that it should be responsible for obtaining the necessary finance from the Ministry of Health and the University Grants Committee. It is also recommended that the Department of Community Health should contain a sub-department of general practice, the function of which would be to help in the organization of general practice in the area, and to encourage the provision of better working conditions for general practitioners and thus better service for patients. The medical school and the teaching hospital should be designed as an entity. The Committee would regard a start in temporary accommodation as both extravagant and undesirable, as it may result in loss to the University of some of the best senior staff—on whom the future of the medical centre depends.

* University of Nottingham. Report of the Medical School Advisory Committee. Pp. 76. (Nottingham: The University, 1965.) 6s.

NATURE CONSERVANCY IN BRITAIN

IN a paper, "Advances in British Nature Conservation", now preprinted from the Society's handbook for 1965*, Mr. E. M. Nicholson, director-general of the

* Society for the Promotion of Nature Reserves. *Advances in British Nature Conservation*. (Preprint from the Society's Handbook for 1965.) By E. M. Nicholson. Pp. 16. (London: Society for the Promotion of Nature Reserves, 1964.)

Conservancy, summarizes some of the recent changes in the concepts, practices and scale of conservation of natural areas in Great Britain in recent years.

Conservation has now ceased to be the affair of a local minority and has become a major nation-wide project. The vague idea that reserves are a good thing and

valuable to naturalists has been replaced by a concept of their role as living museums, and outdoor laboratories, to be fully and systematically studied by scientists, with the view of understanding their ecosystems, biological productivity, population dynamics, successional and land-use history, and so forth. The assumption that any necessary management of reserves can be satisfactorily achieved empirically has been replaced by a comprehensive, two-tier programme of concerted basic and applied ecological researches, designed to elucidate the full range of fundamental principles involved, and to develop the necessary series of techniques and prescriptions for their application. It is also now recognized that not only natural and semi-natural habitats displaying various stages of ecological succession are equally deserving of care and protection in the interests of science, but also modified or induced ecosystems and sites, already used for, or well suited to, ecological investigations. Local distributions are now being systematically and comprehensively mapped, as well as changes and trends in populations. The lack of interest of universities in ecology and conservation has been replaced by a rapid growth of specialized postgraduate courses, and there has been a major increase in the number of students taking doctorates in ecological subjects and a general growth in university field studies, partly at the centres of the Field Studies Council.

The essential role of basic and applied ecology in the training and practice of the land professions, such as forestry, agriculture, fisheries, estate management, land-

scape architecture and town and country planning, is now becoming recognized, as well as the need for increased understanding of the character, scale and causes of human impacts on Nature, both through studies and analysis and through improved communications between the different interests concerned. Applied ecology is now recognized as being the guidance of the proper control of the use of potentially polluting substances, such as toxic chemicals, as well as the promotion, through concerted action and the wise multi-purpose use of land and natural resources, of the general adoption and observance of modern conservation practices.

Mr. Nicholson's thesis is that scientific ecology has now reached the point of transforming the concepts of management of natural areas, and is beginning to make a serious impact on the use and management of land, education, and interests concerned with the use of renewable natural resources. He illustrates this by summarizing the British contribution on an international plane and by reviewing the position in the various counties of England and Northern Ireland. The distinction between conservation movements in Britain and other countries is based far more on the naturalist tradition, on which professional work in biology has lately been superimposed. The centre of gravity of the whole movement is shifting steadily from the saving of species, and even of habitats, to a broader view of conservation as involving the scientific care and good management of man's entire natural environment.

ORIGIN OF ATYPICAL METEORITES FROM THE ARIZONA METEORITE CRATER

By PROF. MICHAEL E. LIPSCHUTZ

Department of Chemistry, Purdue University, Lafayette, Indiana

THE well-known Arizona Meteorite Crater is an impact feature having a diameter of nearly 1 km. Estimates of the mass of the meteoroid which produced it have ranged from 30,000 (ref. 1) to 2.6 million² metric tons. The (spherical) diameters corresponding to these estimates are 20 m and 86 m. The main mass of this meteoroid has never been located. Inasmuch as most of the meteoroid probably vaporized or mixed with the surrounding rock during the explosion, it seems rather unlikely that much of the mass will ever be found. However, a fraction did survive the explosion in the form of many thousands of fragments ranging up to 640 kg in weight. The overwhelming preponderance of these have been 'normal' coarse octahedrites with kamacite band-widths ranging up to 4–5 mm. Less than 12 of the recovered fragments had structures corresponding to those of medium octahedrites. These atypical meteorites have been called Canyon Diablo No. 2, Canyon Diablo No. 3 and Monument Rock³. There is no doubt that these three types differ significantly both from the normal Canyon Diablo meteorites and among themselves⁴ in structure and chemical composition.

Four explanations have been offered which can account for the atypical samples. The first of these is that the meteoroid was not a solid mass on impact with the Earth but consisted of a swarm of much smaller objects⁵. The possibility of such a swarm seems rather remote⁶, and will not be considered further here. A second possibility is that the meteoroid consisted of a main mass of coarse octahedrite structure and several satellites with the medium octahedrite structures³. A third explanation is that there were four distinct falls: a large crater-forming coarse octahedrite mass, followed by three separate medium octahedrite falls³. The fourth possibility is that

all were part of the same mass which had varying physical structures and minor element contents^{6,7}.

Until recently, no definitive results had been obtained as to which of these explanations was correct. On the basis of cosmogenic rare-gas measurements⁸, Heymann was able to show that Canyon Diablo No. 2 was probably buried in the main mass of the meteoroid and exposed to cosmic-ray bombardment for 540 ± 100 million years at a pre-atmospheric depth of 50 cm. The remote possibility, however, existed that it was a distinct fall with a cosmic-ray exposure age of 64 ± 12 million years. The results of the rare-gas measurements on Canyon Diablo No. 3 were rather less conclusive. Either this meteoroid was part of the main mass and had an exposure age of about 1,000 million years or it was originally in a 10^5 kg object having an exposure age of 540 ± 100 million years (either a protuberance on the main mass or as a separate 10^5 kg mass). Similar alternatives⁷ were proposed in order to explain the observed rare-gas contents of sample 24, a normal Canyon Diablo. However, a subsequent $^{40}\text{K}/^{41}\text{K}$ measurement by Voshage⁹ of sample 24 yielded a value in substantial agreement with the exposure age of 540 million years. This result casts considerable doubt on the validity of the exposure age of 1,000 million years for Canyon Diablo No. 3. Most of the known measured medium octahedrites have exposure ages in the 500–600 million year range^{8,9} and it is therefore not possible from Heymann's measurements to decide whether Canyon Diablo No. 3 was located in a 10^5 kg projection from the infinite mass ($\gg 2 \times 10^5$ kg) meteoroid or was a separate fall. A number of recent investigations^{3,7,10} have established the fact that all known normal Canyon Diablo meteorites found on the Crater rim have been shocked to at least 130 kb. Since all three atypical types were recovered



Fig. 1. Comparison between the apparently normal, undeformed Widmannstätten pattern of the moderately shocked Canyon Diablo No. 3 sample (left) with the faint deformed pattern of the heavily shocked Canyon Diablo No. 2 (right). The white areas on the polished surface of the Canyon Diablo No. 3 are ϵ iron

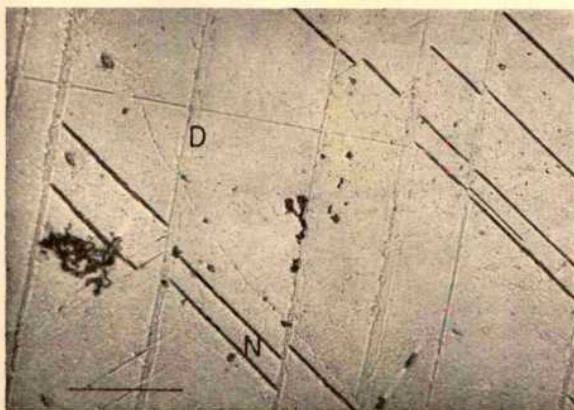


Fig. 2. Microstructure of the lightly shocked Monument Rock meteorite showing deformation bands (D) and Neumann bands (N). The bar in these figures is 0.1 mm

from the north-east rim¹¹ it seemed reasonable to examine them for shock effects in an attempt to resolve the question of their origin.

With the help of Prof. C. B. Moore, curator of the Nininger Meteorite Collection, I obtained samples of Canyon Diablo No. 2 (371.3), Canyon Diablo No. 3 (586.1), and Monument Rock (587.1x). The first two of these are shown in Fig. 1. Monument Rock is illustrated in Plate 22, Fig. 2E of ref. 3. These samples were polished and examined by standard metallographic techniques. The detailed interpretation of shock-induced metallographic changes in meteoritic iron have been described previously^{7,10} and need not be repeated here.

Monument Rock. This meteorite falls into the lightly shocked category (< 130 kb) of Heymann *et al.*⁷. The large number of Neumann bonds (shock twins) in it has previously been noted by Nininger³. The only evidence for any unusual shock is the presence of small deformation bands (Fig. 2) in the kamacite (α -iron). There were no inclusions in the exposed surface which could be studied crystallographically for shock effects.

Canyon Diablo No. 2. This meteorite falls into the heavily shocked category (> 750 kb) of Heymann *et al.*⁷. The Widmannstätten pattern is indistinct (Fig. 1) and the kamacite is entirely recrystallized (Fig. 3). The cohenite (Fe_3C) grains show diffusion borders of pearlite. Some ledeburite-like eutectic is present and the rhabdites (Fe_3P) in the hotter end of the sample are redissolving. The taenite (γ) and plessite ($\gamma + \alpha$) grains are partially or completely clear and there is a secondary kamacite precipitate in some of them. That these thermal effects are due to shock and not to contact with hot ejecta or artificial heating is proved by the crystallo-

graphic character of the cohenite, which has been shocked to about 1,000 kb¹².

Canyon Diablo No. 3. This meteorite falls into the moderately shocked category (130–750 kb) of Heymann *et al.*⁷. The Widmannstätten pattern is quite distinct and undeformed although the macrostructure shows areas of ϵ iron (Fig. 1). Pressure gradients are very common. Some kamacite areas show normal Neumann bands, while others show regions of finely recrystallized kamacite around inclusions, or areas of completely recrystallized kamacite. Some of the patches of ϵ iron are normal, while others are in the process of conversion to polycrystalline kamacite (Fig. 4). The rhabdite and schreibersite (Fe_3P) grains are apparently thermally unaltered. Some cohenite grains show borders of martensite, but most exhibit no carbon diffusion

zones. The taenite and plessite regions are clearing and there is a secondary kamacite precipitate in some taenite bands: the exposed troilite (FeS) nodule of the coarse type 2 variety. A systematic crystallographic study of the cohenite grains in this sample is not yet complete. Those specimens studied thus far fall into the 400–500 kb range so that there seems little doubt that portions of this meteorite were shocked to pressures of at least 500 kb.

It is regrettable that the results from the Monument Rock sample permit no absolute conclusion regarding the origin of this meteorite. It could have been part of

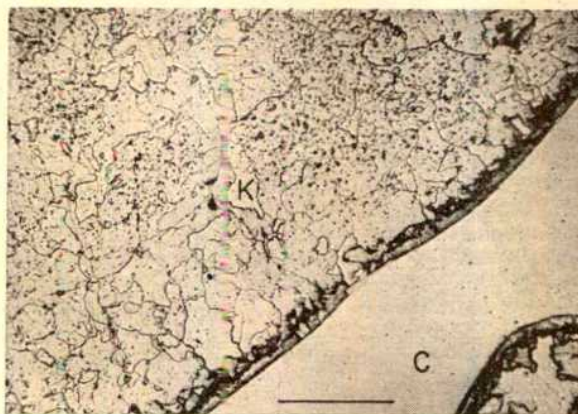


Fig. 3. Microstructure of the heavily shocked Canyon Diablo No. 2 sample showing polycrystalline kamacite (K) and pearlite diffusion border around the cohenite (C)

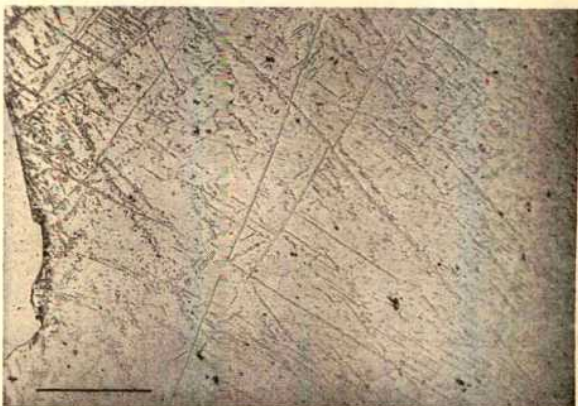


Fig. 4. Microstructure of the moderately shocked Canyon Diablo No. 3 showing ϵ iron beginning to recrystallize, particularly on the left at the interface between the cohenite grain and the iron

the main mass, a satellite of it, or a separate fall. However, it should be pointed out that it is indeed a unique sample. Not only is it the only known specimen of its type found at the Crater site but it is also the only known exception to the observation that rim samples (whether of the normal or atypical varieties) have been moderately to strongly shocked.

An unambiguous conclusion can be reached, however, regarding the origin of both Canyon Diablo No. 2 and Canyon Diablo No. 3. These meteorites were involved in a catastrophic explosion during which they were shocked and therefore heated. The narrow widths of the carbon diffusion borders around the cohenite grains indicate that the duration of reheating was short, at most a few minutes, and that the meteorites cooled quickly to below the γ - α transformation temperature. Thus, their immediate post-shock mass was not considerably larger than their recovered mass. These characteristics are the same as those of the normal shocked Canyon Diablo meteorites. It seems very highly probable, therefore, that both Canyon Diablo No. 2 and Canyon Diablo No. 3 were located in the interior of the meteoroid during the instant

of its explosion and were therefore neither satellites of the main mass nor separate later falls. It seems, therefore, reasonable to regard as proved the earlier suggestion^{6,7} that chemical and structural variations do exist in iron meteorites over distances of less than 100 metres.

This work was supported by grants from the Advanced Research Projects Agency and the National Aeronautics and Space Administration.

¹ Bjork, R. L., *J. Geophys. Res.*, **66**, 3379 (1961).

² Opik, E. J., *Irish Astron. J.*, **5**, 14 (1958).

³ Nünninger, H. H., *Arizona's Meteorite Crater* (American Meteorite Museum, Sedona, Arizona, 1956).

⁴ Goldberg, E. A., Uchiyama, A., and Brown, H., *Geochim. et Cosmochim. Acta*, **2**, 1 (1951).

⁵ Barringer, D. M., *Meteor Crater in Northern Central Arizona* (privately published, 1909).

⁶ Heymann, D., *Nature*, **204**, 819 (1964).

⁷ Heymann, D., Lipschutz, M. E., Nielsen, B. N., and Anders, E., *J. Geophys. Res.* (in the press).

⁸ Voshage, H. (personal communication).

⁹ Vilcek, E., and Wänke, H., *Proc. Symp. Radioactive Dating, Athens, 1962*, 381 (I.A.E.A., Vienna, 1963). Voshage, H., and Hintenberger, H., *ibid.*, 367.

¹⁰ Lipschutz, M. E., and Anders, E., *Geochim. et Cosmochim. Acta*, **24**, 83 (1961).

¹¹ Nünninger, H. H., *Out of the Sky*, 234 (Dover, New York, 1952).

¹² Lipschutz, M. E. (in preparation).

MECHANISM OF STORED-ENERGY RELEASE AT 200° C IN ELECTRON-IRRADIATED GRAPHITE

By PROF. E. W. J. MITCHELL and M. R. TAYLOR

J. J. Thomson Physical Laboratory, University of Reading

THE release of stored energy from reactor-irradiated graphite is of considerable physical and technological interest and has been extensively examined. The results are contained in a large number of papers, most points being covered in the following conference *Proceedings*: the first Geneva Conference on the "Peaceful Uses of Atomic Energy" (1956); the fifth Carbon Conference (1962); the C.E.G.B. Berkeley Conference on "The Properties of Reactor Materials" (1962); and the I.A.E.E. Conference on "Radiation Damage in Reactor Materials" (1963).

For irradiations of low neutron doses the energy is released primarily in the well-known 200° C peak in the warm-up curve, although there is a tail to the curve extending to much higher temperatures. Samples irradiated to higher neutron doses, or at higher irradiation temperatures, release relatively less energy in the 200° C peak, compared with the higher temperature region¹. The energy release has been related to neutron dose and temperature systematically by Bell *et al.*².

The origin of the 200° C peak has remained uncertain. Although there had originally been suggestions that it arose from the recombination of interstitial atoms and vacant sites^{3,4}, more recently it has been generally considered that the energy was associated with a re-arrangement of displaced atoms in which the vacancy concentration was not significantly altered. The types of model which have been considered may be illustrated by referring to those put forward by Bollman⁵ and Iwata and Suzuki⁶. Bollman has suggested that the process involves the recrystallization of small amorphous regions of up to 60 Å diameter which he assumes are produced by neutron irradiation. According to Bollman, the recrystallized regions may contain some dislocation dipoles, but the region is considered to be of much lower energy than the amorphous region.

On the other hand, it has frequently been suggested that the stored energy is released when interstitial atoms re-arrange themselves between graphite planes into a lower energy configuration. In the Iwata-Suzuki model⁶ it is assumed that relatively loose aggregates of C₂ molecules have formed at about room temperature. These

aggregates are assumed to produce the c-axis unit cell expansion and to be loosely held together through the elastic strain, the electronic binding between the C₂ molecules being assumed to be small. At 200° C, Iwata and Suzuki suggest that this loose configuration is converted into the much more stable configuration of the tongue dislocation—not unlike Bollman's dislocation dipole—with a considerable release of energy. Such a process would be accompanied by a recovery in unit cell expansion but the crystal would remain longer than before irradiation.

Each of these models possesses the feature that some disordered arrangement of carbon atoms is converted into a more or less perfect crystal without any interstitial-vacancy recombination occurring. Normal graphite has a relatively large interplanar spacing in the c-direction and the binding between planes is weak. We expect that the differences between elastic strain energies associated with various interstitial combinations will be small. Energy differences between different structures will then arise primarily from the electronic terms. These can be treated as more or less isolated from the rest of the crystal such that a two-dimensional perfect structure between the planes would have an energy not very different from a two-dimensional layer in the perfect crystal. We can therefore expect that the energy gain from the re-arrangement of the interstitials will be less than the energy required to dissociate graphite into free carbon atoms in their *tr*.³ π graphite valence state. Accordingly, from such models the stored energy (*S*) will be expected to be less than the sum of the heat of sublimation (*E_s*) and the promotional energy (*E_p*):

$$S < E_s + E_p = 7.44 \text{ (ref. 7) } + 6.93 \text{ (refs. 8 and 9) eV}$$
 per atom involved in the ordering process, or *S* < about 14–15 eV, there being uncertainty in *E_p*.

In order to decide by how much *S* is less than 14–15 eV on these models we have to specify the electronic state of the distributed interstitials before re-arrangement. If their electronic state were not *tr*.³ π but tended more to the free atomic state ³*P*, then *S* would tend to 7.44 eV (*E_p* → 0). Similarly, if pairs resembling C₂ molecules

between the planes are the starting point of the re-arrangement we should expect $S \approx 7.44 - 3.03 \approx 4.4$ eV/atom, since 3.03 eV is the energy release per atom for the formation of C_2 from carbon atoms in 2P states¹⁰. Thus, while precise values of S cannot be calculated without considerable difficulty for different interstitial re-arrangement models, we can say with fair certainty that $S < 14-15$ eV and in a plausible case we find $S \sim 5$ eV.

The process of radiation damage by reactor irradiation is complex, and it has not been possible to determine accurately from the stored-energy measurements the amount of energy released per atom involved in the process. During the past three years we have been engaged on measuring the energy release in electron irradiated graphite. The objects have been, first, to determine whether the 200° C peak is present—we should not expect to see it if Bollman's hypothesis were correct because of the very much lower mean carbon recoil energy resulting from 2.0-MeV electron irradiation (mean recoil about 131 eV) compared with neutron irradiations (mean about 10⁵ eV). Secondly, if the peak were found we should be able to determine the energy involved per original displacement event, the number of which can be calculated in an electron irradiation with much more certainty than in a reactor irradiation. The experiment has now been completed and in this article we give a preliminary account of the results.

Stored-energy determination. An adiabatic differential power method was used to measure the stored energy released on annealing between 20° C and 400° C. The principle of operation is similar to that of Clarendon et al.¹¹, and to the linear-rise calorimeter of Henson and Mounsey¹². Two specimens, one irradiated, the other unirradiated, were mounted in a cavity which was heated at approximately 6° C/min. Individual heaters mounted axially in each specimen were used to maintain both specimens at the same temperature as the cavity. The difference in the power supplied to the two heaters was measured between 20° C and 400° C. After the initial run the calorimeter was allowed to cool, and then a second run was performed to establish the small power difference which was necessary in the absence of any stored-energy release. The stored-energy release was obtained by subtracting the power difference required in the second run from that required in the first run. Individual runs were reproducible to within ± 0.001 cal deg⁻¹ g⁻¹. The performance of the apparatus was checked by carrying out an experiment in which some small pieces of copper were introduced into one of two unirradiated graphite samples. Full details of the method and the various precautions which have to be taken will be published later.

The specimens, which were of reactor grade A graphite, were irradiated with 2.0-MeV electrons from the University's Van de Graaff accelerator, two specimens at 95° C and the remainder at 65° C. The irradiation flux was $7.7 \mu\text{amp cm}^{-2}$ (4.9×10^{13} e⁻ sec⁻¹ cm⁻²) and the specimens were irradiated in four directions, the maximum dose used being $1.87 \times 10^5 \mu\text{amp min cm}^{-2}$ (7.0×10^{18} e⁻ cm⁻²) in each direction. This was done to ensure as uniform as possible a distribution of damage throughout the thick specimens. Five specimens, irradiated to the different doses shown in Table 1, have been measured.

Calculation of the number of displacements. The concentration of displaced atoms has been determined using

the cross-sections computed by Mitchell and Salisbury¹³ for the displacement energy (E_d) of 60 eV indicated by the electrical resistivity measurements of Lucas and Mitchell¹⁴. The latter experiments showed that the displacement energy must be greater than the commonly assumed 25 eV and their analysis of the energy dependence of the resistivity changes indicated a value of 60 ± 10 eV. In using this cross-section (σ_d , column 20 of Table 1 of Mitchell¹⁵) we have allowed for the production of displacements by the more energetic primary knock-ons¹⁶⁻²⁰. The energy-loss of the electrons in the specimens has been estimated from the range-energy relation as previously described by Clark *et al.*¹⁶. The results are shown in column 2 of Table 1; they correspond to the production in the present conditions of 0.30 displacements cm⁻³ per incident 2.0 MeV e⁻ cm⁻².

It has been suggested by Goggin and Reynolds¹⁷ that close interstitial-vacancy pairs have less effect on the electrical resistance than separated defects. If close pairs were preferentially produced at low energies in the Lucas and Mitchell experiment they would have the effect of making the estimate of $E_d = 60$ eV too high. Current calculations suggest that it would be unlikely that this effect could reduce the value to as low as 50 eV, the effect of which would be to increase the number of defects in the crystal above that calculated ($0.30 \text{ cm}^{-3}/\text{e}^- \text{ cm}^{-2}$) to at most 0.41.

This is one of the various sources of error considered, and their net effect is difficult to estimate precisely. The details of the discussion of errors will be included in the fuller publication. The conclusion is that 0.30 displacements cm⁻³/e⁻ cm⁻² is our best estimate (column 2) and that although the true value might be higher it is unlikely to be as high as 0.41.

Results and discussion. The results of the stored-energy measurements are given in column 3 of the Table in cal/g. Further methods of computing the stored energy in the 200° C peak from the results are being examined, and these may lead to some minor adjustments to the values given here. In subsequent columns the results are expressed as energy in eV per incident electron, and in eV per displaced atom. The slope of the best line of energy versus defect concentration gives a mean value of 13.5 eV per original displacement. (For a displacement energy of 50 eV we would obtain 9.9 eV per original displacement.)

Thus from measurements on electron-irradiated specimens we find that: (a) there is an energy release peak at 200° C; (b) the total energy released in the peak is 3.0 eV per incident 2.0-MeV electron; and that, combining this result with the calculation of the number of displaced atoms, (c) using a displacement energy of 60 eV the energy released in the 200° C peak can be expressed as 13.5 eV per original displaced atom.

The observed energy release in electron-irradiated graphite cannot be explained by Bollman's mechanism. Furthermore, the value of 13.5 eV per displaced atom makes it improbable that the results can be explained by the aggregation of interstitial C_2 molecules for which we have argued that the energy release should be about 4-5 eV. Indeed, it seems impossible to account for the derived value of 13.5 eV per displaced atom without assuming the recombination of interstitial atoms and vacancies. In the following discussion we give reasons for assigning the value of 13.5 eV per displaced atom to interstitial-vacancy recombination.

(1) Lidiard and Perrin²¹ have shown in their analysis of the kinetics of the dimensional changes in neutron-irradiated graphite that recombination has to be taken into account at 150° C.

(2) Using the same cross-sections as those used in calculating the concentrations given in column 2, we find that for the flux used in our experiments of 4.9×10^{13} e⁻ cm⁻² sec⁻¹ we produce 1.5×10^{13} displacements cm⁻³ sec⁻¹. It is generally agreed that interstitial migration occurs between atomic layers in graphite with little diffusion across

Table 1. SUMMARY OF RESULTS FOR ELECTRON-IRRADIATED POLYCRYSTALLINE GRAPHITE

Electron dose (e ⁻ cm ⁻²)	Concentration of displaced atoms ($E_d = 60$ eV) (cm ⁻³)	Stored energy released between 20° and 400° C (cal g ⁻¹)	Stored energy released per incident 2 MeV electron	Stored energy release per original displacement
			eV per incident e ⁻	eV per displacement
1.7×10^{18}	0.53×10^{18}	1.9 ± 0.2	3.4	$15 \pm 3^*$
3.4×10^{18}	0.97×10^{18}	2.4 ± 0.2	2.4	11 ± 2
		2.9 ± 0.25	2.9	13 ± 2
5.3×10^{18}	1.6×10^{18}	5.3 ± 0.1	3.1	14 ± 2
7.0×10^{18}	2.1×10^{18}	6.8 ± 0.1	3.1	14 ± 2

* These are the estimated overall uncertainties.

the layers. In the experiments recorded here, therefore, we expect that displacements were produced at the rate of 5.1×10^5 per cm^2 per interatomic layer per sec.

(3) A single interstitial will diffuse rapidly at 338° K and, using the activation energy of 0.45 eV determined recently by Davies and Mitchell²² from their detailed analysis of the reverse annealing peak, we find that an interstitial will jump at the rate of 2.0×10^6 per sec (assuming a frequency factor of 10^{13} sec⁻¹). For the lower activation energy derived theoretically⁶ we should get an even higher jump frequency.

(4) In electron-irradiated polycrystalline graphite in which the displacements are produced primarily as single interstitial-vacancy pairs, we now consider the obstacles to interstitial aggregation. The most likely obstacles are the grain boundaries the separation of which is about 10^3 Å. In fact, it has often been assumed (for example, Goggin and Reynolds¹⁷) that interstitials cannot pass through a grain boundary for temperatures of about room temperature and below. The grains define an interlayer volume of 3.4×10^{-18} cm³ and an average number of jumps of about 10^5 for an interstitial produced in this volume to reach a boundary.

(5) For an interstitial diffusing with a jump rate of 2×10^6 sec⁻¹ the vicinity of a grain boundary would be reached after 5×10^{-2} sec. This is very much shorter than the time interval between the production of interstitials in the interlayer volume by the irradiation, given by $(5.1 \times 10^5 \times 10^{-10})^{-1}$ or 2×10^4 sec.

Thus in the case of electron irradiation, defects are not produced sufficiently rapidly, within a mutually accessible volume, for diffusive interstitial aggregation to occur. We consider that the interstitials are most likely to be trapped in the vicinity of grain boundaries. Other obstacles or trapping points may be operative and similar conclusions could be drawn for dislocation trapping and impurity trapping (reactor grade A graphite may contain up to 50 p.p.m. of some impurities).

We propose, therefore, that during our irradiation at 65° C interstitial atoms become trapped singly in the vicinity of one or more of the obstacles and that interstitial-vacancy recombination does not occur at 65° C. In the region of the 200° C peak we suggest that these trapped atoms are released and that, predominantly, recombination occurs. This is expected because initially the number of accessible vacancies will be greater than the number of free interstitials. Undoubtedly a small amount of interstitial aggregation could occur. The activation energies involved in the process (a spread of about 0.2 eV around 1.38 eV from our measurements) corresponds to the spectrum of interstitial-obstacle binding energies. Our conclusion is that the 13.5 eV per atom derived from our experiment corresponds to the energy associated with interstitial-vacancy recombination in graphite. From the difference between the detrapping activation energy (1.38 eV) and the interstitial migration energy (0.45 eV) we estimate that the energy to form a Frenkel pair in graphite is $(13.5 + 0.93) \approx 14.4$ eV. (For a displacement energy of 50 eV we would find the formation energy to be 10.8 eV.)

It is interesting to note that Coulson *et al.*⁹ calculated the formation energy of a Schottky defect, in which the ejected atom is on the surface, in graphite to be 10.7 eV. If the atom had been put in an interstitial position the energy of formation would have been higher. Thus, although we should not over-emphasize the agreement, it is encouraging that the value we obtain for the stored-energy release per interstitial vacancy recombination is consistent with Coulson's calculation.

The mechanism described here does not affect the explanation put forward by Goggin and Reynolds¹⁷ of the reverse annealing peak which is found after electron irradiation below 80° K. In this case the interstitial atoms are immobile at the temperature of irradiation so that the interplanar concentration of interstitials may

grow. When the interstitials become mobile simple aggregates may be formed.

Finally, we comment on the significance of our conclusion to the interpretation of the stored-energy release in the 200° C peak in neutron-irradiated graphite. For three low-dose neutron irradiations up to 2.5×10^{18} fast n° cm⁻² Ni scale we have measured the release of stored energy in our apparatus and find, for example, 21.5 cal g⁻¹ at a dose of 2.5×10^{18} , and a mean of 0.9 cal g⁻¹ per 10^{17} fast n° cm⁻². Åstrom²³ reported 5.3 cal g⁻¹ for a thermal dose of 3.8×10^{18} and an estimated fast dose of 3.8×10^{17} (1.4 cal g⁻¹ per 10^{17} fast n°). We cannot be certain about the comparability of the fast dose estimate, and in view of this the two measurements are in reasonable agreement. For our experiment and using the damage function of Thompson and Wright²⁴ we find that the energy release corresponds to about 2 eV per displaced atom. In the reactor irradiation we expect the energy per displaced atom to be less than the value of 14 eV per interstitial-vacancy recombination discussed already because: (1) some multiple interstitial groups are expected to be produced directly by the irradiation; (2) the higher effective damage flux means that there is a much greater chance of interstitial aggregates forming by diffusion. (This is the basis of the homogeneous theory for the nucleation of dislocation loops used by Reynolds and Thrower²⁵ to account for the observed rates of growth in high neutron fluxes.)

Both these factors mean that, relative to the total number of interstitials produced, fewer single interstitials will be trapped by obstacles. Thus only a fraction of the interstitials will be a source of stored energy when they are released in the 200° C peak. Furthermore, continuing re-arrangements of the various interstitial aggregates would yield some stored-energy release on the high-temperature side of the peak. At the highest neutron fluxes the fraction of trapped single interstitials will become progressively smaller and the stored-energy release at 200° C will become a relatively smaller part of the whole.

Conclusions. The stored-energy release in electron-irradiated graphite corresponds to 13.5 eV per displaced atom. The major uncertainty in this value comes from the calculation of the defect concentration which may be too low.

It is shown that, for the electron-beam currents used, an interstitial atom moving between graphite layers could be trapped by grain boundaries, dislocations or impurities long before the production of the next interstitial by the electron in the appropriate volume (for example, interatomic layer in a crystallite). Thus interstitial aggregates will not accumulate and we interpret the measured stored-energy release in the 200° C peak as the recombination of interstitial atoms and vacancies, the interstitials being freed with a range of activation energies around 1.4 eV.

In reactor-irradiated graphite we expect that relatively fewer interstitials are trapped in this way, other interstitial formations occurring because of the higher recoil energies involved and the much higher effective damaging flux. Thus, although the same process of interstitial-vacancy recombination is assumed to be the major contribution to the stored-energy release at 200° C, the energy involved per original displacement is considerably less. This will be true particularly at high fluxes, and the fraction of the total stored energy released in the peak will become progressively smaller as irradiation flux and temperature are increased.

The proposed model therefore accounts for the qualitative features of the energy release in reactor-irradiated graphite. The value of about 14 eV per interstitial-vacancy recombination deduced from electron-irradiated graphite is consistent with the vacancy formation energy of 10.7 eV calculated by Coulson *et al.*⁹. In the latter case the ejected atom has been put on the surface and the defect formation

energy would be higher if the ejected atom remained in an interstitial position.

We wish to thank the Atomic Energy Research Establishment for a grant which enabled this work to be carried out.

- ¹ Bridge, H., Kelly, B. T., and Gray, B. S., *Proc. Fifth Carbon Conf.*, 1, 289 (Pergamon Press, Oxford, 1962).
- ² Bell, J. C., Bridge, H., Cottrell, A. H., Greenough, G. B., Reynolds, W. N., and Simmons, J. H. W., *Phil. Trans. Roy. Soc., A*, 254, 361 (1962).
- ³ Kinchin, G. H., *Proc. Intern. Conf. Peaceful Uses of Atomic Energy*, 7, 472 (U.N., New York, 1956).
- ⁴ Neubert, T. J., and Lees, R. B., *Nuclear Science and Engineering*, 2, 748 (1957).
- ⁵ Bollman, W., *Proc. Fifth Carbon Conf.*, 2, 303 (Pergamon Press, Oxford, 1963).
- ⁶ Iwata, T., and Suzuki, H., *Radiation Damage in Reactor Materials*, 565 (International Atomic Energy Agency, Vienna, 1963).
- ⁷ Knight, H. T., and Rink, J. P., *J. Chem. Phys.*, 29, 449 (1958).
- ⁸ Goldfarb, I. J., and Jaffe, H. H., *J. Chem. Phys.*, 30, 1622 (1959).
- ⁹ Coulson, C. A., Herracz, M. A., Jeal, M., Santos, E., and Senent, S., *Proc. Roy. Soc., A*, 274, 461 (1963).
- ¹⁰ Glockler, G., *J. Chem. Phys.*, 22, 159 (1954).

- ¹¹ Clarebrough, L. M., Hargreaves, M. E., Michell, D., and West, G. W., *Proc. Roy. Soc., A*, 215, 507 (1952).
- ¹² Henson, R. W., and Mounsey, J. A., *U.K.A.E.A. D.E.G. Rep.* 328(W).
- ¹³ Mitchell, E. W. J., and Sallsbury, C. (Limited circulation of folio of "Cross-Section Calculations for Displacement of Atoms by Fast Electrons"; 1933).
- ¹⁴ Lucas, M. W., and Mitchell, E. W. J., *Carbon*, 1, 345 (1964).
- ¹⁵ Mitchell, E. W. J., *Proc. Symp. Radiation Damage in Semiconductors*, 367 (Dunod, Paris, 1964).
- ¹⁶ Clark, C. D., Kemmery, P. L., and Mitchell, E. W. J., *Disc. Faraday Soc.*, No. 31, 96 (1961).
- ¹⁷ Goggin, P. R., and Reynolds, W. N., *Phil. Mag.*, 8, 265 (1963).
- ¹⁸ Lomer, J. N., Mitchell, E. W. J., and Niblett, D. H., *Radiation Damage in Solids*, 1, 205 (International Atomic Energy Agency, Vienna, 1962).
- ¹⁹ Snyder, W. S., and Neufeld, J., *Phys. Rev.*, 103, 862 (1956).
- ²⁰ Kinchin, G. H., and Pease, R. S., *Rep. Prog. Phys.*, 18, 1 (1955).
- ²¹ Lidlard, A. B., and Perrin, R. (Private communication from A.E.R.E. report, 1965).
- ²² Davies, C. B., and Mitchell, E. W. J. (to be published).
- ²³ Åström, H. U., *Ark. Fys.*, 20, 10, 161 (1961).
- ²⁴ Thompson, M. W., and Wright, S. B., *U.K.A.E.A. Rep. AERE—R. 4701* (1964).
- ²⁵ Reynolds, W. N., and Thrower, P. A., *Radiation Damage in Reactor Materials*, 553 (International Atomic Energy Agency, Vienna, 1963).

SOME CHARACTERISTICS OF TROPICAL THUNDERSTORMS

By PROF. S. V. C. AIYA

Department of Electrical Communication Engineering, Indian Institute of Science, Bangalore

THE parameters of atmospheric noise necessary for assessing its interfering effect on the reception of radio signals¹⁻³⁴ have been investigated extensively at Poona (18°31' N., 73°55' E.) and Bangalore (12°58' N., 77°35' E.). Such investigations have resulted in the development of techniques for following the activity of thunderstorms. The data collected are found to be useful for drawing inferences on the characteristics of thunderstorms.

A thunderstorm is a localized thermodynamic process in the atmosphere accompanied by electrical discharges. Its activity is ordinarily dealt with in terms of a 'thundercloud'. If the base area of a thundercloud is idealized to a circle, it could be said that the radius ordinarily lies between 10 and 20 km. The area involved is about 1,000 sq. km or less. Thunderstorms can be classified into various types, namely, local, frontal, etc. The area involved in a local storm may approximate to that associated with a single thundercloud. The area involved in the activity of a frontal type of storm is, at least, ten times larger. That is, the radius of the circle within which there is activity may be 50-100 km. In a frontal type of storm, it is just possible that several thunderclouds in several regions of the large area involved become active; but they may become active during different hours of the day. The duration of activity may vary from one thundercloud to another. At any one time of day, more than one thundercloud may be active but they may be in different stages of activity. All the possibilities indicated could be regarded as random phenomena but they may not be quite independent of each other. Hence, any investigation of the activity of a frontal storm as a whole involves the integrated study of several thunderclouds.

When a thundercloud is active, lightning occurs. This is observed to consist of a series of intermittent flashes over a period of time which may extend from a fraction of an hour to several hours. Lightning flashes are accompanied by electrical discharges, and give rise to electrostatic, induction and radiation fields. At great distances, the radiation field preponderates. Even at close distances, the electrostatic and induction fields can be ignored if measurements are taken over a narrow band at a chosen high frequency. Such measurements enable a study of lightning flashes through their radiation fields only at all distances.

Lightning flashes are known to occur inside the cloud, from cloud to cloud, from the cloud into the air, and from

the cloud to ground. Electrical discharges within the cloud constitute a very essential feature of all types of flashes and they are now known to give rise to the radiation fields at high frequencies. For most flashes, the discharge channel is, for practical purposes, approximately vertical. Some flashes, particularly those of the cloud to cloud type, have long horizontal sections; but even such flashes have been found to give rise to significant vertically polarized radiation. Hence, a simple vertical aerial can be used to pick up the high-frequency radiations from lightning flashes.

At tropical latitudes, the cloud base is ordinarily about 3 km above the mean sea-level. Cloud discharges, therefore, radiate from a height of this order. Direct ray reception of the narrow-band high-frequency radiation can, therefore, be expected for source distances up to about 300 km. This enables the activity of a local frontal storm to be followed by signals unaffected by attenuation due to propagation.

The duration of a flash, the time interval between successive flashes, and the power radiated by a flash are some of the useful parameters for this purpose. The integrated value of the radiation fields due to a flash at a predetermined distance is a measure of the power radiated by a flash. Numerical values of the parameters vary in a random way. Their distributions over a short period (a few minutes) have to be studied so that the statistically computed values can be utilized in following the activity of storms. Statistical studies have shown that the arithmetical average value of a parameter during a 5-min period is adequate. Thus, the time interval between successive flashes can be replaced by the rate of flashing, that is, the number of flashes/min, and the power radiated by a flash, by the arithmetical average value of the integrated radiation fields due to a flash for the 50 flashes of the highest intensity received during the 5-min period and so on.

The growth and decay of the activity of a thundercloud are reflected in corresponding changes of the rate of flashing. In an active thundercloud, a decaying convection cell gives rise to a new cell and this process continues until the thundercloud itself decays. Growth and decay of the cells thus produce a cyclic variation in the rate of flashing. Hence, the lifetime of a thundercloud, the lifetime of a convection cell and the number of such cells developed during the lifetime of the thundercloud, etc., can be determined from studies of the rate of flashing.

The duration of a flash varies with the growth and decay of the activity of a thundercloud and is minimal when the activity has reached its peak. The power radiated by a flash also varies but becomes maximal when the activity has reached its peak. The random variation of the integrated value of the radiation fields arising from a flash when measured at one predetermined distance lies within certain limits. Surprisingly, the range of variation due to changes of distance of the flash even for a locally active frontal storm lies within these limits. Hence, the effect of variations of distances of flashes on field-strength measurements can be neglected.

Radiation from a lightning flash, when picked up by a vertical aerial and fed to a superheterodyne receiver tuned to a high frequency, gives rise to a noise burst at the output of the detector. When measurements are carried out, the receiver is aligned to a selected bandwidth and operates with its automatic gain control switched off. By carefully adjusting the sensitivity of the receiver, it is possible to investigate exclusively the noise bursts arising from either a locally active thundercloud or a locally active frontal type of storm.

A noise burst as described is the counterpart of a lightning flash. The number of noise bursts recorded per minute is a measure of the rate of flashing. An indirect indication of the power radiated by a flash is obtained by measuring the integrated radiation fields for the duration of the corresponding noise burst. The duration of a noise burst corresponds to the duration of a flash and the time interval between successive noise bursts corresponds to the time interval between successive lightning flashes. The activity of thunderstorms can, therefore, be followed by studying the characteristics of the noise bursts arising from them.

Noise-burst measurements have been carried out in three ways, namely, by using (a) a high-sensitivity, high-fidelity level recorder^{14,21}; (b) a noise-burst field-strength meter¹; (c) a noise-burst counter²².

In order to understand the structure of a lightning flash under conditions of intermediate resolution, several records of noise bursts for different periods of time during different hours of day have been obtained using the level recorder at several frequencies. A small portion of a typical record is reproduced in Fig. 1. The background is due to the set noise and other noises of the same order. In this particular case, it is equal to a signal input to the receiver which corresponds to a field strength of $0.8 \mu\text{V/m}$. The noise-burst field strengths are tens of times greater than this. The interval between the time when a burst becomes audible and the time when it ceases to be so is the duration of the burst. This corresponds to AB in Fig. 1 for the burst marked 'P'. The interval between successive audible bursts corresponds to AC in Fig. 1 for the bursts marked 'P' and 'Q'. Data thus obtained have been utilized to investigate the distribution of the duration of lightning flashes and the time interval between them.

In the noise-burst field-strength meter, the noise bursts are fed to a semi-logarithmic amplifier with a vacuum tube voltmeter which has charging and discharging time constants of 10 and 500 msec respectively. The microammeter in this output unit is calibrated by feeding signals modulated 30 per cent by a 400 c/s note from a standard signal generator to the receiver input through a capacity equal to the aerial capacity. The results are reduced to

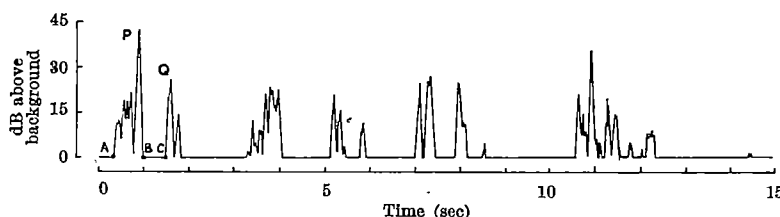


Fig. 1. Noise bursts arising from lightning flashes as recorded on March 26, 1965, between 1600 and 1700 h I.S.T. (frequency, 3 Mc/s; bandwidth, 3.2 kc/s)

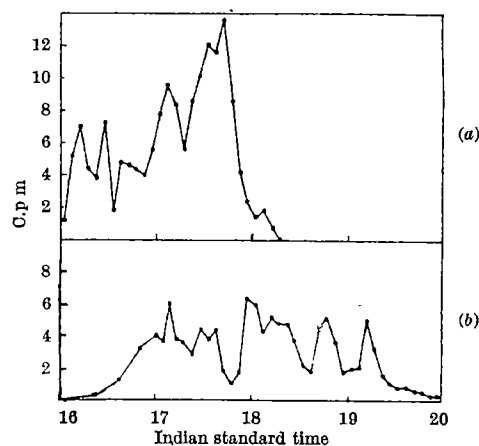


Fig. 2. Flashing characteristics as logged with 10-kc/s bandwidth, 3-Mc/s lightning flash counters. (a) Thundercloud in an overcast sky on May 4, 1962 (counter threshold, $200 \mu\text{V/m}$). (b) Isolated cloud in a clear sky on March 26, 1963 (counter threshold, $100 \mu\text{V/m}$)

equivalent field strengths in $\mu\text{V/m}$ by taking account of the effective height of the aerial. When a noise burst is received, there is a momentary kick of the pointer of the microammeter and the highest scale division it crosses is noted. This value is generally described as the quasi-peak value of the noise-burst field strength. The arithmetical average of the quasi-peak values of the ten highest noise bursts received per minute is described as the 'noise-burst level'.

The noise-burst counter, or the lightning-flash counter, is basically the noise-burst field-strength meter just described. By adjusting the sensitivity of the receiver to a predetermined threshold, it is made to pick up only the noise bursts which produce a field exceeding the threshold. The noise bursts so obtained at the detector are amplified and fed to an impulse stretcher having the same time constants as the vacuum tube voltmeter of the noise-burst field-strength meter. The output of the impulse stretcher is fed to a telephone call register which records the number of bursts received. This instrument has been used to study the variation of the rate of flashing with time.

The parameters of lightning flashes arising from a thundercloud or a thunderstorm exhibit both random and systematic variations. It is the systematic variation which reflects the growth and decay of activity. Random variations can be investigated by making the period of observation as small as possible, but this limits the available population for examining the distribution. The best compromise has been found to lie in the choice of a 5-min period. In the case of any one storm, it is found that the duration of a lightning flash, the time interval between successive flashes and the received noise-burst field-strength have a log-normal distribution. When the activity of a thundercloud is around its peak, the mean value of the duration of a flash is about 200 msec. At other times, it is larger. In the case of a frontal type of storm, the mean value of the duration is generally about 500 msec. The mean value of the noise-burst field-strength is most frequently found to be about 3 dB below the noise-burst level defined earlier.

The activity of several thunderclouds^{18,19} has been studied individually by investigating the variation of the rate of flashing with the time using the lightning-flash counter. A typical unsmoothed characteristic is shown in Fig. 2(a). A feature frequently noticed is the rather sharp fall in the activity during the decay. If the characteristics are smoothed by the method of moving averages, the real crests and troughs in the characteristics can be located. The time interval between successive crests or troughs is a measure of cell life. The cell life is found to be about the same as that reported from

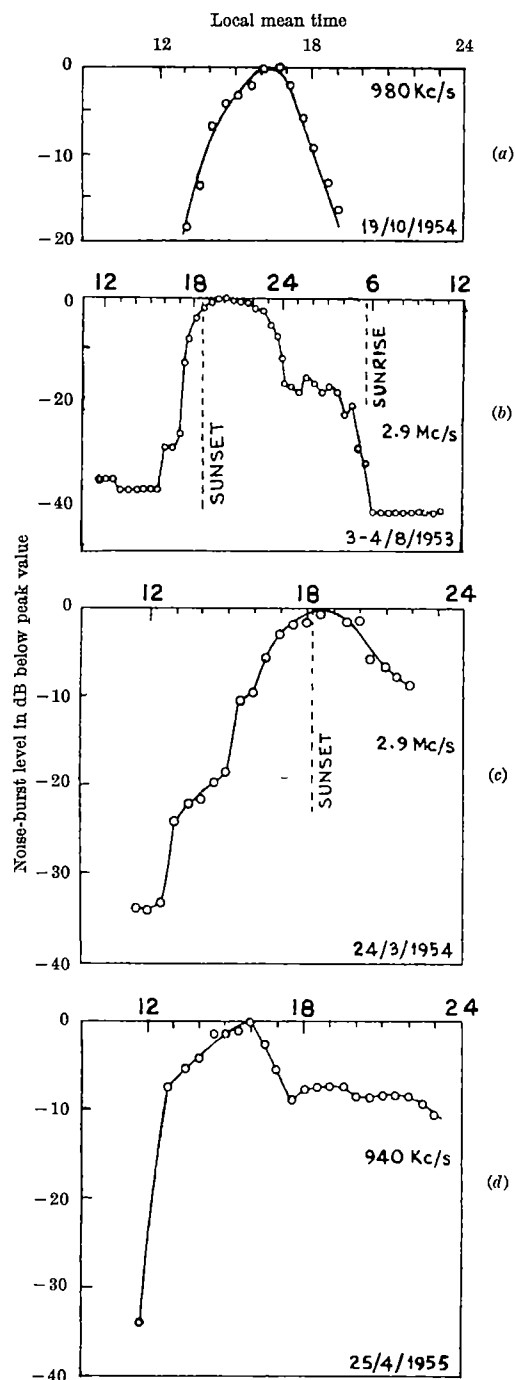


Fig. 3. Storm activity as logged with a 6-kc/s bandwidth noise-burst field-strength meter at Poona at frequencies and on dates shown inside the figure. (a) Local storm over land; (b) and (c) frontal storms over land; (d) storm over the sea.

investigations at higher latitudes, namely, about 30 min. However, the mean duration of the activity of a thundercloud and the mean value of the number of cells developed during the lifetime of a thundercloud are different, namely, about 3 h and 5 respectively. The general characteristics of a thundercloud do not appear to differ with types of storm. The peak activity of a thundercloud has been observed to occur between 1400 and 0400 h L.M.T. The number of thunderclouds showing peak activity after local sunset but before midnight is the largest. A reasonable number of thunderclouds reach their peak activity during late afternoons. At other times during the day, peak activity has not been noticed so very frequently. It must be pointed out that the distribution of the hour of peak activity over the different hours of day varies with the seasons. The

mean value of the peak rate of flashing is 8 and the average value of the rate of flashing during the lifetime of a thundercloud is 3.

There is, quite often, residual activity after the collapse of an active thundercloud. This can go on for even 4 or 5 h. Flashes can be seen and these may be accompanied by thunder also; but the rate of flashing is very low, namely, of the order of one flash in 5 min. Such residual activity of several thunderclouds in a frontal storm can contribute significantly to its rate of flashing.

Whenever the activity of a thundercloud is accompanied by thunder also, there are always some ground strokes. In about 75 per cent of the cases, the activity of thunderclouds is accompanied by thunder. Examined on a long-term basis, the percentage of flashes to the ground appears to be about 10. It has so far not been found possible to distinguish ground flashes from other types of flashes by a study of noise bursts. Attempts made even at such low frequencies as 10 kc/s have been unsuccessful.

On some warm quiet days, there can be one or two isolated clouds in an otherwise perfectly clear sky and these clouds may be at very low heights. Flashes other than ground flashes have been seen in such clouds. A typical flashing characteristic is reproduced in Fig. 2(b). The rate of flashing is invariably low and the noise-burst field-strength received is about 6 dB lower than that for a corresponding thundercloud.

In the case of frontal storms, an examination of the noise data indicates the following characteristics as probable. The activity lasts several hours. During this period, the rate of flashing increases either gradually or sharply to a maximum, remains around this value for a significant period and then decreases. For the greater portion of the duration of the activity, the rate of flashing exceeds the value of 10 and can reach the value of 40; but ordinarily the maximum value is around 25.

The activity of several frontal storms¹⁸ has been followed by studying the variation with time of the noise-burst level, which can be taken as an index of the total power radiated in the form of flashes. Typical curves are reproduced in Figs. 3 and 4. Fig. 3(a) represents a type of storm frequently observed during March-May and September-November. This may be what corresponds to a local storm as observed over tropical land masses. Fig. 3(b) corresponds to the more common one after the monsoon has set in. Fig. 3(c) corresponds to a type of storm which gradually builds up and reaches peak activity a little before sunset. It has not been possible to obtain the complete characteristics of such storms but they appear to decay gradually and last until the early hours of the morning. The storms shown in Fig. 3(b) and 3(c) appear to be typical frontal storms over land. Fig. 3(d) and Fig. 4 give typical characteristics of storms over the sea. A peculiar feature of such storms is a noticeable peak around 1600-1700 h L.M.T.

If a generalization were to be attempted on the basis of the data available, it would be as follows: the activity of a frontal storm commences at about 1200 h L.M.T. In some cases, it gradually builds up to a peak before sunset.

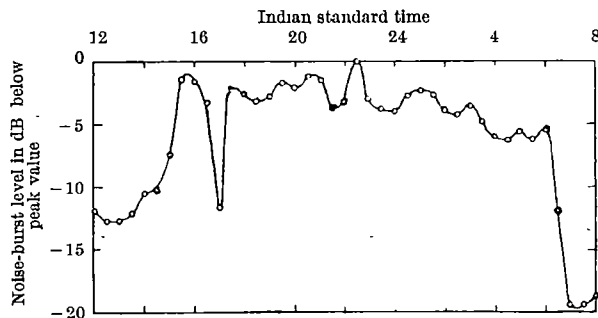


Fig. 4. Storm over the sea as logged at Bangalore on March 19-20, 1965, at 60 kc/s with a bandwidth of 1.8 kc/s.

In others, it rises sharply after about 1600 h L.M.T. and reaches a peak after sunset but before midnight. Generally, the activity is quite significant from late evening until the early hours of the following morning. The fall in activity around the hour of local sunrise is quite sharp and probably corresponds to the collapse of the storm. The activity after midnight is significantly lower than that before midnight.

The features described follow logically from the observed characteristics of thunderclouds including the distribution of their hour of peak activity over the hours of a calendar day on the basis of the description of a frontal storm in terms of thunderclouds as given earlier. The residual activity of individual thunderclouds described previously is also one of the contributing factors.

I thank many of my students for help in various parts of this work, and Sir Basil Schonland for advice.

¹ Aiya, S. V. C., *J. Atmos. Terr. Phys.*, 5, 230 (1964).

² Aiya, S. V. C., *J. Sci. Indust. Res.*, 13, A, 314 (1954).

³ Aiya, S. V. C., *Proc. Inst. Rad. Eng.*, 43, 966 (1955).

⁴ Aiya, S. V. C., *Nature*, 178, 1249 (1956).

⁵ Aiya, S. V. C., *Proc. Inst. Rad. Eng.*, 46, 580 (1958).

⁶ Aiya, S. V. C., *J. Sci. Indust. Res.*, 17, B, 337 (1958).

⁷ Aiya, S. V. C., *Proc. Inst. Rad. Eng.*, 46, 1502 (1958).

⁸ Aiya, S. V. C., *Proc. Inst. Rad. Eng.*, 47, 92 (1959).

⁹ Aiya, S. V. C., *J. Sci. Indust. Res.*, 18, B, 44 (1959).

¹⁰ Aiya, S. V. C., *Proc. Inst. Rad. Eng.*, 48, 955 (1960).

¹¹ Aiya, S. V. C., *J. Sci. Indust. Res.*, 21, D, 203 (1962).

¹² Aiya, S. V. C., and Khot, C. G., *J. Sci. Indust. Res.*, 18, B, 54 (1959).

¹³ Aiya, S. V. C., Khot, C. G., Phadke, K. R., and Sane, C. K., *J. Sci. Indust. Res.*, 14, B, 361 (1955).

¹⁴ Aiya, S. V. C., and Lakshminarayan, K. N., *NBS J. of Res. (Radio Science)* (in the press).

¹⁵ Aiya, S. V. C., Padmanabhan S. V., Phadke, K. R., and Sane, C. K., *J. Sci. Indust. Res.*, 18, B, 47 (1959).

¹⁶ Aiya, S. V. C., and Phadke, K. R., *J. Atmos. Terr. Phys.*, 7, 254 (1955).

¹⁷ Aiya, S. V. C., and Seshagiri, N., *J. Sci. Indust. Res.*, 21, D, 265 (1962).

¹⁸ Aiya, S. V. C., and Sonde, B. S., *Nature*, 200, 562 (1963).

¹⁹ Aiya, S. V. C., and Sonde, B. S., *Proc. Inst. Elec. and Electron. Eng.*, 51, 1493 (1963).

²⁰ Aiya, S. V. C., and Sonde, B. S., *Proc. Inst. Elec. and Electron. Eng.*, 52, 1062 (1964).

²¹ Gupta, S. N., Ph.D. thesis, Ind. Inst. Sci., Bangalore (1965).

²² Joglekar, P. J., Ph.D. thesis, Ind. Inst. Sci., Bangalore (1963).

²³ Lakshminarayan, K. N., *J. Sci. Indust. Res.*, 21, D, 228 (1962).

²⁴ Lakshminarayan, K. N., Ph.D. thesis, Ind. Inst. Sci., Bangalore (1964).

²⁵ Phadke, K. R., *J. Inst. Telecom. Eng.*, 1, 136 (1955).

²⁶ Satyan, M., Ph.D. thesis, Ind. Inst. Sci., Bangalore (1962).

²⁷ Satyam, M., *J. Sci. Indust. Res.*, 21, D, 221 (1962).

²⁸ Satyam, M., *J. Sci. Indust. Res.*, 21, D, 251 (1962).

²⁹ Satyam, M., *J. Sci. Indust. Res.*, 21, D, 260 (1962).

³⁰ Satyam, M., *J. Atmos. Sci.*, 19, 346 (1962).

³¹ Seshagiri, N., *Trans. Inst. Elec. and Electron. Eng.*, EC-12, 413 (1963).

³² Sonde, B. S., Ph.D. thesis, Ind. Inst. Sci., Bangalore (1962).

³³ Sonde, B. S., *Proc. Inst. Elec. and Electron. Eng.*, 51, 1501 (1963).

³⁴ Aiya, S. V. C., and Rangachar, M. J. S. (to be published).

EFFECTS OF PRONOUNCED ELECTRON-CONTENT VARIATIONS ON DOPPLER SHIFT AT LOW ELEVATIONS

By HAIM SOICHER

Institute for Exploratory Research, U.S. Army Electronics Command, Fort Monmouth, New Jersey

THE non-relativistic Doppler shift in the ionosphere is defined as the time derivative of the optical path:

$$\Delta f = \frac{f}{c} \frac{d}{dt} \int_0^{S(p)} n_i ds \quad (1)$$

where Δf is the shift of the emitted frequency f , c is the velocity of light in free outer space, and n_i is the refractive index at the element ds of the curved ray path S , which starts at the satellite position p and terminates at receiver position 0. If we assume an isotropic, time-varying ionosphere and use the high-frequency approximation for the refractive index, equation (1) becomes¹:

$$\Delta f = \frac{f}{c} \left(1 - \frac{k N_p}{f^2} \right) \frac{dS}{dt} - \frac{k}{c} \frac{1}{f} \int_0^{S(p)} \frac{\partial N_i}{\partial t} ds \quad (2)$$

where $k = 4.03 \times 10^7$ cgs units, N_p is the electron density at the satellite, N_i is the electron density along path element ds , and $\frac{dS}{dt}$ is the component of the satellite velocity along the ray path. Equation (2) may be written²:

$$\Delta f = \frac{f}{c} \frac{dS}{dt} - \frac{k}{cf} \frac{dS}{dt} \left[N_p + \frac{1}{dS/dt} \int_0^{S(p)} \frac{\partial N_i}{\partial t} ds \right] \quad (2')$$

The first term describes the Doppler shift in the absence of the ionosphere; the second term includes contribution to the frequency shift from electrons in the vicinity of the satellite and from integrated electron-density fluctuations along the ray path. An additional interpretation of equation (2) can be made. The first term in the right-hand side of equation (2) can be thought of as the contribution of the motion of the satellite and the existence of 'static' (frozen) ionosphere to the Doppler shift. The second term may be thought of as the contribution to the shift of integrated density fluctuations assuming the satellite position is fixed.

Some anomalous Doppler curves describing satellite signal reflexions from ionospheric ionization irregularities³, traversal of the transmitting satellite from a non-illuminated ionosphere into an illuminated one during ground reception⁴, and reception of beyond-the-horizon propagation mode⁵ have been previously described in the literature. However, the anomalous (though continuous) Doppler curve of 1959 Iota I (20-Mc/s beacon frequency, inclination 50°, apogee 1,100 km, perigee 550 km), orbit No. 8274, will be shown to be an example of a large contribution to the Doppler shift from the integrated-electron-density time variations.

Generally, most Doppler irregularities occur at a period when the satellite is near the horizon with respect to the receiving station, since, at this position, the ionospheric sensitive signal has its longest path within the ionosphere, and is incident on the various ionospheric layers at rather low angles. Fig. 1 shows that the Doppler shift from 01:59 to about 240 sec afterwards deviates from the shift extrapolated from the entire observed orbital shift by a

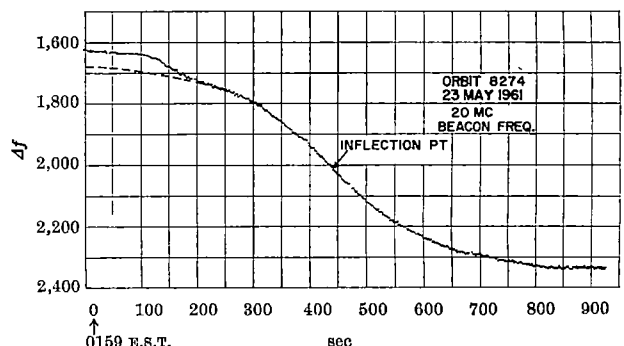


Fig. 1. Anomalous Doppler curve of satellite 1959 Iota I, orbit No. 8274, 23 May, 1961, Δf measured with reference to an injection frequency above the shifted frequency. (Note deviation of observed Doppler curve from extrapolated dashed curve during first 240 sec of orbit reception)

maximum of 50 c/s. The extrapolation that yields the 'normal' (dashed) curve in Fig. 1 was made on the assumption that the Doppler curve is nearly symmetrical with respect to the inflexion point. The orbital zenith angle varies from 85.3° at 01:59 E.S.T. to 66.30° at 02:03 E.S.T. The zenith angles are the complements of the angles the cosines of which determine the magnitude of the component of the velocity of the satellite in the direction of the ray path. However, the foregoing angles cannot deviate sufficiently to cause an anomalous shift of 50 c/s (due to refraction effects of reflexion from ionospheric irregularities). In addition, the fact that the entire satellite reception period is within the geometric horizon of the receiving station rules out beyond-the-horizon indirect reception of the signals of the satellite-ground ionosphere mode or satellite-ionospheric irregularity-ground mode (abrupt discontinuities in Doppler curve). Further, the satellite and the ray path during the time of reception were completely in a non-illuminated ionosphere, which rules out the possibility of effects due to solar illumination of the ionosphere.

Assuming that the electron density at the satellite (N_p) would be the same for a 'normal' Doppler reception as for the anomalous one (shown in Fig. 1) during the first

4 min of reception, and that the refractive effects ($\frac{dS}{dt}$) are the same for both the normal and anomalous modes, we may arrive at a possible mechanism for the observed anomaly. Using equation (2) for the observed and 'normal' shifts, at the time of maximum frequency shift deviation, we obtain:

$$\Delta f_{\text{observed}} - \Delta f_{\text{normal}} = +50 \text{ c/s} = -\frac{k}{cf} \left[\int_{\text{observed}} \frac{\partial N_i}{\partial t} ds - \int_{\text{normal}} \frac{\partial N_i}{\partial t} ds \right] = -\frac{k}{cf} \delta \left[\int_0^{S(v)} \frac{\partial N_i}{\partial t} ds \right] \quad (3)$$

Now, because in this case the frequency deviation is a positive quantity it follows that:

$$\left[\int_{\text{observed}} \frac{\partial N_i}{\partial t} ds - \int_{\text{normal}} \frac{\partial N_i}{\partial t} ds \right]$$

is a negative quantity. The sign of the foregoing integrals is obtained from the continuity equation:

$$\frac{\partial N}{\partial t} = q - L - T$$

where q is the production of electrons term, L is the loss of electrons term and T is the transport term. At night the production term is non-existent. The integrated $L + T$ term, when one considers only vertical transport which is effective mostly at altitudes below that of the satellite, is dominated by the loss term. It follows that in this particular night orbit the integrated electron variation is dominated by the rate of disappearance of electrons due

to attachment and recombination processes and hence is negative. It further follows that:

$$\int_{\text{observed}} \frac{\partial N_i}{\partial t} ds$$

must be more negative than:

$$\int_{\text{normal}} \frac{\partial N_i}{\partial t} ds$$

in order to yield a negative difference. It is concluded, therefore, that the observed rate of loss of electrons along the path is steeper than the one normally expected. One solves for:

$$\delta \left[\int \frac{\partial N_i}{\partial t} ds \right]$$

in equation (3) and concludes that the variation of the electron-density fluctuations along the ray path during the anomalous portion of the Doppler curve is 7.5×10^{11} electrons/cm² sec. This value is an order of magnitude higher than the 'average' change in electron-content fluctuations (frequency scintillations) obtained by Arendt⁶ for data of 1959 *Iota* 1 from March 13 to August 2, 1961. However, Arendt's data, which are based on the average of the root mean square deviations (± 2.54 c/s on 20 Mc/s) of observed Doppler-shift data points with respect to a best fitting curve, do not represent an anomalous Doppler behaviour, but merely point to the uncertainty in precise frequency determination of an ionospherically passing signal.

The termination of the Doppler anomaly at 02:03 need not indicate the end of the electron-content fluctuation. It merely indicates that, with the increase in the angle between ray path and satellite, the first term in equation (2, 2') becomes dominant and the Doppler curve assumes its undisturbed shape. The electron-content fluctuations become the deviations of the observed data points from a best fitting curve. (Note scintillations between 200 and 300 sec after start of orbit in Fig. 1.) At still higher elevations the first term of equation (2, 2') becomes more dominant, and the signal is incident perpendicularly to the various ionospheric layers so that the scintillations become minimal. (Note smooth curve in the vicinity of the inflexion point.)

In conclusion, the observed Doppler anomaly at low elevation is caused by a substantial decrease of the electron density along the ray path. At higher elevation, the Doppler shift is dominated by other factors which overshadow the effects of the density enhancement.

I thank Dr. P. R. Arendt of the Institute for Exploratory Research for advice.

¹ Arendt, P. R., *Proc. Intern. Conf. on the Ionosphere*, London, July 1962, The Inst. of Physics and the Physical Soc., 331 (1962).

² Kelso, J. M., *J. Geophys. Res.*, **65**, 12 (1960).

³ Stanesby, H., *Proc. Inst. Elec. Eng.*, **B**, 105 (Mar 1958).

⁴ Arendt, P. R., and Solcher, H., *Proc. Inst. Elec. Electron. Eng* (Correspondence), **51**, 375 (1963).

⁵ Solcher, H., *Proc. Inst. Elec. Electron. Eng.*, **53**, 405 (1965).

⁶ Arendt, P. R., *Natural Tolerance Limits of Doppler Measurements of Outer-space Radio Signals*, U.S.A.S.R.D.L. Tech. Rpt. 2253 (Feb. 1962).

FRACTIONATION OF RIBONUCLEIC ACIDS IN THE ZONAL ULTRACENTRIFUGE

By DR J. R. B. HASTINGS, DR J. H. PARISH, DR K. S. KIRBY and E. S. KLUCIS

Chester Beatty Research Institute, Institute of Cancer Research, Royal Cancer Hospital, Fulham Road, London, S.W.3

RIBOSOMAL RNA is recognized to have two major components of sedimentation constants about 28s and 18s when the RNA is of mammalian origin¹, while from bacteria the values are a little lower: 23s and 16s. These main components have been isolated in small quantities; but since it is clear that RNA precursors are incorporated into all classes of RNA separated by sucrose

gradient centrifugation, it is important to develop methods whereby recovery of several fractions can be achieved for investigating their physico-chemical properties and bio-synthetic potentialities. We have already shown that RNA from rat liver separated with the rapidly labelled RNA² (method 2) will stimulate the incorporation of amino-acids in a cell-free system (Wilkinson and Kirby,

to be published) and also that the patterns of mRNA on countercurrent distribution are altered: (1) by the action of hormones³, and (2) by carcinogens⁴. Differences were more readily shown by countercurrent distribution than by sucrose density gradient centrifugation.

We have recently had available the Anderson zonal ultracentrifuge⁵ and have found that the separations by sucrose density centrifugation on this instrument are better than on the Spinco model L centrifuge. A much greater quantity of material can be used and recovery at various points along the gradient is good, giving 75–85 per cent total recovery. Moreover, the material from the main peaks is undenatured as, after recovery and drying, it can be centrifuged again to the same position.

Generally, 30–80 mg RNA were dissolved in 0.1 (or 0.15 M) M NaOAc with 2.5 per cent sucrose and centrifuged in a sucrose gradient (5–20 per cent) (0.1 or 0.15 M NaOAc) at 40,000 r.p.m./6 h. The gradients have been based on our experience with separations on the Spinco model L or the M.S.E. 'Superspeed 50' centrifuges.

Fractions (40 ml.) were collected and the optical density recorded automatically at 280 m μ . Fractions were pooled as shown in the figures and RNA was recovered by making the aqueous phase about 10 per cent with respect to NaOAc at pH 6.5 and then adding 'AnalaR' acetone (about 1.5–2 vol.) until two phases are just formed. After standing at 0°/18 h RNA is precipitated in the very small volume of lower phase, from which it can be recovered, washed with ethanol, water, sodium acetate (75:25:2), pH 6.5, and dried.

The radioactive material was counted in a liquid scintillation counter. After suitably diluting an aliquot of the fraction from the sucrose gradient with water so that [Na⁺] was 0.05 M or less, a mixture of butanol, diethyl-dihexylamine and acetic acid was added which extracted all the RNA into the organic phase and this was added directly to the scintillation fluid for counting.

RNA from rat liver. Fig. 1 shows the optical density and radioactive pattern of RNA (34 mg) isolated from rat liver² (method 2) 20 min after injecting 200 μ c. orotic acid/rat. Five fractions were recovered as indicated on the diagram with a total recovery of 85 per cent. The RNA suffered no denaturation or degradation in the density gradient centrifugation or in the process of recovery and drying since dissolution of fraction 1 and of fraction 3 and centrifuging these materials again on sucrose density gradients showed that these fractions corresponded exactly with the 18s and 28s components (Fig. 2). The material from fraction 2 was not a mixture of these components but always contained material of intermediate sedimentation values (Fig. 3). A small additional peak or shoulder in the optical density pattern of the unfractionated material was occasionally observed in the trough between 18s and 28s. The fraction of highest sedimentation values (fraction 5) contained some

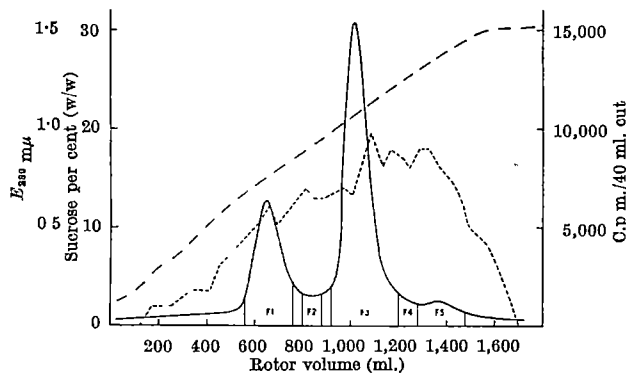


Fig. 1. Ribosomal RNA from normal rat liver, 20 per cent protein diet, 34 mg RNA dissolved initially in 40 ml. of 0.1 M sodium acetate pH 6.5/2.5 per cent w/v sucrose/25° C. Centrifuged 6 h/40,000 r.p.m. ZU-B IV rotor. —, Optical density at 280 m μ ; ---, 7 c.p.m./40 ml. fraction; ·····, sucrose gradient at end of run. Direction of centrifugation: left to right

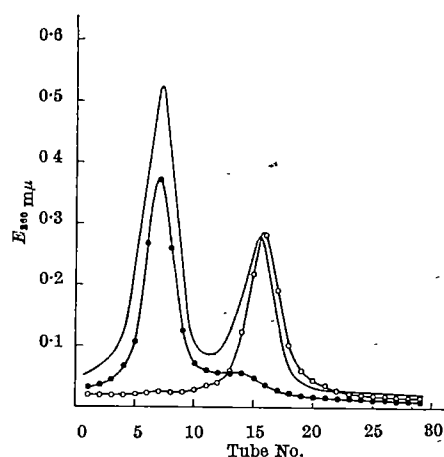


Fig. 2. Ribosomal RNA from normal rat liver. Re-run of fractions 1 and 3 (see Fig. 1) in parallel with unfractionated RNA. Samples dissolved in 0.1 M sodium acetate pH 6.5/1.5 h/25° C and centrifuged (5–20 per cent w/v sucrose gradients) on Spinco model L, S.W.25 for 13 h/22,500 r.p.m./4° C. —, Unfractionated ribosomal RNA (0.8 mg); - - - - - fraction 1 (0.3 mg.); ····· fraction 3 (0.3 mg.). Direction of centrifugation: right to left

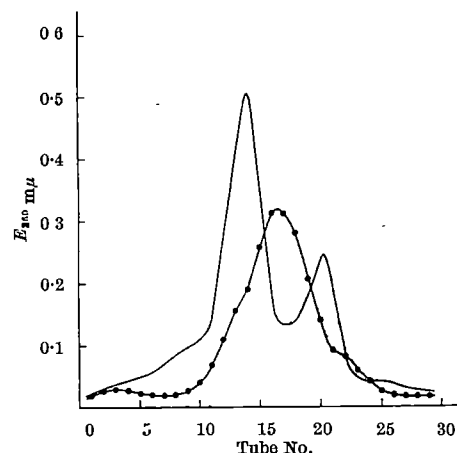


Fig. 3. Ribosomal RNA from normal rat liver. Re-run of fraction 2 (see Fig. 1) in parallel with unfractionated RNA. Samples dissolved in 0.1 M sodium acetate pH 6.5/1.5 h/25° C and centrifuged (5–20 per cent w/v sucrose gradients) on Spinco model L, S.W.25 for 13.5 h/21,000 r.p.m./4° C. —, Unfractionated ribosomal RNA (0.8 mg); - - - - - fraction 2 (0.7 mg). Direction of centrifugation: right to left

28s and 18s and the nature of this material will be considered in the fractionation of the rRNA from *E. coli*.

In each case when a fraction was dissolved and re-centrifuged on the Spinco model L centrifuge a sample of the original, unfractionated material was centrifuged at the same time. The optical density pattern of this material is included in the same graph on which the optical density and radioactive patterns of the various fractions have been drawn.

Previous experiments have shown that hormones and carcinogens can alter the pattern of rapidly labelled RNA isolated from liver after injection of tritiated orotic acid. The changes in patterns were marked in comparisons made by countercurrent distribution but much less by centrifugation in sucrose density gradients. Because of the improved resolution of ribosomal RNA in optical density patterns we have investigated the separation of rapidly labelled RNA in sucrose density gradients by the zonal ultracentrifuge. Fig. 4 shows the optical density and radioactive patterns of RNA from livers of rats which had been on a diet of thioacetamide⁶ for 31 weeks, while Fig. 5 shows those of RNA extracted from liver 60 min after an injection of insulin (20 units/rat). The results confirm the previous experiments, which indicated that the pattern of rapidly labelled RNA was altered by feeding 4-fluoro-4'-dimethylaminoazobenzene (a liver carcinogen) for a protracted period and by various hor-

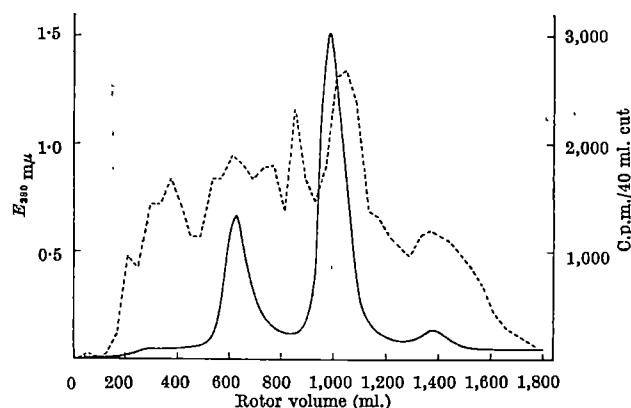


Fig. 4. Ribosomal RNA from thioacetamide-treated rat liver. 40 mg under conditions as in Fig. 1. —, Optical density at 280 mμ; ---, c.p.m./40 ml. fraction. 300 μl. tritiated uridine/rat. Direction of centrifugation: left to right

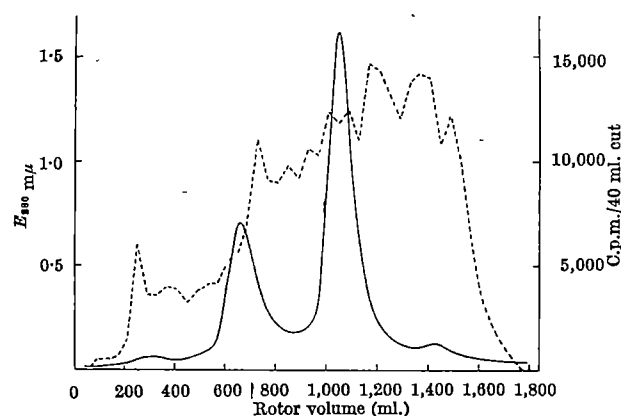


Fig. 5. Ribosomal RNA from insulin-treated rat liver. 40 mg under conditions as in Fig. 1. —, Optical density at 280 mμ; ---, c.p.m./40 ml. fraction. Direction of centrifugation: left to right

mones after relatively short periods. The present method has the advantage that good recoveries make several other investigations possible.

RNA from *E. coli* was isolated from bacteria in the exponential growth phase by extraction with 4-amino-salicylate, triisopropyl-naphthalene sulphonate and a phenol/cresol mixture. RNA was recovered after precipitating DNA with *m*-cresol (method to be published). The material used consisted of 40 mg unlabelled RNA and 5 mg of RNA which had been labelled for 20 sec with tritiated uridine. The optical density patterns of the labelled and unlabelled RNA, centrifuged separately on the Spenco model L centrifuge, were previously shown to be identical. Fig. 6 shows the radioactive and optical density patterns and the mode of fractionation of this material. The recoveries in Table 1 may be compared with those from rat liver RNA. It should be noted that RNA can be effectively recovered even when present in minimal concentration in a region of the sucrose gradient.

Table 1. RECOVERY OF RNA AFTER FRACTIONATION IN THE ZONAL ULTRA-CENTRIFUGE

RNA precipitated by addition of sodium acetate and acetone. Two ml. from each fraction of 40 ml. in the *E. coli* run was used for determination of radioactivity

	Fraction No.	Fraction of rotor effluent (ml.)	RNA (mg)
<i>E. coli</i> (Fig. 6)	1	280-480	2.1
	2	480-640	1.5
	3	640-760	8.0
	4	760-880	4.9
	5	880-1,040	12.6
	6	1,040-1,200	3.0
	7	1,200-1,360	1.9
	Combined recovery = 80 per cent		
Rat liver (Fig. 1)	1	560-760	7.0
	2	800-880	1.2
	3	920-1,200	17.2
	4	1,200-1,280	1.0
	5	1,280-1,480	2.5
	Combined recovery = 85 per cent		

Re-centrifugation of fractions 3 and 5 showed that these were almost pure 16s and 23s components and the radioactive pattern followed the optical density pattern almost exactly. Fraction 4, from the trough between the two ribosomal RNA peaks, differed from the corresponding fraction from rat liver RNA in that the optical density pattern showed the major component to be 16s RNA with a small amount of 23s RNA also present (Fig. 7). The radioactivity followed a similar pattern but was displaced to the heavy side of the optical density pattern. An analogous material was obtained from fraction 6, except that the major component was 23s RNA; the radioactive material again appeared on the heavy side of the optical density pattern.

Fractions 1 and 2 were of interest as they contained a considerable amount of RNA of high specific activity, which was present in two main fractions (A and B). Monier *et al.*⁷ have also reported material of high specific activity from *E. coli* in the 16s-4s region.

The pattern obtained by centrifuging the RNA from fraction 2 is shown in Fig. 8. It will be noted that the optical density pattern is composite and contains some 16s RNA in addition to other lighter components. The radioactive pattern shows that at least three components are present (if the 4s RNA is included). As the radioactive pattern of the 16s RNA from fraction 3 follows almost exactly that of the optical density, composite curves have been included in Fig. 8 on the assumption that the relative specific activity of the 16s and 4s RNAs was 1 and that of components A and B was 6. The curves are good evidence for the presence of these two components of this relative specific activity. Further support was

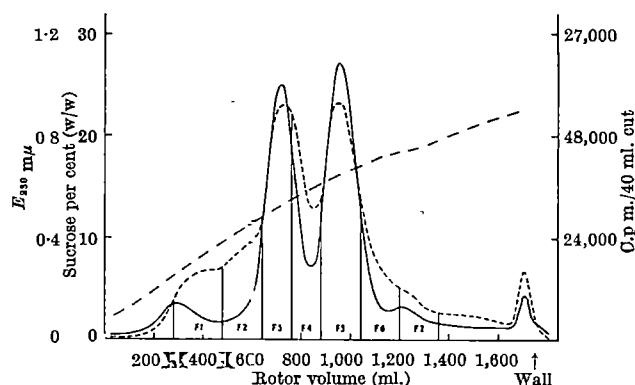


Fig. 6. RNA from *E. coli*. 45 mg dissolved initially in 40 ml. of 0.15 M sodium acetate pH 6.5/2.5 per cent w/v sucrose/1.5 h/2° C. Centrifuged 6 h/40,000 r.p.m. ZU-B IV rotor. —, Optical density at 280 mμ; ---, c.p.m./40 ml. fraction; ·····, sucrose gradient at end of run. Direction of centrifugation: left to right

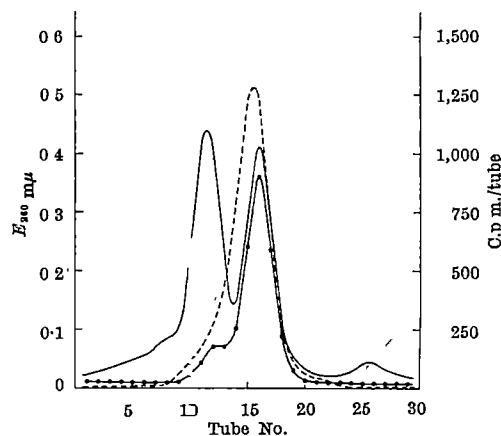


Fig. 7. RNA from *E. coli*. Re-run of fraction 4 (see Fig. 6) in parallel with unfractionated RNA. Samples dissolved in 0.15 M sodium acetate/1.5 h/2° C and centrifuged (5-20 per cent w/v sucrose gradients) on Spenco model L, S W. 25 for 14 h/25,000 r.p.m./4° C. —, Unfractionated RNA (0.8 mg), optical density; - - - - -, fraction 4 (0.4 mg), optical density; ·····, fraction 4, c.p.m. Direction of centrifugation: right to left

obtained by similarly analysing the patterns obtained by centrifugation of fraction 1 (Fig. 9). It will be seen that this fraction contains only a small amount of component B and the bulk of component A is present in fraction 1. It is of considerable significance that, in addition to detecting components A and B by radioactivity, their presence is quite clear from the composite optical density tracings.

The RNA from fraction 7 probably corresponds to the rat liver RNA fraction 5. The re-centrifugation of the *E. coli* material is shown in Fig. 10. It will be noted that the pattern obtained depends on the temperature of dissolution of the fraction. The ratio of the absorbance 260 m μ /absorbance 280 m μ was 2.15 for fraction 7 and

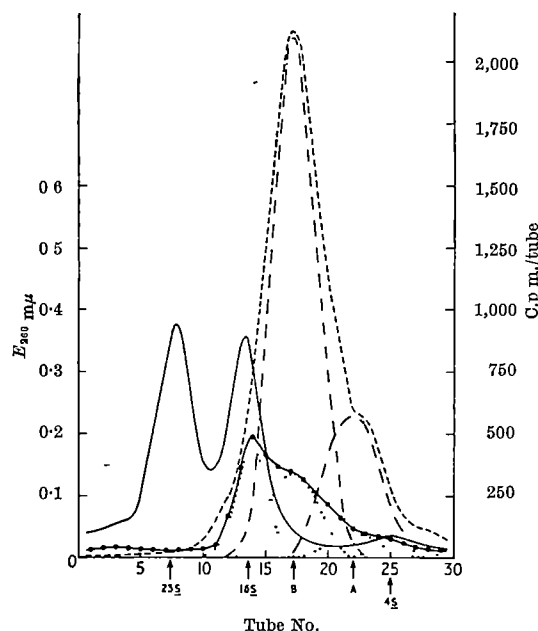


Fig. 8. RNA from *E. coli*. Re-run of fraction 2 (see Fig. 6) in parallel with unfractionated RNA. Samples dissolved in 0.15 M sodium acetate/1.5 h/2° C and centrifuged (5–20 per cent w/v sucrose gradients) on Spinco model L, S.W. 25 for 18.5 h/22,500 r.p.m./4° C. —, Unfractionated RNA (0.7 mg), optical density; —●—●—, fraction 2 (0.5 mg) optical density; ····, fraction 2, composite optical density curves; —·—·—, fraction 2, c.p.m. curves for sub-components A and B. Direction of centrifugation: right to left

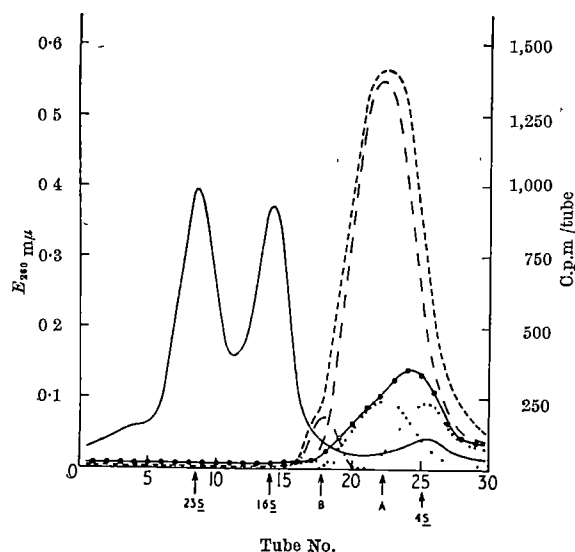


Fig. 9. RNA from *E. coli*. Re-run of fraction 1 (see Fig. 6) in parallel with unfractionated RNA. Samples dissolved in 0.15 M sodium acetate/1.5 h/2° C and centrifuged (5–20 per cent w/v sucrose gradients) on Spinco model L, S.W. 25 for 14 h/24,500 r.p.m./4° C. —, Unfractionated RNA (0.7 mg), optical density; —●—●—, fraction 1 (0.5 mg), optical density; ····, fraction 1, composite optical density curves; —·—·—, fraction 1, c.p.m. curves for sub-components A and B. Direction of centrifugation: right to left

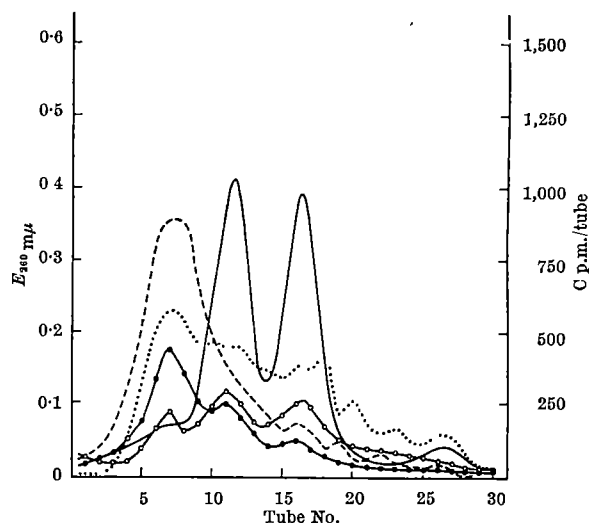


Fig. 10. RNA from *E. coli*. Re-run of fraction 7 (see Fig. 6) in parallel with unfractionated RNA. Samples dissolved in 0.15 M sodium acetate and centrifuged (5–20 per cent w/v sucrose gradients) on Spinco model L, S.W. 25 for 13.5 h/24,000 r.p.m./4° C. —, Unfractionated RNA (0.7 mg), optical density, dissolved 1.5 h/2° C; —●—●—, fraction 7 (0.5 mg), optical density, dissolved 1.5 h/2° C; —·—·—, fraction 7, c.p.m., dissolved 1.5 h/2° C; —○—○—○—, fraction 7 (0.5 mg), optical density, dissolved 1.25 h/2° C, then 0.25 h/25° C; ····, fraction 7, c.p.m., dissolved 1.25 h/2° C, then 0.25 h/25° C. Direction of centrifugation: right to left

also for material at the heavy peak ($\sim 35s$) after re-sedimenting. It is most unlikely then that any protein is present. Since the material gives a greater proportion of components corresponding exactly to the 23s and 16s RNA on dissolution at a higher temperature, it is possible that this heavy fraction (35–40s) may be a deproteinized ribosome and the high specific activity associated with it may indicate that mRNA (or segments of mRNA) also remains bound to this 'skeleton'. Mammalian RNA from the fraction 5 region (Fig. 1) showed a similar tendency to dissociate on redissolution into material sedimenting principally at 18s and 28s.

It is possible then that one factor in the association of the 30s and 50s ribosomal segments could be complementary sequences on the 23s (or 28s) and 16s (or 18s) RNAs, but further work will be required to clarify this point.

Ishihama *et al.*⁸ were able to separate three significant fractions of rapidly labelled RNA from *E. coli* by chromatography on a column of methylated albumin-kieselguhr. Their fraction IV had a peak about 35s on centrifugation in a sucrose gradient and is possibly the same as our fraction 7 (*E. coli*). This fraction was absent when the cells were grown in the presence of chloramphenicol, and it was suggested that the antibiotic may have inhibited the biosynthesis of fraction IV. We would suggest that in the absence of protein biosynthesis incomplete ribosomes (chloramphenicol particles⁹) are made and, in this condition, are unable to bind rapidly labelled RNA (mRNA). Their fractions II and III are not identical to ours but, judging from their sedimentation on gradient centrifugation, are equivalent to part of our fractions 1 + 2 and 2 + 4, respectively.

Scherrer and Darnell¹⁰ and Steele, Okamura and Busch¹¹ have also demonstrated the presence of RNA sedimenting about 35s–45s. Darnell *et al.*¹² were of the opinion that their material from HeLa cells was mainly ribosomal precursor: it had a base composition similar to that of ribosomal RNA and showed only a small degree of hybridization with DNA. It should be noted that after isolating the RNA from HeLa cell polysomes the material with highest specific activity was in the 4s–18s region of the gradient. In addition to 35s material faster sedimenting RNA was present in the extracts from HeLa cells¹⁰ and liver⁹ and, on the analogy that the 35s fraction is 28s + 18s RNA + mRNA, it is possible this 45s fraction consists of deproteinized polysomes.

It is certain therefore that, in addition to the separation of the main components, other fractions of RNA, some of which may be composite, can be isolated by this method, although further characterizations are required to clarify the relationships of the components.

The success of the present method is dependent on the stability of our RNA preparations (in no instance is bentonite required to prevent degradation) and on the ability of the zonal ultracentrifuge to deal with great quantities of RNA and yield better resolution than the standard sucrose density centrifugation.

We thank Prof. A. Haddow for his interest, Dr. N. G. Anderson for making the zonal ultracentrifuge available, and Dr. P. Alexander for co-operation in its use. We also thank Mr. E. A. Cook and Mrs. E. L. Fox-Carter for their assistance, and the Medical Research Council for a grant in support of this work.

This investigation was supported by grants to the Chester Beatty Research Institute (Institute of Cancer

Research: Royal Cancer Hospital) from the Medical Research Council and the British Empire Cancer Campaign for Research, and by research grant CA-03188-08 from the National Cancer Institution, U.S. Public Health Service.

¹ Hall, B. D., and Doty, P., *J. Mol. Biol.*, **1**, 959 (1959).

² Kirby, K. S., *Biochem. J.*, **96**, 286 (1965).

³ Kidson, C. S., and Kirby, K. S., *Nature*, **203**, 599 (1964).

⁴ Kidson, C. S., and Kirby, K. S., *Cancer Res.*, **25**, 472 (1965).

⁵ Anderson, N. G., *J. Phys. Chem.*, **66**, 1984 (1962).

⁶ Fitzhugh, O. G., and Nelsa, A. A., *Science*, **108**, 626 (1948).

⁷ Monier, R., Naono, S., Hayes, D., Hayes, F., and Gros, F., *J. Mol. Biol.*, **5**, 311 (1962).

⁸ Ishihama, A., Mizuno, N., Takai, M., Otaka, E., and Osawa, S., *J. Mol. Biol.*, **5**, 251 (1962).

⁹ Nomura, M., and Watson, J. D., *J. Mol. Biol.*, **1**, 204 (1959).

¹⁰ Scherrer, K., and Darnell, J. E., *Biochem. Biophys. Res. Commun.*, **7**, 486 (1962).

¹¹ Steele, W. J., Okamura, N., and Busch, H., *J. Biol. Chem.*, **246**, 1742 (1965).

¹² Darnell, J. E., Penman, S., Scherrer, K., and Becker, Y., *Cold Spring Harbor Symp. Quant. Biol.*, **28**, 211 (1963).

EFFECT OF STEROIDS AND 'TRITON X-100' ON GLUCOSE-FILLED PHOSPHOLIPID/CHOLESTEROL STRUCTURES

By DR. GERALD WEISSMANN, DR. GRAZIA SESSA and DR. SIEGMUND WEISSMANN

Department of Medicine, New York University School of Medicine, and Materials Research Laboratory, College of Engineering, Rutgers, The State University, New Brunswick, New Jersey

IT has previously been reported that pharmacologically active steroids affect the membranes of lysosomes, mitochondria, and erythrocytes¹⁻⁵. In each of these systems, steroids such as deoxycorticosterone (DOC), etiocholanolone, or progesterone, which do not possess oxygen functions in the 11 or 17 position, appear to disrupt the surfaces of cells or their organelles. In direct contrast, cortisol, cortisone, and their acetates appear to protect membrane-bounded structures against disruption by a variety of agents. Because this property of steroids might account, at least in part, for their pharmacological or physiological activity, the interaction of steroids with lipids common to several biological membranes was examined.

Bangham, Standish and Watkins⁶ have shown that purified phospholipids swell spontaneously in aqueous salt solutions to form liquid crystals, which behave as salt-containing compartments limited by discrete membranes. Such structures appeared as spherulites composed of concentric lamellae, each of which assumed the configuration of a bimolecular leaflet. The spherulites were shown to exhibit permeability characteristics for simple univalent cations, anions, and water, which were qualitatively similar to those occurring across biological membranes. Utilizing such artificial phospholipid structures, we have previously shown that steroids which disrupt lysosomes and other membrane-bounded bodies increase the leakage of trapped cations from their model counterparts⁷. Furthermore, cortisone, cortisol, and their acetates appeared to retard leakage of cations from the artificial structures. Other membrane-disruptive agents such as 'Streptolysin S' and lysolecithin also increased leakage of cations from the spherulites; negatively stained preparations of streptolysin- or lysolecithin-treated spherulites showed that the concentric lamellae had been visibly disrupted⁷.

This report indicates that glucose, like small anions and cations, may be sequestered within artificial phospholipid/cholesterol spherulites, and that membrane-active steroids can modify the permeability of these structures to glucose. Such functional alterations were accompanied by drastic changes in the morphology of the spherulites, viewed by negative-staining techniques in the electron microscope. The action of steroids on these model structures will be

contrasted with changes induced by a non-ionic detergent 'Triton X-100', an alkylphenoxo, poly-ethoxy, surfactant which has previously been shown capable of totally disrupting lysosomes, mitochondria, and erythrocytes¹⁻⁴.

The phospholipid/cholesterol structures, carrying a net negative membrane charge imposed by the anion dicetylphosphate, were prepared by a modification of procedures described in detail elsewhere^{6,7}. Ovolecithin was obtained from General Biochemicals, Chagrin Falls, Ohio, eluted from silicic acid columns in chloroform, and was shown to be free of trace contaminants by thin-layer chromatography. Cholesterol was obtained from Nutritional Biochemicals, Cleveland, Ohio, dicetylphosphate from K and K Biochemicals, Plainview, New York, and steroids obtained from Steraloids, Inc., Pawling, New York. 'Triton X-100' was the gift of Rohm and Haas, Philadelphia, and 'Streptolysin S' of Dr. A. W. Berheimer, New York University.

For each set of experiments, 60 μ moles of ovolecithin, 17.16 μ moles of DCP, and 8.58 μ moles of cholesterol were added in chloroform to a 100-ml. round-bottomed flask. The contents were evaporated in a rotary vacuum-pump apparatus so that the dried lipid was evenly distributed over the lower surface of the vessel. Finally, 6 ml. of glucose solution (0.145 M) were added to the flask, and the lipids were dispersed in the aqueous medium by anchoring the covered flask in contact with a vibrating mixer ('Cyclo-mixer', Clay-Adams Co., New York City) so that small drops of the dispersion were constantly thrown against the side of the flask during vibration (10 min, 23°C). Thereafter, the lipid structures were permitted to complete their spontaneous swelling for 18 h at 4°C. After a second period of vibration (5 min), the lipid particles, which were suspended in aqueous glucose, but now also containing glucose that had been sequestered within lipid lamellae, were placed into Visking dialysis tubing. They were first dialysed for 30 min at 23°C against 900 ml. of an equimolar mixture of NaCl/KCl (total molarity 0.145 M), and dialysis was continued against fresh salt solution until the last dialysate was free of detectable glucose. Usually four, hourly, changes of 900 ml. each were sufficient. One-ml. portions of the dialysed spherulites, now containing only that glucose which had been sequestered, were placed into five.

separate, small dialysis sacs. Test steroids, dissolved in 0.05 ml. of ethanol, or 0.05 ml. of ethanol alone, were dispensed into the sacs and immediately mixed with the suspended spherulites by inversion. To some suspensions, 0.2 ml. of 'Triton X-100' in NaCl/KCl was added to a final concentration of 0.1 per cent v/v. 'Streptolysin S' was dissolved in 0.1 ml. of NaCl/KCl. The sacs were tied, placed into small, narrow-bore test-tubes containing 5 ml. of NaCl/KCl solution, and shaken in a water bath maintained at 37° C. At intervals noted below, the sacs were transferred to fresh test-tubes also filled with NaCl/KCl and the glucose which had escaped through the dialysis casing from the spherulites during these 'leak' periods was determined. After each experiment, aliquots, each of 900-ml. dialysate, of the 'leak' fluids, and of the suspension remaining in the sacs, were analysed for glucose by the glucose oxidase method⁸ ('Glucostat', Worthington Biochemicals, Freehold, New Jersey). Suspensions remaining in the sacs were, however, first boiled for 20 min to destroy the spherulites, centrifuged at 20,000g to remove lipid debris, and the clear supernatants were analysed. In six sets of experiments, total recoveries of added glucose ranged from 98 to 107 per cent. An average of 43.8 per cent of added glucose was trapped within the spherulites after the last 900-ml. dialysis; therefore for 10 μ moles of phospholipid (present in each test suspension) approximately 80 μ moles of glucose were sequestered within the structures.

In Table 1 are shown the results of experiments in which 5-mM concentrations of steroids altered the leakage of glucose from the lipid spherulites. DOC, etiocholanolone, and progesterone, which disrupt naturally occurring membranes (lysosomes, mitochondria, erythrocytes) augmented the leakage of glucose. In contrast, cortisol acetate, which appears to protect biological membranes, diminished the release of glucose from the model structures. In no case did leakage induced by the steroids approach that induced by the non-ionic detergent, 'Triton X-100', which totally disrupts natural membranes. 'Streptolysin S', a bacterial product which lyses cells or their organelles⁹, induced leakage of glucose to roughly the same extent as did the steroids. Fig. 1 shows that leakage was progressive with time, and shows that etiocholanolone-induced release of glucose was relatively delayed compared to DOC, a finding that has since been confirmed for other marker molecules such as phosphate, chromate, and glycine¹⁰.

In an extension of these findings, to be reported in detail elsewhere¹⁰, we have tested the possibility that the interaction of steroids with lipid-bounded structures might regulate enzyme action. Glucose-laden phospholipid cholesterol spherulites were placed in dialysis sacs as stated. However, the sacs were allowed to 'leak' into test-tubes containing not only 0.145 M NaCl/KCl solution, but also the enzymes glucose oxidase, peroxidase, and chromogen. Accelerated formation of reaction product (compared to controls) was seen in tubes with sacs of spherulites to which 'Triton', DOC, or etiocholanolone had been added, as the medium became coloured by chromogen. Tubes to which cortisol acetate had been added showed a delay in the development of colour. These experiments indicated

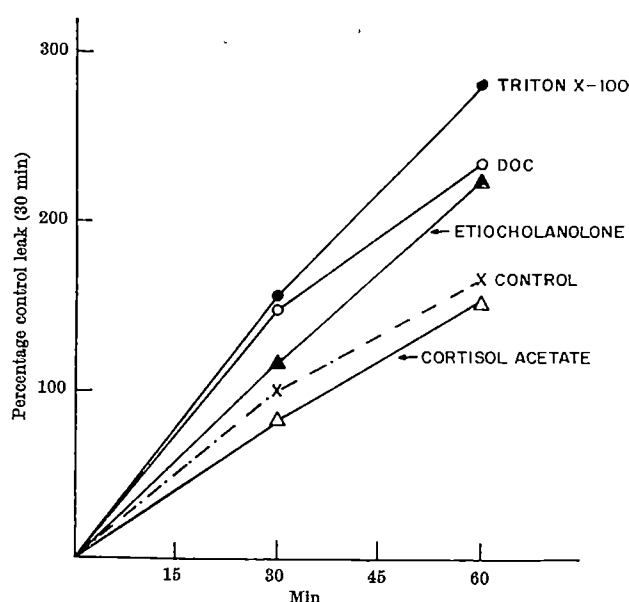


Fig. 1. Release of glucose from artificial phospholipid/cholesterol structures. Results expressed as percentage released from control suspensions at 30 min (10.9 μ moles/ml. suspension). Steroids added at 5 mM concentration, 'Triton X-100' 0.1 per cent v/v

that steroids might regulate enzyme action by modifying the rate at which substrate was made available to the enzyme.

However suggestive these functional studies might be, it was still possible that steroids influenced the attachment or binding of glucose to the lipid membranes, without actually altering their structure or spatial disposition. Therefore the spherulites were studied by negative-staining techniques in the electron microscope in order to identify any possible morphological consequences of steroid action. Aliquots of the spherulite suspensions, prepared as already described, but before dialysis, were exposed to steroids or 'Triton X-100' for 20 min at 23° C. They were kept at 4° C for 3 h (to permit transport), mixed with an equal volume of 3 per cent phosphotungstic acid, and sprayed on a carbon-coated grid by means of an ordinary nebulizer. They were examined in a Hitachi HU 11A electron microscope. Fig. 2a shows a negatively stained preparation of the phospholipid/cholesterol structures, incubated in the presence of ethanol alone. The majority of the figures appeared as spherulites, spread out on the grid, and frequently superimposed on each other. Most measured between 0.2 and 0.8 μ in diameter, although larger, composite figures were occasionally observed. Each spherulite, in appropriate specimens, appeared to be composed of concentric lamellae, usually at a distance of 45–55 Å between layers. Therefore these structures resemble those previously described by Bangham, Standish and Weissmann, with the important difference that the spherulites prepared with 0.45 M glucose are much more discrete, and show considerably less coalescence than did the structures previously described, which were prepared in water. To exclude *moiré* effects as the source for the lamellar appearance, specimens were tilted in the electron beam, but this had no effect on the concentric, lamellar appearance.

When the specimens were incubated with 'Triton X-100', their distinct spherular organization was entirely lost, no lamellar structure could be identified, and the grids appeared filled with deposits of amorphous material (Fig. 2b). However, in contrast to these and to controls, samples which had been incubated with 5 mM DOC showed the emergence of entirely new forms. Although smaller, discrete spherulites could still be identified, the predominant forms were a series of frayed, strand-like structures, loops and swirls of which maintained a lamellar sub-structure (Fig. 2c). These strands were frequently

Table 1

Agent added	No. of expts.	μ moles	Glucose released, Per cent of controls * at 30' \pm S.E.M.
Control	6	18.1	166.3 \pm 5.5
'Triton X-100'	6	30.7	281.0 \pm 27.9
DOC	4	25.5	234.5 \pm 10.9
Etiocholanolone	6	24.4	224.3 \pm 35.2
Progesterone	4	21.0	193.5 \pm 14.1
Cortisol acetate	3	16.8	154.3 \pm 5.7
'Streptolysin S'	2	21.9	201.0

* Control tubes leaked 10.9 μ moles in 30 min. Release of glucose from phospholipid/cholesterol structures. See text for detail of preparation of lipid spherulites composed of o-oleic acid/cholesterol/dicetylphosphate (70:10:20). Each ml. of spherulite suspension sequestered an average of 80 μ moles of glucose before steroids (5 mM), 'Triton X-100' (0.1 per cent v/v) or streptolysin (2,000 haemolytic units) was added. Solvent controls were 0.05 ethanol for steroids, 0.1 ml. NaCl/KCl (0.145 M) in other tubes. Leakage measured after 60 min at 37° C.

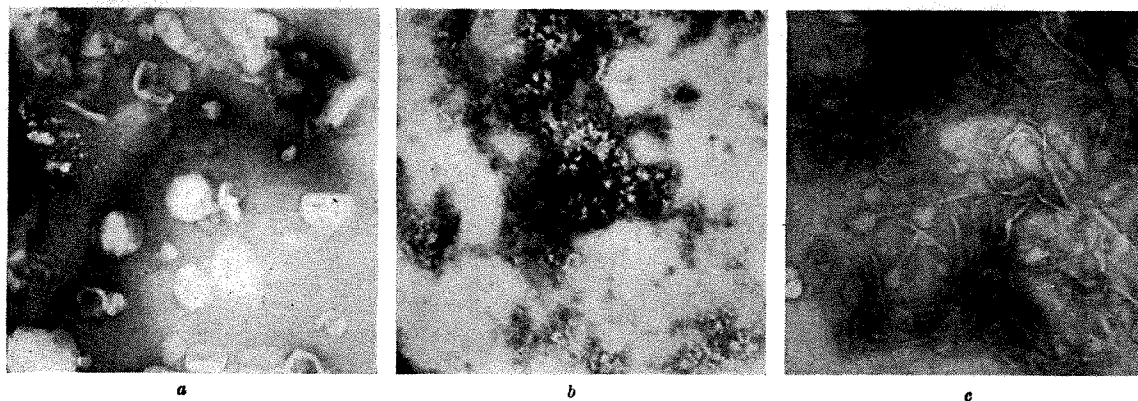


Fig. 2. *a*, Electron micrograph of artificial phospholipid/cholesterol structures. See text for preparation of these spherulites, which have been prepared in 0.145 M glucose and negatively stained with 3 per cent phosphotungstic acid; *b*, preparation as in *2a*, but treated for 20 min at 23°C with 0.1 per cent 'Triton X-100'; *c*, preparation as in *2a*, but treated for 20 min at 23°C with 5 mM DOC (deoxycorticosterone) (\times c. 23,000)

observed as a double- or triple-layered system, sometimes tubular, which in areas appeared to merge with the outermost layers of the native structures. The distance between lamellae was still maintained at approximately 50 Å, but in some three-layered strands a middle, thicker, layer appeared which was somewhat amorphous. Longer exposure to the steroid produced a series of frayed strands and flat sheets.

Negative-staining techniques have previously been used to demonstrate the effects of other membrane-active agents on purified lipid mixtures. Saponin was shown to interact with lecithin/cholesterol dispersions so as to produce a series of new, morphologically distinct forms which could be explained by postulating the interaction of saponin with individual components of the lipid mixture^{11,12}. Lysolecithin and 'Streptolysin S' also brought about morphological changes in phospholipid/cholesterol structures; these differed from those induced by saponin. No new forms appeared, but the net effect was a sort of progressive emulsification: new channels appeared between the internal lamellae of the structures and the surrounding medium⁷. In contrast to lysolecithin or 'Streptolysin', DOC appears to have interacted with phospholipid/cholesterol structures to produce new, strand-like forms at the expense of the spherular arrangement ordinarily preferred by the lipids in 0.145 M glucose. However, because negative-staining techniques do not necessarily show the precise configuration that lipids assume in aqueous dispersion, the extrapolation of these observations to wet states, or to biological systems, is hazardous¹³. Nevertheless, the experiments strongly suggest that steroid-induced changes in the permeability to glucose of phospholipid/cholesterol spherulites are the functional expression of a physical interaction between steroid and membrane lipid. The nature of such an interaction is, at present, only conjectural. Taylor and Haydon¹⁴, testing Willmer's suggestion that steroids, as amphipathic or 'rogue' molecules, might enter into the lipid phase which bounds cells or their organelles¹⁵, found that progesterone entered into phospholipid/cholesterol films at an air-water interface. Since only small amounts of progesterone did, in fact, enter the layers, they were unable to determine whether progesterone inserted vertically, with one polar group at the interface, or, horizontally, with both polar groups at the interface. Munck previously had shown that steroids oriented horizontally at a heptane/water interface¹⁶.

Whatever the position of steroids in lipid membranes proves to be, it would appear that the presence of polar groups at C 11 or C 17 of the sterol radically alters the functional properties of biological membranes exposed to such rogue molecules. Oxygen functions at these positions considerably diminish the lytic effects of DOC, progesterone, and etiocholanolone on lysosomes, mitochondria, and erythrocytes²⁻⁵. Another structural feature is

important in determining membrane action; etiocholanolone and other 5 β -H steroids, the A : B ring junction of which is *cis*, disrupt lysosomes and erythrocytes. In contrast, the planar 5 α -H isomers of these steroids are relatively inert in membrane activity²⁻⁵. Studies with lipid spherulites filled with Na⁺ and K⁺ have shown that these structural 'rules' hold for the model system used here as well. Such observations fit remarkably with the early observations of Selye on the anaesthetic properties of steroids¹⁷. He, too, found that oxygen functions at C 11 or C 17 abolished the anaesthetic effects of etiocholanolone or progesterone, and that 5 α -H isomers were non-anaesthetic. This concordance would support theories of anaesthesia based on the capacity of anaesthetics to enter lipid membranes¹⁸; Bangham has recently found that several local anaesthetics alter the leakage rates of cations from lipid spherulites¹⁹.

We have previously reported that pre-incorporation into lipid spherulites of as little as 1 per cent cortisone, before swelling in salt solutions, produced structures that leaked cations to a lesser extent than did control spherulites on subsequent exposure to lytic agents. It is, therefore, possible that small amounts of steroids may alter the permeability of biological membranes by virtue of their insertion as rogue molecules in bimolecular lipid leaflets. The presence, in an aqueous phase, of a functional protein has been considered an integral feature of biological membranes²⁰. The investigations described here would suggest that at least some membrane phenomena may be explained without considering this protein as more than a coating for a lipid skeleton, the integrity of which may be regulated by amphipathic molecules such as steroids.

One of us (G. W.) is a senior investigator of the U.S. Arthritis Foundation. This work was supported by grants from the U.S. National Institutes of Health (AM-08363) and the Life Insurance Medical Research Fund.

¹ deDruve, C., Wattiaux, R., and Wiblo, M., *Biochem. Pharmacol.*, **9**, 97 (1962).

² Weissmann, G., *Biochem. Pharmacol.*, **14**, 525 (1965).

³ Weissmann, G., and Thomas, L., *Recent Prog. Hormone Res.*, **20**, 215 (1964).

⁴ Weissmann, G., and Keiser, H., *Biochem. Pharmacol.*, **14**, 537 (1965).

⁵ Blecher, M., and White, A., *J. Biol. Chem.*, **235**, 3404 (1960).

⁶ Bangham, A. D., Standish, M. M., and Watkins, J. C., *J. Mol. Biol.*, **13**, 238 (1965).

⁷ Bangham, A. D., Standish, M. M., and Weissmann, G., *J. Mol. Biol.*, **13**, 253 (1965).

⁸ Keston, A. S., *Abst. Papers 129th Meeting, Amer. Chem. Soc.*, 31C (1956).

⁹ Weissmann, G., Keiser, H., and Bernheimer, A. W., *J. Exp. Med.*, **118**, 205 (1963).

¹⁰ Weissmann, G., Sessa, G., and Weissmann, S. (in preparation).

¹¹ Bangham, A. D., and Horne, R. W., *Nature*, **196**, 952 (1962).

¹² Glauert, A. M., Dingle, J. T., and Lucy, J. A., *Nature*, **196**, 953 (1962).

¹³ Bangham, A. D., and Horne, R. W., *J. Mol. Biol.*, **8**, 600 (1964).

¹⁴ Taylor, J. L., and Haydon, D. A., *Biochim. Biophys. Acta*, **94**, 488 (1965).

¹⁵ Willmer, E. N., *Biol. Rev. Camb. Phil. Soc.*, **36**, 368 (1961).

¹⁶ Munck, A., *Biochim. Biophys. Acta*, **24**, 507 (1957).

¹⁷ Selye, H., *Endocrinology*, **30**, 437 (1942).

¹⁸ Shanes, A. M., and Gershfeld, N. L., *J. Gen. Physiol.*, **44**, 345 (1960).

¹⁹ Bangham, A. D. (personal communication).

²⁰ Lehninger, A. L., *Neuroscience Res. Prog. Bull.*, **3**, 25 (1965).

DISTINCTION BETWEEN THE EFFECTS OF ANTIVIRAL AND ANTICELLULAR POLYOMA ANTIBODIES ON POLYOMA TUMOUR CELLS

By INGEGERD HELLSTRÖM

Institute for Tumour Biology, Karolinska Institutet Medical School, Stockholm

SJÖGREN, Hellström and Klein¹ and, independently, Habel², reported in 1961 that mice immunized with polyoma virus as adults were resistant to transplantation with polyoma tumour cells. They attributed this to the presence in polyoma tumours of specific antigens determined by the virus. Sjögren later showed that mice immunized with polyoma tumour cells were also resistant to transplantation of polyoma tumours³. Resistance was also demonstrated in mice immunized with polyoma tumour clones which could not release polyoma virus particles⁴. It was hypothesized that polyoma tumour cells contain an antigen, determined by the polyoma virus but distinct from the antigen of the virus particles⁴.

It was recently demonstrated by Hellström and Sjögren that specific antigenicity of polyoma tumour cells can be detected by an *in vitro* technique in which target cells are incubated with specific isoantibodies and complement and plating efficiency then measured⁵. This method was used for the experiments to be reported. They were performed to distinguish between the effect on polyoma tumour cells of antibodies prepared respectively against the polyoma virus and the hypothetical specific polyoma tumour antigen. The tumour line used for this study, YAA-CI-C3, is a clone derived from YAA-CI, which is a sub-line of the Moloney-virus-induced YAA lymphoma of A/Sn origin that has been subjected to repeated infections with polyoma virus *in vitro* and has acquired the polyoma specific transplantation antigen^{5,6}. It was chosen because of its high sensitivity to antibodies and complement in the test system used. In a control experiment, a YAA-CI line was used which had not yet acquired the polyoma antigen. All the tumour lines tested also contain the Moloney specific antigen⁶.

Two types of anti-polyoma serum were used (Table 1): serum pools obtained from mice immunized with polyoma virus preparations as adults, and pools of 'anti-cellular-antigen' serum, derived from mice which had been inoculated with allogeneic tumours, containing the polyoma transplantation antigen but not releasing detectable polyoma virus particles. Sera obtained from mice

immunized with polyoma virus preparations all contained high levels of anti-polyoma virus antibodies which were detected both by a haemagglutination inhibition technique⁷, and by neutralization of plaque formation by the virus^{8,9}. Sera derived from mice immunized with the polyoma tumour cells did not have detectable antibodies directed against polyoma virus. In addition an A.SW anti-A serum and an A anti-A.SW serum were used. The former was a positive control because of its ability to react with the H-2^a determined antigens of the tumour cells tested. The latter was a negative control, incapable of reaction with the tumour cells.

Trypsinized cell suspensions of transplanted tumours were explanted, propagated approximately 30 days *in vitro*, and used for plating experiments, performed as previously described⁵. These cells were suspended and diluted to a concentration of 4,000 cells/0.1 ml., in conditioned Eagle's medium with 40 per cent foetal calf serum. The use of conditioned medium gathered from YAA-CI-C3 cultures 3-4 days after seeding was found to increase plating efficiency approximately tenfold. Each diluted tumour suspension was added to a tube containing 0.1 ml. undiluted antiserum and mixtures of cells and serum were incubated at 37° C for 20 min with repeated shaking. Then 0.8 ml. undiluted guinea-pig complement was added, and 60 min later 15 ml. of Eagle's medium with 40 per cent foetal calf serum was added. 4-ml. portions were plated in each of three Falcon 50-mm Petri dishes. Some experiments were made with doubled amounts of cells and sera in order to obtain sufficient material for plating in 6 Petri dishes. The dishes were incubated for 7 days at 37° C in a 5 per cent carbon dioxide-air atmosphere and stained with crystal violet, after which the number of colonies was counted.

The findings are presented in Table 2. Treatment of the tumours with A.SW anti-A serum and complement reduced the number of colonies formed after plating by 84-100 per cent, as compared with control groups exposed to an anti-A.SW serum, unable to react against the target cells. Sera from mice immunized against the

Table 1. SAMPLING DATA ON ANTI-POLYOMA SERA USED FOR THE TESTS

Serum pool No.	Immunization		Results of plaque tests†				Serum titre according to HI tests‡
	Mice	Procedure *	Virus dilut.	Plaque numbers (per cent)			
				Control	After treatment with antiser.	Reduct.	
'Anti-polyoma virus'							
1328	<i>A.CA</i> × <i>CBA F</i> ₁	Virus as adults	Undil.	Confluent	0	100	1:10,240
1333		" " "	Dil. 1:10	26	0	100	1:10,240
1348	<i>A.SW</i> × <i>DBA F</i> ₁	" " "					1:10,240
1358	<i>A</i>	" " "					1:5,120
1365	<i>A</i> and <i>A</i> × <i>CBA F</i> ₁	Virus as adults + <i>SEWA-C10 4</i> inocul.					1:1,280
'Anti-polyoma cell antigen'							
1330	<i>A</i>	<i>SEWA-C10 8</i> inocul.					1:160
1345	<i>A</i> × <i>CBA F</i> ₁	<i>SEWA-C10 4</i> inocul.					1:40
1363	<i>A</i> × <i>CBA F</i> ₁	<i>SEWA-C10 4</i> inocul.	Dil.	Confluent	Confluent	0	1:40
1382	<i>A</i> × <i>CBA F</i> ₁	<i>SEWA-C10 5</i> inocul.	Dil. 1:10	26	28	0	1:160

* Mice were immunized as adults by giving them one injection with a high titred polyoma virus, in order to produce antiviral sera. Serum pool 1365 was taken from mice immunized with polyoma virus as adults and given 4 inoculations with the non-releasing polyoma tumour clone SEWA-C10 (ref. 4). Antisera directed against the 'polyoma-cell-antigen' were taken from mice, given 4-8 inoculations of the non-releasing SEWA-C10 polyoma tumour clone (of A.SW origin).

† Plaque tests were performed as described elsewhere (ref. 9). Two different dilutions of a virus-containing tissue culture fluid were treated with antiserum and the reduction in plaque number was observed.

‡ Serum titres were determined according to the HI technique of Eddy *et al.* (ref. 7). Titres of up to 1:100 were considered to depend on non-specific inhibition (ref. 4), and titres of 1:200 or more clearly positive.

Table 2. PLATING EFFICIENCY OF YAA-CI-C3 AFTER EXPOSURE TO VARIOUS ANTISERA. THE NUMBER OF COLONIES FORMED ON EACH PETRI DISH AFTER PLATING OF 1,000 CELLS IS GIVEN. COMPLEMENT WAS ADDED TOGETHER WITH THE SERUM EXCEPT WHEN OTHERWISE STATED

Experiment number	Tumour line	A anti-A.SW serum. Col/plate	A.SW anti-A serum		Anti-polyoma vir. ser.			Anti-polyoma cell ser.				
			Col/plate	% red	Ser. pool No.	Col/plate	% red	Ser. pool No.	With complement. Col/plate	% red	Without complement. Col/plate	% red
1	YAA-CI-C3 (contains polyoma transplant antigen)	90, 130, 128, 80, 94, 98	0, 0, 0, 0, 0, 0	100	1333	126, 116, 118, 112, 130	0	1330	50, 60, 70, 80	39	136, 98, 130, 112, 98, 100	0
2	" "	90, 72, 72	0, 0, 0	100	1333	95, 92	0	1330	50, 53, 41	39		
3	" "	208, 190, 210, 180, 182	0, 0, 0, 0	102	1348	134, 184, 174, 179, 172, 154	12	1345	138, 140, 140, 94, 100, 122	37	200, 204, 184	0
4	" "	120, 110, 124, 100, 98, 102	3, 2, 0, 6, 2, 2	98	1328	68, 74, 78, 64, 64, 66	40	1382	54, 44, 42	60		
5	" "	210, 212, 216	0, 0, 0	100	1358	162, 160, 166	23	1363	164, 152, 132	30		
6	YAA-CI (no polyoma transplant antigen)	138, 138, 148	20, 26, 24	84	1365	134, 142, 134	3	1345	136, 142, 140	1		

hypothetical polyoma-tumour antigen reduced colony numbers by 30–60 per cent although these sera did not contain detectable antiviral antibodies. Complement was necessary for reduction of plating efficiency. The antiviral sera reduced colony numbers only in some experiments. One test was performed with a line of YAA-CI which had not yet acquired detectable polyoma-induced transplantation antigenicity. It was resistant to the action of both anti-cellular and anti-viral polyoma sera but was killed by the A.SW anti-A serum.

These experiments established that the tumour line tested, YAA-CI-C3, containing the polyoma-induced transplantation antigen⁶, is sensitive to treatment *in vitro* with isoantisera directed against polyoma tumour cells but not containing detectable antiviral antibodies. Plating efficiency was only infrequently reduced by strong antiviral sera. The reduction obtained with these sera is probably due to their content of antibodies against cellular polyoma antigen(s) since the virus-immunized mice are resistant to syngeneic polyoma-tumour grafts¹.

The findings reported strongly indicate that transplantation resistance to polyoma tumour cells depends on the presence in the tumours of a new cellular antigen which is induced by polyoma virus, but differs from the antigen of polyoma virus particles.

This work was supported by grants from the Swedish Cancer Society, the Swedish Medical Research Council and by grant CA-04747 to Prof. G. Klein from the National Cancer Institute, U.S. Public Health Service. I also thank Prof. G. Klein for his advice and Mrs. Solveig Humla, Mrs. Britta Jonsson and Miss Maj Britt Sondell for their assistance.

¹ Sjögren, H. O., Hellström, I., and Klein, B., *Cancer Res.*, **21**, 329 (1961).

² Habel, K., *Proc. Soc. Exp. Biol. Med.*, **106**, 722 (1961).

³ Sjögren, H. O., *Virology*, **15**, 214 (1961).

⁴ Sjögren, H. O., *J. Nat. Cancer Inst.*, **32**, 645 (1964).

⁵ Hellström, I., and Sjögren, H. O., *Exp. Cell Res.* (in the press).

⁶ Sjögren, H. O., and Hellström, I., *Exp. Cell Res.* (in the press).

⁷ Eddy, B. E., Rowe, W. P., Hartley, J. W., Stewart, S. E., and Huebner, R. J., *Virology*, **6**, 290 (1958).

⁸ Dulbecco, R., and Freeman, G., *Virology*, **8**, 396 (1959).

⁹ Hellström, I., *J. Nat. Cancer Inst.*, **31**, 1511 (1963).

INTERSPECIES DIFFERENCES IN RESPONSE TO POLYPEPTIDES AS PERMEABILITY FACTORS

By DR. JEAN CARR and PROF. D. L. WILHELM

Prince Henry Hospital, and School of Pathology, University of New South Wales, Sydney

THE ability of bradykinin to induce vasodilatation, increased vascular permeability and leucocytic diapedesis has directed attention to its possible function as a mediator of the early vascular events in inflammation¹. As a permeability factor, bradykinin has outstanding activity in the guinea-pig, being about 80 times more potent in this species than histamine^{2,3}. In the rat, on the other hand, bradykinin and histamine both have relatively low potency², whereas histamine has high activity in the rabbit, comparable to that in the guinea-pig⁴. To our knowledge, however, bradykinin has not been tested in the rabbit.

Bradykinin is a nonapeptide^{5,6} (Fig. 1) to which kallidin is closely related⁶; it differs only in possessing an N-terminal lysine group⁷, thus making kallidin a decapeptide. Although kallidin has pharmacological properties similar to those of bradykinin, there is little evidence concerning the relative potency of the two preparations as permeability factors. Serum kallikrein has a similar potency to bradykinin in the guinea-pig⁸, but the permeability effects of kallikrein may be mainly due to the production of bradykinin⁹.

In the present work, the permeability-increasing potency of synthetic bradykinin, kallidin and elodeisin

has been compared in the skin of guinea-pigs, rats and rabbits. This was done to assess the permeability effects of bradykinin in the rabbit, relative to its known activity in the guinea-pig and rat, to determine the relative potency of bradykinin and kallidin in all three species, and to compare the potency of an unrelated peptide, elodeisin, since the pharmacological activity of polypeptides appears to be affected both by the internal arrangements of their amino-acid residues¹⁰ and their chain-length^{11,12}. Elodeisin is a peptide¹³ containing eleven amino-acid residues (Fig. 1) obtained from the posterior salivary gland of the *Eledone*, a close relative of the genus *Octopus*.

The test preparations were injected intradermally in guinea-pigs, rats and rabbits with Evans blue circulating in their blood, according to the technique of Miles and

Bradykinin

Arg-Pro-Gly-Phe-Ser-Pro-Phe-Arg

Kallidin

Lys-Arg-Pro-Gly-Phe-Ser-Pro-Phe-Arg

Elodeisin

Pyr*-Pro-Ser-Lys-Asp(OH)-Ala-Phe-Ileu-Gly-Leu-Met-NH₂

*Pyr = Pyroglutamyl.

Fig. 1. Structure of bradykinin, kallidin and elodeisin

Miles¹⁴ and Wilhelm *et al.*¹⁵. Twenty min after completing the injections, the animals were killed and the lesions at the test sites were measured on both the upper and under surfaces of the skin. The sites were then excised and their content of exuded dye was determined by homogenizing the skin and extracting the dye by the technique of Clausen and Lifson^{8,16}. The results are recorded in Fig. 2.

Previous work³ in the guinea-pig has indicated that the potency of various permeability factors differs considerably when lesion-diameter is measured on the upper or lower surface of the skin. The procedure of dye extraction is rather tedious as a routine technique, but the results agree more closely with those obtained by measuring lesion diameter on the under rather than the upper surface of the skin².

Although the three polypeptides which were tested each evoke satisfactory responses on both the upper and under surface of rats' skin, the amounts of extracted dye are much less than in the guinea-pig, and the dosage response lines correspondingly unsatisfactory for the evaluation of relative potency. Nevertheless, our dye extraction results for the rat agree with those of other workers^{19,20} using trypan blue as a marker substance in the investigation of the permeability effects of histamine and kallikrein.

In the rabbit, on the other hand, the lesions are not estimable on the upper surface of the skin. The exudation of the dye is confined to the upper layers of the skin and does not extend down to the panniculus carnosus, which in turn is too opaque for the pale overlying lesions to be viewed from the under surface. That the amount of exuded dye is relatively small in the rabbit is confirmed by the dye extraction results (Fig. 2).

As Fig. 2 also illustrates, the amount of exuded dye in the rabbit was even less than in the rat, and the corresponding dosage-response lines were unsatisfactory for the estimation of relative potency of permeability factors.

The foregoing disadvantages in the rat and rabbit were unexpected, particularly in the latter, and hence the interspecies comparison of the three test peptides had to be confined to the results from the upper-surface readings. The outstanding activity of bradykinin in the guinea-pig and its lower activity in the rat are confirmed. In the rabbit, bradykinin has high activity, of the same order as in the guinea-pig. Relative to histamine, bradykinin has a potency of 86 in the guinea-pig and 63 in the rabbit; and relative to 5-hydroxytryptamine, a potency of 0.6 in the rat.

In all three species, kallidin has slightly less activity than bradykinin, but the relative factor is only about 1.3 in the guinea-pig, 2 in the rat, and 3 in the rabbit.

The results for eleodoisin are surprising in that this peptide has high potency in both the guinea-pig and rat, but negligible activity in the rabbit. In the guinea-pig, the activity of eleodoisin is comparable to that of both bradykinin and kallidin, but in the rat it far outstrips any factor we have tested, being 18 times more active than bradykinin and 10 times more active than 5-hydroxytryptamine.

Our results, therefore, indicate that bradykinin has high permeability-increasing activity in the rabbit, as in the guinea-pig and rat, and that in all three species its activity slightly but consistently exceeds that of kallidin. On the other hand, eleodoisin, an unrelated peptide of similar chain-length, has comparable activity in the guinea-pig, outstanding effects in the rat, but negligible

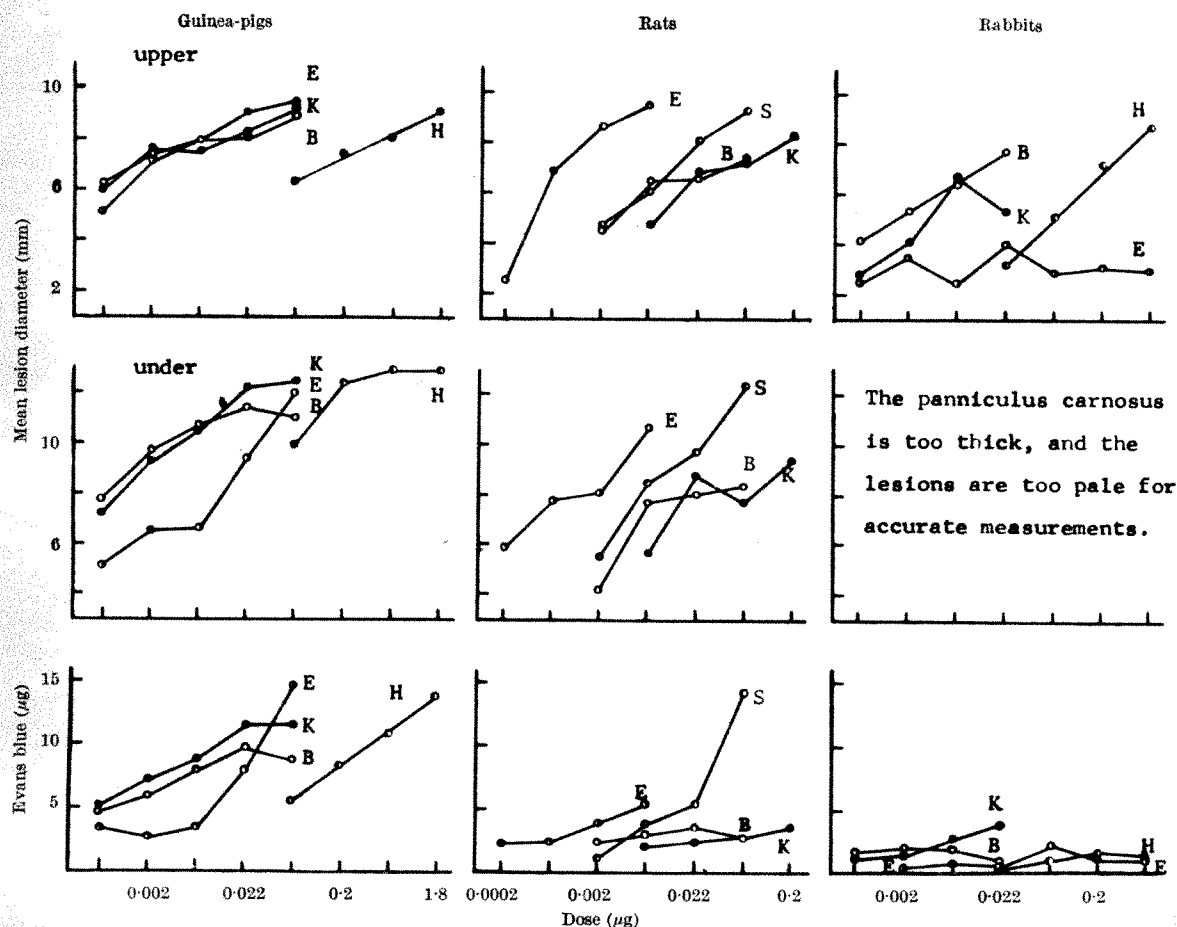


Fig. 2. Permeability-increasing potency of bradykinin (B), kallidin (K) and eleodoisin (E), relative to histamine (H), in guinea-pigs and rabbits, and to 5-hydroxytryptamine (S) in rats. The response lines record the mean lesion diameters on the upper and under surfaces of the skin, and the mean amounts of Evans blue extracted from the same lesions.

potency in the rabbit. Whatever the explanation of this unexpected result with eleodoisin, it seems noteworthy that this is still another example of a biological substance exhibiting wide variations in permeability effects according to the species of test animal³.

We thank Sandoz, Ltd., for supplies of the synthetic peptides, and the Bushells Trust for financial support to one of the authors (J. C.).

¹ Lewis, G. P., *Ann. N.Y. Acad. Sci.*, **104**, 236 (1963).

² Wilhelm, D. L., *Pharmacol. Rev.*, **14**, 251 (1962).

³ Carr, Jean, and Wilhelm, D. L., *Austral. J. Exp. Biol. and Med. Sci.*, **42**, 511 (1964).

⁴ Sparrow, Elizabeth M., and Wilhelm, D. L., *J. Physiol.*, **137**, 51 (1957).

⁵ Konzett, H., and Stürmer, E., *Brit. J. Pharmacol.*, **15**, 544 (1960).

⁶ Lewis, G. P., *Nature*, **192**, 596 (1961).

⁷ Webster, Marion E., and Pierce, J. V., *Ann. N.Y. Acad. Sci.*, **104**, 91 (1963).

⁸ Schachter, M., *Ann. N.Y. Acad. Sci.*, **104**, 108 (1963).

⁹ Margolis, J., and Bishop, Elizabeth A., *Austral. J. Exp. Biol. and Med. Sci.*, **41**, 293 (1963).

¹⁰ Rubin, B., Waugh, Margaret H., Laffan, R. J., O'Keeve, Erika, and Craver, B. N., *Proc. Soc. Exp. Biol.*, **113**, 328 (1963).

¹¹ Spector, W. G., *J. Path. Bact.*, **63**, 93 (1951).

¹² Woolley, D. W., and Merfeld, R. B., *Ann. N.Y. Acad. Sci.*, **104**, 161 (1963).

¹³ Ersamer, V., and Anastas, A., *Experientia*, **18**, 58 (1962).

¹⁴ Miles, A. A., and Miles, E. M., *J. Physiol.*, **118**, 228 (1952).

¹⁵ Wilhelm, D. L., Mill, P. J., Sparrow, Elizabeth M., Mackay, Margaret E., and Miles, A. A., *Brit. J. Exp. Path.*, **39**, 228 (1958).

¹⁶ Clausen, D. F., and Lifson, S., *Proc. Soc. Exp. Biol.*, **91**, 11 (1956).

¹⁷ Rawson, Ruth A., *Amer. J. Physiol.*, **138**, 708 (1942-3).

¹⁸ Ascheim, E., *Nature*, **201**, 1291 (1964).

¹⁹ Judah, J. D., and Willoughby, D. A., *J. Path. Bact.*, **83**, 567 (1962).

²⁰ Spector, W. G., and Willoughby, D. A., *Nature*, **196**, 1104 (1962).

A SIMPLE BIOASSAY FOR ANTIOXIDANTS BASED ON PROTECTION OF *Tetrahymena pyriformis* FROM THE PHOTODYNAMIC TOXICITY OF BENZO(a)PYRENE

By DR. SAMUEL S. EPSTEIN, INNA B. SAPOROSCHETZ, MYRA SMALL, WELDON PARK and NATHAN MANTEL

From the Laboratories of Carcinogenesis and Histology, The Children's Cancer Research Foundation, and the Department of Pathology, Harvard Medical School, at the Children's Hospital, Boston, Massachusetts, and the Biometry Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

ANTIOXIDANTS are widely used in food, as well as in other natural or synthetic products such as petroleum, rubber and paints, to inhibit autoxidative deterioration. This subject has been well reviewed in recent publications^{1,2}. While a voluminous literature exists on chemical tests for antioxidants, biological data on their *in vivo* activity, perforce, have been restricted to some studies on vitamin E biopotency³. The present article describes a simple and rapid biological method for the *in vivo* characterization and assay of antioxidant activity based on the inhibition of the oxygen-dependent phenomenon, photodynamic injury⁴⁻⁷. This approach was stimulated by the finding that vitamin E protects a ciliated protozoan against the photodynamic effects of a polycyclic photosensitizer^{6,7}.

The compounds tested are conventionally grouped, as in Table 1. In general, *primary antioxidants* are considered to function as free-radical acceptors; *secondary antioxidants* act synergistically with primary antioxidants; and *ultra-violet absorbers* block the catalytic effect of ultra-violet and visible light. The *miscellaneous group*, comprised largely of reducing agents with free —SH groups, includes classical radioprotective agents not generally regarded as antioxidants.

From stock solutions of compounds in acetone or water at 0.1 or 0.01 g/ml., serial log dilutions of aqueous suspensions were freshly prepared from 3,000 to 0.3 μ g/ml. The dilution range was appropriately extended, occasionally to as low as 3×10^{-10} μ g/ml., for compounds with high activity. Aqueous suspensions of benzo(a)pyrene (BaP) at 0.1 μ g/ml., freshly prepared from stock acetone solutions of 100 μ g/ml., were used as the standard photosensitizing agent. Cultures of *Tetrahymena pyriformis* var 1, mating type II, were maintained axenically in the dark at 28° C in medium containing 1.5 per cent w/v 'Proteose®' peptone and 0.15 per cent w/v yeast extract at pH 6.8. Suspensions from log-phase cultures, diluted in medium to contain approximately 30 cells, were mixed on single 15 \times 4 mm plastic depression slides with aliquots of BaP and of individual concentrations of antioxidants. These slides, together with a minimum of two antioxidant-free controls, were incubated at 28° C in dark moist chambers for a minimum of 2 h. Two wells were then irradiated simul-

taneously with long-wave ultra-violet light, of 8.750 μ W/cm² at 45 cm, filtered to transmit light from 310 to 400 m μ with a peak at 366 m μ , and observed directly with a binocular stereoscopic microscope at a magnification of $\times 10$. The duration of irradiation required to produce lethality, as indicated by immobilization of 90 per cent of the motile ciliates (LT_{90}), was determined, with an arbitrary observation limit of 60 min. All compounds were tested a minimum of three times, using new stock solutions on at least two occasions.

Protection from photodynamic toxicity was manifest by prolongation of median LT_{90} over median control values (Table 2). Concentrations of compounds producing two-fold prolongation of LT_{90} values and dose-response slopes of these compounds were determined (Table 1 and Fig. 1). Approximately half the compounds tested (58/98) achieved such protection. α -Tocopherol was selected as the standard for apparent relative potency determinations (Table 1). For compounds yielding two-fold or greater protection, the possible role of light absorption was

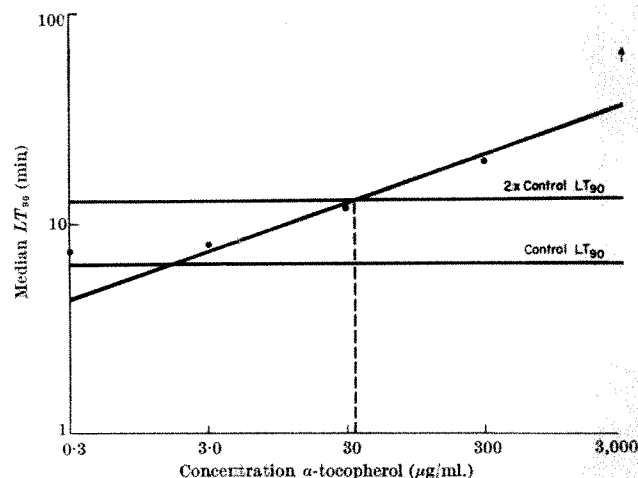


Fig. 1. Method for determining the concentration of α -tocopherol required to produce two-fold prolongation of control LT_{90} values. α -Tocopherol was used as the standard for relative potency determinations. At 3,000 μ g/ml., LT_{90} values exceeding the arbitrary observational limit of 60 min are indicated by the arrow.

Table 1. EFFECTS OF ANTIOXIDANTS ON THE RESPONSE OF *T. pyriformis* TO PHOTODYNAMIC INJURY BY BENZO(a)PYRENE

Compounds*	Inhibition by antioxidants of photodynamic activity of 0.1 µg/ml. of benzo(a)pyrene			
	Conc. (µg/ml.) required for 2-fold prolongation of LT_{50} values†	Apparent anti-oxidant potency relative to α -tocopherol	Dose-response slopes (log/log)	% Photodynamic inhibition attributable to light absorption‡
PRIMARY ANTIOXIDANTS				
Phenolic Compounds				
Phenols				
1 Hydroquinone ¹	> 30	< 1.1	—	—
2 Salicylic acid ¹	> 300	< 0.1	—	—
3 <i>p</i> -Aminosalicylic acid ²	> 300	< 0.1	—	—
4 2,5-Dihydroxybenzoic acid ('Gentiale acid') ²	> 300	< 0.1	—	—
5 3,3',4',5,7-Pentahydroxyflavone ('Quercetin') ⁴	12	2.8	0.32	46
6 Methyl- <i>p</i> -hydroxybenzoate ('Methylparaben') ¹	> 300	< 0.1	—	—
7 Ethyl- <i>p</i> -hydroxybenzoate ('Ethylparaben') ¹	> 30	< 1.1	—	—
8 Propyl- <i>p</i> -hydroxybenzoate ('Propylparaben') ¹	> 30	< 1.1	—	—
9 <i>n</i> -Propylgallate ⁴	> 300	< 0.1	—	—
10 2,4,5-Trihydroxybutyrophene ¹	12	2.8	0.24	0
'Hindered' Phenols				
11 Mixture of 2 and 3- <i>tert</i> -butyl-4-methoxyphenol (BHA) ⁵	> 30	< 1.1	—	—
12 2,6-Di- <i>tert</i> -butyl- <i>p</i> -cresol (BHT) ⁵	> 3,000	< 0.01	—	—
13 <i>tert</i> -Butylhydroquinone ¹	> 300	< 0.1	—	—
14 2,5-Bis(1,1-dimethylpropyl)hydroquinone (<i>tert</i> -amylhydroquinone) ⁴	75	0.4	0.10	0
15 Undefined mixture of guaiacolic acids (gum guaiac) ⁴	2.1	16	0.12	0
16 4,4'-(2,3-Dimethyltetramethylene)dipyrrocatechol (NDGA) ²	1.6 × 10 ⁻²	2.1 × 10 ³	0.14	0
17 0,0-Di- <i>n</i> -octadecyl-3,5-di- <i>tert</i> -butyl-4-hydroxybenzylphosphonate ⁷	15	2.2	0.31	0
18 2,4-Bis-(<i>n</i> -octylthio)-6-(4-hydroxy-3,5-di- <i>tert</i> -butylanilino)-1,3,5-triazine ⁷	11	3.1	0.26	0
19 2,4-Bis-(<i>n</i> -octylthioethylthio)-6-(3,5-di- <i>tert</i> -butyl-4-hydroxyanilino)-1,3,5-triazine ⁷	45	0.7	0.27	0
20 2,4-Bis(<i>n</i> -octylthioethylthio)-6-(4-hydroxy-3,5-di- <i>tert</i> -butylphenoxy)-1,3,5-triazine ⁷	5.4	6.1	0.22	0
21 2,4-Bis(4-hydroxy-3,5-di- <i>tert</i> -butylphenoxy)-6-(<i>n</i> -octylthio)-1,3,5-triazine ⁷	41	0.8	0.26	0
22 4-Hydroxymethyl-2,6-di- <i>tert</i> -butylphenol ('Ionox 100') ⁸	> 3,000	< 0.01	—	—
23 2-(3,5-Di- <i>tert</i> -butyl-4-hydroxybenzylthio)benzothiazole ('Ionox 115') ⁸	150	0.2	0.34	0
24 3,5-Di- <i>tert</i> -butyl-4-hydroxybenzylether ('Ionox 201') ⁸	68	0.5	0.31	0
25 1,4-Bis-(3,5-di- <i>tert</i> -butyl-4-hydroxybenzyl)tetramethylbenzene ('Ionox 242') ⁸	390	0.09	0.34	0
26 2,4,6-Tris-(3,5-di- <i>tert</i> -butyl-4-hydroxybenzyl)phenol ('Ionox 312') ⁸	750	0.04	0.28	0
27 1,3,5-Trimethyl-2,4,6-tris(3,5-di- <i>tert</i> -butyl-4-hydroxybenzyl)benzene ('Ionox 330') ⁸	210	0.2	0.29	0
Tocopherols				
28 α -Tocopherol ³	33	1.0	0.33	0
29 β -Tocopherol ³	13	2.5	0.27	0
30 γ -Tocopherol ³	54	0.6	0.28	0
31 δ -Tocopherol ³	39	0.9	0.27	0
32 5,5'-Bi- α -tocopherol ³	51	0.7	0.33	0
33 D- α -Tocopheryl acid succinate ³	20	1.7	0.33	0
34 D- α -Tocopheryl acetate ³	60	0.6	0.33	0
35 D- α -Tocopheryl polyethylene glycolsuccinate ³	3.6	9.2	0.32	0
Amines				
Aliphatic				
36 2,2',2''-Nitrilotriethanol ('Triethanolamine') ¹	> 3,000	< 0.01	—	—
37 Hydroxylamine hydrochloride ¹	> 300	< 0.1	—	—
Aromatic				
38 Di- <i>o</i> -tolylguanidine salt of dicatechol borate ('Permalux') ⁹	27	1.2	0.12	0
39 Diphenylamine ⁹	> 300	< 0.1	—	—
40 4,4'-Diphenyl- <i>p</i> -phenylenediamine (DPPD) ¹	75	0.4	0.24	0
41 Mixture containing <i>n</i> -phenyl- α -naphthylamine ('Neozone A') ⁹	> 30	< 1.1	—	—
42 Mixture containing <i>n</i> -phenyl- β -naphthylamine ('Neozone D') ⁹	> 300	< 0.1	—	—
43 Mixture containing 4,4'-dimethoxydiphenylamine, DPPD and 'Neozone D' ('Thermoflex') ⁹	4.2	7.9	0.22	0
44 <i>p</i> -Aminobenzenesulphonylguanidine ('Sulphaguanidine') ¹⁰	> 3,000	< 0.01	—	—
Nitrogen heterocyclics				
45 Isonicotinic acid hydrazide ('Isoniazid') ²	> 3,000	< 0.01	—	—
46 2,3:5,6-Dibenzo-1,4-thiazine (phenothiazine) ¹	> 3,000	< 0.01	—	—
47 3,7-Bis(dimethylamino)phenazathionium chloride (methylene blue)	30	1.1	0.25	0
48 3-Amino-1H-1,2,4-triazole ²	> 3,000	< 0.01	—	—
49 6-Ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline (EMQ) ¹¹	60	0.6	0.10	57
50 8-(4-Amino-1-methylbutylamino)-6-methoxyquinoline ('Primaquin') ¹²	11	3.0	0.21	0
51 7-Chloro-4-(4-dimethylamino-1-methylbutylamino)-3-methylquinoline ('Nivaquin C') ¹²	75	0.4	0.20	75
52 7-Chloro-4-(4-diethylamino-1-methylbutylamino)quinoline (chloroquine diphosphate) ¹²	> 3,000	< 0.01	—	—
53 7-Chloro-4-[-4-(ethyl-(2-hydroxyethyl)-amino)-1-methylbutylamino]-quinoline (hydroxychloroquine) ¹²	> 3,000	< 0.01	—	—
Aminoazobenzenes				
54 2,2'-Azonaphthalene ¹³	13	2.5	0.16	16
55 4-Aminoazobenzene ¹³	1.5 × 10 ⁻³	2.2 × 10 ⁴	0.21	0
56 4-Methylaminoazobenzene ¹³	1.0 × 10 ⁻⁶	3.3 × 10 ⁷	0.10	0
57 4-Ethylaminoazobenzene ¹³	4.8 × 10 ⁻³	6.9 × 10 ³	0.15	0
58 4-Dimethylaminoazobenzene ('Butter Yellow') ¹³	6.0 × 10 ⁻³	5.5 × 10 ³	0.16	0
59 4-Diethylaminoazobenzene ¹³	3.1 × 10 ⁻³	1.1 × 10 ⁴	0.11	0
60 4-Ethylmethylaminoazobenzene ¹³	5.3 × 10 ⁻³	6.2 × 10 ³	0.11	0
61 2-Methyl-4-dimethylaminoazobenzene ¹³	1.2 × 10 ⁻⁴	2.8 × 10 ⁴	0.11	0
62 2'-Methyl-4-dimethylaminoazobenzene ¹³	2.1 × 10 ⁻⁶	1.6 × 10 ⁷	0.11	0
63 3-Methyl-4-dimethylaminoazobenzene ¹³	1.0 × 10 ⁻²	3.3 × 10 ³	0.22	0
64 3'-Methyl-4-dimethylaminoazobenzene ¹³	2.1 × 10 ⁻⁶	1.6 × 10 ⁷	0.15	0
65 4'-Methyl-4-dimethylaminoazobenzene ¹³	1.3 × 10 ⁻⁷	2.4 × 10 ⁸	0.12	0
66 4'-Ethyl-4-dimethylaminoazobenzene ¹³	3.6 × 10 ⁻⁴	9.2 × 10 ⁴	0.19	0
67 2',3-Dimethyl-4-aminoazobenzene ¹³	1.2 × 10 ⁻⁴	2.8 × 10 ⁴	0.13	0
68 2-Methyl-4'-ethyl-4-dimethylaminoazobenzene ¹³	1.0 × 10 ⁻³	3.3 × 10 ⁴	0.16	0

Table 1—Continued

Compounds*	Inhibition by antioxidants of photodynamic activity of 0.1 µg/ml. of benzo(a)pyrene			
	Conc. (µg/ml.) required for 2-fold prolongation of LT_{50} values†	Apparent anti-oxidant potency relative to α -tocopherol	Dose-response slopes (log/log)	% Photodynamic inhibition attributable to light absorption‡
SECONDARY OR SYNERGISTIC ANTIOXIDANTS				
69 Boric acid ¹	> 3,000	< 0.01	—	—
70 Formic acid ¹	> 300	< 0.1	—	—
71 Benzoic acid ¹	> 300	< 0.1	—	—
72 Tartaric acid ¹	> 3,000	< 0.01	—	—
73 <i>Trans-trans</i> -2,4-hexadienoic acid (sorbic acid) ⁴	> 300	< 0.1	—	—
74 Ascorbic acid ⁴	> 300	< 0.1	—	—
75 Isoascorbic acid ¹	> 300	< 0.1	—	—
76 Sodium ascorbate ⁴	> 3,000	< 0.01	—	—
77 Ascorbylpalmitate ⁴	0.7	50	0.17	0
78 3,3'-Thiodipropionic acid ⁴	> 300	< 0.1	—	—
79 Dilaurylthiodipropionate ('Milban F') ⁴	90	0.4	0.23	0
80 Lecithin ⁴	4.8×10^{-2}	690	0.18	0
81 Undefined mixture of wheat germ oil and lecithin complex ('Viobin' anti-oxidant) ¹⁴	2.4×10^{-2}	1.4×10^3	0.14	0
MISCELLANEOUS				
Polyenes				
82 β -Carotene ⁴	3	11	0.28	0
83 Xanthophyll ⁴	9.0	3.7	0.28	0
84 Vitamin A ⁴	6.0	5.5	0.26	0
Sulphur-containing Compounds				
85 Cysteine hydrochloride ¹	> 3,000	< 0.01	—	—
86 2,2-Diaminodithiethylsulphide (cystamine) ¹⁵	> 3,000	< 0.01	—	—
87 2-Mercaptoethylamine hydrochloride (cysteamine hydrochloride) ¹⁵	900	0.04	0.10	0
88 2,3-Dimercaptopropanol (BAL) ¹⁵	> 30	< 1.1	—	—
89 <i>S</i> -(2-Aminoethyl)isothiouromium bromide hydrobromide (AET) ¹⁵	> 3,000	< 0.01	—	—
90 Tetraethylthiuramdisulphide ('Antabuse') ²	1.9×10^{-4}	1.7×10^4	0.11	0
91 2-(<i>n</i> -Decylamino)ethanethiol ²	> 30	< 1.1	—	—
ULTRA-VIOLET ABSORBERS				
92 2(2'-Hydroxy-5'-methylphenyl)benzotriazole ('Tinuvin P') ⁷	159	0.2	0.21	100
93 2(2'-Hydroxy-5'- <i>tert</i> .butylphenyl)benzotriazole ⁷	81	0.4	0.23	100
94 <i>p</i> -Octylphenylsalicylate ¹	33	1.0	0.24	0
95 Hexamethylphosphoric triamide ¹	> 3,000	< 0.01	—	—
96 2,4-Dihydroxybenzophenone ¹	> 30	< 1.1	—	—
97 Resorcinol monobenzoate ¹	> 30	< 1.1	—	—
98 4-Dodecyloxy-2-hydroxybenzophenone ¹	12	2.8	0.25	35

* Source: ¹Eastman Chemical Products, Inc., Kingsport, Tenn.; ²Aldrich Chemical Co., Milwaukee, Wis.; ³Distillation Products, Rochester, N.Y.; ⁴Weyerhaeuser Co., Tacoma, Wash.; ⁵Nutritional Biochemicals Co., Cleveland, Ohio; ⁶Will Corp., Rochester, N.Y.; ⁷Geigy Industrial Chemicals, Ardsley, N.Y.; ⁸Shell Chemical Co., New York, N.Y.; ⁹DuPont de Nemours and Co., Wilmington, Del.; ¹⁰K and K Labs., Inc., Long Island, N.Y.; ¹¹Monsanto Chemical Co., St. Louis, Mo.; ¹²Sterling Winthrop Research Inst., Rensselaer, N.Y.; ¹³Dr. J. A. Miller, McArdle Memorial Laboratory, Madison, Wis.; ¹⁴Viobin Corp., Monticello, Ill.; ¹⁵Light and Co., Colnbrook, Bucks, England. We acknowledge generous gifts of samples from the following sources: ¹, ³, ⁴, ⁷, ⁸, ⁹, ¹¹, ¹², ¹³.

† LT_{50} is the time in minutes required to immobilize 90 per cent of *T. pyriiformis*. Where a doubling of the LT_{50} was not achieved at the highest concentration tested, this is indicated by the > sign. In such instances, assay slopes and absorption values could not be determined.

‡ The prolongation of LT_{50} values resulting from interposing solutions of the compounds in a cuvette between the light source and mixtures of cells and BaP is expressed as a percentage of the prolongation of LT_{50} values as determined when the same concentration of compound is tested directly with cells and BaP, in the absence of the cuvette, as in the regular assays.

investigated by interposing a cuvette, containing an acetone or water solution of the compound in a 1-mm light path, between the irradiation source and wells containing cells and BaP only; compounds were generally tested at the minimum concentration producing maximum protection in antioxidant assays. The resulting LT_{50} values were compared with those of controls obtained with acetone or water in the cuvette.

The reproducibility of the assay is good (Table 2), although variation occurs on ageing of stock solutions, particularly with 'Butter Yellow' and 'Antabuse'. Protective activity increases progressively with concentration

of antioxidant (Fig. 1). The assay reveals wide variations in potency, spanning a 10 log range, although this is sometimes limited by cytotoxicity at high concentrations. Dose-response slopes tend to be shallow, and also parallel in the case of close structural variants such as hindered phenols of the triazine series, 'Ionox' compounds, or tocopherols (Table 1).

Comparable potencies obtain for the four tocopherol isomers and acetate or succinate esters of the α -isomer; *in vivo* hydrolysis of the esters to the presumed active free tocopherol is possible. The importance of solubility is suggested by the enhanced activity of the water-soluble glycol-succinate ester, although on a weight basis it contains only about 25 per cent α -tocopherol. Although the dimeric form of tocopherol has relatively feeble vitamin E activity on gestation-resorption bioassay⁸, it protects against photodynamic injury in a similar manner to the monomers. Antioxidants with reported vitamin E biopotency, such as methylene blue, EMQ, DPPD, NDGA, and 'Antabuse', are all active in the photodynamic bioassay; the latter two compounds in particular showing high activity. Phenols and the simple hindered phenols, BHA, BHT, and 'Ionox 100', are generally inactive in contrast to more complex phenols, such as the other

Table 2. PROTECTION OF *T. pyriiformis* FROM PHOTODYNAMIC TOXICITY OF BENZO(a)PYRENE BY VARYING CONCENTRATIONS OF α -TOCOPHEROL

Individual experiments	Time to 90 per cent lethality (min), with 0.1 µg benzo(a)pyrene, as photosensitizer, at specified concentrations, µg/ml., of α -tocopherol					
	3,000	300	30	3.0	0.3	0*
1	> 60	18	12	6.5	5.5	5.5
2	> 60	21	15	8	7.5	6
3	> 60	20	11	7.5	7.5	7
4	> 60	25	17	10	8.5	7
5	> 60	15	11	10	7	6.5
Medians	> 60	20	12	8	7.5	6.5

* Antioxidant-free control times represent means of 2-4 determinations in individual experiments.

'Ionox' antioxidants and the substituted triazines. The antioxidant activity of the aminoazobenzenes, previously described in other systems⁸, was generally found to be extremely high (Table 1), and 'Butter Yellow', for example, is almost one million times more potent than α -tocopherol. As it is unlikely that differences of such magnitude are explicable in terms of free radical acceptance alone, biological factors such as cell uptake, and selective localization or complexing, are probably involved. There is no apparent correlation between the hepatocarcinogenic and antioxidant activities of the aminoazobenzenes. (Hepatocarcinogenic potencies of aminoazobenzenes in rats: + + + +, Nos. 64, 66, 68; + + +, Nos. 56, 58, 60; + +, No. 62; +, Nos. 63, 65, 67; 0, Nos. 55, 57, 59, 61. No. 54 is hepatocarcinogenic in the mouse¹⁰.) Secondary antioxidants, palmitic and dilauryl esters of ascorbic, and 3,3'-thiodipropionic acids, respectively, showed activity in contrast to the acids. Since commercial samples of lecithin may be contaminated with tocopherol, the activities of lecithin and 'Viobin' observed here may reflect antioxidant synergism between lecithin and varying amounts of tocopherol. Apart from the very low potency of cysteamine, radioprotective agents were inactive. The cuvette experiments (Table 1) indicate the role of absorption in the demonstrated activity of primary antioxidants, such as 'Quercetin' and EMQ. Protection by some industrial ultra-violet absorbers, such as 'Tinuvin P', may be due to low transmittance at the 360 m μ region; however, the effects of others are not explicable in this way.

The present assay provides a sensitive biological measure of particular *in vivo* effects of certain antioxidants, but its general applicability and specificity cannot, as yet, be defined. Present experiments¹¹ indicate that, in general, antioxidants yield similar responses in

the photodynamic bioassay irrespective of which particular photosensitizing agent, carcinogenic or otherwise^{12,13}, is used and also that antioxidant activity is inversely related to the concentration of the challenge photosensitizer. Attempts are being made to define the quantitative interaction between antioxidant and photosensitizer, *inter alia*, by determination of inhibition indices, and also the role of cell-uptake in determining activity¹¹. The results of these *in vivo* assays are further being contrasted with those obtained by *in vitro* photodynamic bioassays, using isolated rat liver mitochondria¹¹, and by previously described *in vitro* haemolysis tests¹⁴. In addition, the effects of selected antioxidants on the response of mice to a polycyclic carcinogen are being investigated¹¹. These and other aspects of this work will shortly be reported.

This investigation was supported by Public Health Service research grant C-6516 from the National Cancer Institute.

¹ *Autoxidation and Antioxidants*, 1 and 2, edit. by Lundberg, W. O. (Interscience Publishers, New York, 1961).

² *Symposium on Foods: Lipids and Their Oxidation*, edit. by Schultz, H. W., Day, E. A., and Sinhuber, R. O. (The Avi Publishing Co., Westport, Conn, 1962).

³ Bieri, J. G., in *Progress in the Chemistry of Fats and Other Lipids*, 7 (2), edit. by Holman, R. T. (Pergamon Press, Macmillan Co., New York, 1964).

⁴ Blum, H. F., *Physiol. Rev.*, **12**, 23 (1932).

⁵ Santamaria, L., *Rec. Contrib. Cancer Res. Italy*, **1**, 167 (1960).

⁶ Epstein, S. S., and Burroughs, M., *Nature*, **193**, 337 (1962).

⁷ Epstein, S. S., Burroughs, M., and Small, M., *Cancer Res.*, **23**, 35 (1963).

⁸ Nelan, D. R., and Robeson, C. D., *J. Amer. Chem. Soc.*, **84**, 2963 (1962).

⁹ Rusch, H. P., and Miller, J. A., *Proc. Soc. Exp. Biol. and Med.*, **67**, 140 (1948).

¹⁰ Miller, J. A. (personal communication).

¹¹ Epstein, S. S. (to be published).

¹² Epstein, S. S., Small, M., Falk, H., and Mantel, N., *Cancer Res.*, **24**, 855 (1964).

¹³ Epstein, S. S., Bulon, I., Koplan, J., Small, M., and Mantel, N., *Nature*, **204**, 750 (1964).

¹⁴ Rose, C. S., and Gyorgy, P., *Amer. J. Physiol.*, **168**, 414 (1952).

THREE HAEMOGLOBINS K: WOOLWICH, AN ABNORMAL, CAMEROON AND IBADAN, TWO UNUSUAL VARIANTS OF HUMAN HAEMOGLOBIN A

By DR. N. ALLAN

Subdepartment of Haematology, University College Hospital, Ibadan, Nigeria

AND

D. BEALE, D. IRVINE and DR. H. LEHMANN

Medical Research Council Abnormal Haemoglobin Research Unit, University Department of Biochemistry, Cambridge

HAEMOGLOBIN K was first described by Cabannes and Buhr¹ and is a 'fast' variant of normal adult haemoglobin (haemoglobin A). It moves further towards the anode on electrophoresis at alkaline pH than haemoglobin A, but only just separates from it.

A haemoglobin K with its abnormality in the β -chain has been reported to occur in combination with haemoglobin S (ref. 2). The proportion of S : K was 3 : 2, and there was a mild haemoglobinopathy. The family in which this haemoglobin K was observed came from the West Indies and was of African ancestry. This haemoglobin K was first noted at Woolwich, England, and it will be denoted as K β Woolwich.

We have now found a second instance of the combination of a haemoglobin K with haemoglobin S. By hybridization with canine haemoglobin³ this haemoglobin K could be shown to be a β -chain variant. Unlike haemoglobin K Woolwich, the proportion of this haemoglobin K to haemoglobin S was 3 : 1, similar to that of haemoglobin A in the sickle-cell trait (A + S). There was no haemoglobinopathy. Indeed, the carrier of this K + S combination was found in the course of routine screening of healthy blood donors at University College Hospital, Ibadan, Nigeria. The blood donor, a woman, came from Cameroon, and neither she herself nor her relatives could be examined

further. This new haemoglobin K will be denoted as haemoglobin K β Cameroon (Figs. 1 and 2).

A third sample of haemoglobin K, this time in combination with haemoglobin A, was found in a Yoruba adolescent in Ibadan, in the course of an anthropological survey.

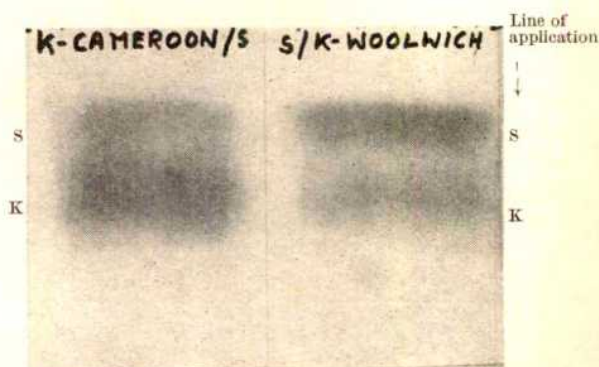


Fig. 1. Paper electrophoresis at pH 8.6 of haemoglobins K Cameroon + S (left) and S + K Woolwich (right). The proportion of K Cameroon to S is that found for A in sickle-cell trait (see Fig. 2).

Hybridization with canine haemoglobin showed the variant to have its amino-acid substitution in the β -chain. The proportion of A : K was 1 : 1, and there was no anaemia. The body was repeatedly examined; but in the members of the family available for examination no further instances of haemoglobin K were found. This haemoglobin K will be denoted as haemoglobin K β Ibadan.

It has previously been discussed that some haemoglobin variants may be recognized as 'abnormal' when in combination with haemoglobin A they are found to form less than half the total haemoglobin⁴. Others may be considered as merely unusual, and they can be recognized by being found in equal proportion to haemoglobin A. The combination A + S (A > S) is an example of the first, abnormal type, and that for A + G Accra (A = G) represents the second unusual type. On two occasions a combination of unusual haemoglobins with haemoglobin S was observed. Haemoglobin S was present as the lesser fraction, as it is found in sickle-cell trait carriers with haemoglobin A (A > S). Haemoglobin J Baltimore⁵ and haemoglobin D Ibadan⁶ were the two unusual haemoglobins which in combination with S formed the greater part of the total pigment. In both instances the carriers of the haemoglobin mixtures had no sickle-cell disease but were sickle-cell trait carriers.

Of our three haemoglobins K, the Woolwich variant can be described as abnormal, and the combination haemoglobin K Woolwich and haemoglobin S (K < S) causes a mild haemoglobinopathy. Haemoglobin K Cameroon together with haemoglobin S results in the sickle-cell trait (K > S), and haemoglobins K Ibadan and A are found in equal proportions (K = A). The two latter haemoglobins K are therefore unusual rather than abnormal. It was of interest to investigate these three haemoglobins which are similar in their electrophoretic properties, and if possible to see which amino-acid substitution in the β -chain changed the physiological value of the resulting variant, and which did not. The haemoglobins were isolated, peptide maps were prepared, and when possible the variant peptides were separated and analysed using the methods recently listed in detail⁶.

Haemoglobin K Woolwich. The peptide map of haemoglobin K Woolwich is shown in Fig. 3. It will be noted that the tyrosine peptide representing β A₁TpXIII and the

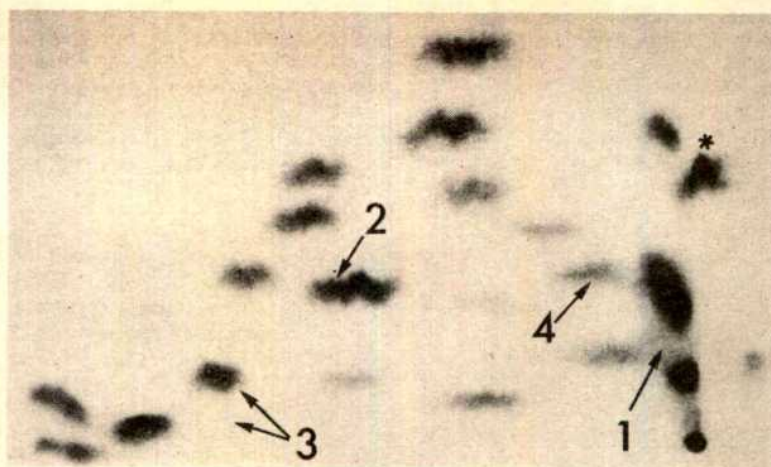
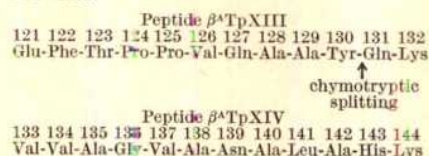


Fig. 3. Peptide map of haemoglobin K Woolwich. Note that at 1 β A₁TpXIII (positive for tyrosine), and at 2 β A₁TpXIV (positive for histidine) are missing. The incidental chymotryptic peptide β 131-132 derived from β A₁TpXIII is also missing at 3 (lower arrow) just below β A₁TpVI 3 (top arrow). A new peptide which was found to represent β A₁TpXIII-XIV can be seen at 4. The starred peptide is β A₁TpV in its usual position—compare with Fig. 6

histidine peptide β A₁TpXIV are missing, as well as a small spot near to β A₁TpVI that is usually present in peptide maps of haemoglobin A. An extended electrophoretogram (Fig. 5) showed these differences more clearly. β TpXIII represents residues 121-132 of the β -chain and β TpXIV residues 133-144.



A mutation affecting the mobility of both these tryptic peptides is likely to involve the lysine residue at position 132 because a change in the nature of this residue, apart from Lys \rightarrow Arg, would result in a peptide bond at β 132 which could not be broken by tryptic hydrolysis. In this case β TpXIII and β TpXIV would form one new peptide containing both tyrosine and histidine. Such a new peptide can be seen in Fig. 3, and it will be noted that it has moved somewhat towards the cathode, thus indicating a slight positive charge. This would eliminate the possibility of a Lys \rightarrow Glu mutation which would have given rise to a negatively charged peptide. This mutation was already unlikely on the basis of the electrophoretic mobility of haemoglobin K Woolwich. The mutation Lys \rightarrow Glu results in the acquisition of two negative charges per half molecule of haemoglobin and this would produce a variant with an electrophoretic mobility resembling that of haemoglobin I or haemoglobin N rather than that of haemoglobin K. The mutation Lys \rightarrow Arg has already been excluded. It would not give rise to a charge change and would not resist the tryptic separation of the β XIII and β XIV peptides. The remaining single mutations permitted by the genetic code⁷ are Lys \rightarrow Gln, Lys \rightarrow Asn, Lys \rightarrow Thr and Lys \rightarrow Met. The amino-acid analysis of the new peptide is given in Table 1. It will be seen that all the residues of β TpXIII and β TpXIV are present except for one lysine residue, and that one additional residue of glutamic acid was found. Glutamine is hydrolysed to glutamic acid when peptides are hydrolysed into their amino-acid constituents and the additional glutamic acid had to come from a glutamine, as the observed electrophoretic mobility of the new peptide demands. The lysine residue in position β 132 of the β -chain had thus been substituted by one of glutamine. Haemoglobin K Woolwich may be described as $\alpha_2\beta_2^{132}\text{Lys}\rightarrow\text{Gln}$. This represents the first observation of a Lys \rightarrow Gln mutation in human haemoglobin. The mutation also explains the absence of the small spot

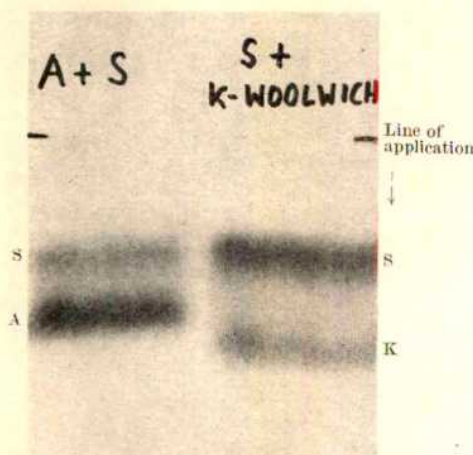


Fig. 2. Paper electrophoresis at pH 8.9 of haemoglobins from sickle-cell trait (A + S) and of haemoglobins S + K Woolwich. The combination of haemoglobin S and K Woolwich shows a greater proportion of S and results in a mild haemoglobinopathy.

Table 1. HAEMOGLOBIN K WOOLWICH

Amino-acid analysis (molar ratio) of the variant peptide $\beta^{\text{K}}\text{Tp}$ (XIII-XIV)				
Known values for haemoglobin A			haemoglobin K	
	βTpXIII	βTpXIV	$\beta\text{TpXIII} + \beta\text{TpXIV}$	βTp (XIII-XIV)
Asp		1	1	0.9
Thr	1		1	1.2
Ser				0.3
Glu	3		3	3.8
Pro	2		2	2.1
Gly		1	1	1.1
Ala	2	4	6	5.8
Val	1	3	4	3.5
Leu		1	1	1.0
Tyr	1		1	0.7
Phe	1		1	0.8
Lys	1	1	2	1.0
His		1		0.9

Low recovery of valine is probably due to slight resistance of Val-Val bond to acid hydrolysis.

usually found near βTpVI . This spot represents a dipeptide $\beta 131-132$ (Gln-Lys) resulting from an incidental chymotryptic splitting of the $\beta 130$ tyrosyl peptide bond during tryptic hydrolysis. No such dipeptide could, of course, arise when $\beta 132$ is Gln instead of Lys because the residues $\beta 131-132$ would remain attached to βTpXIV .

Haemoglobin K Cameroon. Unfortunately there was insufficient material available to make a complete structural investigation, and in spite of all efforts the donor could not be contacted again. The peptide map of this haemoglobin is shown in Fig. 4. It will be seen that the small spot usually found near βTpVI is missing and it is known that this spot represents the dipeptide $\beta 131-132$ (Gln-Lys) arising from an incidental chymotryptic splitting of βTpXIII during tryptic digestion. A very small shift of βTpXIII towards the anode is also noticeable. This alteration in the electrophoretic mobility of βTpXIII was confirmed by an extended electrophoretogram (Fig. 5). The increase in negative charge of βTpXIII is compatible with a haemoglobin K which differs from haemoglobin A by an additional negative charge per half-molecule. This, in βTpXIII , could only be the substitution of a neutral amino-acid residue by Glu or Asp. The absence of the incidental chymotryptic peptide from βTpXIII suggests that there was an inhibition of the chymotryptic splitting of the $\beta 130$ tyrosyl bond. Such an inhibition could be caused by a substitution of the neutral $\beta 129$ Ala residue by an acidic residue. Thus it seems likely that in haemoglobin K Cameroon a Glu or an Asp has replaced the Ala at $\beta 130$. Clearly these observations need substantiating when the opportunity arises.

Haemoglobin K Ibadan. The peptide map of haemoglobin K Ibadan is shown in Fig. 6. It will be seen that

TYROSINE PEPTIDES

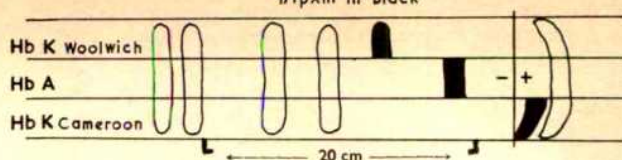
 βTpXIII in black

Fig. 5. Paper electrophoresis (90 min at pH 6.4) of tyrosine containing peptides from haemoglobin A compared with those from haemoglobins K Woolwich and K Cameroon. βTpXIII from haemoglobin K Woolwich is combined with βTpXIV and remains positively charged. βTpXIII from haemoglobin K Cameroon is negatively charged.

the methionine peptide βATpV is missing, and a new methionine peptide has appeared moving further towards the anode with an electrophoretic mobility similar to that of βTpIII . This alteration in electrophoretic mobility of βTpV could be confirmed in an extended electrophoretogram (Fig. 7). βTpV represents residues 41-59 of the β -chain.

βATpV																		
41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59
Phe-Phe	Glu-Ser	Phe-Gly	Asp-Leu	Ser-Thr	Pro-Asp	Ala-Val	Met-Gly	Asn-Pro	Lys									
Splitting with cyanogen bromide																		

Table 2. HAEMOGLOBIN A AND HAEMOGLOBIN K IBADAN

Amino-acid analysis (molar ratio) of peptide βTpV

	βATpV	βKTpV
Asp	3.0	2.9
Thr	1.0	1.0
Ser	2.0	1.8
Glu	1.0	2.0
Pro	2.0	1.9
Gly	1.8	1.0
Ala	1.2	1.0
Val	1.1	0.8
Met	0.8	Present*
Leu	1.1	1.1
Phe	2.4	2.5
Lys	1.2	1.1

* Methionine and sulphone together amounted to about 1 residue.

The amino-acid analysis of the new peptide, from haemoglobin A, is shown in Table 2. It will be seen that the new peptide contains one glycine residue less and one glutamic acid residue more than the haemoglobin A peptide. A mutation Gly \rightarrow Glu would explain the increase in the negative charge of βKTpV and would be compatible with the new variant being a haemoglobin K which has one negative charge per half-molecule more than haemoglobin A. Consistently low recoveries were obtained for phenylalanine. This can be seen also in a previous analysis of βTpV . The Phe-Phe bond may be difficult to hydrolyse because of steric effects. Increasing the time of hydrolysis from 20 h to 40 h at 108° did not improve recovery.

βATpV contains two glycine residues: $\beta 46$ and $\beta 56$. In order to determine which of the two possible glycine residues had been replaced by glutamyl, the methionyl bond at $\beta 55$ was split with cyanogen bromide. The resulting peptides were separated by paper electrophoresis at pH 6.4. βATpV isolated from haemoglobin A was treated similarly. From both βKTpV and βATpV two fragments each were obtained, one charged positively, and one charged negatively. The positively charged peptides obtained from βKTpV and βATpV had the same electrophoretic mobility, whereas of the two negatively charged peptides, that from haemoglobin K had a greater mobility. If the amino-acid substitution Gly \rightarrow Glu in haemoglobin K Ibadan had been at $\beta 56$ no positively charged peptide

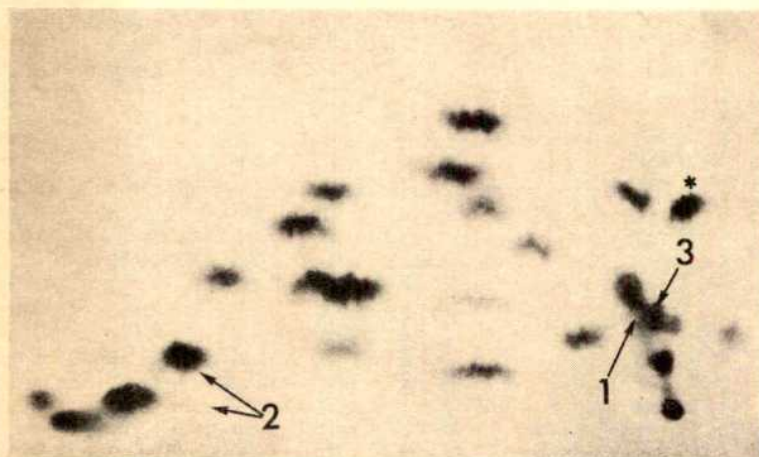


Fig. 4. Peptide map of haemoglobin K Cameroon. Note that a 1 $\beta\text{ATpXIII}$ (positive for tyrosine) is missing. The chymotryptic dipeptide $\beta 131-132$ derived from $\beta\text{ATpXIII}$ is also missing at 2 (lower arrow), just below βATpVI 2 (top arrow). 3 indicates the new peptide $\beta\text{KTpXIII}$. The starred peptide is βATpV in its usual position—compare with Fig. 6.

would have resulted from the cleavage of the methionyl bond at $\beta 55$. Both the positively charged peptides from A and K, presumably representing residues $\beta 56-59$, stained transiently yellow with ninhydrin, indicating the *N*-terminal glycyl. The increased mobility of the negatively charged peptide from haemoglobin K Ibadan indicated that the mutation had occurred at position $\beta 46$. The results of the amino-acid analysis of the four fragments are shown in Table 3. The basic amino-acids were not determined as this was not relevant to the position of the Gly \rightarrow Glu mutation. It will be seen that the positively charged peptides (56-59) from $\beta KTpV$ and $\beta ATpV$ have an identical amino-acid composition. However, the negatively charged peptide representing residues 41-55 from $\beta KTpV$ has one glutamic acid residue more and one glycine residue less than the corresponding negatively charged peptide from $\beta ATpV$. This indicates that of the two glycine residues 46 and 56 the first has been substituted by glutamyl and that the formula of haemoglobin K Ibadan is $\alpha_2\beta_2^{46} \text{Glu} \rightarrow \text{Glu}$. This is the first observation of a Gly \rightarrow Glu mutation in human haemoglobin although a mutation Glu \rightarrow Gly has been observed¹⁰.

Table 3. HAEMOGLOBIN A AND HAEMOGLOBIN K IBADAN
Amino-acid analysis (molar ratio) of peptides arising from splitting βTpV ($\beta 41-59$) at $\beta 55$ methionyl with cyanogen bromide

Electrophoretic mobility at pH 6.4 (Glu, -1.0, Lys, +1.0)	Negatively charged peptides ($\beta 41-55$)		Positively charged peptides ($\beta 56-59$)	
	Hb A	Hb K	Hb A	Hb K
Asp	2.0	2.2	1.0	1.0
Thr	1.0	1.0		
Ser	2.0	1.8		
Homoserine	Present	Present		
Glu	1.2	1.9		
Pro	1.0	1.1	1.0	0.9
Gly	1.0	0.3	0.9	1.0
Ala	1.1	1.0		
Val	1.1	1.0		
Leu	1.0	1.0		
Phe	2.4	2.3		

It has been suggested here that haemoglobin K Cameroon (mutation of $\beta 130$ Ala to Asp or Glu?) and haemoglobin K Ibadan ($\alpha_2\beta_2^{46} \text{Glu}$) are unusual haemoglobins whereas haemoglobin K Woolwich ($\alpha_2\beta_2^{132} \text{Gln}$) is abnormal. It is remarkable that the $\beta 132$ lysine residue, which is substituted by one of glutamine in haemoglobin K Woolwich, is one of the most immutable residues in the evolution of haemoglobin. It is also found in myoglobin, and indeed no haemoglobin has as yet been described in which this $\beta 132$ lysyl is not present. The lysyl in this

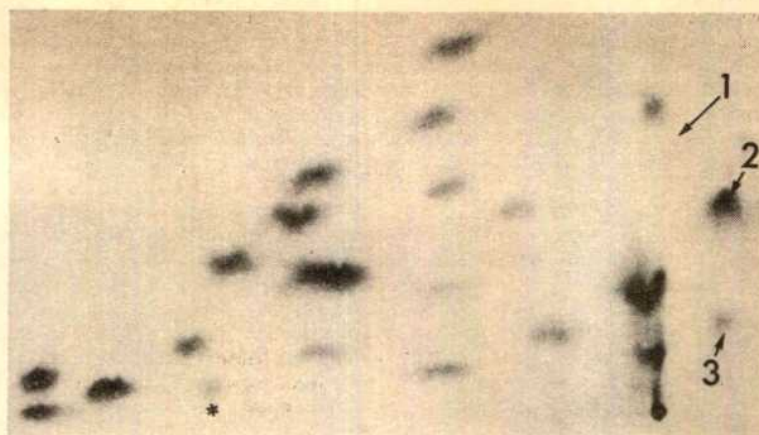


Fig. 6. Peptide map of haemoglobin K Ibadan. $\beta ATpV$ is missing at 1, and a new peptide can be seen at 2, with the electrophoretic mobility of $\beta ATpIII$ (3). The starred peptide is the incidental chymotryptic dipeptide $\beta 31-132$ from $\beta ATpXIII$ which is missing in Figs 3 and 4

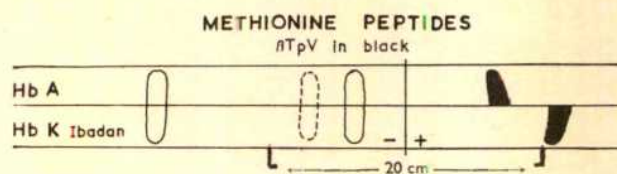


Fig. 7. Paper electrophoresis (90 min at pH 6.4) of methionine containing peptides from haemoglobin A and haemoglobin K Ibadan. Note the increase in mobility of the negatively charged βTpv from haemoglobin K Ibadan

position must obviously serve some purpose in stabilizing the molecule and its replacement by an amino-acid with a neutral side-chain may thus be expected to interfere with the *status quo* within the molecule.

¹ Cabannes, R., and Buhr, L., *Pédiatrie*, **10**, 888 (1955).

² O'Gorman, P., Allsopp, K. M., Lehmann, H., and Sukumaran, P. K., *Brit. Med. J.*, **ii**, 1381 (1963).

³ Huehns, E. R., Shooter, E. M., and Beaven, G. H., *J. Mol. Biol.*, **4**, 323 (1962).

⁴ Lehmann, H., Beale, D., and Boi Doku, F. S., *Nature*, **203**, 363 (1964).

⁵ Charache, S., and Conley, C. L., *Blood*, **24**, 25 (1964).

⁶ Watson-Williams, E. J., Beale, D., Irvine, D., and Lehmann, H., *Nature*, **205**, 1273 (1965).

⁷ Nirenberg, M., Leder, P., Bernfield, M., Brimacombe, R., Trupin, J., Rottman, F., and O'Neal, C., *Proc. U.S. Nat. Acad. Sci.*, **53**, 1161 (1965).

⁸ Bowman, B. H., Oliver, E. P., Barnett, D. R., Cunningham, J. E., and Schneider, R., *Blood*, **23**, 193 (1964).

⁹ Gross, E., and Witkop, B., *J. Biol. Chem.*, **237**, 1856 (1962).

¹⁰ Hill, R. L., Swenson, R. T., and Schwartz, H. O., *J. Biol. Chem.*, **235**, 3182 (1960).

APOFERRITIN SYNTHESIS IN HUMAN ERYTHROID CELLS IN THALASSAEMIA

By DR. EDWIN H. EYLAR* and DR. GASTONE MATIOLI

Departments of Biochemistry and Microbiology, University of Southern California School of Medicine, Los Angeles

A LARGE amount of iron in the form of ferritin and haemosiderin has been revealed in thalassaemic erythropoietic cells by histochemical and electron-optical techniques^{1,2}. One hypothesis claims that ferritin is transferred to the erythroblasts from the macrophages where it is actively synthesized. Recent investigations^{3,4} on rabbit reticulocytes, however, point out that ferritin biosynthesis occurs in erythroid cells. This article describes the biosynthesis of apoferritin by normal and thalassaemic

erythropoietic cells isolated from human bone marrow as well as by reticulocytes. It was found that apoferritin biosynthesis is greatly enhanced in thalassaemia.

Reticulocytes from peripheral blood and erythroblasts isolated from bone marrow were obtained from patients affected by thalassaemia and acute bleeding anaemia. Bone marrow (15-20 ml.) was removed by puncture of the two iliac crests and superior iliac spines. All the procedures described here were carried out at 4°. In order to dissociate cells from the bone marrow fragments, the

*Markle Scholar in academic medicine.

material was gently forced three or four times through the free space of a loosely fitted silicone-coated syringe. The cell suspension was washed three times in cold saline and passed through twenty layers of cheese cloth. The suspension of cells was then centrifuged, and the cells were resuspended in 3 volumes of a 2 per cent solution of fetuin prepared from foetal calf plasma by column chromatography on DEAE-'Sephadex'. The cell suspension in fetuin was applied to a column (2 cm \times 15 cm) containing tightly packed glass wool and the cells eluted slowly by addition of 1 per cent fetuin solution. After again passing the cell suspension through a similar column, the population of cells was represented by red blood cells, reticulocytes and erythroblasts at different stages of maturation, lymphocytes and eosinophils. The other cells (granulocytes, macrophages, megakaryocytes, etc.) were not detected in stain preparations or in phase contrast microscopy. These cells probably remained attached to the glass wool or were destroyed. The eluted cells were washed several times in saline, packed by centrifugation and applied in volumes not exceeding 0.4 ml. per tube to a linear albumin density gradient⁵ ranging between 1.100 and 1.065 and spun at 13,000 r.p.m. in a Spinco model L ultracentrifuge SW 39 rotor at 4°. After spinning for 1 h, the cells were distributed in six rather sharply defined bands. The third, fourth and fifth bands from the top were represented by a mixture of red blood cells (70–75 per cent), reticulocytes (10–20 per cent), and erythroblasts (5–10 per cent) at various stages of maturation, particularly polychromatophylic and orthochromatic erythroblasts. Less than 0.5 per cent eosinophils or lymphocytes were seen. The bands containing the erythroid cells were removed by slicing the tube or by aspiration, and the cells were washed three or four times in cold saline to remove the albumin. The cells were consequently incubated in the media previously described⁴.

Large amounts of peripheral blood (150 ml.) in heparin were collected in siliconized tubes. In order to enrich the reticulocyte population, the blood was centrifuged at 4° at 250*g* for 20 min, and the top layer of cells was removed. The percentage of reticulocytes in different blood samples was increased roughly two-fold by this enrichment process. The buffy coat was removed as completely as possible and the cells were washed in cold saline four times, then incubated as previously reported⁴.

For incubation at 37°, the modified Krebs-Ringer solution was used. For reticulocytes from peripheral blood, the ratio of packed cell volume to incubation media was about 0.4. In every 7 ml. of incubation media, 10 μ c. of ¹⁴C-L-leucine (Volk Chemical Co.) with specific activity of 180 mc./mmole was added. The cell population contained 7–11 per cent reticulocytes and no white cells. The volume of reticulocytes was evaluated by multiplying the haematocrit by the per cent reticulocytes.

For the erythroblast preparations, approximately 6 ml. media was used for every 1 ml. packed cells which contained about 5–10 per cent erythroid cells with the reticulocyte: erythroblast ratio varying from 1:1 to 2:1 to 1:2 for different preparations. About 7.5 μ c. of ¹⁴C-L-leucine was added per 6 ml. incubation media. Under these conditions no perceptible haemolysis occurred even up to 3 h. The volume of erythroblasts was computed by multiplying the haematocrit by the volume per cent of erythroblasts assuming spherical cells and a volume ratio of 2.3:1 for erythroblasts to erythrocytes⁶.

After haemolysis of the reticulocyte or erythroblast preparations, the solution was centrifuged at 20,000*g* for 20 min to remove stroma and mitochondria under conditions described previously⁴. Very little haemoglobin was left in the precipitate after washing once with 0.15 M sodium chloride at pH 8.5. The ferritin was isolated from the combined supernatant and wash solutions by adding an excess of antiserum⁴ obtained from rabbits. Immunization of the rabbits⁴ was carried out with human ferritin prepared from liver which had been obtained from cases

of severe haemachromatosis. It was crystallized three times with 20 per cent cadmium sulphate and contained no traces of contaminating proteins as revealed by starch-gel electrophoresis at pH 8.6, ionic strength 0.02.

The incorporation of ¹⁴C-leucine into ferritin of reticulocytes from peripheral blood is shown in Fig. 1. The biosynthesis of ferritin was found in all the patients, and from Fig. 1 it is clear that ferritin synthesis in thalassaemia (curves A and B) is greatly increased over the controls. Our technique measures only the activity of the soluble ferritin and apoferritin of the 15,000*g* supernatant fluid, and thus the activity associated with haemosiderin or ferritin of the mitochondria, nuclei, or stroma would be missed. The apoferritin synthesis shown in curve A (Fig. 1) is about 37-fold increased over the controls (curves C and D) after 30 min. Much variation was found in the abilities of the reticulocytes from different patients with thalassaemia to synthesize ferritin as shown by curves A and B. The maximal synthesis was found with a 24-month-old girl with very severe clinical symptoms. The variation in general in reticulocytes seemed to correlate roughly with the severity of the anaemia as judged clinically. None of the patients of this investigation had been transfused within one year or longer, and all thalassaemic patients had 75–95 per cent haemoglobin F.

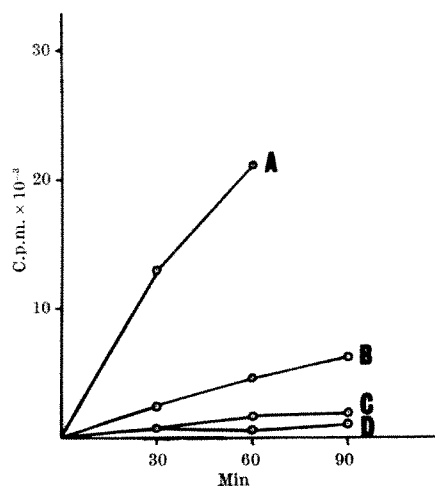


Fig. 1. Incorporation of ¹⁴C-L-leucine found in isolated ferritin, measured as c.p.m./ml. packed reticulocytes, is plotted as a function of time of incubation. Curves A and B represent cases of thalassaemia and curves C and D represent cases of acute bleeding anaemia. The incubation conditions at 37° and isolation of the ferritin are described in the text.

The incorporation of ¹⁴C-leucine into ferritin of erythroblasts isolated from bone marrow is shown in Fig. 2. Again, as with the reticulocytes, the ferritin synthesis in thalassaemia is greatly elevated over the control. Here, also, there is much variation between patients. This variation, however, unlike the situation with the reticulocytes, could not be correlated directly with the severity of the clinical symptoms. For example, curve A represents a case of mild thalassaemia (judged clinically) while curve C, which is only slightly above the normal, represents very severe anaemia.

The incorporation of ¹⁴C-leucine into apoferritin in human reticulocytes and erythroblasts in thalassaemic and non-thalassaemic cases agrees with earlier results⁴ which showed that rabbit reticulocytes were able to synthesize apoferritin. These results offer direct proof for the intracellular origin of ferritin, and initiate new questions concerning the interrelationships of iron-containing macromolecules within the cell. In thalassaemia the ferritin synthesis is greatly elevated as seen from Figs. 1 and 2 in both reticulocytes and erythroblasts. A comparison of the ratio of ferritin to haemoglobin synthesis shown in Table 1 illustrates more validly and dramatically the

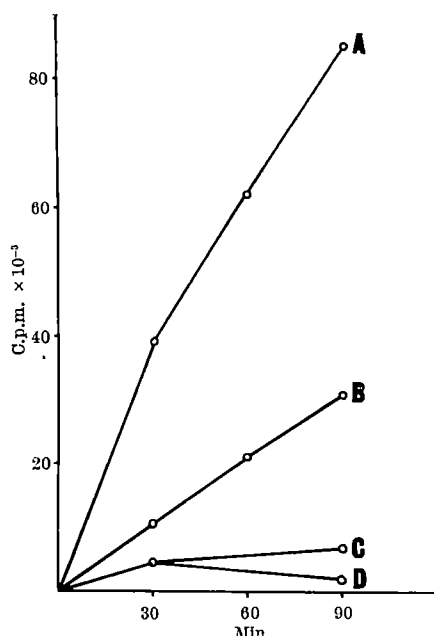


Fig. 2. Incorporation of ^{14}C -L-leucine into the isolated ferritin, measured as c.p.m./ml. packed erythroblasts, is plotted against time of incubation at 37° as described in the text. Curve A represents a case of thalassaemia with mild clinical symptoms whereas curves B and C represent severe thalassaemia. Curve D was obtained with erythroblasts from a case of acute bleeding anaemia.

enhanced ferritin synthesis in thalassaemia. The possible objections to comparison of different cell populations because of variation due to factors like polysome concentration, rate of amino-acid transport, age of cell, and environmental differences are obviated. Furthermore, the biosynthetic abilities of reticulocytes and erythroblasts correlate with electron microscopic results which show greatly elevated levels of ferritin and haemosiderin in thalassaemia. A rough calculation can be made from the results shown in Fig. 1 (curve A) to show that thalassaemic reticulocytes synthesize ferritin at the rate of $0.7 \mu\text{g}$ per 48 h per cell. For erythroblasts, the *in vitro* synthesis of ferritin (curve A, Fig. 2) is calculated to be $3\text{--}6 \mu\text{g}$ of ferritin per 48 h per cell in thalassaemia. These figures compare favourably with the $2\text{--}3 \mu\text{g}$ of ferritin and haemosiderin estimated to be present in the thalassaemic erythroblasts⁷. The calculated rate of ferritin synthesis represents a minimal value since only soluble ferritin of the $15,000g$ supernatant fluid was measured. The specific activity of the leucine is probably reduced by residual leucine in the intracellular pool, and some messenger RNA (mRNA) is probably destroyed as the reticulocytes mature. It is apparent nevertheless that the erythroid cell is capable of synthesis of much of its ferritin and haemosiderin, if not all of it. There would be little reason, therefore, for macrophages to transfer ferritin to the erythroid cell as suggested by Bessis and Breton-Gorius². Rather the macrophage system may acquire it by transfer

from erythroblasts, reticulocytes and erythrocytes in the interest of iron conservation. Furthermore, the iron of the ferritin normally found in reticulocytes and erythroblasts in thalassaemia represents 5–7-fold greater iron⁷ than found in the haemoglobin of a normal cell. It appears, therefore, that ferritin in erythropoietic cells need not be used as an iron depot for haemoglobin synthesis⁸ and may function in other biological roles.

Most of the thalassaemic patients of this investigation had never been transfused; consequently, it cannot be claimed that the increased ferritin biosynthesis is due to iron overloading. Variations in both haemoglobin and ferritin biosynthesis occurred among different cases. With reticulocytes the variation in ferritin synthesis roughly paralleled the severity of the thalassaemia. With erythroblasts, the variation in ferritin synthesis could reflect, in addition to the other factors mentioned above, the age and type of the erythroblasts. It is certainly not likely, however, that the variation between cell populations from normals and thalassaemics could account for the greatly increased ferritin synthesis in thalassaemics since similar conclusions were derived from results with reticulocytes, which represent a more homogeneous population than erythroblasts. In order to eliminate the variation between cell populations from different patients, the ratio of synthesis of ferritin to haemoglobin for both reticulocytes and erythroblasts are tabulated in Table 1. Although average haemoglobin synthesis is reduced in the thalassaemic condition in our work—about 50 per cent in erythroblasts and 30 per cent in reticulocytes—the haemoglobin synthesis nevertheless serves as a valid frame of reference to judge the ferritin synthesis because the latter is so greatly elevated. Table 1 shows a 10–40-fold increase (1,000–4,000 per cent) in the ratio of ferritin to haemoglobin synthesis in thalassaemics with respect to the normals for erythroblasts and reticulocytes respectively. Correction for lowered haemoglobin synthesis gives a figure of 5–28-fold increase of ferritin synthesis of thalassaemic erythroblasts and reticulocytes over normals. Although the cells from the 2 thalassaemic cases in Table 1 showed widely different abilities to synthesize protein (curves A and B of Fig. 1), the ratios are in good agreement. Table 1 also illustrates that erythroblasts show a greater ferritin to haemoglobin ratio than reticulocytes in both the normal and thalassaemic state. These results would be anticipated on the basis of microspectrophotometric data⁹, which showed that ferritin synthesis precedes haemoglobin synthesis in the pronormoblast and basophilic erythroblast stages.

In β -thalassaemia, the average amount of haemoglobin per cell is reported to be about 15–25 per cent less than the normal⁹. It would appear logical that the enhanced production of ferritin in thalassaemia could account in large measure for this reduction. The early increase of synthesis of ferritin in young erythroblasts in thalassaemia could lead to a decrease in the cellular iron available for haemoglobin production⁴. There are no evidences that ferritin iron is utilized for haemoglobin synthesis⁸. Apoferritin molecules bind approximately 500 times as many iron atoms as haemoglobin, and its increase could reduce haemoglobin synthesis, which is known to be responsive to levels of iron concentration, by a negative feedback. Another alternative, of course, is that the ferritin biosynthesis is increased as a consequence of hypothesis of haemoglobin. Because iron induces both proteins, the existence of a block in the biosynthesis of haemoglobin would allow more iron to induce apoferritin synthesis and react with apoferritin to make ferritin. Against this possibility, however, stands the very high concentration of ferritin found both by cytochemical and biochemical methods, which cannot be accounted for by the difference between the iron used for production of haemoglobin in normal erythrocytes and thalassaemic cells. In fact, while normal erythrocytes use $0.1 \mu\text{g}$ of iron to make $30 \mu\text{g}$ of haemoglobin a large proportion

Table 1. A COMPARISON OF THE RATIOS OF RADIOACTIVITY IN ^{14}C -LEUCINE FOUND IN ISOLATED FERRITIN AND TOTAL HAEMOGLOBIN IN RETICULOCYTES AND ERYTHROBLASTS

	Ratio of c.p.m. in ferritin $\times 10^4$ to the c.p.m. in haemoglobin/ml. cells	
	Thalassaemia	Normal
Reticulocytes	120	2.5
	110	
Erythroblasts	230	21
	170	

The conditions of the incubation and the isolation of the ferritin are described in the text. The amount of radioactivity in the haemoglobin was determined as follows: the $20,000g$ supernatant fluid was adjusted to 50 per cent saturation with ammonium sulphate (pH 7.0). After setting 1 h at room temperature, the solution was centrifuged, and the precipitate was washed once with 50 per cent ammonium sulphate. The combined supernatant fluid and wash solution were combined with an equal volume of 10 per cent trichloroacetic acid. The precipitate was washed, and the radioactivity determined as described elsewhere (ref. 4).

of thalassaemic erythrocytes make 15 μg of haemoglobin, leaving then 0.05 μg of iron for ferritin⁷. The amount of iron bound to ferritin, however, is 5–7 times larger (that is, 0.250–0.350 μg or more). It is obvious that the total lack of information concerning the expression of the ferritin gene makes it very difficult to discuss the high production of ferritin within the genetic framework of present interpretations of thalassaemia. Ferritin is present in concentrations comparable to those found in thalassaemia in erythroblasts of patients with refractory anaemias with sideroblastic marrow. The fact that 30 per cent of these patients have haemoglobin F sometimes in high amounts (12 per cent) with no evidence for a hereditary basis¹⁰ may open the question of existence of causal relationship between anaemia, production of haemoglobin F and high synthesis of ferritin outside of a rigid hereditary sequence.

We thank Dr. Thomas Bothwell, University of Witwatersrand, Johannesburg, South Africa, for supplying

us with antiserum to human ferritin, and the staff of the Clinica Medica, Cagliari, Sardinia, for the use of laboratory facilities. We also thank Miss M. Washington and Miss T. Laico for assistance and Mr. Larry Gaeta of Hyland Laboratories for a gift of foetal calf serum.

This work was supported by grants AM-07395-02 and AM-08197-01 from the U.S. Public Health Service and the Hastings Foundation.

¹ Maitoli, G., *Haematol. Latina*, 3, 95 (1959).

² Bessis, M. C., and Breton-Gorius, J., *Blood*, 19, 635 (1962).

³ Eylar, E. H., Bekhor, I., and Maitoli, G., *Fed. Proc.*, 23, 1174 (1964).

⁴ Maitoli, G., and Eylar, E. H., *Proc. U.S. Nat. Acad. Sci.*, 52, 508 (1964).

⁵ Lief, R., and Vinograd, J., *Proc. U.S. Nat. Acad. Sci.*, 51, 520 (1964).

⁶ Thorell, B., *Studies on the Formation of Cellular Substances During Blood Cell Production* (H. Kimpton, London, 1947).

⁷ Maitoli, G., Bahr, G., Zeitler, E., and Baker, R., *J. Ultrastructure Res.*, 13, 85 (1965).

⁸ Zail, S. S., Charlton, R. W., Torrance, J. D., and Bothwell, T. H., *J. Clin. Invest.*, 43, 680 (1964).

⁹ Rucknagel, D., *Ann. New York Acad. Sci.*, 119, 436 (1964).

¹⁰ Beaven, G. H., Ellis, M. J., and White, J. C., *Brit. J. Haematol.*, 6, 201 (1960).

FORMATION OF INTRA-MITOCHONDRIAL GLUCOSYL-OLIGOSACCHARIDES FROM GLUCOSE BY INTACT NOVIKOFF ASCITES HEPATOMA CELLS

By DR. V. N. NIGAM and DR. G. DE LAMIRANDE

Montreal Cancer Institute, Notre Dame Hospital, and University of Montreal, Canada

AN amylomaltase able to synthesize glucosyl-oligosaccharides from maltose has been found in both animal and plant tissues^{1–3}. The presence of endogenous glucosyl-oligosaccharides has been reported in rat liver⁴ and their formation from ¹⁴C-glucose observed in rat diaphragm⁵. Of particular interest are the results of Sie *et al.*⁶, who obtained higher specific activity in oligosaccharides as compared with glycogen soon after NaH¹⁴CO₃ was administered to starved rats treated with cortisol. They concluded that glucosyl-oligosaccharides may be involved in the mechanism of glycogen synthesis. Olavarria⁷, on the other hand, using a partially purified glycogen synthetase of rat liver, has been able to obtain oligosaccharides of higher specific activity than glycogen only at longer periods of incubation, and concludes that they are formed by the degradation of newly formed exterior chains of glycogen by a contaminating enzyme. However, the two experimental systems are different from each other and the results from the purified system do not imply that glycogen formation *in vivo* cannot proceed through the formation of smaller intermediates. The priming action of glucosyl-oligosaccharides, when present in higher concentrations, has been demonstrated for glycogen synthetase⁸.

Our interest in the mechanism of glycogen synthesis originated from the observed inability of tumours to store glycogen. Although initial investigation has revealed that in Novikoff ascites hepatoma phosphoglucomutase activity is greatly reduced (it is 3.0 μmole G1P conversion to G6P per min per ml. packed cells; rat liver has 43 similar units per g wet weight) and glycogen synthetase activity is also diminished (it is 0.88 μmole ¹⁴C-glucose incorporated from UDP-G-¹⁴C per min per ml. packed cells as compared with 2.8 similar units per g wet weight of rat liver), the tumour cell is nevertheless able to synthesize glycogen at rates higher than those observed for rat liver slice. Fig. 1 shows the time-course of glucose incorporation into glycogen by the washed intact Novikoff ascites hepatoma cells⁹, suspended in the incubation medium described by Wu and Racker¹⁰. It can be seen that the rate of glycogen synthesis approximates to 1.1 μmole per min per ml. packed cells (rat liver slice has 0.2–0.4

μmole per min per g wet tissue). Watanabe¹¹ working with AH-130 hepatoma cells, and Alpers *et al.*¹² with HeLa cells, have also reported significant glycogen-synthesizing capacities in these tumours. Thus, it appears that in spite of a severe decrease in enzymatic activity of certain key enzymes the glycogen synthesizing activity still persists in the tumour.

These facts, taken together with the work of Sie and Fishman, and of Beloff-Chain, suggest the possibility of an alternative pathway for glycogen synthesis. The results reported here tend to support the conclusion of Sie *et al.*⁶ that an oligosaccharide route to glycogen may exist.

Novikoff ascites tumours cells were prepared as described before⁹. They were suspended for 15 min in ice-

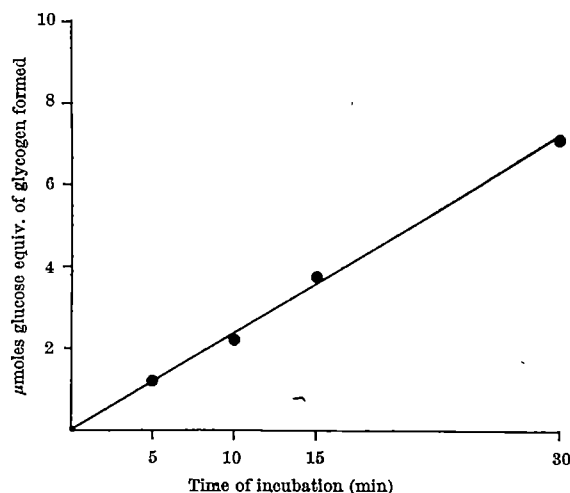


Fig. 1. Time-course of glucose incorporation into glycogen by intact Novikoff ascites hepatoma cells. The digest consisted of 0.9 ml. cell suspension (0.2 ml. packed cells) in *tris*-incubation medium and 0.1 ml. solution in *tris*-incubation medium of 10 μmoles glucose-1-¹⁴C (100,000 c.p.m.). The reaction was carried out in 20-ml. beakers kept in a Dubnoff shaker flushed with oxygen at 37°. Glycogen was isolated by the KOH method and counted.

cold 1/4-strength *tris*-wash medium of Wu and Racker¹⁰ free of phosphate buffer, to allow easy breakage of the tumour cells later¹³. They were centrifuged in the cold and the sedimented cells were mixed with 2.0 ml. ice-cold distilled water and transferred to a 100-ml. Erlenmeyer flask. The thick suspension was rapidly brought to room temperature and 75 μ moles of glucose-1-¹⁴C (1×10^6 c.p.m.) was added (0.5 ml.) and the mixture was shaken in the presence of oxygen for 60 sec. After this short incubation, the cells were chilled and five volumes of cold water added; the cells were homogenized as described by Hawtree and Silk¹³. To the homogenate enough 1 M sucrose was added to make the mixture 0.25 M with respect to sucrose concentration. The homogenate was subjected to differential centrifugation and the mitochondrial fraction was isolated¹⁴. It was washed twice with cold 0.25 M sucrose. The second wash showed only traces of radioactivity. The packed mitochondrial fraction was then suspended in ice-cold water and a fraction was placed on the planchet, dried and counted using a Picker X-ray counter. Table 1 shows the number of counts obtained in the mitochondrial fraction from cells treated with ¹⁴C-glucose in the absence and presence of 2,4-dinitrophenol. It can readily be seen that glucose incorporation into the mitochondrial fraction takes place only when oxidative phosphorylation is possible.

Table 1. RETENTION OF RADIOACTIVITY BY TUMOUR MITOCHONDRIA ISOLATED AFTER INCUBATION OF INTACT TUMOUR CELLS WITH GLUCOSE-1-¹⁴C, IN THE PRESENCE AND ABSENCE OF 0.25 mM DINITROPHENOL

The counts are corrected for internal absorption due to traces of sucrose. Experimental details are described in the text. Total c.p.m. in the mitochondrial fraction/ml. packed cells

Exp. No. Additions	1	2	3
None	4×10^3	7.5×10^3	7.2×10^3
DNP	450	750	780

The identity of the labelled product in the mitochondrial fraction was established as follows. The washed mitochondrial fraction was extracted with 50 per cent ethanol. It was centrifuged and the supernatant removed. The precipitate was washed once more with 50 per cent ethanol and the alcoholic solution was recombined with the first supernatant. The solution was evaporated to dryness *in vacuo*. The material obtained was extracted with methanol (5 ml.), centrifuged to remove the precipitate, and re-evaporated to dryness *in vacuo*. The sugars were dissolved in 0.4 ml. methanol. In various experiments, 20–40,000 c.p.m. were recovered as sugars associated with the mitochondrial fraction per 4–5 ml. packed cells. The methanolic solution of the sugars was subjected to chromatography on paper as described by Giri and Nigam¹⁵. The chromatogram was developed with aniline diphenylamine phosphate¹⁵ whereon the presence of glucosyl-oligosaccharides became apparent. Fig. 2 shows a facsimile of the chromatogram obtained, together with a reference mixture of glucose, sucrose, maltose, maltotriose and maltotetraose. The lower bands and maltose gave the typical blue colour indicative of 1–4 glucosidic linkages¹⁶. When the bands corresponding to oligosaccharides and glucose were eluted the percentage distribution of radioactivity was roughly of the following order: glucose, 35–45; maltose, 10–15; maltotriose, 20–25; maltotetraose, 20–25; maltopentaose, 10–15.

In control experiments when labelled glycogen (obtained after incubation of tumour cells with glucose-1-¹⁴C and isolated by the KOH method, 150,000 c.p.m.) was added to the tumour homogenate and the mitochondrial fraction isolated and washed as described here, no detectable counts remained with the mitochondrial fraction. Similarly, added labelled glucose was not adsorbed on the mitochondria even after 30 min incubation at room temperature.

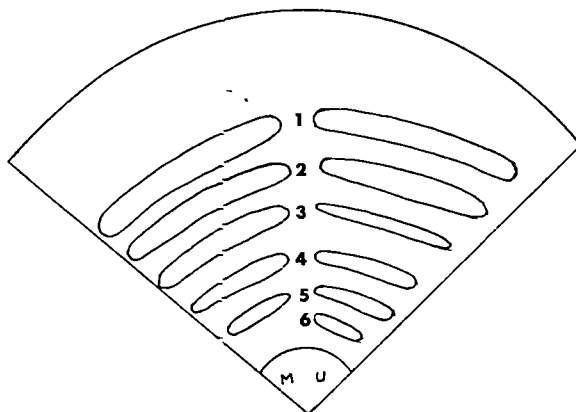


Fig. 2. Sector of a circular-paper chromatogram showing the presence of glucose (band 1), maltose (band 3), maltotriose (band 4), maltotetraose (band 5) and maltopentaose (band 6) in the mitochondrial extract of Novikoff ascites hepatoma cells (U). Presence of sucrose (band 2) is due to the use of sucrose after homogenization. Band 2 has little radioactivity. M represents a synthetic mixture of glucose, sucrose, maltose, maltotriose and maltotetraose, numbered 1 to 5 in the order of decreasing R_f values. Developing solvent: acetone-butanol-water (7 : 2 : 1). Spraying reagent: aniline diphenylamine phosphate

The presence of oligosaccharides in the mitochondrial fraction raises two questions. Are they the degradation product of glycogen by amylase when both sediment with the mitochondrial fraction, or are they in the process of being built into glycogen? It appears improbable that these saccharides of low molecular weight will sediment with the mitochondria. Furthermore, all operations after the incubation are carried out at 0°–5°, and immediately after the mitochondrial fraction is obtained 50 per cent ethanol is added. Under these conditions amylase-mediated degradation of glycogen appears unlikely.

We interpret our results as indicative of an initial reaction of glucose at the mitochondrial level in the respiring intact cell which leads to the formation of a high-energy glucosyl precursor of oligosaccharides and glycogen. Such a compound may have the capacity to accept glucosyl residues from suitable donors to form short or long glucosidic chains, depending on the period and conditions of incubation. Since the amount of glycogen formed by intact cells subjected to hypotonic treatment is small and the formation of oligosaccharides relatively significant, it seems likely that the oligosaccharides are on the route to glycogen. They may have a high turn-over rate perceptible only when the rate of glycogen formation is reduced by hypotonicity. Whether they act solely as primers or their presence reveals a step-wise build-up of glycogen molecules has yet to be ascertained. Extremely low content of glycogen in the freshly prepared tumour cells may suggest that they also could act as primers. This investigation has been supported by a grant to Dr. A. Cantero from the National Cancer Institute of Canada. We thank Dr. W. H. Fishman for assistance.

¹ Giri, K. V., Nagabhushanam, A., Nigam, V. N., and Bejavadi, B., *Science*, **121**, 898 (1955).

² Nigam, V. N., and Giri, K. V., *J. Biol. Chem.*, **235**, 947 (1960).

³ Miller, K. D., and Copeland, W. H., *Biochim. Biophys. Acta*, **22**, 193 (1956).

⁴ Fishman, W. H., and Sie, H. G., *J. Amer. Chem. Soc.*, **80**, 121 (1958).

⁵ Beloff-Chain, A., Cantanzaro, R., Chain, E. B., Masl, I., Pocchiarl, F., and Rossi, C., *Proc. Roy. Soc., B*, **481** (1955).

⁶ Sie, H. G., Ashmore, J., Ehler, R., and Fishman, W. H., *Nature*, **184**, 1380 (1959).

⁷ Olavarria, J. M., *J. Biol. Chem.*, **235**, 3058 (1960).

⁸ Goldberger, S. H., *Biochim. Biophys. Acta*, **56**, 357 (1962).

⁹ Nigam, V. N., *Nature*, **196**, 478 (1962).

¹⁰ Wu, R., and Racker, E., *J. Biol. Chem.*, **234**, 1029 (1959).

¹¹ Watanabe, M., *Science Reports Res. Inst., Hokoku Univ.-C.*, **11**, 203 (1963).

¹² Alpers, J. B., Wu, R., and Racker, E., *J. Biol. Chem.*, **238**, 2274 (1963).

¹³ Hawtree, A. O., and Silk, A. H., *Biochem. J.*, **74**, 21 (1960).

¹⁴ Umbreit, W. W., Burris, R. H., and Stauffer, J. F., *Manometric Techniques*, fourth ed., 183 (Burgess Publishing Co., Minneapolis, 1964).

¹⁵ Giri, K. V., and Nigam, V. N., *J. Indian Inst. Sci.*, **36**, 259 (1954).

¹⁶ Giri, K. V., Nigam, V. N., and Srinivasan, K. S., *Nature*, **173**, 953 (1954).

GUANASE ACTIVITY OF THE SYMBIONTS AND FAT BODIES OF THE COCKROACH, *Leucophaea maderae*

By DR LEON L. PIERRE

Department of Biology, Long Island University, Brooklyn I, New York

GUANASE is an enzyme which is commonly found in the tissues of animals, as evidenced by the conversion of guanine (a degradation product of nucleic acids) to xanthine. Anderson¹ found the enzyme in high concentration in the fat bodies of the cockroach, *Periplaneta americana*, the armyworm, *Prodenia eridania*, and the mealworm, *Tenebrio molitor*. Similarly, Lisa and Ludwig² found it in the fat body of the normal cockroach, *Leucophaea maderae*. The purpose of this investigation is to determine whether this enzyme is produced by the symbionts, the fat body, or both in the cockroach, *L. maderae*.

The symbionts and fat bodies used in these experiments were cultivated or prepared according to the method of Pierre³. Recent modification of this method facilitates an easier isolation of the symbionts by exposure of the bared abdominal cavity once to the 70 per cent ethanol for 5–10 min, and then substitution of the sterilizing solution for ethanol in each case afterwards. 500 mg material obtained from the symbionts (cultures 24–48 h old) and aposymbiotic adult and nymphal fat bodies (sixth to eighth instar nymphs) were used in the isolation of guanase. The material was homogenized with a motor-driven homogenizer in 8 ml. of distilled water, and allowed to stand for 25 min to permit further extraction. It was then subjected to high-speed centrifugation at 15,000 r.p.m. *in vacuo* at 0° C for 25 min, and further extraction was performed according to the method of Kalckar⁴ as modified by Lisa⁵.

The substrates guanine and xanthine were prepared by dissolving 1 mg of each singly or together in 1 ml. of 0.01 N NaOH. 1 ml. of each substrate or the mixture of both substrates and 1 ml. of the particular enzyme fraction were placed in the main portion of a Thunberg tube, evacuated for 5 min, and then immersed in a constant temperature water-bath at 38° C for 2 h. This temperature is most favourable for the activity of guanase while it inhibits traces of xanthine which invariably accompany the isolation of guanase. At the end of incubation, the proteins in the mixtures were destroyed with 10 per cent perchloric acid, and the deproteinized mixtures were centrifuged at 2,000 r.p.m. for 5 min. 0.5 ml. of the supernate were added to 9.5 ml. of 0.1 N NaOH, and 3 ml. of the mixture were assayed against 3 ml. of 0.1 N NaOH in the Beckman DU spectrophotometer according to the method of Block and Johnson⁶. Verification of the results obtained by this method was evidenced by an assay of incubated mixtures after 1 and 2 h periods according to the method of Roush and Norris⁷. Final confirmation of both methods of assay was obtained by subjecting the deproteinized mixtures to ascending chromatographic studies. Strips of Whatman No. 1 chromatographic paper (6 × 35 cm) were spotted at one end with one lambda portion of the mixtures. One end of the paper was immersed in the solvent (50 ml. butanol + 15 ml. ethanol + 35 ml. distilled water), and the other hung loosely in a cylindrical jar. The top of this jar was sealed and the papers were left for a day. At the end of 24 h, the papers were taken out and air-dried. Spots appearing on the papers were discovered by means of an ultra-violet lamp. These spots were circled with a lead pencil, cut out and eluted in 10 ml. of 0.1 N NaOH. The eluted mixtures were read spectrophotometrically at 260 and 280 mμ. The *R_F* values of the substrates were obtained from the position of the spots on the chromatographic paper. At least five determinations of the activity of substrate and enzyme were performed at a time.

The results of this investigation, performed by the methods of Block and Johnson⁶, and Roush and Norris⁷,

and by chromatographic procedure, revealed that guanine was completely converted to xanthine by each fraction of the enzyme guanase. A noticeable shifting of the minimum and maximum absorption peaks of guanine (240 and 270 mμ) to those of xanthine (260 and 275 or 280 mμ) occurred when this substrate was incubated with the enzyme at 38° C for 2 h according to the method of Block and Johnson⁶ (Fig. 1). A similar activity of the enzyme on the substrate was observed when assays were made according to the method of Roush and Norris⁷ (Fig. 2), and by chromatographic studies (Fig. 3). No change in the absorption peaks of xanthine occurred when this substrate was incubated with the enzyme. Similarly, no change in the absorption peaks of guanine occurred when this substrate was incubated without the enzyme fractions. Mixtures of xanthine and guanine incubated with the enzyme and then assayed also showed evidence of the conversion of guanine to xanthine.

Assays made according to the method of Block and Johnson⁶ revealed that the enzyme fractions from the symbionts, adults and nymphs lowered the optical density of the substrate to the extent of -0.150, -0.148, -0.94 respectively. Corresponding values for this lowering of the optical density when assays were made after the 1-h period of incubation according to the method of Roush and Norris⁷ were -0.140, -0.150 and -0.53 respectively. Similarly, the respective values obtained for the decrease in density when guanine and xanthine were incubated together with the enzyme were -0.110, -0.110 and -0.98. The overall extent in the decrease in density over a 2-h period was 33.3 per cent.

The results of these experiments reveal a high activity of the enzyme guanase in the symbionts and fat body of the cockroach, *L. maderae*. The amount of enzyme

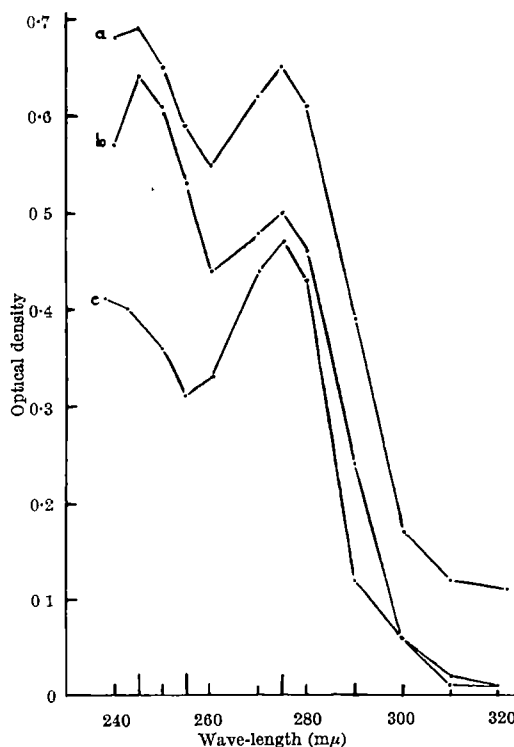


Fig. 1. Activity of guanase isolated from (a) adults, (b) symbionts; (c) nymphs, and incubated with guanine over a 2-h period

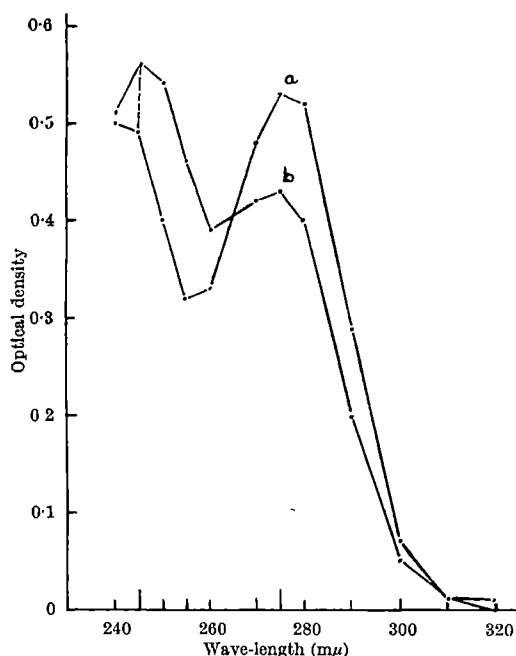


Fig. 2. Activity of guanase isolated from the symbionts and incubated with the substrate (a) xanthine, and (b) guanine over a 1-h period. This graph is identical with those obtained with the adult and nymphal extracts similarly treated.

extracted varied from time to time, and thus accounted for higher densities occasionally. Possibly the slightly lower values obtained when both substrates were incubated together with the enzyme can be accounted for in this fashion: the addition of the reaction product xanthine to the added substrate xanthine tended to shift the equilibrium of the reaction.

The 33.3 per cent lowering of the optical density of guanine by guanase over a 2-h period compares favourably with the 30 per cent decrease obtained by Lisa and Ludwig² with the fat body of the normal insect. Similarly, the R_F values of 0.030 and 0.087 obtained in these experiments for guanine and xanthine respectively also compare

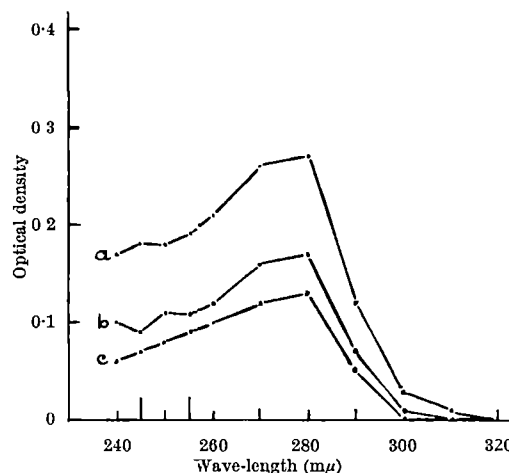


Fig. 3. Disappearance of guanine and the simultaneous production of xanthine when the substrate was subjected to chromatographic studies, then eluted and assayed. (a) Activity of the symbiont enzyme; (b) activity of the adult enzyme; (c) activity of the nymphal enzyme.

favourably with the respective values of 0.000 and 0.062 obtained by those workers. However, the high activity obtained by these workers for the enzyme of the normal fat body could be attributed to the enzyme present in both the fat body and the symbionts. No difference in guanase activity was observed in the enzyme extracted from the symbionts or the adult fat body. However, the activity of the nymphal extract was much slower than that of the symbionts or adults. Possibly, the nymphs depend on the symbionts for guanase activity at some stage of their lives.

I thank Dr. Daniel Ludwig, of Fordham University, for his advice.

¹ Anderson, A. D., Ph.D. thesis, Cornell University (1953).

² Lisa, J. D., and Ludwig, D., *Ann. Entomol. Soc. Amer.*, **52**, 548 (1959).

³ Pierre, L. L., *Nature*, **193**, 904 (1962).

⁴ Kalkar, H. M., *J. Biol. Chem.*, **167**, 461 (1947).

⁵ Lisa, J. D., Ph.D. thesis, Fordham University (1958).

⁶ Block, W. D., and Johnson, D. V., *J. Biol. Chem.*, **217**, 43 (1955).

⁷ Roush, A., and Norris, E. E., *Arch. Biochem.*, **29**, 124 (1950).

DIFFERENT MOLECULAR SPECIES OF MOUSE INTERFERON INDUCED BY STATOLON

By PROF. T. C. MERIGAN

Department of Medicine, Stanford University
School of Medicine, Palo Alto, California

DR. W. J. KLEINSCHMIDT

AND
Lilly Research Laboratories, Indianapolis,
Indiana

STATOLON, an anionic polysaccharide produced by *Penicillium stoloniferum*, achieves its antiviral action by stimulating interferon production, both in the whole animal and in tissue culture^{1,2}. The antiviral substance produced by chick embryo fibroblasts after statolon stimulation was demonstrated to be interferon by its host cell species specificity, acid stability, trypsin sensitivity, and a characteristic heat inactivation curve. Additional biological data suggesting the identity of the active portion of viral and statolon-induced interferons are provided by the experiments of Baron³. These showed that the *in vivo* antiviral protection with mouse interferon induced by statolon against three viruses (encephalomyocarditis virus, vesicular stomatitis virus, and vaccinia) is similar to that provided by the interferon induced by Newcastle disease virus (NDV). A number of other agents, including bacteria⁴, endotoxin^{5,6}, rickettsia⁷ and phytohaemagglutinin⁸, have also been reported to induce interferon production. The interferon produced by these non-viral agents has been characterized primarily by

biological methods. Viral-induced interferon, prepared *in vitro* or *in vivo*, has been shown to be indistinguishable for a given cell species⁹. In a particular species, completely unrelated viruses can induce formation of interferon that cannot be differentiated on the basis of biological and physical properties. In contrast, a single virus can be shown to induce the formation of physically different interferons in cells of different animal species⁹.

We have compared the characteristics of molecular size and charge of non-viral- versus viral-induced activities, as prepared both *in vitro* and *in vivo*. Mouse serum interferon was prepared by intravenous injection of mice with NDV (ref. 10). The serum was held at pH 2 for five days (4° C) to destroy residual virus. Chick interferon was prepared by influenza infection of 9–11 day embryonated eggs¹¹, and purified by zinc precipitation and 'CM-Sephadex' column chromatography¹². Statolon-induced mouse serum interferon was prepared by collecting serum from 12 to 14 g mice after statolon injections intraperitoneally of 2.5 mg or intravenously of 0.72 mg.

Table 1. APPEARANCE OF INTERFERON IN MICE AT DIFFERENT TIMES AFTER THE INJECTION OF STATOLON

Inhibition titre of serum pool (hours after injection)	Hours	Titre
	1	0
	2	258
	6	8,192
	12	4,096
	24	1,024
	48	128
	72	128

Appearance of interferon in mice at different times after the injection of statolon. Seven groups of 20 mice of 12–14 g were injected intravenously with 0.72 mg of statolon. The mice were bled at the times indicated after injection and the serum prepared. Interferon activity was measured by a plaque-inhibition of vesicular stomatitis virus on confluent monolayers of MCN cells. Titre is expressed as the reciprocal of the highest dilution of serum giving 100 per cent inhibition.

Weights of statolon used are based on the non-dialysable solids present in preparations prepared as described previously¹³. The time course of induction of interferon by intravenous administration of statolon in mice was determined (Table 1). For the mouse serum interferon used in these investigations, the mice were bled at 6 h following statolon injection. Statolon-induced interferon was prepared *in vitro* by applying 0.53 mg of statolon to a 6.5 × 13.5 cm monolayer of primary mouse embryo fibroblasts and collecting the supernatant fluids after 24 h. This interferon preparation was concentrated fifty-fold by dialysis against polyethylene glycol 6000 prior to use.

For estimation of interferon titre, a plaque-inhibition assay⁹ was used with a 50 per cent inhibition end-point. To minimize the antiviral effects produced by any residual statolon in the sample, short interferon adsorption periods (6 h) were used. After adsorption of interferon to confluent monolayers of primary mouse or chick embryo fibroblasts growing in tissue culture plates, the cells were challenged with vesicular stomatitis virus.

Mouse and chick interferons are highly specific for cell species^{9,14}. Because each could be assayed in the presence of the other, the method of co-chromatography⁹ previously used to compare viral-induced interferons was used. To determine the molecular weight of the various types of mouse interferon, chromatography was carried out on a long column of 'Sephadex G-100' using both standard chick interferon and phenol red as internal markers. The molecular weight of the statolon-induced mouse serum interferon was also determined by ultracentrifugation in a density gradient. Samples were centrifuged by the method of Martin and Ames¹⁵ in a 2–12 per cent sucrose concentration for 16 h at 105,000g in the Spinco SW 39 rotor, and position compared with that of an egg white lysozyme standard.

The polyacrylamide disc electrophoresis system of Ornstein and Davis¹⁶ was used for electrophoresis of interferon¹³, with the pH 4.3 acetate buffer of Reisfield¹⁷, and a methyl green internal standard. After electrophoresis, the gels were sliced into 1–2-mm sections and placed in tissue culture medium containing 0.05 per cent bovine serum albumin. They were eluted at 4° C overnight before assay of interferon activity in the individual aliquots of medium.

The statolon tissue culture mouse interferon had a molecular weight (Table 2) and electrophoretic mobility ($R_F = 0.35$) similar to mouse viral-induced interferon ($R_F = 0.35$). However, the statolon-induced interferon, as it appeared in mouse serum, had a greater molecular weight—approximately 90,000 (Fig. 1, Table 2). The electrophoretic migration of statolon-induced serum interferon ($R_F = 0.63$) is different from that of statolon-

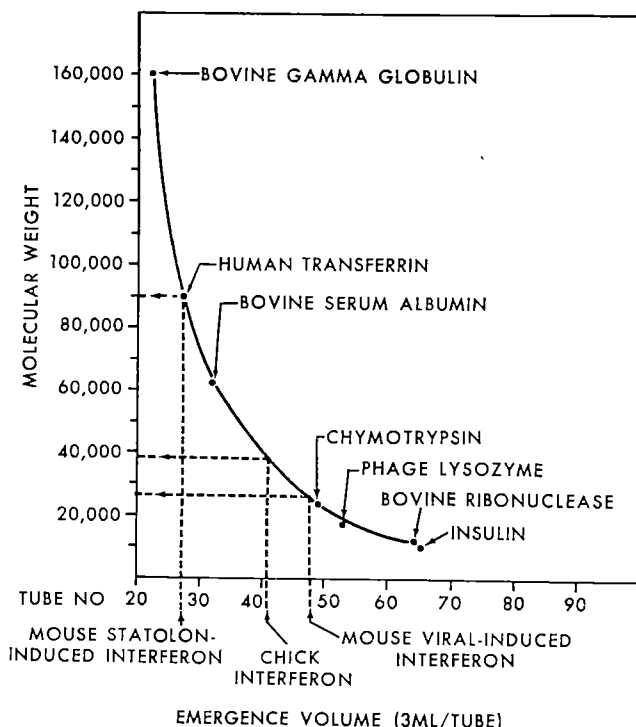


Fig. 1. Molecular weight determination of various mouse serum interferons on 'Sephadex G-100'. Protein standards of known molecular weight were individually chromatographed on the same column. Chromatography of both interferon and protein standards was carried out at 4° C in phosphate-buffered saline on a 120 × 12 cm column of 'Sephadex G-100'. By this same method, Hallum, Stinebring and Youngner¹⁹ have demonstrated an identical molecular weight for NDV induced mouse plasma interferon which has not been exposed to pH 2 but which has had residual NDV virus inactivated by exposure to anti-NDV serum.

induced tissue culture interferon ($R_F = 0.35$). The sedimentation velocity of statolon-induced mouse serum interferon in a sucrose density gradient confirmed its high molecular weight (approximately 85,000). This differs from the value others¹⁸ have found for virus-induced mouse serum interferon (13,000–25,000).

Disaggregation of the heavy interferon was attempted with sodium dodecyl sulphate (SDS) (0.02 per cent), alkali (pH 11) and 5 M sodium chloride. Each was added to the serum by dialysing the sample at 4° C overnight against 50 vol. of the given reagent in 0.1 M salt. Before electrophoresis or chromatography, the reagents were removed by dialysis against 50 vol. of buffer appropriate for the electrophoretic or chromatographic procedure. Unfortunately, the SDS destroyed the activity of the statolon-induced mouse serum interferon. The electrophoretic migration of statolon serum interferon was not influenced by exposure to pH 11 ($R_F = 0.61$) or the 5 M sodium chloride ($R_F = 0.68$).

Previous investigations¹² demonstrate viral-induced chick interferon to be a covalently bonded structure of molecular weight about 30,000 and slightly acidic in charge. It has disulphide bridges, though the number of polypeptide chains is unknown^{12,20}. The viral-induced interferons of chick, mouse and man all have a similar molecular weight and charge¹². The molecular weights have been determined by both sucrose density gradient ultracentrifugation and 'Sephadex G-100' column chromatography^{11,12,18,21–23}. Mouse and chick interferons have indistinguishable charges by gradient ion exchange co-chromatography on 'CM-Sephadex'. Chick, mouse and human interferons are eluted from the 'Amberlite XE-64' resin over the same pH range, whether prepared *in vivo* or in tissue culture¹². For example, human interferon induced in tissue culture by Newcastle disease virus chromatographically resembles human serum interferon induced during immunization with live measles vaccine²⁴.

Table 2. MOLECULAR WEIGHT ON 'SEPHADAX G-100' OF VARIOUS MOUSE INTERFERONS

Type	Inducing agent	Molecular weight
Tissue culture interferon	Chikungunya virus	28,000
Serum interferon	Newcastle disease virus	28,000
Tissue culture interferon	Statolon	34,000
Serum interferon	Statolon	90,000

Comparison of molecular weights of various interferons as determined on 'Sephadex G-100'.

Mouse ($R_F = 0.35$), chick ($R_F = 0.29$) and human ($R_F = 0.32$) interferon all have relatively similar electrophoretic migrations with respect to a methyl green marker¹², in polyacrylamide gels at pH 4.3. The charge and size properties of mouse interferon induced in primary mouse embryo fibroblasts by statolon resemble those of viral-induced interferons.

On the other hand, statolon mouse serum interferon has both different size and charge characteristics. It appears to be similar to the endotoxin-induced heavy mouse interferon described by Hallum, Stinebring and Youngner¹⁹. Statolon differs from endotoxin in its relative lack of toxicity and higher potency of *in vivo* antiviral activity²⁵.

The fact that interferon induced in tissue culture by statolon is similar to viral-induced interferon in both charge and size suggests that interferon induced *in vivo* by statolon is an aggregated form of the smaller molecule. This association could occur either by polymerization with itself or by aggregation or covalent bonding to another molecule. It is possible that the two activities reside in entirely unrelated molecules.

Schonne²⁶ has observed that Sindbis virus in tissue culture stimulates the production of rat interferon, with a molecular weight range of 29,000–100,000. Others^{27,28} have noted viral interferons to form complexes with bovine serum albumin: hence procedures known to break up protein complexes might demonstrate the structural identity of *in vivo* and *in vitro* statolon-induced interferon. In contrast with chick interferon¹², both statolon and viral-induced mouse interferons are inactivated by high concentrations of urea. Therefore, they cannot be investigated with electrophoretic procedures in urea-containing buffers which have been successfully used with chick interferon¹².

Although *in vivo* statolon-induced interferon is similar to that induced by endotoxin in molecular weight and charge, the time of appearance of circulating interferon after injection of statolon is different from that seen with endotoxin (Table 1). The interferon induced by endotoxin had a peak titre at 2 h and had virtually disappeared at 24 h (ref. 5). The time pattern of appearance of statolon-induced interferon is similar to the pattern observed with viral-induced interferon, with the highest titre occurring at 5 h (ref. 10) or at 12 h (ref. 4).

In summary, statolon-induced mouse serum interferon was found to differ from virus-induced mouse interferon in molecular size and charge. The significantly higher molecular weight (90,000) of the former was observed with both 'Sephadex G-100' column chromatography and sucrose density ultracentrifugation. The mouse interferon induced by statolon in tissue culture, however, resembled the viral-induced interferon in molecular weight (30,000) and in electrophoretic mobility in polyacrylamide gels.

We thank Mrs. C. Winget, Miss C. Dixon, Mr. E. B. Murphy and Mr. E. L. Hayes for technical assistance. This work was supported in part by U.S. Public Health Service grant AI-05329-03.

¹ Kleinschmidt, W. J., Cline, J. C., and Murphy, E. B., *Proc. U.S. Nat. Acad. Sci.*, **52**, 741 (1964).

² Kleinschmidt, W. J., and Murphy, E. B., *Virology* (in the press).

³ Baron, S. (in preparation).

⁴ Youngner, J. S., and Stinebring, W. R., *Science*, **144**, 1022 (1964).

⁵ Stinebring, W. R., and Youngner, J. S., *Nature*, **204**, 712 (1964).

⁶ Ho, M., *Science*, **146**, 1472 (1964).

⁷ Hopps, H., Kohno, S., Kohno, M., and Smadel, J. E., *Bact. Proc.*, **5**, 144 (1964).

⁸ Wheelock, E., *Science*, **145**, 310 (1965).

⁹ Merigan, T. C., *Science*, **145**, 811 (1964).

¹⁰ Baron, S., and Buckler, C. E., *Science*, **141**, 1061 (1963).

¹¹ Lampson, C. P., Tytell, A. A., Nemes, M. M., and Hilleman, M. R., *Proc. Soc. Exp. Biol. and Med.*, **112**, 468 (1963).

¹² Merigan, T. C., Winget, C. A., and Dixon, C. B., *J. Mol. Biol.* (in the press).

¹³ Kleinschmidt, W. J., and Probst, G. W., *Antibiot. Chemotherapy*, **12**, 298 (1962).

¹⁴ Baron, S., Barban, S., and Buckler, C. E., *Science*, **145**, 813 (1964).

¹⁵ Martin, R. G., and Ames, B. N., *J. Biol. Chem.*, **236**, 1872 (1961).

¹⁶ Ornstein, L., and Davis, J. L., *Disc. Electrophoresis*, by Distillation Prods. Industries, N.Y. (1962).

¹⁷ Reisfeld, R. A., Lewis, U. S., and Williams, D. E., *Nature*, **195**, 281 (1962).

¹⁸ Rotem, Z., and Charlewood, P. D., *Nature*, **198**, 1066 (1963).

¹⁹ Hallum, J. V., Stinebring, W. E., and Youngner, J. S., *Virology* (in the press).

²⁰ Fantes, K. H., and O'Neill, C., *Nature*, **203**, 1048 (1964).

²¹ Kreuz, L. E., and Levy, A. H., *Nature*, **200**, 883 (1963).

²² Phillips, A. W., and Wood, R. W., *Nature*, **201**, 819 (1964).

²³ Jungwirth, C., and Bodo, G., *Biochem. Z.*, **339**, 39 (1964).

²⁴ Merigan, T. C., Gregory, D., and Petralli, J. (in preparation).

²⁵ Probst, G. W. (personal communication).

²⁶ Schonne, E. (personal communication).

²⁷ Davis, A., *Biochem. J.*, **92**, 29 (1964).

²⁸ Fantes, K. H., O'Neill, C., and Mason, P. J., *Biochem. J.*, **91**, 20P (1964).

AUTORADIOGRAPHIC IDENTIFICATION OF THE D (13–15) CHROMOSOME RESPONSIBLE FOR D₁ TRISOMIC PATAU'S SYNDROME

By DR. F. GIANNELLI

Paediatric Research Unit, Guy's Hospital Medical School, London, S.E.1

IT has recently been shown that the pattern of DNA synthesis of human chromosomes is complex and appears to be relatively chromosome specific, but the mechanism responsible for the time sequence of chromosome synthetic events is unknown.

This article reports an investigation of the labelling patterns of the D group chromosomes based on a case of Patau's syndrome, which is known to be a D₁ interchange trisomic.

The patient (PRU No. 1492), a girl who died in the neonatal period, was considered in life and at necropsy to have the typical features of Patau's syndrome generally caused by primary D₁ trisomy¹.

Blood and skin cultures were analysed in the Paediatric Research Unit by Dr. A. I. Taylor. Forty-five cells were counted and the modal number was 46. Formally, one D (13–15 Denver) chromosome was missing and an extra chromosome resembling a No. 3 was present. In the light of the clinical findings, the patient was thought to be a case of interchange D₁ trisomy, and it was considered likely that two chromosomes of the D group were involved in the interchange, or else that the additional chromosome

resembling a No. 3 was an isochromosome of the long arm of a D chromosome. An additional 156 complete metaphases were analysed; each contained 46 chromosomes and showed the chromosome complement already described here. Cytological analysis of the patient's parents showed that they had normal chromosome complements.

Blood, cultured by a micromethod developed in this Unit², was labelled by adding 0.25 μ c. of tritiated thymidine (Radiochemical Centre, Amersham, spec. act. 2 c./mmole) to each ml. of culture for the last 4 h of incubation, so as to differentially label the very late synthesizing chromosome regions. Desacetylmethylcholchicine ('Colcemid', Ciba) was added 1.5 h prior to termination.

Skin cultures were set up by the technique described by Hsu and Kellog³ and labelled at the third passage by adding 0.25 μ c. of tritiated thymidine (Amersham, spec. act. 2 c./mmole) to each ml. of culture medium for the last 7 h of incubation, and treated with 'Colcemid' for the last 3 h. Slides were prepared by air drying.

Autoradiographs of blood culture slides were prepared at once by a stripping film technique⁴, but skin culture slides were first stained for 3 min in 2 per cent lacto-

acetic orcein and well-spread metaphases were photographed. Then the slides were rinsed in several changes of xylol and absolute alcohol, hydrolysed for 2 min in 4 N hydrochloric acid at 18° C and coated with Kodak 'AR 10' autoradiographic stripping film. After exposure for 5 days, they were developed and stained with azurmethylen-blue⁵.

Qualitative analysis of DNA labelling patterns. The accuracy of the results of the examination of chromosome labelling patterns is limited by, among other factors, the random nature of tritium radiations and by the resolution of the available autoradiographic techniques. Therefore, errors of classification based on single cells are likely to occur and the pairing of morphologically similar chromosomes requires a statistical approach which involves the analysis of numerous individual cells. In this study, in order to avoid bias, each of the chromosomes under consideration has been given a letter code according to its labelling pattern and only in the final conclusions have these patterns been related to the different chromosome pairs.

Blood cultures were used to give information on the very last part of the chromosome DNA synthesis and skin cultures to examine a longer period of DNA synthesis and try to resolve more clearly the differences in the synthetic patterns of the *D* group chromosomes.

(A) **Labelling patterns of the *D* group chromosomes.** In blood culture the five *D* chromosomes showed three labelling patterns (Fig. 1): the first (coded type *A*) was marked by a pattern of dense labelling of the distal part of the long arm, while the proximal part of the long arm and the centromere were unlabelled; the second (type *B*) showed diffuse labelling, which was often denser over the proximal part of the long arm and the centromere; and the third (type *C*) was unlabelled or had less than five autoradiographic grains per chromosome.

The *D* chromosomes in the skin cultures were distinguished as follows: type *A*¹ either completely labelled or with a small unlabelled gap in the long arm near the centromere, or with short arm and centromere region unlabelled; type *B*¹ with dense labelling over the centromere region and proximal half to two-thirds of the long arm; type *C*¹ with the short arm and centromere labelled but with the long arm almost completely unlabelled (Fig. 1). The difference between the *B*¹ and *C*¹ types was more quantitative than qualitative. At times one or more *D* chromosomes showed only few randomly scattered auto-

Table 1. CELL DISTRIBUTION IN RELATION TO THE LABELLING PATTERNS OF THE *D* CHROMOSOMES

Blood culture	No. of cells with each combination	Skin culture	No. of cells with each combination	Total
Combination of labelling patterns		Combination of labelling patterns		
0A+1B+4C	2	0A ¹ +2B ¹ +3C ¹ or C ²	1	2
1A+2B+2C	12	0A ¹ +0B ¹ +5C ²	2	14
1A+1B+3C	11	1A ¹ +2B ¹ +2C ¹ or C ²	57	68
1A+3B+1C	1	1A ¹ +1B ¹ +3C ¹ or C ²	7	18
1A+0B+4C	14	1A ¹ +3B ¹ +1C ¹ or C ²	5	6
2A+1B+2C	1	1A ¹ +4B ¹ +0C ¹ or C ²	7	7
2A+2B+1C	4	1A ¹ +0B ¹ +4C ¹ or C ²	5	19
2A+0B+3C	1			1
Cells with one or more <i>D</i> chromosomes overlapped	14	Cells with one or more <i>D</i> chromosomes overlapped	10	24
Cells with all <i>D</i> chromosomes unlabelled	2			2
Total	62	Total	94	156

Table 2. CELL DISTRIBUTION IN RELATION TO THE LABELLING PATTERNS OF THE LARGE METACENTRIC CHROMOSOMES RESEMBLING CHROMOSOME No. 3

Combination of labelling patterns	Blood culture	Skin culture	Total
2 Z or Z ¹ +1 A/A or A ¹ /A ¹	36	51	87
3 Z or Z ¹	4	6	10
1 Z or Z ¹ +1 A/A or A ¹ /A ¹			
+1 overlapped	9	2	11
2 Z or Z ¹ +1 overlapped	6	4	10
2 chromosomes overlapped or unidentifiable	7	31	38
Total	62	94	156

radiographic grains. This is considered to be a pattern equivalent to a *C*¹ type in cells which became exposed to tritiated thymidine late in the DNA synthetic period and we will refer to this pattern as *C*². The cell distribution according to the labelling patterns of the *D* chromosomes is shown in Table 1.

(B) **Labelling patterns of the large metacentric chromosomes resembling No. 3.** In blood culture, two out of the three large metacentric chromosomes resembling chromosome No. 3 (Denver) showed a diffuse light labelling over both arms, often with a dense cluster of autoradiographic grains over the centromere region (type *Z*). By contrast the third metacentric chromosome (type *A/A*) showed heavy and symmetrical labelling over the distal part of both arms while the centromere region was generally unlabelled.

In skin cultures these large metacentric chromosomes were usually completely labelled, but two had denser labelling of the centromere and sometimes lighter labelling of the middle part of one or both arms (type *Z*¹), while the third had a lightly labelled centromere region (type *A*¹/A¹) (Fig. 1). The cell distribution relative to the labelling patterns of these chromosomes is shown in Table 2.

Quantitative analysis of DNA labelling. In the experiments recorded here the labelling pattern of a chromosome is related to the position of the late synthesizing regions, while the number of autoradiographic grains is a function of the number, size and rate of DNA synthesis, of the late synthesizing regions. Therefore, the grain count has been used to distinguish further between the different *D* group chromosomes, to separate the normal chromosomes No. 3 from the morphologically similar *D/D* chromosome, and to examine its structure. In blood culture, the mean grain count over the type *A/A* chromosome has been compared with that over the two of type *Z* and the mean of half the grain count over the type *A/A* with the mean grain count of the type *A* chromosome and the most densely labelled non-*A/D* chromosome (hot-non-*A*). The *A* and hot-non-*A* chromosomes were also

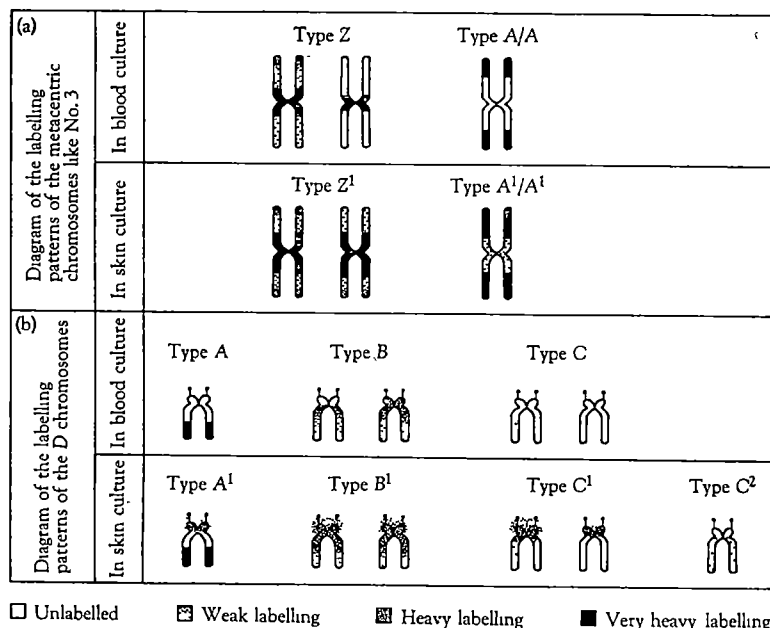


Fig 1

Table 3

Type of culture	Null hypothesis	Mean grain counts	Test results	Probability
Blood culture	1. $A/A = Z$	$A/A = 20.543$	$\chi^2_{100} = 8.73$	$P < 0.001$
	2. $\frac{A/A}{2} = A$	$Z = 10.414$ $\frac{A/A}{2} = 10.486$	$\chi^2_{70} = 0.24$	$P > 0.8$
	3. $\frac{A/A}{2} = \text{hot-non-}A$	$A = 10.553$ $\frac{A/A}{2} = 10.516$	$\chi^2_{12} = 6.07$	$P < 0.001$
	4. $A = \text{hot-non-}A$	$\text{hot-non-}A = 5.844$ $Z = 10.553$ $\text{hot-non-}A = 5.368$	$\chi^2_{11} = 7.51$	$P < 0.001$
Skin culture	5. $A^1/A^1 = Z^1$	$A^1/A^1 = 28.578$ $Z^1 = 17.353$	$\chi^2_{150} = 13.18$	$P < 0.001$
	6. $\frac{A^1/A^1}{2} = A^1$	$\frac{A^1/A^1}{2} = 14.532$	$\chi^2_{95} = 2.69$	$0.01 > P > 0.005$
	7. $\frac{A^1/A^1}{2} + X = A^1$	$A^1 = 16.959$ $\frac{A^1/A^1}{2} + X = 15.864$	$\chi^2_{95} = 1.214$	$0.400 > P > 0.200$
	8. $\frac{A^1/A^1}{2} = \text{hot-non-}A^1$	$A^1 = 16.959$ $\frac{A^1/A^1}{2} = 14.532$	$\chi^2_{95} = 1.84$	$0.1 > P > 0.05$
	9. $\frac{A^1/A^1}{2} + X = \text{hot-non-}A^1$	$\text{hot-non-}A^1 = 13.122$ $\frac{A^1/A^1}{2} + X = 15.864$	$\chi^2_{95} = 3.327$	$P < 0.001$
	10. $A^1 = \text{hot-non-}A^1$	$\text{hot-non-}A^1 = 13.122$ $A^1 = 16.959$ $\text{hot-non-}A^1 = 13.122$	$\chi^2_{95} = 3.955$	$P < 0.001$

Bold type (for example, A/A) signifies the mean grain count over the relevant chromosomes (for symbols of these, see text). $X = 7.5$ per cent of the grains counted over the A type D chromosome.

compared between themselves. In the skin culture similar comparisons were made. The findings are summarized in Table 3.

Because of asynchrony of cellular DNA synthesis, it was decided to standardize the grain counts over each arm of the A/A or A^1/A^1 type metacentric chromosome and the D group. This was done as follows:

In each cell the counts over each arm of the A/A and A^1/A^1 type chromosome and the D chromosomes were multiplied by a factor obtained by dividing the mean grain count of each chromosome group for all the cells by the grains counted over the corresponding group in each individual cell. In this way the counts for each chromosome are adjusted relative to the group's total counts and comparison is thus made possible. For this analysis only the D chromosomes of 82 skin culture cells (where grains could be counted with sufficient accuracy) were used. Blood culture cells had far too many unlabelled chromosomes and were, therefore, discarded. The chromosomes of the D group were classified in each cell according to their grain counts. Then the frequency distribution curves of the adjusted grain counts over both arms of the A/A and A^1/A^1 chromosome and of all the five D chromosome classes were plotted (Figs. 2 and 3).

Conclusion and discussion. Two of the three large metacentric chromosomes (type Z or Z^1) completed DNA synthesis first in the median part of one or both their arms, then in the terminal parts of both arms and finally in the centromere region, and have been interpreted as the normal pair No. 3, known to have a similar synthetic pattern^{6,7}. The third metacentric chromosome (A/A or A^1/A^1) showed a very symmetrical pattern of DNA synthesis; the centromere and the proximal part of each arm completed their DNA synthesis early while the middle and distal parts did so late. This chromosome was interpreted as the D/D chromosome. Consistently, only one A (or A^1) type chromosome was present in the patient's cells and it had a pattern of DNA synthesis similar to that of each arm of the presumptive D/D chromosome and markedly different from the remaining four D chromosomes. A small region of the long arm of this type A element, near the centromere, was the first to complete synthesis followed by the centromere and short-arm region, while the middle and distal parts of the long arm synthesized their DNA very late. The other four

D chromosomes, in those cells in which only one D chromosome of type A was present, could be classified into two different pairs (B or B^1 , and C or C^1 or C^2), according to their labelling patterns. These differences, however, were not invariably clear enough to allow confidence in the precise allocation of these four chromosomes. There was an excess of type C chromosomes, particularly in blood cultures, because this type includes a pair of early synthesizing D chromosomes (type C proper) plus all the unlabelled or weakly labelled D chromosomes belonging to

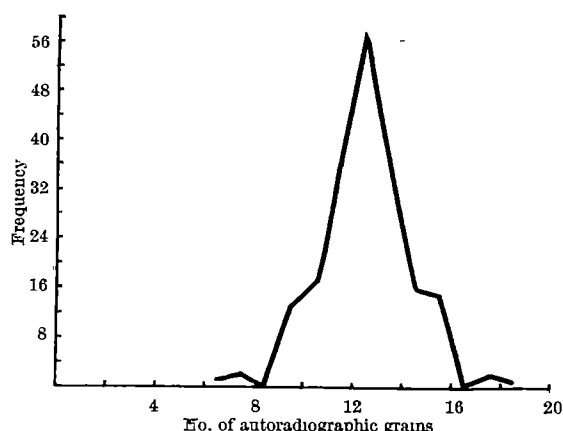


Fig. 2. Adjusted grain counts frequency distribution of the two arms of the A/A and A^1/A^1 type metacentric chromosome

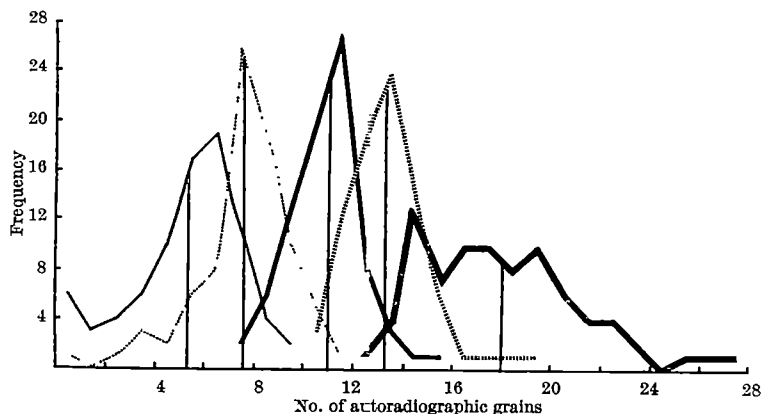


Fig. 3. Adjusted grain counts frequency distribution of the D chromosomes

the other two types found in those cells which had almost completed DNA synthesis before tritiated thymidine was added to the cultures. All four non-*AD* group chromosomes had a similar sequence of synthetic events, but one pair (*C*, *C*¹ or *C*²) was often ahead of the other pair (*B* or *B*¹) in its DNA synthesis. These all completed DNA synthesis first in the distal part of the long arm, then in the proximal part, centromere and short-arm regions, and were interpreted as two pairs of homologous chromosomes with some degree of intrapair asynchrony which was thought to contribute to the blurring of the difference in their DNA synthesis. The labelling over the short arms of these chromosomes was much denser in the skin culture than in the blood culture. This is probably due to the different periods of time for which the cells in the two tissues were exposed to tritiated thymidine but a specific tissue difference is not excluded.

The grain count analysis showed that in the fraction of the DNA synthetic period studied in blood and skin cultures, the two presumptive No. 3 chromosomes synthesized a significantly smaller amount of DNA than the presumptive *D/D* chromosome (Table 3: 1 and 5), thus confirming the distinction made on the basis of the labelling patterns. The *D* chromosome of the *A* or *A*¹ type synthesized significantly more DNA than any of the other *D* chromosomes (Table 3: 4 and 10). The frequency distribution of the adjusted grain counts of the two arms of the *D/D* chromosome did not show any sign of bimodality (Fig. 2). Therefore, the mean grain counts over each of these two arms should not be greatly different from each other, and the results in Table 3: 2 and 3 and 7 and 9 (see following) can be taken as evidence that in blood cultures each arm of the *D/D* chromosome synthesized an amount of DNA equal to that synthesized by the *A* or *A*¹ group chromosome and significantly higher than that synthesized by any of the other *D* chromosomes.

While, as indicated, in blood cultures the *A* type *D* chromosome had completed DNA synthesis in the region of the short arm and centromere, in skin cultures the *A*¹ type was often still synthesizing DNA in this zone. The grains counted over this part of the chromosome were 15 per cent of the total count over the same chromosome. Since the *D/D* chromosome is at least without short arm and centromere region of one of the *D* chromosomes which form it (assuming that it is the result of an interchange), it is not surprising that, in contrast to the observations made on blood cultures, in skin cultures the mean of half the grain counts of the presumptive *D/D* chromosome was significantly smaller than the mean grain count over the *A*¹ type *D* chromosome (Table 3: 6) and was not significantly larger than the mean grain count over the hot-non-*A* type *D* chromosome (Table 3: 8). However, if 7.5 per cent of the counts over the *A*¹ type *D* chromosome is added to half the grain counts of the presumptive *D/D* chromosome to account for the loss of one short arm and centromere region, the skin culture findings are similar to those in blood cultures (Table 3: 7 and 9).

Finally, the five frequency distribution curves of the adjusted grain counts of the *D* chromosomes fall quite clearly into three groups (Fig. 3): two curves with low mean grain counts, two curves with intermediate mean grain counts, and only one curve with high mean grain count. Type *C*¹ and *C*² chromosomes contributed mainly to the first and second curve, type *B*¹ to the third and fourth, and type *A*¹ almost exclusively to the fifth curve. Therefore, I feel justified in dividing the five normal *D* chromosomes present in this patient into one late replicating chromosome, two intermediate replicating and two early replicating chromosomes, having respectively type *A*¹, *B*¹ and *C*¹ or *C*² labelling patterns.

The *D/D* chromosome has both arms with a labelling pattern and grain count very similar to the *A* type *D* chromosome, and this suggests either that it is formed by the union of two homologous *A* type *D* chromosomes or that it is an isochromosome for the long arm of this

chromosome. In either event, the patient would be effectively trisomic for the type *A* chromosome. However, it could be argued that the interchange chromosome, if this is indeed what the *D/D* chromosome is, is formed by two non-homologous *D* chromosomes and one of them carries factors capable of inducing a new pattern of DNA synthesis in the other. This is in contrast with our experience in other autosomal interchanges of similar type^{8,9}. Moreover, the complex pattern of human chromosome DNA synthesis suggests that it is controlled perhaps by more than one factor influencing discrete chromosome regions and it seems to us unlikely, therefore, that in an interchange of the centric fusion type between two non-homologous chromosomes, one of them could control the pattern of synthesis of the other. Finally, if the *D/D* chromosome were formed by non-homologous chromosomes, one being a type *A* and the other a type *B* or *C*, the patient would be trisomic for one of the latter chromosomes because he carries already two normal type *B* or *C* chromosomes. This would be in contrast with the clinical description and our findings in a primary *D*₁ trisomic subject with Patau's syndrome in whom three *A* type *D* chromosomes were present¹⁰, and with two similar cases reported by Yunis *et al.*¹¹, where again three *A* type *D* chromosomes were found.

The suggestion has been put forward⁷ that in trisomics one of the chromosomes present in triplicate might be delayed in its DNA synthesis. Our findings in this patient and in one primary *D*₁ trisomic subject¹⁰ indicate a great similarity in the pattern of DNA synthesis and a fair degree of synchrony between the three presumptive homologues.

The chromosomes responsible for *D*₁ trisomy have large late synthesizing regions^{10,11} and the same may be true of the chromosome responsible for *E*₁ trisomy^{12,13} and Down's syndrome^{7,14}. Late DNA synthesis in the *X* chromosome has been correlated with genetic inactivation. Yunis¹⁵ and Stalder *et al.*¹⁶ have suggested that the viability of the *D*₁, *E*₁ and perhaps 21 trisomic zygotes is due to the presence of large late synthesizing and, by inference, genetically inert chromosome regions. However, this functional interpretation of the patterns of autosome DNA synthesis is a matter of conjecture based on analogy with the late synthesizing *X* chromosome which may not apply to the autosomes. In fact, late autosome DNA synthesis is concordant in all members of a chromosome pair while one *X* chromosome does not show late synthesis and is always out of step with the other (or the others). Therefore, while the idea of the relative genetic inertness of the autosomal late synthesizing regions is tempting, direct genetic evidence is wanted to attempt safely a functional interpretation of the autosomal patterns of DNA synthesis. On the grounds of these results, it is clear that what I have called *A* type *D* chromosome is responsible for *D*₁ trisomy and that the pattern of DNA synthesis is not altered by the trisomic state.

I thank Prof. P. E. Polani and Mr. J. L. Hamerton for their advice, Dr. J. A. Fraser Roberts for his advice on the statistical treatment of the data, Miss D. A. Baker for the illustrations, and Miss V. M. McGuire, Miss G. Olley, Mrs. B. Scott and Mr. E. Nicholson for their assistance. This work was supported by the Spastics Society.

¹ Taylor, A. I., and Polani, P. E., *Guy's Hosp. Rep.*, 113, 231 (1964).

² McGuire, V. M. (in preparation).

³ Hsu, T. C., and Kellog, jun., D. S., *J. Nat. Cancer Inst.*, 25, 221 (1960).

⁴ Giannelli, F., *Lancet*, i, 863 (1963).

⁵ Klinger, H. (personal communication).

⁶ Giannelli, F. (unpublished results).

⁷ Schmid, W., *Cytogenetics*, 2, 175 (1963).

⁸ Giannelli, F. (unpublished results).

⁹ Yunis, J. J., Alter, M., Hook, E. B., and Mayer, M., *New Engl. J. Med.*, 271, 1133 (1964).

¹⁰ Giannelli, F. (unpublished results).

¹¹ Yunis, J. J., Hook, E. B., and Mayer, M., *Lancet*, ii, 935 (1964).

¹² German, J., *J. Cell. Biol.*, 20, 37 (1964).

¹³ Yunis, J. J., Hook, E. B., and Mayer, M., *Lancet*, ii, 286 (1964).

¹⁴ Yunis, J. J., Hook, E. B., and Mayer, M., *Lancet*, i, 465 (1965).

¹⁵ Yunis, J. J., *Nature*, 205, 311 (1965).

¹⁶ Stalder, G. R., Buhler, E. M., and Buhler, V. K., *Humangenetik*, 1, 307 (1965).

LETTERS TO THE EDITOR

ASTROPHYSICS

Measured Velocities of Interplanetary Dust Particles

An interplanetary dust particle experiment was flown on the *OGO-I* satellite launched in September 1964. The primary purpose of the experiment was to measure the speeds and directions of arrival of dust particles within 120,000 km of the Earth's surface, and thus determine the orbits of these particles. If sufficient orbits had been measured, the nature of the apparent dust cloud around the Earth¹ could have been determined. Troubles were encountered with both the spacecraft and the experiment, however, and only three probable dust particle impacts were recorded. This communication briefly describes the instrument and the limited amount of results obtained.

The detector array consists of four tubular detectors aligned along the three body axes of the spacecraft. One detector looks radially away from Earth ($-Z$ direction) and the other three look in a plane normal to the Z direction. Each detector accepts particles with an apparent radiant not more than 6° removed from the detector axis (Fig. 1). An impacting micrometeoroid first encounters the front thin-film sensor which comprises two thin films and a grid. The plasma generated by the passage of the particle through these films starts a 2 mc/s clock which is stopped when the particle impacts on the rear sensor 10 cm down the tube. Thus, the time of flight over 10 cm is measured and the particle velocity following the penetration of the front sensors is determined. The deceleration suffered by a 10^{-10} g iron particle passing through the front sensor at speeds around 3 km/sec has been experimentally determined to be about 10 per cent.

The rear sensor performs two main functions. First, there is a thin-film capacitor deposited on a glass plate. This capacitor momentarily shorts when a particle impacts and the resultant signal stops the time-of-flight clock. Second, a lead zirconate transducer bonded to the back of each glass plate provides a measure of the momentum of each impacting particle. Knowing the velocity, the particle mass can be determined. Thus each tubular detector can provide a measure of the velocity, direction of arrival, and mass of an impacting dust particle.

Three particle impacts were recorded on one tube during the first ten days of operation. Spacecraft trouble was such that the experiment was only turned on for 56 h of this time, and data were received for only 4 h of these 56. Unfortunately, the rear sensor capacitors can suffer a permanent short when subjected to particle impact with the power off the experiment. There was no provision for clearing such a short on this particular experiment, and we believe that three of the four capacitors were dead before experiment data were received. The experiment

was again turned off for a short period after the third impact had been recorded, and although a great quantity of data has been received since, no more impacts have been noted. This suggests that the fourth sensor was shorted by impact when the experiment was turned off on the tenth day after launch.

In view of the paucity of data (the three impacts total only ten bits of information with no redundancy) we had to look rather closely into the statistical reality of the data, considering both experiment noise and telemetry noise. There is no history of the former in our experience with the instrument flown. The unit is well shielded electrically, and also, a time-of-flight measurement requires two independent events to occur within a very restricted time-interval. There were no spacecraft commands sent at the time of the three events, nor did the neighbouring telemetry words show any signs of radio-frequency interference. We have considered the possibility of telemetry noise indicating false impacts and treated the first three weeks of data in considerable detail. The probability of all three impacts being false is less than 2 parts in 10^5 , and the probability of any two of the three being false is less than 3 parts in 10^3 .

The details of the three impacts are shown in Table 1. The first two were recorded within 27 min of each other at altitudes in excess of several Earth-radii, and the observed velocities were in the range 30–50 km/sec. These velocities preclude any possibility that the particles were in closed orbits around the Earth. The third impact indicates a low-velocity particle which impacted at a much lower altitude and which may have had a mass of an order of magnitude greater than the masses of the first two.

Table 1. IMPACT PARAMETERS OF PARTICLES OBSERVED BY THE COSMIC DUST EXPERIMENT ON *OGO-I*

	Time of impact (U.T. 1964)	Altitude (km)	Observed velocity (km/sec)	Geocentric velocity (km/sec)	Mass (g)
Hit 1	257 ^d 03h 45 ^m	22,400 km	$40 < V_0 < 50$	$40 < V_g < 70$	$< 7 \times 10^{-13}$
Hit 2	257 ^d 04h 12 ^m	28,700 km	$33 < V_0 < 40$	$30 < V_g < 60$	$< 1 \times 10^{-11}$
Hit 3	259 ^d 18h 35 ^m	2,900 km	$4.7 < V_0 < 4.8$	$8 < V_g < 15$	$< 7 \times 10^{-11}$

The uncertainty in the observed velocity arises partly from the necessity to digitize the time of flight for telemetry and partly from the uncertainty in the actual deceleration through the front sensor. The maximum deceleration allowed in computing the possible geocentric velocities was 30 per cent. The attitude of the spacecraft at the times of the three impacts was unfortunately not known, so that the range of geocentric velocities possible for each impact was broadened even further to the values given in Table 1. Even with the true geocentric velocities being subject to such wide limits and with only the spin axis of the spacecraft known, it has been possible to deduce something about the probable orbits of these three particles before impact. The first two were probably in high-eccentricity heliocentric orbits of high inclination to the ecliptic plane; in fact the orbits were probably retrograde. For masses less than 10^{-11} g, one would not expect the particles to remain in these orbits for periods much greater than 10^3 or 10^4 years, so probably they were of relatively recent origin, perhaps from a long-period comet. It is worth noting that the direction of the spin axis of the spacecraft precludes any possibility that the two high-speed particles came from the Moon. They must have been in heliocentric orbits.

The third particle could have been either in a closed geocentric orbit or in a low eccentricity orbit of low inclination. The lack of spacecraft attitude information prevents any further delineation. Finally, the flux based

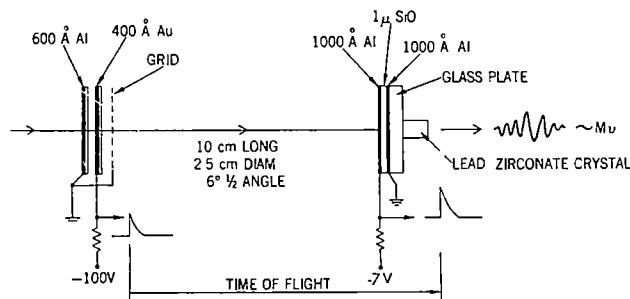


Fig. 1. The *OGO-I* micrometeoroid sensor which determines particle mass and velocity

on these three impacts in 4 h is in excellent agreement with the microphone data recorded by previous near-Earth satellites².

One of the authors (C. S. N.) has been supported in this work by a National Academy of Sciences—National Aeronautics and Space Administration Research Associateship.

C. S. NILSSON C. W. McCracken
W. M. ALEXANDER O. E. BERG
L. SECRETAN

Goddard Space Flight Center,
National Aeronautics and Space Administration,
Greenbelt, Maryland.

¹ Whipple, F. L., *Nature*, **189**, 127 (1961).

² Alexander, W. M., McCracken, C. W., Secretan, L., and Berg, O. E., in *Space Research III*, edit. by Priester, W., 891 (Amsterdam, North-Holland Publishing Co., 1963).

PHYSICS

A Dense Packing of Hard Spheres with Five-fold Symmetry

SUPPOSE a plane of hard spheres is constructed such that the spheres form concentric pentagons with an odd number of balls per pentagon side. A second plane of hard spheres is now constructed such that the spheres form concentric pentagons with an even number of spheres per pentagon side. If this second plane is placed in intimate contact with the first, with their five-fold axes coincident, there results a layer which, within the plane of the layer, can be continuously packed to infinity (Fig. 1). Identical layers can then be stacked one on another, with their five-fold axes coincident, to give an infinite packing along the five-fold axis. An infinite structure can thus be constructed the nucleus of which is a pentagonal dipyrmaid of seven spheres.

Following the foregoing packing sequence with polygons other than the pentagon results in other, well-known structures. The same sequence with squares yields cubic close packing¹ and, with hexagons, primitive hexagonal. A difficulty arises when attempts are made to apply the exact sequence to triangles, because concentric triangles with an even (or odd) number of spheres per side cannot be made coplanar. It is important that, of these polygons, only the pentagon cannot form a regular tessellation and therefore, although it can be packed to infinity, it has a unique axis, the single five-fold rotation axis.

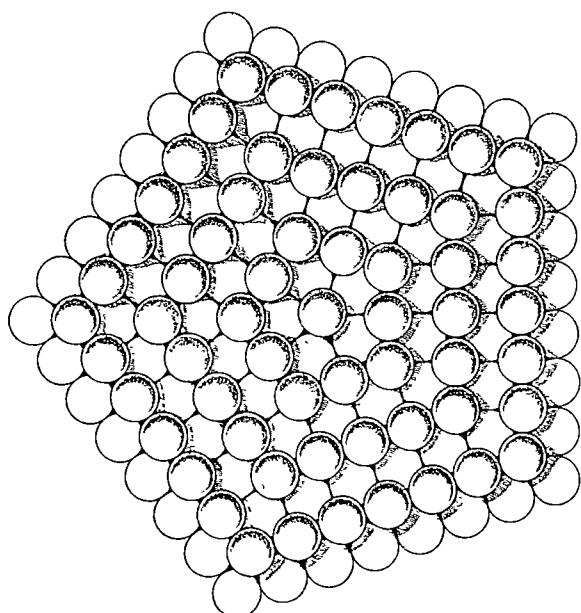


Fig. 1. A layer of hard spheres based on a packing sequence of concentric pentagons.

An alternative way of generating the same pentagonal structure is as follows: Construct $n(n = 1, 2, 3, \dots \infty)$ pentagonal pyramidal shells of hard spheres such that each face is an equilateral triangle of side length n (spheres). If shell 1 is placed in the cavity of shell 2 there results a pentagonal dipyrmaid of seven atoms. Likewise when shell 3 is placed on the structure there results a pentagonal dipyrmaid of twenty-three spheres. In fact, as each subsequent shell is placed on the growing structure there always results a pentagonal dipyrmaid bounded by close-packed planes, each face of which is an equilateral triangle with n (shell number) spheres to a side. This pentagonal dipyrmaid consists of five distorted tetrahedral the edges parallel to the five-fold axis being expanded by 5.15 per cent. Within each tetrahedron the structure is body-centred orthorhombic with cell dimensions chosen such that the pentagonal dipyrmaid faces will be close packed and two adjacent tetrahedra will be joined by a coincidence boundary. These conditions yield a body-centred orthorhombic cell with dimensions (diameter of sphere = 1.000), $a = 1.000$, $b = \cot 36^\circ = 1.3764$, $c = (2^2 - \csc^2 36^\circ)^{1/2} = 1.0515$. Thus this pentagonal structure has a density independent of position of 0.72357. This density is slightly lower than that for close packing (0.74048), but higher than body-centred cubic (0.68017) or icosahedral shell packing (0.68818) (ref. 2). The co-ordination is 10 at a distance of 1.000 and 12 at a distance of 1.052. This structure is an example of G_2^1 type symmetry, that is, a one-dimensionally periodic group in three dimensions, and its symmetry group is $5mP2ml$ (Niggli's³ nomenclature).

Structures which have the symmetry described here have been observed experimentally. Gedwill, Altstetter and Wayman⁴, using optical microscopy, observed five-fold symmetry in cobalt crystals produced by the hydrogen reduction of cobaltous bromide. Wentorf⁵, also using optical microscopy (external morphology), observed five-fold symmetry in synthetic diamonds. Ogburn, Paretskin and Peiser⁶, using X-rays, found pentagonal symmetry in copper [110] dendrites grown by electrodeposition. The most striking examples, however, are the sub-micron whiskers of nickel, iron and platinum grown from the vapour by Melmed and Hayward⁷. These whiskers, 50–200 Å in diameter, had a five-fold rotational symmetry observed by field emission microscopy. The five-fold symmetry was found not to be limited to the surface, as no change in symmetry was observed in the continuous reduction in length of several iron whiskers.

In all these cases the structure was explained as a quintuple twin ((111) twinning plane) with five face-centred cubic individual crystals about a common [110] axis, the $7^\circ 20'$ difference between $5 \times 70^\circ 32'$ and 360° being made up with lattice strain or imperfections. It is unlikely, however, that a twinning mechanism could generate a structure having the small size (50–200 Å) and atomic perfection (at the five-fold axis) of Melmed and Hayward's⁷ whiskers. On the other hand, it appears that the formation of a pentagonal dipyrmaid nucleus and its subsequent growth is a more probable and simpler mechanism for the formation of this structure. Furthermore, if a twinning mechanism were responsible for the five-fold symmetry one would expect [110] to be an observed whisker orientation in normal, non-pentagonal, whiskers. This is indeed the case for nickel and platinum, but the observed orientation for face-centred cubic iron is [100] (ref. 8).

It is also to be noted that the pentagonal nucleus for the structure described here has the same form as one of the configurations which has been proposed as an important element of liquid structure by Bernal^{9,10}. It is evident from the foregoing discussion that crystallization can occur by the growth of such a configuration.

I thank Profs. F. C. Frank, C. S. Smith, and D. Turnbull for their advice, and the Xerox Corporation for a fellowship. This work was supported in part by the Office of Naval Research under contract Nonr 1866 (50), and by the

Division of Engineering and Applied Physics, Harvard University.

B. G. BAGLEY

Division of Engineering and Applied Physics,
Harvard University,
Cambridge, Massachusetts.

¹ Coxeter, H. S. M., *Illinois J. Math.*, **2**, 746 (1958).

² Mackay, A. L., *Acta Cryst.*, **15**, 916 (1962).

³ Niggli, A., *Zeit. Krist.*, **111**, 288 (1959).

⁴ Gedwill, M. A., Altstetter, C. J., and Wayman, C. M., *J. App. Phys.*, **35**, 2266 (1964).

⁵ Wentorf, R. H., jun., in *The Art and Science of Growing Crystals*, edit. by Gilman, J. J., 192 (Wiley, New York, 1963).

⁶ Ogburn, F., Paretzkin, B., and Pelser, H. S., *Acta Cryst.*, **17**, 774 (1964).

⁷ Melmed, A. J., and Hayward, D. O., *J. Chem. Phys.*, **31**, 545 (1959).

⁸ Melmed, A. J., and Gomer, R., *J. Chem. Phys.*, **34**, 1802 (1961).

⁹ Bernal, J. D., *Nature*, **183**, 141 (1959).

¹⁰ Bernal, J. D., *Nature*, **185**, 68 (1960).

GEOFYSICS

Damping of S Waves

THE modified Lomnitz law of imperfection of elasticity makes the strain under constant stress P :

$$\epsilon = \frac{P}{\mu} \left[1 + \frac{q}{\alpha} \{ (1 + at)^\alpha - 1 \} \right] \quad (1)$$

α is probably such that at is large for $t = 1$ second. For longer times the relevant constants are the rigidity μ , α , and qa^α . The data used to estimate α and qa^α are the damping of the 14-monthly variation of latitude and the fact that S at a distance of about 80° is clear and attains half the value for pure elasticity in about 2 sec. An alternative, possibly better, is to take the ratio as $\frac{1}{2}$. These lead to estimates as follows¹:

I	α	$10^4 qa^\alpha \times (1 \text{ sec})^\alpha$	
$\frac{1}{2}$	0.256	3.247	(1)
$\frac{1}{4}$	0.236	4.415	

These lead to no contradictions with other data, such as have been found in attempts to apply the elasto-viscous law. Since estimates of damping have now been made from surface waves, it appears interesting to compare the results with those found from the foregoing law. In this communication values are found for S waves.

The exponent in the complex representation of a travelling harmonic wave is:

$$zt - \frac{zx}{\beta_0} - \frac{1}{2} \frac{qx}{\beta_0} (\alpha - 1)! a^\alpha z^{1-\alpha} \quad (2)$$

where z is to be taken as imaginary. With $z = i\gamma$, $\gamma > 0$, this is:

$$\begin{aligned} & i\gamma \left(t - \frac{x}{\beta_0} \right) - \frac{1}{2} \frac{qx}{\beta_0} (\alpha - 1)! a^\alpha \gamma^{1-\alpha} e^{\frac{1}{2}\pi i(1-\alpha)} \\ &= i \left[\gamma \left(t - \frac{x}{\beta_0} \right) - \frac{1}{2} \frac{qx}{\beta_0} (\alpha - 1)! a^\alpha \gamma^{1-\alpha} \sin \frac{1}{2}\pi(1-\alpha) \right] \quad (3) \\ & \quad - \frac{1}{2} q \frac{x}{\beta_0} (\alpha - 1)! a^\alpha \gamma^{1-\alpha} \cos \frac{1}{2}\pi(1-\alpha) \end{aligned}$$

For $z = -i\gamma$ the imaginary part is reversed and the real part unaltered. The latter gives a damping, with exponent proportional to the distance travelled.

In the calculations the travel time x/β_0 was taken as 200 sec, corresponding to a distance for S of about 7° . The real part of the exponent is denoted by $-k$, and results are given for different periods of the wave, $2\pi/\gamma$, in sec.

Period	k	
	$\alpha = 0.256$	$\alpha = 0.236$
1	0.176	0.251
2	0.105	0.148
5	0.053	0.073
10	0.032	0.043
20	0.019	0.025
50	0.0086	0.013
100	0.0057	0.0074
1,000	0.0010	0.0013

For distance 20° these must be multiplied by nearly 3. But it is clear that the results do not account for the fluctuations of amplitude in S (or in P) at distances up to 20° , by factors of order 100. 5 sec can be regarded as a typical period of the first swing. I should expect results for surface waves to be comparable.

The data used sample the whole of the Earth's shell (now, rather unfortunately I think, usually called the mantle) and therefore refer to average properties. We should like to know to what extent they refer to the outer tenth of the radius. The amplitudes of both P and S decrease greatly to about 10° and rise to strong maxima about 20° . This has been attributed alternatively to variation in the rate of change of velocity with depth and to absorption; these could both produce similar effects on amplitudes. It seems that if the average imperfection of the shell is applicable to the outer tenth absorption is inadequate. Conversely, if the variations of amplitude in earthquake waves are attributed to absorption, this must be in a very thin layer.

The additional imaginary part of the exponent in equation (3) would imply a delay in travel time, proportional to $(\text{period})^\alpha$, and therefore would have little effect for short periods.

The calculations in this communication were made by Miss A. A. Houston at the Mathematical Laboratory, Cambridge.

Estimates of damping of free oscillations are given by Alsop, Sutton and Ewing², in the form:

$$A(t) = A(t_0) \exp \left[- \frac{\pi(t-t_0)}{QT} \right] \quad (4)$$

For the ${}_0S_2$ node they give the period T as 53 min, $Q = 370$. As this mode affects all the shell it should be comparable with the foregoing, with x about 3,000 km, and the expected damping coefficient would be of order 5×10^{-3} . Thus their value is of the order that would be expected from this rough comparison.

HAROLD JEFFREYS

160 Huntingdon Road,
Cambridge.

¹ Jeffreys, H., and Crampin, S., *Mon. Not. Roy. Astro. Soc.*, **121**, 571 (1960).

² Alsop, L. E., Sutton, G. H., and Ewing, H., *J. Geophys. Res.*, **66**, 2911 (1961).

Cassiterite as a Carrier of Palaeomagnetism

IN an attempt to extend the scope of palaeomagnetism when investigating endogenous geological processes^{1,2} the magnetic properties of high-temperature minerals were investigated. The main objective was to find other minerals not yet examined from this point of view which would be amenable to palaeomagnetic examination. The Japanese work on synthetic ferrites³ suggested to us that certain natural high-temperature minerals (possessing a high isomorphic miscibility of elements) might have similar lattice structures and in consequence be ferromagnetic and capable of retaining a stable thermoremanent magnetization. The investigation proved successful. Garnets were the obvious minerals for initial examination. All the garnets examined possessed a weak ferromagnetic moment of the order 10^{-5} E.M.U./c.c., but were found to be unstable and so of little use in palaeomagnetism. Of a number of other minerals examined, cassiterite was found to possess unique palaeomagnetic properties.

Cassiterites obtained from Czechoslovakia (Horní Slavkov, Cínovec), as well as foreign localities (Cornwall, south-west England, Altenberg, Eastern Germany, Swaziland, the Congo, Mexico and New England), showed values of natural remanent magnetization (J_n) ranging from 10^{-7} to 10^{-3} E.M.U./c.c. Of a total of 44 samples examined, 18 had values of J_n lying between 10^{-4} and 10^{-3} E.M.U./c.c., which are values comparable to those exhibited, for example, by Central European basalts.

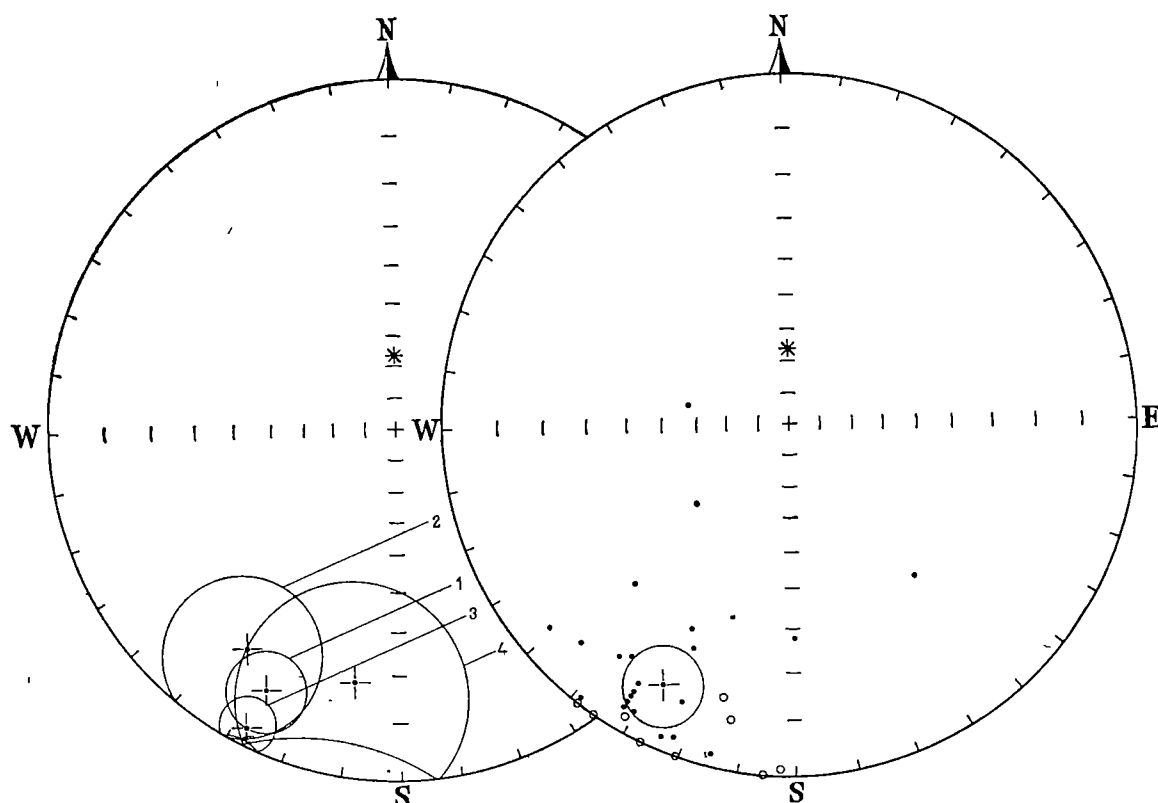


Fig. 1. Stereographic projection of J_n directions of oriented samples of the greisen body 'Huber', Horní Slavkov. ●, ○, Projection of the J_n direction into the lower and upper hemispheres; —•—, projection of the mean direction of J_n ; ★, direction of the present-day theoretical dipole field. Left-hand side: mean J_n directions and confidence circles calculated at the probability level of 95 per cent⁴ for samples collected at three distance localities of the same worked deposit (Nos. 2, 3 and 4); No. 1 stands for the mean direction and confidence circle for all measurements. Right-hand side: projection of J_n direction of all the greisen samples investigated, the mean direction and corresponding confidence circle

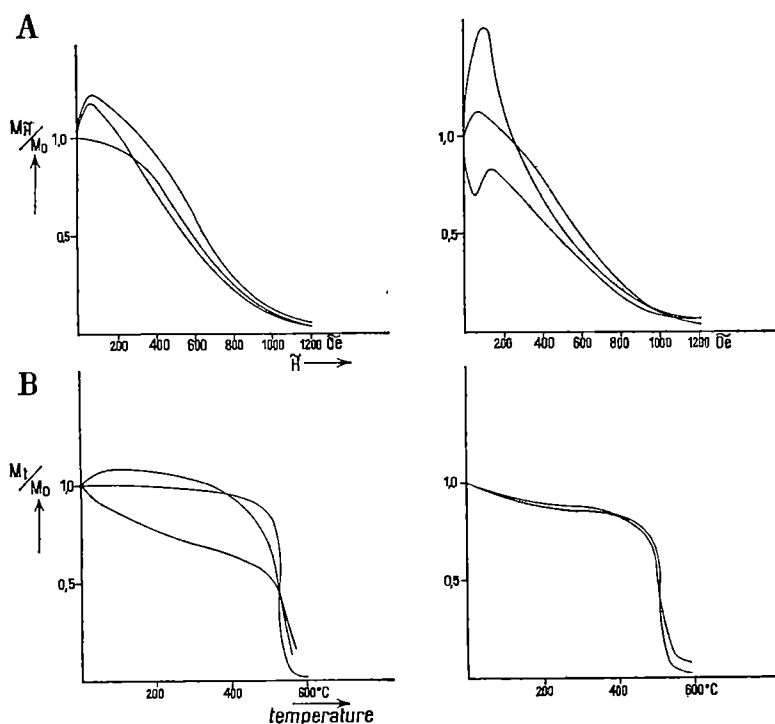


Fig. 2. a.c. (A) and thermal (B) demagnetization of greisen samples, the J_n directions of which are shown in Fig. 1. M_n , Magnetic moment of a sample demagnetized by an H field (oersted); M_t , magnetic moment of a sample demagnetized at temperature t (in centigrade); M_0 , natural magnetic moment

Another characteristic of all the cassiterites is their high Q_n -coefficient (the ratio of remanent to inductive magnetization measured in the Earth's magnetic field) which is usually of the order of 10^1 and frequently is greater than 50. In the course of a.c. demagnetization the direction of remanent magnetization did not change significantly from the J_n direction and only in strong fields (1,200 oersted) did the magnetic moment drop to 2–20 per cent of its original value. A similar resistance was shown by cassiterite samples during thermal demagnetization. At about 525°C ($\pm 25^\circ\text{C}$) their magnetic moment dropped sharply to zero; this temperature is close to the curie point of manganoferrite⁴.

To test the stability of magnetization of cassiterite oriented samples were collected from the greisen body 'Huber' in Horní Slavkov (western Czechoslovakia), which contains grains of cassiterite. Their J_n directions shown in Fig. 1 differ markedly from the direction of the present-day geomagnetic field and are similar to the directions of the magnetization found in rocks of Permo-Carboniferous age from that region. Fig. 2 shows a.c. and thermal demagnetization curves for six and five greisen samples, respectively, their J_n directions being included in Fig. 1. The a.c. and thermal demagnetization curves of the greisen samples are similar to those from cassiterite monocystals and

GEOLOGY

Isotopic Ages and Andean Uplift

aggregates. Tables 1 and 2 summarize the data derived from measurements during demagnetization of samples, the demagnetization curves of which are shown in Fig. 2. Laboratory tests show a high palaeomagnetic stability of cassiterite. The J_n directions corresponding to those of other Permo-Carboniferous rocks are in agreement with the generally accepted Permo-Carboniferous age of cassiterite-wolframite formation of western Czechoslovakia, which represents a genetic analogue of the ore mineralization in Cornwall.

Table 1. MEAN DIRECTIONS OF REMANENT MAGNETIZATION OF SIX GREISEN SAMPLES FROM HORNÍ SLAVKOV, A.C. DEMAGNETIZATION

Peak value of a.c. demagnetizing field (oe)	Declination (deg)	Inclination (deg)	α (deg)	k	R
0	210.0	5.0	5.76	136.04	5.96
50	212.4	6.1	7.75	75.75	5.93
100	211.0	7.2	7.23	88.72	5.94
200	213.0	4.6	7.00	92.68	5.95
300	209.8	3.8	6.03	124.25	5.96
500	204.0	6.3	7.84	74.00	5.93
800	195.6	9.4	12.94	27.74	5.82
1,200	181.6	16.6	21.41	10.74	5.53

Table 2. MEAN DIRECTIONS OF REMANENT MAGNETIZATION OF FIVE GREISEN SAMPLES FROM HORNÍ SLAVKOV, THERMAL DEMAGNETIZATION

Temperature (°C)	Declination (deg)	Inclination (deg)	α (deg)	k	R
20	211.9	-8.4	12.08	41.09	4.90
100	210.3	-7.2	13.06	35.27	4.89
200	209.9	-7.7	12.80	36.35	4.89
300	209.8	-7.7	12.09	41.00	4.90
400	209.9	-6.7	13.06	35.29	4.89
500	209.1	-2.6	14.00	30.00	4.87

The magnetization of cassiterites was described by Ramdohr⁶, who attributed it to an admixture of columbite. The samples of pure columbite which were examined, however, had a much lower remanent magnetization than magnetic samples of cassiterite in which columbite is only a minor component. Microscopic and X-ray analysis did not show the presence of any heterogeneous mineral admixture even in the strongly magnetic samples. On the contrary, the identical properties of cassiterite (in particular, the curie point, the course of a.c. demagnetization, high value of the Q_n -coefficient) shown in samples from various localities point against heterogeneous admixtures of various ferromagnetics as principal bearers of magnetization of cassiterite. The results of quantitative spectral analysis (the constant-temperature arc method) showed that samples with higher magnetization had a generally higher content of iron not exceeding 0.6 per cent. It is assumed that iron is bound in cassiterite to the structural lattice which is in agreement with the finding of Stumpff⁷ based on assays with an electronic microsonde. It is probable that many minerals generally considered as 'non-magnetic' can bind to their structural lattice elements in such a way as to render them naturally ferromagnetic.

We thank Dr. K. Tuček, director of the Mineralogical Department of the National Museum in Prague, for samples of cassiterite and other minerals used in the investigation.

V. HANUŠ

Geological Institute of the Czechoslovak
Academy of Sciences,
Prague.

M. KRS

Institute of Applied Geophysics,
Prague, Czechoslovakia.

¹ Hanuš, V., and Krs, M., *Geophys. J. Roy. Astro. Soc.*, **8**, 82 (1963).

² Krs, M., *Pure and App. Geophys.*, **57**, 96 (1964).

³ Takel, Takeshi, *Ferrity* (translation from Japanese into Russian, 'Metal-lurgia', Moscow, 1964).

⁴ Bozorth, R. M., *Ferromagnetism* (D. Van Nostrand Co., Inc., Toronto, New York, London, 1951).

⁵ Fisher, R. A., *Proc. Roy. Soc., A*, **217**, 295 (1953).

⁶ Ramdohr, P., *C.R. Soc. Géol. Finlande*, **33**, 473 (1961).

⁷ Stumpff, E. F., *Neues Jahrb. Mineral., Monatsh.*, **88** (1963).

AN outline of the research programme undertaken from University College, London, to elucidate the history of Andean Uplift in northern Chile has recently been presented in *Nature*¹. Locally, in the San Bartolo area, the post-Cretaceous geological history has been fairly clearly established from work carried out in 1961 (ref. 2). The chronology established, however, was entirely relative, in the absence of fossil evidence in this suite of continental sediments and volcanic rocks. Thus the San Bartolo ignimbrite formation, which is part of the Formation Liparítica of Bruggen³, was regarded by him as Miocene or younger. Other workers, however, have suggested chronologies which imply a late Pliocene or Pleistocene age⁴.

Previous age determinations on the ignimbrites by the Branch of Isotope Geology of the U.S. Geological Survey⁵ gave a minimum age of 5 million years and practically eliminated the possibility of a Pleistocene age.

The field work^{2,6} has shown that the deposition of the ignimbrite formation, which consists of a number of flows separated by gravels, bears a close relation to the tectonic activity which warped the early Tertiary planation surface and which produced much of the present Andean relief. In fact, the deformation of the originally planar surfaces of the ignimbrite flows provides much of the evidence of tectonic activity. The latter was episodic; it began shortly before and continued after the period of ignimbrite eruption. The possibility arises, therefore, not only of dating particular events, but also of determining the time span of ignimbrite eruption and therefore of determining the rate of Andean uplift.

The ignimbrites contain large biotite phenocrysts and provide admirable material for isotopic age determination by the potassium-argon method. The ages obtained can be linked with every confidence to the time of consolidation of the flows.

Four specimens were selected for the preliminary investigation, which has been carried out at Cambridge. Two specimens, 868 and 869, come from prominent flows close to the bottom and top of the ignimbrite formation according to the succession established in the San Bartolo area². The Puripicar tuff (869) has a large aerial extent of about 1,000 km² and is supposed to have originated from fissure eruptions now covered by the Plio-Pleistocene volcanics of the high Andes.

Table 1. RESULTS OF POTASSIUM-ARGON AGE DETERMINATIONS ON BIOTITES FROM NORTH CHILEAN IGNI MBRITES

Specimen No.	K ₂ O* (%)	Vol. of radiogenic ⁴⁰ Ar (mm ³ /g of sample)	% atmos. contain.	Age and error (m.y.)	Average age and error
869	7.58	1.654 × 10 ⁻³	83.2	4.2 ± 0.5	4.24 ± 0.05
		1.633 × 10 ⁻³	82.5	4.29 ± 0.24	
867	7.07	1.640 × 10 ⁻³	85.1	6.95 ± 0.33	6.89 ± 0.07
		1.610 × 10 ⁻³	86.7	6.82 ± 0.12	
866	5.87	1.434 × 10 ⁻³	61.9	7.58 ± 0.13	7.53 ± 0.05
		1.461 × 10 ⁻³	72.7	7.48 ± 0.20	
868	7.29	2.335 × 10 ⁻³	59.6	9.6 ± 0.5	10.0 ± 0.4
		2.226 × 10 ⁻³	51.2	10.4 ± 0.4	

$$\lambda\beta = 4.72 \times 10^{-10} \text{ yr}^{-1}, \lambda_a = 0.584 \times 10^{-10} \text{ yr}^{-1}.$$

* K₂O analyses by A. E. S. Mayer at Univ. Coll., Lond.

The age determinations of these two specimens show that the ignimbrite eruptions date from Upper Miocene⁷ and continued through much of the Pliocene. Some six million years were therefore available for the accompanying tectonic activity. A considerable part of Andean uplift must have been accomplished during this time. Tectonic activity (and less significant ignimbrite eruption) continued after the eruption of the Puripicar tuff, however, and a considerable proportion of Andean uplift must also be late Pliocene or Pleistocene^{2,6}. If the total period of Andean uplift is taken as approximately the ten million years from late Miocene time, the average rate of uplift to the 4,000 m of the Puna surface (which, in fact, was episodic²) is less than 0.5 mm/y. Again, it may be con-

firmly inferred that this part of the Andean region was one of low relief until Late Miocene time. The general history of Andean uplift is therefore probably much more similar to that of other Tertiary mountain chains⁸ than has often been supposed.

Specimen 866 comes from a hill of rhyolite at Copacoya which forms an inlier in the Puripicar tuff. Its similarity in petrographic character to the ignimbrites suggests that it may well be a late-stage viscous extension of the degassed magma from a fissure vent which had previously erupted ignimbrites in the succession below the Puripicar tuff. Its age of 7.53 ± 0.05 m.y. conforms with this inference.

Specimen 867 represents a flow from the Chaxcas volcanic dome which erupted ignimbritic material from a central vent within the main area of ignimbrite deposition. The age relations of these central eruptions to the main ignimbrite flows were not certainly known from the field evidence⁹ but the age determination places at least one flow firmly within the main period of ignimbrite eruption.

The determinations presented here effectively show that the eruption of the Formación Liparítica covered a wide time span. Flows of this formation occur interbedded with continental sediments in the Salar de Atacama and in the basin of the River Loa¹. It is hoped to undertake further work on these flows to provide a radiometric time-scale for the geological history of these regions also.

R. W. R. RUTLAND
J. E. GUEST

Department of Geology,
University College,
London.

R. L. GRASTY

Department of Geodesy and Geophysics,
University of Cambridge.

¹ Hollingworth, S. E., *Nature*, 201, 17 (1964).

² Rutland, R. W. R., and Hollingworth, S. E., "Post-cretaceous Evolution of the San Bartolo Area, Northern Chile" (in the press).

³ Bruggen, J. M., *Fundamentos de la Geología de Chile. Instituto Geográfico Militar, Santiago, Chile*, 374 (1950).

⁴ Harrington, H., *Geology of Parts of Antofagasta and Atacama Provinces, N. Chile, Bull. A.A.P.G.*, 45, 169 (1961).

⁵ Dingman, R. J., *Cuadrángulo Tular, Provincia de Antofagasta. Carta No. 11. Inst. de Invest. Geol. Chile* (1963).

⁶ Rutland, R. W. R., and Guest, J. E., "Tectonic Pattern of the Region between Tococone and San Pedro de Atacama, N. Chile" (in preparation).

⁷ *Quart. J. Geol. Soc. Lond.*, 120s, 280 (1964).

⁸ de Sitter, L. U., *Amer. J. Sci.*, 250, 297 (1952).

⁹ Guest, J. E., thesis, Univ. London (1964).

CHEMISTRY

Nomadic Behaviour of Yttrium Ions and their Structure in Solution

Two basic problems have long been predominant in the solution chemistry of the rare earth elements: the directional change of sequential properties at gadolinium, and the nomadic behaviour of the yttrium ion. Treatment of the 'gadolinium break' appears amenable to the ligand field approach, but the adoption by yttrium of a serial position differing with the nature of the menstruum has not been so classically explained. The inception of ion exchange techniques for separation of the rare earth elements has indeed tended to distract attention from this problem which was ever-present in the older classical methods of separation by fractional crystallization or precipitation.

Nomadism of the yttrium ion in a separating rare earth sequence has, in the past, been attributed to functional covalency or an 'elastic' property of the yttrium ion¹ or even to the possible formation of a 'yttryl' ion². Now, however, the ability of yttrium ions to locate at varying sites in the lanthanon series is found attributable to its participation in hydroxy polymer formation. The structures formed are of the type shown by Salmon and Wall³

and Randall and Staveley⁴ to be formed by aluminium ions, and by Pokras and Kilpatrick⁵ to exist in scandium solutions. Suggestive evidence for such polymer systems in yttrium solutions can be found in the literature and was emphasized in the current work by preliminary studies of solvent extraction and reaction velocities. Final definition has, however, been made by X-ray diffraction studies on yttrium solutions.

The technique employed was virtually that used by Brady *et al.*⁶. Because ions of different scattering power were involved, radial distribution functions were calculated in terms of electron density by the generalized Fourier approach:

$$\Sigma 4\pi r^2 K_m \rho_m(r) = 4\pi r^2 \rho_0 \Sigma Z_m + 2r/\pi \int_0^\infty s i(s) \sin r s ds$$

Warren, Krutter and Morningstar⁷ and, more recently, Brady⁸ have adequately described this procedure.

The formation of polymers based on hydroxyl bonded yttrium ions necessitates the existence of a Y-O association. Analysis of diffraction patterns and radial distribution functions shows clearly that this exists in sulphate and nitrate solutions, and can occur to a minor extent in chloride solutions through the development of Cl-O bonds, but does not occur in perchlorate solutions (Figs. 1-4).

Two types of Y-O bonds are, however, seen: that forming with the associated anion (Y-O)' and that essentially responsible for polymer formation, formed with a hydroxyl bridge (Y-O)". The proportion of (Y-O)' and (Y-O)" bonds in a solution depends on the associated anion and pH. Neither type of association occurs in perchlorate solutions. (Y-O)' type bonds form in chloride solution only at low pH—high acidities appear to degrade (Y-O)"

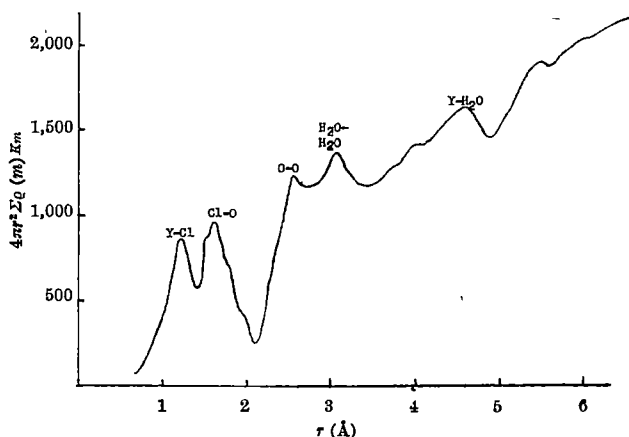


Fig. 1. Radial distribution in 2 M $Y(ClO_4)_3$ solution

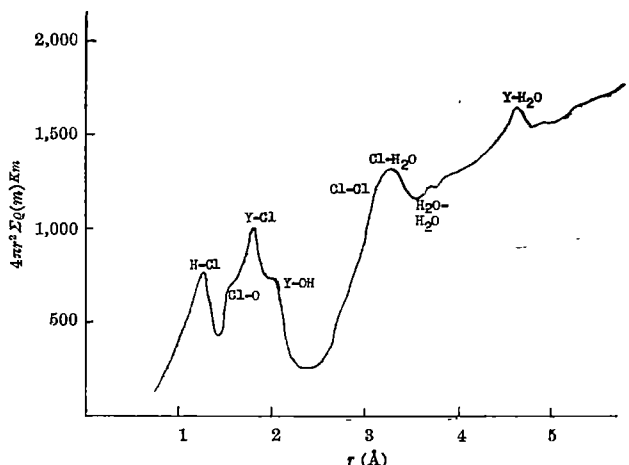
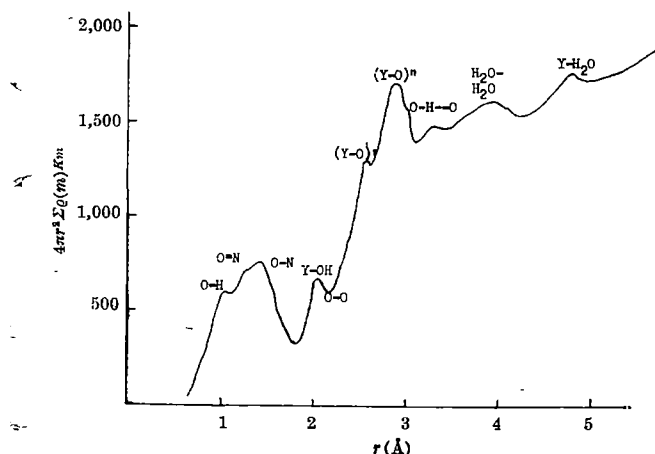
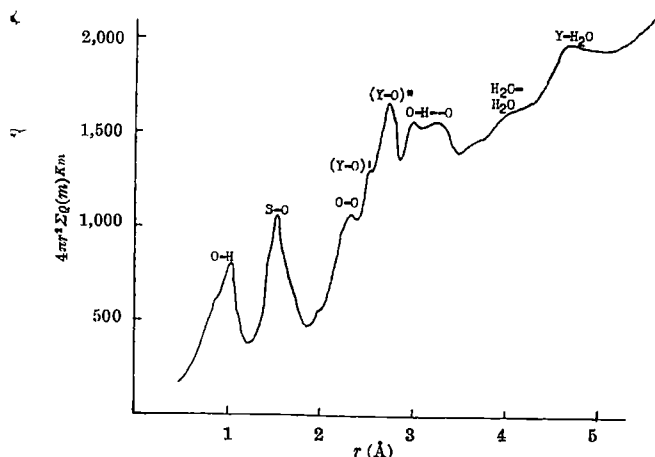


Fig. 2. Radial distribution in 2 M YCl_3 solution

Fig. 3. Radial distribution in 2 M $\text{Y}(\text{NO}_3)_3$ solutionFig. 4. Radial distribution in 1 M $\text{Y}_2(\text{SO}_4)_3$ solution

type bondings. The proportion of $(\text{Y}-\text{O})''$ to $(\text{Y}-\text{O})'$ bonds is greater in sulphate than in nitrate solution, and only species containing $(\text{Y}-\text{O})'$ bonds extract into an organic phase from aqueous solution.

Reaction velocity studies have permitted the rough calculation of activation energies (E_{In}) for associated yttrium ions. These increase generally as:

$\text{Y}(\text{ClO}_4)_3$: 63.5 YCl_3 : 74.3 $\text{Y}(\text{NO}_3)_3$: 88.7 $\text{Y}_2(\text{SO}_4)_3$: 92.6 and we may relate the differing values of E_{In} to the degree of hydroxy-polymer formation by taking the hydroxyl bridge bonding energy as ~ 5 kcal (ref. 8).

This, together with modular analysis, shows that in chloride solution, the polymer is, at the most, dimeric; in nitrate solutions it is probable that quadri- or sexa-polymers are formed, with a penta-polymer representing the average situation. The polymers formed in sulphate solution appear to hold six bridging groups.

In contrast, Brady⁶ has shown that erbium solutions are completely ordered, with no evidence indicated for the formation of polymers. Solvent extraction and reaction velocity studies have provided additional confirmation of the non-existence of aquo-polymeric cations of the other lanthanons although lanthanum itself appears to exhibit a very slight tendency to polymer formation under specific conditions.

Degradation of the yttrium hydroxy polymers is found to depend on the polarizing effect of an added reactant. This effect appears to relate not only to the total ionic charge developed on dissociation of the reactant molecule, but also to a summation of the ionic susceptibilities of the added ions:

$$P = Z_e \sum_{\text{zan}}^{\text{cat}}$$

A close relationship has been found between P and reaction velocities.

We can consider then the position of yttrium in a lanthanon series in terms of polymer formation in which yttrium ions, as hydroxyl-bridged moieties, possess co-ordination numbers greater than 6 (that of the lighter lanthanons). The sequential separation location of yttrium is then determined by the degradation of these polymers of effective C.N. 12 to C.N. 9 (dimers) or C.N. 6 (monomers) according to the polarization factor of the reactant system. Where little or no degradation takes place, yttrium will be located close to lanthanum (as in the double ferricyanide precipitation); where breakdown to dimers is effected, yttrium will be positioned in the neodymium-terbium region (as in hydroxide, nitrate, cobalticyanide or simple ferricyanide fractionations); where complete monomerization obtains, yttrium will adopt its 'normal' ionic radius position near holmium (ion exchanger, double sulphate, bromate separations).

In addition to presenting a rationale for the nomadic behaviour of yttrium ions, this identification of yttrium hydroxy-polymers has broader implications. As well as in scandium and aluminium solutions (*vide supra*), hydroxyl bridged polymers have been observed in indium solutions by Irving, Rossoti and Williams⁹. Although no studies appear to have been reported on the structures of gallium or thallium solutions, Magnusson¹⁰, in assessing the short-range interactions of aqueous ions on a thermodynamic basis, shows a generic association (in terms of hydration-free energy) of Al^{3+} , Sc^{3+} , and Y^{3+} with Ga^{3+} , In^{3+} and Tl^{3+} rather than with the trivalent lanthanons.

It may be strongly suggested then that polymer formation in aqueous solution is an attribute of Group III elements other than the lanthanons. Whether the $4f$ shell electron complement contributes to this effect presents an interesting area for the ligand field approach.

This note summarizes for the immediate benefit of scientists in this field the results of a broad research programme which has recently been completed. A series of individual papers is in preparation which will present in greater detail experimental data obtained for each phase of the work.

R. C. VICKERY

P.O. Box 563,
Okauchee, Wisconsin.

¹ Marsh, J. K., *J. Chem. Soc.*, 118, 1084 (1947)

² Vickery, R. C., *Chemistry of Yttrium and Scandium* (Pergamon Press, London, 1960).

³ Salmon, J. E., and Wall, C. C. L., *J. Chem. Soc.*, 1128 (1958).

⁴ Randall, T., and Staveley, L. A. K., *J. Chem. Soc.*, 472 (1959).

⁵ Pokras, L., and Kilpatrick, M., *J. Electrochem. Soc.*, 100, 85 (1953).

⁶ Brady, G. W., et al., *J. Chem. Phys.*, 26, 1371 (1956); 27, 304 (1957); 28, 404 (1958); 30, 1079 (1960)

⁷ Warren, B. E., et al., *J. Amer. Ceram. Soc.*, 19, 202 (1936).

⁸ Pauling, L., *Nature of the Chemical Bond*, second ed. (Cornell, 1948).

⁹ Irving, H., et al., *J. Chem. Soc.*, 1906 (1955).

¹⁰ Magnusson, L. B., *J. Chem. Phys.*, 39, 1353 (1963).

Use of Infra-red Spectroscopy to determine the Orientation of Pyridine sorbed on Montmorillonite

ORGANIC molecules sorbed on layer silicates generally adopt one single orientation. For those molecules for which the assignment of the absorption bands to the different vibrational modes has been well established, this association presents a favourable case for analysis by infra-red spectroscopy. The orientation of the molecules can be inferred from the observed dichroism of specific absorption bands.

This communication reports an analysis of the orientation of pyridine sorbed on montmorillonite. The use of polycrystalline aggregates, in which the orientation is not perfect, restricts the information to a qualitative nature. However, this information is of great use, especially when combined with the results obtained by other techniques.

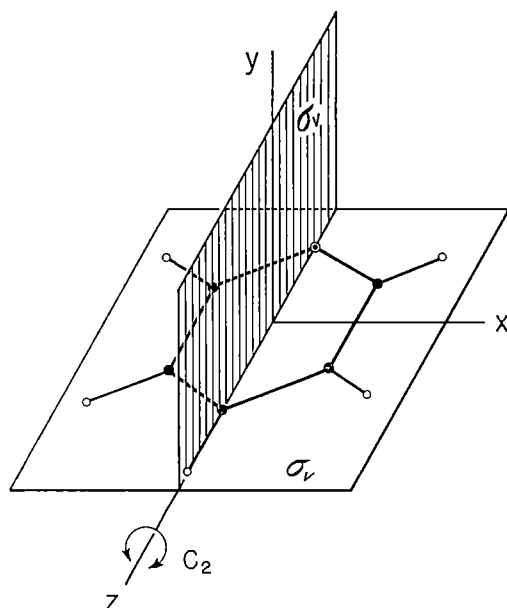


Fig. 1. Symmetry elements of the pyridine molecule, point group C_{2v} .

Pyridine belongs to the point group C_{2v} (Fig. 1), for which the principal axes and the directions of the changes of the dipole moment coincide. The infra-red-active vibrations are classified, according to their symmetry, in the following classes:

- (1) Symmetry class A_1 , that give dipole moment changes parallel to the z axis (C_2 axis).
- (2) Symmetry class B_1 , that give dipole moment changes parallel to the x axis.
- (3) Symmetry class B_2 , that give dipole moment changes parallel to the y axis.

A_1 and B_1 are in-plane vibrations, while B_2 are out-of-plane vibrations.

The infra-red spectrum of pyridine has been extensively studied and the assignment of most of the observed bands is well established^{1,2}. Some of these bands appear in regions that are well resolved from the silicate absorptions, permitting their separate observation in the spectrum of the silicate-pyridine complex.

According to Greene-Kelly³, pyridine, and several related molecules, adopt two kinds of orientation depending on the surface concentration: for high surface concentrations the plane of the ring is perpendicular to that of the silicate sheet, while at lower surface concentrations the planes of the molecules are parallel to that of the silicate sheet.

Both kinds of complexes have been prepared, the first one by sorbing neutral pyridine molecules on Na-montmorillonite, and the second by introducing the pyridine as a cation. The films of each complex were prepared such that silicate layers were in strongly preferred orientation in the plane of the film.

Pyridine sorbed on Na-montmorillonite exhibits absorptions at 703, 748, 1,217, 1,443, 1,492, 1,596 cm^{-1} and others around 3,000 cm^{-1} . These absorptions are only slightly modified with respect to those of liquid pyridine. We have selected for observation those the assignation of which is unequivocal and are in regions free of silicate absorptions.

Fig. 2 illustrates these absorption bands for two different inclinations of the film. Only the 1,443- cm^{-1} band (class B_1 , dipole moment change parallel to the x -axis) shows significant increase with the incidence angle. The

ratios are: 1.04 (703 cm^{-1} band), 1.04 (748 cm^{-1} band), 1.60 (1,443 cm^{-1} band), and 1.08 (1,492 cm^{-1} band). These results indicate that the molecules are disposed with their planes perpendicular to the silicate layers and with the principal axis (C_2 axis) parallel to the layers.

The infra-red absorption spectrum of the pyridinium ion, which also belongs to the point group C_{2v} , is somewhat modified from that of the sorbed pyridine. Bands have been observed at 677, 748, 1,340, 1,492, 1,550, 1,625, 1,640 cm^{-1} and several others above 3,000 cm^{-1} , in the pyridinium-montmorillonite complex. The position of the bands coincides with those reported previously for the pyridinium ion by other authors⁴⁻⁶.

Although the assignment of the bands for the pyridinium ion is not so well established, it is logical to assign the 677 and 748 cm^{-1} bands to out-of-plane vibrations and those between 1,300 and 1,700 cm^{-1} to in-plane vibrations or combination modes. The 1,492 cm^{-1} band which is present at the same frequency as in pyridine must have the same origin.

In Fig. 3 are represented the absorption bands of the pyridinium ion adsorbed in montmorillonite for two inclinations of the film. Only the 677 and 748 cm^{-1} bands (out-of-plane vibrations, class B_2) with dipole moment changes perpendicular to the molecule plane show significant increase in intensity with the angle of incidence. The

$$\frac{\log \left(\frac{I_0}{I} \right)_{40^\circ}}{\log \left(\frac{I_0}{I} \right)_0}$$

ratios are: 1.80 (677 cm^{-1} band), 1.68 (748 cm^{-1} band), 1.00 (1,492 cm^{-1} band), and 1.01 (1,550 cm^{-1} band). This indicates that the molecules are oriented with their planes parallel to the silicate sheets.

The orientations arrived at by infra-red spectroscopy agree with those inferred by Greene-Kelly from X-ray spacing measurement and one-dimensional Fourier syntheses⁷. They also confirm Greene-Kelly's analysis of the change of birefringence in preferred pyridine-montmorillonite complexes⁷. Our Na-montmorillonite-pyridine gives a spacing of 14.8 Å (8 orders observed) while the montmorillonite-pyridinium gave a spacing of 12.5 Å (6 orders observed).

It is interesting to note the utility of infra-red dichroism measurements of molecules sorbed on layer silicates in determining the symmetry species of the vibrations

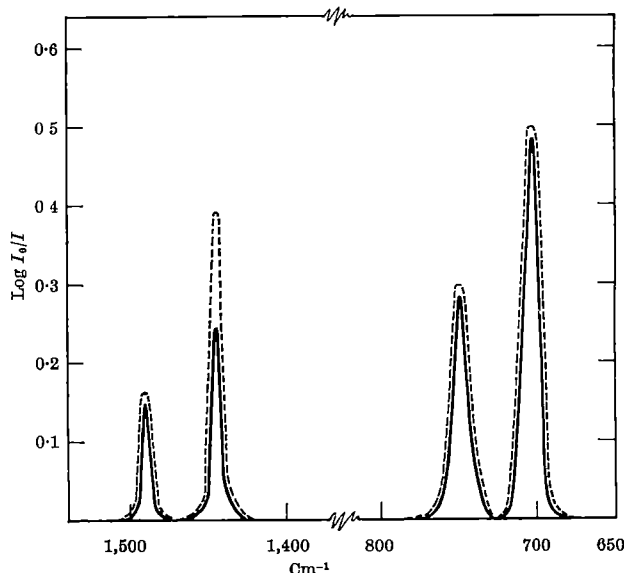


Fig. 2. Infra-red absorption bands of pyridine sorbed on Na-montmorillonite for two incidence angles, — 0°; --- 40°

$$\frac{\log \left(\frac{I_0}{I} \right)_{40^\circ}}{\log \left(\frac{I_0}{I} \right)_0}$$

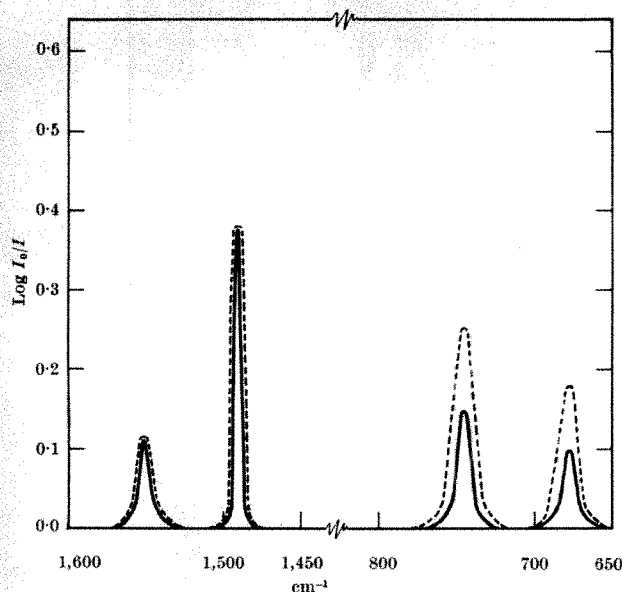


Fig. 3. Infra-red absorption bands of pyridinium ion sorbed on montmorillonite for two incidence angles, — 0°; --- 40°

provided that the orientation of the molecules has been established by other methods.

This work was supported in part by a U.S. Public Health Service contract 8T1-ES-30-02.

J. M. SERRATOSA

Environmental Health Engineering,
University of Texas,
Austin.

¹ Kline, C. H., and Turkevich, J., *J. Chem. Phys.*, **12**, 300 (1944).

² Corsin, L., Fax, B. J., and Lord, R. C., *J. Chem. Phys.*, **21**, 1170 (1953).

³ Greene-Kelly, R., *Trans. Faraday Soc.*, **51**, 412 (1955).

⁴ Cook, D., *Canad. J. Chem.*, **39**, 2009 (1961).

⁵ Gill, N. S., Nuttall, R. H., Scaife, D. E., and Sharp, D. W. A., *J. Inorg. Nucl. Chem.*, **18**, 79 (1961).

⁶ Swoboda, A. R., and Kunze, G. W., *Clay and Clay Minerals*, **13** (Pergamon Press, London, in the press).

⁷ Greene-Kelly, R., *Clay and Clay Minerals*, **10**, 469 (Pergamon Press, London, 1963).

Relaxation Effects in the Electrophoresis of Disperse Systems

It was shown by Henry¹ that the movement of spherical particles in an electric field depends on the size of the particle and the ionic strength and that the relationship between mobility and zeta-potential depends on the radius of the particle, a , and the Debye-Hückel double layer thickness, $1/\kappa$. It was pointed out by Overbeek² that, in addition, the relaxation of the ionic atmosphere of the particle must be considered. Overbeek² and Booth³ treated this problem theoretically and obtained analytical expressions for the mobility, which included corrections for retardation and relaxation, in terms of an incomplete power series in zeta-potential the coefficients of which were functions of κa . The limitation of the equations of Overbeek and Booth was that the expressions were only valid for zeta-potentials less than 25 mV. More recently Wiersema⁴ has obtained a numerical solution of the problem which gives mobility as a complete power series in zeta-potential and is valid for potentials greater than 25 mV.

Experimentally, however, little evidence has been accumulated which substantiates the occurrence of relaxation effects in electrophoresis. von Stackelberg *et al.*⁵ examined the mobility of some emulsion droplets in the κa range 10–2,000 and obtained qualitative evidence for relaxation. An indirect observation has also been made by Overbeek⁶ with reference to work carried out by Troelstra and Kruyt⁷ on the electrophoresis of silver iodide

particles in bariura nitrate solutions. No systematic observations appear to have been made using well-characterized, monodisperse solid particles, covering a range of particle diameters and electrolyte concentrations. This communication presents a preliminary report of a detailed examination of the variation of electrophoretic mobility as a function of particle size and electrolyte concentration with such a system.

Using an emulsion polymerization technique, with hydrogen peroxide as the initiator, a series of monodisperse polystyrene latex particles have been prepared with diameters ranging from 600 to 4230 Å. The details are recorded in Table 1. The initial styrene-water emulsion was stabilized by sodium laurate. The latter was later removed by dialysis leaving polystyrene particles with carboxyl surfaces. A mobility against pH curve for latex D, obtained after exhaustive dialysis, is given in Fig. 1. This curve indicates that the surface groupings have a pK of about 4.4. The presence of carboxyl groups has also been confirmed by infra-red spectroscopic studies on the latices.

Table 1

Latex	Diameter in Å
A	600 ± 96
B	1030 ± 82
C	2425 ± 130
D	3680 ± 170
E	4230 ± 160

From the computations of Wiersema⁴ it can be shown that the electrophoretic mobility as a function of κa , at constant electrokinetic potential, gives curves of the form shown in Fig. 2 for 1:1 electrolytes. Thus particles with the same zeta-potential, but different radii, can have different mobilities according to the κa region in which they fall. The mobilities of the latex particles of different sizes were determined in sodium chloride solutions, of constant ionic strength, at pH 7.5. The results at sodium chloride concentrations of 5×10^{-5} M, 10^{-2} M and 5×10^{-2} M are plotted against $\log \kappa a$ in Fig. 2. At a salt concentration of 5×10^{-5} M the results for latices A, B and C fall on the theoretical curve which corresponds to a zeta-potential of 59 mV. A minimum occurs in the mobility values at a κa value close to 2, in agreement with the position of the minimum on the curves derived from Wiersema's computations.

At a salt concentration of 10^{-2} M, with latices A, B and C, the minimum disappears and the results lie on an ascending curve. A further increase of electrolyte concentration to 5×10^{-2} M shifts the curve to higher κa values and decreases the slope of the curve. At both salt concentrations the correspondence of the experimental results with the theoretical curves is good. Latices D and E clearly have higher surface potentials than A, B and C, but the shift produced by changing the salt

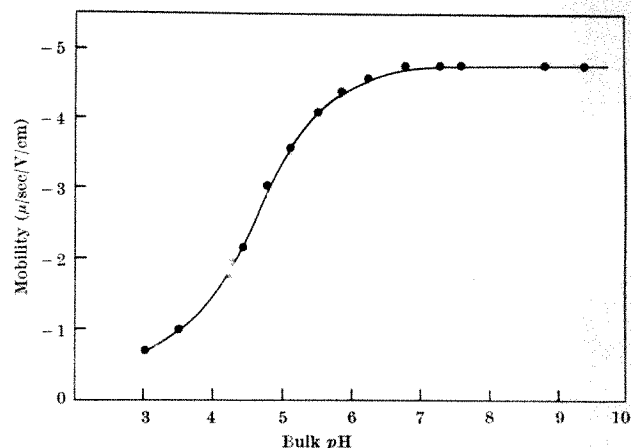


Fig. 1. Electrophoretic mobility against pH for latex D after exhaustive dialysis in 5×10^{-4} M sodium chloride solution

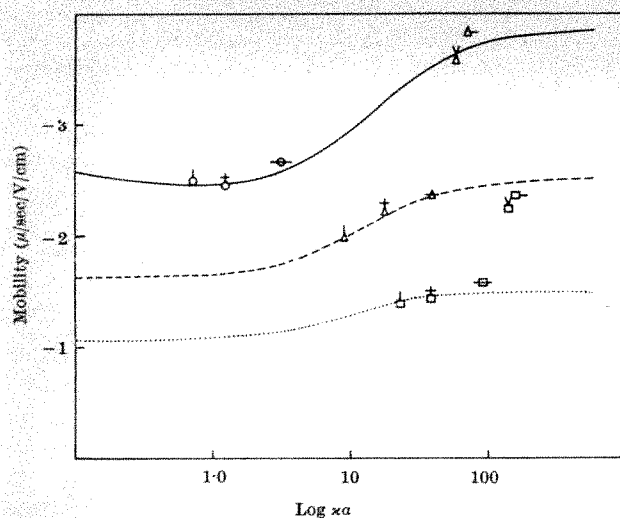


Fig. 2. Mobility against κa . Theoretical curves for zeta-potentials of 50 mV, —; 32 mV, ----; and 20 mV, Results obtained in sodium chloride solutions: ○, 5×10^{-3} M; Δ, 10^{-3} M; □, 5×10^{-3} M. Latices, A, ○; B, Δ; C, □; D, +; E, -.

concentration is consistent with that expected on the basis of relaxation. The pattern of the results obtained in sodium chloride solutions has also been confirmed with 2:1 and 3:1 electrolytes.

The minimum in the mobility results for latices A, B and C in 5×10^{-3} M salt solution and the behaviour of the mobility against κa curves with increasing ionic strength are all indications that relaxation is a real effect in the electrophoresis of colloidal particles. The results obtained, moreover, appear to agree quite closely with the behaviour predicted by Wiersema⁴. Comparison of mobilities, as a function of κa , at constant zeta-potential and ionic strength can be made from the experimental data obtained, but this would not constitute an independent test of the theory unless the zeta-potential was measured by a completely independent method.

A more detailed account of the electrophoretic properties of the latices as well as studies on their stability and viscous properties will appear in due course.

We would like to thank I.C.I., Ltd., Blackley, for financial support of this work, and Prof. J. Th. G. Overbeek and Dr. P. H. Wiersema for a number of discussions on the relaxation effect.

J. N. SHAW
R. H. OTTEWILL

Department of Physical Chemistry,
University of Bristol.

¹ Henry, D. C., *Proc. Roy. Soc., A*, **133**, 106 (1931).

² Overbeek, J. Th. G., *Kolloid-Beih.*, **54**, 287 (1943).

³ Booth, F., *Proc. Roy. Soc., A*, **203**, 514 (1950).

⁴ Wiersema, P. H., Thesis, University of Utrecht, 1964.

⁵ von Stackelberg, M., Heindze, H., Wilke, F., and Doppelfeld, R., *Z. Electrochem.*, **61**, 781 (1957).

⁶ Overbeek, J. Th. G., *Colloid Science*, edit. by Kruyt, H. R., **1**, 232 (Elsevier, 1952).

⁷ Troelstra, S. A., and Kruyt, H. R., *Kolloid-Z.*, **101**, 186 (1942).

Ultra-violet Absorption Spectra of Aromatic Nitrenes and Dinitrenes

NITRENES, molecular fragments with an electron sextet on nitrogen, are known to be the primary products of the decomposition of organic azides¹⁻³. Their existence as separate intermediates and their triplet character were recently demonstrated by electron spin resonance spectroscopy⁴. We have investigated the photolysis of several aromatic azides and diazides in organic glasses at 77° K in an attempt to trap the nitrenes presumably formed. Three types of behaviour were observed.

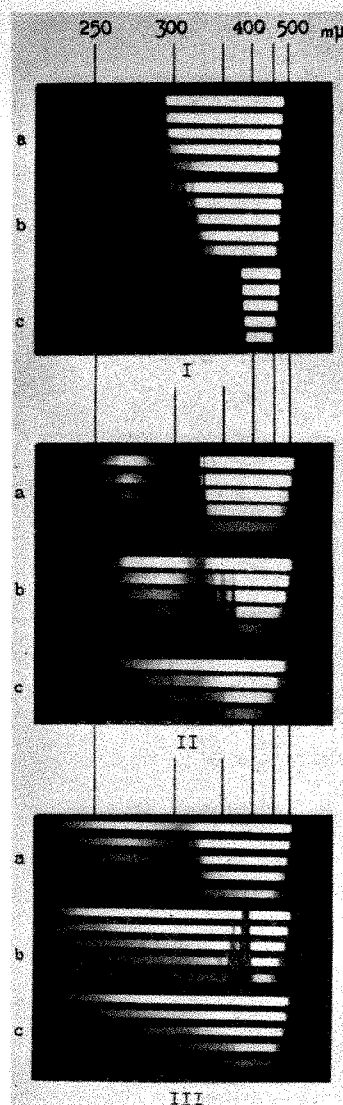


Fig. 1. Absorption spectra of nitrenes. a, b, c refer to the azides, the nitrenes, and the final products after melting, respectively: I, phenyl azide; II, α -naphthyl azide; III, 4,4'-diazidobiphenyl.

Phenyl azide and its derivatives gave, on irradiation with ultra-violet light, intermediates in sufficient concentration for their absorption spectra to be observed. In Fig. 1, Ib, the long wave-length bands of phenyl nitrene are clearly visible and are, incidentally, very similar to the corresponding absorption bands of the benzyl radical⁵. The nitrene is, however, destroyed by prolonged irradiation and only partial conversion of the azide to the nitrene is achieved. With α -naphthyl azide (Fig. 1, II) and the diazides of more extended conjugated systems (for example, 4,4'-diazidobiphenyl in Fig. 1, III) high conversion yields or complete conversion were obtained.

Molecules where the azido group is attached to a heterocyclic ring with two heteroatoms did not give detectable intermediates.

o-Azidobiphenyl is a special case; at 77° K in the solid matrix the azide is changed directly into the final stable product, carbazole.

Where detectable intermediates were formed they were stable in the organic glass at liquid nitrogen temperature, but disappeared on melting or on slightly softening the glass. The absorption of the intermediates was independent of the solvent used; spectra taken in glasses of methylcyclohexane, 3-methylpentane, and EPA, a mixture of diethyl ether, isopentane and ethyl alcohol 5:5:2, were identical. The intermediates react with oxygen even at

77° K with the formation of a transient which absorbs in the visible and disappears on melting. It is felt that these results, together with the bulk of the chemical evidence, justify the assignment of the observed spectra to nitrenes.

A. REISER
V. FRAZER

Research Laboratory,
Kodak, Ltd., Wellesbourne,
Harrow, Middlesex.

¹ Boyer, J. H., and Cantor, F. C., *Chem. Revs.*, **54**, 1 (1954).

² Smolinsky, G., *J. Org. Chem.*, **26**, 4108 (1961).

³ Horner, L., and Christman, A., *Angew. Chemie. (Intern. Ed.)*, **2**, 599 (1963).

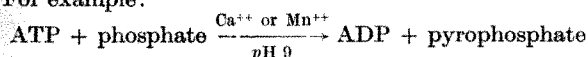
⁴ Smolinsky, G., Wasserman, E., and Yager, W. A., *J. Amer. Chem. Soc.*, **84**, 3220 (1962).

⁵ Norman, I., and Porter, G., *Proc. Roy. Soc., A*, **230**, 399 (1955).

BIOCHEMISTRY

Transphosphorylation of Adenosine Di- and Tri-phosphates in the Presence of Calcium Phosphate Precipitates

It has been discovered by Lowenstein^{1,2} that in dilute aqueous solution in the presence of certain divalent cations there is a tendency for the terminal phosphate group of the adenosine triphosphate ion (ATP) to be transferred to inorganic phosphate or other suitable acceptor. For example:



where ADP represents the adenosine diphosphate ion. Krane and Glimcher³ have recently shown that a similar non-enzymatic transphosphorylation occurs on the surface of calcium phosphate crystals with hydroxyapatite structure when they are in contact with aqueous solutions of ATP. In this reaction the acceptor is phosphate belonging to the crystal. The rate of reaction is faster and less dependent on pH than that of Lowenstein.

It has now been found, during experiments on the interactions of calcium, phosphate, and certain polyphosphate ions in aqueous solution (to be reported in detail later), that a mixture containing freshly precipitated, apparently amorphous, calcium phosphate is an efficient transphosphorylating medium for ATP and also ADP; especially if the nucleotide is initially in acid solution and is then co-precipitated by adding alkali. The pH dependence of transphosphorylation resembles that of Krane and Glimcher³, but the rate is slower. In a typical experiment a mixture containing trisodium phosphate (0.04 M), calcium chloride (0.07 M), and the sodium salt of ADP (0.02 M) was acidified to pH 4 with hydrochloric acid. Sodium hydroxide (1.0 N) was then added to pH 7.7 and the mixture made up to standard volume to give the concentrations in brackets. The precipitate contained initially 75 per cent of the ADP, 75 per cent of the phosphate, and 93 per cent of the calcium. The whole mixture was incubated at 38° C and at intervals portions were analysed chromatographically^{1,4}. Fig. 1, in which the ordinates give molar percentages referred to the initial ADP concentration, shows that during incubation the ADP concentration (Curve 1) decreased. At the same time an almost equivalent amount of adenosine monophosphate (AMP) (Curve 2) and a smaller amount of pyrophosphate (Curve 3) were formed. There was a much smaller decrease in ADP concentration in a control mixture containing no phosphate (Curve 4). No pyrophosphate was formed, although a small amount of AMP was formed, probably by hydrolysis of ADP. Very little pyrophosphate was formed in another control mixture containing no ADP.

The effect of the incubation of ATP in place of ADP under the foregoing conditions is shown in Fig. 2. Initially, the precipitate contained 59 per cent of the ATP, 74 per cent of the phosphate, and 93 per cent of the calcium. The ATP concentration (Curve 1) decreased rapidly and ADP

(Curve 2) and pyrophosphate (Curve 3) were formed. AMP (Curve 4) was also formed, although more slowly, presumably from ADP as in Fig. 1. The ATP concentration in a control mixture containing no phosphate decreased appreciably (Curve 5), probably by hydrolysis in the presence of calcium ions⁵, although there was little formation of pyrophosphate or AMP.

These results were reproducible and were unaffected by addition of chloroform or thymol to prevent biological growth. It is therefore concluded that non-enzymatic transphosphorylation from ADP and ATP to phosphate occurred in the presence of the calcium phosphate precipitates. Transphosphorylation appeared to be more closely associated with the precipitates than with the solution. Thus incubation of supernatant solutions after centrifuging off the precipitate yielded much less pyrophosphate than would have been expected if transphosphorylation occurred only in solution. Other experiments with ATP in which different conditions of precipitation were used showed that the extent of pyrophosphate formation increased as the proportion of ATP in the precipitate increased. Pyrophosphate formation was 30 per cent lower after 48 h when ATP was added to freshly precipitated calcium phosphate instead of being co-precipitated. At ATP concentrations less than 0.005 M, all the ATP precipitated and the initial rate of pyrophosphate formation (0.03 mole/mole of precipitated phosphate/h at 38°) was almost independent of ATP concentration—as might be expected for heterogeneous reaction.

It therefore appears that the environment provided by freshly precipitated slightly alkaline calcium phosphate is

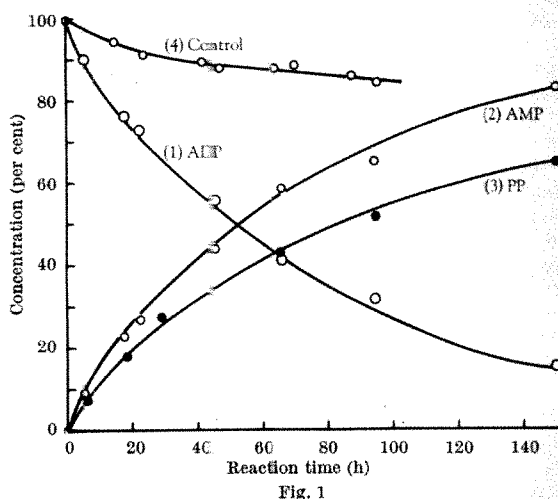


Fig. 1

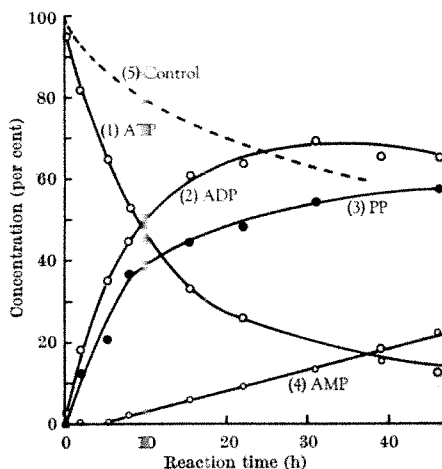


Fig. 2

favourable for non-enzymatic transphosphorylations. Preliminary electron diffraction and X-ray diffraction measurements showed that the precipitates were amorphous and did not change on incubation.

I thank Dr. G. H. Taylor, Dr. H. J. Kisch, and Dr. J. Donderdale of the C.S.I.R.O. Division of Coal Research for diffraction measurements and interpretations; and Mr. R. F. Sloggett for assistance with analyses.

R. W. BURLEY

Physical Chemistry Unit,
C.S.I.R.O. Division of Food Preservation,
Biochemistry Department,
University of Sydney, Australia.

¹ Lowenstein, J. M., *Biochem. J.*, **70**, 222 (1958).

² Lowenstein, J. M., *Biochem. J.*, **75**, 269 (1960).

³ Krane, S. M., and Glimcher, M. J., *J. Biol. Chem.*, **237**, 2991 (1962).

⁴ Wood, T., *J. Chromatog.*, **8**, 475 (1962).

⁵ Tetas, M., and Lowenstein, J. M., *Biochemistry*, **2**, 350 (1963).

An *in vitro* Action of Human Growth Hormone in the Presence of Actinomycin D

THE *in vitro* addition of growth hormone to isolated diaphragm from the hypophysectomized rat stimulates the uptake of glucose¹⁻³, the incorporation into protein of natural amino-acids³⁻⁵ and the accumulation in the tissue in an unchanged state of certain natural and unnatural amino-acids^{6,7}. These effects are similar to those of insulin on isolated diaphragm from the normal or the hypophysectomized rat⁸. Eboue-Bonis *et al.*⁹ have recently found that the action of insulin *in vitro* in promoting glucose uptake and the incorporation into protein of label from labelled natural amino-acids is still seen in the presence of actinomycin D—a powerful inhibitor of the synthesis of nucleic acid¹⁰. We have investigated the influence of actinomycin D on the effectiveness of human growth hormone in promoting the incorporation into protein of ¹⁴C from glycine-¹⁴C-U and into nucleic acid of ¹⁴C from orotic acid 6-¹⁴C, when the hormone is added *in vitro* to isolated diaphragm from hypophysectomized rats.

The general methods were those of Manchester and Young³ except that glucose was estimated by a glucose oxidase method¹¹ and the amounts of radioactive carbon incorporated into protein and into nucleic acid were estimated by a method involving a combination of those used by Manchester¹² and Wool¹³. For this, material derived from a hemidiaphragm, which had been incubated with labelled glycine and labelled orotic acid simultaneously, was used. Preliminary experiments showed that under the conditions used the labelling of protein from labelled orotic acid was less than 10 per cent of that from labelled glycine, and that the labelling of nucleic acid from labelled glycine was less than 10 per cent of that from labelled orotic acid. Because the labelling of nucleic acid, though not of protein, was diminished when glucose was present in the medium, glucose was omitted from the medium in those experiments which were not concerned with a determination of the uptake of glucose by the isolated diaphragm. Actinomycin D was added to the medium in some experiments at a concentration of 10 µg/ml. of medium, the diaphragm being soaked in buffer containing this concentration of actinomycin before transfer to the incubation flask.

Paired hemidiaphragms were incubated in the presence of 1 mM glycine-¹⁴C-U (0.1 µc./ml.) and 0.01 mM orotic acid 6-¹⁴C (0.1 µc./ml.), and after 2 h were homogenized in 5 ml. of 10 per cent trichloroacetic acid at 0° C. The insoluble material was washed repeatedly with trichloroacetic acid at 0° C and fat-soluble material removed by extraction with 96 per cent ethanol at 0° C. Nucleic acid was then extracted into 10 per cent aqueous sodium chloride at 100° C and the sodium nucleates estimated by the method of Wool¹³. Protein was extracted from the

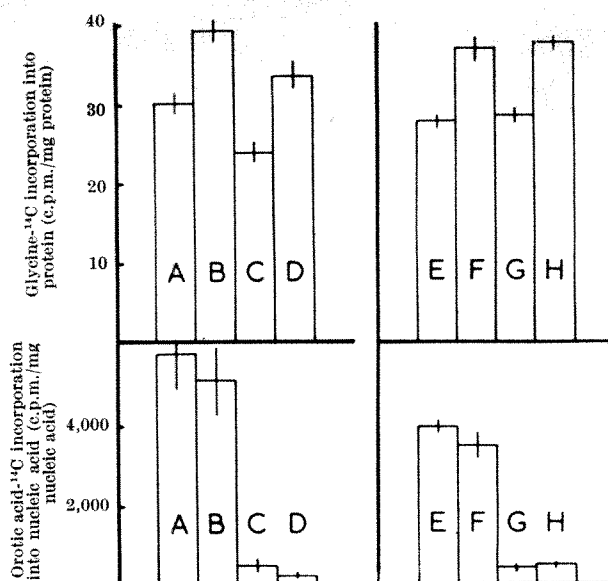


Fig. 1. The *in vitro* effect of human growth hormone and actinomycin D on the incorporation of glycine-¹⁴C and orotic acid-¹⁴C into protein and nucleic acid respectively. In experiments B and D growth hormone at 50 µg/ml. was present *in vitro* while in F and H the concentration was 10 µg/ml. In experiments C, D, G and H actinomycin D was present in a concentration of 10 µg/ml. The results illustrated represent the mean results for four groups of paired hemidiaphragms. The vertical lines at the top of the columns represent the standard errors of the mean value.

sodium chloride-insoluble material into 0.4 N sodium hydroxide, and precipitated by 7 per cent perchloric acid which was then heated to 95° C for 20 min. The protein was then estimated by the method of Manchester¹².

As was to be expected³, growth hormone did not promote the uptake of glucose, or the incorporation into protein of label from glycine when the hormone was added *in vitro* to diaphragm from normal rats, but did so with diaphragm from hypophysectomized rats. These results are not illustrated. The observations illustrated in Fig. 1 show that the addition of human growth hormone to the medium in a concentration of 50 µg/ml. or 10 µg/ml. increased the incorporation into protein of label from glycine to a statistically significant extent, whether or not actinomycin was present in the medium. On the other hand, in the presence of actinomycin the incorporation into nucleic acid of the label from orotic acid was reduced to 5–10 per cent of that in its absence. Under the conditions of these experiments no evidence was seen of a significant change in the rate of nucleic acid synthesis when growth hormone was added *in vitro*.

Wool^{13,14} suggested that insulin may exert a primary action on the synthesis of ribonucleic acid, the latter acting as 'messenger RNA'¹⁵ with respect to the promotion of protein biosynthesis under the influence of this hormone. The evidence provided by Eboue-Bonis *et al.*⁹ did not support this idea. The results recorded here likewise do not agree with any idea that growth hormone promotes protein biosynthesis by way of a primary action on the production of messenger RNA. Since, under the conditions of our experiments, actinomycin did not completely suppress the incorporation of label from orotic acid into nucleic acid, though much of this residual incorporation may be the terminal labelling of 'transfer RNA'¹⁶, no firm statement can be made that growth hormone continued to promote protein biosynthesis when all RNA synthesis had been abolished.

We have sought, but failed, to find evidence that growth hormone stimulates protein biosynthesis by a primary action on the production of messenger RNA.

The results of these investigations are represented in Fig. 1. The growth hormone preparation showed a statistically significant stimulation of amino-acid incorporation at both 50 µg/ml. and 10 µg/ml., and it is seen

that the stimulation is unaffected by actinomycin, which has reduced the incorporation of orotic acid into nucleic acid to 5–10 per cent of the control values. The results presented here do not enable us to report any effect of growth hormone on nucleic acid synthesis, although experiments designed to study this more closely may reveal such effects.

During the writing of this communication Goodman¹⁶ reported that Knobil has also evidence that the action of growth hormone in stimulating protein biosynthesis still occurs when the production of new nucleic acid in the muscle cell is inhibited.

We thank Dr. A. Stockell Hartree for purified human growth hormone, Miss M. B. Thomas for the hypophysectomies and Mr. D. C. Gardiner for general assistance.

T. E. MARTIN
F. G. YOUNG

Department of Biochemistry,
University of Cambridge.

- ¹ Park, C. R., Brown, D. H., Cornblath, M., Daughaday, W. H., and Krahling, M. E., *J. Biol. Chem.*, **197**, 151 (1952).
- ² Ottaway, J. H., *Biochim. Biophys. Acta*, **11**, 443 (1953).
- ³ Manchester, K. L., and Young, F. G., *J. Endocrinol.*, **18**, 381 (1959).
- ⁴ Kostyo, J. L., and Knobil, E., *Endocrinology*, **65**, 525 (1959).
- ⁵ Manchester, K. L., and Wallis, M., *Nature*, **200**, 888 (1963).
- ⁶ Kostyo, J. L., Hotchkiss, J., and Knobil, E., *Science*, **130**, 1653 (1959).
- ⁷ Kostyo, J. L., *Endocrinology*, **75**, 113 (1964).
- ⁸ Manchester, K. L., and Young, F. G., *Vit. Horm.*, **19**, 95 (1961).
- ⁹ Eboue-Bonis, D., Chambaut, A. M., Volfin, P., and Clauser, H., *Nature*, **199**, 1183 (1963).
- ¹⁰ Franklin, R. M., *Biochim. Biophys. Acta*, **72**, 555 (1963).
- ¹¹ Huggett, A. St. G., and Nixon, D. A., *Biochem. J.*, **66**, 12P (1957).
- ¹² Manchester, K. L., *Biochem. J.*, **81**, 135 (1961).
- ¹³ Wool, I. G., *Biochim. Biophys. Acta*, **68**, 28 (1963).
- ¹⁴ Wool, I. G., and Munro, A. J., *Proc. U.S. Nat. Acad. Sci.*, **50**, 918 (1963).
- ¹⁵ Brenner, D., Jacob, F., and Meselson, M., *Nature*, **196**, 576 (1961).
- ¹⁶ Goodman, H. M., *Endocrinology*, **76**, 1134 (1965).

Long-spacing Segments from Renatured $\alpha 1$ Sub-units of Collagen

THE mammalian collagen molecule is thought to be composed of two $\alpha 1$ and one $\alpha 2$ peptide chains. Alpha 1 and $\alpha 2$ have approximately the same molecular weight but differ in their amino-acid composition¹. A mixture of $\alpha 1$ and $\alpha 2$ in the ratio of 2:1 (produced by the denaturation of neutral salt-soluble rat skin collagen) re-forms, under certain conditions suitable for renaturation, as much as 50 per cent of the rod-like collagen molecules². It is of interest to learn whether the triple helix of the collagen molecule can also be formed from three $\alpha 1$ chains. Recently, Piez and Carrillo³ have investigated the renaturation of the isolated sub-units ($\alpha 1$, $\alpha 2$, and β_{12}) of collagen at 15°. According to our experience these conditions are not optimal for the formation of the triple helix⁴.

We isolated the $\alpha 1$ component from neutral salt-soluble calf skin collagen according to the method of Piez *et al.*¹. Its homogeneity was checked by means of ultracentrifugation and starch-gel electrophoresis. Renaturation was achieved by incubating at a collagen concentration of 2.6 mg/ml. for 100 h at 22° in 0.25 M sodium citrate buffer, pH 3.7. The denaturation curve of the renatured solution gave a value of 30° for T_m (defined according to v. Hippel *et al.*⁵). Renatured acid-soluble collagen from calf skin has a T_m of 33° after renaturation under identical conditions.

When examined in the ultracentrifuge the renatured material showed, in addition to $\alpha 1$, a second, slower sedimenting peak which, during the course of the renaturation, grew at the expense of the $\alpha 1$ peak. Following the addition of pepsin (weight ratio of collagen: enzyme of 10:1; temp., 25°) the $\alpha 1$ component disappeared while the slower, pepsin-resistant peak, although not quite so sharp as the native molecule (Fig. 1), sedimented at approximately the same rate.

It was possible to produce long-spacing segments (Fig. 2) from the pepsin-resistant material, which accounted for

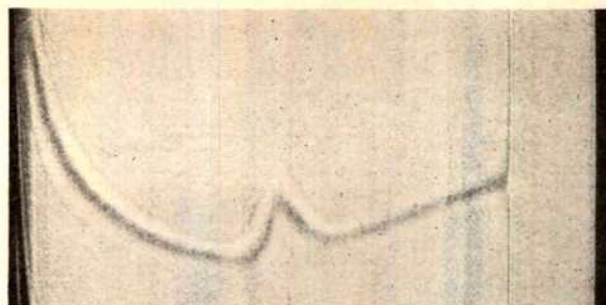


Fig. 1. Sedimentation diagram of renatured $\alpha 1$ after 18-h pepsin treatment; concentration prior to pepsin addition 2.6 mg collagen/ml. in 0.25 M citrate buffer, pH 3.7; temp., 22°; 59,000 r.p.m.; phase angle, 66°; photographed after 150 min.

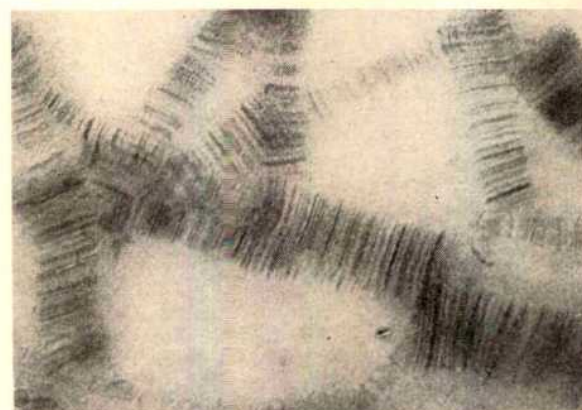


Fig. 2. Long-spacing segments from renatured $\alpha 1$ sub-units, produced by dialysis of the pepsin-treated solution in 0.05 per cent acetic acid against 0.4 per cent ATP solution, pH 3.5; stained with phosphotungstic acid and counterstained with uranylacetate.

almost 40 per cent of the starting material. The cross-striation pattern appeared to be the same with respect to the position of the individual cross-striations when compared with normal collagen. There appears to be some difference in the intensities of the individual bands. An exact photometric evaluation of the cross-striation pattern remains to be done.

These preliminary experiments indicate that it is possible to build a native-like triple helix from three $\alpha 1$ chains. The cross-striation pattern of the segments thus obtained from $\alpha 1$ argues against the collagen model of Hodge⁶ in which the $\alpha 1$ chain would be built up from five identical sub-sub-units and the $\alpha 2$ chain from seven identical sub-sub-units. If this were the case, then the cross-striation pattern of the long-spacing segments formed from $\alpha 1$ should be divided into five equal periods. The fact that no repeating period at all is evident suggests that it is very unlikely that $\alpha 1$ is composed of several identical smaller sub-units.

This investigation was supported by the Deutsche Forschungsgemeinschaft.

KLAUS KÜHN
CHRISTIAN TKOCZ
BODO ZIMMERMANN
GUNDOLF BEIER

Max-Planck-Institut für Eiweiss- und Lederforschung,
Munich, Germany.

- ¹ Piez, K. A., Eigner, E. A., and Lewis, M. S., *Biochemistry*, **2**, 58 (1963).
- ² Kühn, K., and Zimmermann, B., *Arch. Biochem. Biophys.*, **109**, 534 (1965).
- ³ Piez, K. A., and Carrillo, A. L., *Biochemistry*, **3**, 908 (1964).
- ⁴ Kühn, K., Engel, J., Zimmermann, B., and Grassmann, W., *Arch. Biochem. Biophys.*, **105**, 387 (1965). Beier, G., Engel, J., and Grassmann, W., *Biophysiker-Tagung Wien*, 14-16.9.1964, Tagungsbericht, p. 101.
- ⁵ Hippel, P. H. v., and Wong, K. Y., *Biochemistry*, **2**, 1399 (1963).
- ⁶ Hodge, A. J., *Proc. U.S. Nat. Acad. Sci.*, **51**, 871 (1964).

Glycogen in Yeast Form of *Paracoccidioides brasiliensis*

Paracoccidioides brasiliensis is the causative agent of South American blastomycosis. McKinnon and Vinelli¹, and Carbonell *et al.*², suggested in their histochemical investigations that the yeast form of this fungus may contain glycogen. The present investigation deals with the biochemical identification of glycogen in the yeast form of *P. brasiliensis*. The fungus used (strain No. 8159 of the Instituto Nacional de Tuberculosis, Caracas, Venezuela) was originally isolated from a lymph node in a human systemic infection and cultured on brain heart infusion agar (Baltimore Biological Laboratory) with human blood (10 per cent) and antibiotics (penicillin, 20 units per ml.; streptomycin, 400 µg per ml.) at 37° C. The fungus was collected on the fifth day of culture, and washed 4 times with distilled water by centrifugation (500 g, 5 min). After extracting the lipids by means of a mixture of ethanol and ether (3:1), the fungus, suspended in distilled water, was disintegrated in a Branson sonifier at 20 kc/s for 20 min. The disruption of the cell material was completed by treatment in a Waring blender for 1 h. The polysaccharide was isolated according to the method of Abdel-Akher and Smith³, that is, extraction with boiling water, removal of proteins by trichloroacetic acid, and purification by repeated precipitation from aqueous solution with ethanol. From 7.04 g of vacuum-dried fungus material, a polysaccharide fraction (126 mg), acetone-soluble lipids (977 mg) and acetone-insoluble lipids (110 mg) were obtained.

The isolated polysaccharides dissolved easily in water, showing opalescence. A reddish brown colour was produced with iodine. After hydrolysis in N HCl for 3 h in a boiling-water bath, 64 per cent of the weight was liberated as glucose which was determined enzymatically by the combined system of hexokinase and glucose-6-phosphate dehydrogenase⁴. This value was almost equal to that of 65 per cent obtained after the digestion by 'Diazyme' (Miles Chemical Company). 'Diazyme' contains amylase and amyloglucosidase and is used in quantitative determinations of glycogen⁵. After complete hydrolysis by phosphorylase and amyloglucosidase, the ratio of glucose-1-phosphate to glucose was determined to be 10:9 according to the method of Bueding and Hawkins⁶. This result implies that the polyglucose contained in the fungus represents glycogen and not starch.

The polysaccharide fraction contained, besides glycogen, other compounds which were demonstrated by chemical analysis. The total sugar content of the dry material, evaluated by the anthrone method⁷ with glucose as standard, was found to be 85 per cent. The material contained 1.7 per cent nitrogen⁸, 3.2 per cent proteins⁹ with bovine serum albumin as standard, 0.4 per cent total phosphorus¹⁰, less than 1.2 per cent glucosamine¹¹, and less than 2.0 per cent pentoses¹² with xylose as standard. Ketosugars¹³ and uronic acids¹⁴ could not be detected.

The results reported here suggested that most of the nitrogen was not derived from glucosamine but probably from proteins. In order to remove the proteins, part of the polysaccharide fraction was treated with 30 per cent potassium hydroxide for 2 h in a boiling-water bath and purified by repeated precipitation (3 times) from aqueous solution with ethanol. This alkali-treated polysaccharide fraction contained as much as 80 per cent glycogen, as found after acid hydrolysis or digestion by 'Diazyme' or phosphorylase. The ratio of glucose-1-phosphate to glucose was 10:5. This alkali-treated polysaccharide fraction contained the following substances: sugars (as glucose), 95 per cent; nitrogen, less than 0.2 per cent; proteins, less than 0.5 per cent; pentoses, less than 0.5 per cent.

The difference between the glucose content as found by specific enzymatic methods and the total sugar content as obtained by the anthrone method is apparently due to the presence of other sugars. After hydrolysis in N sulphuric

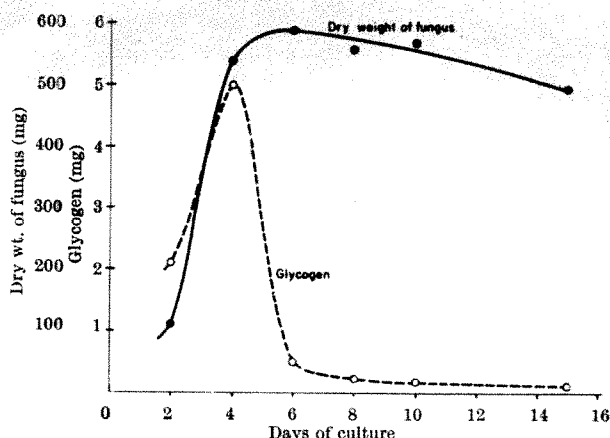


Fig. 1. Glycogen content and growth of *Paracoccidioides brasiliensis* cultured on blood agar

acid for 4 h in a boiling-water bath and after neutralization with barium carbonate, mannose was found by paper chromatography, using various solvent systems; phenol¹⁵, *s*-collidine¹⁵, and ethyl acetate-water-acetic acid (3:3:1)¹⁶. Mannose may exist in the polysaccharide fraction as mannan in combination with glycogen.

The relationship between glycogen content and age of culture was studied as follows: the fungus, collected after various periods of culture, was washed 4 times with distilled water and, after extraction of lipids, the glycogen-containing fractions were prepared by the method described here. To remove glucose and glucose-6-phosphate, the concentrated fractions were dialysed against cold distilled water for 24 h instead of treating them with trichloroacetic acid and ethanol. The fractions containing less than 2 mg of polysaccharides were digested with 2 mg of 'Diazyme' at 40° C for 2 h in a total volume of 5 ml. of acetate buffer (0.02 M, pH 5.0). After digestion, the liberated glucose was determined enzymatically. As shown in Fig. 1, the glycogen synthesized during the lag and logarithmic growth phases is consumed rapidly after the onset of the stationary phase. This finding is in agreement with earlier histochemical results².

We thank Dr. K. Gaede for his advice and Mr. J. Rodriguez for his assistance in the culture of the fungus.

This work was partially supported by the Research Office of the U.S. Army (grant DA-ARO-49-092).

FUMINORI KANETSUNA
LUIS M. CARBONELL

Departamento de Patología Experimental,
Instituto Venezolano de Investigaciones Científicas,
Caracas, Venezuela.

¹ McKinnon, J., and Vinelli, H., *An. Fac. Med. Montevideo*, **34**, 461 (1949).

² Carbonell, L. M., Castilejón, H., and Pollak, L., *J. Histochem. Cytochem.*, **12**, 413 (1964).

³ Abdel-Akher, H., and Smith, F., *J. Amer. Chem. Soc.*, **73**, 994 (1951).

⁴ Slein, M. W., in *Methods of Enzymatic Analysis*, edit. by Hans-Ulrich Bergmeyer, 117 (Academic Press, New York and London, 1963).

⁵ Johnson, J. A., Nash, J. D., and Fusaro, R. M., *Anal. Biochem.*, **5**, 379 (1963).

⁶ Bueding, E., and Hawkins, J. T., *Anal. Biochem.*, **7**, 26 (1964); **9**, 115 (1964).

⁷ Scott, T. A., and Melvin, E. H., *Anal. Chem.*, **25**, 1656 (1953).

⁸ Johnson, M. J., *J. Biol. Chem.*, **137**, 575 (1941).

⁹ Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. Biol. Chem.*, **193**, 265 (1951).

¹⁰ Fiske, C. H., and Subbarrow, Y., *J. Biol. Chem.*, **66**, 375 (1925).

¹¹ Belcher, E., Nutton, A. J., and Sambrook, C. M., *Analyst*, **79**, 201 (1954).

¹² Fernell, W. R., and King, H. K., *Analyst*, **78**, 80 (1953).

¹³ Bryson, J. L., and Mitchell, T. J., *Nature*, **167**, 864 (1951).

¹⁴ Gee, M., and McCready, R. M., *Anal. Chem.*, **29**, 257 (1957).

¹⁵ Partridge, S. M., *Biochem. J.*, **42**, 238 (1948).

¹⁶ Jermyn, M. A., and Isherwood, F. A., *Biochem. J.*, **44**, 402 (1949).

PHYSIOLOGY

Plasma Growth-hormone Levels in Chronic Starvation in Man

BOTH fasting and induced hypoglycaemia are powerful stimuli to secretion of growth hormone in man¹⁻³, but the effect of prolonged starvation on secretion of growth

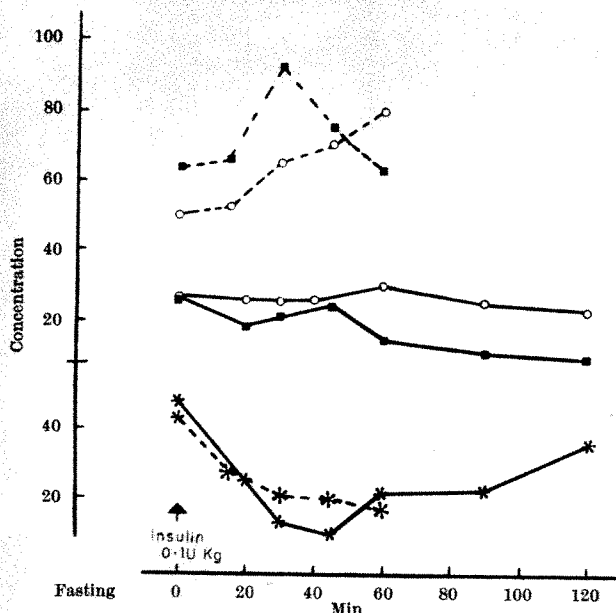


Fig. 1. Concentrations of growth hormone, cortisol and glucose before and during an intravenous insulin-tolerance test in two young female subjects with chronic starvation due to anorexia nervosa. Case 1 shown by continuous lines; Case 2 by interrupted lines. The test in Case 2 was terminated with glucose at 60 min because of severe neuroglycopenic symptoms. ○, Cortisol, μg/100 ml; *, glucose, mg/100 ml; ■, growth hormone, μmg/ml.

hormone is unknown. It has been suggested, on indirect evidence⁴, that secretion of growth hormone is impaired in chronic malnutrition.

We have measured levels of growth hormone in plasma by a sensitive and specific radioimmunoassay technique⁵ in two young female subjects with profound chronic under-nutrition due to primary anorexia nervosa before and during insulin-induced hypoglycaemia. Each subject was a typical example of the condition, had recently lost two stone or more in weight and, at the time of examination, weighed less than 70 lb. Total daily dietary intake was unknown but estimated to be less than 400 calories. Since food and even minimal exercise may profoundly alter the level of growth hormone in the blood², assays were carried out with the subjects in bed after an overnight fast and during the course of a standard intravenous insulin-tolerance test (0.1 U/kg body-weight). Glucose was measured by glucose oxidase, cortisol by fluorimetry.

The results, shown in Fig. 1, indicate high levels of growth hormone in the plasma at rest (upper limit of normal less than 10 μmg/ml.) with no response in one subject, and a minimal non-sustained increase in the other in response to an induced hypoglycaemic stimulus adequate to produce neuroglycopenic symptoms. Plasma cortisol responses were similar; fasting-levels were high (upper limit of normal, less than 25 μg/100 ml.) and increased minimally, or not at all, during induced hypoglycaemia.

The suggestion is made that the stimulus to growth hormone produced by starvation is, or is near, maximal and the high level achieved thereby is responsible for prevention of death from fasting hypoglycaemia, but, because of limited reserve capacity, resistance to induced hypoglycaemia is reduced⁶.

VINCENT MARKS
N. HOWORTH

The Area Laboratory,
West Park Hospital, Epsom, Surrey.

F. C. GREENWOOD

The Imperial Cancer Research Fund,
Lincoln's Inn Fields, W.C.2.

¹ Roth, J., Glick, S. M., Yalow, R. S., and Berson, S. A., *Metabolism*, **12**, 577 (1963).

² Hunter, W. M., and Greenwood, F. C., *Brit. Med. J.*, **i**, 804 (1964).

³ Frantz, A. G., and Rabkin, M. T., *New Eng. J. Med.*, **271**, 1375 (1964).

⁴ Monckberg, F., Conoso, G., Oxman, S., Pak, N., and Monaghelli, J., *Pediatrics*, **31**, 58 (1963).

⁵ Hunter, W. M., and Greenwood, F. C., *Biochem. J.*, **81**, 43 (1964).

⁶ Cooke, J. N. C., James, V. H. T., Landon, J., and Wynn, V., *Brit. Med. J.*, **i**, 662 (1964).

Fasting Levels of Growth Hormone in Men and Women

THE finding of a twenty-fold difference in fasting sera between adult females and males, as reported by Unger *et al.*¹, is intriguing. It may serve to explain the higher free fatty acid levels observed in both healthy and diabetic female subjects compared with males², for the effect of growth hormone both *in vitro* and *in vivo* on enhancing the lipolytic activity of adipose tissue is well known³. On the other hand, the possibility that at least part of this sex-difference may be attributed to the presence of prolactin must be considered. This might occur in either or both of the following ways: (1) the growth hormone antigen used contained some contaminating prolactin; (2) human prolactin cross-reacts with anti-human growth hormone antisera. The biochemical similarity between these two polypeptides is well known⁴ and the *in vivo* similarity between them has also been noted⁵. The problem of immunological specificity is also open to some question, as recently discussed by Berson *et al.*⁶. Indeed, Hayashida⁷ has considered the evidence that human prolactin and growth hormone represent different activities of the same molecule. Greenwood⁸ has also reported that he has noted a cross-reacting substance in plasma throughout pregnancy, and also in placental extracts. This presumably is related to the placental lactogen of Josimovich, which has been shown to be immunologically similar to growth hormone⁹.

In conclusion, it appears that no definite statement can be made at the present time regarding the significance of this sex difference in serum growth-hormone-levels. The possibilities are either that this has a true physiological significance or that the higher levels in females simply represent cross-reacting prolactin. Some of this controversy may be resolved by comparing the levels of growth hormone in the pre-pubertal age group.

C. FAIDMAN*

Department of Physiology,
University of Manitoba.

* Medical Research Fellow.

¹ Unger, R. H., *et al.*, *Nature*, **205**, 804 (1965).

² Moorhouse, J. A., Steinberg, J., and Rosen, N. J., *J. Clin. Endocrinol.*, **23**, 1080 (1965).

³ Hartog, M., *Postgrad. Med. J.*, **40**, 470 (1964).

⁴ Ferguson, K. A., *Metabolism*, **13**, 985 (1964).

⁵ Beck, J. C., Gonda, A., Eamid, M. A., Morgen, R. O., Rubinstein, D., and McGarry, E. E., *Metabolism*, **13**, 1108 (1964).

⁶ Berson, S. A., Yalow, R. S., Glick, S. M., and Roth, J., *Metabolism*, **13**, 1135 (1964).

⁷ Hayashida, T., *Endocrinology*, **70**, 846 (1962).

⁸ Greenwood, F. C., *Metabolism*, **13**, 1169 (1964) (in discussion).

⁹ Josimovich, J. B., and MacLaren, J. A., *Endocrinology*, **71**, 209 (1962).

Antidiuretic Hormone and Bile Flow

DURING experiments related to the excretion of manganese¹, variations in flow of bile in the rat suggested the possibility that bile flow is sensitive to the action of the antidiuretic hormone.

Intravenous injections of 'Pitressin' (Parke-Davis 0.3-0.6 pressor units/100 g) into rats induced a sudden short-lived diminution of bile flow. In this investigation the bile was delivered directly on to filter-paper strips via polyethylene catheters and the diameter of the round spots was measured with callipers to the nearest 0.1 mm. The standard curve shown in Fig. 1 relates this measurement to the volume of bile. Collections lasted between 10 and 150 sec, producing spots between 11 and 21 mm in diameter.

The sharp fall of the bile flow after pitressin is shown on Fig. 2. This fall had a mean and standard deviation of 70 ± 10 per cent in the seventeen animals tested. The basal level oscillated around a fairly constant mean, even if the experiments lasted for 3 h. These oscillations were present regardless of the animals' state of hydration.

The pattern shown in Fig. 2 could be induced again following the initial response by injections of 'Pitressin' 30 min later. It could be prolonged by giving the second injection 5 min after the first. Minimal flow rates were attained about 4 min after injection, and full restitution of bile flow was reached during the next 16–20 min. The pattern was the same in markedly overhydrated and in dehydrated animals, in spite of the fact that the preinjection level of the bile flow ranged between 8 and 12 $\mu\text{l./min}$ in the overhydrated group and between 1 and 3 $\mu\text{l./min}$ in the dehydrated animals. Animals were given 5–5 ml. of 12 per cent ethanol by stomach tube to inhibit secretion of antidiuretic hormone. These showed initial flow rates between 10 and 16 $\mu\text{l./min}$, which, however, responded to the pitressin in an identical manner to that described.

The rapidity of this response and its independence from the animal's state of hydration suggested that a smooth muscle coat was being constricted by the hormone. Wicks impregnated with the same hormone solution applied to the blood vessels of the liver had no effect on the bile flow*. By contrast, the usual response was elicited when these wicks were applied to the bile duct. Intravenous injections of 10 μg of L-epinephrine per rat or 1.6–2.4 mg of cyclic AMP (adenosine 3,5-monophosphate)^{2,3} induced the same response. This could be blocked by the local application of 'Novocaine' to the common bile duct. Indeed, the duct was found to possess a circular smooth muscle layer on histological examination. The sum of these observations was interpreted as indicating that the intravenously injected 'Pitressin' had caused a constriction of the muscular coat of the common bile duct proximally to the insertion of the catheter.

An additional hormonal action, namely on the reabsorption of water by the columnar epithelium lining these ducts, had been suggested by the results of the experiments with ethanol. Ballooned bile ducts could not be tested *in vitro*⁵. Therefore, evidence for water reabsorption was sought in intact animals by determining the osmolality of their bile. Two samples of bile from the ligated, ballooned bile ducts of two animals hydrated in the standard fashion showed 316 and 317 mOs/l. of bile respectively. Three samples were analysed from each of a

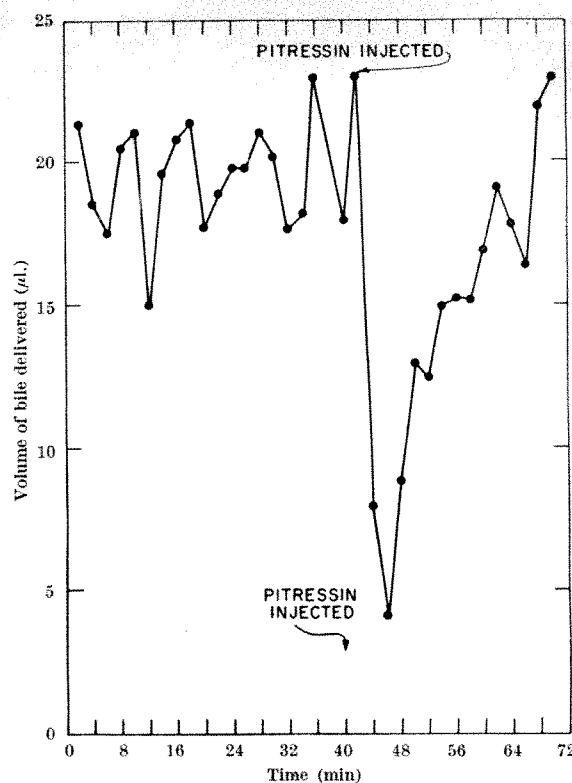


Fig. 2. Effect of intravenously injected 'Pitressin' on the bile flow of the rat

series of non-obstructed animals, as shown by the following means and standard deviations: dehydrated, 316 ± 6 mOs/l. (three animals); standard hydration, 298 ± 0 mOs/l. (four animals); water loaded, 276 ± 3 mOs (two animals). These preliminary results indicated that the osmolality of the bile did reflect the animals' state of hydration as had the bile flow. Furthermore, prolongation of the contact of the bile with the histologically intact epithelium of the ligated common duct appeared to result in more concentrated bile.

In summary, rapid sampling of rat bile showed the bile flow to oscillate. Intravenously injected 'Pitressin' induced a sharp diminution of the flow, which was ascribed to contraction of the smooth muscle surrounding the large bile ducts. In addition, there was collected suggestive evidence for a hormonal effect on the permeability of the bile ducts.

This work was supported by the U.S. Atomic Energy Commission.

EARL HOMSHER*
GEORGE C. COTZIAS

Medical Research Center,
Brookhaven National Laboratory,
Upton, Long Island,
New York.

* Summer student from the University of Pittsburgh.

¹ Cotzias, G. C., and Papavasiliou, P. S., *Nature*, **201**, 828 (1964).

² Orloff, J., and Handler, J. S., *Amer. J. Med.*, **36**, 686 (1964).

³ Davoren, P. R., and Sutherland, E. W., *J. Biol. Chem.*, **238**, 3009 (1963).

⁴ Brauer, R. W., Leong, G. F., and Hollaway, R. J., *Amer. J. Physiol.*, **177**, 103 (1954).

⁵ Schwartz, I. L., Rasmussen, H., Schoessler, M. A., Silver, L., and Fong, C. T. O., *Proc. U.S. Nat. Acad. Sci.*, **46**, 1288 (1960).

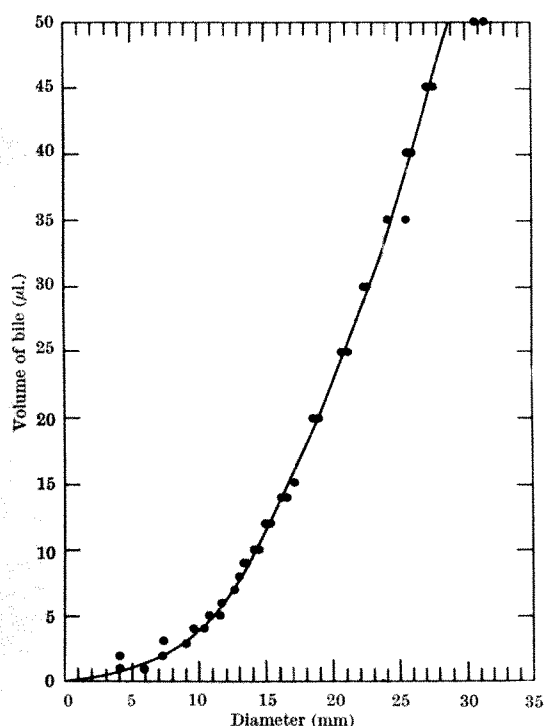


Fig. 1. Relation of volume of rat bile to the diameter of spots produced on a filter paper

Alcohol and Evoked Potentials in the Cat

In our previous communication¹, we reported the relative effects of ethanol on the midbrain reticular formation and one site within the somatosensory cortex. It was found that the cortex was depressed by ethanol to a

greater degree than the reticular formation. Further investigations indicated that the response of the somatosensory cortex to alcohol depended essentially on the site of the recording electrode. In the earlier work the electrode was located on or near the coronal suture line for recording the cortical response on stimulation of the sciatic nerve. In the present report the electrode placement was in the region of the cruciate fissure.

Acute experiments were performed on healthy adult cats ranging in weight from 2.2 to 3.6 kg body-weight. Chloride silver ball electrodes (1 mm diameter) were placed under visual inspection in the region of the cruciate fissure with a medial location. The stereotaxic co-ordinates for the electrodes placed in the reticular formation were the same as described before. In each experiment, the electrode placements in the reticular formation were verified by histological examination of unstained frozen sections of the brainstem as described by Morillo and Baylor². Those experiments where the electrode placements were in doubt were discarded. In six animals the responses were recorded from the reticular formation at the mid-collicular level and the cruciate fissure of the somatosensory cortex contralateral to the stimulated sciatic nerve. In all the experiments following intravenous administration of alcohol (1 g/kg) both responses showed marked depression at 2 min. Recovery process was evident in each structure 15 min after alcohol administration. At 60 min the better part of the cortical response had recovered, but the reticular formation still exhibited marked degree of depression. Typical results of these experiments are shown in Fig. 1 which clearly indicate that the reticular formation exhibited a greater degree of depression throughout the entire period of observation.

The results are contrary to those presented earlier¹. The discrepancy was found to be due to the areas of recording sites in the somatosensory cortex. Moreover, experiments performed to examine the simultaneous effects of alcohol on the two cortical areas further reinforced the conclusion of the greater variability of the responses recorded from the coronal suture line as well as the lesser sensitivity of the cruciate site to the depressant effect of alcohol¹. Similarly the simultaneous responses of VPL and somatosensory cortex also disclosed that the cortical area was depressed as already described, whereas the VPL was scarcely affected by the dose of alcohol used in these studies. These results and those with two other sensory modalities (vision and audition) will be published in detail elsewhere. Obviously, the degree of depression of the somatosensory cortex by alcohol varies with the cortical

locus. Thus the relationship between reticular and cortical depression is a complex one and varies with the recording site in the somatosensory cortex.

ARLENE K. SCHWEIGERDT*

ANANT R. DRAVID

ANN H. STEWART

HAROLD E. HIMWICH

Thudichum Psychiatric Research Laboratory,
Galesburg State Research Hospital,
Galesburg, Illinois.

* Present address: Louisiana State University, Baton Rouge, Louisiana.

¹ Dravid, A. R., DiPerri, R., Morillo, A., and Himwich, H. E., *Nature*, **200**, 1328 (1963).

² Morillo, A., and Baylor, D., *Electroenceph. Clin. Neurophysiol.*, **15**, 455 (1963).

Proposed Physiological Function for Plasma Cholinesterase

THE physiological function of plasma, or non-specific, cholinesterase, although receiving some attention¹, has not been established. This report deals with the possibility of its role as a regulator of free choline in the plasma. Several experiments were conducted *in vitro* to determine: (1) the inhibitory effects of free choline on the synthesis of acetylcholine and the activity of serum cholinesterase; (2) whether acetyl-S-coenzyme A (AcCoA) and choline acetylase were capable of synthesizing acetylcholine from another choline ester.

Rabbits were killed, and their brains removed and immediately dropped into liquid nitrogen. The brains were pulverized using a mortar and pestle at the temperature of dry ice. Acetone-cried powder was obtained by double extraction with cold acetone² and the choline acetylase was extracted by the method of Nachmansohn, Hestrin and Voripaieff³. Cholinesterase was prepared from horse plasma by the method of Oliver and Funnell⁴. Acetyl-S-coenzyme A was obtained from General Biochemicals, Ltd., Chagrin Falls, Ohio; propionylcholine iodide from Distillation Products Industries, Rochester, New York; and choline chloride from Anachemia Chemical Co., Toronto.

Solutions were prepared in Nachmansohn extraction buffer³, except that plasma cholinesterase was in *tris* buffer, pH 7.4. All reaction mixtures were made up to a final volume of 4 ml.

Acetylcholine formation was based on the change in absorbance at 232 mμ due to the hydrolysis of AcCoA⁵, and cholinesterase activity was determined by the method of Kellow and Lindsay⁶. In both cases, activity was measured at 26° C using a Beckman 'DK2' recording spectrophotometer. Propionylcholine was selected as the fatty acid choline ester because, at the wave-length used, its absorbance does not change with hydrolysis and of all those tested only its molar extinction was low enough to permit reliable interpretation.

In the first series, absorbance/time studies were made of the following reactions: (a) AcCoA + choline acetylase; (b) AcCoA + choline acetylase + choline; (c) AcCoA + choline acetylase + propionylcholine; (d) AcCoA + choline acetylase + propionylcholine + cholinesterase; (e) AcCoA + choline acetylase + cholinesterase. In each case, AcCoA was added at time zero.

A second series using AcCoA, choline acetylase and choline was carried out to determine the effect of varied choline concentration on acetylcholine formation. A third series using cholinesterase, benzoylcholine and varied choline concentration was investigated to find the effect of choline on the activity of cholinesterase.

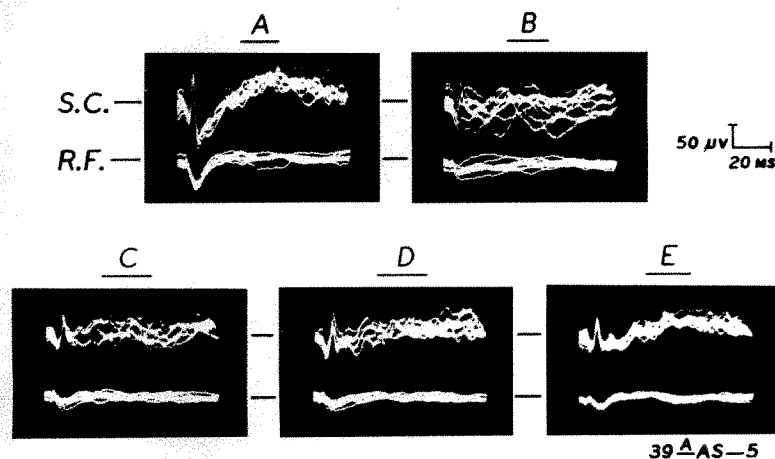


Fig. 1. Evoked responses to electrical stimulation of the sciatic nerve recorded from the contralateral midbrain reticular formation (R.F.) and from the cruciate fissure in the somatosensory cortex (S.C.). A, Control; B, C, D and E, respectively, 2, 15, 30 and 60 min after intravenous administration of alcohol (1 g/kg). A depression in both responses is evident. (B) The reticular formation showing a greater degree of depression. Partial recovery of the cortical response can be seen at 60 min (E) while a marked depression still persisted in the reticular formation.

Trials (a), (c), and (e) of Series 1 showed that AcCoA was not hydrolysed when free choline was absent (Fig. 1). On the other hand, AcCoA was hydrolysed when choline was supplied directly (trial b) or indirectly through the action of cholinesterase on propionylcholine (trial d). The effects of varied concentrations of choline on the hydrolysis of AcCoA (Series 2) indicated that a peak activity occurred at approximately 10^{-5} M choline (Fig. 2). Cholinesterase activity was sensitive to a range of choline concentrations (Series 3) and, although inhibition was slight at 10^{-6} M choline, this inhibition increased to approximately 23 per cent at 10^{-3} M choline (Fig. 3)—the optimum concentration for acetylcholine formation (Fig. 2).

The results of Series 1 indicated that, at least for acetylcholine synthesis under the experimental conditions described, choline must be supplied in the free form. The concentration of free choline in the plasma of a number of species is held 'remarkably constant' at about 10^{-5} M (refs. 7 and 8) and increases in choline concentrations up to one-hundred-fold are followed by a rapid return to normal, partly through kidney excretion and partly due to a 'homeostatic mechanism'. Since, in the present study, the inhibition of plasma cholinesterase by choline occurred at a much lower concentration than the inhibition of choline ester synthesis (Figs. 2, 3), it is conceivable that serum cholinesterase could constitute a part of this homeostatic mechanism. That is, as the concentration of choline increases above the reported plasma concentration, 10^{-5} M, its return to within a normal range would be favoured by: (1) a decrease in choline ester hydrolysis caused by increased inhibition of cholinesterase; (2) an increase in choline ester synthesis caused by activation of the synthesizing mechanism, characterized in this study by choline acetylase and AcCoA. Both these effects would

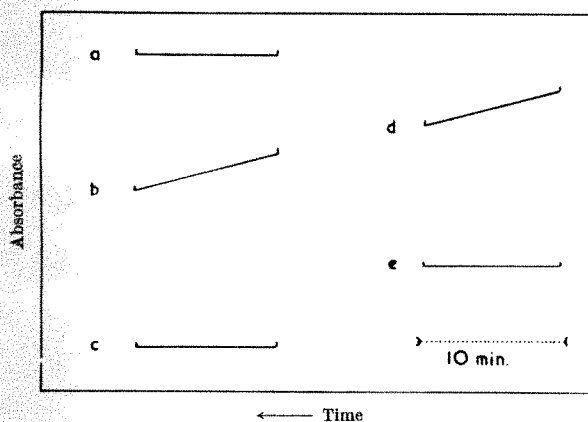


Fig. 1. Re-drawing of hydrolysis curves obtained in Series 1. Reaction time 10 min

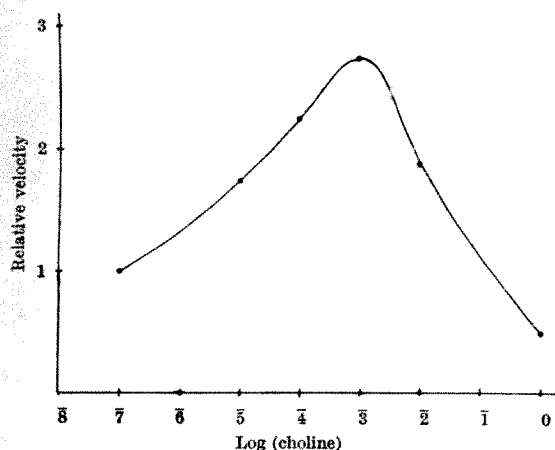


Fig. 2. Effect of choline concentration on acetylcholine synthesis. Velocity at 10^{-7} M choline expressed as unity

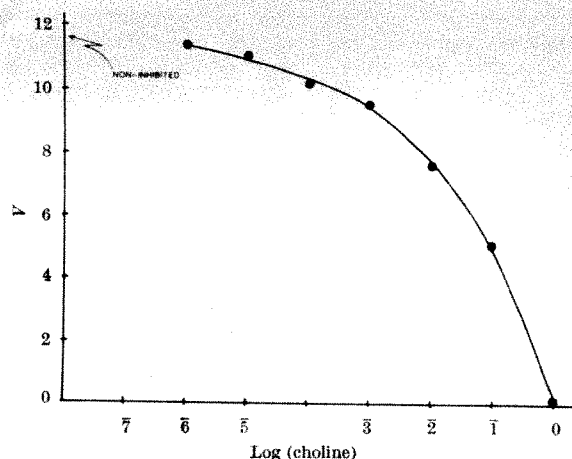


Fig. 3. Effect of choline concentration on cholinesterase activity. Velocity expressed as micromoles benzoylcholine hydrolysed per h

produce an increase in conjugated choline. Conversely, if the free choline concentration drops below normal, plasma cholinesterase activity is increased and esterification activity is decreased, thereby resulting in an increase in free choline concentration. Since a variety of choline esters occurs in plasma, the balance of conjugated/free choline could be maintained, in part, by the various isozymes of choline-, aliphatic-, and aryl-esterases^{9,10}, each utilizing a preferential choline ester substrate.

Thus, the homeostatic mechanism existing for the control of free choline in plasma⁷ may depend on two opposing systems, one (the plasma cholinesterases) responsible for choline ester hydrolysis, and the other responsible for choline ester formation; choline acts as an auto-regulator in the mechanism by its complementary effects on these two opposing systems.

H. S. FUNNELL

Division of Pharmacology,
Ontario Veterinary College,
University of Guelph, Canada.

W. T. OLIVER

Food and Drug Directorate,
Tunney's Pasture,
Ottawa, Canada.

¹ Clithrow, J. W., Mitchard, M., and Harper, N. S., *Nature*, **199**, 1000 (1963).

² Nachmansohn, D., and John, H. M., *J. Biol. Chem.*, **158**, 157 (1945).

³ Nachmansohn, D., Hestrin, S., and Voripaleff, H., *J. Biol. Chem.*, **180**, 875 (1949).

⁴ Oliver, W. T., and Funnell, H. S., *Amer. J. Vet. Res.*, **24**, 32 (1963).

⁵ Stadtman, E. R., in *Methods in Enzymology*, edit. by Colowick and Kaplan, **3**, 935 (Academic Press Inc., New York, 1959).

⁶ Kalow, W., and Lindsay, H. A., *Canad. J. Biochem. Physiol.*, **33**, 568 (1955).

⁷ Bligh, J., *J. Physiol.*, **117**, 234 (1952).

⁸ MacIntosh, F. C., *Canad. J. Biochem. Physiol.*, **41**, 2555 (1963).

⁹ Oki, Y., Oliver, W. T., and Funnell, H. S., *Nature*, **203**, 605 (1964).

¹⁰ Oki, Y., Oliver, W. T., and Funnell, H. S., *Canad. J. Physiol. Pharmacol.* (in the press).

Introduction of a Permanent Electrode into the Pituitary Gland of the Cow: Influence of Electrical Stimulation on Milk Ejection

PROCEDURES involving intervention with the pituitary gland or the hypothalamus, in the investigation of their influence on lactation, have mainly been carried out on small laboratory animals. Few experiments of this kind have been performed on ruminants; most of these were on goats and the remainder on sheep. This type of procedure has been extended to the cow by a process which we have described elsewhere¹. Essentially, the method consists of inserting a specially adapted needle through the skin and through the muscular part of the tongue to the pharynx, where the operating worker, with his hand in the oral cavity of the animal, places the tip of the needle in a defined position on the sphenoid bone. By means of

a drill which operates through the guiding needle, he drills through the sphenoid bone to the pituitary gland. In this way it is possible to carry out the application of various substances to the pituitary with the view of destroying it, or of carrying out biopsy of the pituitary gland, or of collecting blood from the sinus cavernosus.

In the next stage of our work we devised a method of introducing and placing permanent electrodes for direct stimulation. The electrodes used were made of platinum, or some other suitable metal, in the form of an insulated wire, which was conducted through a plastic cannula of polyvinylchloride and a cuff of stainless steel, and fixed in the sphenoid bone. The metal cuff was fixed in place with a ring of polyvinylchloride. The electrodes were passed through the application needle, which had the same diameter as the metal cuff of the electrode. The metal cuff, which was approximately 2 cm long, formed a continuation of the application needle, and with the aid of this needle it was firmly embedded in the sphenoid bone. When the electrode was so placed in the sphenoid bone it was not usually possible to pull it out. After fixing the metal cuff into the sphenoid bone, we pulled the wire carefully through the oral cavity, and with the help of a rubber probe we brought it out over the meatus nasi ventralis through the nostrils of the animal. Then we inserted a strong hypodermic needle into the wall of a nostril, in the free space between the cartilages parietales; we slid the wires leading to both electrodes through the needle, and after removing the latter, fastened the wires, which were taken out, with one or two stitches on to the skin of the regio lateralis nasi. It is possible to connect the stimulator here. Meanwhile, we controlled the exact placing of the electrodes radiographically and by dissection. The state of health of the animal and its physiological functions were not affected by the intervention, and the operation could be repeated at will.

A description of access to the pituitary gland of cattle was published by L. E. Donaldson and W. Hansel² and similarly of sheep by L. Z. McFarland, M. T. Clegg and W. F. Ganong³. In both cases access is over the foramen ovale. We consider that our way of reaching the pituitary gland is more reliable, because the needle introduced over the foramen ovale as a rule passes the pituitary gland at the posterior border.

We have used this method of stimulating the pituitary gland in connexion with further work on the neurohumoral regulation of lactation, particularly the mechanism of oxytocin action and ejection reflex. According to preliminary results, we have succeeded in inducing milk ejection by a series of square wave impulses. The mechanism of this effect is the subject of further investigation.

B. HYNĚK
J. BÍLEK
M. LIS

Laboratory of Animal Physiology and
Genetics of the Czechoslovak Academy of Sciences,
Uhřetěves, near Prague, Czechoslovakia.

¹ Hyněk, B., Bílek, J., and Lis, M., *Archiv. für experimentelle Veterinärmedizin* (in the press).

² Donaldson, L. E., and Hansel, W., *J. Dairy Sci.*, **47**, 98 (1964).

³ McFarland, L. Z., Clegg, M. T., and Ganong, W. F., *Proc. Soc. Exp. Biol. and Med.*, **103**, 538 (1960).

PHARMACOLOGY

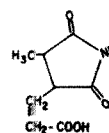
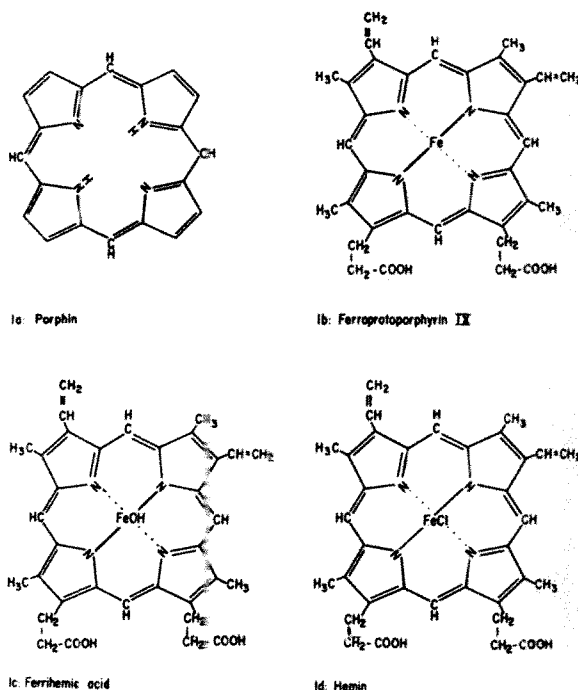
Chloroquine Resistance and the Nature of Malarial Pigment

THE appearance of strains of human *falciparum* malaria resistant to chloroquine¹ has stimulated investigations on the mode of action of this drug and on the mechanism of resistance to it. Although no morphological differences have been described between chloroquine-resistant and ordinary *Plasmodium falciparum*, a striking difference was observed in the mouse malaria *P. berghei*; in the re-

sistant strain, pigment (haemozoin) was not visible by light microscopy, and even with the electron microscope only a few minute grains of pigment-like material could be detected². This finding and the hypothesis advanced independently by Seaueler and Cantrell³ and Cohen *et al.*⁴, that the binding of chloroquine by ferrihaemic acid might account for chloroquine resistance, direct attention to haemoglobin digestion and its waste product haemozoin as being possibly of significance in both the mode of action of chloroquine and in resistance to it.

Although haemozoin was described in 1847, before malaria parasites themselves had been seen, its chemical nature and its role in protein metabolism of the parasite are not well understood. Deegan and Maegraith⁵ showed clearly that haemozoin is not solely the iron protoporphyrin portion of haemoglobin, as had been naïvely assumed, but that it contains also a proteinaceous moiety. This conclusion was fully confirmed and extended by Sherman and Hull⁶. Since the terminology of derivatives of haemoglobin is a confusing one, as is well illustrated by errors in the recent literature (see following), some definitions are in order.

Haematin compounds are iron complexes of porphyrins and similarly constituted tetrapyrrolic substances. Porphin has the formula $C_{20}H_{14}N_4$ and contains 4 pyrrole-like rings welded together in a complicated ring system by 4 methene groups (Fig. 1a) (ref. 7). Haem is (ferrous) iron protoporphyrin IX, the designation IX indicating the side-chain configuration (Fig. 1b). Haematin, also called oxyhaemin, ferrihaemic acid or ferrihaem hydroxide (Fig. 1c), and haemin, or ferrihaem chloride, both have iron in the ferric state. Haemoglobin is a red conjugated protein of blood consisting of haem and a water-soluble protein, globin. On treatment with hot sodium chloride



1e. Hematinic acid

Fig. 1

and acetic acid it decomposes to yield haemin. Treatment of haemin with sodium hydroxide produces ferrihaemic acid (haematin, oxyhaemin or ferrihaem hydroxide), whereas treatment of haemin by dilute acids results in the removal of the iron giving protoporphyrin IX. If one hydrolyses haemoglobin carefully with hydrochloric acid the molecule is cleaved into 2 fragments: haemin (4 per cent) and globin (96 per cent). Drastic reduction of haemin with hydrogen iodide and red phosphorus gives a mixture of 4 pyrroles variously substituted with methyl and ethyl groups plus haematinic acid (Fig. 1e) (ref. 8). Oxidation of haemin with chromic acid yields haematinic acid⁹. The definitions and synonymy alone provide reasons sufficient for confusion.

Schueler and Cantrell³ have shown that ferrihaemic acid (haematin) can inactivate chloroquine to some extent both *in vivo* and *in vitro*, and on this basis have suggested that chloroquine-resistant parasites might produce more ferrihaemic acid than non-resistant strains. They state: "A singular intermediate product in the formation of malarial pigment is ferrihaemic acid (a ferrihydroxide form) which results from the oxidation and hydroxylation of haemin (a ferrous chloride form)". To our knowledge, ferrihaemic acid has never been identified as an intermediate in haemozoin formation (nor has any other intermediate been identified). Moreover, haemin is not a ferrous form (see foregoing definitions); only the haem as it exists in undenatured haemoglobin has iron in the ferrous state. The hypothesis that haemoglobin metabolism is somehow related to chloroquine resistance may still be correct. Certainly, the finding that chloroquine-resistant *P. berghei* produce very little, if any, haemozoin would fit this hypothesis. Unfortunately, however, too little is yet known to permit a detailed theory. In discussing the digestion of haemoglobin, Peters *et al.*² state: "It is possible that haemoglobin is split into globin and a porphyrin fraction, haem, although there is no direct proof that this is so. Haem is said to be oxidized first to haematinic acid and later to haematin, a toxic substance which is rendered both safe and insoluble by conjugation with a nitrogenous moiety to form the typical malaria pigment". They continue: "... workers have shown that haematinic acid can form a complex with chloroquine". Haematinic acid and ferrihaemic acid are not identical (see foregoing definitions), as these authors suggest. Furthermore, they state: "... the failure of RC (= chloroquine-resistant *P. berghei*) parasites to produce normal pigment ... can also be interpreted as a failure of the RC parasites to conjugate haematin with a nitrogenous moiety". Haemozoin is indeed a complex of haematin with a proteinaceous (perhaps denatured) moiety, but there is no shred of evidence that free haematin is ever formed.

What evidence there is indicates the contrary. Thus the early work of Moulder and Evans¹⁰ showed that extracts of *P. gallinaceum* contained a proteolytic enzyme capable of producing amino nitrogen from denatured haemoglobin. One of us (Sherman, unpublished results) has found a similar enzymatic activity in extracts of *P. lophurae*. Furthermore, amino-acid analyses of haemozoin of *P. lophurae* and *P. berghei* and of duck and mouse haemoglobins indicate that haemozoin is a product of partial proteolysis of haemoglobin (Tables 1 and 2).

For these analyses the avian malaria *P. lophurae* was isolated from duck red cells⁶, the free parasites lysed by alternate freezing and thawing the lysate centrifuged at 20,000*g* at 4° C for 1 h, the insoluble pellet (haemozoin) collected and washed three times in distilled water. *P. berghei* haemozoin was prepared from the livers of infected mice (7 days after inoculation) by grinding in a glass homogenizer followed by washing and collecting the insoluble residue by differential centrifugation. The isolation was followed by phase-contrast microscopy. Crystalline duck haemoglobin was prepared according to the method of Drabkin¹¹ and globin by the method of Anson and Mirsky¹². Haemozoin was cleared of colour

(removal of haem) and protein precipitated as described by Fulton and Grant¹³. Samples were hydrolysed and analysed for amino-acids according to the methods described for the Spinco 120B amino-acid analyser. Because the amino-acids found in crystalline duck haemoglobin were the same as duck globin it seemed unnecessary to prepare crystalline mouse haemoglobin. As can be seen from the figures in the last column, the amino-acids in haemozoin vary from those found in haemoglobin. The data are consistent with the idea that in the formation of haemozoin from haemoglobin there is: (1) denaturation and insolubilization of haemoglobin; (2) oxidation of the iron; (3) partial proteolysis of the globin. Haemozoin, although electrophoretically immobile on paper, stains with bromphenol blue⁶. In addition, the presence of amino-acids after extraction with trichloroacetic acid, ethanol and ether are definitive proof of its proteinaceous nature. This is true not only of the form from the red cells but also of that from the liver. Without the demonstration of plasmodial enzymes which break and reform haem to protein bonds it seems unlikely that haemoglobin is first split into haem and this then re-conjugated to another protein.

Haemozoin is not a chemically characterizable compound in a strict sense, and this property is reflected in varying amino-acid analyses. Although it is possible that adsorbed proteins may modify our results from isolation to isolation it seems more reasonable that the variations are real. We believe that the haemozoin one isolates is an array of partially degraded and denatured haemoglobins. These represent the point at which the investigator halted parasite proteolysis. Its amino-acid composition reflects the amino-acid sequence in the haemoglobin and the mechanism of cleavage of the haemoglobin by the plasmodial proteolytic enzymes.

Unfortunately, amino-acid changes from host-cell haemoglobin to haemozoin offer no real understanding of the kind and amounts of amino-acids used for parasite protein synthesis. In order to determine the amino-acids utilized, sequential analyses of substrate and residue are necessary as well as *in vitro* production of haemozoin by a

Table 1. THE AMINO-ACID COMPOSITION OF DUCK HAEMOGLOBIN AND *Plasmodium lophurae* HAEMOZOIN (MALARIA PIGMENT) (Expressed as moles per cent of recovered amino-acids)

	Globin		Crystalline haemoglobin	Haemozoin		Per cent change
	Sample 1	Sample 2	Sample 1	Sample 1	Sample 2	H ₂ vs. Hb
Lysine	7.5	7.7	7.1	8.9	8.4	+12
Histidine	5.1	5.5	6.9	8.6	11.5	+78
Arginine	3.2	3.2	3.6	5.4	4.5	+55
Aspartic acid	9.1	8.8	8.9	10.5	9.0	+9
Threonine	4.0	4.0	4.1	3.7	3.3	-17
Serine	2.2	2.1	2.1	3.4	2.8	+44
Glutamic acid	7.7	7.9	8.2	10.0	8.5	?
Proline	4.0	4.1	4.5	4.4	4.5	+10
Glycine	6.6	6.6	6.0	6.8	10.8	?
Alanine	14.1	13.1	13.2	8.1	7.1	-55
Cystine						
Valine	9.1	8.8	8.7	5.7	5.3	-61
Methionine	1.3	1.1	1.2	1.2	1.3	0
Isoleucine	4.5	4.2	4.5	7.6	6.5	+62
Leucine	13.1	14.1	12.6	8.8	8.9	-32
Tyrosine	2.3	2.4	2.3	3.5	3.3	+45
Phenylalanine	5.9	6.3	6.1	4.2	4.4	-30

Table 2. AMINO-ACID COMPOSITION OF MOUSE HAEMOGLOBIN AND *Plasmodium berghei* HAEMOZOIN (Expressed as moles per cent of recovered amino-acids)

	Mouse Hb	<i>P. berghei</i> haemozoin		Per cent change
		Sample 1	Sample 2	H ₂ vs. Hb
Lysine	8.1	7.3	6.6	-15
Histidine	7.1	2.5	2.3	-67
Arginine	2.3	5.4	5.6	+140
Aspartic acid	10.3	9.2	9.4	-7
Threonine		4.1	4.7	0
Serine		6.9	4.1	-40
Glutamic acid		5.3	11.0	+98
Proline		3.4	4.8	+85
Glycine		8.5	5.0	?
Alanine		13.9	8.3	-60
Cystine		0.93	1.12	0.95
Valine		8.7	7.4	-16
Methionine		0.93	2.2	+150
Isoleucine		5.6	5.4	?
Leucine		9.7	9.5	?
Tyrosine		3.3	3.1	?
Phenylalanine		4.6	4.1	?

plasmodial proteolytic enzyme. The mechanism of chloroquine resistance may be related to ferrihaemic acid; but this has not been demonstrated, nor can haemozoin be called haematin, haematinic acid or ferrihaemate dimer. Reduced quantities of haemozoin in chloroquine-resistant strains of malaria cannot be due to ferrihaemic acid alone since the pigments are not identical.

This investigation was supported by a grant from the U.S. National Institute of Allergy and Infectious Diseases (AI 05226) and in part by a grant (AP 71-02) from the Division of Air Pollution, Bureau of State Services, U.S. Public Health Service.

IRWIN W. SHERMAN

Department of Life Sciences,

J. BRIAN MUDD

Department of Biochemistry,
University of California,
Riverside.

WILLIAM TRAGER

The Rockefeller Institute,
New York 21, N.Y.

¹ Young, M. D., and Moore, D. V., *Amer. J. Trop. Med. and Hyg.*, **10**, 317 (1961). Degowin, R. L., and Powell, R. D., *Amer. J. Trop. Med. and Hyg.*, **14**, 519 (1965).

² Peters, W., *Nature*, **203**, 1290 (1964). Peters, W., Fletcher, K. A., and Stäubli, W., *Ann. Trop. Med. and Parasit.*, **59**, 126 (1965).

³ Schueler, F. W., and Cantrell, W. F., *J. Pharmacol. Exp. Therap.*, **143**, 278 (1963).

⁴ Cohen, S. N., Phifer, K. O., and Yielding, K. L., *Nature*, **202**, 805 (1964).

⁵ Deegan, T., and Maegraith, B. G., *Ann. Trop. Med. and Parasit.*, **50**, 194 (1956).

⁶ Sherman, I. W., and Hull, R. W., *J. Protozool.*, **7**, 409 (1960).

⁷ Lemberg, R., and Legge, J. W., *Hematin Compounds and Bile Pigments* (Interscience, 1949).

⁸ Fieser, L. S., and Fieser, M., *Organic Chemistry* (Reinhold, 1956).

⁹ Bentley, K. W., *The Natural Pigments* (Interscience, 1960).

¹⁰ Moulder, J., and Evans, jun., E. A., *J. Biol. Chem.*, **164**, 145 (1946).

¹¹ Drabkin, D. L., *Arch. Biochem. Biophys.*, **21**, 224 (1949).

¹² Anson, M. L., and Mirsky, A. E., *J. Gen. Physiol.*, **13**, 469 (1929-30).

¹³ Fulton, J. D., and Grant, P. T., *Biochem. J.*, **63**, 274 (1956).

Mepacrine- and Primaquine-resistant Strains of *Plasmodium berghei*, Vincke and Lips, 1948

THE demonstration that the mechanism of pigment formation in the rodent malaria parasite, *Plasmodium berghei*, could be disturbed when the parasites became resistant to the antimalarial drug, chloroquine^{1,2}, has since been confirmed by Thompson *et al.*³. The same phenomenon has now been described by Jacobs in a quinine-resistant strain⁴. In continuation of our own investigations we have developed two further strains of *P. berghei*, one of which, the *P* strain, has a high level of primary resistance to the 8-aminoquinoline compound, primaquine, and the second, the *M* strain, a high level of primary resistance to the familiar 9-aminoacridine derivative, mepacrine. The methods followed for the induction of drug-resistance in these strains and their subsequent testing for sensitivity to various drugs are identical to those used with the chloroquine-resistant (*RC*) strain and have been described elsewhere⁵. Preliminary examination of the ultrastructure of parasites of the *P* and *M* strains has been made in association with Dr. W. Stäubli, following the techniques already published².

Intraerythrocytic parasites of the 43rd passage of the *P* strain *P. berghei* survived in albino mice which received 30 mg/kg of primaquine diphosphate daily as a subcutaneous injection, for 55 days from the day of infection. In untreated mice the infection runs a slower course than that with normal (*N* strain) parasites. While the latter kill all untreated mice within 6-9 days, the animals may survive on the average from 14 to 19 days when infected with the *P* strain. The total dose of primaquine diphosphate (once daily subcutaneous for 4 consecutive days) which inhibits development of the *N* strain by 50 per cent, that is, the *ED*₅₀, is 9 mg/kg and the *ED*₅₀ is 24 mg/kg. The corresponding *ED*₅₀ of *P* strain passage 61 is 48

mg/kg while the *ED*₅₀ can be extrapolated graphically⁶ as approximately 400 mg/kg. A total dose of 1,650 mg/kg failed to eradicate parasitaemia from all mice infected with *P* passage 43, but permitted some of the animals to survive, presumably by giving them sufficient time to develop their own defence mechanisms. *P* strain *P. berghei* examined by light microscopy readily are seen to contain malaria pigment. The presence of pigment grains somewhat smaller than those of normal parasites was confirmed by electron microscopy which also demonstrated the presence in numerous trophozoites of more than one food vacuole, a feature we have previously associated with our chloroquine-resistant *RC* strain². Some difficulty was experienced in preparing ultra-thin sections in which this pigment was demonstrable since, although we used the identical technique with which we had previously been able to show the presence of normal haemozoin², the *P* strain pigment dissolved out very readily during the standard staining procedures. The pattern of cross-resistance of our *P* strain is still being investigated. Preliminary studies show that the trophozoites are resistant to the dihydrotriazine metabolite of proguanil (as we had earlier observed when testing our 'triazine'-resistant *B* strain of *P. berghei*⁶). The *P* strain also exhibits resistance to pyrimethamine, while sensitivity to chloroquine and mepacrine so far is within normal limits. Details of these tests will be published elsewhere. The resistance to primaquine is unstable and some change towards normal sensitivity to this compound was noted in parasites passaged from an untreated 14-day-old infection with *P* passage 43 into clean untreated mice. During the course of the infection in the donor mice the pigment granules, as seen by light microscopy, appear to become darker and somewhat larger from about the fourteenth day of infection onwards.

M strain parasites show a similar level of resistance against mepacrine methanesulphonate. The *ED*₅₀ and *ED*₉₀ of the *N* strain for this compound are 15 and 26 mg/kg respectively. Parasites of *M* passage 32 continued to develop in mice treated with a subcutaneous dose of 50 mg/kg mepacrine methanesulphonate daily for 14 days from the day of infection, a total of 700 mg/kg. (At this point all the mice succumbed to overdosage of the drug.) Parasites of *M* passage 44 survived in several of a further group of mice given 30 mg/kg subcutaneously for 35 days or a total of 1,050 mg/kg. By light microscopy the *M* strain trophozoites and schizonts present a very similar appearance to that described for chloroquine-resistant *P. berghei*². No pigment grains are seen in parasites in mepacrine-treated mice. Granules (0.03-0.07 μ mean diameter) within the cytoplasm of the parasites can be demonstrated by electron microscopy. Normal pigment grains measure 0.1-0.3 μ (ref. 2). Multiple food vacuoles are also common and afford a further point of similarity between this and our *RC* strain. *M* strain parasites on preliminary testing have been found to be resistant also to chloroquine and quinine but sensitive to primaquine, pyrimethamine, 'triazine', sulphadiazine and diaminodiphenylsulphone. The resistance to mepacrine is extremely labile. Pigment reappeared by the end of 7 days in untreated mice infected with *M* passage 44. At the same time, the parasites reverted to a normal sensitivity to mepacrine.

In an earlier note¹ I suggested that my observations on pigment changes associated with the development by *P. berghei* of resistance to chloroquine could afford supportive evidence for the hypothesis of Schueler and Cantrell⁷ and Cohen, Phifer and Yielding⁴. I thank Prof. Yielding (personal communication) for pointing out an error in my terminology. All references to 'haematinic acid' should have read 'ferrihaemic acid'. Deegan and Maegraith in 1956 (ref. 9) suggested that malaria pigment consists of the porphyrin, haematin, combined with a nitrogenous moiety. In the preceding communication Sherman, Mudd and Trager¹⁰ now confirm that the

haemozoin of normal *P. berghei* is a heterogeneous mixture of porphyrin-peptide compounds derived from the degradation of the host erythrocyte haemoglobin at various points in its chain by proteolytic enzymes of the parasite. Whether a part of the haemoglobin is completely degraded in the process to yield a soluble porphyrin is not known but would not be beyond the realms of possibility. The work of Ball *et al.*¹¹ with *P. knowlesi* would, however, suggest that, at least in this species, "the parasite retains within its cell all of the heme that it splits off from hemoglobin". (These authors were, of course, of the then current opinion that the porphyrin and globin portions were completely split by the malaria parasite.) It is difficult to visualize, therefore, how such a mechanism as complexing of antimalarial drugs by ferrihaemic acid can account for the development of drug resistance by *P. berghei* although it may play a part once resistance has developed. If, then, free porphyrin is released by the parasite following the more complete digestion of haemoglobin in the food vacuoles that apparently occurs in our *RC* and *M* and Jacobs's⁴ quinino-resistant strains, it might conceivably inactivate some of the drug with which it is in contact in the artificially high concentration that is used in such experimental conditions in mice. Some other, so far undefined mechanisms must underlie the ability of the parasites to survive such a normally lethal environment. This order of drug concentration is certainly never likely to be encountered in the human subject with *P. falciparum* malaria.

Of immediate practical importance is the possibility that primaquine may prove to be relatively ineffective for the treatment of infection by other species of malaria parasites such as *P. falciparum* that are resistant to pyrimethamine, proguanil or its dihydrotriazine metabolite.

W. PETERS

Research Laboratories,
Pharmaceutical Division,
Ciba, Ltd., Basle,
Switzerland.

- ¹ Peters, W., *Nature*, **203**, 1290 (1964).
- ² Peters, W., Fletcher, K. A., and Stäubli, W., *Ann. Trop. Med. and Parasit.*, **59**, 126 (1965).
- ³ Thompson, P. E., Bayles, A., Olszewski, B., and Waitz, J. A., *Science*, **148**, 1240 (1965).
- ⁴ Jacobs, R. L., *J. Parasit.*, **51**, 481 (1965).
- ⁵ Peters, W., *Exp. Parasit.*, **17**, 80 (1965).
- ⁶ Peters, W., *Exp. Parasit.*, **17**, 90 (1965).
- ⁷ Schueler, F. W., and Cantrell, W. F., *J. Pharmacol. Exp. Therap.*, **143**, 278 (1964).
- ⁸ Cohen, S. N., Phifer, K. O., and Yielding, K. L., *Nature*, **202**, 805 (1964).
- ⁹ Deegan, T., and Macgrath, B. G., *Ann. Trop. Med. and Parasit.*, **50**, 194 (1956).
- ¹⁰ Sherman, I. W., Mudd, J. B., and Trager, W. (preceding communication).
- ¹¹ Ball, E. G., McKee, R. W., Anfinsen, C. B., Cruz, W. O., and Gelman, Q. M., *J. Biol. Chem.*, **175**, 547 (1948).

HAEMATOLOGY

[Haptoglobin Johnson in Australian Aborigines

THE rare haptoglobin phenotype, Johnson, discovered by Giblett¹ in a Negress and her daughter, has since been described in Chinese, Jewish and Caucasian populations². During the course of a population study of the blood groups and serum proteins of Australian Aborigines of Central Australia³, two sera taken from members of the Pintubi tribe were found to have this phenotype. Afterwards, samples from the family of one of these were obtained. While there is some White admixture among Central Australian Aborigines, the propositus himself is, so far as can be ascertained from his blood groups and general appearance, wholly Aboriginal.

The haptoglobin phenotypes of the members of the pedigree are indicated in Fig. 1. The starch-gel pattern designated H (for hypohaptoglobinaemia) was very similar to that of the serum of a member of the pedigree reported by Ramot *et al.*⁴ in which the parents were also Hp 2-1

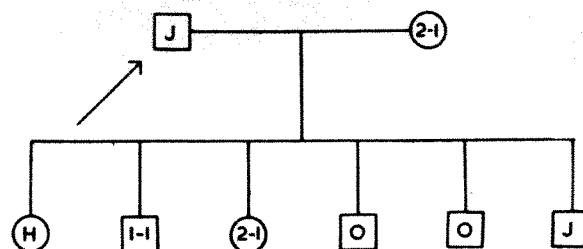


Fig. 1. Pedigree of an Australian Aboriginal family involving the Johnson haptoglobin type

and Johnson. Their pedigree and that reported here are in agreement with the previously proposed hypothesis⁵ that Johnson is genotypically $Hp^{2J}Hp^1$. Further, both pedigrees agree with the suggestion⁴ that the genotype $Hp^{2J}Hp^2$ is either hypohaptoglobinaemic or ahaptoglobinaemic.

We thank Dr. R. L. Kirk for confirming our Johnson typing and for the gift of a Johnson reference sample. We also thank Mr. H. C. Giese, director of Welfare, Northern Territory Administration, for his kindness in facilitating visits to Papunya, and to the Australian Institute of Aboriginal Studies for a grant in support of this investigation.

D. W. COOPER

Department of Genetics,
University of Adelaide,
Adelaide,
South Australia.

H. B. M. LEWIS*

Red Cross Blood Transfusion Service,
Adelaide,
South Australia.

E. M. NICHOLLS

Department of Human Genetics,
University of New South Wales,
Kensington,
New South Wales.

* Present address: Aberdeen and North-East of Scotland Blood Transfusion Service, Royal Infirmary, Aberdeen, Scotland.

¹ Giblett, E. R., cited by Smithies, O., and Connell, G. E., in *Biochemistry of Human Genetics* (Ciba Foundation Symp.), 178 (Churchill, London, 1959).

² Smithies, O., Connell, G. E., and Dixon, G. H., *Nature*, **196**, 232 (1962).

³ Nicholls, E. M., Lewis, H. B. M., Cooper, D. W., and Bennett, J. H., *Amer. J. Hum. Genet.*, **17**, 293 (1965).

⁴ Ramot, B., Kende, G., and Arnon, A., *Nature*, **196**, 176 (1962).

⁵ Smithies, O., cited by Giblett, E. R., *Vox Sang.*, **6**, 513 (1961).

Low Concentrations of Serum Haptoglobin in Mothers of Children with Haemolytic Disease of the Newborn

THE ante-partum prognosis of foetal haemolytic disease is very difficult. The presence and titre of antibodies discovered in pregnancy are not always comparable with the seriousness of the clinical manifestations in the newborn; in the case of an incompatibility in the ABO system not even this guide is available, so that for this purpose new methods are persistently being sought¹.

In foetal erythroblastosis the foetus is attacked by antibodies. The damage found after delivery varies in degree from anaemia² to hydropic foetus and, in the extreme, to stillbirth. The disintegration products of foetal erythrocytes are supposed to be transported out of the foetus by the placenta. The transmission of unconjugated bilirubin through the placenta was directly shown by Schenker *et al.*³ in the guinea-pig foetus. This is compatible with placental transmission of free haemoglobin molecules, which obviously represent the main fraction of the foetal extracorporeal haemoglobin in the absence of haptoglobin—traces of which are only seldom found in the cord blood by special methods⁴. The transmission of free foetal haemoglobin into the maternal

Table 1

		Controls				Affected mothers				
		(1) Post-partum mg% HbBC	(2) 6 days post-partum mg% HbBC	A HbBC mg%	A %		(1) Post-partum mg% HbBC	(2) 6 days post-partum mg% HbBC	A HbBC mg%	A %
Hp (1-1)	Rot	123	*							
Hp (2-1)	Spa	128	202	74	58	Klu	81	163	82	101
	Sic	87	208	121	139	Doč	134	185	51	38
	Voj	86	168	82	95	Jel	125	*		
	Sme	162	202	40	25	Zdr	152	217	65	43
	Ned	62	171	109	176	For	137	173	36	26
	Elb	156	215	59	38					
	Mean value	113			89		126			52
Hp (2-2)	Vlk	97	150	52	53	Val	34	*		
	Suc	94	200	107	114	Kar	63	167	104	165
	Boč	125	238	113	91	Mar	53	*		
	Sko	95	130	35	37	Tur	60	195	135	225
	Mean value	104			74		52			195
Mean value		109			+83		93			+100

Analysis of variance of the samples 1 of the Hp (2-2) group: $F = 12.35$ for $v_1 = 1$, $v_2 = 3$; $F_{0.05} = 10.12$.

* Not obtained.

circulation ought to be followed by a depression of the serum haptoglobin concentration according to Laurell and Nyman⁵, and as suggested by certain haemolytic disorders⁶.

The present experiments were designed to show whether there is a lower serum haptoglobin level in mothers whose children were to be treated by exchange transfusion, as compared with the mothers of healthy children. The blood samples were collected within a day post-partum. The second sample was drawn from each mother six days after delivery, that is, when the haptoglobin eliminated as a complex with haemoglobin would be fully replaced⁵. The haptoglobin concentration values obtained by three repeated determinations by the method of Owen, Better and Hoban⁷ are shown in Table 1 according to the haptoglobin serum groups identified by starch-gel electrophoresis.

Six days after parturition all the haptoglobin values are significantly elevated ($t = 9.73$ for $v = 15$) without significant difference between affected mothers and controls.

The mean haptoglobin values of the samples collected in the first 24 h post-partum are not significantly different in the unclassified populations, or in the groups Hp (2-1). Comparison of the haptoglobin concentration values of the group Hp (2-2) sera, however, shows a lower mean value in mothers whose children, suffering from a haemolytic disease caused by an incompatibility of the blood groups in the ABO or Rh systems, were treated by exchange transfusion. The statistical significance of the difference was confirmed by an analysis of variance.

With respect to the elevation of the haptoglobin concentration after delivery, previously observed by Lacomme⁸, the lower haptoglobin concentration values found immediately after delivery may be regarded as a persistence of the lower haptoglobin level in the prenatal period. The haptoglobin concentration values in the group Hp (2-1) cover a wide range. According to Nyman⁵, the dispersion is as great as that of the whole unclassified population. This is presumed to be the reason for the non-significant results (above) in the whole population and in the group Hp (2-1); the greater homogeneity on a lower level of haptoglobin concentration values of the sera of the Hp (2-2) group⁹ would be the reason why these showed a significant difference. The depression of the haptoglobin is evidently caused by the same factors as were noted in the experiment of Laurell and Nyman⁵ and in some haemolytic disorders⁶. Changes in the haptoglobin concentration may prove to be of greater diagnostic and prognostic value than the concentration of extracorporeal haemoglobin itself, which is permanently eliminated from the circulating blood in the form of a haptoglobin-haemoglobin complex, while the formation of haptoglobin is not accelerated by saturation of haptoglobin by haemoglobin only¹⁰.

The results described here serve as a starting point for a more detailed investigation of the individual haptoglobin concentration changes in the prenatal period.

JAN PINTERA

Transfusion Station,
Purkyně University,
Brno,
Czechoslovakia.

¹ Walker, W., *Abstr. Tenth Cong. Intern. Soc. Blood Transf.* (Stockholm, 1964).

² Mollison, P. L., and Cutbush, M., *Brit. Med. J.*, **1**, 123 (1949).

³ Schenker, S., Dawber, N. A., and Schmid, R., *J. Clin. Invest.*, **43**, 32 (1964).

⁴ Fine, J. M., Imperato, C., Battistini, A., and Moretti, J., *Nouv. Rev. fr. d'Hémat.*, **1**, 72 (1961).

⁵ Laurell, C. B., and Nyman, M., *Blood*, **12**, 493 (1957).

⁶ Allison, A. C., and Rees, W., *Brit. Med. J.*, **ii**, 1137 (1957).

⁷ Owen, J. A., Better, F. S., and Hoban, J., *J. Clin. Pathol.*, **13**, 163 (1960).

⁸ Nyman, M., *Scand. J. Clin. Lab. Invest.*, **39**, II Supp. (1959).

⁹ Nyman, M., *Clin. Chim. Acta*, **3**, 111 (1958).

¹⁰ Jayle, M. F., Moretti, J., and Mouray, H., *Nouv. Rev. fr. d'Hémat.*, **2**, 473 (1962).

HISTOCHEMISTRY

Cholinergic Excitation and Inhibition in the Cerebellar Cortex

The cholinergic character of mossy fibres and especially that of their endings in the parenchyma islets of the cerebellum has been repeatedly demonstrated both biochemically¹ and histochemically²⁻⁶. There is also evidence for the cholinergic nature of Golgi axons, synapsing with granule cell dendrites in the cerebellar glomeruli⁷, although it appears that at this site an adrenergic mechanism is involved as well. The experiments reported here were performed on the cerebella of guinea-pigs; they suggest that cholinergic mechanisms are involved both in the parallel axon-Purkinje dendritic spine synapses (regarded as excitatory junctions⁸) and in the basket axon-Purkinje cell-body synapses (supposed to be inhibitory in their character⁹).

The photomicrographs (Figs. 1, 2 and 3) were obtained from sagittal and frontal sections of the guinea-pig cerebellum, stained for acetylcholinesterase according to the Gerebtzoff modification of Koelle's thiocholine technique. It appears that the entire length of granule cell axons (both the ascending parts and the T-shaped bifurcated branches) displays acetylcholinesterase activity, most marked at the varicosities (beads) of these fibres, which are supposed to synapse with Purkinje and Golgi dendritic spines. Phase-contrast microscopy actually reveals the presence of cholinesterase-negative dendritic spines intruding the enzyme-active axons. On the other hand, there is a major concentration of enzymatically active nerve fibres at the bases of Purkinje cell bodies, eventually becoming arranged

as a cuff around the origin of the Purkinje axon. These structures are undoubtedly identical with the 'basket-formations' of the inner stellate (basket) cells. The acetylcholinesterase activity of the parenchyma islets (glomeruli) consisting of axonic endings of mossy fibres and Golgi cells, and of the short dendritic branches of granule cells, show an acetylcholinesterase activity similar to that already described in the rat, rabbit and cat. The climbing fibres, around the primary and secondary Purkinje dendrites, can also be identified as strongly acetylcholinesterase-active structures.

It appears, therefore, that in the guinea-pig, all synapses in the cerebellar cortex are operated by a mechanism involving acetylcholinesterase activity. It should be stated that we have found (unpublished) that at all these synapses the enzyme is located presynaptically, as are also the synaptic vesicles in these junctions. Assuming that the presence of synaptic vesicles and of acetylcholin-

esterase proves a cholinergic mechanism, it would appear that both the excitatory synapses (mossy fibre-granule cell; parallel axon-Purkinje dendrite; parallel axon-Golgi cell; parallel axon-basket cell; climbing fibre-Purkinje dendrite) and the inhibitory junctions (Golgi axon-granule cell; basket axon-Purkinje cell) are operated by the same chemical mediator substance. This, in turn, would indicate that—at least in the cerebellar cortex—it is not the chemical structure of the mediator substance, but rather the molecular structure of the underlying post-synaptic membrane that is responsible for the excitatory or inhibitory effect of the nerve impulse.

P. KÁSA
B. CSILLIK

Department of Anatomy,
University Medical School,
Szeged, Hungary,
and
Department of Anatomy,
Université de Liège,
Belgium.

¹ Hebb, K., *Int. Rev. Neurobiol.*, **1**, 163 (1959).

² Koelle, G. B., *J. Comp. Neurol.*, **100**, 211 (1954).

³ Gerebtzoff, M. A., *Cholinesterases* (Pergamon Press, New York, 1959).

⁴ Tewari, H. B., and Bourne, G. H., *Acta Anat.*, **51**, 349 (1962).

⁵ Csillik, B., Joó, F., and Kása, P., *J. Histochem. Cytochem.*, **11**, 113 (1963).

⁶ Kása, P., Joó, F., and Csillik, B., *J. Neurochemistry*, **12**, 31 (1965).

⁷ Kása, P., and Csillik, B., *Second Intern. Cong. Histochem. Cytochem.* (Frankfurt am Main, 1964).

⁸ Eccles, J. C., *The Physiology of Synapses* (Academic Press, New York, 1964).

PATHOLOGY

Immunological Tests in Helminthic Diseases

THERE is an increasing tendency to use immunological tests when considering helminthic diseases to avoid the difficulty inherent in the finding of the worm or its ova. Though such a procedure is obviously of value both in epidemiology and in clinical investigations, it seems necessary to sound a word of caution in interpretation.

It should not be necessary to point out that different parameters are being measured, and that, in fact, they are in some cases estimating factors in opposition. Whatever technique is being used to estimate the immune response the latter is likely to be paralleled to some extent, however minor, by a protective mechanism.

Theoretical considerations reveal some interesting phenomena which must be taken into consideration in survey work of any kind, and in the clinical interpretation of immunological results.

If a is the proportion of a population infected in unit time, and b that of individuals escaping, then the age-distribution of infections will be the expansion of the binomial $(a+b)^t$. Thus, the proportion of individuals infected at age t will be $1-b^t$, but of these a^t will have been infected t times, ta^{t-1} will have been infected $t-1$ times, and so on.

If we make the not unwarranted assumption that the acquisition of protection is some function of the number of infections and their duration, we can derive a series of curves reflecting the age distribution of apparent infection in a population. Fig. 1 shows the time-distribution curve of manifestation for an endemicity of $a = 0.20$ and for several values of w , which may be defined as the parasitoduration required to induce protection. The smaller the value of w , the greater the immune response, and where $w = \infty$ there is no development of immunity. Superimposed is the proportion of protected (immune) individuals.

The similarity of the 'manifest' curves to the age-distributions obtained for many parasites is immediately apparent; on the other hand, the proportion of immunes,

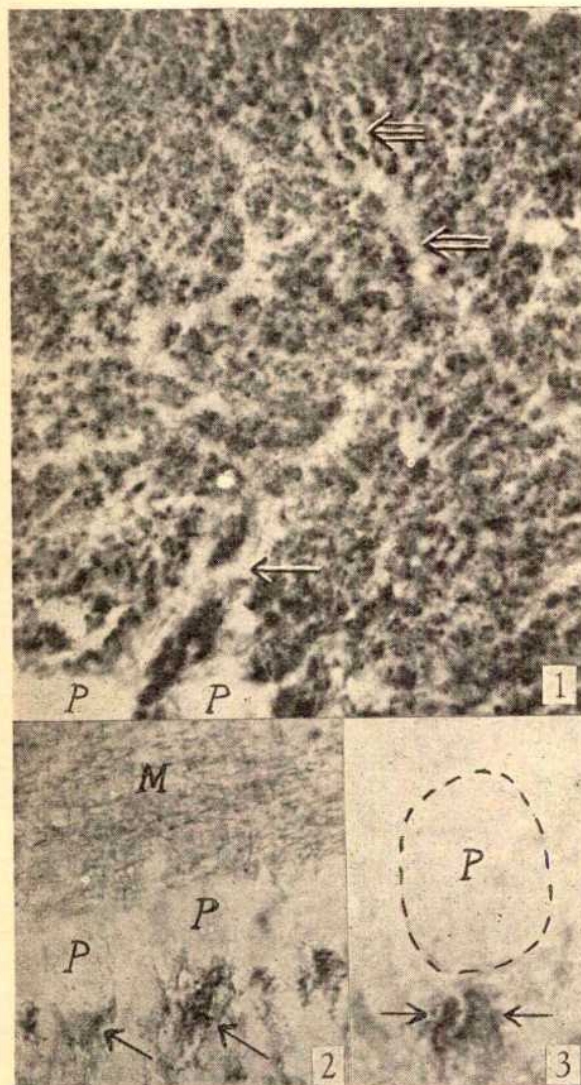


Fig. 1. Sagittal section of guinea-pig cerebellum. Acetylcholinesterase reaction, pH 5.8. Incubation, 2 h. Cell bodies and dendrites (arrows) of Purkinje cells (P) are unstained, whereas the vast amount of parallel fibres (cut transversely) react strongly. Double arrow, secondary dendrite; triple arrow, tertiary dendrite. Note the dendritic spines (non-reacting) of the tertiary dendrites, surrounded by heavily stained parallel fibre junctions.

Fig. 2. Coronal section of guinea-pig cerebellum; acetylcholinesterase reaction, pH 5.8. Incubation, 1 h. Note the strongly reacting basket cell endings (arrows) at the bases of the unstained Purkinje cells (P). In the molecular layer (M) parallel fibres are stained.

Fig. 3. As Fig. 2, but pH 5.2. Note the strong reaction of the basket-cell 'cuff' (arrows around the axon origin of the Purkinje cell, P).

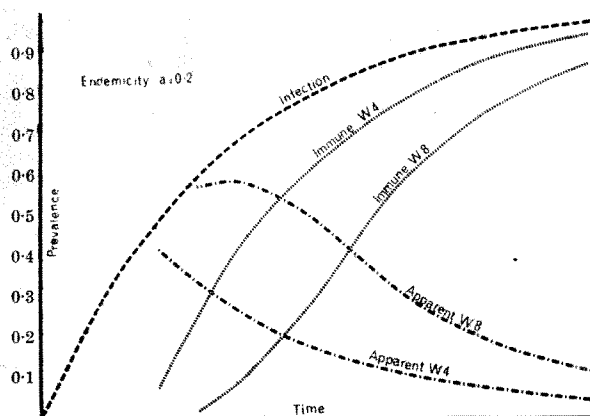


Fig. 1. Effect of differing immune pressure on manifestation

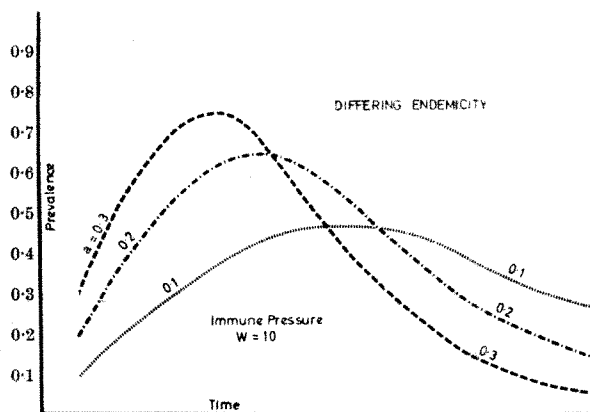


Fig. 2. Effect of differing endemicity on manifestation

presumably parallel to those detected by immunological tests, follows an entirely different curve, emphasizing the fact that not only are different parameters being measured, but also that they are, at greater values of t , operating in opposition.

Though this concept should be obvious, many investigators are assessing the value of this, that or the other immunological approach by comparison with the finding of ova or the like.

A further startling fact is shown by Fig. 2, which shows the curves, derived by applying different endemicities (values of a) to the same protection pressure. It will be seen that the higher the endemicity the lower the incidence at longer exposures. This merely re-emphasizes the caution required in interpretation of survey figures. If, for example, the presence or number of ova is being used as a parameter, then examination of too old an age-group would give an entirely fallacious estimate of endemicity. On the other hand, the presence of immune bodies might be a better measure.

To clinicians, this hypothesis is an additional warning to treat the parasite and not the immunological test.

These considerations do not take into account such phenomena as immune tolerance or antibody saturation, both of which may have to be considered in the interpretation of survey results.

I thank J. de V. Clarke, of the Bilharzia Research Laboratory at Salisbury, for the stimulus which provoked these thoughts.

R. ELSDON-DEW

Amoebiasis Research Unit*,
Institute for Parasitology,
Durban.

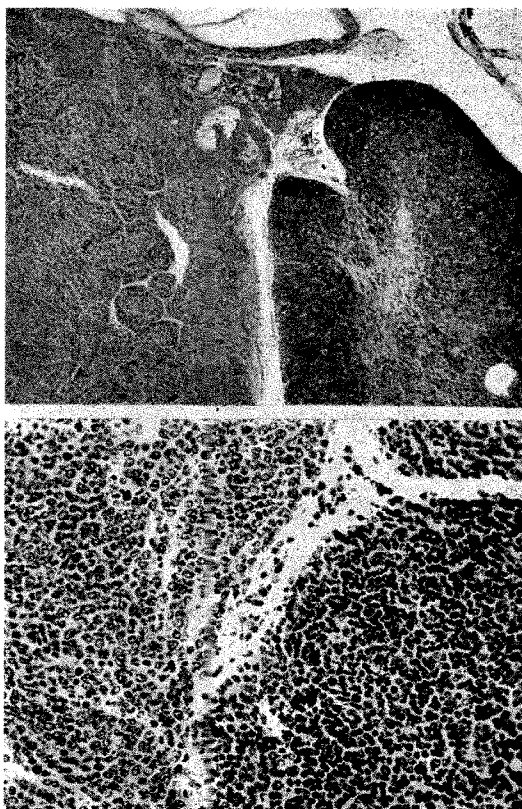
* The Amoebiasis Research Unit is sponsored by the following bodies: the South African Council for Scientific and Industrial Research, the University of Natal, the Natal Provincial Administration, and the U.S. Public Health Service (grant AI 01592).

Lymphatic Neoplastic Transformation of Thymus in Mice with Myeloid Virus-induced Leukaemia

In a recent study¹ it was found that in mice of certain strains inoculated with Graffi virus, removal of thymus had practically no effect in modifying the incidence of leukaemia while it favoured to a considerable extent the shift of neoplastic transformation from lymphoid to myeloid tissues. In addition, it was noted that in intact mice with generalized myeloid leukaemia the thymus appeared free from disease, or in some cases showed leukaemic changes of the lymphatic type. It was then considered of interest to study more systematically the morphology of thymic tissue in animals which developed myeloid leukaemia following inoculation of Graffi virus at birth.

C57BL mice received subcutaneously, within 48 h after birth, 0.05 ml. of cell-free extract containing Graffi virus, prepared as previously reported¹. They were observed for development of leukaemia and killed when moribund. Among the 61 leukaemic mice examined both histologically and haematologically, the type of leukaemia was lymphatic in 35 and myeloid in 26. Mice with myeloid leukaemia showed grossly in nearly all cases a characteristic green colour of the enlarged lymph nodes, while the thymus appeared pearly white and normal or slightly increased in size. The difference in colour was useful in outlining the contour of the whitish thymus surrounded by the green mediastinal lymph nodes.

Of the 26 mice with myeloid leukaemia five had atrophic thymus, composed of non-neoplastic lymphocytes. In the other 21 cases the thymus was involved by the leukaemic process, but the type of neoplastic cells replacing thymic tissue was clearly lymphoid. Thus, tumour tissues of both lymphoid and myeloid type were microscopically recognizable side by side, separated only by the thin capsules of mediastinal nodes and thymus (Figs. 1a and b).



Figs. 1a and b. Myeloid leukaemia with lymphatic neoplastic transformation of thymus. Medial lymph nodes on left, thymus on right. (Haematoxylin-eosin, a, $\times 60$; b, $\times 200$)

In mice with lymphatic leukaemia, thymus appeared markedly enlarged and showed neoplastic lymphoid changes indistinguishable from those observed in the other organs involved.

The autonomous neoplastic transformation of thymus in myeloid leukaemia induced by Graffi virus was also confirmed in another series of experiments to be reported in detail elsewhere. Virus-injected thymectomized ($C3Hf/Gs \times CBA/H-T6T6$) F_1 mice bearing subcutaneous thymus graft from normal new-born $C3Hf/Gs$ donors developed either lymphoid or myeloid leukaemias. In a small number of these animals with myeloid leukaemia, the grafted thymus exhibited neoplastic modification of the lymphoid type. The co-existence of two different neoplastic tissues was further proved by the successful transfer of lymphoid thymus and myeloid nodes from the same leukaemic animal to syngeneic mice, leading to local development of both lymphoid and myeloid growths.

Subsequent or simultaneous proliferation of two different types of haemopoietic tissues has also been described in other leukaemic murine diseases²⁻⁴. However, on the basis of these results, it would seem that the thymus has a peculiar cytological character such that leukaemic transformation within this organ is restricted to the lymphoid cell-type.

This work was supported by grant DRG-808 from the Damon Runyon Memorial Fund for Cancer Research, and by a grant from Consiglio Nazionale delle Ricerche, Roma. One of us (G. T.) also holds a fellowship from the Lady Tata Memorial Fund.

L. CHIECO-BIANCHI
L. FIORE-DONATI
G. TRIDENTE
N. PENNELLI

Istituto di Anatomia Patologica,
Divisione di Cancerologia Sperimentale,
Università di Padova, Italy.

¹ Fiore-Donati, L., and Chieco-Bianchi, L., *J. Nat. Cancer Inst.*, **32**, 1083 (1964).

² Metcalf, D., Furth, J., and Buffett, R. F., *Cancer Res.*, **19**, 52 (1959).

³ Rauscher, F. J., *J. Nat. Cancer Inst.*, **29**, 515 (1962).

⁴ Stansly, P. G., and Soule, H. D., *J. Nat. Cancer Inst.*, **29**, 1083 (1962).

IMMUNOLOGY

Histocompatibility Antigens in Transplanted Mouse Eggs

THE hypotheses advanced to account for the success of the foetus as an intra-uterine homograft in pregnancy have recently been reviewed by Billingham¹. Three possible mechanisms have been suggested to explain the apparent lack of immunological reaction by the pregnant mother against the genetically foreign offspring with which she is in intimate contact²: (a) the antigenic immaturity of the foetus; (b) the immunological inertness of the mother; and (c) some form of anatomical separation of the foetus from the mother. It has, however, been shown that transplantation antigens appear early in embryonic life and that the mother, though pregnant, is fully capable of reacting against these antigens³⁻⁵. In addition, it has been shown that the uterus is not a specially privileged recipient site for prolonged homograft survival^{6,7}.

We have previously suggested that the trophoblast may be an immunologically privileged anatomical barrier between mother and foetus, ensuring foetal survival^{8,9}. Fertilized hybrid mouse ova were transplanted heterotopically beneath the renal capsule of recipients of the maternal strain. In this site, the ova developed into agglomerates of trophoblastic giant cells which did not provoke demonstrable specific sensitivity to subsequent paternal skin grafts. In addition, trophoblastic proliferation did not seem to be inhibited even when the recipients had previously been immunized by paternal skin grafts.

The present experiments were designed to elucidate further the previously postulated deficiency in effective histocompatibility antigens in mouse trophoblast. Ova, derived from inbred matings, homozygous with respect to histocompatibility antigens, were transplanted into highly immunized recipients.

The mice used were of the inbred strains, $C3H/HeJ$ and $C57BL/6$, obtained from the Jackson Memorial Laboratories, Bar Harbor, Maine. Adult male $C57$ mice were divided into three experimental groups of recipients. Group I received no treatment prior to ova transplantation; the animals in Group II were grafted with full-thickness $C3H$ skin grafts by the standard method of Billingham and Medawar¹⁰; transplants of ova were carried out one to two months after these skin grafts. The animals in Group III received two consecutive full-thickness $C3H$ skin grafts, with an interval of three weeks between grafts; in addition, these animals received 8-12 weekly intraperitoneal injections of 25-30 million dissociated spleen cells. Ova transplants were performed less than one week following the last injection. A fourth group of syngeneic recipients consisted of $C3H$ adult male mice.

Fertilized $C3H$ ova were expressed from the oviduct 1½-2½ days post-coitus as previously described⁸. Two ova were individually placed beneath the capsule of each recipient $C57$ kidney through a finely drawn Pasteur pipette. Both kidneys were routinely used, each recipient thus receiving four ova. Ten days after transplantation, the animals were bled, and the serum was collected and frozen. Haemagglutinin titres were later carried out by the method of Stimpfling¹¹. All animals were then killed, and the kidneys were inspected for haemorrhagic tumours characteristic of trophoblastic proliferation. All suspect areas were removed for individual histological study; the tissues were fixed in formalin, embedded in paraffin and sectioned at 7µ. Haematoxylin and eosin staining was normally performed.

The results are summarized in Table 1. Trophoblastic tumours were found in most (80 per cent) of the previously untreated $C57$ recipients of $C3H$ ova as well as in 84 per cent of the syngeneic recipients. Histologically, these haemorrhagic tumours consisted of abnormally large trophoblastic giant cells with vesicular nuclei. There was a marked haemorrhagic reaction, host blood filling the intercellular spaces of the cellular network.

Table 1. TROPHOBLASTIC PROLIFERATION FROM FERTILIZED $C3H$ MOUSE OVA IN IMMUNIZED AND NON-IMMUNIZED MALE MICE

Recipient animal	Immunization	No. recipient animals	No. animals with trophoblast	Titre anti- $C3H$ haemagglutination
$C3H$ male	None	19	16	<1/20
$C57$ male	None	20	16	<1/20
$C57$ male	One $C3H$ skin graft	19	5	<1/20
$C57$ male	Two $C3H$ skin grafts and 8-12 $C3H$ spleen cell injections	16	0	1/80-1/640

Only 25 per cent of the $C57$ male mice which had previously rejected one $C3H$ skin graft supported $C3H$ trophoblastic proliferation under the kidney capsule. Histologically, these growths did not differ significantly from those grown in unsensitized recipients. A characteristic inflammatory response involving mononuclear cells was not consistently present. There was no detectable haemagglutinating activity in the sera of untreated and singly skin-grafted recipients.

No evidence of trophoblastic proliferation could be found ten days after $C3H$ ova implantation into those $C57$ recipients hyperimmunized by $C3H$ skin grafts and multiple $C3H$ spleen cell injections. In these, haemagglutinin titres ranged from 1:80 to 1:640.

Several investigators have shown that the ectopic implantation of fertilized mouse ova results in the proliferation of trophoblastic tumours even in xenogeneic combinations¹²⁻¹⁴. We have previously demonstrated

that proliferation of hybrid (*CBA* × *A*) mouse trophoblast is possible in mice of the maternal strain (*CBA*) which have previously rejected paternal (*A*) skin grafts. In order to determine if this failure of antigenic expression was due to an absolute deficit of antigens or to some other characteristic of trophoblastic growth, the present experiments utilized inbred ova homozygous with respect to histocompatibility determinants. In addition, implants were made into recipients hyperimmunized against the histocompatibility antigens of the parental donor with resulting high titres of haemagglutinin activity. A definite inhibition of trophoblastic growth was observed and was proportional to the degree of pre-existing immunity. It is therefore apparent that histocompatibility antigens are present in trophoblast or in the pre-trophoblastic tissues of ectopically implanted mouse ova.

Quantitation of the number of antigenic determinants per cell, or per square unit of cell surface, is, of course, beyond the reach of the present techniques. Nevertheless, the likelihood of a relative deficiency in the expression of histocompatibility antigens in trophoblast, as compared with other tissues, still appears to be substantial from the present extension of our previous experiments. The fact that homozygous donor cells were used in the more recent experiments makes it possible that their more obvious susceptibility to appropriate prior sensitization may reflect a gene-dose effect.

This work was supported by research grant CA-07466-01, awarded by the National Institutes of Health.

RICHARD L. SIMMONS
PAUL S. RUSSELL*

Department of Surgery,
Columbia University College of Physicians and Surgeons,
New York.

* Present address: Massachusetts General Hospital, Boston.

¹ Billingham, R. E., *New Eng. J. Med.*, **270**, 667 and 720 (1963).

² Medawar, P. B., *Evolution*, **7**, 320 (1953).

³ Woodruff, M. F. A., *Proc. Roy. Soc., B*, **143**, 68 (1957).

⁴ Terasaki, P. I., *J. Embryol. Exp. Morphol.*, **7**, 409 (1959).

⁵ Haskova, V., in *Biological Problems of Grafting*, 95, edit. by Albert, F., and Medawar, P. B. (Blackwell Scientific Publications, Oxford, 1959).

⁶ Schlesinger, M., *J. Nat. Cancer Inst.*, **23**, 927 (1962).

⁷ Poppa, G., Simmons, R. L., David, D. S., and Russell, P. S., *Transplantation*, **2**, 496 (1964).

⁸ Simmons, R. L., and Russell, P. S., *Ann. N.Y. Acad. Sci.*, **99**, 717 (1962).

⁹ Simmons, R. L., and Russell, P. S., *Amer. J. Obstet. Gynec.*, **85**, 583 (1963).

¹⁰ Billingham, R. E., and Medawar, P. B., *Brit. J. Exp. Biol.*, **28**, 385 (1951).

¹¹ Stimpfling, J. H., *Transpl. Bull.*, **27**, 109 (1961).

¹² Simmons, R. L., and Russell, P. S., *Transplantation*, **2**, 551 (1964).

¹³ Kirby, D. R. S., *J. Embryol. Exp. Morph.*, **10**, 496 (1962).

¹⁴ Kirby, D. R. S., *Nature*, **194**, 785 (1962).

RADIOBIOLOGY

Tests on *Drosophila* for the Production of Mutations by Irradiated Medium or Irradiated DNA

(1) *Irradiated medium.* Ionizing radiations are likely to find increasing use in industry for prolonging the useful storage-life of food. Before the method is declared safe and becomes widely acceptable, however, it is necessary to ensure that consumption of irradiated food is free from genetic ill-effects. Mutation rates have been shown to increase when bacteria are grown in ultra-violet-irradiated substrate¹. Bacterial medium irradiated with ionizing radiations, however, has not been tested for its mutagenic effects. Information about the indirect genetic effects of ionizing radiations in higher organisms is scanty. Investigations of the cytological effects in root meristems of plants grown in X- and γ -ray irradiated potato mash² and fruit juices³ have shown that the aberration frequencies in treated roots were strikingly higher than in corresponding controls. In order to test for similar effects in animals, these studies were extended to *Drosophila*. It was found that the rate of sex-linked lethals was increased

slightly, but significantly, in the progeny of flies raised on irradiated food⁴. In view of the obvious importance of this observation it was thought necessary to re-test it, scoring both autosomal and sex-linked lethals. The former were included because it has been shown^{5,6} that feeding of unirradiated calf thymus DNA has a very specific and pronounced mutagenic effect on chromosome II of *Drosophila*, while the X-chromosome remains largely refractory to this treatment. Induced dominant lethality was also investigated in view of the chromosome breakage observed in plant material grown on irradiated medium. The results of these investigations are reported in this communication.

Food consisting of 10 per cent dried killed yeast, 10 per cent sucrose and 3 per cent agar in water was irradiated by the Radiation Research Laboratory, Wantage. The total dose of 1 mrad of γ -rays was delivered in a single exposure lasting 70 min from a 14,000-c. annular cobalt-60 source. The Oregon-K (Or-K) stock for these experiments has been used in this laboratory for mutation experiments for a long time and has shown a spontaneous rate of about 0.3 per cent sex-linked lethals in periodic checks. In the first experiment 24- and 48-h-old larvae were transferred to the irradiated food about 36 h after the termination of radiation exposure, and the frequency of sex-linked and second chromosome (autosomal) lethals was determined simultaneously in both treated males and females. In order to keep the experiment manageable and because of the known spontaneous mutation rate of the stock, controls were not included in this experiment. In a second experiment the usual maize meal-molasses food was used. Young Or-K females, mated 24 h earlier, were transferred to the irradiated food for egg laying immediately on termination of the irradiation exposure, so that the developing flies should be exposed to the full effect of the treatment including that due to any transient radicals. Males which had developed on irradiated food were tested for sex-linked lethals. Comparable controls were included in this experiment. Scoring was done by the usual techniques, namely, hatchability for dominant lethals, Muller-5 test for sex-linked lethals and *Cy/L* test for second chromosome lethals.

The results are summarized in Table 1. Since no difference was found between 24- and 48-h old larval transfers, data from these two sets of the first experiment have been pooled. It will be seen that the values for sex-linked lethals in experiment 1 are within the range usually found for the stock used. Those for autosomal lethals are twice as frequent, and this is as expected from the length relationship between the X- and the second chromosome. No difference apart from that inherent in the difference of sex⁷ was observed in tests on treated males and females, thus ruling out any influence of germinal selection against sex-linked lethals in the hemizygous males. Tests for dominant lethality, replicated twice, likewise gave entirely negative results, the mean percentage of hatchability in control and treated series being 87.7 and 87.2 respectively. In the second experiment a higher mutation rate might have been expected because of the action of transient radicals which would have been missed in the first experiment. Actually, mutation frequency was even lower here than in experiment 1 and not higher than in the controls.

These experiments, therefore, do not provide any evidence for a mutagenic substance being produced in irradiated food. Similar negative results have been

Table 1. FREQUENCY OF LETHALS IN *Drosophila melanogaster* RAISED ON IRRADIATED OR UNIRRADIATED FOOD

Experiment No.	Sex of the treated flies	Chromosome	No. of chromosomes tested	Lethals (%)
1	Males	X	1,653	0.256
		II	1,438	0.556
	Females	X	1,458	0.137
		II	1,378	0.290
2	Males	X (treated)	2,044	0.097
		X (control)	1,328	0.150

obtained by Moutschen (personal communication). On the other hand, Swaminathan *et al.*⁴ and more recently Rinehart (personal communication) have obtained small, but statistically significant, increases of mutation rates in *Drosophila* raised on irradiated food, indicating that in certain circumstances marginal effects in mutation rate may be produced. In contrast to this, the previously mentioned experiments on plant chromosomes had shown a striking increase in the frequency of chromosome breaks in root meristems of wheat and barley grown on irradiated water⁵, potato mash² and fruit juices³. The difference between the striking cytological effects in plants and the marginal effects in *Drosophila* may be due to some basic differences in the two systems. Obvious among these are: (1) In plants the treatment is applied directly to the tissue in which the effects are looked for, so that the cells can take up the reactive principle fairly quickly and without its undergoing metabolic changes. In feeding experiments with *Drosophila*, on the other hand, the irradiated food has to undergo a series of metabolic changes in the digestive system of the fly. The radiomimetic principle produced by irradiation will thus reach the gonads in a greatly modified form which may be mutagenically ineffective. (2) While chromosome breaks in plants are scored in cell cycles immediately following the treatment, *Drosophila* germ cells are sampled many cell generations after treatment. Also, aberrations in treatments with fruit juices consisted entirely of chromosome breaks and no evidence for translocations indicating rejoining was obtained. Such chromosome breaks, if induced in *Drosophila*, can produce dominant lethals only, and these, if produced in pre-meiotic cells, are likely to be eliminated during meiosis. In order to create experimental conditions for *Drosophila* that are more similar to those used in plants, one might determine dominant lethals in mature sperms of adult *Drosophila* males that have had irradiated glucose or fruit juices administered to them by drinking or injection. Experiments on these lines have now been started.

(2) *Irradiated DNA*. While these experiments on the effect of irradiated food were in progress, Parkash published results of an experiment in which 1.8 per cent of irradiated fish sperm DNA had been mixed into the food of *Drosophila* larva. The emerging males had 36 lethals in 635 tested X-chromosomes, a frequency of 5.7 per cent⁶. In a second experiment¹⁰, 9.6 per cent autosomal lethals were obtained, but there was no control and it is not clear whether pre-existing lethals had been excluded. This seemed such a striking and important observation that I repeated the experiment, using herring sperm DNA but otherwise the same procedure as Parkash. The irradiation with 100,000 r. of γ -rays was performed by the Radiation Research Laboratory, Wantage (exposure time 31 min). The irradiated DNA was mixed with *Drosophila* food, and Oregon-K flies were allowed to feed and lay eggs on this food. Food mixed with unirradiated DNA served as control. Males that had developed on DNA-treated food were tested for sex-linked recessive lethals according to the Muller-5 method. The experiment was repeated thrice with negative results. In a total of 5,705 control and 5,762 treated chromosomes the percentage incidence of sex-linked lethals was 0.29 and 0.35 respectively. Dr. Parkash (personal communication to Dr. C. Auerbach) suggested that the discrepancy between our results might be due to the difference in medium used. Because of a possible competition between the irradiated DNA and the nucleic acids of yeast, he had prepared his medium entirely without yeast, according to the following formula: sugar, 7 g; bran, 7 g; maize meal, 6 g; and agar, 0.8 g; cooked in 100 ml. water. Another experiment was carried out, using the aforementioned formula. The rest of the experimental procedure was the same as described earlier. The development of the flies on this food was considerably delayed, first emergence taking 14 days as against 9–10 days at 25° C on the food used in this laboratory. In

1,089 chromosomes from 91 males developing on food mixed with unirradiated DNA and 2,220 chromosomes from 167 males developing on food containing irradiated DNA, the percentage of sex-linked lethals was 0.18 and 0.22 respectively. My experiments therefore give no support to the conclusion that, in general, irradiated DNA is mutagenic for *Drosophila*. It is difficult to visualize which special conditions led to the positive results in the experiment carried out by Dr. Parkash.

I thank Dr. C. Auerbach for her advice. This work was undertaken during the tenure of a Commonwealth scholarship.

V. L. CHOPRA

Institute of Animal Genetics,
Edinburgh.

¹ Stone, W. S., Wyss, O., and Haas, F., *Proc. U.S. Nat. Acad. Sci.*, **33**, 59 (1947).

² Swaminathan, M. S., Chopra, V. L., and Bhaskaran, S., *Rad. Res.*, **16**, 182 (1962).

³ Chopra, V. L., Natarajan, A. T., and Swaminathan, M. S., *Rad. Bot.*, **3**, 1 (1963).

⁴ Swaminathan, M. S., *et al.*, *Science*, **141**, 637 (1963).

⁵ Gershenson, S., *C.R. (Dokl.) Acad. U.R.S.S.*, **26**, 601 (1940).

⁶ Mathew, C., *Genet. Res.*, **6**, 163 (1965).

⁷ Auerbach, C., *J. Genet.*, **41**, 255 (1941).

⁸ Natarajan, A. T., and Swaminathan, M. S., *Indian J. Genet.*, **18**, 220 (1958).

⁹ Parkash, O., *Nature*, **205**, 312 (1965).

¹⁰ Parkash, O., *Naturwiss.*, **52**, 142 (1965).

Mutagenic Effect of Irradiated and Unirradiated DNA in *Drosophila*

THE treatment of food with ionizing radiation is known to be an effective means of prolonging the useful storage-life of food. However, in view of the possible health hazards which might be involved in the treatment of food with ionizing radiation, a working party, appointed by the Ministry of Health, has evaluated the possible dangers, and the controls required, in the use of ionizing radiation for this purpose¹.

One consideration of importance is the possible production of chemical mutagens by the action of the ionizing radiation on the food material¹. The available evidence for a mutagenic effect of irradiated food on animal tissues is based on experiments where *Drosophila* larvae have been cultured on a medium which has previously been exposed to a heavy dose of ionizing radiation. Although it has been reported² that there is a slight, but significant, increase in the rate of sex-linked recessive lethals in *Drosophila* larvae reared on an irradiated medium, other workers³ have not been able to detect an increase in mutation under similar conditions. However, it has been claimed in recent communications by Parkash^{4,5} that the irradiation of DNA (a component of most foodstuffs) prior to its addition to a *Drosophila* culture medium produces a pronounced increase in mutation in the larvae compared with the unirradiated (and non-mutagenic) DNA sample. This observation, and its relation to the assessment of the hazards involved in the sterilization of food by ionizing radiation, obviously requires an independent verification of this effect.

The present communication describes experiments designed to detect a mutagenic effect of DNA toward *Drosophila* larvae after the DNA had been exposed to a heavy dose of ionizing radiation prior to its addition to a chemically defined and axenic *Drosophila* medium. Although unirradiated DNA is found to be mutagenic under these conditions, the prior irradiation of the DNA does not change the mutagenic effectiveness of the DNA compared with the unirradiated DNA sample.

Irradiation of calf-thymus DNA (British Drug Houses, Ltd.), as a dry powder, was by γ -irradiation (100,000 r.) delivered in a single exposure lasting 6 min 12 sec from a 40,000-c. cobalt-60 source at the Molteno Institute, Cambridge. Both the unirradiated and the irradiated DNA's were added as powders to the chemically defined medium (see Table 1 for the composition) when the temper-

ature of the medium was 55° C. The media were shaken until homogeneous, and dispensed as 25-ml. portions into bottles; cultures which showed signs of microbial contamination over the period of treatment were discarded.

Newly emerged Oregon-K flies were fed for 3 days on a generous supply of yeast, and allowed to lay for 3 h on to a roughened agar surface. The collected eggs were then sterilized using Sang's method⁶, and spread evenly over a sterile agar surface. After incubation at 25° C for 20 h, the newly hatched larvae were transferred under aseptic conditions on to the freshly prepared treatment media, to give a larval density of 100 larvae per 25 ml. of medium. The cultures were then placed at 25° C until the adults emerged.

On emergence, males were individually mated to two virgin Muller-5 females for 3 days for the detection of sex-linked recessive lethal mutations⁷; and, for the detection of autosomal recessive lethal mutations⁷, to females from a stock carrying one second chromosome with the dominant markers, Curly wing and Lobe eye, and the other second chromosome with the dominant marker, Plum eye.

Table 2 illustrates the results obtained when larvae are cultured in a medium containing either 5 per cent unirradiated DNA, or 5 per cent irradiated DNA. Survival was high on each treatment medium, and the developmental rates were essentially the same as the control (no DNA). Both DNA experiments show an increase in the frequency of sex-linked recessive lethal mutation compared with the control (the spontaneous mutation rate of the Oregon-K stock is usually in the range 0.1–0.3 per cent sex-linked recessive lethal mutations). Although DNA appears to be significantly mutagenic under these conditions, there is no significant difference between the irradiated and unirradiated DNA treatments.

Table 3 illustrates the autosomal recessive lethal mutation rates obtained from further experiments, where larvae were cultured on media containing either 5 per cent unirradiated DNA or 5 per cent irradiated DNA. A control (no DNA) is essential when scoring autosomal recessive lethals, since pre-existing lethals may be present in either sex, and care has to be taken not to score them as lethals induced by the treatment. Also, since larval treatments affect early stages of germ cell development (primary spermatocytes, and spermatogonia), tests of allelism between lethals arising from the same male are necessary because of the clonal bunching of mutations from these germ cell stages.

Table 1. COMPOSITION OF THE CHEMICALLY DEFINED *Drosophila* MEDIUM

Compound	Amount (g)
Casein ('Genatosan-low vitamin')	5.00
Fructose	0.75
Cholesterol	0.03
Lecithin	0.04
Agar ('Oxoid No. 3')	3.00
NaHCO ₃	0.14
KH ₂ PO ₄	0.183
Na ₂ HPO ₄	0.189
Aneurine	0.0002
Riboflavin	0.0010
Nicotinic acid	0.0012
Calcium pantothenate	0.0016
Pyridoxine	0.00025
Biotin	0.000016
Folic acid	0.001
Water to 100 ml.	

Table 2. SEX-LINKED RECESSIVE LETHAL MUTATION FREQUENCIES IN *Drosophila* MALES AFTER LARVAL FEEDING TREATMENTS WITH EITHER UNIRRADIATED DNA (5 PER CENT), OR WITH IRRADIATED DNA (5 PER CENT) IN A CHEMICALLY DEFINED AND ASEPTIC MEDIUM

	Control (no DNA)	Unirradiated DNA	Irradiated DNA
Duration of treatment	Entire larval life	Entire larval life	Entire larval life
Survivors (per cent)	98	94	93
No. males examined	46	47	48
Completely sterile <i>F</i> ₁ cultures (per cent)	2	8	4
No. chromosomes examined	440	403	480
No. lethal chromosomes	0	3	3
Lethal frequency (per cent)	0.0	0.7	0.6

Table 3. AUTOSOMAL RECESSIVE LETHAL FREQUENCIES (*F*₁ COMPLETE LETHALS AND *F*₁ LETHAL-MOSAICS) IN *Drosophila* MALES AFTER LARVAL FEEDING TREATMENTS WITH EITHER UNIRRADIATED DNA (5 PER CENT) OR WITH IRRADIATED DNA (5 PER CENT), IN A CHEMICALLY DEFINED AND ASEPTIC MEDIUM

	Control (no DNA)	Unirradiated DNA	Irradiated DNA
Duration of treatment	Entire larval life	Entire larval life	Entire larval life
Survivors (per cent)	97	90	89
No. males examined	46	51	63
Completely sterile <i>F</i> ₁ cultures (per cent)	2	15	13.7
No. chromosomes examined	560	376	644
No. lethal chromosomes	2	4	9
Lethal frequency (per cent) (<i>F</i> ₁ complete lethals)	0.36	1.10	1.40
No. non-lethal <i>F</i> ₁ cultures examined	47 arising from 47 males	42 arising from 42 males	41 arising from 41 males
Average No. of females examined per culture	10	10	10
No. non-lethal <i>F</i> ₁ cultures yielding at least one lethal in the <i>F</i> ₂	2	6	5
Cultures showing mosaicism (per cent) (<i>F</i> ₁ lethal-mosaics)	4.2	14.3	12.1
Total No. <i>F</i> ₂ females examined	490	432	410
No. lethal-bearing <i>F</i> ₂ females	2	6	5
Lethals in <i>F</i> ₂ (per cent)	0.41	1.39	1.22
Total lethal frequency (per cent) (<i>F</i> ₁ complete lethals + <i>F</i> ₁ lethal-mosaics)	4.56	15.40	13.50

Both DNA experiments show a significant increase in *F*₁ complete lethals (detected in the *F*₂ generation) compared with the control, but again there is no significant difference between the mutation rates in the unirradiated and the irradiated DNA experiments. Several bunches of identical (allelic) lethals were detected in the progeny of individual males in both DNA experiments, and each bunch was scored as a single lethal.

The standard autosomal recessive lethal test in these experiments has been extended to an *F*₂ generation by sampling one non-lethal *F*₁ culture arising from each *P*₁ male. This procedure detects those lethals which are not completely established throughout the gonad of the *F*₁ male or female (and detected as *F*₁ complete lethals), but are lethal mutations which occur after a delay, and are established in only a fraction of the gonad of the *F*₁ male or female (*F*₁ lethal-mosaics). The genetical basis of sex-linked recessive lethal-mosaicism is explained in ref. 8; it should be noted, however, that although *F*₁ sex-linked recessive lethal-mosaicism is detected in the *F*₂ generation⁸, *F*₁ autosomal recessive lethal-mosaicism is detected in the *F*₂ generation. Both the irradiated and unirradiated DNA experiments show a significant increase in *F*₁ autosomal recessive lethal-mosaicism compared with the control.

Although the overall autosomal recessive lethal frequencies (*F*₁ complete lethals + *F*₁ lethal-mosaics) for both the unirradiated and irradiated DNA experiments show a significant increase in mutation compared with the control, the mutation rates of the unirradiated DNA and the irradiated DNA experiments are not significantly different from one another.

Unirradiated DNA. The results reported here with unirradiated DNA allow interesting comparisons to be made with the observations of other workers. Thus, Gershenson⁹ has claimed that when *Drosophila* larvae are reared on a medium containing calf-thymus DNA (10 per cent, and later 13 per cent), various types of heritable visible mutations are produced, but no sex-linked recessive lethal mutations. Other workers^{10,11} were also unable to detect an increase in sex-linked recessive lethals after feeding calf-thymus DNA to *Drosophila* larvae, although some phenotypic abnormalities were observed.

Renewed interest in the mutagenic effect of DNA on *Drosophila* larvae arose when it was later claimed¹² that, although DNA does not produce sex-linked recessive mutations, various visible mutations are produced, and these are found to be located on the second chromosome. This observation suggested that DNA, administered by

larval feeding, might have a preferential effect on the second chromosome. This assumption received further general support from experiments in which DNA was injected into adult *Drosophila* males^{13,14}, where it was found to produce autosomal *Minutes*, but no sex-linked recessive lethal mutations.

On the basis of these observations, Mathew¹⁵ has studied the effect of DNA on *Drosophila* larvae reared on a yeast-sugar medium supplemented with 10 per cent (and later with 13 per cent) calf-thymus DNA. In keeping with the findings of previous workers, he found that the sex-chromosome was refractory to the treatment. However, he did observe a pronounced mutagenic effect on the second chromosome, both in the induction of F_1 complete recessive lethals and in F_1 recessive lethal-mosaics¹⁵.

The experiments reported here (Tables 2 and 3) support Mathew's observations that calf-thymus DNA is mutagenic towards the second chromosome of *Drosophila* larvae, in the production of both complete and delayed recessive lethal mutations. However, our experiments show that the sex-chromosome is not refractory to the mutagenic activity of calf-thymus DNA; indeed, it appears to be equally as sensitive as the second chromosome. (Since the second chromosome has approximately twice the genetic material as the sex-chromosome, the spontaneous and induced mutation rates on the second chromosome are expected to show about twice the rate of the sex-chromosome.)

To explain the variance of our DNA-induced sex-linked recessive lethal mutation rates with those of other workers, we might suggest that our more refined culturing technique allows the detection of the slight mutagenic effect which is produced on the sex-chromosome.

Irradiated DNA. Tables 2 and 3 show that the irradiation of calf-thymus DNA, prior to its addition to the larval treatment medium, does not produce a significant increase in mutation over that of the unirradiated DNA. These experiments thus lend no support to Parkash's observations^{4,5} that irradiated DNA produces a significant increase in mutation when fed to *Drosophila melanogaster* larvae.

We thank Prof. J. M. Thoday for supplying the laboratory facilities, and the Pakistan Atomic Energy Commission (A. H. K.), the British Council (A. H. K.) and the British Empire Cancer Campaign for Research (T. A.) for financial support.

A. H. KHAN
T. ALDERSON

Department of Genetics,
University of Cambridge.

¹ Rep. Working Party on Irradiation of Food (H.M.S.O., 1964).

² Swaminathan, M. S., Nirula, S., Natarajan, A. T., and Sharma, R. P., *Science*, **141**, 637 (1963).

³ Chopra, V. L. (preceding communication).

⁴ Parkash, O., *Nature*, **205**, 312 (1965).

⁵ Parkash, O., *Naturwiss.*, **52**, 142 (1965).

⁶ Sang, J. H., *J. Exp. Biol.*, **33**, 45 (1956).

⁷ Auerbach, C., *Mutation*, Part I, *Methods* (Oliver and Boyd, Edinburgh and London, 1962).

⁸ Alderson, T., *Nature*, **207**, 164 (1965).

⁹ Gershenson, S., *Visti Acad. Nauk. U.R.S.S.*, **9/10**, 83 (1939).

¹⁰ Rapoport, J. A., *C.R. (Dokl.) Acad. Sci. U.R.S.S.*, **27**, 1033 (1940).

¹¹ Muller, H. J., *Cold Spring Harb. Symp. Quant. Biol.*, **9**, 151 (1941).

¹² Gershenson, S., and Kiselyeva, I. A., *C.R. (Dokl.) Acad. Sci. U.R.S.S.*, **123** (3), 554 (1958).

¹³ Fahmy, O. G., and Fahmy, M. J., *Nature*, **191**, 776 (1961).

¹⁴ Fahmy, O. G., and Fahmy, M. J., *Nature*, **196**, 873 (1962).

¹⁵ Mathew, C. Ph.D. thesis (Edinburgh Univ.), and *Genet. Res.*, **6**, 163 (1965).

Lack of Mutagenic Effect of Irradiated *Drosophila* Medium

THE possibility that irradiated food may be mutagenic gains great importance from the widespread use of radiation for the preservation of food. Stone *et al.*¹ have found increased mutation frequencies in *Staphylococcus aureus* which had been cultured in ultra-violet-treated medium.

More recently, there have been reports²⁻⁵ on cytological abnormalities in plant roots that had been kept in X-rayed culture fluid. Recent experiments on *Drosophila*⁶ have indicated that the incidence of sex-linked recessive lethals and visible mutations may be enhanced by feeding the larvae on irradiated medium. In view of the practical importance of this possibility, a large-scale experiment was carried out to test the genetic effects of irradiated food on *Drosophila melanogaster*.

Bottles with basic medium containing water, glucose, agar, yeast and propionic acid in the proportion of 100 : 10 : 3 : 10 : 0.4 in g were irradiated from a cobalt-60 source at the Atomic Energy Establishment, Trombay, Bombay. Two doses of 150,000 and 300,000 rads were given at a dose rate of 25,000 rads/min. Control bottles were made up in the same way. Treated and control bottles were stocked with 10 young males and 20 virgin females of the wild-type Oregon-K strain. When sufficient eggs had been laid, the parents were discarded. The emerging males, which had undergone their development on irradiated medium, were divided into two groups. In one group, the males were mated with virgin females of genotype $y^{sc}sc$ *In* 49 sc^s ; *bw*; *st* and the progeny was scored for recessive sex-linked lethals and translocations. In the second group, the males were mated with virgin females of an attached-X strain and the progeny was scored for sex-linked visible mutations and large deletions. In both groups, the test was extended over four broods of three days each. Table 1 summarizes the results.

Table 1

Dose (rads)	Sex-linked lethals	Translocations	Sex-linked visibles	Large deletions
0	9/2971 = 0.3%	0/3560	—	—
150,000	10/3532 = 0.25%	0/4031	0/49,056	0/46,871
300,000	8/3766 = 0.21%	0/4160	0/45,300	0/48,014

Clearly, irradiated medium in these experiments had not produced any genetical effects. It is difficult to account for the difference between our results and those obtained by Swaminathan⁶. The strain of males used, the composition of the medium and the radiation dose in our first experiment were the same as in Swaminathan's experiments; in the second experiment, we used an even higher dose of radiation. As regards sex-linked lethals, the difference between our results and those of Swaminathan is only on the borderline of significance for the treated series (χ^2 about 4 for 1 D.F.); the main difference lies in the control rate, which in Swaminathan's experiments was unusually low for the Oregon-K strain. Much more striking is the complete absence of visibles in our experiment as compared with the high frequency of visibles reported by Swaminathan. However, most of Swaminathan's visibles were not tested genetically and may have been phenotypic changes; moreover, it is not clear whether the visible changes in the F_2 of the sex-linked lethal tests occurred in the proportions expected if they had been due to the presence of chromosomes from the irradiated P_1 males. Without further tests, the claim that irradiated medium produces genetic changes of any kind in *Drosophila* does not seem justified.

O. S. REDDI
G. M. REDDY
J. J. RAO
D. N. EBENEZER
M. S. RAO

Radiobiological Research Unit,
Osmania University,
Hyderabad 7, India.

¹ Stone, W. S., Wyss, O., and Haas, F., *Proc. U.S. Nat. Acad. Sci.*, **33**, 59 (1947).

² Natarajan, A. T., and Swaminathan, M. S., *Indian J. Genet.*, **18**, 220 (1958).

³ Natarajan, A. T., *Riso Report*, **16**, 39 (1960).

⁴ Swaminathan, M. S., Chopra, V. L., and Bhaskaran, S., *Rad. Res.*, **16**, 182 (1962).

⁵ Chopra, V. L., Natarajan, A. T., and Swaminathan, M. S., *Rad. Bot.*, **3**, 1 (1963).

⁶ Swaminathan, M. S., Nirula, S., Natarajan, A. T., and Sharma, R. P., *Science*, **141**, 637 (1963).

BIOLOGY

Sexual Dimorphism in *Tilapia*

OBSERVATIONS have shown that the growth rate of males in the *Tilapia* genus is faster than that of the females.

Tilapia aurea (formerly known as *Tilapia nilotica* L.) and *Tilapia galilaea* usually reproduce during the second year of life. Cultivation of *Tilapia aurea* in its second year of life and *Cyprinus carpio* produced low yields of harvestable *Tilapia*, due to the spawning of *Tilapia*.

Mono-sex culture of male *Tilapia aurea* was successful and yielded large fish. Yashouv and Hefetz¹ showed that sex can be differentiated by differences of the openings in the genital papilla. The males have two openings and the females three. Pruginin and Shell² sexed *Tilapia aurea* with a mechanical grader.

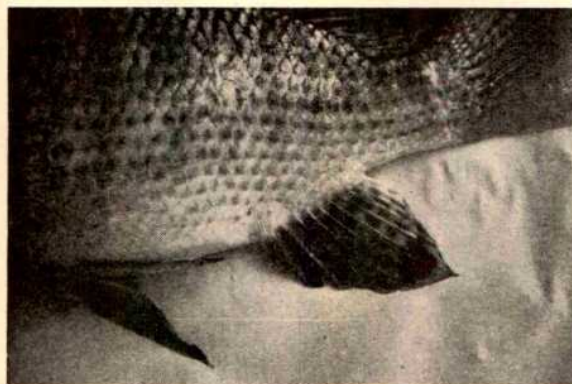


Fig. 1. The anal fin in *Tilapia aurea*. Upper, male; lower, female

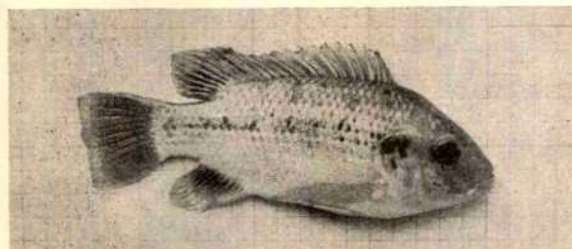
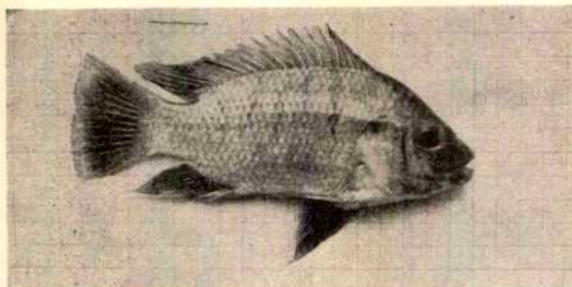


Fig. 2. The anal and dorsal fins in *Tilapia galilaea*. Upper, male; lower, female

While working with *Tilapia aurea* and *Tilapia galilaea* it was noticed that sexing can be accomplished either according to the genital papilla, or according to the shape of the dorsal and anal fin (Figs. 1, 2).

In males above 60 g the dorsal and anal fins are pointed, while in the females the dorsal and anal fins are rounded and more expanded. In bigger fish these differences are more distinct. These secondary sexual characteristics may be helpful in sexing *Tilapia aurea* and *Tilapia galilaea*.

J. CHERVINSKI

Fish Culture Research Station,
Dor, Israel.

¹ Yashouv, A., and Hefetz, A., *Bamidgeh*, 11, 36 (1959).

² Pruginin, Y., and Shell, E. W., *Prog. Fish-cult.*, 24, 37 (1962).

Presumed Sensory Cells in Fish Epidermis

METHYLENE blue, injected subcutaneously, will stain particular elongated cells, spindle-shaped or flask-shaped, in the epidermis of various teleost fish. Such cells were found by Whitear¹ in the minnow (*Phoxinus laevis*), and afterwards in several other species, both freshwater and marine; that paper should be consulted for references to the scanty earlier literature on the subject. It was suggested that these cells are receptors for the common chemical sense, but as it seems preferable to describe them by a term not indicative of a supposed function, they will be called 'spindle cells' here. For technical reasons, the exact relationship of the spindle cells to the surface of the skin, and to nerve fibres, could not be ascertained by means of the light microscope.

Epidermal spindle cells have now been identified in sections of minnow skin, examined by electron microscopy. Skin from the operculum of *Phoxinus laevis* was fixed in buffered osmium tetroxide, stained in bulk with alcoholic phosphotungstic acid, and embedded in 'Araldite'.

Examination of electronmicrographs has confirmed that the spindle cells do constitute a distinct cell type, which cannot be confused with general epithelial cells, mucous (goblet) cells, or with other secretory cells present in the epidermis. They have been found in sections of skin taken from both inner and outer surfaces of the operculum; methylene blue staining showed them to be present on the chest and gular region. Their distribution is not entirely even, as they tend to occur in groups of three or four, although the spindle cells are not usually contiguous. The adjacent epithelial cells are unmodified, which is one of the characters distinguishing a group of spindle cells from a taste-bud.

Fig. 1 shows the appearance of a spindle cell in *Phoxinus*, as seen in electronmicrographs. The nucleus usually lies at the level of the second or third layer of epidermal cells from the outside, and a neck-like process extends to the surface. Sometimes a pointed process extends inwards also. Usually the outer end of the distal process is slightly bulbous, and it is invariably outlined by a zonula occludens² 0.5–0.75 μ deep. A single microvillus, with no definite internal structure, projects from the spindle cell beyond the level of the skin surface, but it is not clear whether it is always present. Nor is it certain what relationship the microvillus has to the thin cuticle which covers the surface of the skin, but which is easily detached during preparation.

The spindle cells can be recognized in ultra-thin sections, even if the distal process is not included, by the numerous rounded bodies, 0.075–0.1 μ in diameter, which pack the cytoplasm. In phosphotungstic acid stained material, most of these bodies are electron-dense, but some are paler and have the appearance of vesicles. They resemble the inclusions described by Trujillo-Cenóz³ in the sensory cells of fish taste-buds, and their distribution within the cell is also similar. They are most abundant immediately distal to the nucleus, least so proximal to it. Similar bodies do occur in ordinary epithelial cells of the

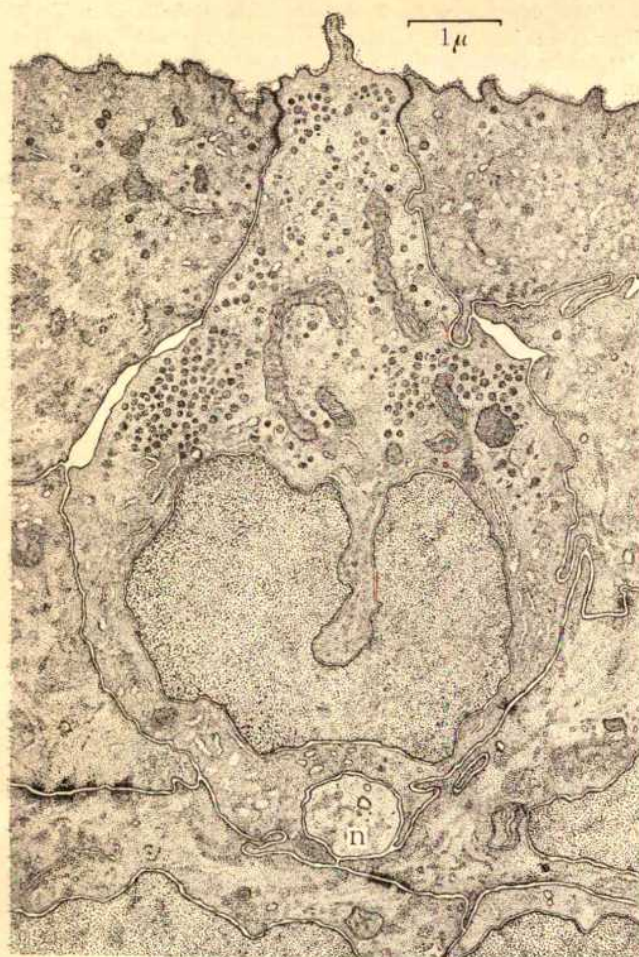


Fig. 1. Drawing traced from electronmicrographs of three serial sections of a spindle cell in the epidermis on the outer side of the operculum of *Phoxinus laevis*. The cuticle is missing from the surface of the skin

minnow, but not in such numbers as in the spindle cells. The spindle cells often contain a few large electron-dense inclusions, up to 0.5μ in diameter, which, again, may also be found in epithelial cells. Otherwise the cytoplasmic structure of the spindle cells is quite normal. There are a number of elongated mitochondria in the neck region, an endoplasmic reticulum with channels and vesicular profiles, and often some multivesicular bodies. A Golgi complex and a centrosome, not shown in the figure, lie distal to the nucleus. Desmosomes occur between the spindle cell and the adjacent epithelial cells, but they are smaller and scarcer than elsewhere in the epidermis.

In several series of sections, profiles which appear to be of a nerve fibre have been found associated with a spindle cell. One such is shown in Fig. 1, *n*. Here the fibre is indented into the base of the spindle cell. In some cases the apparent nerve fibre lay against the side of the spindle cell or was partially wrapped around the base of its neck. No special synaptic structures have been made out as yet. In some series the nerve fibre appeared to end on, or invaginate into, the spindle cell. In one case a fibre appeared to pass on from one spindle cell to a neighbouring one. It must be admitted that it is not certain that these profiles are of nerve fibres, for epidermal nerve fibres lack many of the structures by which nerves are normally identified, such as sheath cells or prominent neurofilaments. There is a possibility of confusing the profile of a nerve fibre with that of a process from one of the amoeboid cells which are present in the epidermis.

The appearance of some of the spindle cells suggests that they, like the general epithelial cells and the goblet cells,

are deciduous. If they are shed, they are presumably replaced, but how is not clear.

It can now be considered established that the spindle cells do exist in minnow skin. While the function of a cell type cannot be ascertained absolutely from histological studies, the evidence from electron microscopy tends to reinforce, rather than otherwise, the hypothesis that the spindle cells are epithelial sensory cells, and probably chemosensory, for in their fine structure, as well as their general appearance, they resemble the sensory cells of taste-buds. An electron microscope study of the skins of other fishes known to possess spindle cells is in progress.

MARY WHITEAR

Department of Zoology,
University College, London.

¹ Whitear, M., *Quart. J. Microsc. Sci.*, **93**, 289 (1952).

² Farquhar, M. G., and Palade, G. E., *J. Cell. Biol.*, **17**, 375 (1963).

³ Trujillo-Cenóz, O., *Z. Zellforsch. mikrosk. Anat.*, **64**, 654 (1961).

Taxonomic Investigation of Several *Brassica* Species using Serology and the Separation of Proteins by Electrophoresis on Acrylamide Gels

IN recent years, attention has been drawn to the usefulness of biochemical data in studies of angiosperm taxonomy^{1,2}. Some of these data refer to proteins, and two methods of investigation which have produced interesting results are the serological analysis of plant proteins³ and the separation of proteins on acrylamide gels⁴.

So far as is known, these two methods have not been applied to the same plant material, and the present note is a preliminary account of the application of the methods to the seed proteins of *Brassica nigra* (L.) Koch, *Brassica oleracea* L. and *Brassica campestris* L. On morphological and cytological grounds, these plants are regarded as distinct species, and as a preliminary to an investigation of some disputed aspects of taxonomy within the genus, the two methods of protein analysis have been applied to this well-accepted system.

In the serological section of the work (J. G. V. and A. W.), rabbit antisera to saline extracts of the seeds of the three species were prepared using a series of subcutaneous injections of the seed extract incorporating Freund's adjuvant. Immuno-electrophoretic analyses of the three antisera indicated in all cases a large number of antigen-antibody systems as shown by precipitin arcs. Because of the large number of arcs it was difficult, before absorption, to make an accurate comparison of the three species except in the case of a strong arc formed to a portion of the globulin protein fraction of the seed (Fig. 1, *x*). Against the antisera of all three species, the protein forming this arc in an extract of *B. nigra* was found to migrate towards the negative pole, in contrast to the behaviour of similar proteins in *B. campestris* and *B. oleracea*.

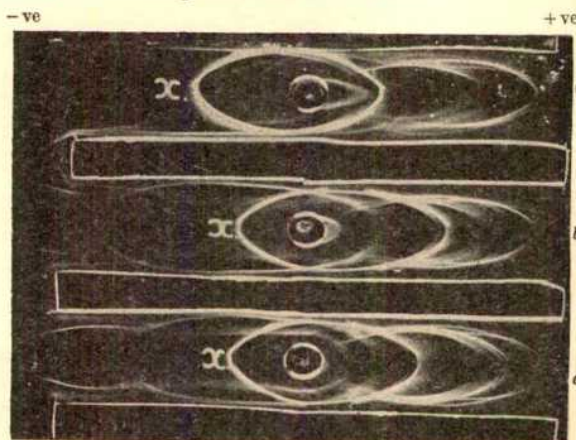


Fig. 1. Immuno-electrophoretic analysis of extracts of: (a) *B. nigra*; (b) *B. oleracea*; (c) *B. campestris* against *B. campestris* antiserum

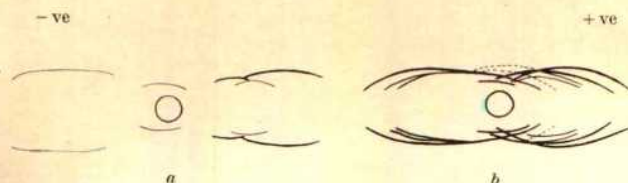


Fig. 2. Immuno-electrophoretic analysis of *B. campestris* extract against its homologous antiserum. (a) Absorbed with *B. oleracea* extract, (b) absorbed with *B. nigra* extract (*B. campestris* has more proteins different from *B. nigra* than it has from *B. oleracea*)

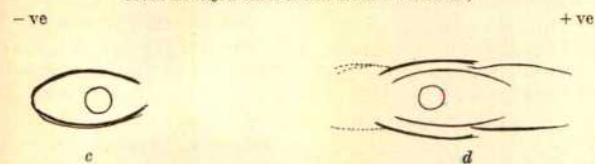


Fig. 3. Immuno-electrophoretic analysis of *B. oleracea* extract against its homologous antiserum. (c) Absorbed with *B. campestris* extract, (d) absorbed with *B. nigra* extract (*B. oleracea* has more proteins different from *B. nigra* than it has from *B. campestris*)

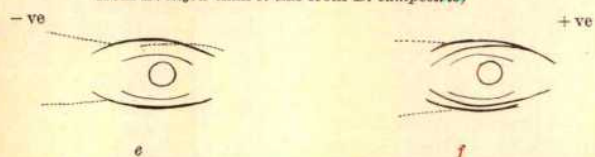


Fig. 4. Immuno-electrophoretic analysis of *B. nigra* extract against its homologous antiserum. (e) Absorbed with *B. campestris* extract, (f) absorbed with *B. oleracea* extract (*B. nigra* has a similar number of proteins different from both *B. campestris* and *B. oleracea*)

Absorption of the antisera provided a clearer taxonomic comparison (Figs. 2-4). On the basis of the number of antigen-antibody systems, *B. campestris* and *B. oleracea* would appear to be closer to each other than either is to *B. nigra*. This observation was confirmed using the Ouchterlony double diffusion technique.

In the acrylamide section of the work (D. B. and S. W.), seeds were extracted with 5 per cent (w/v) potassium sulphate and the extracts were subjected to electrophoresis by the method of Ornstein and Davis⁵ using the apparatus described by Laycock, Thurman and Boulter⁶. A taxonomic assessment of the three species was based on the appearance and R_f values of the protein bands obtained from the seed extracts (Fig. 5), the results of which could be summarized as follows: (a) four protein bands common to all three species; (b) three protein bands common to *B. oleracea* and *B. campestris* only; (c) two bands common to *B. nigra* and *B. campestris* only; (d) no bands common to

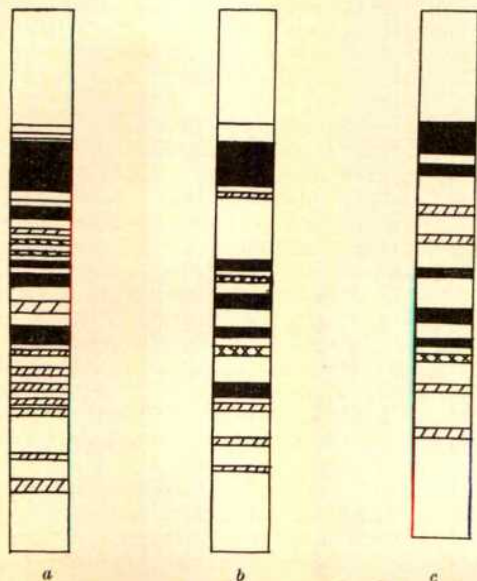


Fig. 5. Diagram of gels of electrophoresed proteins of: (a) *B. oleracea*; (b) *B. campestris*; and (c) *B. nigra*

B. nigra and *B. oleracea* only; (e) one band found in *B. campestris* only; (f) six bands found in *B. oleracea* only; and (g) five bands found in *B. nigra* only.

Both serology and acrylamide-gel separation, based on seed proteins, support the classification of the plants into three taxa. The evidence of these techniques also suggests a closer relationship between *B. campestris* and *B. oleracea* than between either of these species and *B. nigra*. This supports the classification by Schulz⁷ on morphological grounds of the three species into two sections—*Brassicotypus* (*B. campestris* and *B. oleracea*) and *Melanosinapis* (*B. nigra*). Acrylamide-gel separation also provides evidence for *B. nigra* being closer to *B. campestris* than it is to *B. oleracea*.

On the basis of the results obtained, it is felt that serology and acrylamide-gel separation might prove useful in the investigation of *Brassica* taxonomy. In this example, the acrylamide method had the greater resolving power and also has the added advantage that it is a quick and simple technique. It is intended to publish the details of this investigation elsewhere.

We are indebted to the Agricultural Research Council for its support of the serological section of the work.

J. G. VAUGHAN
A. WAITE

Biology Department, Queen Elizabeth College
(University of London),
Campden Hill Road, London, W.8.

D. BOULTER
S. WAITERS

Hartley Botanical Laboratories,
University of Liverpool.

¹ Swain, T., *Chemical Plant Taxonomy* (London and New York, 1963).

² Alston, R. E., and Turner, B. L., *Biochemical Systematics* (New Jersey, 1963).

³ Gell, P. G. H., Hawkes, J. E., and Wright, S. T. C., *Proc. Roy. Soc., B*, **151**, 364 (1960).

⁴ Fox, D. J., Thurman, D. A., and Boulter, D., *Phytochemistry*, **3**, 417 (1964).

⁵ Ornstein, L., and Davis, E. J., *Disc Electrophoresis* (Distillation Product Industries, Eastman Kodak Co., Rochester, N.Y., 1962).

⁶ Laycock, M. V., Thurman, D. A., and Boulter, D., *Clin. Chim. Acta*, **11**, 98 (1965).

⁷ Schulz, O. E., *Cruciferae-Brassicaceae*, in Engler's *Das Pflanzenreich*, **70**, 1 (1919).

Seasonal Changes in the Histological Structure of the Spinal Column of *Sorex araneus* (L.)

MORPHOLOGICAL measurements¹ have shown that the spinal column of the shrew (*Sorex*) contracts during the winter and lengthens again in the spring. This lengthening is noticeably greater than the winter contraction. This study shows a relation between these changes and the histological structure of the spinal column.

The material comprised 35 specimens of *Sorex araneus* (L.); 17 were young individuals of the same year's brood, captured in July–October; 11 were wintering animals, captured in January–March, and 7 were wintered adults, 6 of which were captured in May and one in September. Three vertebrae of the lumbar region of the spinal column of each individual were fixed in formol and then decalcified in nitric acid. The preparations were treated by the usual wax embedding method, cut into 10- μ sections, and stained with Lillie's² allochrome method with aldehyde-fuchsin.

Clear seasonal changes were found in the intervertebral disks and in the nucleus pulposus. In young shrews the latter was large and apparently contained much liquid (Fig. 1a), while in the wintering ones it was considerably flattened longitudinally (Fig. 1b), and in the wintered adults again at least as large as in the young ones (Fig. 1c). The annulus fibrosus, surrounding the nucleus pulposus, was without folds in the young specimens; in the wintering ones, particularly those captured late in winter, it was clearly folded at the margins, apparently corresponding to the flattening of the nucleus pulposus. In wintered adults the annulus fibrosus was again unfolded. According to preliminary measurements

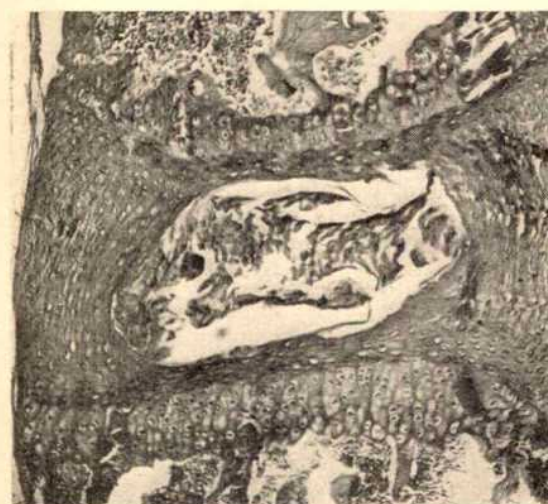
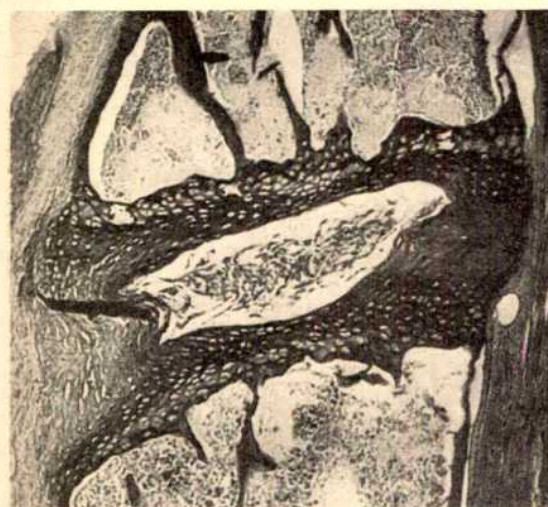
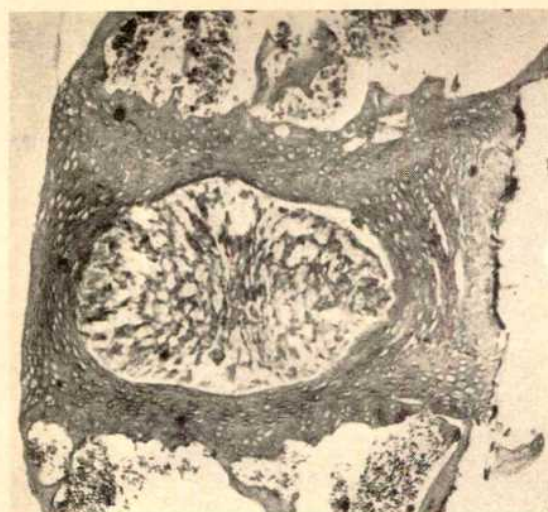


Fig. 1. Longitudinal sections through intervertebral disks and nucleus pulposus of (a) a young shrew; (b) a wintering shrew; (c) a post-wintering adult.

the young and the wintering individuals do not show any significant differences in the thickness of the cartilaginous layer situated in the surface of the annulus fibrosus towards the bone pulp. On the contrary, all the wintered adults show vigorous growth in this layer, which therefore is considerably thicker than in the other groups investigated.

The growth of the cartilage begins in the hyaline cartilage bordering the annulus fibrosus (visible as a light layer in Fig. 1b). The substantia compacta at the surface of the hyaline cartilage seems to break and possibly also disappear to some degree when the cartilage grows. The new cartilage is rather loose and weakly PAS-positive, and the cells are hypertrophic. In the wintered shrew captured in the following autumn the cartilage was already strongly PAS-positive, and numerous elastic fibres were found in it and in the annulus fibrosus.

The results presented here show that the contraction of the spinal column of *Sorex araneus* during winter is apparently caused, at least in the main, by the flattening of the nucleus pulposus. As the latter again increases in size in the spring, this flattening in winter cannot be caused by ageing, which, however, also has been found to cause a volume decrease in the nucleus pulposus¹. The vigorous and rapid lengthening of the spinal column in the spring is caused by the volume increase in the nucleus pulposus and by the considerable growth in the cartilage. This growth in the cartilage explains the fact that the spinal column of the wintered specimens is longer than of the specimens before wintering.

LAURI SAURE
HEIKKI HYVÄRINEN

Department of Zoology,
University of Oulu, Finland.

¹ Siivonen, L., *Ann. Acad. Sci. Fenn.*, IV, 21, 1 (1954).

² Gurr, Edward, *Staining Animal Tissues*, 130 (London, 1962).

³ Ham, A. W., and Leeson, T. S., *Histology* (London, 1961).

Effects of Starvation and Protein Depletion on Mercury Retention in Two Strains of Chickens

Two strains of White Leghorn chickens designated as *R* and *S* have been developed from common parentage at this station¹. When chickens of these two strains were injected intramuscularly with 3 mg mercury as phenylmercuric acetate (PMA) or mercuric chloride/kg/body-wt., 2-4 times more mercury was retained by the livers and kidneys of the *R* strain chickens than was retained by the same organs from the *S* strain chickens^{2,3}. Lillis *et al.*⁴ reported a similar differential retention when chickens from these strains were injected with 9.1 mg copper as cupric acetate/kg/body-wt.

Starvation and/or deprivation of water were selected as possible methods for altering the mercury retention pattern in the organs of these chickens. Feed, water or both were withheld from about forty chickens of the *R* and *S* strains from the time of injection of 3 mg mercury as PMA/kg/body-wt. until decapitation of the chickens 96 h later. The control group received both commercial feed and water *ad lib*. The livers and kidneys of the killed chickens were analysed for mercury^{5,6}.

The effects of deprivation of feed and water on the liver and kidney mercury retention pattern may be seen in Fig. 1. The point represents the mean value of the percentage retention of the injected dose by the livers or kidneys, and the bracket enclosing each point represents the 95 per cent confidence intervals. The values in the livers from the two strains of control chickens were widely separated, indicating statistical significance, as were the values in the kidneys from the two strains. The chickens offered feed only maintained the significant difference in retention. However, the values in the *S* liver and *R* liver overlapped when the chickens were offered water only or neither feed nor water. Thus starvation altered the differences by increasing retention in the *S* strain liver more than the *R* strain liver.

Further diet investigations were predicated on the results of the starvation experiment and on the work of Surtshin⁷ and Surtshin and Yagi⁸. They had demonstrated a change in the renal distribution of mercury following mercuric chloride injections in rats when a sucrose and vitamin diet was substituted for commercial feed.

Approximately 100-day-old *R* and *S* strain chicks were given a commercial feed until 8 weeks of age. The chickens to be depleted were fasted for 24 h and offered, *ad lib.*, granulated sugar and drinking water containing 3.0 ml. of a vitamin preparation, 'ABDEC' drops (Parke, Davis and Co.), per 800 ml. of water*. When the protein-depleted chickens had lost approximately 30 per cent of their body-weight, they were injected intramuscularly with mercuric chloride or PMA at the rate of 3.0 mg mercury/kg/body-wt. The control chickens continued on the commercial ration during the entire experiment and were injected with the mercurials at the same time as the experimental chickens.

Some of the experimental chickens remained on the sugar and vitamin solution diet after injection (unfed chickens (*UF*)), while others were returned to the normal commercial ration (fed chickens (*F*)). At the end of the 96 h all chickens were decapitated and their livers and kidneys excised and analysed.

Fig. 2A graphically illustrates the relationship of the percentage of mercury retained in the liver and kidneys between the *R* strain and *S* strain control chickens, the *R* strain and *S* strain depleted chickens and the *R* strain and *S* strain depleted and repleted chickens after injection with 3.0 mg mercury as PMA/kg/body-wt. The values in the livers and in the kidneys from both lines of the control chickens were widely separated, designating statistical significance. However, in the protein-depleted group, the *R* liver and the *S* liver confidence intervals overlapped, as did the *R* kidney and *S* kidney confidence intervals. Thus, as shown by lack of statistical significance between similar organs from chickens of the two previously different strains, alteration in the retention of mercury by protein depletion

has occurred. Even the percentage retention values of the organs from the protein-depleted *S* chickens were significantly higher than the retention values of similar organs from the *R* control chickens. The confidence limits of the depleted and afterwards repleted chickens again showed a statistical difference. Thus the refeeding with the commercial ration, even for a short time, counteracted to a large degree the change in the retention patterns brought about by the protein-free diet.

The graphic relationship of mercury retention in the organs of the *R* and *S* chickens, depleted and controls, after mercuric chloride injection is illustrated in Fig. 2B. A significant difference may be noted in the mercury retention between the livers and between the kidneys of the *R* and *S* control groups. The mercury retention in the livers of the unfed *R* and *S* chickens, the livers of the fed *R* and *S* chickens and the kidneys of the unfed *R* and *S* chickens were altered by the protein-free diet, so that within each group the significant differences no longer occurred. Only in the case of the *R* and *S* kidney values from the fed chickens did the significant difference remain. The kidneys of the *R* and *S* fed group still continued to show the significant difference in mercury retention. Thus, alteration of the mercury retention patterns of these two strains of chickens, whether injected with PMA or mercuric chloride, may be accomplished by limiting their protein intake.

The work was supported in part by the Public Health Service research grant EF-00177, from the Division of Environmental Engineering and Food Protection.

DONNA V. LARKIN

V. L. MILLER

G. E. BEARSE

C. M. HAMILTON

Departments of Agricultural Chemistry,
Animal Science and Veterinary Medicine.
Washington State University,
Western Washington Experiment Station,
Puyallup, Washington.

* Bearse, G. E., Becker, W. A., and Hamilton, C. M., *Poultry Sci.*, **42**, 110 (1963).

* Miller, V. L., Bearse, G. E., Kimura, Y., and Csonka, Elizabeth, *Proc. Soc. Exp. Biol. and Med.*, **100**, 301 (1959).

* Miller, V. L., Bearse, G. E., and Hammermeister, K. E., *Poultry Sci.*, **38**, 1037 (1959).

* Lillis, Donna V., Miller, V. L., Bearse, G. E., and Hamilton, C. M., *Toxicol. and App. Pharmacol.*, **5**, 22 (1963).

* Polley, D., and Miller, V. L., *Anal. Chem.*, **27**, 1162 (1955).

* Kimura, Y., and Miller, V. L., *Anal. Chim. Acta*, **27**, 325 (1962).

* Surtshin, A., *Amer. J. Physiol.*, **190**, 271 (1957).

* Surtshin, A., and Yagi, K., *Amer. J. Physiol.*, **192**, 405 (1958).

ANATOMY

Ultrastructure of the Phrenic Nerve

A COMPLETE ultrastructural analysis of the phrenic nerve at a particular level is of interest in view of recent light and electron microscope observations which suggest that there are extensions of subarachnoid space along the length of peripheral nerves¹⁻³. The present observations were made during the course of quantitative investigations on fibres and vessels in the phrenic nerve and on the tissues ensheathing them.

A portion of the left phrenic nerve 5 mm in length was removed from an adult male cat under 'Nembutal' anaesthesia. The specimen was fixed in 2.5 per cent osmium tetroxide buffer, according to the method described by Richardson⁴, dehydrated in acetone and embedded in 'Araldite'. Complete transverse sections were cut with a glass knife on an L.K.B. microtome and mounted over a 750- μ aperture in a copper grid coated with lead hydroxide after Karnowsky⁵ and examined in a Siemens 'Elmiskop F' electron microscope. The image of the entire cross-section of the nerve was photographed

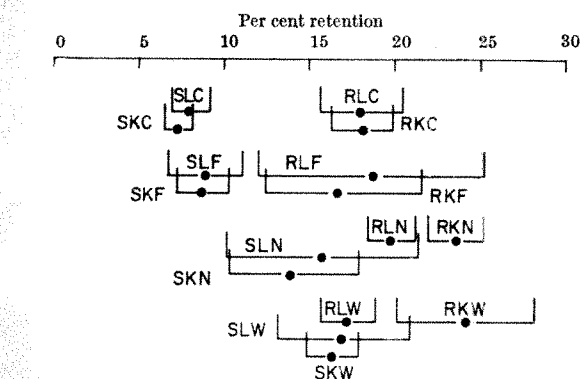


Fig. 1. Effect of the lack of feed, water or both on the percentage of mercury in the livers and kidneys of two strains of chickens 96 h after injection with 3.0 mg mercury as phenylmercuric acetate per kg body-weight. Brackets indicate 95 per cent confidence-levels. *R* and *S*, Strains of chickens; *L*, liver; *K*, kidney; *C*, control chickens; *F*, feed only; *W*, water only; and *N*, no feed or water

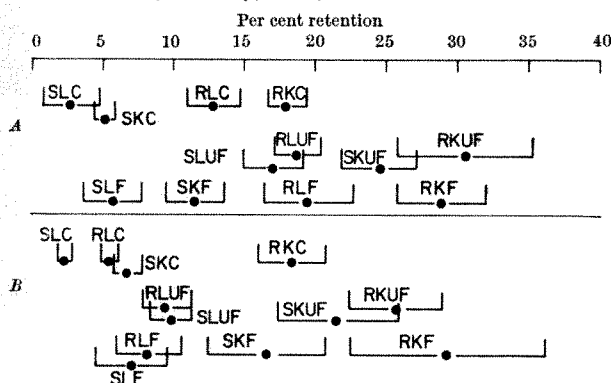


Fig. 2. The effect of diet on the percentage retention of mercury in the livers and kidneys of two strains of chickens 96 h after injection with 3.0 mg mercury as phenylmercuric chloride (A) or mercuric chloride (B) per kg body-wt. Brackets indicate 95 per cent confidence-levels. *R* and *S*, Strains of chickens; *L*, liver; *K*, kidney; *C*, control chickens; *F*, protein-depleted and -repleted chickens; *UF*, protein-depleted chickens

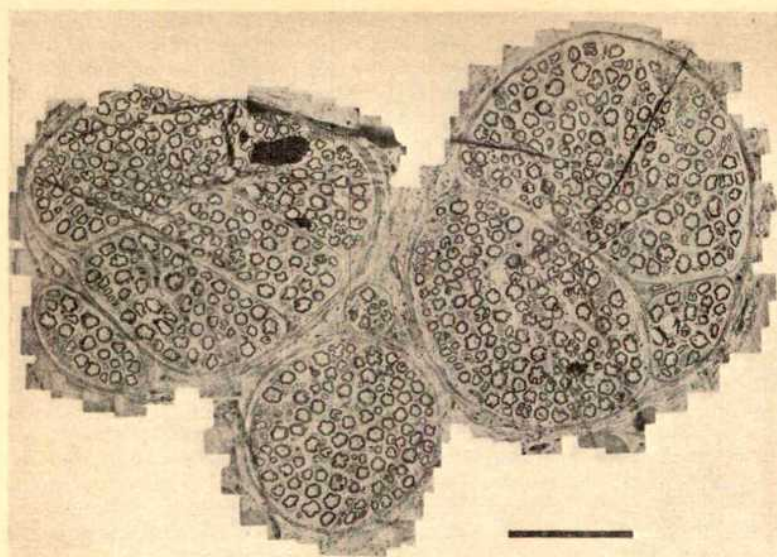


Fig. 1. Transverse section of phrenic nerve from a cat (original montage: $c. \times 4,500$). Scale = 100μ

and a montage constructed, the total magnification being 4,500 (Fig. 1).

At the level examined, 4 nerve fasciculi were present of which the two larger ones comprised 3 and 6 subfasciculi respectively. The sheaths surrounding the fasciculi and subfasciculi are referred to here as the perineurium. In all, 802 myelinated fibres, ranging from 4 to 20μ in external diameter, and 2,094 unmyelinated fibres were

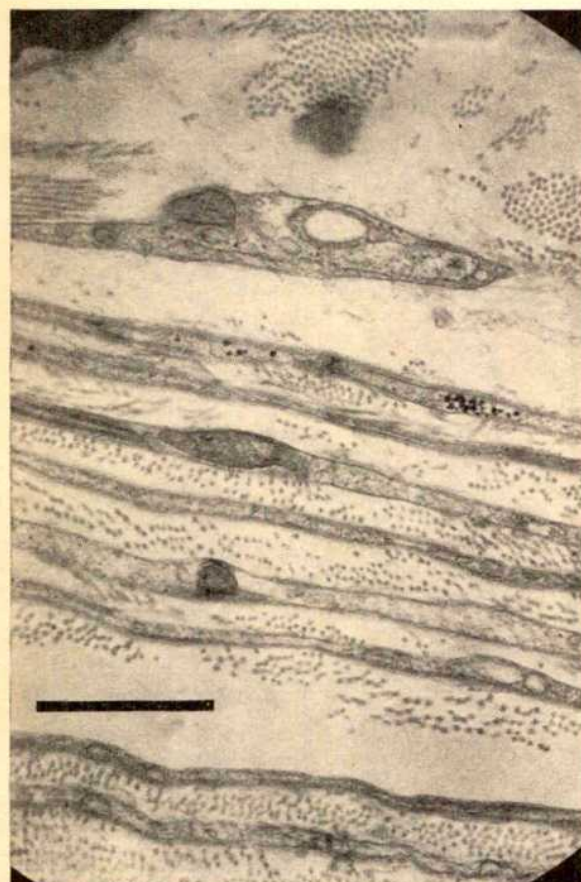


Fig. 2. Transverse section of part of the perineurium showing alternate layers of fibroblasts and collagen fibres ($c. \times 42,000$). Scale = 1μ

present with an apparently random distribution of the myelinated fibres throughout the section. The unmyelinated fibres were aggregated in groups within the fasciculi; the groups in peripheral regions of the fasciculi contained more unmyelinated fibres (50–200) than those nearer the centre (5–20). Each group of unmyelinated fibres was associated with a small number of myelinated fibres ranging from 0.5 to 2μ in external diameter.

Surprisingly few blood or lymphatic vessels were seen throughout the section and they were all less than 10μ in diameter. A total of 23 such vessels was found within the fasciculi and a further 5 were situated in the extra-fascicular connective tissue immediately adjacent to the perineurium. Unlike the observations made by Shanthaveerappa and his colleagues, no extensions of 'perineurial epithelium' enveloping intrafascicular vessels were seen.

Alternating layers of cells and intercellular 'spaces' containing numerous collagen fibres formed the basis of the perineurial layers (Fig. 2). The cells were identified as fibroblasts because of their

close proximity to collagen fibres, some of which lay in invaginations of the cell membrane—a relationship which has also been found in other situations (Porter and Pappas⁶; Wessel⁷).

The possibility that the cells of the perineurium, identified here as fibroblasts, correspond to those lining the subarachnoid space was investigated in cats. The results obtained indicate that the meningeocytes of both the pia mater and arachnoid in the region of spinal nerve roots generally form a limiting membrane to the subarachnoid space and are only rarely separated from it by tissue containing collagen fibres.

The present observations, therefore, do not support the view that there are extensions of the subarachnoid space along peripheral nerves.

ANDREW S. WILSON
DOUGLAS G. SILVA

Department of Anatomy,
Monash University,
Melbourne, Australia.

¹ Shanthaveerappa, T. R., and Bourne, G. H., *Z. Zellforsch.*, **61**, 742 (1964).

² Shanthaveerappa, T. R., et al., *Acta Anat. (Basle)*, **52**, 193 (1963).

³ Shanthaveerappa, T. R., et al., *J. Anat. (Lond.)*, **96**, 527 (1962).

⁴ Richardson, K. C., *J. Anat. (Lond.)*, **96**, 427 (1962).

⁵ Karnovsky, M. J., *J. Biophys. Biochem. Cytol.*, **11**, 729 (1961).

⁶ Porter, K. A., and Pappas, G. D., *J. Biophys. Biochem. Cytol.*, **5**, 153 (1959).

⁷ Wessel, W., *Virchows Arch. Path. Anat.*, **332**, 224 (1959).

Electron Microscopic Observations on the Carotid Body

ELECTRON microscopic investigations of the carotid body¹⁻⁶ have provided some basic information concerning the cell types present and the characteristics of nerve fibres and blood vessels. Since the early work of De Castro^{7,8}, the glomus cells have been widely assumed to be the chemoreceptor component of the carotid body. Lever, Lewis and Boyd⁵ found that nerve fibres containing a concentration of mitochondria and microvesicles terminated near glomus cells. Ross⁴, on the other hand, found that the few terminal axons in apposition with glomus cells did not contain mitochondria or synaptic vesicles. In neither investigation was specialization of opposing cell membranes observed. In the examination of the carotid body recorded here, further characteristics of both the nerve endings on the glomus cells and vascular endothelium have been determined.

Cats were anaesthetized with sodium pentobarbitone (30 mg/kg) intraperitoneally. The trachea was cannulated

and the larynx and pharynx reflected. The carotid body was perfused for 1–2 min via the common carotid artery with 5 per cent glutaraldehyde in Sorensen's phosphate buffer at pH 7.3–7.4. The carotid body was then removed, cut into small blocks and fixed for 5 h in glutaraldehyde. The tissue was washed in phosphate buffer for 45 min, post-fixed in osmium tetroxide for 16 h, dehydrated in ethanol and embedded in araldite. Ultra-thin sections were cut on an L.K.B. microtome, stained with lead and uranyl acetate and examined in a Siemens 'Elmiskop I' electron microscope.

Nerve endings were frequently seen and occurred in intimate relationship with cells containing osmiophilic granules. These cells have been variously referred to as glomus cells by Lever, Lewis and Boyd⁵, chemoreceptor cells by Ross⁴ and Type 1 cells by De Kock^{6,9}. The nerve endings have been identified by their content of mitochondria and vesicles, the latter with a diameter of approximately 500 Å. At times two nerve endings abutted directly on an individual cell. The plasma membranes of the axon and the glomus cell were separated by a distance of 85–170 Å. Along opposing cell surfaces, specialized electron-dense zones (0.2–0.5 µ in length) were found, usually more than one at each axon–glomus junction. At these specialized sites, the plasma membranes were a further 50–100 Å apart and the intervening cleft was of increased density, though the distinct lamination that occurs in desmosomes was not observed (Fig. 1). Cytoplasmic densities, which sometimes appeared to be fibrillary, occurred in the adjoining cytoplasm and extended up to 500 Å into the glomus cell and axon. These electron-dense structures were observed only between the nerve endings and glomus cells, and in several electron micrographs vesicles within nerve endings were concentrated at these sites. No comparable concentration of osmiophile granules or other vesicles was seen in the glomus cell. Many nerve endings contained dense particles approximately 150 Å in diameter. Mostly these occurred in clusters having an overall diameter of up to 500 Å. Though there was some intermingling with synaptic

vesicles, the particles were often segregated in areas of cytoplasm of diminished density and encircled by mitochondria. These particles may be similar to the 'solid' particles described by Gray¹⁰ and Taxi¹¹. In addition, within the nerve endings, there was an occasional vesicle (up to 0.1 µ in diameter) with a central electron-dense core similar to those described in other nerve endings^{10,11}.

Blood vessels were numerous and sinusoidal, and in cross-section were always lined by at least three endothelial cells. The walls of some vessels were thin and occasional pericytes were seen; others were composed of one or two layers of muscle fibres, but none contained elastic tissue. As well as the usual cytoplasmic constituents, two additional features were observed in the endothelial cells. Fenestrations (500–700 Å in diameter) occurred in attenuated segments of endothelial cells in thin-walled blood vessels. These have not previously been observed in the carotid body. Secondly, dense-walled or coated vesicles and caveolae were found in the endothelium. These specialized micropinocytotic vesicles are believed to be concerned with protein uptake^{12–14}. Though they have been reported in frog endothelium, this appears to be the first time their presence has been recorded in mammalian endothelium. They were also seen in glomus and supporting (satellite or Type 2) cells.

The specialization at the axon–glomus cell junction described in this investigation bears a close resemblance to the synaptic contacts which are known to occur in the central nervous system and believed to be sites of chemical transmission^{10,11,15–17}. The nerve endings in the carotid body could have a similar function. For many years it has been considered probable that chemical transmission is concerned in the generation of nerve impulses in the carotid body (see Heymans and Neil¹⁸ and Anichkov and Belen'kii¹⁹ for reviews of this problem), and recently Eyzaguirre, Koyano and Taylor²⁰ have presented evidence that an acetylcholine-like substance may be involved in this process. However, we have not seen vesicles of any sort accumulating on the glomus cell side of the dense areas of intercellular contact, and only occasionally on the axon side—a feature of synapses where chemical transmission is believed to occur. Furthermore, there is no evidence that the nerve endings described here are related to chemoreceptor afferent fibres in the sinus nerve.

We thank Dr. E. H. Mercer for providing electron-microscope facilities and Miss N. Carroll and Miss J. Leightwood for their assistance.

This work was supported by the National Heart Foundation of Australia.

T. J. BISCOE

W. E. STEHBENS

Departments of Physiology and Experimental Pathology,

Australian National University, Canberra.



Fig. 1. Cat carotid body. Electron micrograph showing, above, a nerve ending and, below, a part of a glomus cell. The nerve ending contains mitochondria surrounding large electron-dense particles. Vesicles are concentrated towards the junction of the nerve ending with the glomus cell where two areas of increased density are present. Membrane bound osmiophilic granules are seen in the glomus cell. (\times 28,860)

¹ Engström, H., Hamberger, C.-A., Holmer, M., and Rignell, N. S., *Acta otolaryng.*, **48**, 379 (1957).

² Garner, C. M., and Duncan, D., *Anat. Rec.*, **130**, 691 (1958).

³ Hoffman, H., and Birrell, J. E. W., *Acta Anat.*, **32**, 297 (1958).

⁴ Ross, L. L., *J. Biophys. Biochem. Cytol.*, **6**, 253 (1959).

⁵ Lever, J. D., Lewis, P. R., and Boyd, J. D., *J. Anat. (Lond.)*, **93**, 478 (1959).

⁶ De Kock, L. L., and Dunn, A. E. G., *Nature*, **202**, 821 (1964).

⁷ De Castro, F., *Trab. Lab. Invest. Biol. Univ. Madr.*, **24**, 365 (1926).

⁸ De Castro, F., *Trab. Lab. Invest. Biol. Univ. Madr.*, **25**, 331 (1927).

⁹ De Kock, L. L., *Acta Anat.*, **21**, 101 (1954).

¹⁰ Gray, E. G., in *Electron Microscopic Anatomy*, edit. by Kurtz, S. M., 369 (Academic Press, New York, 1964).

¹¹ Taxi, J., *C.R. Acad. Sci., Paris*, **252**, 174 (1961).

¹² Roth, T. F., and Porter, K. R., *J. Cell. Biol.*, **20**, 213 (1964).

¹³ Rosenbluth, J., and Wissig, S. L., *J. Cell. Biol.*, **23**, 307 (1964).

¹⁴ Stehbens, W. E., *Nature*, **207**, 197 (1965).

¹⁵ Gray, E. G., in *Electron Microscopy in Anatomy*, edit. by Boyd, J. D., Johnson, F. R., and Lever, J. D., 54 (Arnold, London, 1961).

¹⁶ De Lorenzo, A. J., *Bull. Johns Hopk.*, **108**, 258 (1961).

¹⁷ Hamlyn, L. H., *J. Anat. (Lond.)*, **96**, 112 (1962).

¹⁸ Heymans, C., and Neil, E., *Reflexogenic Areas of the Cardiovascular System*, 190 (Churchill, London, 1958).

¹⁹ Anichkov, S. V., and Belen'kii, M. L., *Pharmacology of the Carotid Body Chemoreceptors*, translator by Crawford, R., 147 (Pergamon, London, 1963).

²⁰ Eyzaguirre, C., Koyano, H., and Taylor, J. R., *J. Physiol. (Lond.)*, **178**, 463 (1965).

MICROBIOLOGY

Timing of Protein Synthesis during Germination and Outgrowth of Spores of *Bacillus cereus* Strain T

THE emergence of a vegetative cell from a dormant bacterial spore represents one of the most direct examples of intracellular differentiation. During this transition, progressive changes in the pattern of RNA synthesis¹, classes of ribosomal particles², metabolic activity^{3,4} and structures^{5,6} are observed. Although the complete differentiation process is dependent on RNA and protein synthesis, not all the individual stages depend on new protein synthesis. It has been generally assumed that this transition involves two stages: (a) germination, which is characterized by degradative reactions and breaking of the dormant state, and (b) outgrowth, which is dependent on biosynthesis of new cellular components. The metabolic activity observed after the addition of germinating agents is probably due to a combination of two responses: activation or 'unmasking' of some dormant enzyme system and/or early biosynthesis. It is the purpose of the present report to determine whether these two stages are separated in time and to determine whether transcription and translation are ordered events during outgrowth.

Examination of the nature of increases in an essential system for energy, such as glucose oxidation, provides a useful model for distinguishing between the phenomena of activation during germination and biosynthesis during outgrowth. To distinguish between the two processes, the effect of inhibitors of protein synthesis on outgrowth was examined. Spores of *Bacillus cereus* strain T were heat-activated at 65° C for 2 h, centrifuged, and washed, and germination was initiated as described in Table 1. After 10 min, the germinated spores were centrifuged, resuspended in *tris*-phosphate buffer and the effects of actinomycin D, chloramphenicol and puromycin on amino-acid incorporation and initial rates of glucose oxidation were examined (Table 1). Leucine incorporation during outgrowth was inhibited by more than 93 per cent by inhibitors of protein synthesis, whereas these agents had no effect on the activity of the glucose oxidizing system.

If the protein synthesizing system during outgrowth is dependent on new mRNA synthesis, then one would expect that the half life of amino-acid incorporation in the presence of actinomycin D would be of the same order (a few minutes) as that observed in vegetative cells⁷. To test this possibility, heat-activated spores were germinated (see Table 1) in the presence of ¹⁴C-L-leucine. At intervals, 20 µg/ml. of actinomycin D was added, and the incorporation of radioactivity into protein was followed. As shown in Fig. 1A, addition of actinomycin D with the germinating agents completely blocks leucine incorporation, lending further support to the view that dormant spores are devoid of stable mRNA (ref. 8). Five minutes after the addition of germinating agents, protein synthesis is initiated. The rate of leucine incorporation rises gradually for the next 30 min. When actinomycin D was added 10 or 19 min after the onset of germination, leucine incorporation continued in each case for 3 min and then ceased. The apparent half-life of protein synthesizing ability (1.5 min) is in agreement with the

Table 1. EFFECT OF INHIBITORS OF PROTEIN SYNTHESIS ON AMINO-ACID INCORPORATION AND GLUCOSE OXIDATION BY GERMINATED SPORES

Incubation conditions	Leucine incorporation µmoles/mg spores	Q _{O₂} µl O ₂ /mg spores/h
Control	0.39	28.2
Actinomycin D (20 µg/ml.)	0.020	27.3
Chloramphenicol (40 µg/ml.)	0.012	28.8
Puromycin (40 µg/ml.)	0.028	25.7

Spores were germinated at 30° C in 0.04 M *tris* buffer, pH 8.3, containing 10⁻³ M KH₂PO₄, 0.5 mg adenosine/ml., 2.5 mg L-alanine/ml. and 0.5 per cent glucose. Outgrowth conditions: 1 mg spores/ml. in *tris*-phosphate buffer containing 0.5 per cent glucose, 30° C. Radioactive leucine (1 µg/µg/ml.) incorporation was followed for 40 min. The respiratory activity (Q_{O₂}) was the initial rate of glucose oxidation.

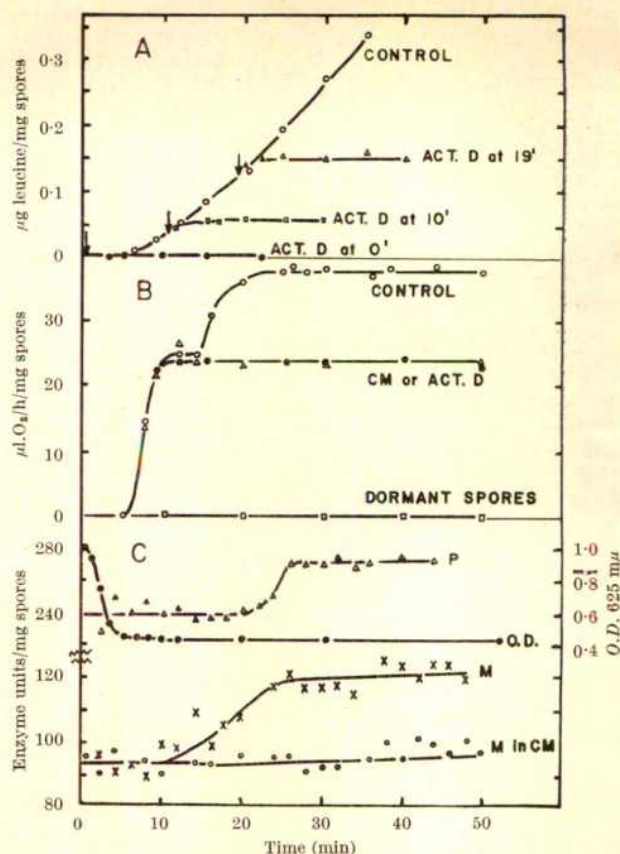


Fig. 1. A suspension of spores (1 mg/ml.) was heat-activated at 65° C for 2 h, centrifuged, washed and resuspended in the synthetic medium of Nakada¹¹. Germination was initiated by the addition of 0.5 mg adenosine/ml., 2.5 mg L-alanine/ml. and 0.5 per cent glucose at 30° C. (A) 2-¹⁴C-L-leucine (1 µg/µg/ml.) was added at time zero to each flask. Actinomycin D (20 µg/ml.) was added at 0, 10 and 19 min as indicated. Incorporation of radioactivity was followed in the hot TCA-insoluble fraction. (B) Respiratory activity was followed during germination by measuring the rate of O₂ uptake in the presence or absence of 30 µg/ml. of actinomycin D or 40 µg/ml. chloramphenicol. The glucose oxidation by dormant spores is included as a control. (C) Enzyme synthesis during germination. At intervals, aliquots were removed into pre-chilled buffer containing 100 µg/ml. chloramphenicol to stop further enzyme synthesis. The cells were washed, disrupted and assayed for alkaline phosphatase (P) or α-glucosidase (M) in the presence of chloramphenicol as described elsewhere (ref. 8). In a control flask, α-glucosidase activity was followed in a culture germinated in the presence of 100 µg/ml. chloramphenicol. The units of enzyme activity for P and M are 0.05 µmoles and 0.1 µmoles substrate hydrolysed/h respectively.

half-life of pulse labelled RNA (2 min) during outgrowth^{8,9}. These and other data¹⁰ support the conclusion that during outgrowth protein synthesis is dependent on transcription.

The increase in the respiratory activity during germination and outgrowth is shown in Fig. 1B. This increase is biphasic. The first appearance of respiratory activity coincides with the initial decrease in optical density accompanying germination (Fig. 1C) and reaches a plateau at the time (10 min) when germination is complete. Five minutes later, a second rise in respiratory activity occurs which continues for 11 min and leads to a 50 per cent increase in the rate of glucose oxidation. Chloramphenicol (CM) and actinomycin D inhibit the second rise but have no effect on the initial rise in respiratory activity. Therefore, germination leads to an activation of respiratory enzymes, and this is followed during outgrowth by the synthesis of components of this system.

Comparison of Figs. 1A and 1B suggests that following germination the entire genome may not be available for transcription. Synthesis of components of the respiratory system is initiated 10 min after the onset of protein synthesis and lasts for only a brief period. Furthermore, ordered transcription during outgrowth is evidenced by following two enzymes synthesized during outgrowth (Fig. 1C). Both α-glucosidase and alkaline phosphatase

are synthesized in steps, and each step occupies only a small fraction of the time required for the first signs of cell division (220 min). Analysis of acrylamide-gel electrophoretic patterns of cell extracts at various times during outgrowth showed that different classes of proteins were synthesized at each time interval⁸. Similarly, sequential synthesis of three enzymes during synchronous division of vegetative cells following outgrowth has been observed⁸.

Two conclusions are apparent from these observations. First, the conversion of a dormant spore to a vegetative cell involves two distinct stages, germination and outgrowth. Germination does not involve protein synthesis but leads to an activation of enzymes in the spore, whereas outgrowth is ordered. During this period mRNA has a half-life of a few minutes. Therefore, the sequential synthesis of proteins must reflect differences in the time of transcription of corresponding portions of the genome.

This investigation was supported by research grants from the National Institutes of Health (5T1-GM-686, AI-01459 and GM-12332).

W. STEINBERG
H. O. HALVORSON

Department of Bacteriology,
University of Wisconsin, Madison.

A. KEYNAN

Israeli Institute of Biological Research,
Ness-Ziona, Israel.

E. WEINBERG

Rockefeller Institute,
New York.

¹ Dol, R. H., and Igarashi, R. T., *Proc. U.S. Nat. Acad. Sci.*, **52**, 755 (1964).

² Woese, C. R., Langridge, R., and Morowitz, H. J., *J. Bacteriol.*, **79**, 727 (1960).

³ Levinson, H. S., and Hyatt, M. T., *J. Bacteriol.*, **72**, 176 (1956).

⁴ Goldman, M., and Blumenthal, H. J., *J. Bacteriol.*, **87**, 377 (1964).

⁵ Fitz-James, P. C., *Canad. J. Microbiol.*, **1**, 525 (1955).

⁶ Kawata, T., Inoue, T., and Takagi, A., *Japanese J. Microbiol.*, **7**, 23 (1963).

⁷ Levinthal, C., Keynan, A., and Higa, A., *Proc. U.S. Nat. Acad. Sci.*, **48**, 1631 (1962).

⁸ Kobayashi, Y., Steinberg, W., Higa, A., Halvorson, H. O., and Levinthal, C., in *Spores III*, edit. by Campbell, L. L., and Halvorson, H. O., 200 (Amer. Soc. Microbiol., Ann Arbor, 1965).

⁹ Higa, A. Ph.D. thesis, Massachusetts Institute of Technology (1963).

¹⁰ Balassa, G., *Biochim. Biophys. Acta*, **72**, 497 (1963).

¹¹ Nakada, H. M., *J. Bacteriol.*, **88**, 1522 (1964).

VIROLOGY

Nucleic Acid of Infectious Laryngotracheitis Virus

THERE is now considerable evidence that halogenated derivatives of the nucleoside 2'-deoxyuridine inhibit the growth of viruses that have DNA as their nucleic acid constituent but not of those containing RNA^{1,2}. Inhibition occurs at a stage of nucleotide assembly and the thymidine analogue 5-iodo-2'-deoxyuridine (IUdR) has been shown to be incorporated into the DNA of vaccinia virus³. For cells infected with herpes simplex, this inhibition is partly reversible when thymidine is added together with the drug⁴.

Close similarities in the structure and development of the virus particles of infectious laryngotracheitis (ILT) and those of the herpes group of viruses were noted in a previous report⁵. A more recent study⁶ has demonstrated marked similarities in the ether sensitivity and fine structure of these particles and in the type of cytopathic effect produced when they are propagated in cultured chicken embryo kidney (CEK) cells. While these observations suggest a close resemblance between members of the herpes virus group and ILT virus, they are not sufficient to allow chemical similarities to be presumed. In an effort, therefore, to obtain information as to the type of nucleic acid which is present in ILT virus, experiments were performed to determine the effects of IUdR on the growth of ILT and herpes simplex virus (HS) in CEK monolayer cultures.

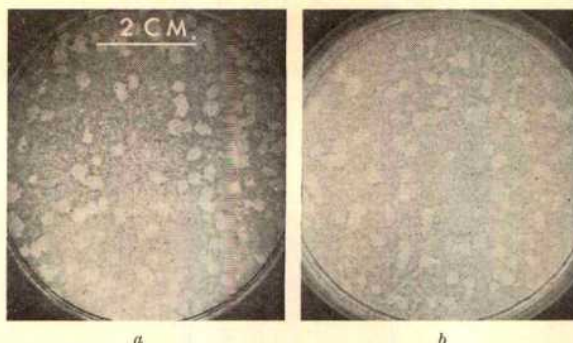


Fig. 1. Plaques produced in CEK monolayers by (a) ILT and (b) HS

A plaque system previously described for ILT⁷ was used for the assay of both viruses. Modifications to this method included the use of a growth medium described elsewhere⁸ for chicken fibroblasts and 6-cm plastic Petri dishes specially coated for use in cell culture (Falcon Plastics, Baltimore Biological Coy., Md., U.S.A.). The cultures were stained with neutral red diluted 1:10,000 in phosphate buffered saline (PBS), 5 days after inoculation with ILT, and 7 days after inoculation with HS. Plaques were counted the day after the addition of stain. The morphology of plaques produced by both viruses was similar, but those due to ILT appeared sooner. Plaques produced by both types of virus are shown in Fig. 1.

In the growth experiments the HFEM strain of HS was used and was obtained as yolk-sac propagated material from Mr. I. Jack of the Royal Children's Hospital, Melbourne. It was passaged twice through CEK and gave a titre of 1.57×10^7 plaque-forming units (P.F.U.) per ml. A lyophilized preparation of the Queensland strain of ILT was used, an egg-propagated strain of moderate virulence with a plaque titre of approximately 10^6 P.F.U. per ml.

Cell monolayers, washed with PBS, were inoculated with 1.0-ml. amounts of each virus and allowed to adsorb for 1 h at 37° C, in a humidified incubator gassed with 5 per cent CO₂. Unadsorbed virus was then removed by rinsing 3 times with PBS. Five ml. of a maintenance medium, which differed from the growth medium in that it contained only 2 per cent calf serum, was added. Concentrations of IUdR of 0, 10, 100, or 1,000 γ /ml. were included in the medium, the latter concentration being the threshold of toxicity for established monolayers of other cells⁹. All cultures were then returned to the incubator.

Whole cultures containing each drug-level were collected 24, 48 or 72 h after infection. Cells were removed from the culture dish with a rubber 'policeman' and, after pipetting several times, the cell clumps were treated for 1 min by a Mullard ultrasonic generator—a procedure found to break up cells effectively and to release more virus than could be obtained by freezing and thawing. Cultures containing no drug were treated in a similar fashion immediately after

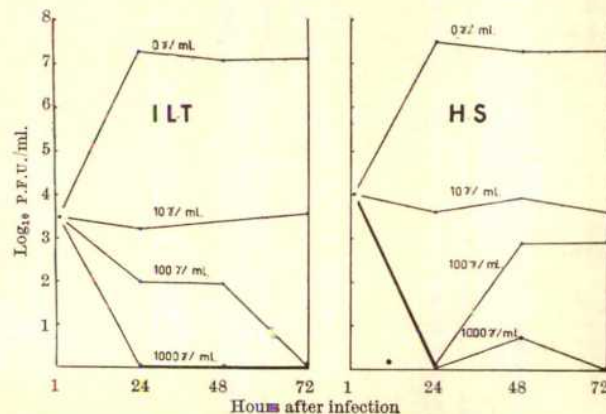


Fig. 2. The growth curves of ILT and HS in CEK monolayers in the presence of varying concentrations of IUdR

adsorption and washing, to provide an estimate of the 'background' level of virus associated with cells during the eclipse phase of the growth cycle. All samples were snap frozen and stored at dry ice-box temperatures until assays could be carried out.

Results of one experiment are shown in Fig. 2. This indicates that considerable inhibition of both viruses occurred at all concentrations of the analogue studied. The recovery of HS at higher drug concentrations after treatment for 24 h may have been due to the survival of IUdR resistant mutants⁹ of HS and their subsequent replication in later cycles of growth.

The inhibition of both viruses by IUdR, when grown under identical conditions, indicates that the nucleic acid of ILT is DNA, and provides further evidence for the inclusion of this virus within the herpes virus group.

Note added in proof. Since this letter was submitted for publication, evidence has come to hand that some halogenated derivatives of 2'-deoxyuridine, notably 5-bromo-2'-deoxyuridine, inhibit the multiplication of certain RNA viruses which require the participation of DNA at an early stage of multiplication (Bader, *Virology*, **22**, 462; 1964; Thormar, *ibid.*, **26**, 36; 1965). However, in conjunction with other reports of similarities between ILT and HS, a virus known to contain DNA, the parallel inhibition of both viruses by IUdR must be regarded as further evidence that the nucleic acid of ILT is DNA.

G. A. TANNOCK

Viral Products Laboratory,
National Biological Standards Laboratory,
Parkville N.2, Victoria, Australia.

¹ Salzman, N. P., *Virology*, **10**, 150 (1960).

² Herman, E. C., jun., *Proc. Soc. Exp. Biol. and Med.*, **107**, 142 (1961).

³ Prusoff, W. H., Bakhle, Y. S., and McCrea, J. F., *Nature*, **199**, 1310 (1963).

⁴ Roizman, B., Aurelian, L., and Roane, P. R., jun., *Virology*, **21**, 482 (1963).

⁵ Watrach, A. M., Vatter, A. E., Hanson, L. E., Watrach, M. A., and Rhoades, H. E., *Amer. J. Vet. Res.*, **20**, 537 (1959).

⁶ Fitzgerald, J. E., and Hanson, L. E., *Amer. J. Vet. Res.*, **24**, 103, 1297 (1963).

⁷ Howes, D. W., Tannock, G. A., and Sinkovic, B., *Proc. Twelfth World's Poultry Congress, Sydney*, Sect. Papers, 344 (1962).

⁸ Rubin, H., *Virology*, **10**, 29 (1960).

⁹ Buthala, D. A., *Proc. Soc. Exp. Biol. and Med.*, **115**, 69 (1964).

SOIL SCIENCE

Measurement of Exchangeable Aluminium in Acid Soils

MANY acid soils are known to contain exchangeable aluminium, but no satisfactory quantitative method for its determination has hitherto been available. This has proved to be a handicap in investigations of the degree of saturation with metal cations, suspected toxicity of adsorbed aluminium to crop plants as well as other soil properties influenced by exchangeable aluminium.

Neutral solutions of various salts will displace a definite amount of aluminium under standard experimental conditions. The quantity of aluminium released in this way may exceed the cation exchange capacity of the soil, so that some way must be found for distinguishing between the exchangeable and non-exchangeable components present in the extract. A technique for estimating this latter function is proposed here.

Exchangeable aluminium is displaced with difficulty by other cations, but it can be effectively removed by a long series of successive extractions. If it is assumed that the amount of non-exchangeable aluminium dissolved during each extraction in the series is constant, then the sum of the contributions from this source can be subtracted from the total extractable aluminium to give an estimate of exchangeable aluminium. For this approach to be successful, experimental conditions with respect to soil-to-solution ratio, temperature and period of contact must be kept constant during extraction.

In accordance with a Nernst type distribution, it may be expected that with each successive extraction, a steadily decreasing amount of exchangeable aluminium

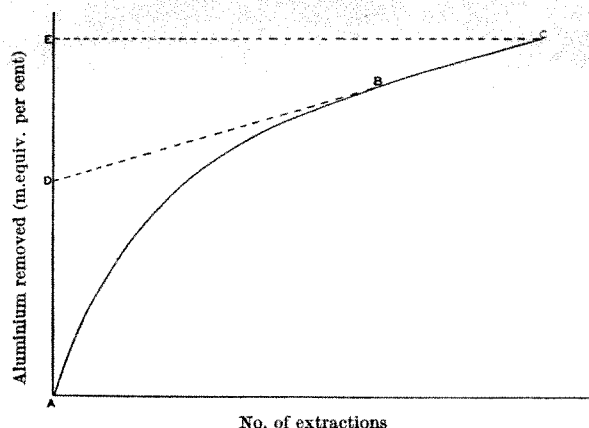


Fig. 1. Characteristic curve for the progressive extraction of aluminium from acid soils

will be released together with a constant quantity of non-exchangeable aluminium. The Nernst curve should thus become linear if continued beyond the point at which all exchangeable aluminium has been removed.

In Fig. 1, the curve *AB* represents the progressive removal of both forms of aluminium and the linear part *BC* that of non-exchangeable aluminium only. The amount of the latter removed per extraction will determine the slope of *BC* which will also depend largely on the solubility of the solid phase aluminium compounds. When *BC* is extrapolated, *AD* will represent exchangeable and *DE* non-exchangeable aluminium.

The experimental procedure is as follows. Place 5 g air-dry soil in a tared 100-ml. centrifuge tube and add 50 ml. extracting solution adjusted to the field pH of the soil; shake for exactly 2 min in a reciprocating shaker. Centrifuge and decant the clear supernatant into a suitable container. Re-weigh the tube so as to obtain the weight of the occluded solution; add a further 50 ml. of extractant, shake, centrifuge and decant. This procedure is repeated 24 times, decanting into a separate container each time. Determine the aluminium present in each extract and plot the cumulative data as shown in Fig. 1.

Exchangeable aluminium measured by this technique appears to be a characteristic of the particular soil and is reasonably constant irrespective of the nature of the extractant as shown in Table 1.

Table 1. EXCHANGEABLE AND NON-EXCHANGEABLE ALUMINIUM EXTRACTED BY DIFFERENT SALT SOLUTIONS

Solution	Aluminium removed (m.equiv. per cent)		
	Total aluminium	Non-exchangeable aluminium*	Exchangeable aluminium
0.2 N KCl	5.7	0.075	3.9
0.2 N NH ₄ Cl	6.0	0.079	4.0
0.2 N CaCl ₂	5.4	0.074	3.6
0.2 N NH ₄ NO ₃	6.0	0.087	3.8
N NaCl	6.1	0.090	3.8

* Per extraction.

Aluminium was determined by the method of Frink and Peech¹.

The sample used was from the *A*₁ horizon of a highly weathered acid ferrallitic soil with a pH value of 4.5 in water and 3.8 in N potassium chloride. It has a cation exchange capacity of 6.0 m.equiv. per cent with a base saturation of 20 per cent (excluding aluminium). The five extracting solutions were all adjusted to pH 4.0, which was regarded as the field pH value of the soil. Twenty-four separate aluminium determinations are required for each estimate of exchangeable aluminium, so that differences between the five extractants could be due to experimental error. Exchangeable hydrogen probably accounts for the difference between cation exchange capacity and total bases including exchangeable aluminium.

J. B. SKEEN
M. E. SUMNER

Department of Soil Science,
University of Natal, Pietermaritzburg.

¹ Frink, C. R., and Peech, M., *Soil Sci.*, **93**, 317 (1962).

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, November 15

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 10.30 a.m. and 2.30 p.m.—Colloquium on "Phase Measurement Throughout the Spectrum".

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Dr. D. E. Stevenson: "The Assessment of Possible Health Hazards Associated with the Use of Pesticides".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. J. Ralph Audy (U.S.A.): "Red Mites and Typhus. V. Old and New Horizons".*

INSTITUTION OF MECHANICAL ENGINEERS, MANUFACTURE AND MANAGEMENT GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "The Engineer and the Law".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Prof. Collin Cherry: "The Nature of Human Communication". (First of three Cantor Lectures on "World Communication").

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (at the College of Further Education, Hatfield Road, St. Albans), at 7.30 p.m.—Dr. J. S. Gourlay and Dr. C. K. Warren: "The Chemist's Contribution to the Building Industry of the Future".

Tuesday, November 16

UNIVERSITY OF LONDON (at Imperial College of Science and Technology, London, S.W.7), at 1.30 p.m.—Mr. E. F. Schumacher: "A Map of Knowledge: Seeing Where Everything Belongs".*

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, Westminster, London, S.W.1), at 5.30 p.m.—Mr. P. A. Scott: "Port of Tema".

PARLIAMENTARY AND SCIENTIFIC COMMITTEE, GENERAL COMMITTEE (in Committee Room 12, House of Commons, Westminster, London, S.W.1), at 5.30 p.m.—Discussion on "Scientific Aspects of Food Additives", opened by Prof. R. A. Morton, F.R.S., Prof. A. C. Frazer, C.B.E., and Dr. L. Golberz.

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. F. J. C. Roe: "Factors Involved in Lung Cancer Causation". (Sixth of Fifteen Lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)*

INSTITUTION OF MECHANICAL ENGINEERS, PROCESS ENGINEERING GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Application of Non-Destructive Testing to Process Plant Inspection".

Wednesday, November 17

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 2 p.m.—Prof. I. H. Mills: "The Relationship of Adrenal Androgen to Cortisol Secretion".*

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 5 p.m.—Mr. David Oates: "Tell Al Rimah—The Temple and Ziggurat".

ROYAL METEOROLOGICAL SOCIETY (at 49 Cromwell Road, London, S.W.7), at 5 p.m.—Mr. E. J. Williamson and Mr. J. T. Houghton: "Radiometric Measurements of Emission from Stratospheric Water"; Mr. A. W. Brewer and Mr. A. W. Wilson: "The Measurement of Solar Ultraviolet Radiation in the Stratosphere"; Mr. D. O. Staley: "Radiative Cooling in the Vicinity of Inversions and the Tropopause".

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, RADAR GROUP (at 9 Bedford Square, London, W.C.1), at 5.30 p.m.—Dr. P. G. F. Caton: "The Use of Doppler Radar in Meteorological Research".

WORTHFUL SOCIETY OF APOTHECARIES OF LONDON, FACULTY OF THE HISTORY OF MEDICINE AND PHARMACY (at Black Friars Lane, Queen Victoria Street, London, E.C.4), at 5.30 p.m.—Dr. T. D. Whittet: "The Apothecaries in the Great Plague of London, 1665" (Sydenham Lecture).

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 6 p.m.—Prof. Erwin Meyer: "Electroacoustics".

INSTITUTION OF MECHANICAL ENGINEERS, INTERNAL COMBUSTION ENGINES GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Mr. F. J. Wallace and Mr. E. J. Wright: "Characteristics of a Two-Stroke Opposed-Piston Compression-Ignition Engine Operating at High Pressure".

SOCIETY OF ENVIRONMENTAL ENGINEERS (in the Mechanical Engineering Department, Imperial College, Exhibition Road, London, S.W.7), at 6 p.m.—Mr. R. Blanchflower: "Damping Properties of Engineering and Viscoelastic Materials".

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 6 p.m.—Prof. Leon Festinger (Stanford): "The Role of Efference in Visual Perception". (Further lectures on November 18 and 19.)*

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (at the Angel Hotel, 109 High Road, Ilford), at 7.30 p.m.—Dr. H. G. Rains: "The Chemistry of Paint Formulation".

Thursday, November 18

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (joint meeting with the British Society for Parasitology, at Manson House, 26 Portland Place, London, W.1), at 2.30 p.m.—Symposium on "The Pathology of Parasitic Diseases". 7.30 p.m.—Laboratory Meeting at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1.

OPERATIONAL RESEARCH SOCIETY (at the Royal Aeronautical Society, 4 Hamilton Place, London, W.1), at 4.30 p.m.—Mr. H. Boothroyd and Mr. M. F. Shutter: "Beyond Stock Control".

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. T. C. Askwith, Mr. A. Cameron, Mr. R. F. Crouch and Mr. R. Gohar: "The Surface Chemistry of Hydrodynamic Lubrication of Point Contacts".

INSTITUTION OF MINING AND METALLURGY (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Mr. A. A. T. Finn: "Tailing Dam Construction at Mufuhura Copper Mines, Ltd., Zambia"; Mr. J. Pereira and Mr. C. J. Dixon: "Evolutionary Trends in Ore Deposition".

LONDON MATHEMATICAL SOCIETY (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Annual General Meeting. Prof. A. G. Walker: "Harmonic Spaces in Differential Geometry" (Presidential Address).

INSTITUTE OF PETROLEUM, INFORMATION DISCUSSION GROUP (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. Leslie Wilson: "Information for the Technologist: Prospects for Improved Services".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Mr. H. S. Wolff: "Problems of Advanced Technology in Medicine". (Seventh of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)*

INSTITUTE OF REFRIGERATION (at Lloyd's Register of Shipping, 71 Fenchurch Street, London, E.C.3), at 6 p.m.—Informal Discussion on "The Refrigeration Industry and the Metric System".

SOCIETY OF CHEMICAL INDUSTRY, ROAD AND BUILDING MATERIALS GROUP (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Mr. G. E. Bessey: "Current Developments Affecting the Design and Use of Mortars for Building Purposes".

Friday, November 19

INSTITUTE OF NAVIGATION (at the Royal Aeronautical Society, 4 Hamilton Place, London, W.1), at 5.30 p.m.—Mr. T. G. Thorne: "Multi-purpose Airborne Radar".

INSTITUTION OF ELECTRICAL ENGINEERS, CONTROL AND AUTOMATION DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Instrument Scale Graduator" opened by Mr. L. B. S. Golds.

ROYAL INSTITUTE (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. J. M. Bruckshaw: "The Channel Tunnel".

Friday, November 19—Saturday, November 20

ZOOLOGICAL SOCIETY OF LONDON; MAMMAL SOCIETY OF THE BRITISH ISLES; and ASSOCIATION FOR THE STUDY OF ANIMAL BEHAVIOUR (in the Meeting Room of the Zoological Society of London, Regent's Park, London, N.W.1), at 10.30 a.m. daily—Symposium on "Play, Exploration and Territory in Mammals".

Saturday, November 20

INNER LONDON EDUCATION AUTHORITY (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Mr. R. D. MacCuaig: "Modern Methods of Locust Control".*

Monday, November 22

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 5 p.m.—Major N. J. D. Prescott, R.E.: "The Geodetic Satellite—SECOR".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. H. Braunsteiner (Innsbruck): "Clinical Significance of Triglyceride Determinations, Especially in Relation to Age, Body Weight, Different Forms of Diabetes and Thyroid Disease".*

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Prof. Collin Cherry: "The Communication Explosion". (Second of three Cantor Lectures on "World Communication").

SOCIETY FOR VISITING SCIENTISTS (at The English-Speaking Union, Dartmouth House, 37 Charles Street, London, W.1), at 7.30 p.m.—Discussion Meeting on "The Retrieval of Scientific Information". Chairman: Dr. H. T. Hookway. Speakers: Prof. E. Kaiser, Dr. W. Batten and Mr. A. St. Johnston.

Monday, November 22—Tuesday, November 23

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2)—Conference on "U.H.F. Television".

Monday, November 22—Friday, November 26

INSTITUTE OF METALS (at the Royal Commonwealth Society, Craven Street, London, W.C.2)—Third International Conference on "Plutonium".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER or ASSISTANT LECTURER IN STATISTICS IN THE EDUCATION DEPARTMENT, to be concerned with research with the work of advanced students and with teaching—Prof. B. A. Peel, Department of Education, The University of Birmingham, Birmingham 15 (November 17).

LECTURER (with a research bias and a physics/electronics background) IN THE DEPARTMENT OF ELECTRICAL AND CONTROL ENGINEERING—The Secretary, Battersea College of Technology, Battersea Park Road, London, S.W.11 (November 19).

LECTURERS or ASSISTANT LECTURERS IN MATHEMATICS in the School of Mathematics and Physics—The Registrar, University of East Anglia, Earlham Hall, Norwich, NOR 88C (November 20).

READERS, SENIOR LECTURERS, LECTURERS or ASSISTANT LECTURERS IN PHYSICS in the School of Mathematics and Physics—The Registrar, University of East Anglia, Earlham Hall, Norwich, NOR 88C (November 20).

DEMONSTRATOR (preferably honours graduate in agriculture or agricultural botany) IN AGRICULTURE (Crop Agronomy)—The Registrar, University of Nottingham, Nottingham (November 22).

RESEARCH ASSISTANT (preferably with a veterinary degree) IN VETERINARY MICROBIOLOGY, to join a group working on the pathogenesis of enteric infections—The Registrar, The University, Liverpool, 3, quoting Ref. CV/328/N (November 22).

CHAIR IN STATISTICS—The Secretary, Royal College of Advanced Technology, Salford 5, Lancashire (November 25).

LECTURER or ASSISTANT LECTURER IN GEOGRAPHY, with particular reference to physical geography—The Assistant Secretary, London School of

Economics and Political Science, Houghton Street, Aldwych, London, W.C.2 (November 25).

CHAIR OF PHYSIOLOGY—The Secretary of the University Court, University of Glasgow, Glasgow (November 26).

LECTURER IN ANALYTICAL OR PHYSICAL CHEMISTRY—The Clerk to the Governors, Woolwich Polytechnic, London, S.E.18 (November 26).

EXPERIMENTAL OFFICER (interested in the design and construction of equipment for a wide range of psychological experiments, and preferably with a knowledge of electronics) IN THE DEPARTMENT OF PSYCHOLOGY—The Registrar, The University, Manchester, 13, quoting Ref. 195/65 (November 30).

LECTURER (Grade II) IN MATHEMATICS (Pure or Applied)—The Registrar, University Senate House, Bristol, 2 (November 30).

READER AND LECTURERS (2) IN ANATOMY; and a LECTURER (with a degree in pharmacology, physiology or pharmacy) IN PHARMACOLOGY at Makerere University College, Uganda (University of East Africa)—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (November 30).

READER (preferably, but not necessarily, qualified in a branch of classical applied mathematics) IN APPLIED MATHEMATICS—The Secretary, Battersea College of Technology, Battersea Park Road, London, S.W.11 (November 30).

LECTURER (Grade II) (preferably with special interests in one of the following fields: invertebrate functional morphology, respiratory physiology or fish physiology) IN ZOOLOGY—The Registrar, University Senate House, Tyndall Avenue, Bristol, 2 (December 1).

SENIOR LECTURER IN VETERINARY MEDICINE (small animals)—The Secretary, The Royal Veterinary College (University of London), Royal College Street, London, N.W.1 (December 1).

CHAIR OF MATHEMATICS at the London School of Economics and Political Science—The Academic Registrar, University of London, Senate House, London, W.C.1 (December 2).

SENIOR LECTURERS, LECTURERS and ASSISTANT LECTURERS IN THE DEPARTMENT OF STATISTICS—The Secretary, University of Edinburgh, Edinburgh (December 10).

LECTURER (suitably qualified graduate with special qualifications in educational foundations (history and theory) or in educational sociology) IN EDUCATION at the University of Otago, Dunedin, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, December 15).

SENIOR TECHNOLOGICAL OR TECHNOLOGICAL (with experience in museum work and photography, in addition to histopathology) IN PATHOLOGY in the Faculty of Veterinary Science—The Registrar, University of Khartoum, P.O. Box 321, Khartoum, Sudan (December 22).

VISITING PROFESSOR IN VETERINARY PATHOLOGY—The Registrar, University of Khartoum, P.O. Box 321, Khartoum, Sudan (December 22).

CHAIR OF AGRONOMY at the University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Australia, December 31.)

CHAIR OF LOGIC AND METAPHYSICS—The Secretary, University of St. Andrews, College Gate, St. Andrews, Fife, Scotland (December 31.)

CHAIR OF PHARMACEUTICS at the University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, December 31.)

CHAIR OF SOCIOLOGY at Victoria University of Wellington, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, December 31.)

CHAIR OF VETERINARY ANATOMY at the University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Australia, December 31.)

SECOND CHAIR OF BIOLOGY—The Secretary, University of Lancaster, Bailrigg House, Lancaster (December 31.)

FISHERIES OFFICER (national of the United Kingdom or the Republic of Ireland, with a good science degree and experience of fisheries management, including marketing and processing) IN MALAWI, to collect statistics and develop and demonstrate to local fishermen improved methods of fishing appropriate to local conditions—The Appointments Officer, Ministry of Overseas Development, Room 301, Eland House, Stag Place, London, S.W.1, quoting Ref. RC.289/134/01.

HEAD OF THE DEPARTMENT OF MATHEMATICS (Grade IV)—The Clerk to the Governing Body, Northern Polytechnic, Holloway, London, N.7.

LECTURER OR ASSISTANT LECTURER (well-qualified physicist and preferably with experience of the application of physics in industry) IN PHYSICS—The Registrar, Bradford Institute of Technology, Bradford, 7.

LECTURER (well qualified in physics and preferably experience in some branch of electronics) IN THE DEPARTMENT OF PHYSICS—Clerk to the Governing Body, Northern Polytechnic, Holloway, London, N.7.

MICROBIOLOGISTS—Dr H. B. Noel, Chairman, Department of Biology, University of Waterloo, Ontario, Canada.

PROFESSOR OF STATISTICS AND OPERATIONAL RESEARCH—The Registrar, Bradford Institute of Technology, Bradford, 7.

PROFESSOR IN PHYSICAL CHEMISTRY—Prof Harry E. Gunning, Head, Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada.

RESEARCH ASSISTANT (qualified in chemical engineering, physics, chemistry or other related discipline) IN THE DEPARTMENT OF CHEMICAL ENGINEERING, to work on a Ministry of Technology research contract investigating the liquid-liquid extraction of metal ions—The Academic Registrar, Loughborough College of Technology, Loughborough, Leicestershire, quoting Ref. 37/2/AF.

RESEARCH TECHNICIAN (with qualifications, not necessarily a degree, or experience in microbiology, biochemistry or chemistry) IN THE DEPARTMENT OF BIOCHEMISTRY for work in bacterial enzymology—The Registrar, The University, Liverpool, 3, quoting Ref. 329/N.

TECHNICAL ASSISTANT (preferably with some experience in radioactive counting techniques) to work with a whole-body radioactivity counter used in clinical research—The Secretary, Department of Radiotherapeutics, Addenbrooke's Hospital, Cambridge.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Medicine in 1815. (An Exhibition to commemorate the 150th Anniversary of the end of the Napoleonic Wars, 15 April to 31 December 1965) Pp. 43. (London: The Wellcome Historical Medical Museum and Library, The Wellcome Building, 1965.) [10]

Commonwealth Mycological Institute. Mycological Papers, No. 100: Index to Mycological Papers, Nos. 1-99 (1925-1965) Compiled by Joan

E. P. Hickman. Pp. 59. 10s. net. Phytopathological Papers, No. 6: A List of Plant Pathogenic and Other Fungi of Cyrenaica (Libya). By J. Kranz. Pp. 24. 7s. 6d. net. (Kew. Commonwealth Mycological Institute, 1965.) [110]

Britain's Part in the New Scientific Industrial Revolution. By Prof. J. D. Bernal. (Forty-third Earl Grey Memorial Lecture delivered at the University of Newcastle upon Tyne, 17th May, 1964.) Pp. 19. (Newcastle upon Tyne: The University, 1965.) 3s. [110]

Society of Instrument Technology. Careers with Instruments. Pp. 24. (London: Society of Instrument Technology, 1965.) 2s. 6d. [110]

The British Glass Industry Research Association. Tenth Annual Report, 1965. Pp. 42. (Sheffield: The British Glass Industry Research Association, 1965.) [110]

The Library Association. Special Subject List No. 46. Religion and Science. By A. Balthy. Pp. 36. (London: The Library Association, 1965.) 10s. (L.A. Members 7s. 6d.) [110]

Other Countries

Vertebrate Paleontology in Alberta: Report of a Conference held at the University of Alberta, August 29-September 3, 1963. Pp. 76. (Edmonton: University of Alberta, 1965.) [410]

United States Department of the Interior: Geological Survey. Bulletin 1201-C: Geology of the Wayland Quadrangle, Stephens and Eastland Counties, Texas. By Donald A. Myers. Pp. v+63+plates 1-3. Bulletin 1214-B: Humate in Coastal Sands of Northwest Florida. By Vernon E. Swanson and James G. Palacas. Pp. iv+29. Water-Supply Paper 1594-C: Artificial Recharge in Oregon and Washington, 1963. By Don Price, D. H. Hart and B. L. Foxworthy. Pp. iv+63+plates 1 and 2. Water-Supply Paper 1644: Quality of Surface Waters of the United States 1959. Parts 7 and 8: Lower Mississippi River Basin and Western Gulf of Mexico Basins. Prepared under the direction of S. K. Love. Pp. xi+508. 1.75 dollars. (Washington, D.C.: Government Printing Office, 1965.) [410]

United States Department of the Interior: Geological Survey. Water-Supply Paper 1770-U: Geology and Ground-Water Resources of Dane County, Wisconsin. By Denzel R. Cline. Pp. v+64+plates 1-8. Water-Supply Paper 1809-L: Reconnaissance of the Ground-Water Resources in Parts of Larimer, Logan, Morgan, Sedgwick, and Weld Counties, Colorado. By William G. West, Jr. Pp. iv+24+plate 1. (Washington, D.C.: Government Printing Office, 1965.) [410]

Zoologica Africana, Vol. 1, No. 1: Proceedings of a Symposium on African Mammals held at Salisbury, Rhodesia, 26-28 September, 1963. Edited by M. K. Rowan. Pp. 265. (Cape Town: Zoological Society of Southern Africa, 1965.) R. 2 50; 25s. [410]

Organization for Economic Co-operation and Development: European Nuclear Energy Agency. World Uranium and Thorium Resources. Pp. 22. (Paris: Organization for Economic Co-operation and Development, 1965.) [410]

United States Department of Commerce: National Bureau of Standards. Technical Note No. 18-22: Quarterly Radio Noise Data, March-May, 1964. By W. Q. Crichlow, R. T. Disney and M. A. Jenkins. Pp. 96. (Washington, D.C.: Government Printing Office, 1965.) 50 cents [410]

United States Department of the Interior: Fish and Wildlife Service. Bureau of Sports Fisheries and Wildlife. Circular No. 211: Distribution of Alaskan Mammals. By Richard H. Manville and Stanley P. Young. Pp. iv+53. 60 cents. Circular No. 212: Characteristics of Fish Populations in Upper Mississippi River Backwater Areas. By Lyle M. Christensen and Lloyd L. Smith. Pp. iv+53. (Washington, D.C.: Government Printing Office, 1965.) [410]

Educational Services, Inc., Watertown, Mass. ESI Quarterly Report, Summer-Fall 1965. Pp. 267. (Watertown, Mass.: Educational Services, Inc., 1965.) [410]

United States Department of Commerce: Weather Bureau. Weather and Climate Modification. By Donald L. Gilman, James R. Hibbs and Paul L. Laskin. Pp. v+41. (Washington, D.C.: Government Printing Office, 1965.) [410]

Australia: Commonwealth Scientific and Industrial Research Organization. Techniques for Measuring Oceanic Primary Production Using Radioactive Carbon. By N. Dyson, H. B. Jitts and B. D. Scott. Pp. 12. (Melbourne: Commonwealth Scientific and Industrial Research Organization, 1965.) [410]

Transactions of the American Philosophical Society. New Series, Vol. 55, Part 4. David Ramsay, 1749-1816—Selections from his Writings. Edited with Introduction and Notes by Robert L. Brunschow. Pp. 250. (Philadelphia: The American Philosophical Society, 1965.) 6 dollars. [410]

Royal Society of New Zealand. Bulletin No. 7, Vol. 3: An Index to the Collembola. By Dr. J. T. Salmon. Pp. 645-651. (Wellington: Royal Society of New Zealand, 1965.) [410]

Annals of the South African Museum. Vol. 48, Part 7: A New Species of Flat Fish, *Mancopsetta mifordii*, from South Africa, with Notes on the Genus *Mancopsetta*. By M. J. Penrith. Pp. 181-188+1 plate. Vol. 48, Part 8: *Ctenogobius cloatis* Smith, 1960, a Synonym of *Ctenogobius saldanha* (Barnard, 1927). By Frank Hamilton Talbot and Mary-Louise Penrith. Pp. 189-193. Vol. 48, Part 9: Isopoda and Amphipoda Collected by the Hough Island Scientific Survey. By K. H. Barnard. Pp. 195-210. Vol. 48, Part 10: Studies on the South African Clitellidae. I. Description of a New Species of *Pavoclitellus*, and Redescription of *Gynoclitellus rotundifrons* (Barnard). By Mary-Louise Penrith. Pp. 211-217. Vol. 48, Part 11: Contribution to the Knowledge of the South African Species of the Genus *Ceramus* Latreille (Hymenoptera: Masaridae). By F. W. Gess. Pp. 219-231. Vol. 48, Part 12: The Russian Dinocerophan *Deuterosaurus*. By L. D. Boonstra. Pp. 233-236. (Cape Town: South African Museum, 1965.) [410]

Editorial and Publishing Offices of "NATURE"

MACMILLAN (JOURNALS) LIMITED

4 LITTLE ESSEX STREET, LONDON, W.C.2

Telephone Number: Temple Bar 6633. Telegrams: Phusis London W.C.2

Annual subscription: Inland £14, Overseas £13 15s., payable in advance, postage paid to any part of the world.

Advertisements only should be addressed to

T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2.

Telephone Number: Holborn 4743

Registered as a newspaper at the General Post Office

Copyright © Macmillan (Journals) Limited, November 13, 1965

NATURE

No. 5012

SATURDAY, NOVEMBER 20, 1965

Vol. 208

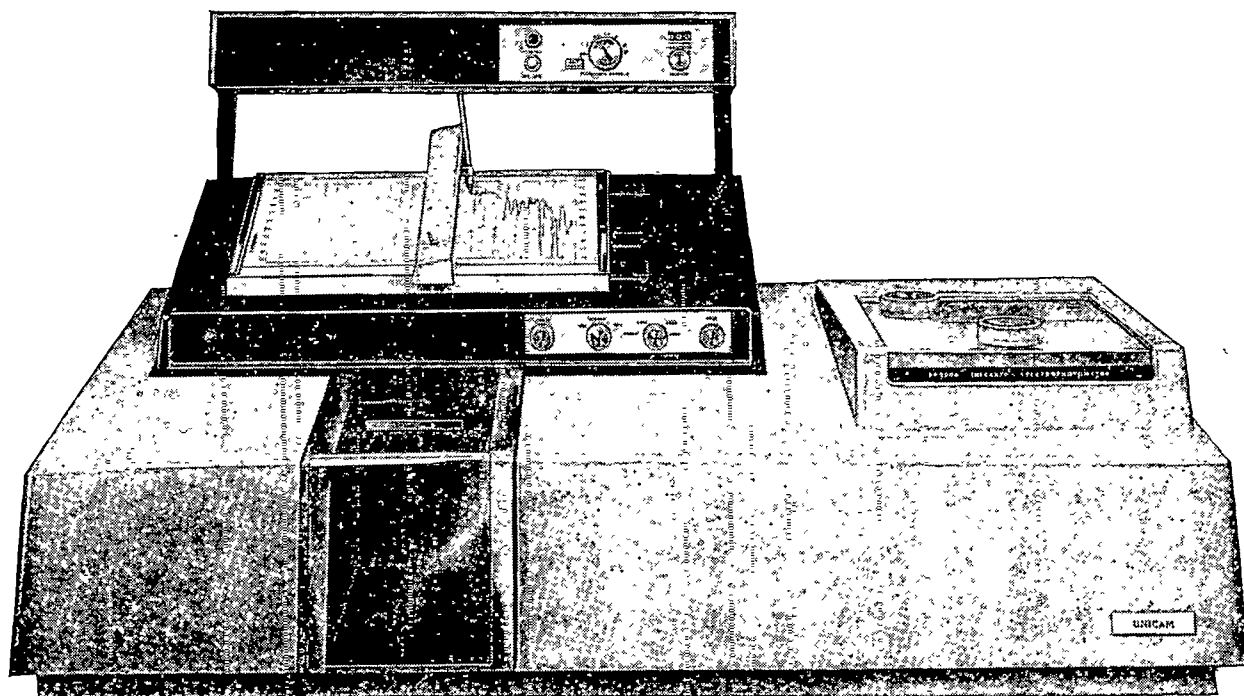
Published by
MACMILLAN (JOURNALS) LTD.

CONTENTS

	<i>Page</i>
TOWARDS A UNIVERSITY OF THE AIR	715
CITATION INDEXING. By C. W. Cleverdon	717
COMMUNICATION, THE SOCIAL WEB. By Prof. Colin Cherry	717
SOIL SCIENCE. By Dr. A. M. Smith	718
MANUFACTURE OF WOOD PULP. By Dr. Julius Grant	719
SEMICONDUCTOR CONTROLLED RECTIFIERS. By Prof. W. Fishwick	719
ALKALINE EARTH METALS. By Prof. Eleanor Zaimis	720
RESEARCH IN PHOTOGRAPHY. By K. O. Ganguin	720
A HUMANISTIC TECHNOLOGY. By Vice-Admiral H. G. Rickover, C.B.E.	721
MATERIALS SCIENCE AND ENGINEERING AT THE MASSACHUSETTS INSTITUTE OF TECHNOLOGY	726
THE WALTER AND ELIZA HALL INSTITUTE OF MEDICAL RESEARCH, MELBOURNE	729
OBITUARIES:	
PROF. T. P. HILDITCH, C.B.E., F.R.S. By P. N. Williams	730
PROF. C. B. PURVES. By Henry I. Bolker	731
NEWS AND VIEWS	732
ENZYMES NOMENCLATURE	736
INTERNATIONAL COMMITTEE ON LABORATORY ANIMALS. By Prof. J. A. B. Gray	738
BASIC COMPONENT OF SOLAR RADIO EMISSION AT CENTIMETRE AND DECIMETRE WAVELENGTHS. By Dr. M. K. Das Gupta and D. Basu	739
STRUCTURE OF THE RADIO CONTINUUM BACKGROUND AT HIGH GALACTIC LATITUDES. By Dr. M. J. S. Quigley and Dr. C. G. T. Haslam	741
AGE OF THE WEARDALE GRANITE. By F. J. Fitch and Dr. J. A. Miller	743
RECENT FINDS IN THE UPPER CARBONIFEROUS OF SOUTH-WEST ENGLAND AND THEIR SIGNIFICANCE. By Dr. H. G. Reading; Dr. J. E. Prentice	745
APPLICATION OF AN AGAR-AGAR DIFFUSION PROCEDURE TO PESTICIDE RESIDUE ANALYSIS AND TO THE CHOLINESTERASE SCREENING OF CANDIDATE PESTICIDES. By Dr. K. I. Beynon and G. Stoydin	748
EVALUATION OF ION EXCHANGE CARTRIDGES FOR FIELD SAMPLING OF IODINE-131 IN MILK. By R. H. Johnson, jun., and T. C. Reavey	750
EXAMINATION OF A POLYSACCHARIDE FLOCCULANT AND FLOCCULATED KAOLINITE BY ELECTRON MICROSCOPY. By Dr. Arnold Audsley and Anita Fursey	753
EFFECTS OF HYDROCORTISONE SUCCINATE ON THE COMPLEMENT SYSTEM. By Dr. Henry Gewurz, Paul R. Wernick, Dr. Paul G. Quie and Prof. Robert A. Good	755
CORRELATION BETWEEN UNIT ACTIVITY AND SLOW POTENTIAL CHANGES IN THE UNANESTHETIZED CEREBRAL CORTEX OF THE CAT. By A. D. J. Robertson	757
BLOOD FLOW IN TURTLES. By Fred N. White and Gordon Ross	759
BINDING OF XENON TO HORSE HAEMOGLOBIN. By Dr. B. P. Schoenborn	760
POLYMORPHISM AND MITOCHONDRIAL ACTIVITY IN SLEEPING SICKNESS TRYPANOSOMES. By Dr. Keith Vickerman	762
ANCESTRY OF SEA-STARS. By Dr. G. M. Philip; Prof. H. Barraclough Fell	766
NATURE OF THE NON-HAEM IRON IN FERREDOXIN AND RUBREDOXIN. By Dr. R. D. Gillard, Dr. E. D. McKenzie, Prof. R. Mason, S. G. Mayhew, Dr. J. L. Peel and J. E. Stangroom	769

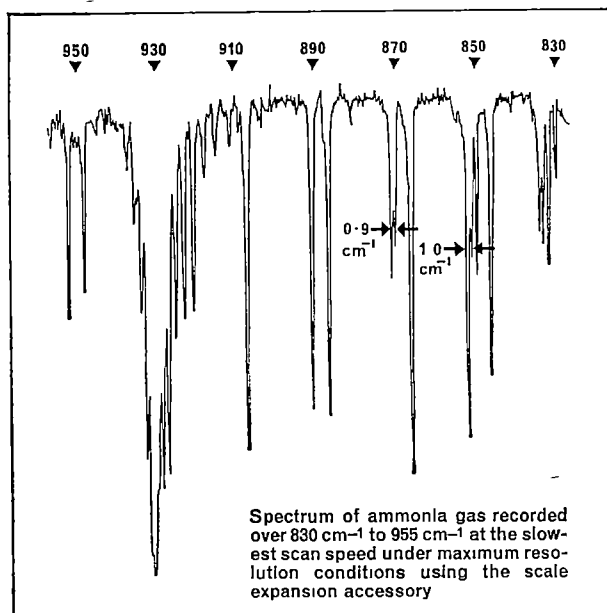
(Continued overleaf)

NEW grating SP200G infrared spectrophotometer



high resolution

Unicam now offers all the advantages of a grating filter monochromator combined with the proved features of the SP200 prism spectrophotometer. The first order of the NPL grating is isolated by four interference filters, brought into play by an automatic mechanism exclusive to Unicam. The resolution is as high as 3 cm^{-1} at 3000 cm^{-1} and stray light is less than 1% throughout most of the range and never exceeds 2%. The wavenumber accuracy is better than 2 cm^{-1} at 1000 cm^{-1} and 4 cm^{-1} at 3000 cm^{-1} . To this performance are added the advantages of the flat bed recorder, the accessible sample compartment and the convenient operation of the SP200. The new SP200G is constructed and finished to the high degree of precision for which Unicam instruments are famous. Descriptive literature is available on request.



Precision Spectrophotometers

UNICAM

Unicam Instruments Limited • York Street • Cambridge • Telephone: Cambridge 61631 • Telegrams: Unicam Cambridge • Telex: 81215

LETTERS TO THE EDITOR

	Page		Page
GEOPHYSICS		HISTOCHEMISTRY	
Antimatter and Tree Rings.—V. S. Venkatavaradan	772	A Lymphocyte-stimulating Factor produced <i>in vitro</i> .— Dr. J. Gordon and Prof. L. D. MacLean	795
PHYSICS		IMMUNOLOGY	
Delayed Fluorescence from Organic Crystals.—G. F. Moore and Dr. I. H. Munro	772	Fluorochrome Stains for Histological Diagnosis of Visceral Mycoses.—G. Bader, D. Stiller and K. Ruffert	796
Transmission through a Tapered Quartz Tube in the Laser Near Field.—H. J. Caulfield	773	PATHOLOGY	
Surface Charges on Arc Track Oxide Layers.—Dr. W. P. Jayasekara, Dr. A. E. Guile and Dr. K. A. Naylor	774	Inhibition of Growth of Malignant Cells <i>in vitro</i> by a Component of Normal Adult Connective Tissue.— Prof. Mary Stearns Parshley and Ines Mandl	800
METEOROLOGY		Isolation of <i>Cryptococcus neoformans</i> from Pigeon Habitats in London.—Dr. H. S. Randhawa, Dr. Y. M. Clayton and Dr. R. W. Riddell	
Determination of Cloud Altitudes from <i>Gemini-Titan-5</i> . —F. Saiedy, W. A. Morgan and Dr. D. Q. Wark	775	801	
Water Balance and Evaporation Studies.—M. Rama Rao	776	BIOLOGY	
CHEMISTRY		Activation of Spores of <i>Penicillium roqueforti</i> .—R. C. Lawrence	
Diperoxodiethylenetriamminechromium(IV) 1-hydrate: a New Chromium(IV) Peroxo Compound.—Dr. D. A. House and Prof. Clifford S. Garner	776	801	
Infra-red Spectra of Quaternary Ammonium Com- pounds adsorbed on Silica-gel 'Aerosil'.—Dr. L. C. F. Blackman and R. Harrop	777	Warted Pollen Grains of the Epacridaceae.—Dr. J. M. Matthews	
Inhibition of Corrosion of Copper in Chromic Acid.— K. F. Lorking	778	803	
Effect of Environment on the Orientation of the Nitrate Ions in Crystals.—Prof. N. R. Kunchur	778	Relationships between Constituents of Cones and Male Flowers of the Hop (<i>Humulus lupulus</i> , L.).— Dr. R. D. Hartley and Dr. R. A. Neve	
Ultrasonic Method of Determining the Critical Micelle Concentration of Surfactants.—J. Kuppusami and C. V. Suryanarayana	780	804	
Radiolysis of Sodium Tetrathionate Dihydrate.—Prof. R. L. Eager and Dr. D. S. Mahadevappa	781	Radiation-induced Sex Reversal in <i>Ecballium elaterium</i> . —César Gómez-Campo and Manuel Casas-Builla	
BIOCHEMISTRY		805	
Denaturation of Lactic Dehydrogenase Isozymes and its Clinical Application.—Dr. Aarne Konttinen and Seppo Lindy	782	Ingestion of Soil by Sheep in New Zealand in Relation to Wear of Teeta.—W. B. Healy and T. G. Ludwig	
Ultra-violet Spectroscopy in the Analysis of Chromato- grams.—Dr. Denis M. Abelson	784	806	
Accumulation and Incorporation of Amino-acid in Rat Intestine <i>in vitro</i> .—Dr. J. R. Bronk and Dr. D. S. Parsons	785	ENTOMOLOGY	
Changes in Amino-nitrogen Content of Solutions of γ -Keratose from Wool Keratin.—Dr. R. S. Asquith and P. A. Watson	786	Changes in Size of the Corpus Allatum in a Polymorphic Insect.—Dinah White	
Binding of Tetracyclines to Bone.—Dr. D. D. Perrin	787	807	
PHYSIOLOGY		Periodic Aggregating and Take-off in Anthophorid Bees, <i>Anthophora sraensis</i> Fab.—Prof. L. K. H. Goma	
Determination of Nicotine in Smokers' Urine by Gas Chromatography.—Dr. Neal L. McNiven, Kanaya H. Raisiniani, Sanford Patashnik and Prof. Ralph I. Dorfman	788	807	
Diurnal Variations in Ovarian Ascorbic Acid Content of Pseudopregnant Immature Rats used in Parlow's Test.—Dr. M. de la Lastra and Dr. H. Croxatto	789	Relationship between the Removal of the Frontal Ganglion and Protein Starvation in <i>Locusta migra- toria</i> L.—Dr. K. U. Clarke and C. Gillott	
Intracellular Chloride Activity of Crayfish Giant Axons.—Dr. A. Strickholm and B. G. Wallin	790	808	
PHARMACOLOGY		MICROBIOLOGY	
Effects of Propylthiouracil and Thyroid Feeding on the Response of Mice to Injected Convulsant Barbiturate.—Prof. Arthur J. Prange, jun., and Prof. Morris A. Lipton	791	Isolation of Bacteria able to metabolize Fluoroacetate or Fluoroacetamide.—Dr. M. Kelly	
Epileptogenic Effect of Extradural and Extracranial Cobalt.—Hushang Payan, Dr. Ralph Strebel and Prof. Seymour Levine	792	809	
Formation of Amines in the Gastric Mucosa of the Rat.—Rolf Håkanson	793	CYTOLOGY	
HAEMATOTOLOGY		Functional Similarities between the Cytoplasmic Organelles of Melanocytes and the Mitochondria of Hepatocytes.—Dr. Melvin H. Van Woert, Alexander R. Nicholson and Dr. George C. Cotzias	
A Factor stimulating DNA Synthesis derived from the Medium of Leucocyte Cultures.—Dr. Shinpei Kasakura and Prof. Louis Lowenstein	794	810	
		VIROLOGY	
		Growth and Cytopathogenicity of <i>H</i> -viruses in Human and Simian Cell Cultures.—Dr. H. Toolan and Dr. N. Ledinko	
		812	
		Stimulation of Sendai Virus Multiplication by Puro- mycin and Actinomycin D.—Dr. David O. White and Ian M. Cheyne	
		813	
		GENETICS	
		A Lactate Dehydrogenase Variant in the Mouse.— Mrs. Linda Riles	
		814	
		PSYCHOLOGY	
		A General Theory for the Evolution of Intelligent Behaviour.—Dr. David Stenhouse	
		815	
		Effect of Foster-mothers' Strain and Pre-natal Experi- ence on Adult Behaviour in Rats.—Dr. J. M. Joffe	
		815	

**FOR
PERIODS
UP TO
1 hour
24 hours
7 days**

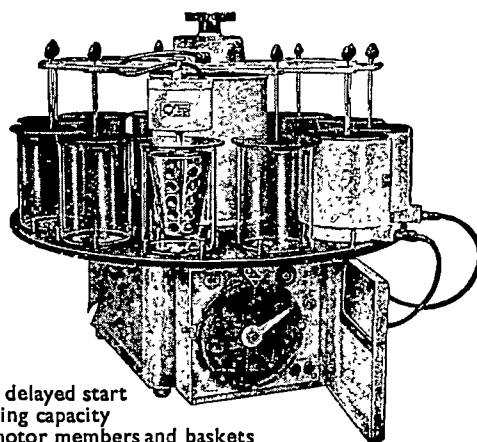
The

Histokinette

stays on the job

**Histokinette
special features
include:—**

- Up to 24 hours delayed start
- Double processing capacity
- One or more motor members and baskets
- Unbreakable tissue basket and containers



Diagnostic reports to hand hours earlier than they would be with manual processing and in far greater numbers—the release of a valuable technician for other duties... these are the outstanding advantages of the Histokinette Automatic Tissue Processing machine. Once the Histokinette has been put into operation, it will *without supervision* go through the entire cycle of fixation, dehydration, impregnation in wax, or alternatively can be set up for the washing or staining of tissues. It can handle large numbers of specimens at the same time and while staff is off duty—speed up almost any technique by ensuring quicker and more thorough penetration by reagents.

A 24-hour clock is fitted as standard. Change over to 1 hour or 7 day cycles (the latter of great value in double embedding and bone decalcification techniques) is simple and rapid.

Hendrey Relays

Write for illustrated brochure

The Hendrey Histokinette is in general use at home and overseas
... to quote a few in U.K. ... and some of our exports

Aberdeen	Dundee	London	Argentina	Eire	Italy
Bangor	Edinburgh	Manchester	Australia	Egypt	Japan
Belfast	Exeter	Newcastle	Austria	Finland	Mexico
Birmingham	Glasgow	Oxford	Belgium	France	New Zealand
Bristol	Gloucester	Sheffield	Brazil	Ghana	Nicaragua
Cardiff	Leeds	Southampton	Canada	Greece	Norway
	Liverpool	Swansea	Ceylon	Holland	Pakistan
			Chile	Hong Kong	S. Africa
			China	Hungary	Singapore
			Colombia	India	Sweden
			Cuba	Iran	Switzerland
			Denmark	Israel	Thailand

HENDREY RELAYS LTD. 390-394 BATH ROAD. SLOUGH. BUCKS. Telephone: Burnham 609/612
MANUFACTURING ELECTRICAL ENGINEERS. CONTROL & LABORATORY APPARATUS. Telegrams: Hendrelay, Slough
Sole Export Distributors: Measuring & Scientific Equipment Ltd., 25-28 Buckingham Gate, London, S.W.1.



PRECISION INSTRUMENTS

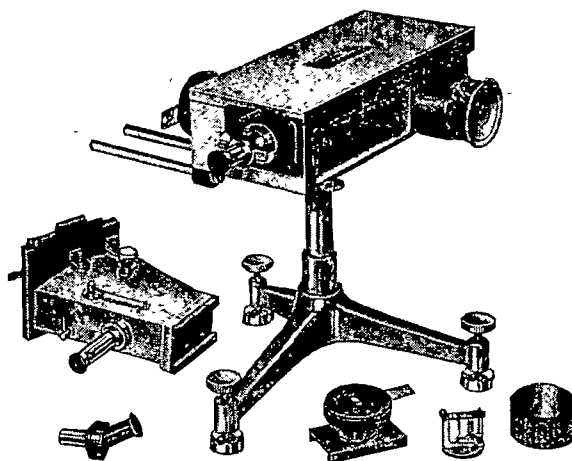
Direct Reading Wavelength Spectrometer

This is just one of the many fine instruments of our manufacture that can be found in daily use throughout the world.

As a large range of accessories can be supplied with this spectrometer it is a really universal instrument.

We will be pleased to recommend the equipment most likely to meet special requirements.

Please write for full details to Dept. N



SOLE DISTRIBUTORS IN U.K. OF SCHOTT INTERFERENCE FILTERS

Bellingham & Stanley Limited
71 HORNSEY RISE, LONDON, N.19. PHONE: ARCHWAY 2270

TOWARDS A UNIVERSITY OF THE AIR

IN the last of nine papers presented to Section L (Education) of the British Association for the Advancement of Science at its meeting in 1964, Dr. K. Adam discussed the responsibility of television: this responsibility has since been sharpened by the continuing discussions on the feasibility and the scope for a 'university of the air'. To that suggestion, indeed, Dr. Adam made passing reference, though he had reservations on the ground of costs. In his closing remarks he insisted that, however well such a development went, broadcasting, in the face of the imperious needs of education, was an aid to teaching and not a substitute for it, an ancillary and not a principal, a satellite but not a saviour. In this paper he was content to urge that television research was an essential part of the joint responsibility of the provider and the community he serves. It needed to be enlarged and given depth and we should no longer be asked to accept facile and unsupported opinions.

Beyond this, Dr. Adam suggested that the unique quality of television transmission was the transmission of experience. News was no longer 'then' but 'now'. Here, he thought, lay the special responsibility of broadcasting, and particularly of television. Among the most important services which television could undertake, he suggested, were those that could be rendered to the emergent and developing countries and in the field of science. Not only, in this way, could such services extend our comprehension of a dramatically changing world, but also they could show man himself, in the person of the scientist, as a growing creature, whose birthright is progress, and who, whether his subject is crystallography or cosmology, is a human being under the weight of his invention and his experiment. This kind of comprehension or reassurance in a world which is always more of man's making, and less the work of Nature, contributes directly to the permanent education which we need to keep us in a state of balance and of wonder.

If, therefore, this is the unique contribution of television, and one which it does supremely well because it is uniquely qualified for the purpose, we should be careful that other demands are not allowed to deflect it from such a function. It follows that this is a joint responsibility of the producer and of the community. It was thus strange to find that in a series of twelve articles on "The B.B.C.'s Duty to Society", published in *The Listener* during June–September 1965 (to which Dr. Adam himself contributed), almost no reference was made to this aspect. Indeed, only Baroness Barbara Wootton appeared to refer to the way in which broadcasting and television could contribute to spreading awareness of the social revolution of our time. She went on, in fact, to comment that broadcasting programmes gave her no sense of the march of science as the great intellectual adventure of our time. Neither these programmes nor the earlier contributions to the series of Sir Hugh Greene or Dr. Adam appeared to her to convey any sense of the universality of the process by which cumulative additions to knowledge are made, or of the unique opportunity of broadcasting to equip ordinary people with the mental tools, and to help them to form the mental beliefs appropriate to the age in which they live.

Such a suggestion is contradictory to Sir Hugh's affirmation that the British Broadcasting Corporation has

a duty to take account of the changes in society and to be ahead of public opinion rather than always to wait on it, and even against his admission that, at least in the secular and scientific fields, to-day's heresies often prove to be to-morrow's dogmas. That, however, was his only reference to science, which was not even mentioned by Dr. Adam—though he reiterated the point about news being 'now' which he made in his earlier paper. Furthermore, an editorial comment in *The Listener* on September 2 covering the whole series seems to indicate that the Corporation has missed the whole point of Baroness Wootton's criticism or even of Dr. Adam's address to the British Association.

In the light of all this we may now turn to consider the proposal for a university of the air, of which the Prime Minister said at Guildhall, London, in September, that, although much work remained to be done, the Government had gone a long way towards working out the educational functions and content. A high-powered educational advisory committee had reached agreement on an outline plan and had recommended that the university should offer primary courses leading to degrees and cover professional, technical and refresher courses. He was satisfied that the educational problems could be overcome, but admitted that there were big technical and financial problems. He hoped that discussions with the broadcasting authorities would start in the near future. Use of a fourth channel, perhaps on a shared or partnership basis, was not excluded.

In this context, research interest attaches to the statement, "University of the Air", issued in June by the University of Strathclyde. This discusses some aspects of the structure and organization of a national university of the air: arguments in favour of the establishment of such a university are regarded as overwhelming. Our normal educational resources are already so over-strained as to be incapable of fostering a dynamic and flexible method of educating and re-educating adults as well as children to fit new roles in a rapidly changing society. However, it would be unrealistic to suggest that these urgent and growing demands should be met by the British Broadcasting Corporation or the Independent Television Authority. Their output is different in essence and intent from that envisaged from a university of the air. While it can be argued that the 'University of the Air' should be independent also of existing academic institutions to give it the desired freedom to experiment, it would, for the time being, be wise to limit the new organization to a selected group of existing educational institutions. Such an arrangement would enable it to draw from them the co-operation and support necessary to further its experiments and validate their results.

It is suggested, accordingly, that, as a practical step, the Government should delegate responsibility for local development of the University of the Air to regional boards, on which would be represented educational interests sympathetic to the idea. The work of the regional boards would be co-ordinated under a United Kingdom Educational Television Authority. This Authority would also be responsible for establishing a national library of filmed programmes, and for gradually taking over the construction and maintenance of transmitter

stations. The production studios should remain in the control of regional boards and educational institutions. A structure of this kind should permit the advantages of centralization in cutting certain overheads in ensuring a common purpose, as well as those of decentralization in ensuring the maximum utilization of regional resources in teaching skills and technical facilities. Production of programme courses should be spread over those institutions best able to deal with particular subjects. It would be necessary for each regional board to undertake the preparation and dissemination of preparatory courses which would lead students to uniform entrance standards for the degree courses provided by the University of the Air.

The statement from the University of Strathclyde rejects as undesirable and uneconomic any attempt at 100 per cent coverage by broadcast television. This would be an inefficient exploitation of the four broadcast channels likely to be available for educational television. As the service provided by a University of the Air would attract at any time only a very small (although important) fraction of the whole population, it is suggested that it would be better to devise a means of reaching all parts of the country by a combination of methods, confining broadcast television to those areas and to those purposes for which it is best fitted. Accordingly, it is proposed that only the major conurbations, containing 60–70 per cent of the population, should be covered by broadcast television, using a small number of low-power transmitters, and serving the rest of the country by distributed film prints or programmes which could be projected in schools or halls or homes. This would make it possible to conserve the channels now available in the very-high-frequency band to the point where four or more channels could be made available at each transmitter station for simultaneous broadcasting.

Acceptance of 60–70 per cent coverage in this way does not mean that the public beyond the range of television and sound transmission would be unable to enrol at the University of the Air. It is proposed that all the programmes provided for television by the University should be recorded and prints of the original transmission can readily be circulated anywhere for viewing as already indicated. All the courses would be supported by carefully prepared lecture notes and reading lists, and each week all enrolled students would be required to submit exercises for marking and comment by tutors. Whereas in areas of sparse population, broadcasting becomes more expensive than supplying film prints, apart from the additional disadvantage of the inefficient use of broadcasting channels which are scarce, in areas of dense population the cost per student of providing film prints for group or individual viewing would far exceed that of reaching the students by broadcasting television. Experience gained at the University of Strathclyde suggests that total production costs of the 16-mm films would be about £100 per hour in the universities, as compared with £5,000 per hour quoted for the British Broadcasting Corporation's studios. Preliminary surveys carried out on behalf of the University of Strathclyde have already confirmed that local transmitters in Band IV with omnidirectional aerials sited at the centres of large cities and towns would only require low power of the order of 1 kW, or 4 kW of effective radiated power. The cost of such a transmitter is estimated at about £30,000.

This statement is obviously more optimistic than is Mr. B. Jackson, director of the National Extension College

in Cambridge—the experimental teaching unit set up in 1963 to pilot the concept of a University of the Air. Work began with 'Dawn University'—a series of first-year lectures from Cambridge broadcast before breakfast and limited to television teaching. This and later experience, such as the six-week television experiment called College of the Air on Anglia Television, lead Mr. Jackson to suggest, first, that the University of the Air should be designed as a research-based project at least for the next three years. Next, he suggests that considerable caution is desirable as to the audience or students to be attracted, quite apart from the question of numbers. Furthermore, his experience seems to indicate that the crucial element in a University of the Air would be correspondence tuition, and although he thinks a fourth channel might help, he does not regard it as ideal. It would be preferable to have the equivalent spread over rural radio and television channels, and he suggests that, looking at the problem from a primarily educational point of view, there are strong reasons for avoiding the confines of a separate television channel.

Mr. Jackson's caution does not necessarily invalidate the Strathclyde view, which fully recognizes the importance of further enquiry and that the steps recommended would only be experimental. It should be noted, however, that Mr. Jackson is insisting, above all, that decisions should be concerned with students and not simply with techniques. His caution is tinged with a realism which is distrustful of political idealism which does not rest firmly on ascertained facts and is merely wishful thinking. Some of those facts, moreover, lie outside the field of the Authority responsible for programmes for any such University of the Air. This is evident from a preliminary memorandum on "Broadcast Adult Education and Public Libraries"—the implication of which the Library Association has accepted.

This memorandum by Mr. J. F. W. Bryon points out that students enrolling for such courses will need access to a wide range of literature for which they will look largely to the public library. For that reason alone, liaison is desirable between those planning the courses and librarians, so that the implications for the provision of books may be taken into account from the earliest stages. So far as numbers of potential users are concerned, Mr. Bryon suggests it might help if enrolment forms were distributed and collected by public libraries. The memorandum also notes that the public library might frequently offer a convenient centre for viewing. It is, however, in regard to the content of reading lists that consultation between lecturers and librarians is particularly desirable, but it is not forgotten that many students may require instruction or guidance in the use of a library.

What emerges from all three contributions is the grave danger of allowing any project for a University of the Air to become the plaything of party politicians. The resources required are far too large for Britain to engage in any idealistic project which is not closely related to demands which have definitely been ascertained and for which adequate resources can be committed. This is an inescapable responsibility of Governments, but a responsibility nevertheless rests alike on the broadcasting and the educational authorities, including the universities, to see that assets and resources are not wasted and that programmes are directed to practical and realizable objectives. This is the responsibility of producer and community to which Dr. Adam referred.

CITATION INDEXING

Science Citation Index 1964 Annual Cumulation

An International Interdisciplinary Index to the Literature of Science and Technology in Eight Parts. Part 1: Citation Index, A-Duke, Cols. 1-3168, Part 2: Citation Index, Duke-Kofe, Cols. 3169-6584, Part 3: Citation Index, Koff-Roes, Cols. 6585-10000, Part 4: Citation Index, Roes-Zzeb and Anonymous, Cols. 10001-13518, Part 5: Citation Index, Patent Citation Index and Acknowledgements, Cols. 13519-16404, Part 6: Source Index, Anonymous and A-Geye, Cols. 1-2658, Part 7: Source Index, Geye-Ogre, Cols. 2659-5316, Part 8: Source Index, Ogre-Zz, Cols. 5317-7893. Editor: E. Garfield. (Philadelphia: Institute for Scientific Information, Inc., 1965.) 1,250 dollars.

THE *Science Citation Index* for 1961 was reviewed in *Nature*, 203, 446 (1964), when an account was given of the technique for using a citation index. The *Index* for 1964 is an improvement in many ways. It was published on a quarterly basis during the year, and the volumes reviewed here represent the annual cumulation. Whereas the *Index* for 1961 appeared as the result of a research grant from the National Science Foundation and the National Institutes of Health, the present index can be considered as the first to be issued on a production basis, and it is obviously an advantage that the publication delay has been reduced.

The other major change has been the inclusion of a list of all the citing papers. This greatly improves the convenience of using the *Index*, since, as is inevitable in this type of index, many of the citing papers will prove to be quite irrelevant for the subject of the search. With the earlier *Index*, this point could only be established by checking the actual journal; now, by referring to the list of citing papers, from the titles one can correctly reject many of the non-relevant papers. The physical price to be paid for this is that the number of volumes has increased from five to eight; the financial price is that the cost has risen to 1,250 dollars. Against this, there has been an increased coverage; the source journals have increased only to 700 from 613, but the separate journal papers have risen from 102,000 to 151,000. In addition, for the first time, patents are included, and the 47,000 patents issued in the United States in 1964 bring the total of source items up to 199,000. It is a disappointment to find that the standard computer print-face, severely reduced in size, is still being used, and also that the *Index* is still heavily biased towards the life sciences. It is to be hoped that the balance will be redressed in the *Index* for 1965, when, it is stated, more than 1,000 journals will be covered.

The present value and future potentialities of the *Science Citation Index* are difficult to assess. The cost is such that it can be considered prohibitive for individuals, yet for a library it would represent only a fraction of the cost of one human indexer. The usefulness of the *Index* would be greater if its coverage were wider, yet this would result in an increase in cost unless there was a corresponding increase in the number of purchasers. Before placing a subscription, a librarian needs to be reasonably satisfied that the citation index is not only a useful tool, but also that it will not cease publication within a year or so, for it is only when the library shelves have a run covering at least five years that the majority of users of the library will be prepared to accept it as a method of retrieving information. Only then will it be possible to assess how usefully it can fulfil its basic purpose and find whether its obvious technical weaknesses are obviated by the flexibility and ease of compilation. The use of a computer for this purpose makes possible a number of interesting alternatives. Already an Automatic Subject Citation Alert (ASCA) service is being offered, by which subscribers can obtain regularly citations to papers by selected authors. There is no reason why cumulations covering selected

subject fields should not be issued at a price which would appear more reasonable to prospective purchasers. There are also, of course, known ways of improving the operational performance of a citation index; some of these, by increasing the complexity, would raise the cost, but improvement in the citing habits of authors would do more than anything else to increase the usefulness of a citation index. Perhaps the time is coming when editors of scientific journals should take the same active steps to obtain a high quality of citing as many have recently been doing for abstracts and titles. C. W. CLEVERDON

COMMUNICATION, THE SOCIAL WEB

Communication Processes

Edited by Frank A. Geldard. (NATO Conference Series, Vol. 4.) Pp. x+293. (London and New York: Pergamon Press, 1965.) 100s. net.

COMMUNICATION Processes is Volume 4 of the NATO Conference Series of volumes, being the *Proceedings* of a symposium held in Washington in 1963, under the sponsorship of the NATO Advisory Group on Human Factors. This military sponsorship should not mislead one, however, because the contents will be of interest to a wide civil readership and, indeed, have, on the whole, no limitation to military situations at all, but relate to many kinds of formal social institution.

The book is divided into five principal sections: (1) data presentation; (2) data transmission; (3) language barriers and language training; (4) group communication; (5) man-computer communication. Each consists of an introductory paper with very extensive attendant discussions. About one-fifth of the whole contents is written in French. The conference was concerned essentially with human factors of communication and not at all with electronics or engineering; of course, human factors are themselves very important to engineers. The participants may be regarded as being the most prominent psychologists and sociologists concerned with communication processes—an impressive galaxy of stars from the various NATO countries, representing a wide range of interests and opinions.

At least a third of this book is devoted to 'pragmatic' aspects of communication; that is, to the importance of personal prejudices, to people's positions in a social hierarchy, to their cultural backgrounds and to non-cognitive factors of language; the subtle connotations of language, one's image of oneself and other determinants of human communication. This aspect of the book is valuable, for such vital matters are not always evident to those engaged in the development of technical means of communication, and they are ignored by those who take a strictly logical view of semantics, a view which seems so prevalent to-day. A 'yes' can be turned to a 'no' by a smile, or by one's knowledge of the speaker's reputation. Meanings are not properties of words but are relationships, between words and people, in various situations and conditions. These pragmatic factors can be of particular importance in cross-cultural communication, already hampered by the strict untranslatability of languages. However, John Blæk, of Ohio State University, plays down these factors, in his paper "Language Barriers and Language Training", and quotes several historical examples of people, drawn from different cultures, nevertheless getting on together quite well; Columbus, for example, among the natives, and Marco Polo. But his examples are very special ones and, besides, the fact that such people were not promptly murdered does not mean that they were understood correctly. I cannot help quoting the old story of Captain Cook who, when he asked a native "What's that silly-looking animal called?", was given the answer: "Eangaroo", which means in Aborigine "I don't know" (so I am told!). Of course, the 'word

barrier', in this context, is itself open to misinterpretation. Languages are not barriers in the sense of 'walls', but rather in the sense of 'ambiguous signposting'. But Prof. Black is a professor of speech and is concerned with such things as morphemes, syntax and phonemes, rather than with the *rapprochement* between two human beings engaged in discussion. Other discussants, especially psychologists, emphasized the conceptual difficulties involved in translation, without in any way denying the value of Prof. Black's experimental work.

In his opening paper at the fourth session on "Group Communication", M. Didier Anzieu also brings out the importance of these language 'barriers' in various social groups. Such barriers can be strong between people, or groups, of the same nominal language, by reason of personal status in a group hierarchy, or by personal non-explicit connotations of words and phrases. (How important is language to the maintenance of class divisions in Britain!) Lack of feed-back in an organization means one-way communication (orders, directives) and can lead to hostility. Our social behaviour and attitudes in a group depend not only on 'message received' but also on our unconscious adjustments based on assumptions about other people's social behaviour in like circumstances. Behaviour is not absolute but relative. There may also be conflict between the needs of the individual and the needs of the group.

Other papers presented dealt with more strictly stimulus/response, mechanistic, aspects of communication and control, relating especially to man-computer systems. It is interesting, however, to hear psychologists' views of such 'formal' aspects which are more commonly discussed by engineers. Questions of human 'matching' to machines were considered, signal codings, sensing, identifying and interpreting. The papers, though presented against a military background, contain much of quite general interest to other situations to-day: in industry, for example. As said earlier, this military emphasis seems a little unfortunate.

COLIN CHERRY

SOIL SCIENCE

Experimental Pedology

Proceedings of the Eleventh Easter School in Agricultural Science, University of Nottingham, 1964. Edited by E. G. Hallsworth and D. V. Crawford. Pp. xi+414. (London: Butterworths Scientific Publishers, 1965.) 85s.

Forty years ago the word 'pedology' was being used somewhat tentatively in Great Britain, where the examination of soils had, in the main, been an application of the analytical skills of chemists. Indeed, in the middle of last century Thompson and Way had discovered the property of cation exchange; but their results were inexplicable until the laws of mass action and the properties of colloidal particles were established. Then followed a revival of interest in the subject, and by the 1920s the theory of cation exchange seemed to offer a simple explanation of the natural development of soil acidity and the ability of the soil to release nutrients for the plant.

Meantime, the pedologists in Europe and North America had been accumulating observations in the field on profile characteristics. They had tended to rely heavily on geology, at least in the preliminary stages of soil classification, and it was obvious that both physical and biological sciences were required to provide explanations for the mobility of ions and colloidal material responsible for horizon differentiation, for the attack on litter by micro-organisms and small animals in search of energy, and for the modification of the soil by the products of decomposition and deposition.

Responses in biological activity to fluctuations in habitat, resulting from moisture and temperature changes

and the redistribution of components, inevitably increase the complexity of a soil system. However, the precision of recently developed analytical techniques has stimulated the study of slow biochemical reactions, and rapid progress has been made in all the sciences applied to soil. Communications within and between the various disciplines have also been assisted since 1927 by congresses and commission meetings of the International Society of Soil Science and by numerous national and regional conferences.

The 1964 Easter School at the University of Nottingham was an opportunity of reviewing the present position with respect to the experimental approach to pedology, and the *Proceedings* comprise 29 papers. They fall into five sections, dealing with: (1) geochemistry and weathering of soil minerals; (2) redistribution of inorganic substances; (3) biological aspects of soil formation; (4) pedo-genetic studies; (5) demonstrations. The discussions of groups of the papers are most informative in revealing differences of opinion and promising lines of further investigation.

The nature of the surfaces of fine particles is discussed by J. J. Fripiat and the organic geochemistry of sedimentary rocks by W. D. Evans. J. Rausell-Colom *et al.* describe detailed studies on the weathering of mica, by X-ray diffraction and spectrographic analysis and by electron probe, while R. M. S. Perrin has assessed the rate of weathering from the composition of drainage waters. In two papers, S. Hénin and G. Pedro, and S. Caillère and S. Hénin, examine the sub-division and dissolution of rocks by weathering speeded up in the laboratory by Soxhlet extraction, and the formation of clay minerals by the chemical treatment of specific minerals, the transition being followed by thermal loss-of-weight curves. J. van Schuylenborgh discusses the mechanism of the formation of sesquioxides and the cementing agents in different horizons, while D. Acquaye and J. Tinsley deal with the solubility of silica and its movement through soils under various conditions. The mobility of phosphate, as observed in radioautographs of crumbs previously immersed in carrier-free $H_3^{32}PO_4$ solution, is described by D. Gunary *et al.*, and D. H. Yaalon deals with the distribution of anions under arid conditions, and C. B. Wells with the deposition of calcium carbonate.

In the biological group of papers, G. V. Jacks reviews ideas on the 'organic' weathering of solid rock by bacteria, fungi and algae at the start of soil formation. J. D. Ovington deals with the accumulation of nutrients in woodlands, D. S. Jenkinson with the decomposition of carbon-labelled ryegrass, R. Bétrémieux and S. Hénin with the products of fermentation in profile development, and N. A. Burges with the various micro-organisms involved in the attack on plant tissues. The effects of animal activity on the litter and on soil structure are described respectively by J. van der Drest and G. W. Heath, while C. Jeanson and G. Monnier discuss the influence of soil fauna on the stabilization of structure. From the numerous experimental data presented, it is clear that much remains to be done to clarify the effects of the numerous factors met with in the field and the interdependence of the various groups of organisms involved.

In section 4, C. Bloomfield reviews the present position of theories on the mechanism of podsolization, D. V. Crawford presents evidence of the complex interaction of physical, chemical and biological processes which cannot be examined in isolation, while H. P. Blume and E. Schlichting emphasize that the basic problem is, in fact, to sort out the various soil-forming processes. V. B. Proudfoot deals with the mutual benefits to archaeologist and pedologist from the examination of prehistoric sites, D. G. McKinlay gives a detailed account of the physics of fluvial transport and deposition, and T. W. Walker advances analytical data in support of phosphorus as a key element in pedogenesis because of its role in the

ecological sequence. How far the various aspects of weathering, transport and deposition are related to soil classification is discussed by E. G. Hallsworth in a scheme based on leaching sequence and conforming with the conclusions reached by experimental pedology.

Three short papers on demonstrations of pyrochromatography, watertable studies, and the effect of stones on soil transport, conclude the volume, which has author and subject indexes, has been most carefully edited and very well produced.

A. M. SMITH

MANUFACTURE OF WOOD PULP

Pulping Processes

By Dr. Sven A. Rydholm. Pp. ix+1269. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, Inc., 1965.) 285s.

THE author of *Pulping Processes* is a Swede and, judged by the introduction, a disciple of the late Erik Hagglund, professor of cellulose technology and wood chemistry at the Royal University of Technology, Stockholm. He has spent his career of some 20 years in the Swedish pulp industry, principally on industrial research, and is at present assistant research director of one of the foremost Swedish pulp and paper mills. In this volume he has produced one of the most comprehensive books on pulping processes yet available, and it is written in faultless English. Indeed, the work may be described as almost monumental, and it will undoubtedly serve as a standard reference work on wood pulping for many years to come.

The book commences with a survey of the forests of the world and includes and discusses data on wood species, the gross and minute structures of trees and woody fibres, and the chemical composition and reactions of their components. The operations involved in the preparation of cellulose pulp from the forest to the finished product are then described and discussed. The section headings comprise the manufacture of unbleached pulp and of bleached pulp, including the preparation of the chemicals required. A final chapter surveys the properties and uses of pulp and the world pulp industry. The treatment of the subject is such that the physical and chemical processes involved are emphasized, rather than the purely technological aspects. In this way the influences of process variables on the yields and properties of pulps are demonstrated and analysed. The chemical reactions involved, the preparation and recovery of pulping chemicals, and the manufacture of organic by-products are also dealt with.

It is inevitable that even a book having such a high standard of attainment should also have its weak points, if only because its wide and detailed scope places a considerable strain on the expert knowledge of a single individual. The introduction suggests that one of these limitations is apparent to the author, namely, the fact that the time involved in writing such a book means inevitably that some of it is soon out of date. This applies with particular force to the statistical data and the author's discussion of the pulping industry of the world, which are based on 1960 figures and facts; a great deal of importance has happened in this connexion in the past 5 years. A further, and perhaps more important, limitation arises from the fact that, to the author, pulp means almost exclusively wood pulp and there is scant and not always wholly accurate treatment of fibrous pulping materials other than wood. Quantitatively and on a world-wide basis, such pulps may be of relatively small significance at the present time as compared with wood pulp. However, to developing countries they are often the only source of available paper-making material, and as time goes on they will certainly increase in importance; they certainly deserve more than 16 pages in a book of this size.

It should be emphasized that the foregoing criticisms should be viewed in their correct perspective, especially as the latter refers only to non-woody fibres. So far as wood pulping processes are concerned the book can be recommended highly and without reserve.

JULIUS GRANT

SEMICONDUCTOR CONTROLLED RECTIFIERS

Semiconductor Controlled Rectifiers

Principles and Applications of *p-n-p-n* Devices. By F. E. Gentry, F. W. Gutzwiller, N. Holonyak, jun., and E. E. Von Zastrow. (Series in Solid State Physical Electronics.) Pp. xv+383. (Englewood Cliffs, N.J., and London: Prentice-Hall International, 1964.) 120s.

DURING the ten years following its discovery in 1949, the transistor developed, at least in principle, to the point where it could replace 'hard' thermionic valves in all but three fields, namely, for generating high powers at radio frequencies, in electrometer applications, and in micro-wave applications. The field effect transistor now permits replacement of the valve in electrometers, and various so-called 'solid state klystrons' now becoming available are rapidly encroaching on the micro-wave field. However, the eclipse of 'hard' valves by the transistor is as nothing compared with the rapidity and thoroughness with which the thyristor, or semiconductor controlled rectifier, has swept the 'soft' valve into history. The only field left now for the thyatron (and its various relatives) is a very tenuous grasp on applications in which working voltages are too high for present-day semiconductor devices. Indeed, thyristors now also seriously threaten the survival of the magnetic amplifier, which clings to a place in contemporary technology only by virtue of economic factors and industrial inertia, both of which are likely to be only of transient significance.

Needless to say, the rapid coming to maturity of thyristor technology has led to the production of a spate of books expounding the essentials both of device design and circuit application. *Semiconductor Controlled Rectifiers* is a good example. It is well produced, thorough in its treatment, extensive and reliable. In that it describes recent and relatively sophisticated developments, it is a second-generation book—no pioneering text, but likely to be useful for a considerable period. The emphasis is clearly forward application, rather than device design, although the latter is covered sufficiently well to give an adequate introduction, and certainly should make it relatively easy to understand why available devices have the properties that they have.

However, two criticisms must be made. First, one-sixth of the book is taken up with a summary of the elementary theory of semiconductors and the *p-n* junction. This is really inexcusable. Many good text-books on this subject are now available and, in any event, the fundamentals are a necessary part of the education of every electrical engineer to-day. To me it seems quite possible that it was only included because of the present fashion in the United States and elsewhere not to write or publish short books. The present volume is not a particular sinner in this respect, but certainly could have been reduced in length by at least one-third without loss. Secondly, circuit technique using thyristors is expounded in a 'cookery book' fashion. This is still customary in electronics text-books. The outstanding systematic exposition of the technique of circuit design is still awaited for reasons which are not clear. Perhaps the subject has lacked the intellectual cachet necessary to attract attention of a sufficiently serious kind. For whatever reason, electronic circuit design has yet to find its Mendeleev, and can scarcely be considered a science in

the sense, say, of the definition used by Prof. P. B. Medawar in a recent article (*Encounter*, August 1965). To sum up, the book is thorough, reliable, and with a wide range of proven circuit arrangements described in adequate detail; sufficient, in fact, to enable the engineer to develop the skill necessary for the design of new configurations.

W. FISHWICK

ALKALINE EARTH METALS

Handbuch der Experimentellen Pharmakologie
Begründet von A. Heffter. Fortgeführt von W. Heubner.
Erganzungswerk. Herausgegeben von O. Eichler und A. Farah. Band 17: Ions Alcalino-Terreux. Teil 1: Systèmes Isolés. Sous-Editeur: Zénon-Marcel Bacq. Pp. xx+574. 148 D.M. Teil 2: Organismes Entiers. Pp. xviii+1068. 198 D.M. (Berlin: Springer-Verlag, 1963, 1964.)

IN these two volumes the editors have brought together twenty Belgian authors who have, between them, contributed seventeen chapters. The first volume deals with the action of the alkaline earth metals studied in isolated preparations, the second with work on whole organisms.

Z.-M. Bacq opens the first volume with an interesting account of the importance of the alkaline earth metals in living matter. In the second chapter, the chemistry and physical chemistry of magnesium, calcium, strontium and barium are discussed by E. Fredericq. The next four chapters deal with the action of the alkaline earths on simple enzymes (L. Massart and R. Vercauteren), blood coagulation (Y. Bounameaux and J. Lecomte), complex enzyme systems (C. Liébecq) and micro-organisms (Z.-M. Bacq). The intra- and extra-cellular distributions of Ca and Mg are discussed by E. Schoffiels. This is followed by chapters dealing with the action of alkaline earth metals on tissues of invertebrates (Z. M. Bacq), on nerve fibres and nerve cells (J. A. Cerf and I. Leusen), on neuromuscular transmission (J. E. Desmedt), on skeletal (X. Aubert), cardiac (J. G. Henrotte) and smooth muscle (Z.-M. Bacq and R. Charlier) and blood elements (J. Lecomte and Y. Bounameaux). The first volume ends with a most useful review by E. Schoffiels and Z.-M. Bacq on the factors which control the states of equilibrium of the various cations.

The second volume concentrates on the many metabolic processes in which the alkaline earth metals are involved in the whole organism. The first two chapters are written by M. J. Dallemagne and deal in great detail with calcium in the circulation, bones and teeth. The third chapter is devoted to the cerebrospinal fluid and the central nervous system (I. Leusen) and to the aqueous humour (Z.-M. Bacq). Subsequent chapters deal with problems concerning the kidney (G. Barac), milk (G. Peeters and R. Vercauteren), sweat (Z.-M. Bacq), gastrointestinal secretions (H. Brabant and J. van Geertruyden), blood vessels and allergy (J. Lecomte). The metabolism of magnesium (M. J. Dallemagne) and that of strontium and radiostrontium (J. Goffart) follow, and the second volume ends with a chapter by J. Lecomte on the toxicity of alkaline earth metals.

This outline of the contents of the two volumes gives some indication of the scope of the work. Prof. Z.-M. Bacq has done his work of planning and co-ordination admirably and is to be congratulated on having persuaded so many distinguished Belgian workers to join him in this enterprise. All the chapters are of high quality and present in a clear manner a wealth of information on a field of wide interest. There are extensive lists of references at the ends of the chapters which add greatly to the usefulness of the book. Moreover, each of the two volumes is self-contained, with its own author and subject index. The drawings are excellent and the volumes beautifully produced.

The coverage is so wide that workers in a great number of disciplines will find these two volumes very useful indeed. However, in order to enjoy them to the full, the reader should have a competent knowledge of French, all chapters being in that language. Moreover, if he wishes to have the two volumes on his own shelves, he needs a respectable bank balance, the price of the two volumes being £30 5s. 8d.

ELEANOR ZAIMIS

RESEARCH IN PHOTOGRAPHY

Mitteilungen aus den Forschungslaboratorien der Agfa-Gevaert AG, Leverkusen-München
Band IV. Pp. viii+474. (Berlin, Heidelberg and New York: Springer-Verlag, 1964.) D.M. 96.

VOLUME 4 of *Mitteilungen aus den Forschungslaboratorien der Agfa-Gevaert AG*, like its predecessors, comprises a selection of papers originating from the Agfa Laboratories in Leverkusen and Munich. It represents a cross-section of the work in recent years of the Agfa research workers in the field of photographic and related science and technology.

A total of 35 individual papers, 13 of which have previously been published in other journals, cover the following ground: thermo-dynamic studies of the basic photographic process and a review of the present theories of spectral sensitization; investigations of the growth and crystal habit of silver halide microcrystals and of the composition and stability of the latent image. Other papers give predominantly theoretical treatment of the mechanism of various reactions which are relevant to the photographic process and of development. Another section deals with the properties of the processed photographic image and includes papers on the sensitometry as well as on statistical investigations of tolerances of reversal colour films. This part also includes no less than six papers in a field of growing importance which describe investigations into the performance characteristics of photographic layers in terms of the modulation transfer theory.

Papers in the field of organic chemistry range widely: the reaction of coupling compounds with *p*-phenylene-diamines, resulting in the formation of colourless products and of dyes; optical sensitizing dyes; new developer substances; reactive dyes for gelatine and analytical chemistry. Two further contributions are concerned with the preparation and properties of magnetic tape. Finally, there are contributions in the camera and equipment field; on the spectral sensitivity distribution of photo-electronic receivers and on the efficiency of illumination systems. The standard of the experimental work and of its presentation is high throughout. Among the many experimental techniques used, electron-microscopic photography deserves special acknowledgment.

The volume has been edited by E. Klein and R. Matejec. Prof. Klein, who is the research director of the Agfa part of Agfa-Gevaert, is co-author of several of the papers. Other contributors include such well-known names as R. Matejec, E. Moisar, J. Eggers, W. Behrendt, G. Langner and W. Püschel.

This volume is dedicated to F. Gajewski, who initiated Agfa research in the photographic field in the 1930's. It is in this context that one may understand the reasons for publishing volumes of this kind which cover such a wide range of subject-matter; that is, to demonstrate within and outside the company the value of research work which does not always become apparent in the company's products. By the nature of their work, most scientists in the field would be interested only in some section or sections of this volume, and the majority would be equally well served if the individual papers could appear in the appropriate journals, as many of them have done already.

K. O. GANGUIN

A HUMANISTIC TECHNOLOGY*

By VICE-ADMIRAL H. G. RICKOVER, C.B.E.†
U.S. Navy

CHANGE is now part of life in all industrially advanced countries—continuous, rapid, all-pervading change. The ultimate cause of this unsettling situation is the explosion of science: factual knowledge doubles every decade or so. Its direct cause is the technological revolution: new knowledge is put to practical use about as rapidly as knowledge itself expands.

The impact of technology on individuals and on society at large is profoundly affected by the attitude of the public and of its leaders toward technology; that is, by prevailing concepts of what technology is and what purpose it should serve.

When technology is believed to be a force with a momentum of its own that puts it beyond human direction or restraint, it may become a Frankenstein destroying its creator. But when it is viewed humanistically, in other words, as a means to human ends, it can be made to produce maximum benefit and do minimum harm to human beings, and to the values that make for civilized living. It may even enable man to become more truly human than it has ever been possible for him to be. Of technology it can be truly said that it is not "either good or bad, but thinking makes it so".

I propose to show there is a tendency in contemporary thinking to regard technology as an irresistible force rather than a tool. The tendency is more pronounced in some countries than in others but is observable wherever there is rapid technological progress. Since it encourages the use of technology in ways that on balance are harmful, this point of view should be replaced by a humanistic attitude—an attitude which looks on technology as an instrument created for no other purpose than to serve man.

There is no need to belabour the point that technology, properly used, can be of great benefit. But there is need to bring out the potential harm which technology, improperly used, may cause. My concern is with attitudes, for I believe it is attitudes that determine what we do with technology.

The part played in the formation of popular attitudes by all media of communication—books, newspapers, journals, radio, television—is too obvious to need comment. Apart from formal education, which for most people ends at the threshold of maturity, these media bear the respon-

sibility of supplying the factual information men must have if they are to arrive at rational judgments on issues that interest them as individuals, or concern them as citizens of a democratically organized society. Many of these issues contain technological elements. In presenting them to the public, it is important to assign these technological elements their appropriate place. Therefore, those involved with communications (using the term in its broadest connotation) must themselves have a clear conception of the nature of technology and of its proper role.

Technology has been defined as "covering the field of how things are commonly done or made", and, somewhat more broadly, as "what things are done or made". It is a modern term, but we are in the habit of using it retroactively. We apply it to the techniques of a pre-industrial metal worker no less than to those of a modern metallurgist. Yet modern technology differs significantly from that of the past in being largely based on science, that is, founded on accurate knowledge of the workings of Nature. Earlier techniques, arts, skills were almost entirely empirical. Because of his knowledge of Nature, man, through technology, is now able to alter his material environment, the material conditions of life. If these changes are to be beneficial, not harmful, technology must be managed as a humanistic enterprise.

By boring into the secrets of Nature, scientists discover keys that can be used to unlock powerful forces. Technology is concerned with putting these forces to practical use. The apparatus set up for this purpose is huge and complex, difficult for laymen to understand. Yet the basic nature and purpose of technology are not beyond the comprehension of ordinary citizens.

Technology is tools, techniques, procedures, things; the artefacts fashioned by modern industrial man to increase his powers of mind and body. Marvellous they are, but let us not be overawed by these artefacts. Certainly they themselves do not dictate how we should use them, nor, by their mere existence, do they authorize actions that were not anteriorly lawful. We alone bear responsibility for our technology. In this, as in all our actions, we are bound by the principles governing human behaviour in our society. Ethics, I need scarcely say, are not only personal but also social.

This surely must be obvious to any reasonable man. Yet it cannot be over-emphasized, for a considerable body of opinion propagates what comes close to being the opposite view. The notion is widespread that, having wrought vast changes in the material conditions of life, technology perforce renders obsolete traditional concepts of ethics and morals, as well as accustomed ways of arranging political and social relationships. Earnest debates are at present taking place as to whether it is possible to act morally in the new technological society, and proposals have been made—quite seriously—that science must now replace traditional ethics. We have here a confusion of means with ends that should be cleared up.

The laws disclosed by science must of course be heeded by those who wish to exploit scientific discoveries; in his technological activities man is bound by the laws of science. But it does not follow that he is bound by the laws of science in his purely human relations as well. "Science", wrote Vannevar Bush, "has come a long way, in delineating the probable nature of the universe that surrounds us, of the physical world in which we live, of our own structure, our physical and chemical nature.

* Substance of a Granada Lecture of the British Association for the Advancement of Science, delivered in Guildhall, London, on October 27. This Lecture does not necessarily reflect the view of the U.S. Secretary of the Navy or the Department of the Navy.

† Vice-Admiral Hyman G. Rickover is head of the Division of Nuclear Propulsion in the U.S. Navy Bureau of Ships and director of the Division of Naval Reactors, U.S. Atomic Energy Commission.

During the Second World War he was head of the Electrical Section of the Navy's Bureau of Ships, and at its conclusion was assigned to the Atomic Submarine Project. He took the lead in the design, development and construction of nuclear propulsion systems for submarines and other naval ships, the first of which, the submarine *Nautilus*, was launched in 1954. To-day the U.S. Navy has three nuclear-powered surface vessels, including the giant aircraft carrier *Enterprise*, and a large number of nuclear-powered hunter killer and *Polaris* submarines. Although well past retiring age, Admiral Rickover has been retained in service and remains personally responsible for the whole of this nuclear propulsion programme. He has also been charged with the responsibility of developing large-scale atomic power plants to generate electricity.

In addition to a direct concern with every detail of these nuclear-power programmes, Admiral Rickover has given much time and thought to problems of American education, which he has compared with school systems in other countries, including Great Britain, in three books and in several reports to the House of Representatives Appropriations Committee.

Among the many honours and awards which have come to Admiral Rickover are the very rare Congressional Gold Medal, which he received in recognition of his work in the nuclear-power programme field, and the Enrico Fermi Award, where his name joins a distinguished list including John von Neumann, Ernest Lawrence, Glenn Seaborg, and J. R. Oppenheimer. Among many honorary degrees which he has received is a D.Sc. of the University of Birmingham; he was made an honorary C.B.E. in 1948.

It even enters into the mechanism by which the brain itself operates. *Then it comes to the question of consciousness and free will—and there it stops.* No longer can science prove, or even bear evidence. Those who base their personal philosophies or their religion on science are left, beyond that point, without support”.

Through technology man has been relieved of much brutal, exhausting, physical labour as well as boring routine work; he has been provided with numerous mechanical slaves who do certain kinds of work faster, cheaper and more efficiently than people. Why should the ease and affluence made possible by technology affect precepts that have guided Western man for centuries? This may brand me as old-fashioned, but I have not yet found occasion to discard a single principle that was accepted in the America of my youth. Why should anyone feel in need of a new ethical code because he is healthier or has more possessions or more leisure? Does it make sense to abandon rules one has lived by because one has acquired better tools for doing one's work?

Tools are for utilizing the external resources at our disposal; principles are for marshalling our inner, our human resources. With tools we alter our physical environment; principles serve to order our personal life and our relations with others. The two have nothing to do with each other.

It disturbs me to be told that technology ‘demands’ an action the speaker favours, that ‘you cannot stop progress’. It troubles me that we are so easily pressured by purveyors of technology into permitting so-called ‘progress’ to alter our lives, without attempting to control it—as if technology were an irrepressible force of Nature to which we must meekly submit. If we reflected, we might discover that not everything hailed as progress contributes to happiness; that the new is not always better nor the old always outdated.

Perhaps we are receptive to these arguments because we tend to confuse technology with science. Not only in popular thinking but even among the well-informed the two are not always clearly distinguished. In consequence, characteristics pertaining to science are attributed to technology. The etymology of the word may contribute to this confusion. Its suffix lends to technology a false aura—as if it signified a body of accumulated, systematized knowledge, when in fact the term refers to the apparatus through which knowledge is put to practical use. The difference is important.

Science has to do with discovering the facts and relationships of observable phenomena in Nature, and with establishing theories which serve to organize masses of verified data concerning these facts and relationships. Because of the care scientists take to verify the facts supporting their theories, and their readiness to alter theories when new facts prove an established theory to be imperfect, science has great authority. What the scientific community accepts as proved is not questioned by the public. No one disputes that the Earth attracts the Moon, or that atomic fission produces energy.

But technology cannot claim the authority of science. It is properly a subject of debate, not alone by experts but by the public as well. It has proved anything but infallibly beneficial. Much harm has been done to man and Nature because technologies have been used with no thought for the possible consequences of their interaction with Nature. A certain ruthlessness has been encouraged by the mistaken belief that to disregard human considerations is as necessary in technology as it is in science. The analogy is false.

The methods of science require rigorous exclusion of the human factor. They were developed to serve the needs of scientists, whose sole interest is to comprehend the universe; to know the truth; to know it accurately and with certainty. The searcher for truth cannot pay attention to his own or other people's likes and dislikes, or to popular ideas of the fitness of things. This is why

science is the antithesis of ‘humanism’, despite the fact that historically modern science developed out of and parallel to the humanism of the Renaissance.

What scientists discover may shock or anger people—as did Darwin's theory of evolution. But even an unpleasant truth is worth having; besides, one can choose not to believe it. It is otherwise with technology. Science, being pure thought, harms no one; therefore it need not be humanistic. But technology is action, and often potentially dangerous. Unless it is made to adapt itself to human interests, needs, values, and principles, more harm than good will be done. Never before, in all his long life on Earth, has man possessed such enormous power to injure his human fellows and his society as has been put into his hands by modern technology.

This is why it is important to maintain a humanistic attitude toward technology; to recognize clearly that, since it is a product of human effort, technology can have no legitimate purpose but to serve man—man in general, not merely some men; future generations, not merely those who at present wish to gain advantage for themselves; man in the totality of his humanity, encompassing all his manifold interests and needs, not merely some one particular concern of his. Humanistically viewed, technology is not an end in itself but a means to an end, the end being determined by man himself in accordance with the laws prevailing in his society.

A word may be in order concerning the disparate meaning of the word ‘law’, depending on whether it is used in the ordinary sense—which is also the original sense of the word—or by scientists. Law, as commonly understood, refers to those rules of human conduct prescribed and enforced by society. The scientists have appropriated the term. They use it to describe regularities exhibited by physical phenomena—the rules by which the cosmos governs itself. In the transition, the word has taken on a new meaning.

Law that governs human society is not the result of scientific method but of wisdom and experience, of consensus as to what is just and fair. In autocracies, law is what the ruler wishes it to be and what he is able to enforce by naked power. The purpose of human law is to resolve conflicts by the application of definitive rules. These rules are always debatable and can be changed when there is public demand for a change or when the rule-maker desires them to be changed.

From the layman's point of view, what the scientist calls law is fact, rather than law—immutable fact. Or, if you prefer, it is law operating in a sphere where man exercises no influence. He cannot alter the laws of the cosmos; he can only discover them. It would be pointless for him to debate these laws; he must accept them. A law of science expresses mechanical regularity where no choice of action, no free will, comes into play; it deals with constancy of behaviour in Nature; it has relevance for man only because it makes the universe more comprehensible to him and, by disclosing how Nature works, allows him to utilize the forces of Nature for his own purposes.

When we make use of these forces, we must of course heed the laws of science which describe their behaviour; they are laws we cannot bend to our will. But we must likewise heed the man-made laws of our society, for technology is action which affects fellow human beings. Technology straddles, as it were, the law of the cosmos and the law of man; it is subject to both. Much confusion in popular thinking arises from this fact. The two laws are confounded. Or, to put it differently, they are thought to be part of a single system of law so that one or the other must perforce take precedence.

Ever since science discovered that the Earth is not the centre of the cosmos, as had been maintained by the highest human authorities, we have been learning painfully that the laws of science cannot be overturned by human fiat. To-day, acceptance of duly authenticated

scientific theories or laws is common practice in enlightened countries. Occasionally, an attempt is made to muzzle scientists whose findings contravene accepted dogma—as when the U.S.S.R. forced geneticists to conform to the party line, or when some backward community in the West forbids the teaching of evolution because this conflicts with the community's religious dogma. But these are rare cases, and no reasonable person condones them.

It has taken a long time to attain this rational attitude toward science, and we are conscious of the consequences of intolerance in the past. Perhaps this is why we have been excessively tolerant toward those who claim the right to use technology as they see fit, and who are wont to treat every attempt by society to regulate such use in the public interest as if it were a modern repetition of the persecution of Galileo.

Assuredly, we have the right to use the instrumentality of law and of government to protect ourselves against technological injury. Yet this simple truth is obscured by the effective way in which opponents of protective measures play on the layman's respect for science—in a conscious or unconscious attempt to brainwash the public so it will accept their argument without debate. When attacking legislation that would restrain the user of technology, it is common practice to argue as if at issue were acceptance of a law of science. Yet what is being discussed is not science but the advisability or legality of the technological exploitation of science. The public would not be deceived by such arguments if it clearly understood the fundamental difference between science, which is pure knowledge, and technology, which is action based on knowledge.

To guard against being misled, one should cultivate an attitude of scepticism whenever the word science is used. Is it science that is being discussed or is it technology? If technology, the question at once arises whether the proposed action is legally permissible and socially desirable. These are matters which lie outside science. Just as the law of the cosmos cannot be overturned by human fiat, so is human law supreme within its own proper sphere of operation. Technology must therefore conform to that most basic of all legal maxims, the 'mutuality of liberty'; the principle that one man's liberty of action ends where it would injure another. Without this maxim, freedom would be a barren privilege.

Humanistically viewed, technology can have no legitimacy unless it inflicts no harm. Granted this premise, the prerequisite for users of technology is—or ought to be—that they comprehend and respect the laws of science applicable to their particular technology; that they exercise a prudent man's care in assessing the probable consequences of this technology; and, should it be potentially harmful, that they abstain from using the technology until they have found ways to render it harmless.

Whether or not a particular technology has harmful potentialities ought not to be decided unilaterally by those who wish to use it. Destructive technologies are often highly profitable for those promoting them. They have a vested interest in the technology; it may give them money, reputation, power. They are an interested party to the conflict between private and public interest which every potentially harmful technology poses. Moreover, they are nearly always practical men more knowledgeable about efficiency in using a technology than about the legal and scientific implications of such use.

A broader range of intellectual power should be brought to bear on the whole question of technological exploitation of scientific knowledge. Purely practical considerations should be supplemented with scholarly knowledge of long-range consequences; private interest in efficiency with public interest in safety. The automatic discipline of a free market—where it still exists—does not include side-effects and long-range consequences; it merely reflects consumer preference for a product that appears useful and is reasonably priced. The consumer is too ill-informed about safety to make his opinion felt.

I think one can fairly say that the practical approach to a new scientific discovery and its utilization through technology is usually short-range and private, concerned only with ways to put the discovery to use in the most economical and efficient manner, little thought being given to its ultimate consequences. The scholarly approach—if I may use this term—is long-range and public; it looks to the effects which a new technology may have on people in general, on the nation, on the world; on present and future generations. Of course there are men who combine the two approaches and you find them among people whose primary interest is practical, no less than among those whose primary interest is scholarly. Both approaches are necessary to illuminate the problem and help solve it. To exclude the one or the other prevents finding the way to a humanistic technology.

I can best illustrate what I want to bring out by a simple example. Commercial deep-sea fishing can be done so efficiently with modern techniques that a few enterprises could rapidly sweep the oceans free of commercial fish: and this is what the fishermen of all nationalities wish to do. As practical men they are interested only in using technology to increase their catch, preserve it and get it to market as speedily as possible. In pursuing this short-range private objective, they have been quite ingenious. Figuratively speaking, the world's marine scholars have stood by wringing their hands at the fishermen's 'practical' folly. To the scholars it has been incomprehensible that rational human beings should fail to see that in the end more can be taken from the sea if fishing conforms to sensible conservation measures which permit the species to reproduce itself.

We witness at the moment the end of one of the saddest stories of misuse of technology by greedy fishing interests. Unless "the myopic stupidity of commerce"—as the *New Scientist* calls it—is curbed by really effective international action, the great whales—the blue, the finback, the sperm—will soon disappear forever, victims of man's 'practical' folly.

These and other whales once populated the high seas in immense numbers. For hundreds of years whaling remained a reasonably fair contest between man and the intelligent, swift-moving mammals he hunted. Modern technology has turned it into brutal genocide. The culprits are the whaling fleets of Norway, Japan and the U.S.S.R., last remnants of what once was a large company of whaling fleets from many nations. One by one they ruined themselves by destroying the marine resources on which their livelihood depended. Yet nothing has been learned from the lessons of the past.

The International Whaling Commission, in 1964, advised that, unless the catch were drastically reduced for a number of years, the whales would not reproduce themselves in sufficient numbers to support continued commercial fishing. The whalers rejected the warning (the Japanese called it "over-emphasized biology") and agreed among themselves to catch twice the limit proposed by the Commission, only to find the stocks already so depleted that they could not even meet their own 'limit'. Blindly pursuing what they doubtless consider an eminently practical objective, maximum profit to-day, they are wiping out the very resources that could insure them a profit to-morrow.

Practical considerations apart, is anyone—are fifteen fishing fleets, 250 ships in all—justified in using technology to exterminate a species which has existed on this Earth for millions of years—the largest animal the world has ever seen? Do we know that our descendants will not at some future time have need of these mammals? R. A. Piddington, in his book *The Limits of Mankind*, remarks that nobody knows what the biological consequences of the extermination of such whales are likely to be. "But if nearly a million of these huge animals, with their enormous appetites, can be removed in a single generation from the balance of marine life without causing violent repercussions,

all our previous experience of this subject has given us the wrong answers.' He notes that the sperm whale is the only creature eating "that nightmarish monster the giant squid", and suggests that one consequence of exterminating this huge whale may be a tremendous increase in the population of giant squid and their penetration close to our shores—not a pleasant prospect.

Technological damage to deep-sea fisheries happens to be an international problem, therefore particularly difficult to solve. But quite as disastrous in its ultimate consequences is the discharge of poisonous waste products by industrial plants using rivers and lakes as their private sewers. Valuable national fisheries have been ruined, not to speak of harm done to those who may eat fish poisoned by the waste products of new technologies. Detergents pose a similar problem. They are cheaper to manufacture than soap, hence more profitable to the producer, and they are preferred by consumers for their superior cleansing capacity. But, discharged into waterways, their organically undissolvable chemicals have proved particularly intractable pollutants.

Irretrievable damage has been done by those who use technology without giving thought to its effect on our environment. Waste products, carelessly emitted, create a massive problem of soil, water and air pollution: we may be damaging the atmosphere permanently by changing its chemical composition. Wholesale slaughter of wild animals upsets the ecology with consequences we cannot even fathom as yet. In some places the contours of the land are being changed by strip mining which gouges out chunks of earth and rock with their top soil and vegetation, leaving behind a desolate lunar landscape.

In so far as damage is still remediable—and much of it can never be undone—it must be remedied by public action, at taxpayers' expense. The total cost to the public of private carelessness and wilfulness in the use of technology will be enormous.

Experience shows that, by itself, the legal maxim of 'the mutuality of liberty' will not prevent premature commitment to technologies that may later prove harmful. The maxim must be implemented by preventive public action—action of the kind that has long been operative in the field of public health. There is need for laws requiring that before a particular technology may be used, reliable tests must have been made to prove it will be useful and safe. A few such laws are already on the statute books; more are needed. But we rarely get positive action until a human tragedy dramatizes the need for protection. So was it once, too, with preventive public health measures.

It will be remembered that they received their major impetus from the great cholera invasions of the past century. These were themselves a result of new technologies in transportation which enormously speeded the movement of persons and goods. The time-interval for travel from the areas of endemic cholera in the Far East to Europe and America was reduced below the incubation period of the disease. Without strong measures, the West could not protect itself against disease carriers coming from the East. These measures were bitterly fought because they impeded the movements of people and merchandise. It took repeated major epidemics to compel action.

In the United States there are preventive laws protecting the public against dangerous drugs. The first law, bitterly fought by the pharmaceutical interests, did not pass until a major tragedy had occurred—the death of a considerable number of patients who had taken a drug which was effective in the treatment of their particular disease, but had unforeseen side-effects proving fatal to some. After much procrastination, a second, more stringent law was passed, but not until the uproar over the thalidomide babies caused the legislators to respond with alacrity.

Though a technology may clearly harm paramount human values, restraining laws will not be forthcoming unless public demand is sufficiently vocal and persistent to wear down the opposition of those with a vested interest in the harmful technology. Opposition tactics follow a pattern that is monotonously repeated whenever the attempt is made to regulate a technology in the public interest. It is well to familiarize oneself with the pattern.

I have mentioned efforts to confuse the issue by arguing as if a law of science were at issue when in fact the proposed legislation deals with technology, not science. If this argument fails, the need for the proposed law is then categorically denied. Warnings of scientists are rejected as 'unproved' and 'exaggerated'. Later, when these prove to have been entirely correct, the argument shifts from the substantive question of whether a technology is harmful to an attack on the legitimacy of any kind of protective legislation. Such legislation would violate basic liberties, it is claimed; it would establish government tyranny and subvert free democratic institutions. If all this proves futile and legislation is imminent, there will be urgent demands that it be postponed until 'more research' can be undertaken to establish the appositeness of the proposed law.

These delaying tactics are highly effective. It takes firm commitment to a humanistic technology to push through needed legislation as well as thorough understanding of the filibustering tactics of opponents, and great skill in combating these tactics. No wonder public opinion and the law have nowhere fully caught up with those who misuse technology. Often as not they escape with impunity, no matter how gravely they injure man or society.

I suggest that, as a special public service, those trained in the law take on the task of working for better protection against technological injury. This is a new and fruitful area in which lawyers could make important contributions to human welfare—an area which requires no revolutionary change in the political or economic structure of society, merely greater precision and fuller implementation of the traditional principle that injuring the health or causing the death of human beings is unlawful. The term health should not be limited to physical health but should include psychic health and protection of the human personality as well. New technologies based on the uncertain 'science' of the social sciences involve snooping into the inner recesses of the human mind, personality testing and pseudo-scientific manipulation of human beings. When they are imposed as conditions of employment or otherwise partake of an element of compulsion, these technologies should be regulated or outlawed entirely.

Much more thought should be given to technological interference with the balance of Nature and its consequences for man, present and future. Let me give one more example of the harm such interference may cause.

To-day we have new technologies for the destruction of insect pests and weeds. Their use is profitable for the manufacturers of pesticides and weed killers; it is helpful to farmers who are able to get better crops, reduce human labour, and produce at greater profit; it benefits consumers who are offered a wider variety of food at less cost. Here is a classic case of what technology can do for us. Unfortunately, we have left out of consideration the balance of Nature. If used improperly, these pesticides and weed killers will poison soil, crops, birds, animals, fish and eventually man. In her book *Silent Spring*, Rachel Carson spoke out eloquently against committing this ecological sin.

Ecologists constantly warn us that when the balance of Nature is upset, everything in Nature is threatened, including man himself. It seems certain that unless he sets limits to his destructive instinct, man will ultimately exterminate all wild life. He will then be left alone on Earth with his domesticated animals and with swarms of insects and germs; alone in a world he has fashioned in the image of his technology.

Insects have flourished on this Earth for 350 million years or more and outnumber man by a factor of hundreds of thousands. They are better adapted to survive new technologies than larger animals. A few specimens always survive whatever poison may be administered to them. Multiplying with fantastic rapidity, they then present man with a harder strain which he must attack with still more poisonous pesticides. The true victims of this endless battle are not the insects but other living creatures, our natural allies in the war against insects. Piddlington closes his chapter on the balance of Nature with a synthetic proverb which doubtless makes no sense to practical men but will be readily understood by those who comprehend the ABCs of ecology: "Whoever destroys an elephant creates a thousand rats, or a million flies".

The examples I have given show that to make technology safe, we must have protective laws; that enactment of such laws depends on a humanistic attitude toward technology on the part of significant segments of the public and its leaders. But more is needed. Law and public opinion nearly always lag behind the swift development of new technologies. Therefore, what is additionally needed is more informed and responsible thinking among those who manage technologies. This can best be brought about by 'professionalizing' the decision-making process in technology. Experience has shown that in the hands of professional persons technology is managed with greater concern for human welfare than when it is controlled, as at present, by non-professionals. The classic example is medicine.

Of all technologies, that of the physician has benefited man most and harmed him least. The stringent standards set by the profession and by society for the education and professional conduct of physicians accounts for this happy circumstance. Not only is no one permitted to practise who has not given proof of his competence, but physicians must also be broadly, liberally, humanistically educated men and women. This gives them perspective in evaluating their professional actions, an ability to see these actions against a humanistic background. Moreover, they operate under a code of ethics which requires them to place the needs of patients above all other considerations.

We owe to Greece the noble idea that special knowledge and skill ought to be used humanistically, instead of merely for personal aggrandizement or power, or as a means of extracting maximum gain from those in need of the services of men possessing special expertise. It was a novel idea at the time, and remains unknown to this day in many regions of the world. Even among the people of Western civilization the precept is rarely followed outside medicine and a few other 'learned' or liberal professions. Most human affairs are conducted on the old Roman maxim of *caveat emptor*.

I have long believed that we should come appreciably closer to a humanistic technology if engineering were practised as a humanistic profession, and if, in consequence, engineers were accorded the professional independence granted members of liberal professions. I feel certain engineers would then find it possible to act with the same sense of professional responsibility and service to humanity which is characteristic of good physicians. It may be that in some countries engineering has already attained the status of a truly liberal or humanistic profession. But I doubt if this is the case in Great Britain or the United States.

Engineering now stands at the threshold; there is no reason why it should not enter the liberal professions. It has as its theoretical foundation a body of systematic knowledge, an academic discipline as rigorous and extensive as that of other learned professions. It has a highly developed technique for applying this specialized knowledge to practical problems. But to-day there is no absolute requirement that an engineer must be a liberally educated man, nor has engineering adopted the kind of ethical code that governs the older professions of medicine and law.

It is because of the 'professional' characteristics I have here stated that members of the 'learned' professions demand and are accorded professional independence. "The essence of professions," wrote Abraham Flexner, an expert in this field, "resides in the application of free, resourceful, unhindered intelligence to the comprehension of problems." Service ceases to be professional if it has in any way been dictated by the client or employer.

The role of the professional man is to lend his special knowledge, his well-trained intellect, his dispassionate habit of visualizing problems in terms of fundamental principles to whatever specific task is entrusted to him. Professional independence is not a special privilege but rather an inner necessity for the true professional man; it is a safeguard for his employer and for the public as well. It is what chiefly sets him apart from the skilled technician.

This independence of professional judgment has not yet been accorded the engineer. He still has to win it for himself. Engineers are nearly always salaried employees rather than self-employed, which makes it all the more essential that they gain professional status. Where engineers and physicians work in the same organization, it happens not infrequently that the most experienced engineer's professional judgment will be overruled by a lay superior, while no one would think of dictating to a physician, no matter how young and inexperienced he might be. Yet the university-trained engineer is as competent a professional in his field as is the physician. The difference lies in the determination of the medical profession to resist lay interference and its success in winning this point, while the engineering profession has shown little determination, and therefore has had little success.

I speak of this with feeling. As is known, my work is in one of the new technologies: one that is dangerous unless properly handled. I am frequently faced with the difficulty of convincing administrative superiors that it is not safe for them to overrule their technical experts. Here is a case in point.

A superior once asked me to reduce radiation shielding in our nuclear submarines. He said the advantage of getting a lighter-weight reactor plant was worth risking the health of personnel. It was not possible to make him see that such a concept could not be accepted; that, moreover, where radiation is involved, we are dealing not just with the lives of present-day individuals but with the genetic future of all mankind. His attitude was that we did not know much about evolution and if we raised radiation exposures we might find the resulting mutations to be beneficial—that mankind might 'learn to live with radiation'. As one may surmise, I did not reduce the thickness of the shielding.

The British and the Americans are fortunate to live in countries so organized politically that individuals enjoy the greatest possible freedom consistent with their obligations as members of a civilized society. But freedom always comes at a cost. As citizens of a free, self-governing society, we are individually and severally responsible for the quality of our society. The values making for civilized life are neither created nor preserved without continuous effort. In a democracy it is the people themselves who must make this effort.

A final word needs to be said concerning the impact of technology on the political institutions of a free society.

By making it possible for affluence and leisure to be spread over large segments of the population—theoretically over all the people—technology gives support to democratic institutions. We are approaching a situation comparable to that of Athens 2,500 years ago, where every citizen was an active participant in the governance of his city State. He would not have had the leisure to do this had there not been slavery. To-day, each of us has many more mechanical slaves than the Athenian had live

ones. We have at least as much leisure as he had to devote to public affairs.

The Athenian, however, dealt with public issues which were not beyond the comprehension of ordinary citizens. Modern democratic citizens, on the other hand, are faced with issues which are extremely difficult for laymen to understand. They must depend, to an extraordinary degree, on the advice of experts. Whether such advice is competent as well as impartial is often hard to judge. Much of the difficulty arises from the complexities technology introduces into modern life. To the extent that it renders public issues incomprehensible to ordinary citizens, technology undermines democratic institutions.

Technologically created affluence and leisure make it possible for Great Britain and the United States—in fact all advanced industrial countries—to socialize the cost of education, thus giving every citizen a chance to become as educated as his God-given talents and his determination allow him to be. The opportunity is there, but will it be seized?

We do not yet know whether people may not prefer to live the life of the idle rich—as they imagine it to have been—pursuing fun and games, not bothering about becoming educated or meeting their public responsibility, or whether they will decide to emulate those—and there were many in all ages—who considered material comfort and leisure a trust, to be used for improvement of self and society. We have a choice, but unless democratic electorates raise their competencies to a higher plateau, they will discover that they cannot effectively control either their government or their technology.

It is obvious that a society's technological level determines the range of occupational skills for which there is effective demand. The higher the level, the smaller the demand for unskilled labourers, the greater that for intelligent, well-educated professional persons, semi-professionals, skilled technicians. Less obvious is the fact that technology also sets a lower limit to the educated intelligence citizens must have if they are to meet their public responsibilities—a sort of Plimsoll mark. Those who fall below this mark are precluded from participating in the public dialogue through which consensus is formed in free societies; they are precluded simply because they do not understand public issues involving technology. Democracy is not viable if too many fall below this mark.

In an oversimplified way, one might say that in a free society citizens have private liberties and public responsibilities; they safeguard their private liberties by faithfully discharging their public duties. Any diminution,

whatever its cause, of the individual citizen's ability to think independently about matters that determine the shape of his society, any lessening of his participation in its governance, makes society less free, democracy less viable.

How we use technology affects profoundly the shape of our society. In the brief span of time—a century or so—that we have had a science-based technology, what use have we made of it? We have multiplied inordinately, wasted irreplaceable fuels and minerals and perpetrated incalculable and irreversible ecological harm. I have thought much about this, and I can find no evidence that man contributes anything to the balance of Nature—anything at all. On the strength of his knowledge of Nature, he sets himself above Nature; he presumes to change the natural environment for all the living creatures on this Earth. Do we, who are transients and not overly wise, really believe we have the right to upset the order of Nature, an order established by a power higher than man?

These are complicated matters for ordinary citizens to evaluate and decide. How in future to make wiser use of technology is perhaps the paramount public issue facing electorates in all industrial democracies; a problem difficult enough in itself but rendered still more so by the strategies of those who wish to continue using harmful technologies.

As I said at the beginning, the communications media could render great service by providing the public with a balanced view of the issue. Reportage which consistently takes a humanistic attitude toward technology would be of immense help. So would consistent stress on the propriety of laws to prevent technological damage. It cannot be said too often that Government has as much a duty to protect the land, the air, the water, the natural environment of man against such damage, as it has to protect the country against foreign enemies and the individual against criminals; conversely, that every citizen is duty-bound to make an effort to understand how technology operates and what are its possibilities and limitations. All this is necessary if technology is to be assigned its proper place in human affairs, if it is to be made humanistic.

A free society centres on men. It gives paramount consideration to human rights, interests and needs. But, once ordinary citizens come to feel that public issues are beyond their comprehension, a pattern of life may develop where technology, not man, would become central to the purpose of society. If we permit this to happen, the human liberties for which mankind has fought, at so great a cost of effort and sacrifice, will be extinguished.

MATERIALS SCIENCE AND ENGINEERING AT THE MASSACHUSETTS INSTITUTE OF TECHNOLOGY

WITH the official opening and dedication of the new interdisciplinary laboratory of the Center for Materials Science and Engineering (Fig. 1) on October 1, 1965, a new phase in the long-standing development of research and teaching in this field was inaugurated at the Massachusetts Institute of Technology. The principal speaker at the dedication of the new building was Dr. William O. Baker, vice-president of research, Bell Telephone Laboratories. The building was named the Vannevar Bush Building in recognition of the outstanding contributions to science and engineering and to their utilization to meet urgent national needs of Dr. Vannevar Bush, honorary chairman of the Corporation of the Massachusetts Institute of Technology.

Other speakers at the dedication included President Julius A. Stratton, Dean Jerome B. Wiesner of the School of Science, Dean Gordon S. Brown of the School of Engineering and Dr. James R. Killian, jun., chairman

of the Corporation, who presided. Representing the Advanced Research Projects Agency of the Department of Defense was its director, Dr. Charles M. Herzfeld. It was a happy combination of support from the Advanced Research Projects Agency and the Massachusetts Institute of Technology Second Century Fund with special assistance in financing from the Alfred P. Sloan Foundation of New York which made the building possible.

Materials science and engineering, both in their fundamental and practical aspects, have been studied for many years at the Institute. The Departments of Mechanical, Electrical and Chemical Engineering and the Department of Metallurgy have for many years been investigating the properties of traditional metals, ceramics, glasses and other materials used in the course of their professional activities, and more recently new materials such as semiconductors and polymers have been added to the list. The basic science departments, especially physics and chemistry,

have long had as part of their programme fundamental investigations of the cohesive properties of solids and of the phenomena of electrical and heat conduction in various materials as well as of their magnetic and optical properties. There is now scarcely a department within the Schools of Science, Engineering and Architecture which is not involved in materials research and teaching in a major way.

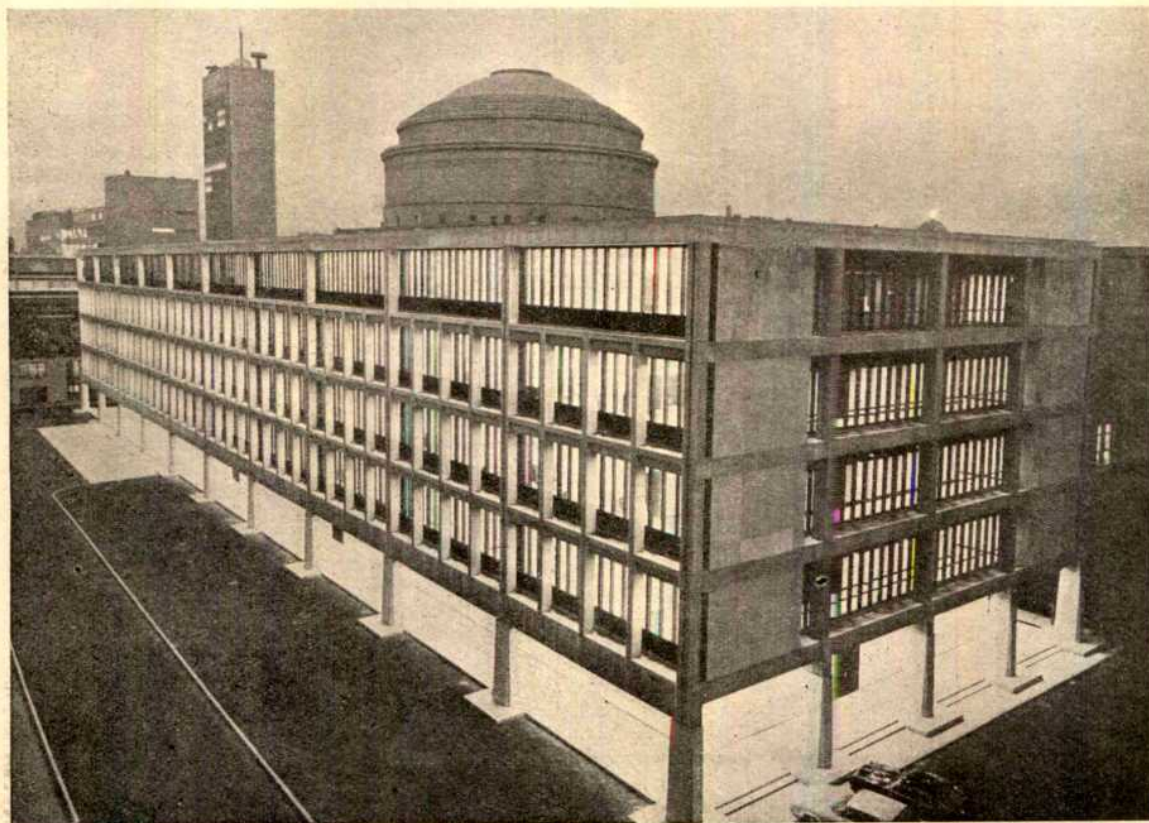
To give a focus for all this activity the Center for Materials Science and Engineering has been established, and also to take advantage of the interdisciplinary character of much materials research. This arose from a strong desire to strengthen the programme of materials research to meet the urgent demands of modern technology both through research achievement and through the production of well-trained engineers and scientists in this field. The benefit obtained by contact of research workers from different disciplines is well recognized in a number of great industrial and Government laboratories; the Interdisciplinary Laboratory for Materials Science and Engineering has been built to encourage such interaction in a university environment.

The new laboratory building provides a number of central facilities which have always been envisaged as the cement which would bind the Center together. Modern equipment for materials research is expensive, and centralization is the only way to provide some of this equipment. The facilities include equipment for preparation, purification and evaluation of various groups of materials. This equipment is usually in charge of a professor, and four main groups of materials are provided for at the moment: metals, insulators, semiconductors and composite materials such as ceramics. A central analytical laboratory provides up-to-date chemical methods of evaluation, while physical methods include X-ray analysis, optical and electron microscopy, and electron micro-probe analysis. Some expensive pieces of

equipment used primarily by individual professors for their own research are also available some of the time for use by others. These facilities are also backed by the close association of the laboratory with other laboratories of the Institute, such as the Laboratory for Nuclear Science, which provides facilities for the use of neutron scattering, the National Magnet Laboratory, which provides very high magnetic fields, and the Spectroscopy Laboratory with its excellent instruments and wide variety of optical techniques.

The research programme in the new laboratory is supported by a number of Government agencies and industrial organizations through individual contracts and grants to professors, but a substantial part is supported by the Advanced Research Projects Agency (ARPA). This research covers a wide variety of topics, too numerous to be detailed here, but falling generally into the following categories: basic physics and chemistry of solids, study of materials of interest in electronics and optics, research into the factors that determine the ultimate strength and mechanical properties of materials under a wide variety of conditions. Of particular interest are materials for use at high temperatures and pressures. Special materials such as high-field superconductors, and laser materials, are also included.

Although the research programme to be carried out in the new building represents a substantial fraction of the total materials research effort at the Institute, it is only part of a considerably larger activity in various departments and in other laboratories. It is planned to concentrate a good deal of the more fundamental research on materials science and engineering in the new laboratory. This has been achieved by bringing together some of this work from a number of departments and by starting new programmes of basic research. A number of these new research projects are already well under way and are producing results which may ultimately have a profound



(Massachusetts Institute of Technology photograph)

Fig. 1. Center for Materials Science and Engineering, Massachusetts Institute of Technology

effect on technology. The main impact of these new facilities, however, is likely to be through the body of scientists and engineers who will receive an advanced training in materials research through their association with the Interdisciplinary Laboratory.

The laboratory is staffed largely by faculty members drawn from a number of academic departments, at present mainly from physics, chemistry, electrical engineering and metallurgy, together with their graduate students. These are supported by technicians and engineering assistants. A small number of senior research staff holding appointments with the Division of Sponsored Research are also employed, together with an increasing number of young post-doctoral research staff continuing a research programme for two to three years after graduation. This form of further training has been growing in importance in the physical sciences and is now also becoming more common in engineering research. The laboratory is therefore largely an academic one, the director, Prof. R. A. Smith, C.B.E., F.R.S., being also a professor in the Physics Department. The director is supported by a steering committee, of which he is chairman, consisting of the deans of science and engineering and a number of senior faculty members.

The laboratory building was designed by the architectural firm Skidmore, Owings and Merrill of Chicago and has some novel features. It is a ferro-concrete structure built on five floors, giving a gross area of about 150,000 square feet. Since the building is joined to the main building of the Institute and forms a new north façade, the architects were faced with the problem of providing a functional and modern building which would fit in with the severely 'classical' form of the existing building. In this they have been successful. The new building is pleasing, yet distinctly modern, in appearance and in function. The design of the building is based on the concept of central vertical service cores around which the individual laboratories are arranged in groups of six on each floor. Each vertical shaft has cross-section 9 ft. \times 14 ft. and carries supply lines for hot and cold water, drains, various kinds of gas and compressed air, exhaust ducts for fume cupboards, heat canopies, etc. The laboratories are arranged in a double line on each side of the central plane of the building, though in a few instances particularly large laboratories are made by joining two so as to have twice the normal width. The access corridors run along the whole length of the building and on each of the long sides between them and the outside walls is a generous provision of offices and small conference rooms. The laboratories have no windows on to the corridors and are dependent entirely on the air-conditioning system for ventilation and on artificial lighting. The offices, on the other hand, have large windows forming a significant part of the outer walls. The provision of offices is such that all staff have office space in daylight, in general shared, except for senior faculty members. Light services are carried above false ceilings in the corridors. This design dictated full air-conditioning for the entire building which is supplied by means of a forced-flow air system through heat exchangers fed by steam brought in from the outside for heating and through installed chillers for cooling.

Many of the laboratories were designed with known needs in mind, but have been made sufficiently flexible so as to accommodate changes of programme. Electric power enters each laboratory from the vertical service cores in a 3 in. \times 5 in. metal raceway at bench height having ample capacity to distribute many current outlets around the walls, and to give a great deal of versatility.

The windows of the building, which are set in heavy wooden frames, are each 8 ft. high and made of heat-absorbing glass. They are set back 2.5 ft. from the external columns of the building. This provides shading and gives a reduction of sun-glare from the large expanse of window glass. These windows are one of the distinctive

architectural features of the building and give it its dominant external characteristic, as will be seen from the photograph.

Before the official opening of the new building a symposium on "Materials Science and Engineering" was held on September 30 and on the morning of October 1. The symposium, under the chairmanship of Prof. R. A. Smith, supported as session chairmen by Prof. A. H. von Hippel and Prof. N. J. Grant, covered a wide variety of topics. Speakers were drawn from the wider community of the Massachusetts Institute of Technology including the Lincoln Laboratory and the National Magnet Laboratory.

The first session was given over to basic research in solid-state physics. Prof. G. B. Benedek discussed some fascinating experiments on the scattering of light by thermally generated acoustic waves in liquids and solids. Of particular interest were some new results on scattering from liquids near their critical point. These experiments depend on the availability of highly monochromatic light sources of considerable power and illustrate well the use of lasers as powerful tools in physics research. Prof. D. P. Shoemaker described the use of low-energy electron diffraction techniques in the study of the arrangement of atoms on the surfaces of crystals and emphasized the importance of such studies in helping to clarify such processes as heterogeneous catalysis, corrosion, passivation and epitaxial growth. Prof. C. G. Shull described the use of polarized beams in neutron diffraction studies of ferromagnetic crystals particularly in determining the distribution of magnetization throughout the unit cell in such crystals. He also described some new experiments on the scattering of neutrons by type II superconductors, which show effects due to electron spin pairing and field penetration below the superconducting transition temperature. Prof. B. L. Averbach then showed how spin correlations have been determined above the critical temperature by studies of diffuse neutron scattering in single crystals of iron and MnO.

The second session opened with a review by Prof. J. C. Slater of what can be done at present in explaining the properties of solids in terms of quantum theory. He dealt particularly with band structure, including the shape of the Fermi surface in metals, and with the interpretation of experiments on solids in the presence of magnetic fields. Prof. B. Lax, National Magnet Laboratory, discussed the use of high magnetic fields in such experiments including the observation of helicon and Alfvén waves in solid-state plasmas. He also considered the use of high fields in the study of such phenomena as magnetic breakdown and the role of open orbits in conduction in metals in a high magnetic field. This session also included discussion of research on materials of interest in electronics. Prof. G. W. Pratt described his recent theoretical investigations of the lead salts, PbS, PbSe and PbTe, and showed how much better understanding of the band structure of these materials is now possible through the inclusion of relativistic effects in the calculations. He was able to calculate the variation of band structure with pressure and to predict the possibility and range of tuning of lasers made from these materials by application of pressure. Dr. R. H. Rediker of Lincoln Laboratory discussed laser action in semiconductors and showed how PbSe laser diodes had been tuned through the range 8–22 μ by use of the pressure effect, in some recent experiments carried out in collaboration with Prof. W. Paul of Harvard University. He also reported that lasers made with all three salts, PbS, PbSe and PbTe, had been made to operate through the use of optical excitation.

The third session of the symposium opened with a discussion by Prof. Morris Cohen of the essential phenomena which underlie the flow and fracture strength of various types of crystals including metallic, ionic and covalent crystals. The contributions and limitations of

the theory of dislocations were considered and the rather greater attention now being given to such things as precipitation hardening was emphasized. Prof. W. D. Kingery then discussed the control of microstructure in ceramics and showed the great importance of investigations of such structure in interpreting measurements of diffusion coefficients and related quantities in such materials. He emphasized the need for careful preparation of materials in order to obtain reproducible results. Prof. H. C. Gatos described some new studies of the surfaces of semiconducting compounds such as InSb and underlined the importance of such studies in understanding chemical reactions which take place at the surfaces of such semiconductors. The symposium concluded with a discussion by Prof. John Wulff of the metallurgy of hard superconductors. This type of material is becoming of great technological interest and it is important to understand the part played by the effects of processing on microstructure and on impurity content. Interaction of oxygen has been shown to have a marked effect on the upper critical field, and combination of thermal

and mechanical treatment have been shown to have effects which in turn depend on the impurity concentration.

During the symposium a luncheon and a banquet were held. At the former Dr. William Shockley spoke on the contrast between the teaching of materials science when he was a graduate student as compared with what is now taught to students and on the importance of imaginative terminology, illustrating his point with such terms as 'hot electrons' and 'optical pumping'. At the banquet the principal speaker was Dr. Harold Brown, now secretary of the Air Force. Dr. Brown emphasized the vital importance of materials research in all areas of technical development whether military or civil, and showed that there is scarcely an advanced project not limited by failure of materials to meet the ever-increasing demands made of them. He also spoke of the importance of new ventures in the utilization and management of federal funds for research and of the opportunities and responsibilities which academic institutions assumed in participating in federal support.

THE WALTER AND ELIZA HALL INSTITUTE OF MEDICAL RESEARCH, MELBOURNE

Sir Macfarlane Burnet, O.M., F.R.S.

ON September 1, 1965, Sir Macfarlane Burnet retired from the directorship of the Walter and Eliza Hall Institute, Melbourne, a position he had held with increasing distinction for twenty-one years.

Frank Macfarlane Burnet was born at Traralgon, Victoria, in 1899, and graduated in medicine from the University of Melbourne in 1922. After a resident year at the Melbourne Hospital, Dr. Charles Kelloway, then the director of the Walter and Eliza Hall Institute, recognized Burnet's research potential and persuaded him to join the staff of the Institute. Thus, with the exception of two study periods in London, Burnet was associated with the Walter and Eliza Hall Institute for forty-one years.

Burnet's first interest was in bacteriophage virus multiplication, and his early studies on one-step growth curves and genetic mutations in phage remain classics to this day. Immunology soon joined virology as a primary interest, and in 1930 Burnet studied staphylococcal toxin and produced an effective toxoid.

A period of study at the National Institute of Health in London, under Henry Dale, proved a turning-point in Burnet's career. This was the time of the discovery of ways of growing the influenza virus. Burnet soon mastered and developed the Goodpasture technique of virus culture in the egg embryo and, over the next twenty years, used this model for an extensive investigation of influenza virus multiplication. Burnet collaborated with Gottschalk and Ada on biochemical aspects of viral multiplication, leading to the discovery that influenza was an RNA virus and to the identification of the viral enzyme neuraminidase.

Immunological theory was a continuing interest of Burnet's, and in 1948 he and Fenner proposed the 'self-marker' theory of antibody formation, with its prediction of the phenomenon of immunological tolerance. This monograph deeply influenced the work of Billingham, Brent and Medawar; when these workers made immunological tolerance an experimental fact, a new era of cellular immunology began. For their work on immunological tolerance, Burnet and Medawar shared the Nobel Prize for Medicine and Physiology in 1960.

In 1957 Burnet found that the field of virology no longer excited him so much as it had done in the past. He took a courageous decision: the whole of the work of

the Walter and Eliza Hall Institute was to be switched progressively to immunology. The next three years saw the emergence of the clonal selection theory of antibody formation, the single cell approach to immunology by Nossal and Lederberg, and the increasing analysis of clinical auto-immune mechanisms by Wood and Mackay.

It was typical of Burnet that his Nobel Lecture in Stockholm in 1960 contained not an analysis of past achievements but a major prediction for the future. Burnet predicted that the thymus would prove of crucial importance to the understanding of the immune process. He returned to Melbourne to study the thymus of auto-immune mice—the extraordinary New Zealand Black strain. He encouraged Metcalf to pursue his studies of thymic influence on lymphopoiesis and leukaemogenesis in the cancer research laboratories of the Walter and Eliza Hall Institute. The extraordinary impact that the work on the thymus, performed over the past four years, has had on immunobiology led to a very fitting culmination of Burnet's long period as director of the Walter and Eliza Hall Institute. To mark his retirement, the Ciba Foundation organized a symposium on "The Thymus: Experimental and Clinical Studies", which was held in Melbourne in late August.

Burnet's honours are almost too numerous to recount. He was elected a Fellow of the Royal Society in 1942, was awarded its Royal Medal in 1947 and the Society's highest honour, the Copley Medal, in 1958. He was a Foundation Fellow of the Australian Academy of Science, and was elected its president in 1965. Burnet was knighted in 1951, and in 1958 was received in audience by Her Majesty Queen Elizabeth II, who conferred on him the Order of Merit. In 1960 he shared the Nobel Prize with Prof. P. (now Sir Peter) Medawar. Over the years he has received numerous honorary doctorates and lectured widely on his work and theoretical ideas.

No one who is familiar with Burnet's boundless enthusiasm, amazing capacity for work, and intense dedication to the cause of science will be surprised to hear of his retirement plans. Apart from his important duties as president of the Australian Academy of Science, he has joined the interim council of Melbourne's third university, La Trobe University (see *Nature*, 208, 434; 1965) and has become chairman of the New Guinea Medical Research Advisory Committee of the Australian Government. He has also accepted a position

as Rowden-White Research Fellow in the Department of Microbiology of the University of Melbourne, and will continue to work actively on the pathology and genetics of auto-immunity in mice. He hopes to write at least two major text-books—a major thesis on modern immunological theory, and a more popular work. We can thus look forward to a continued flow of enlightened speculation and inspiration from Burnet's richly productive pen.

Prof. G. J. V. Nossal

PROF. G. J. V. NOSSAL, formerly deputy-director of the Walter and Eliza Hall Institute, has been appointed to succeed Sir Macfarlane Burnet as director. Prof. Nossal was educated at St. Aloysius College, Sydney, and the University of Sydney, where he graduated B.Sc. (Med.) 1952 in bacteriology, and M.B., B.S., 1954, with first-class honours and a University Medal. In 1957 he joined the staff of the Walter and Eliza Hall Institute, graduating Ph.D. in experimental medicine in 1959. In 1960–61 he was assistant professor in the Department of Genetics, Stanford University School of Medicine, and visiting professor of immunology, University of Michigan, in 1961. He then returned to the Walter and Eliza Hall Institute.

Since then, he has been a visiting scientist to various institutes, notably the Children's Cancer Research Foundation, Boston, in 1962, and Irvington House Institute, New York University, in 1964. Dr. Nossal went to the Walter and Eliza Hall Institute when the emphasis was switching from virus work to immunology. Under the stimulus of Burnet and also Prof. Joshua Lederberg, then a visiting professor, Nossal was the first to demonstrate that isolated lymphoid cells (plasma cells) could liberate antibody. In doubly immunized animals the great majority of cells produced one type of antibody only. He also made important contributions to the understanding of immunological tolerance, and while at Stanford University investigated in detail the kinetics of cellular proliferation in lymph nodes stimulated by antigen injection. He has since made significant contributions to the study of antigen distribution in lymph nodes, thus opening up an important field in the relationship of antigen to the induction of antibody formation. His reviews on immunological topics have played a major part in the development of important aspects of the genetic aspects of immunity. On succeeding Sir Macfarlane Burnet he was appointed professor of medical biology in the University of Melbourne.

OBITUARIES

Prof. T. P. Hilditch, C.B.E., F.R.S.

PROF. T. P. HILDITCH, professor of industrial chemistry in the University of Liverpool during 1926–51, who was mainly responsible during those years for the great advance in our knowledge of the chemical constitution of natural oils and fats, died at his home in Oxtou, Birkenhead, on August 9.

Thomas Percy Hilditch was born in London in 1886 and educated at Owen's School, Islington. He proceeded to University College, London, in 1904, where he graduated B.Sc. with first-class honours in chemistry. In 1908 he was awarded an 1851 Research Exhibition, which enabled him to work for two years at Jena under Prof. L. Knorr and for one year at Geneva under Prof. Ph. Guye. He returned to University College to continue research with Prof. S. Smiles. His major interests in these years were the aromatic sulphones, sulphonic acids, and the relationship between optical activity and unsaturation. In 1911 he was awarded the D.Sc. of the University of London. By the time he was twenty-five years old he had published some forty papers and his reputation as a brilliant organic chemist was well established.

In 1911 Hilditch joined Joseph Crosfield and Sons, Ltd., Warrington, as research chemist. Crosfield's had recently put into operation the first successful plant for the hydrogenation of oils and fats. The hydrogenation process and catalysts for it were the subjects of major investigations carried out by Hilditch during his fourteen years at Warrington. The results of these were published, in conjunction with E. F. Armstrong, in a series of papers in the *Proceedings of the Royal Society*. This was not by any means the only field of investigation in which Hilditch worked successfully at Crosfield's. During the First World War, for example, he was intimately connected with a Government project at Warrington for the industrial production of acetaldehyde and acetic acid from ethyl alcohol.

In 1925 Hilditch was appointed to the newly created Campbell Brown chair of industrial chemistry in the University of Liverpool, a post he held until his retirement in 1951. It is by the results of his work during that period that he will always be remembered.

Only a year before Hilditch went to Liverpool, E. F. Armstrong had entitled his presidential address to the Society of Chemical Industry "A Neglected Chapter of

Chemistry: Fats". Hilditch's and his students' work at Liverpool between 1925 and 1951 completely removed any justification for the title of Armstrong's address to be used again.

Hilditch always considered that oils and fats were a branch of organic chemistry, and it was with this attitude that he commenced and carried out his work so successfully. Little quantitative information was available in 1925 on the component fatty acids of natural oils and fats, and not any on the component glycerides. Furthermore, the techniques for obtaining these were, for the first, inadequate and, for the second, non-existent. By 1951 not only had Hilditch and his students experimentally obtained this information for many oils and fats, but their work had stimulated others all over the world to work in this field. His classic book, *The Chemical Constitution of Natural Fats*, in the fourth (1964) edition shows that we now know quantitatively the component fatty acids of some 1,450 fats and the component glycerides of about 100.

He always believed that there was a relationship between the component fatty acids and glycerides, and the order of the evolutionary development of the parent organisms from which the fats were derived. This relationship was the basis on which he wrote his classic book. However, even in 1964 he felt that gaps in our knowledge remained, particularly in the component glyceride field, but he anticipated that with the aid of recent techniques such as gas-liquid chromatography, controlled enzyme hydrolysis and thin-layer chromatography, these would be quickly filled.

The success of Hilditch's research school at Liverpool became known throughout the world, so that by his retirement he had had students from very many countries. Not only did he successfully conduct his own Department, but he was also a colleague valued by many others on the University staff, and his services to the general administration were greatly appreciated.

During his retirement Hilditch continued his activities and interest in his subject and wrote two further editions of *The Chemical Constitution of Natural Fats*, as well as for some years acting as consultant to two industrial firms. He wrote five other books during his lifetime, three of which had two or more editions. These were text-books on physical and organic chemistry, and

A Concise History of Chemistry, written in his early years, while the later ones were *Industrial Catalysis* and *Industrial Fats and Waxes*.

Hilditch was elected a Fellow of the Royal Society in 1952. He was also a Fellow of the Chemical Society and the Royal Institute of Chemistry, and a member of the Society of Chemical Industry: he served the last two Societies as a council member and as vice-president. Up to the end of his life he continued to give great service to their Liverpool sections, by whom he will be greatly missed.

Hilditch was frequently consulted by Government bodies, and for his services to the Colonial Products Research Council he was awarded the C.B.E. in 1952.

During his retirement he received many other honours. In 1962 the Lampitt Medal from the Society of Chemical Industry, in 1964 the Chevreul Medal from the Grouperment Technique Des Corps Gras, France, and this year the American Oil Chemists' Society held in his honour a special symposium on "Glycerides", and their North Central Group gave him the Alton E. Bailey Award. Unfortunately, Prof. Hilditch was unable to receive these last two personally.

At a first meeting with Hilditch, even in his younger days, one had the immediate impression of a highly disciplined man with authority and of a somewhat austere mien, but on longer acquaintance something would occur to produce that kindly smile by which all his old friends affectionately remember him. The affection in which he was held was evinced by the many visitors to his home, particularly old students, and the honour which they felt when in turn he visited them, as he frequently did.

Hilditch always put his energies first into the duties of his position at the time, whether as student, industrial research chemist, or professor, but he was not without outside interests. He delighted in working in his garden or watching a cricket match and for many years served All Saints Church, Oxtou, as churchwarden. It was at this church that the final tribute was paid to him in the presence of a large number of his former colleagues, students and friends.

His widow, who greatly helped him in his work, survives him, and also three married daughters of a previous marriage.

P. N. WILLIAMS

Prof. C. B. Purves

PROF. CLIFFORD B. PURVES, chairman of the Department of Chemistry, McGill University, Montreal, died on September 30. As E. B. Eddy professor of industrial and cellulose chemistry he was also head of the Wood Chemistry Division of the Pulp and Paper Research Institute of Canada.

He leaves a widow, three sons, and three daughters.

Although honours embarrassed him, Dr. Purves's distinction as an educator and scientist was recognized through the award of two honorary degrees, one from Lawrence College, Appleton, Wisconsin (1944), and the other from Windsor University, Windsor, Ontario (1964). In 1960 the Chemical Institute of Canada, of which he had been president in 1956-57, presented him with its medal for distinguished service to the sciences of chemistry and chemical engineering in Canada. The American Chemical Society, in 1963, honoured him with the Anselme Payen Award for his contributions to the field of wood chemistry.

Dr. Purves was a man of modesty, warmth, generosity, fairness and inflexible honesty. Though he never failed to remember important things, true to the character of a professor he was absent-minded about trivia and often made his absent-mindedness the butt of his own wit. His sense of humour was strong; he had a large fund of anecdotes, always apt to the occasion, and, unlike many professors, he never ruined their point in the telling.

His strongest personal characteristic was concern. He was always concerned about each of his colleagues and students as individuals and, however busy he may have been, he never turned away a caller who might be troubled by a problem, personal or scientific. He always helped to the utmost of his power and probably felt his deepest frustrations on those occasions when his help was to no avail.

His concern extended far beyond his immediate colleagues to the profession of chemistry in general. His active participation in the Corporation of Professional Chemists of Quebec (he was its president in 1964) arose from his feeling of the need to strengthen the position of chemists as professionals.

Outside the realm of chemistry, Dr. Purves had other concerns. In his Convocation Address at the University of Windsor he spoke fervently of the need for educators, in their anxiety to cater for the demands of college-oriented youngsters of IQ 110 and higher, who constitute only 20-30 per cent of the general population, not to overlook the plight of the 20 per cent of IQ less than 80. He felt that in an increasingly technological society the lives of these latter people were becoming more and more aimless, and devoid of satisfactions.

In his Anselme Payen Award Lecture in 1963, Dr. Purves had also spoken of education. He felt that the greatest single deficiency of modern primary education in North America was its failure to teach elementary arithmetic, and he held up the Scottish education system as a model to be followed.

Born and educated in Scotland, Dr. Purves went to Canada in 1943 to succeed Prof. Harold Hibbert on the latter's retirement from the chair of industrial and cellulose chemistry. He arrived well prepared, having obtained his doctorate in 1929 in the field of carbohydrate chemistry under Sir James Irvine. During 1926-29 he had also worked as a Commonwealth Fund Fellow attached to the Polarimetric Division of the U.S. Bureau of Standards, in Washington, D.C., where C. S. Hudson had been his chief.

After two more years in Scotland, Dr. Purves moved to the United States in 1931, and became a research associate at the Chemical Foundation of New York (now the U.S. National Institutes of Health). During 1936-43 he was an associate professor of organic chemistry in the Massachusetts Institute of Technology.

At McGill, he built a strong research group, and continued his investigations of the chemistry of carbohydrates. A large part of his work was directed at the location of substituents in such cellulose derivatives as the acetates, xanthates and nitrates (see *Chemistry in Canada*, 25-29; December 1960). During this period he also began to interest himself in the lignin component of wood, and in 1947 published a method for the isolation of lignin by the mild oxidation of carbohydrates with potassium periodate (Ritchie and Purves, *Pulp and Paper Mag. Canada*, 48, No. 12, 74; 1947; Wald, Ritchie and Purves, *J. Amer. Chem. Soc.*, 69, 1371; 1947). This isolated lignin formed the basis of much subsequent work on the mechanisms of reactions related to the processes of the pulp and paper industry.

At McGill alone, more than a hundred students received their doctorate degrees under the tutelage of Dr. Purves. Many of them have now themselves achieved prominence in universities, government and industry.

Dr. Purves assumed the chairmanship of the Department of Chemistry in 1961, and under his administration the Department grew in the number and quality of its staff. But his chief task during the past four years was to supervise the planning and construction of the new Chemistry Building, named in memory of his long-time colleague, Prof. Otto Maass. That building now stands as a memorial to both of them.

HENRY I. BOLKER

NEWS and VIEWS

The Editor of NATURE

It is with deep regret that we announce the sudden death, on November 15, of L. J. F. Brimble, editor of *Nature*.

Jack Brimble and *Nature* have been so closely associated for so many years that we find it impossible as we now go to press to assess the loss to the journal and to the world of science.

An appreciation will appear next week.

The National Physical Laboratory:

Sir Gordon Sutherland, F.R.S.

At the end of October 1964 Sir Gordon Sutherland resigned from the directorship of the National Physical Laboratory to become Master of Emmanuel College, Cambridge. He joined the Laboratory in 1956 from the University of Michigan, where he had been professor of physics and director of the Biophysics Research Centre since 1949. His earlier work at Cambridge had brought him recognition as a leading authority on infra-red spectroscopy. As director of the National Physical Laboratory, Sir Gordon was responsible for many important developments in the work and facilities of the Laboratory. In 1958 he reorganized part of its divisional structure on a more functional basis, concentrating the metrological activities into the Applied Physics, Light and Standards Divisions and creating the Basic Physics Division to pioneer developments in molecular physics which were likely to have potential importance to industry. New buildings and facilities for the Ship Division were opened at Feltham by the Duke of Edinburgh in 1959, a new laboratory with special equipment for the mechanical working of refractory metals was provided for the Metallurgy Division in 1963, and the Basic Physics and Autonomics Divisions occupied new buildings in 1964. Glazebrook Hall, a former wind-tunnel laboratory reconstructed as a conference and social centre with catering facilities for the staff and visitors and including a lecture theatre seating 380, was opened in 1961; its name commemorates the first director of the Laboratory. A Van de Graaf accelerator and a *KDF 9* computer, respectively, were added to the facilities of the Applied Physics and Mathematics Divisions, and new equipment was installed for the work of the Aerodynamics Division on supersonic flight and industrial aerodynamics. During the period under review the total staff increased from 1,080 to 1,460, and the number of scientific officers, including Research Fellows, rose from 155 to 255. Sir Gordon was especially interested and successful in fostering contacts with the universities which were of mutual benefit, leading on one hand, for example, to better success in the recruitment of highly qualified graduates to the National Physical Laboratory, and, on the other, to a considerable expansion in the number and value of research contracts placed by the Laboratory with universities. The announcement of the award of his knighthood was made in the Queen's Birthday Honours List of 1960.

Dr. J. V. Dunworth, C.B.E.

DR. J. V. DUNWORTH has been appointed director of the National Physical Laboratory in succession to Sir Gordon Sutherland. Dr. Dunworth was born in Manchester and educated at Manchester Grammar School and Clare College, Cambridge, where he graduated with first-class honours in 1937. In the same year he joined Lord Rutherford's nuclear physics research team at the Cavendish Laboratory, and was later elected a Fellow of Trinity College, Cambridge. During the Second World War he

was a member of Sir John Cockcroft's team working on radar, and in 1944 he was seconded to the National Research Council of Canada, again with Sir John Cockcroft, to work on the development of atomic energy. At the end of the War, Dr. Dunworth returned to the University of Cambridge as a lecturer in physics. In 1947 he became a member of the staff of the Atomic Energy Research Establishment at Harwell, where in due course he became head of the Reactor Division. He was alternate United Kingdom member on the Organizing Committee of the United Nations Atoms for Peace Conferences held in Geneva in 1955 and 1958. Dr. Dunworth was appointed deputy director of the National Physical Laboratory in 1962 and has been acting as director since the resignation of Sir Gordon Sutherland in 1964. During 1965 he was responsible for implementing the decision to merge the National Chemical Laboratory into the National Physical Laboratory. He is president of the British Nuclear Energy Society, a member of the Council of the Institute of Physics and the Physical Society, and a member of the Institution of Electrical Engineers. He was elected a Fellow of the American Nuclear Society in 1960. Dr. Dunworth was appointed a C.B.E. in 1955.

It has also been decided by the Ministry of Technology to replace the Executive Committee of the National Physical Laboratory by a smaller Steering Committee. The first chairman of the new Committee will be Prof. B. H. Flowers, Langworthy professor of physics in the University of Manchester.

Electronic Engineering in the University of Hull:

Prof. D. A. Bell

IN appointing Prof. D. A. Bell to the newly established chair of electronic engineering, the University of Hull has added to its staff an engineer of international reputation with a wide and varied range of interests. Prior to his appointment, Prof. Bell was visiting professor of telecommunications at McGill University. Prof. Bell is perhaps best known for his work on noise, and his many contributions to the investigation of this subject have crystallized in his book, *Electrical Noise*. His knowledge of communications is also shown by many papers, and the titles of his other books—*Information Theory and its Engineering Applications*, *Statistical Methods in Electrical Engineering* and *Intelligent Machines*—show not only some of his interests, but also his constant endeavour to relate theoretical developments to engineering realities and his deep concern with the social consequences of engineering progress. He has always been anxious to work and study with social scientists and has long argued in favour of the inclusion of social science in the engineering curriculum. But his interest in education has not been limited to the universities, nor his knowledge of science to the physical sciences and the technologies based on them. Indeed, his outstanding characteristic is a scientific curiosity, to satisfy which he has worked at many problems in many fields and taken part in many controversies. His new colleagues will find him stimulating and vigorous and can rely on him not only to lead in his special field, but also to take an active part in the whole life of the University.

A. Harden (1865–1940)

BORN in Manchester on October 12 a century ago, Arthur Harden was first noted as an inspired lecturer and demonstrator at Owen's College, where he collaborated with Henry Roscoe on the writing of text-books. Harden graduated at Erlangen in 1888, spent nine years at

Manchester, and in 1897 took a post at the Jenner Institute of Preventive Medicine (which later became the Lister Institute). There he began his fruitful investigations of fermentation, and in particular of the yeast juices first prepared by Buchner. Although when kept warm the enzymes from yeast liquefied cell walls while the autolysed liquid still contained zymase, it was expected that, on heating, the enzymes would be destroyed. Harden, however, showed that boiled fluid or extract could increase fermentation due to the presence of a 'co-enzyme' containing phosphates: on adding potassium phosphate a vigorous fermentation set in. He was thus able to show how cell-free juice could be separated into co-zymase and apo-zymase. From this he developed the concept of ferment and co-ferment. He went on to show the relation between a hexose and phosphate—the enzyme reacting with phosphate being named 'phosphatase'—and discovered several ester-like 'sugar phosphates'. Apart from directing the biochemical division of the Lister Institute and holding a chair at London, Harden edited the *Biochemical Journal* and wrote many text-books. He was awarded the Davy Medal and shared the Nobel Prize with Euler-Chelpin, who carried on Harden's work on heat-stable co-enzymes and purified the co-enzyme of zymase.

Science in France

THE October–November 1964 issue of *Nouvelle Frontière* (No. 8) is of exceptional interest for a series of articles on the politics of science. G. Palewski writes on science and national independence; A. Lebarthe on a crusade for science; and F. Le Lionnais on the pattern of research. The organization of research in France is described by P. Frédet, and P. Piganiol writes on international scientific co-operation. Under the title "Research and Power", F. Maintenon discusses the duel between the United States and the U.S.S.R. P. Cognard deals with research and planning, and R. Goussault with manpower problems under the title "Investment in Man", while P. Aigrin deals with research and defence. There are also short articles on nuclear research, space research and research in biology and in electronics. Some problems of scientific policy are also considered in an article in *Le Progrès Scientifique* for May 1965 (No. 84), the preceding issue of which (No. 83, April 1965) is devoted mainly to a review of the situation and perspectives of biological and medical research in France, but includes also the text of a survey of biological and medical research in Europe by R. P. Grant, C. P. Huttner and C. C. Metzner, published in *Science* for October 23, 1964.

The British Glass Industry Research Association

THE tenth annual report of the British Glass Industry Research Association has recently been published (Pp. 42. Sheffield: The British Glass Industry Research Association, 1965). It records some important economic and technical developments for the year under review, not the least of which is the materially improved financial position of the Association, due to substantial sums of money emanating from the Department of Scientific and Industrial Research, the Ministry of Aviation, and the Electricity Council. The latter two sponsor special research projects: the former on the mechanism of strengthening of glass, the latter on investigating ways of facilitating the introduction of electric melting. On the technical side, the chief subjects considered in the report of the director of research, Dr. R. G. Newton, are: improvement of furnace life; heat utilization in furnaces; improvements in methods of founding glass; investigation of thermal conditions during glass-forming operations; improved strength in glass and glass articles; analysis of glasses and raw materials; and service behaviour of glass articles. One research achievement is emphasized in this report; this concerns the mechanism of upward-drilling

of refractories which has now been elucidated so convincingly that a ciné-film has been prepared to show exactly how it takes place in furnaces; this subject is described in some detail under the heading "Improvement of Furnace Life" in the director's report. The Association continues to maintain its valuable information service and, besides research, its miscellaneous work includes some consultative testing, testing of safety glass, surveys of lighting conditions in factories, and statistical analyses.

The Wildfowl Trust

THE annual report of the Wildfowl Trust for 1963–64 is a record of remarkable growth and success (Edited by Hugh Boyd. Pp. 136+32 photographs. Illustrated by Peter Scott. Slimbridge, Gloucestershire: The Wildfowl Trust, 1965). In four years the number of visitors has increased by 50 per cent. Finances are satisfactory, and the facilities for research continue to improve and increase in extent. The number of imaginative ventures to further the aims of the Trust increases each year, and, as an example, the report describes the identification competition for schools. This was held at the New Grounds on March 7 in bitterly cold weather. Sixty-four boys and girls from sixteen schools took part. Leighton Park again entered several strong teams and had three teams in the first five places in the Senior Competition; their A team won the first prize from Monmouth, thus reversing the previous year's result. In the middle age-group a team from Acklam Hall, who had travelled 260 miles from Middlesbrough, came first, with Winchester second. The junior prize was won by Gloucester Girls' High School by one point from Beaudesert Park. Besides full details of the collections, the report describes research and conservation initiated and carried out by members of the staff. It also contains articles by distinguished contributors on research and conservation in various countries overseas. The photographs and drawings are of the usual high standard, although, perhaps, Peter Scott has excelled all his former efforts with his coloured cover drawing; it deserves separate publication.

Careers with Instruments

Careers with Instruments is a pamphlet prepared by the Society of Instrument Technology and is, in fact, a guide to both school-leavers and graduates who may be considering a working life in the fields of measurement, automatic control, data handling and computation, all implying training and competence in the use of modern instruments (Pp. 24. Society of Instrument Technology, 20 Peel Street, London, W.E, April 1965. 2s. 6d.). The underlying theme of this pamphlet is that measurement and control serve and are served by all sciences and technologies; in effect, there is a two-way relationship between such disciplines as physics, mathematics, chemistry, electronics, nucleonics, chemical-, civil-, electrical-, mechanical- and production-engineering, also medicine, and measurement and control. The training pattern and prospects in this vocation are clearly set out and described, whether the starting-point be a secondary modern school, grammar school, or apprenticeship in the industry; it takes care of advancement via colleges of further education, technical colleges, colleges of advanced technology, and university qualifications. Thus are fashioned craftsmen, technicians, or persons capable of filling responsible technical posts, and so on, subject to acquisition of City and Guild Final Certificate, Higher National Certificate or Diploma, or institutional recognition, whichever best fits the particular course of higher education chosen by the individual until he or she achieves his or her ultimate goal. The instrument world is, at the present time, well co-ordinated in Britain by such organizations as the Worshipful Company of Scientific Instrument Makers; the Society of Instrument Technology; various associations of manufacturers, such as the British Industrial Measuring and Control

Apparatus Manufacturers' Association, the Scientific Instrument Manufacturers' Association, the Scientific Instrument Research Association, and the United Kingdom Automation Council. The work of the British Standards Institution, with its many standards and excellent codes of practice, conceived and executed in close co-operation with these various bodies, is a vital part of this closely knit organization, and is rightly acknowledged in this informative publication.

Cerebrovascular Accidents

THE booklet entitled *Cerebrovascular Accidents as a Public Health Problem—Selected Recent Abstracts II*, is the second of two publications on this important subject which are issued free from the School of Hygiene and Public Health, Johns Hopkins University (Pp. 64. Baltimore, Md.: Research in Public Health Administration Project, School of Hygiene and Public Health, Johns Hopkins University, 1965). It provides abstracts of recent papers by numerous authors on this disease and on problems related to it. After an introduction by Prof. C. M. Wylie, successive chapters deal with problems of diagnosis, epidemiology and statistics, preventive aspects, and medical and surgical care. The main aim of the booklet is to help readers to find original papers which they may have overlooked, and both *Abstracts I* and *II* should be valuable to everyone who is interested in advances in knowledge of these afflictions, or who is in any way associated with their practical care. Publication of the booklet has been financed by a grant from the W. K. Kellogg Foundation.

Aquatic Microbiology Group

A NUMBER of workers engaged in various fields of aquatic microbiology have for some time felt that it would be well worth while to call into being an informal aquatic microbiologists group. Such a group would be useful for the interchange of ideas and for a discussion of the problems encountered in this field. The autumn meeting of the Society for General Microbiology held in Aberdeen, on September 16–18, included a symposium on marine microbiology, and afforded an obvious opportunity to convene such a group. Accordingly, the group met for the first time at Torry Research Station, with Dr. Lucas, director of the Marine Laboratory of the Department of Agriculture and Fisheries for Scotland, in the chair. The meeting was entirely informal and was concerned more with work in progress, or with problems facing workers in this field, than with the accounts of completed work which are presented at the familiar formal scientific meeting. The first discussion was concerned with sea-water as a culture medium, and reference was made particularly to the suspended matter found in natural waters, which microbiologists usually remove when making their media. It was suggested that by so doing they changed the physical and chemical environment sufficiently to prevent some organisms from growing, and that by leaving the suspended matter in the medium it may be possible to culture certain micro-organisms that cannot, at the moment, be cultured by traditional techniques. The second discussion dealt with the 'dark growth' of certain phototrophic algae which also have the ability to grow heterotrophically in the dark. Much of the discussion was concerned with the interpretation of saturation constants and doubling times found for a number of algae and bacteria under various experimental conditions. These suggest that in the natural environment the bacteria would soon outstrip the algae in the utilization of the available nutrients. Possible mechanisms to explain these phenomena were put forward and examined. The third session was devoted to an account of the work, now in progress in various laboratories, on the bactericidal effect of light on *Escherichia coli* in sea-water. This led to a more general discussion of the effects of light in the

visible range of bacteria, and on the possible protective effect of carotenoid pigments. At the end of the discussion it was decided that further meetings of a similar type should be convened, the first to be held in about a year.

Fertilizer Corporation of India, Ltd.

THE Planning and Development Division of the Fertilizer Corporation of India, Ltd., publishes a quarterly bulletin entitled *Technology*, which is concerned with both academic and practical problems in the expanding field of fertilizer production and use. The contents of a recent issue indicate the scope of this journal (*Technology*, 2, No. 1; January–March 1965. Pp. 69, including separate subject index, Vol. 1, January–December 1964. Fertilizer Corporation of India, Ltd., Sindri, Bihar). The chief subjects discussed are: X-ray study of calcium ammonium nitrate, by S. K. Ghosh, V. K. Srinivasa and B. K. Banerjee; physical investigation of calcium ammonium nitrate, by S. Mukherjee, M. Samaddar and H. Roy; removal of nitrogen from fertilizer factory effluent by biochemical nitrification and denitrification, by A. C. Das, J. A. Khan and B. K. Dutta; trivalent arsenic compound as an inhibitor of corrosion of stainless steel in sulphuric acid, by A. K. Roy and K. M. Verma; extraction and estimation of humic and fulvic acids from soil and their fractionation by paper chromatography, by A. Sinha and R. N. Shukla; use of air from a nitric acid turbo-compressor for de-riming cold boxes in air-liquefaction units of an ammonia plant, by B. S. Kalia and C. L. Kaul; bulk blending and compatibility of fertilizers, by S. Varma; and reprography, by M. R. Roy. The subjects of short communications range from solubility of arsenic trioxide in aqueous ammonia at 40° and 50° C, desensitizers for ammonium nitrate, determination of total pyridines and quinolines in ammoniacal liquor by ultra-violet spectrophotometry, to testing of gas-mask canisters. Other features of this bulletin are technical digests, notes, news and statistics.

Organo-mineral Complex in Soils

THE complex of mineral and organic matter in soils has been intensively investigated both by chemists and by biologists. The problem presents great difficulties, since the organic matter is of complex constitution and the surfaces of the mineral matter vary from those of relatively well-recognized micro-crystalline clay minerals to those of amorphous oxides and hydroxides. A liberally documented review of present knowledge, entitled 'Interaction between Clays and Organic Compounds in Soils'. Part 1: 'Mechanisms of Interaction between Clays and Defined Organic Compounds', by D. J. Greenland, is presented in *Soils and Fertilizers* (28, No. 5, 415; 1965). Free organic material may be separated from weakly bonded material by flotation techniques in liquids of suitable density (1.8–2.0) following satisfactory dispersion. Except in very sandy soils, most of the total organic matter is bonded with inorganic material, the uncombined fraction being in an early stage of decomposition—the bulk of humic acids, for example, seems to be present in the complex. It would also appear that the clay-size mineral matter is mainly associated with the complex, while sand and silt are mainly 'free'. The forces concerned in the interaction may be Coulombic attractions, or Van der Waals forces, but there may be a large number of major and minor interactions between different parts of the surface of the mineral and the organic molecule. The relative importance of the various processes has been investigated by measuring the adsorption of well-defined organic compounds by montmorillonite, clays with mica-type lattices, kaolinite, and oxides. In general, organic compounds of molecular weight less than 150 are not adsorbed unless they can take part in ion-exchange, but larger molecules, with or without a charge, may compete

effectively with water molecules, and uncharged polymers are strongly adsorbed.

Carbon and Graphite Group

A CARBON and Graphite Group has been created by the Institute of Physics and the Physical Society. The aim of the new Group is to provide a meeting-ground for physicists who are interested in carbon and graphite materials, and to collaborate with similar groups in sister societies, in order to further the work of an informal group which has been meeting regularly in London. The meetings are expected to interest theoretical physicists and those working with these materials in industry, academic institutions and Government laboratories. Joint meetings with other societies on topics of common interest are also envisaged. Prof. A. R. Ubbelohde and Dr. G. S. Parry have been appointed chairman and honorary secretary, respectively, of the provisional Committee, and the remainder of the members who signed the request for the formation of the Group will be invited to serve on it. It is anticipated that the first elected Group Committee will be nominated and elected by the Group members in the summer of 1966. Further information regarding membership of the Institute and Society and of its Carbon and Graphite Group can be obtained on request from the Registrar, Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

The Emmie Clough Scholarship

THE Royal Horticultural Society has announced that it is offering a scholarship provided out of a bequest from the late Mrs. Emmie Clough, to enable a girl to take a course of training in horticulture which would otherwise be beyond her means. The scholarship, valued at £380 a year, will commence in September 1966. It will be open to women between the ages of eighteen and thirty, and will be tenable for two years at Waterperry Horticultural School, Nr. Wheatley, Oxford. Candidates will be required to have attained a standard of education which will permit them to take full advantage of the course of study, and should normally have completed or be prepared to undertake such periods of practical training in horticulture as may be considered necessary. Further

information can be obtained from the Secretary, Royal Horticultural Society, Vincent Square, London, S.W.1.

University News:

London

PROF. W. MORRIS-JONES, professor of political theory and institutions in the University of Durham, has been appointed director of the Institute of Commonwealth Studies and professor of commonwealth affairs from January 1. Dr. G. S. W. Organe has been appointed to the chair of anaesthetics, tenable at Westminster Medical School. The following readers have also been appointed: Dr. S. Rosenblat (theoretical mechanics, tenable at the Imperial College of Science and Technology); Dr. J. S. Tooms (applied geochemistry, tenable at the Imperial College of Science and Technology). The following titles have been conferred: *Professor*, Dr. H. Spencer (morbid anatomy, in respect of his post at St. Thomas's Hospital Medical School); Dr. J. W. Smith (chemistry, in respect of his post at Bedford College); *Reader*, Dr. T. J. Chandler (geography, in respect of his post at University College); Dr. J. R. May (bacteriology, in respect of his post at the Institute of Diseases of the Chest).

Announcements

MR. H. WILLIAMSON (Canada) has been appointed chairman of the Executive Council of the Commonwealth Agricultural Bureaux in succession to Mr. C. S. McMorris (Jamaica) as from January 1, 1966. Mr. J. A. Afari (Ghana) has been appointed vice-chairman, from the same date.

A SYMPOSIUM on "The Distribution and Biochemistry of Latex in Plants", organized by the Phytochemical Group, will be held in the School of Pharmacy, London, on January 6. Further information can be obtained from Mr. A. H. Williams, Research Station, Long Ashton, Bristol.

A MEETING on "Management Control and Computers", arranged by the Central London Productivity Association, in association with the British Productivity Council, will be held in London during December 9-10. Further information can be obtained from Mr. L. Fuller, Arthur Andersen and Co., St. Alphage House, 2 Fore Street, London, E.C.2.

THE NIGHT SKY IN DECEMBER

All times are in Universal Time

MOON		CONJUNCTIONS WITH THE MOON	
New Moon	22d 21h	Venus	26d 04h, 5° N.
Full Moon	8d 17h	Mars	25d 09h, 3° N.
		Jupiter	9d 12h, 2° S.
		Saturn	1d 07h, 4° N. 23d 17h, 3° N.

PLANETS

Times of Rising (R) and Setting (S) during the month

Name	R/S	Beginning	Middle	End	Mag.	D_p (10 ⁶ miles)	Zodiacal position
Mercury	R	Unfavourable	6h 05m	7h 00m	—	80	—
Venus	S	18h 55m	19h 10m	19h 00m	-4.4	42	—
Mars	S	18h 05m	18h 05m	18h 15m	+1.4	199	Sagittarius→Capricornus
Jupiter	R	16h 55m	15h 50m	—	-2.3	384	Taurus
Saturn	S	23h 30m	22h 30m	21h 35m	+1.2	911	Aquarius

D_p is the distance of planet from the Earth on the 15th of the month

OCCULTATIONS OF STARS BRIGHTER THAN MAGNITUDE +6 AT GREENWICH

Star	R/D	Time	Mag.
30 Psc	D	2d 18h 36.2m	+4.7
57 Gem	R	10d 21h 26.2m	+5.1
× Gem	D	11d 06h 27.0m	+3.7
× Gem	R	11d 07h 05.0m	+3.7
46 Leo	R	14d 00h 05.3m	+5.7
89 Psc	D	31d 21h 15.9m	+5.3

(D, disappearance; R, reappearance)

METEORS

Name	Active period	Date of maximum	Radiant	Remarks
Ursids	20d-22d	22d	217° R.A. + 76° Dec.	Favourable

OTHER PHENOMENA

8d 17h	Penumbra Eclipse of Moon:	Moon enters penumbra	15h 07m
		Mid eclipse	17h 10m
		Moon leaves penumbra	13h 12m
22d 00h	Mercury 7° N of Antares		
22d 02h	Winter Solstice		

ENZYME NOMENCLATURE

THE Commission of Editors of Biochemical Journals (J. T. Edsall (*president*), W. V. Thorpe (*secretary*), A. Dillmann, W. A. Engelhardt, Y. Raoul, E. C. Slater), appointed by the International Union of Biochemistry (IUB), wishes to direct attention to the recently published *Enzyme Nomenclature*¹, which is the report of the IUB Standing Committee on Enzymes.

The draft of this report was considered by a joint meeting of the Standing Committee and the IUB Commission of Editors of Biochemical Journals in Rome in February 1964. The version agreed to by that joint meeting was adopted by the Council of the IUB at its meeting in New York on July 27, 1964, and designated *Recommendations (1964) of the IUB on the Nomenclature and Classification of Enzymes*.

The report of the Standing Committee on Enzymes is based on the report of the IUB Commission on Enzymes², adopted by the General Assembly of the IUB in Moscow on August 16, 1961. The changes made by the Standing Committee in the report of the Commission on Enzymes are of four types: (a) additions of new enzymes, and, where necessary, new sub-groups to accommodate them; (b) correction of definite errors in the first edition; (c) changes in the nomenclature itself to meet criticisms which had been put forward; (d) addition of systematic names in some cases where the original Commission put forward only trivial names.

The chapter on the nomenclature of the cytochromes was revised by a special committee set up for this purpose. The chapter in the new report includes proposals for the nomenclature of haem compounds and haemoproteins in general.

Since the publication of the Report of the Commission on Enzymes in 1961, many of its recommendations have been widely used in scientific journals and text-books. Most biochemical journals urge authors to follow most of the recommendations even if they do not insist on all. Some journals already require the procedure suggested in Chapter 6, p. 29, that when an enzyme is the main subject of a paper or abstract, its code number (preceded by the letters *EC*), systematic name and source should be given at its first mention; thereafter the trivial name may be used. Enzymes which are not the main subject of the paper or abstract should be identified at their first mention by their code numbers. When the paper deals with an enzyme which is not yet in the Enzyme Commission's list, the authors may introduce a new systematic name and/or a new trivial name, both formed only according to the recommended rules, but a number should be assigned only by the IUB.

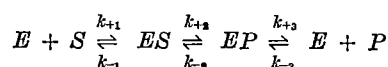
An addition to the new report is the inclusion in the index of names which have been in frequent use but which are no longer recommended. It was often difficult to find in the old report the new name of an enzyme known to the reader only by its old name. Many enzymologists may note with regret that the name by which they have long known a favourite enzyme is printed in italics in the index, indicating that it is not recommended. For example, fumarase (*EC* 4.2.1.2) is replaced by fumarate hydratase as trivial name (systematic name, L-malate hydro-lyase). Those who are irritated by this change should perhaps pause to think how many students first coming across the name fumarase might legitimately think that it catalyses the hydrolytic splitting of fumaric acid. Those who shed muramidase-containing tears on reading the first report may now rejoice that the old name lysozyme (*EC* 3.2.1.17) has been restored, whereas *muramidase* is now relegated to the list of disapproved names.

The chapter on enzyme units has received only one alteration. In the first report a standard temperature of

25° C was suggested, but this is now changed to 30° C because of the ambient laboratory temperature prevailing in many countries. No biochemical journals insist on the use of the Enzyme Commission's unit (*U*) of enzyme activity (the amount which will catalyse the transformation of 1 μ mole of the substrate per min under standard conditions). However, this unit is to be strongly recommended and some journals suggest conversion of data in terms of the new unit when the paper has to be returned to the author for other revisions. The derived units specific activity (*U*/mg) and molecular activity (*U*/ μ mole enzyme) are also to be recommended. Where inconvenient numbers would otherwise be involved, terms such as milli-unit (*mU*), kilo-unit (*KU*) or, for those who specialize in small activities, nano-unit (*nU*) or pico-unit (*pU*).

The IUB Commission of Editors of Biochemical Journals would particularly like to direct the attention of authors to the recommendation that enzyme assays be based wherever possible on measurements of initial rates of reaction in order to avoid complications due, for example, to reversibility of reactions or to the formation of inhibitory products. Many papers are submitted in which kinetic parameters are calculated on the basis of data in which the initial rate was not measured. The substrate concentration should be, wherever possible, sufficient for saturation of the enzyme, so that the kinetics in the standard assay approach zero order. Where a distinctly sub-optimal concentration of substrate must be used, the Michaelis constant should be determined where feasible so that the observed rate may be converted into that which could be obtained on saturation with substrate.

The chapter on the symbols of enzyme kinetics is unchanged. The recommended symbols, *v* (velocity), *V* (*v* at infinite substrate concentration), *K_m* (Michaelis constant, that is, substrate concentration where *v* = *V*/2), *K_s* (substrate constant, that is, dissociation constant of the reaction *E* + *S* \rightleftharpoons *ES*), *K_i* (inhibition constant, that is, dissociation constant of the reaction *E* + *I* \rightleftharpoons *EI*), and *k* for rate constant are widely used. The recommended numbering of rate constants for enzyme systems involving consecutive steps, namely:



has not been widely adopted, and editors are still reluctant to request authors to make the extensive alterations to the typescript which would often be necessary.

The chapter on the classification and nomenclature of cytochromes has been completely rewritten. The term cytochromoid, introduced in the previous report to describe haemoproteins with haemoglobin-like structure and a reactivity with ligands which do not react with cytochrome *c*, has been set aside. It is now proposed that these non-haemochrome haemoproteins should be considered as variant *c*-type cytochromes. To indicate that a haem *c* prosthetic group is not in a haemochrome linkage, a dashed symbol, *c'*, is recommended. This chapter also defines a number of haem compounds and contains much useful information on the chemistry of these compounds and of haemoproteins. The individual cytochromes are now described in greater detail and some cytochromes appearing in the previous list have been dropped. Cytochromes *c₄* and *c₅* are now brought under cytochrome *c₂*. Cytochrome *f* is given the name cytochrome *c₆*, although no doubt it will continue to be called cytochrome *f* as well. Cytochrome *d₁* (*a₄*) and a number of *C* cytochromes have been dropped. Indeed, the capital letters, introduced in the first report to describe a cyto-

chrome at a certain stage of the investigation, have been dropped.

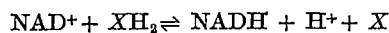
The chapter on the terminology of enzyme formation does not appear in the new report. Part of it (formation from precursors) has been added to the chapter on classification and nomenclature of enzymes.

The chapter on the nomenclature of the nicotinamide nucleotide co-enzymes is an abbreviated version of part of the chapter on the nomenclature of coenzymes in the first report. The sections on ubiquinone or coenzyme Q and on coenzyme A have been omitted, since these compounds have been considered by the IUPAC (International Union of Pure and Applied Chemistry)-IUB Joint Commission on Biochemical Nomenclature, which maintains close contacts with the IUB Commission of Editors of Biochemical Journals. Ubiquinone (coenzyme Q) has been considered in a report on the nomenclature of quinones with isoprenoid side-chains (see, for example, ref. 3). This report makes two alternative recommendations for the naming of ubiquinone (coenzyme Q), namely: (1) the name be ubiquinone-*n* and the abbreviation *Q_n*, where *n* is the number of isoprenoid units in the side-chain; (2) the name be ubiquinone *Q_n* and the abbreviation *Q_n*. No changes in the name coenzyme A (CoA, CoASH) are proposed.

One of the more controversial recommendations of the Enzyme Commission was the use of the name nicotinamide adenine dinucleotide (NAD) and nicotinamide-adenine dinucleotide phosphate (NADP) instead of DPN and TPN. Many criticisms were received by the Standing Committee on Enzymes. These received careful consideration, but the Committee decided that the original arguments as set out in Chapter 4 of the *Report of the Commission on Enzymes* were sufficient to warrant no interference being made with their decision.

The editorial boards of some biochemical journals have encountered strong opposition from their authors to the replacement of the DPN-TPN nomenclature. Although the IUB Commission of Editors of Biochemical Journals has endorsed the new nomenclature, two of the larger journals represented in the Commission have been unable to enforce it, and have permitted the two systems to stand side by side.

In the first report, the Commission on Enzymes recommended two alternative systems of designating the reduced forms of NAD and NADP acting as substrates for enzyme reactions. The two systems were formulated $\text{NAD}^+ \rightarrow \text{NADH} + \text{H}^+$, and $\text{NAD} \rightarrow \text{NADH}_2$. The latter formulation was used in the enzyme list. In the new report the two forms are referred to simply as 'NAD' and 'reduced NAD' in the enzyme names and in the chemical equations illustrating the reaction catalysed by the enzyme in question. On the other hand, the IUPAC-IUB Commission on Biochemical Nomenclature has recommended that the abbreviations NAD and NADP should be used only when the state of oxidation of the compounds need not be specified. The oxidized and reduced forms of the coenzymes should be designated by NAD^+ (NADP^+) and NADH (NADPH), respectively. These may be used in an equation as follows:



For this reason, some journals will permit and even prefer the designation of an enzyme such as EC 1.6.99.3 by NADH: (acceptor) oxidoreductase (systematic name) and NADH dehydrogenase (trivial name) rather than by the names reduced-NAD: (acceptor) oxidoreductase and reduced NAD dehydrogenase, respectively, which appear in the new report. This is in conformity with current practice.

Because of difficulties with indexing, the use of chemical formulae in enzyme names has been prohibited, for example, EC 1.11.1.6 (catalase), which was given the systematic name $\text{H}_2\text{O}_2 : \text{H}_2\text{O}_2$ oxidoreductase in the first edition, has now been changed to hydrogen-peroxide:

hydrogen-peroxide oxidoreductase. (Some journals may object to placing a hyphen between the two parts of a chemical name, which, according to the conventions of chemical nomenclature, do not have a hyphen in the English language.)

On the other hand, standard abbreviations for compounds of importance in biochemistry, as accepted by the IUPAC-IUB Commission on Biochemical Nomenclature, have been used in enzyme names, for example, ATPase (EC 3.6.1.3 and 3.6.1.8). Indeed, more use could possibly have been made of standard abbreviations, and editors will not object when these are used in enzyme names, for example, glutathione: hydrogen-peroxide oxidoreductase (EC 1.11.1.9) could be written GSH: hydrogen-peroxide oxidoreductase, and the systematic name of glutathione reductase (EC 1.6.4.2) can, in the opinion of the Commission of Editors, be legitimately written $\text{NAD(P)H} : \text{GSSG}$ oxidoreductase instead of the longer name, reduced $\text{NAD(P)} : \text{oxidized-glutathione oxidoreductase}$.

The new report repeats the statement of the first report that abbreviations for names of enzymes, for example, GDH, should be strongly discouraged. While the Commission of Editors endorses this statement, and many journals rigorously enforce the prohibition of abbreviations for the names of enzymes, it must be recognized that such abbreviations are widely used, especially in clinical chemistry. It may soon be necessary to rationalize and standardize this practice rather than to ban it.

The most important change in the enzyme list is the reclassification of hydrogenases (Group 1.12), oxygenases (Group 1.13) and hydroxylases (Group 1.14). Errors in the first list have been corrected and many new enzymes added. The list now contains 875 enzymes.

It is obvious that the further purification of enzymes and advances in our knowledge of the mechanism of reactions catalysed by specific enzymes may soon make the recommended nomenclature no longer acceptable in certain cases. The present basis of classification is functional because sufficient chemical knowledge is absent. When more becomes known about the nature of active sites and amino-acid sequences, a chemical classification may become possible.

It is also clear that not everyone will agree with the classification and nomenclature of all the 875 enzymes. Editors of biochemical journals will carefully and sympathetically consider reasoned requests by an author to depart from the recommended nomenclature, and will forward them to Prof. E. C. Webb, who has been designated by the Council of the IUB to assemble such comments. Indeed, the Standing Committee on Enzymes received and considered many criticisms from authors which were transmitted by the editorial boards of various biochemical journals. If the editorial board agrees with the arguments brought forward by an author, it will allow him to depart from the recommendations of the enzyme report. It would be desirable to state the reasons for this departure in the text of the paper or in a footnote.

It should be added, however, that the experience of editors is that many authors have not grasped the basis of the nomenclature recommended by the Commission on Enzymes, namely that an enzyme should be named according to the reaction which it catalyses. Since the specificity of enzymes is not absolute, some arbitrariness in naming the substrate is inevitable. The principles followed by the Commission on Enzymes in choosing between different possibilities are given in Rule 14, p. 32, of the new report. Since it appears that few authors are fully aware of the implications of this rule, it might be useful to consider it in more detail. The long-known enzyme succinate dehydrogenase (EC 1.3.99.1) is given the systematic name succinate: (acceptor) oxidoreductase, even though it also catalyses the oxidation of a number of α -monosubstituted succinates. On the other hand, alcohol dehydrogenase (EC 1.1.1.1) is named alcohol: NAD oxido-

reductase, because it acts on a wide range of alcohols. Lactate dehydrogenase (*EC* 1.1.1.27) is named L-lactate : NAD oxidoreductase, even though it reacts quite rapidly with NADP as well as with NAD. However, the most commonly occurring glutamate dehydrogenase (*EC* 1.4.1.3) is named L-glutamate:NAD(P) oxidoreductase (deaminating), because it reacts readily with both NAD and NADP (see Rule 16). The aldehyde dehydrogenases give special difficulties. No less than 18 are listed in Group 1.2.1 (with NAD or NADP as acceptor). Of these, 14 are named in terms of a specific hydrogen donor, while in the others the donor is given simply as aldehyde. This should not be taken to mean that the 14 are absolutely specific for a single aldehyde. Of the 18 enzymes, NAD is given as acceptor for 8, NADP for 6 and both nucleotides for 4.

There are many discrete enzymes, differing in amino-acid composition, physical properties and enzyme kinetics, all of which have to be named aldehyde : NAD oxidoreductase (*EC* 1.2.1.3). At present these must be distinguished by source, such as organism, tissue and cell component. The IUB Commission of Editors of Biochemical Journals has set up a sub-committee to consider the problems of nomenclature posed by recent research on the nature of isoenzymes and enzyme sub-units.

¹ *Enzyme Nomenclature. Recommendations (1964) of the International Union of Biochemistry on the Nomenclature and Classification of Enzymes, together with their Units and the Symbols of Enzyme Kinetics.* Pp. v + 219. (Elsevier Publishing Co., Amsterdam, 1965). 2.50 dollars.

² *Report of the Commission on Enzymes of the International Union of Biochemistry, 1961* (Pergamon Press, Oxford, 1961).

³ *Biochim. Biophys. Acta*, **107**, 5 (1965).

INTERNATIONAL COMMITTEE ON LABORATORY ANIMALS

THE International Committee on Laboratory Animals held its third international symposium at Dun Laoghaire, near Dublin, during September 6-17 under the general title, "The Husbandry of Laboratory Animals".* The Committee, while well known to those professionally engaged in laboratory animal science, appears to be largely unknown to the users of laboratory animals. It was, however, the users, who, acting through their international unions, set up the Committee. It seems appropriate, therefore, that a general report of the Committee and its work should be made to laboratory animal users on this occasion.

History. Ten years ago two independent initiatives towards an international organization were being made. One was by the International Union of Biological Sciences and the other by the Council for International Organizations for Medical Sciences working in association with Unesco. In December 1956 these organizations met and recommended the establishment of the International Committee on Laboratory Animals. The International Union of Physiological Sciences joined soon after, and the Committee was established as an inter-union committee; experts, who were heads of laboratory animal centres, were co-opted. Unesco provided most of the financial support and was represented on the Committee by an observer. Two other unions have since joined—the International Union against Cancer and the International Union of Biochemistry.

The growth of the Committee's activities was such that by 1961 a revised constitution was necessary. Laboratory animal centres or committees had been established in many countries to co-ordinate work in the field and act as information centres. It seemed important to associate these bodies with the International Committee. The Committee now consists of the union members and national representatives, numbering twenty-seven, coming from all parts of the world. The final authority rests with the governing body, which consists of all the union representatives together with an equal number of national representatives; the World Health Organization is represented by an observer. In effect this is an equal division between the users and the laboratory animal experts. The detailed work is done by an executive committee of individual experts.

The financial support from Unesco was only temporary and in 1962 it ended. The Committee is most grateful to Unesco. The World Health Organization has now undertaken to support the work of the Committee, and to that Organization also the Committee is deeply grateful.

Activities. Surveys of the production and utilization of laboratory animals have been carried out and published in relation to twenty-one countries. This has enabled the

problems to be assessed and has provided a strong stimulus to the development of national centres dealing with problems connected with laboratory animals. Another major factor in such development has been the visits, made under Committee auspices, of the officers and other experts to many countries to give advice on laboratory animal problems. Up to the present time this advice has been mainly concerned with the development of healthy supplies of small mammals. Similarly, other activities have been concentrated in this direction. Thus, efforts have been made to improve technician training, and notes on training courses have been published in the *International Committee on Laboratory Animals Bulletin* which is published twice a year. Scholarships have been awarded to a number of workers in the field, and this has enabled them to work for a time in one of the established centres, in this way helping the development of new centres elsewhere.

Two previous international symposia have been held on "Living Animal Material for Biological Research" and "The Problem of Laboratory Animal Disease". These, together with that just held, have provided a forum for the discussion of a range of scientific problems connected with the supply of healthy animals, in particular small mammals.

Besides the basic problems of improving the health, nutrition and handling of animals, two other general fields have always been on the Committee's programme. The first of these concerns more specialized questions related to the breeding of small mammals. This side of the work has included a bibliography (now taken over by the publishers of the *Zeitschrift für Versuchstierkunde*—Gustav Fischer Verlag, Jena, German Democratic Republic) and the preparation of lists of agreed definitions. These are slow steps towards the difficult task of designating primary type colony centres and listing the primary type colonies being maintained. The difficulty in this task lies in specifying strains in such a way that the designations have real meaning.

The Committee is one concerned with laboratory animals of all kinds and not simply small mammals. One of its early aims was to establish world lists of sources of laboratory animals, especially lower vertebrates and invertebrates. This aim has so far proved very difficult to achieve and, with the concentration of effort on standards of care in animal houses for small mammals, little has yet been done in this field. This has caused some concern, and the governing body has now put on record its hope that some advance in the non-mammalian field may take place in the next three years.

In all these fields of activity this international organization is primarily a channel of communication between national organizations, and the latter have, as one of their functions, the giving of information not only to those

* The proceedings are being published by Academic Press.

actively involved in laboratory animal care and production, but also to any user of experimental animals. In Great Britain the centre is the Laboratory Animals Centre (director, Mr. J. Bleby), Medical Research Council Laboratories, Carshalton, Surrey, and the national

representative on the International Committee is Dr F. J. C. Roe, Department of Experimental Pathology, Chester Beatty Research Institute, Institute of Cancer Research, Fulham Road, London, S.W.3.

J. A. B. GRAY

BASIC COMPONENT OF SOLAR RADIO EMISSION AT CENTIMETRE AND DECIMETRE WAVE-LENGTHS

By DR. M. K. DAS GUPTA and D. BASU

Institute of Radio Physics and Electronics, University of Calcutta

IT is now well established that at centi- and decimetre wave-lengths the solar radio emission, apart from the more rapidly varying features like bursts of various types, consists of: (1) a basic component remaining constant over a considerable period of time; (2) a slowly varying component showing a slow day-to-day variation in intensity. Of these two, the first is the radiation from the Sun when the disk is completely free from any active region while the slowly varying component is the excess radiation due to localized active zones on the disk. The method of separating the two components from the total solar flux was first introduced by Pawsey and Yabsley¹, who plotted the total flux against sunspot area and extrapolated the best-fitting line to the condition of zero area on the assumption that the radio flux depends on sunspots only. However, it is now confirmed² that the regions corresponding to sunspots continue to emit radio waves in cm- and dm-wave-lengths for a considerable period even after the visible sunspots have disappeared and also that these start emitting much earlier than the actual appearance of the sunspots. There are thus present on the solar disk, at any instant, some active regions that are strong sources of radio emission but are not at all associated with the then visible sunspots. As such, the basic component cannot be segregated from the total flux by taking into account sunspots alone.

Attempts were made independently by Allen³ and Covington and Harvey⁴ to determine the basic component with this new consideration in view. However, Allen's method seems to be too complicated and does not appear to be readily applicable from a practical point of view. Regarding the analysis by Covington and Harvey, on the other hand, the assumption that the contribution from radio regions not associated with sunspots is proportional to the number of sunspot groups is only a rough approximation. We were thus prompted to examine the aspect of the evaluation of the basic component of solar radio emission in relation to recent data.

As has already been mentioned, the slow day-to-day variations of the solar radio flux would be due to the presence of active regions, visible or invisible, on the solar disk. These variations exhibit roughly a 27-day period of recurrence which corresponds to the apparent period of rotation of the Sun about its vertical axis. It may, however, be mentioned in this connexion that such variations in the solar radio flux exist⁴ even at zero sunspot number condition which can thus be attributed to active regions other than those associated with sunspots. It is obvious that there will be no variation at all of the daily values of solar flux if the disk is free from any active radio region, and hence, so far as the basic component is concerned, the amplitude of such variation will be zero.

In our analysis we considered solar radio flux data at frequencies 1,000, 2,000, 2,800, 3,750 and 9,400 Mc/s. The daily values of the flux at each of these frequencies were plotted separately for the years 1958 (sunspot maximum) and 1964 (sunspot minimum). All these curves showed substantially similar variation for the individual

years and indicated pronounced maxima and minima following roughly the 27-day period. Fig. 1 shows such plots for the frequency 3,750 Mc/s for the years 1958 and 1964 as well as 1961. An additional striking feature exhibited by the daily mean flux curves is that the pronounced maximum-to-minimum amplitude of this variation seems to change rather systematically with the mean value of the flux. Thus the amplitude is highest for 1958 when the mean flux is also maximum, and it is lowest for 1964 when the mean flux is minimum, while for 1961 the condition is intermediate between the two. We were thus led to examine the matter in some detail. For any frequency we determined the maximum-to-minimum amplitude of variation of the daily mean flux corresponding to each of the 27-day cycles (henceforth called variation amplitude or VA) individually for both the years. The mean values of the solar radio flux for each of the periods corresponding to these cycles were also determined. For any particular frequency and for any particular year, we thus obtained a set of VAs and the corresponding values of mean solar radio flux. These flux values were plotted against corresponding variation amplitudes. Fig. 2 shows the plots for the frequencies 1,000, 2,000, 3,750 and 9,400 Mc/s separately for 1958 and for 1964. The linear relationship between the solar flux and the amplitude of variation is at once evident. On extrapolating to zero value of VA the best-fitting line is found to cut the flux

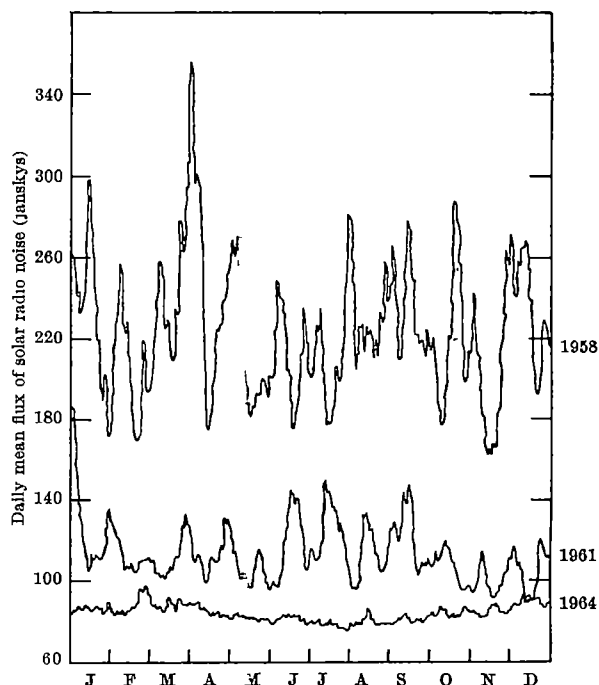


Fig. 1. Plots showing the variation with time of the daily mean solar radio flux at 3,750 Mc/s for 1958, 1961 and 1964

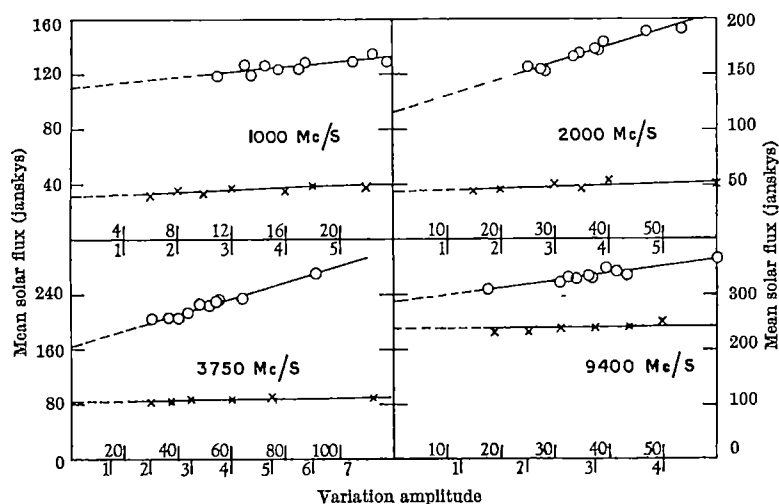


Fig. 2. Plots showing the linear relationship between the mean values of solar radio flux and the amplitudes of variation of the flux for periods corresponding to the so-called 27-day cycles at frequencies 1,000, 2,000, 3,750 and 9,400 Mc/s for the years 1958 (O) and 1964 (x). The upper scale in each case indicates the values for 1958, while the lower one indicates those for 1964.

axis with a positive intercept. This value of the solar flux, corresponding to zero value of VA, can thus be attributed to the basic component of solar radio emission at that particular frequency. The total solar flux at any of these frequencies can thus be represented by:

$$F = F_0 + KV$$

where F is the total flux, F_0 is the basic component, V is the amplitude of variation of flux corresponding to the 27-day cycle and K is a constant. The basic component was similarly calculated for all the five frequencies separately for both the years. It should, however, be noted that in determining the 27-day cycles sufficient care was taken to be sure that each of these exhibits pronounced maxima and minima very clearly. Any ambiguous cycle was rejected altogether from considerations. Further, if the same value of VA appeared more than once in a year, the average value of the corresponding mean solar fluxes was taken. This method of determining the basic component would thus eliminate the effect of all sorts of active regions on the solar disk, visible or invisible. It should be noted in this connexion that the present method of evaluating the basic component will be possible only for the frequency range 470–29,000 Mc/s for which the characteristic 27-day variations in received solar radio flux exist, as we have shown⁴.

Table 1 shows the values of the basic component, F_0 , of solar radio emission at different frequencies individually for the years 1958 and 1964, corresponding to the maximum and minimum periods of solar activity respectively, and also the ratios $(F_0)_{\max}/(F_0)_{\min}$ (designated henceforth as ρ).

The flux values at different wave-lengths of the basic component F_0 , converted to corresponding equivalent black-body temperatures, have been plotted in Fig. 3 both for sunspot maximum and minimum years. For comparison, the continuous curve representing the experimentally obtained values compiled by Ellison⁵ has also been shown. It is quite evident from the $(F_0)_{\max}$ and $(F_0)_{\min}$ plots that the basic component varies in course of a solar cycle. The factor ρ by which it changes from maximum to minimum years is found to depend on frequency as has been shown in Fig. 4. The variation

Table 1. BASIC COMPONENT OF SOLAR RADIO EMISSION AT DIFFERENT FREQUENCIES IN JANSKYS FOR SUNSPOT MAXIMUM AND SUNSPOT MINIMUM YEARS

	1,000 Mc/s	2,000 Mc/s	2,800 Mc/s	3,750 Mc/s	9,400 Mc/s
1958 (max)	110.3	115.3	172.2	163.8	286.0
1964 (min)	31.4	42.7	67.7	83.2	238.6
ρ	3.5	2.7	2.5	2.0	1.2

appears to be negligible at wave-lengths less than 3 cm and seems to increase with increasing wave-length. It appears that ρ is unity at frequencies above 10,000 Mc/s (10,810 Mc/s to be exact), which implies that the radio flux of the quiet sun originating at the base of the chromosphere, as also from the photosphere, undergoes no change in intensity in course of a solar cycle. This corroborates the results obtained earlier by Van de Hulst⁶ and also by Christiansen and Hindmann⁷.

It should, however, be noted here that the factor ρ obtained at dm-wave-lengths is slightly greater than that proposed by Van de Hulst from considerations of a change in the coronal electron density determined from brightness variation. This might possibly be due to the fact that the activity of the present solar cycle, for which this analysis has been made, was far more prominent than the earlier ones. As such, the maximum-to-minimum variation in the coronal electron density, and hence that in the radio flux from the quiet sun, is possibly greater than that observed by previous workers.

As regards the metre wave region, however, the predictions of Van de Hulst and also the investigations of Christiansen and Hindmann suggest no change in the basic component from sunspot maximum to minimum conditions. Lack of continuous data at frequencies less

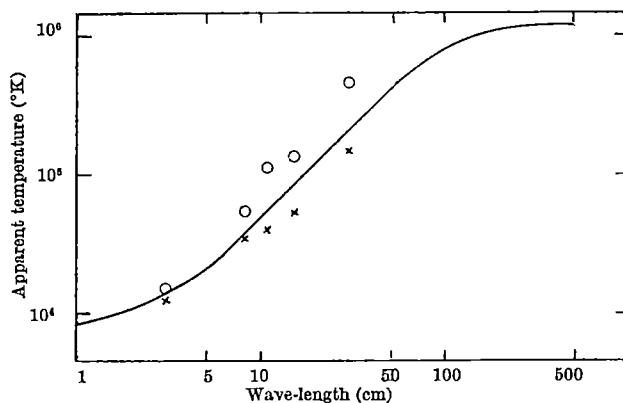


Fig. 3. Apparent temperatures of the quiet sun at different wave-lengths obtained from the present analysis for the years 1958 (O) and 1964 (x). The solid line indicates the spectrum determined experimentally by interferometer technique.

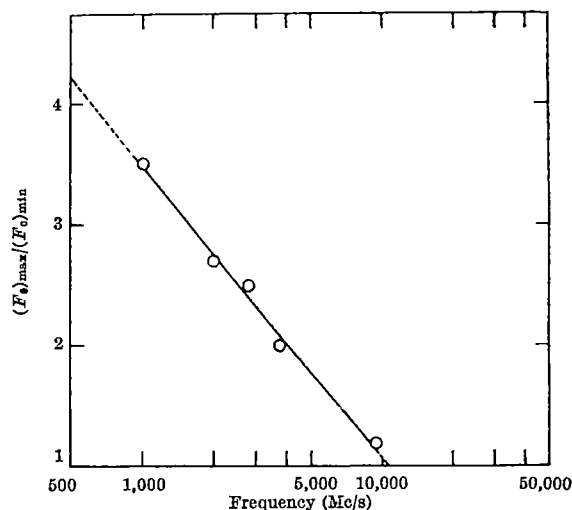


Fig. 4. Curve showing the variation with frequency of the ratio of basic component at sunspot maximum to that at sunspot minimum.

than 1,000 Mc/s did not permit us to extend the present analysis at higher wave-lengths. However, the spectra of the basic component (Fig. 3) both at sunspot maximum and minimum years seem to follow appreciably the experimental curve, and hence it can perhaps be logically assumed that at higher wave-lengths, where the curve becomes asymptotic, $(F_0)_{\max}$ to $(F_0)_{\min}$ variations would again be negligible.

On accepting this, one may conclude that the variation in basic component of solar radio emission with the phase of the solar cycle is only confined to a specific band of wave-lengths—cm and dm regions. Curiously enough, this is also the band in which the slowly varying component is found to exist. It is perhaps worth investigating whether the two might have a common cause. The exact nature of the year-to-year variation and precise evaluation of the band over which the basic component actually varies in

course of a solar cycle will be extremely important. Detailed analysis to this effect is being carried out and will be reported in due course.

We thank Prof. J. N. Bhar and Dr. S. Basu for their advice. Data of solar-radio flux at 2,800 Mc/s belong to the National Research Council, Ottawa, and those at 1,000, 2,000, 3,750 and 9,400 Mc/s to the Research Institute of Atmospherics, Nagoya. We thank Mr. A. E. Covington of Ottawa and Dr. H. Tanaka of Nagoya for sending us their data before publication.

¹ Pawsey, J. L., and Yabsley, D. E., *Austral. J. Sci. Res.*, A, 2, 198 (1949).

² Covington, A. E., and Harvey, G. A., *Astrophys. J.*, 132, 435 (1960).

³ Allen, C. W., *Mon. Not. Roy. Astro. Soc.*, 117, 174 (1957).

⁴ Das Gupta, M. K., and Basu, D., *Nature*, 203, 626 (1964).

⁵ Ellison, M. A., *The Sun and its Influence*, 188 (Routledge and Kegan Paul, London, 1959).

⁶ Van de Hulst, H. C., *B.A.N.*, 11, 135 (1950).

⁷ Christiansen, W. N., and Hindmann, J. V., *Nature*, 167, 635 (1951).

STRUCTURE OF THE RADIO CONTINUUM BACKGROUND AT HIGH GALACTIC LATITUDES

By DR. M. J. S. QUIGLEY and DR. C. G. T. HASLAM

Nuffield Radio Astronomy Laboratories (University of Manchester), Jodrell Bank, Macclesfield, Cheshire

THE purpose of this article is to summarize the results of an investigation into the origin of the continuum radio emission in directions away from the Milky Way, which has been conducted at Jodrell Bank. It now seems possible to reach a general conclusion about the structure of the emission.

The existence of the *North Polar Spur* has been known for some years¹ and, by using data from different observatories, it has been shown² that the *Spur* conforms closely to a small circle for much of its length. A detailed investigation³, which is in course of publication, shows that the complicated structure associated with the *Spur* is almost entirely enclosed within this circle. The diameter of the circle is found to be $113^\circ \pm 3^\circ$ and its centre is at $l_{II} = 330^\circ \pm 2^\circ$, $b_{II} = 19.5^\circ \pm 2^\circ$.

In a series of observations which have been described previously², it was shown that there exists a second high-latitude feature, lying in the southern galactic hemisphere. Because of its situation and appearance we have referred to this as the *Cetus Arc*. The *Cetus Arc* is also found to lie closely along a small circle, although, as with the *Spur*, there is some structure within the *Arc* which falls into no obvious geometrical pattern. With more extensive data than before, we have constructed a stereogram of the *Cetus Arc* in new galactic co-ordinates. This is shown in Fig. 1. The track of the *Arc* was determined from various maps by finding the midpoint of the half-power levels, at various places along the *Arc*. The surveys used were at frequencies of 240 Mc/s (ref. 2), 400 Mc/s (ref. 5) and 178 Mc/s (ref. 6). The points have been plotted as filled circles, open circles and triangles, respectively, and the width to half-power points is indicated by bars. An attempt has been made to fit a circle to the plotted points. Four such circles are shown in Fig. 1. Their parameters are given in Table 1. For most of its length the *Arc* is reasonably well fitted by circle No. 2, but for $b_{II} < 30^\circ$ at $l_{II} \sim 45^\circ$ it diverges from this circle. This may be a real effect or it could arise from either of the following causes:

(1) The *Arc* is being obscured and distorted by the strong emission near the galactic plane.

(2) The position of the ridge is not well defined in this area. For example, on the 400-Mc/s map at latitude -25° , the mean of the longitudes of the 10-unit-contour is at $l_{II} = 40^\circ$, that of the 12-unit contour at $l_{II} = 44^\circ$, and that of the 14-unit contour at $l_{II} = 49^\circ$. This last figure would place the ridge precisely on circle No. 2.

Apart from this anomaly, the *Arc* fits circle No. 2 to within $\pm 2^\circ$ of arc over a total length of 133° . Noting the spread of the values listed in Table 1, we consider that between the points $l_{II} = 40^\circ$, $b_{II} = 35^\circ$ and $(152^\circ, 25^\circ)$ the *Arc* is described by a circle having the following parameters:

Centre (on sphere) at $l_{II} = 100^\circ \pm 3^\circ$, $b_{II} = -33^\circ \pm 3^\circ$.

Diameter, $92^\circ \pm 3^\circ$

In an inspection⁴ of a number of maps of the radio continuum another feature has been identified which seems similar to those described here. We refer to Fig. 2, which is a reproduction of a 240-Mc/s map published by us previously²: full details of the observations are to be found in the reference. The co-ordinate system is the new galactic and the contours are marked in $^\circ K$ above an arbitrary reference-level. Since the beam-width is 1° , and the scans were separated by intervals of 4° in galactic latitude, the map will only represent large-scale features.

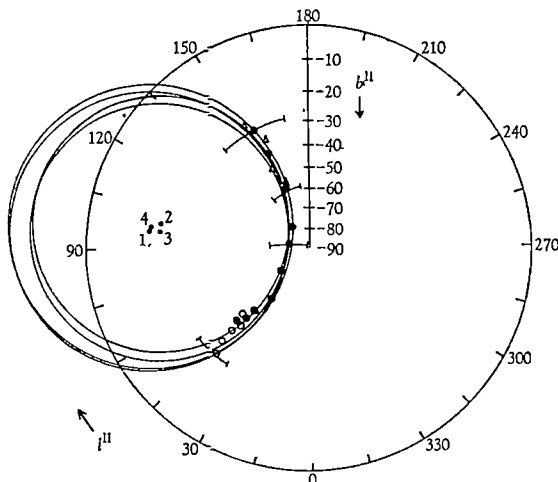


Fig. 1. A stereographic projection of the *Cetus Arc* in new galactic co-ordinates. The south galactic pole is at the centre of the stereogram

Table 1

Circle No.	Co-ords of centre on stereogram		Co-ords of centre on sphere		Diameter (deg.)
	l_{II}	b_{II}	l_{II}	b_{II}	
1	97.0	-18.0	97.0	-30.5	95
2	100.0	-22.0	100.0	-33.0	92
3	97.0	-22.0	97.0	-33.0	91
4	98.0	-19.0	98.0	-32.0	97

Apart from the steep rise in intensity at low latitudes, the obvious structural features in the region are the projections from the borders of the map at latitude $b_{II} \sim 40^\circ$, which are joined by a band of emission at least 6° K brighter than its surroundings. For the right-hand projection there is some indication that it issues from the galactic plane, but the area of the survey is insufficient to include all the feature.

The 240-Mc/s map may be compared with more extensive surveys, such as those of Seeger *et al.*⁵ at 400 Mc/s, and of Turtle and Baldwin⁶ at 178 Mc/s, which confirm the existence of the large-scale structure apparent in Fig. 2. They also indicate that the projecting spurs at latitude 40° can be traced back to the galactic plane at longitudes $l_{II} \sim 87^\circ$ and $\sim 157^\circ$. We are therefore observing a structure similar to the *Cetus Arc* and the *North Polar Spur*, that is, a belt which projects from the galactic plane, and returns at a different longitude. The resemblance is strengthened by the fact that the belt becomes weakest at its furthest point from the galactic plane. However, it is distinguishable above the background at all points. Its minimum temperature (measured above adjacent areas of sky) at 240 Mc/s is about 6° K and the maximum 25° K. We propose to consider this belt as a coherent feature, and we shall refer to it as *Loop III*, the numeral indicating that this is the third high-latitude feature to be identified. It is also the least bright of the three. Its existence has been noted independently in a recent paper by Seeger *et al.*⁷

As with the *Spur* and the *Cetus Arc*, the geometry of *Loop III* has been investigated by plotting it on a galactic stereogram, which is reproduced in Fig. 3. The position of the *Loop* was measured from the same surveys, and in the same manner, as was that of the *Arc*. The figure shows three circles which have been fitted to the *Loop*. Their parameters are listed in Table 2.

Table 2

Circle No.	Co-ords. of centre on stereogram		Co-ords. of centre on sphere		Diameter (deg.)
	l_{II}	b_{II}	l_{II}	b_{II}	
1	124	2	124	11.5	71
2	124	6	124	14.5	71
3	123	3.5	123	13.0	72

For most of the *Loop*, circle No. 1 gives the best fit, but the bulge at $l_{II} = 155^\circ$ to 160° , $b_{II} \sim 30^\circ$, is more closely fitted by circle No. 2. The deviations of the *Loop* from circle No. 1 are at most $\pm 3^\circ$ of arc, so we adopt this as the best fit to the plotted points and assert that the *Loop* is reasonably well described by a circle which has the following parameters:

Centre (on the celestial sphere) at $l_{II} = 124^\circ \pm 1^\circ$,
 $b_{II} = 11.5^\circ \pm 2^\circ$.

Diameter, $71^\circ \pm 2^\circ$.

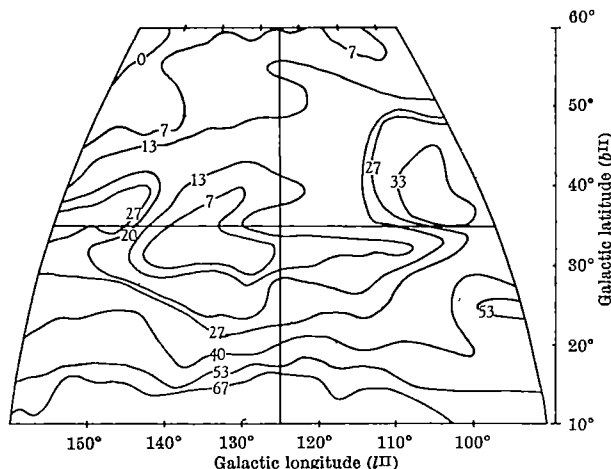


Fig. 2. 240-Mc/s isophotes of a region in the north galactic hemisphere. The brightness temperatures are marked in deg. K above an arbitrary level

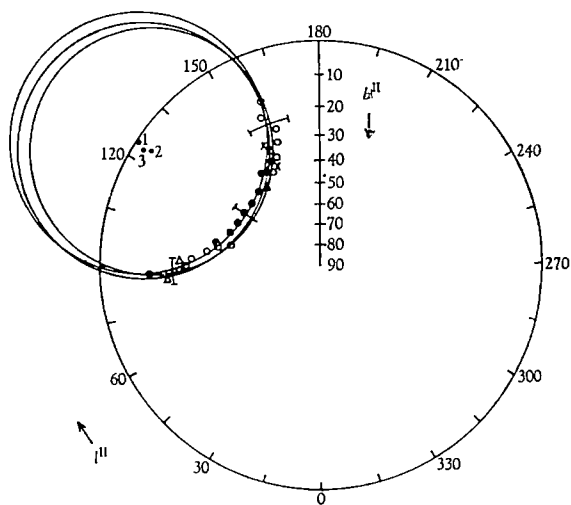


Fig. 3. A stereographic projection of *Loop III* in new galactic co-ordinates. The north galactic pole is at the centre of the stereogram

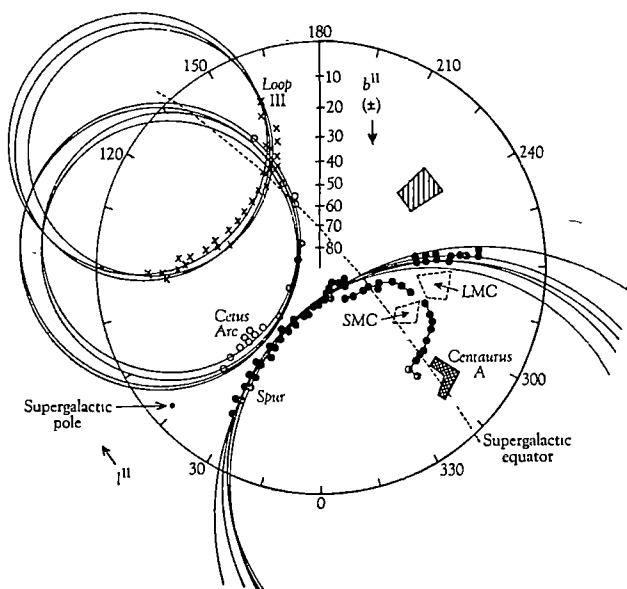


Fig. 4. A stereographic projection of the celestial sphere, in new galactic co-ordinates. Objects which lie wholly or predominantly in the northern galactic hemisphere have been projected from the south galactic pole and vice versa. The *North Polar Spur*, the *Cetus Arc*, and *Loop III* are outlined by filled circles, open circles, and crosses, respectively. Various preferred directions have been inscribed, as follows: the supergalactic pole (ref. 9) and equator (NGH only); the *Centaurus A* extended radio source (ref. 9); the Large and Small Magellanic Clouds (SGH). The least bright area in the northern galactic hemisphere is shown as a shaded patch in the upper right-hand corner

The errors in the foregoing values are estimated from the spread of values in Table 2 and from the deviations of the *Loop* from the circles.

It is interesting to represent the *Spur*, the *Cetus Arc*, and *Loop III* together in the same stereogram to see if they bear any relation to each other or to the galactic plane, points of minimum halo emission, etc. It is difficult to draw all three of the features on a straightforward stereogram because of the rapid expansion of the scale outside the primitive. However, we may adopt the device of projecting objects which lie wholly or predominantly in the northern galactic hemisphere from the south galactic pole, and those in the southern hemisphere from the north pole. This procedure has been followed in constructing Fig. 4. The *Spur*, the *Cetus Arc*, and *Loop III* are outlined by filled circles, open circles, and crosses, respectively. Marked also are a number of directions to which some interest is attached. These are detailed in the caption.

We have inspected the figure and have been unable to find any special geometrical relationship among the dispositions of the three arcs, or between them and any of the various preferred directions. However, using our results, Meaburn⁶ has demonstrated a remarkable positional coincidence between *Loop III* and a number of high-velocity neutral hydrogen clouds. The three arcs do seem to have one feature in common: their brightest parts lie near to the galactic plane, and the brightness diminishes with increasing latitude. This fact may be significant for their interpretation.

The three arcs and their associated features appear to account for a very large proportion of the structure observed in the radio continuum away from the galactic plane, and perhaps also for a substantial part of the total

halo emission. It is hoped in the future to carry out more detailed mapping of the *Cetus Arc* and *Loop III*, which so far have not been examined at high resolution.

¹ Hanbury Brown, R., Davies, R. D., and Hazard, C., *Observatory*, **80**, 191 (1960).

² Large, M. I., Quigley, M. J. S., and Haslam, C. G. T., *Mon. Not. Roy. Astro. Soc.*, **124**, 405 (1962).

³ Large, M. I., Quigley, M. J. S., and Haslam, C. G. T., *Mon. Not. Roy. Astro. Soc.* (In the press).

⁴ Quigley, M. J. S., thesis, Univ. Manchester (1965).

⁵ Seeger, C. L., Stumpers, F. L. H. M., and van Hurek, N., *Philips Tech. Rev.*, **21**, 317 (1960).

⁶ Turtle, A. J., and Baldwin, J. E., *Mon. Not. Roy. Astro. Soc.*, **124**, 459 (1962).

⁷ Seeger, C. L., Westerhout, G., Conway, R. G., and Hoekema, T., *Bull. Astro. Inst. Neth.*, **18**, 1 (1965).

⁸ Meaburn, J. E., *Nature*, **207**, 179 (1965).

⁹ de Vaucouleurs, G., *Sov. Astr. A. J.*, **3**, 897 (1960).

AGE OF THE WEARDALE GRANITE

By F. J. FITCH

Department of Geology, Birkbeck College, University of London

AND

Dr. J. A. MILLER

Department of Geodesy and Geophysics, University of Cambridge

THE Weardale granite was encountered at 1,281 ft. depth beneath unconformable Carboniferous Limestone in the Durham Colleges Geology Department Rookhope borehole (NY 35/937,428), West Durham, in 1960. The possibility that a high-level granite might exist beneath the Alston block was first suggested by Dunham in 1934, when he described the concentric pattern of the zonal mineralization in the area¹. A regional gravity survey by Bott and Masson-Smith gave support to this suggestion and indicated the probable presence of a granite with cupolas close beneath the mineralized area². As the mineral veins of West Durham are found in Carboniferous strata and bear a distinct petrographical resemblance to those of Devon and Cornwall, it was thought at that time that the postulated granite might be Variscan in age.

The first results of the research borehole put down by Dunham *et al.* to test these hypotheses appeared in 1961, when they reported that, although granite had been found, it was seen to be unconformably overlain by Lower Carboniferous strata, and must therefore be pre-Carboniferous in age³. At the same time isotopic age investigations of two samples of this newly discovered granite were reported by Dodson and Moorbath⁴. Their conclusions from these investigations were: (1) that the Weardale granite was emplaced in Middle or Late Devonian times around 362 ± 6 m.y.; (2) that it is significantly younger than the Shap granite, but possibly contemporaneous with the Skiddaw granite. We find it difficult to accept the conclusions of Dodson and Moorbath and have made a further

isotopic age investigation of the granite as part of an extended programme of geochronology of the rocks of north-east England.

A full description and discussion of the geological results of the Rookhope borehole has recently been presented to the Geological Society of London by Dunham *et al.*⁵. Both in the discussion of this paper and in the discussion of an earlier paper by Brown, Miller and Soper on the intrusions of the English Lake District⁶, the same unresolved difference of opinion can be found. One school of thought (K. C. Dunham, Miller, Brown, Bowie) believed that the Late Devonian age obtained for the Weardale Granite by Dodson and Moorbath should be treated with some caution: (a) because of the probable effects of later mineralization; (b) because abnormal variations in the radioactivity of the granite and in the Rb/Sr ratios have been noted, suggesting inhomogeneity, and the possibility that not all the rock crystallized at the same time. In the other view (A. C. Dunham, Soper) a late Devonian age was accepted because it was thought possible that late potassic differentiates of the British Caledonian 'Newer Granite' magma could have been emplaced in Middle, or even Upper, Devonian times.

Four new potassium-argon age determinations are reported in Table 1. Specimen FM 271 is a muscovite-alkali-granite-gneiss which has suffered several phases of metasomatic alteration and intermittent cataclasis. Specimen FM 272 is a chlorite (biotite)-muscovite-alkali-granite-gneiss less affected by subsequent metasomatism. Specimen R/14 is a muscovite concentrate supplied by

Table 1. NEW POTASSIUM-ARGON AGES FROM THE WEARDALE GRANITE

Samples from the Rookhope Borehole, Co. Durham	Refs.	Instrument and method	Data			Age and error (m.y.)
			K ₂ O (%)	% Atmos. contamination	Vol. * ⁴⁰ Ar Wt. sample (g)	
Weardale granite	FM 272	Omegatron	0.12	31.2	0.1313	392 ± 6
Muscovite 1312' 9"	160 KA/804	Isotope dilution				
Weardale granite	FM 271	Omegatron	0.78	13.3	0.1279	359 ± 5
Muscovite 1395' 6"	159 KA/803	Isotope dilution				
Weardale granite	FM 272	Omegatron	2.70	82.6	0.0244	255 ± 12
'Chlorite' 1312' 9"	160 KA/850	Isotope dilution				
Weardale granite	R/14	Mass spec.	0.96	13.9	0.1323	364 ± 12
Muscovite 1396'	KA/276	Isotope dilution				

$\lambda = 0.584 \times 10^{-10} \text{ y}^{-1}$, $\lambda_{\beta} = 4.72 \times 10^{-10} \text{ y}^{-1}$.

* (mm)³ radiogenic argon N.T.P.

the Oxford Isotope Laboratory, and is similar material to that on which the original age work was done by Dodson and Moorbath⁴.

The analytical methods used at Cambridge were similar to those described by Miller and Brown⁷.

Table 2 summarizes the relevant age data at present available. Geologically, the Weardale granite can be said to be pre-Viséan. The rocks into which it is intruded are not seen in the borehole, but by analogy with the nearby Cross Fell Inlier the youngest rocks which are involved as host-rocks would most probably be late Silurian in age⁸.

Table 2. SUMMARY OF RELEVANT AGE DATA ON WEARDALE GRANITE

Sample	Method	Age and error (m. y.)	Ref.
1396'	Rb/Sr muscovite	360 ± 12	Dodson and Moorbath, 1961
1396'	Rb/Sr muscovite	365 ± 12	Constants used:
1396'	K/Ar muscovite	370 ± 10	$^{87}\text{Rb} = 0.1475 \times 10^{-10} \text{ y}^{-1}$
1314'	K/Ar muscovite	356 ± 12	$\lambda_{\beta} = 4.72 \times 10^{-10} \text{ y}^{-1}$
			$\lambda_{\alpha} = 0.584 \times 10^{-10} \text{ y}^{-1}$
1396'	K/Ar muscovite	364 ± 12	This article
1395' 6"	K/Ar muscovite	359 ± 5	This article
1312' 9"	K/Ar muscovite	392 ± 6	This article
1312' 9"	K/Ar 'chlorite'	255 ± 12	This article

Beneath the Carboniferous unconformity, granite was continuously exposed in the Rookhope borehole from 1,281 ft. to 2,650 ft. Dunham *et al.*⁶ described it as having a low-dipping foliation in the upper part, becoming less obvious below 2,225 ft. depth. Mineralogically it is said to contain biotite, abundant muscovite, low albite, orthoclase, microcline and quartz. Many mineral veins cut the granite: pyrrhotine is present in these veins below 1,355 ft., and the source of mineralization is presumed to lie beneath the granite, which has acted as a channel for the rising fluids.

The specimens of Weardale granite which have been used so far in age investigations all come from the foliated upper portion of the granite. Further analysis of specimens from the lower part of the borehole should be undertaken in the future.

The geological history of the foliated portion of the granite is complex, but can be largely understood by careful study of thin sections: and appears to be as follows:

(A) *Initial crystallization* of muscovite-biotite alkali-granite.

(B) *Foliation and partial high-temperature metasomatism*: during this first subsequent event shearing and cataclasis converted the granite above 2,225 ft. to a granite-gneiss. Original quartz grains were completely granulated and reduced to (now recrystalline) lensoid masses: primary biotite and muscovite were streaked out along shear planes. Accompanying this strong mechanical metamorphism, but more restricted in its distribution, was a wave of metasomatic recrystallization. In the rocks most strongly affected by this metasomatism, original feldspar is strongly attacked along its margins by the growth of myrmekite fringes, large new muscovites grow in random orientation, overprinting earlier textures and mineral fabric, new medium- and fine-grained quartz and albite appear, and there is partial and, in places, complete, recrystallization of early quartz, micas and feldspar. This first subsequent event was probably the result of renewed intrusive activity at depth, only partially reactivating the consolidated upper facies of the granite pluton.

(C) *Low-temperature metasomatism*: one or more episodes of low-temperature metasomatism associated with 'Variscan' mineralization can also be distinguished. During these events the granite suffered mild brecciation in places, and any primary biotite that was still present was largely converted to chlorite and iron ore. Sericitization and the appearance of other secondary minerals, such as calcite, was widespread, especially close to mineral veins.

It was suggested by Dodson and Moorbath⁴ that the coincidence of apparent isotopic ages obtained by both the K/Ar and Rb/Sr methods makes it probable that the weighted mean of their results (362 ± 6 m.y.) was close to the true age of the emplacement of the Weardale granite.

This is an invalid argument when applied to a mineral separation from rock material with a complex metasomatic history. Analysis of the same contaminated mineral sample by different methods may well produce similar apparent ages within the experimental errors, and the fact that almost identical results were obtained in this case does not necessarily imply that they reflect the age of original crystallization.

Examining first the K/Ar results from muscovite, it can be seen that each muscovite sample extracted from the granite will be a mixture of muscovites of different ages. They will each contain varying proportions of some or all of the following: (1) original primary muscovites; (2) original primary muscovites giving low ages because of subsequent cataclasis or partial recrystallization; (3) large secondary muscovites of the early high-temperature metasomatism; (4) sericite of later low-temperature metasomatism(s); (5) secondary muscovites or sericite giving low ages because of mild cataclasis and other effects associated with the subsequent low-temperature metasomatism(s).

Analysis of these samples will produce average age results none of which will be the true age of any of the events that affected the granite. As the largest muscovite fractions present in the rock, however, are those occurring under (2) and (4) above, the average results should normally fall somewhere between the ages of (2) and (4). Thus the highest K/Ar result (392 ± 6 m.y.) can be accepted as a good minimum age for the gneissification of the granite and the lowest result (356 ± 12 m.y.) can be taken as being greater than the age of subsequent low-temperature metasomatism(s). The Rb/Sr results must be interpreted similarly. Each muscovite sample used is a mixture of muscovites which crystallized in a different isotopic environment. None of the subsequent metasomatisms which have affected the Weardale granite would have produced isochemical recrystallization. When secondary muscovite crystallized in the rock, its initial strontium-isotope ratio would have been related to the new metasomatic environment and not to the average strontium-isotope composition of the rock (as is assumed under conditions of isochemical metamorphism). Thus the Rb/Sr results (365 ± 12 m.y. and 360 ± 12 m.y.) indicate, as do the K/Ar results, an average age somewhere between the age of gneissification and that of the 'Variscan' mineralizations. Taking into consideration the considerable variability in the proportions of true primary (1) and secondary muscovite (3) in the samples, one possible inference from the low total spread of both K/Ar and Rb/Sr results from muscovite (392 ± 6 m.y.- 356 ± 12 m.y.) would be that the age of primary crystallization cannot be very much before that of gneissification. A Pre-Cambrian age of initial crystallization would not appear to be likely for this granite. The results definitely suggest initial intrusion during the main late Silurian/Lower Old Red Sandstone Caledonian orogenic/magmatic event (say within the period 425-400 m.y.) followed by re-activation and gneissification of this early facies by further intrusive activity before 392 ± 6 m.y. This interpretation is consistent with other work on the 'Newer Granite' suite published recently^{9,10}.

The ages of the subsequent low-temperature metasomatisms, associated with 'Variscan' mineralization, can only be indicated as less than 356 ± 12 m.y. from the muscovite results alone, but further evidence is obtained from the one 'chlorite' result. This 'chlorite' sample contains a small amount of sericite and muscovite impurity, but is largely composed of chlorite, which crystallized during the widespread mineralizing episode. As the muscovite impurity will raise the apparent age, the age of 255 ± 12 m.y. obtained from this fraction is a maximum age for the last major mineralizing event in this area. Two widespread early episodes of the so-called 'Variscan' mineralization are known throughout England about 290 m.y. and 225 m.y., and metasomatism associa-

ted with these episodes can be shown to cause partial recrystallization and markedly to influence the K/Ar ages of earlier igneous rocks¹¹. It is thought most probable that the Weardale granite acted as a channel for the upward migration of metasomatizing fluids during both these episodes and possibly also during later much less intense mineralizing events.

We thank Prof. K. C. Dunham and Sir Edward Bullard for their advice and for providing samples of granite from the Rookhope borehole; also Miss D. E. Bate for the careful work involved in separating the small amount of 'chlorite' from sample FM 272.

- ¹ Dunham, K. C., *Quart. J. Geol. Soc., Lond.*, **90**, 689 (1934).
- ² Bott, M. H. P., and Mason-Smith, D., *Geol. Mag.*, **90**, 127 (1953); *Quart. J. Geol. Soc., Lond.*, **113**, 93 (1957).
- ³ Dunham, K. C., Bott, M. H. P., Johnson, G. A. L., and Hodge, B. L., *Nature*, **190**, 899 (1961).
- ⁴ Dodson, M. H., and Moorbath, S., *Nature*, **190**, 900 (1961).
- ⁵ Dunham, K. C., Dunham, A. C., Hodge, B. L., and Johnson, G. A. L., *Proc. Geol. Soc., Lond.*, **94**, 1617 (1964).
- ⁶ Brown, P. E., Miller, J. A., and Soper, N. J., *Proc. Yorks. Geol. Soc.*, **34**, 331 (1964).
- ⁷ Miller, J. A., and Brown, P. E., *Adv. Sci. (Lond.)*, **20**, 527 (1964).
- ⁸ House, M. R., *Quart. J. Geol. Soc., Lond.*, **120** S, 332 (1964).
- ⁹ Miller, J. A., and Brown, P. E., *Geol. Mag.*, **102**, 106 (1965).
- ¹⁰ Fitch, F. J., Miller, J. A., and Brown, P. E., *Nature*, **208**, 275 (1964).
- ¹¹ Fitch, F. J., and Miller, J. A., *Quart. J. Geol. Soc., Lond.*, **120** S, 159 (1964).

RECENT FINDS IN THE UPPER CARBONIFEROUS OF SOUTH-WEST ENGLAND AND THEIR SIGNIFICANCE

RECENT field work by myself and colleagues, and two new faunal discoveries, have led to a re-appraisal of the stratigraphy and structure of the Upper Carboniferous of south-west England. The evidence suggests that, instead of two simple successions younging towards the axis of a synclorium, there may be a series of blocks each with its individual stratigraphical succession and separated from each other by major thrust faults.

Our present knowledge of the stratigraphy stems from the work of Owen^{1,2} in the south, and Prentice³⁻⁵ in the north, who both suggested that the structure was broadly that of a synclorium with the succession younging southwards in the north and northwards in the south.

In the southern portion of the synclorium the accepted succession of formations has been²: Crackington Measures → Bude Sandstones → Welcombe Beds.

Goniates^{2,6} prove a Namurian (E_2 to R_{2b}) for the Crackington Measures and a Lower Westphalian age (G_2) for the Welcombe Beds^{7,8}. However, the contacts of the Bude Sandstones with the neighbouring formations are faulted and there is no unequivocal field evidence that they lie between the Crackington Measures and the Welcombe Beds. In fact, Ashwin⁹ suggested on structural grounds that the Welcombe Beds formed the core of an anticlinorium and underlay the Bude Sandstones.

The Bude Sandstones have been given a Lower Westphalian age because of plants identified by Crookall¹⁰ as belonging to the Lower Coal Measures; but no molluscs had been found until in 1963 I discovered goniates and lamellibranchs on the coast at Sandy Mouth (SS 202100) in a black sulphurous shale 10 yd. north of the steps leading down to the beach. Specimens were sent to Dr. W. H. C. Ramsbottom, who made further collections on the foreshore and identified the goniates as *Weidyceras* sp. nov. and the lamellibranchs as *Dunbarella* and *Caneyella*. Dr. Ramsbottom considers that a horizon in the Lower Westphalian "just above the *G. listeri* Marine Band" is indicated.

Thus the precise age of part of the Bude Sandstones is established. Field work by myself and Mr. J. P. B. Lovell¹¹ suggests that the goniatic horizon is about 1,000 ft. above the base of the Bude Sandstones, which are about 4,000 ft. thick altogether. The Bude Sandstones are therefore undoubtedly younger than the Crackington Measures, but unfortunately their relationship to the Welcombe Beds is still uncer-

tain, as it is not clear where in the Lower Westphalian the Welcombe Beds should be placed.

In the northern part of the synclorium recent work began with the important pioneer work of Prentice³⁻⁵, who suggested that the stratigraphical succession passed from turbidites of the Instow Beds into a 'coal measure' facies, the Northam and Abbotsham Beds, and continued through 'slumped' Greencliff Beds into more turbidites, the Cockington Beds. The central part of Prentice's succession has been modified by De Raaf, Reading and Walter¹² and Walker¹³, and now stands as follows:

Bideford Group	Cockington Beds 700 ft.	'Identifiable gastrioceratids'	'Turbidite'
	Greencliff Beds 500 ft.	<i>C. communis</i> at base	'Slump'
	Abbotsham Formation 1,200 ft.	<i>A. lensulcata</i> near top	6 paralic cycles
	Northam Formation 1,450 ft.	<i>C. (?) bellula</i> near the base	3 paralic cycles
	Westward Ho! Formation 1,660 ft. +	No fossils	Laminated silty mudstones with turbidites
	Instow Beds 1,500 ft.	<i>G. listeri</i> near top	'Turbidite'
	Limekiln Beds 100 ft.	R_1 , R_{2a} goniatices	Siliceous siltstones and black shales

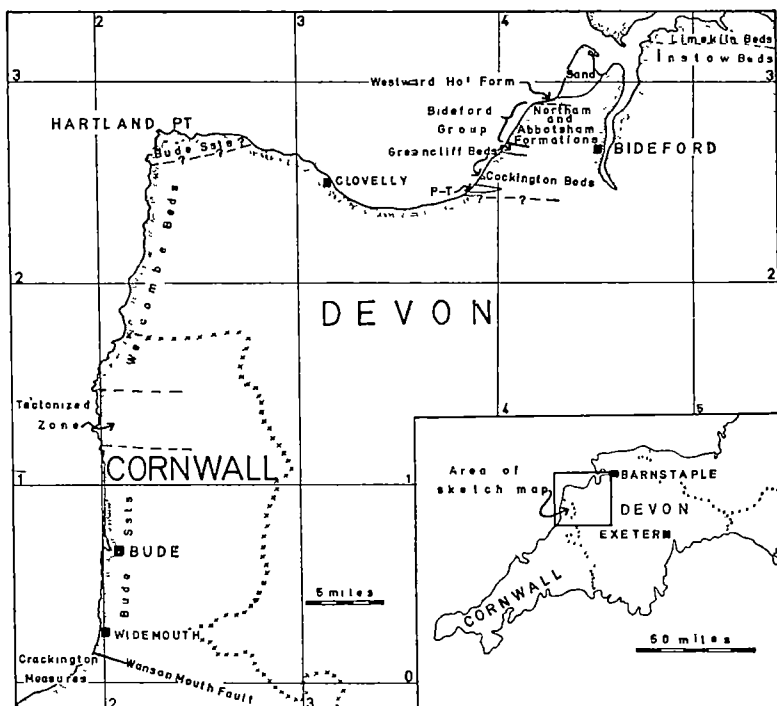


Fig. 1. Sketch map of localities and Upper Carboniferous formations in the coastal regions of north Devon and Cornwall

Recently, Dr. D. Boschma and Dr. R. G. Walker found lamellibranchs in loose slabs of sandstone, undoubtedly fallen from a horizon 80 ft. above the base of the Abbotsham Formation in the top of the first paralic cycle. The locality is on the coast west of Bideford (SS 411278). Dr. R. M. C. Eagar and Mr. M. A. Calver have examined the lamellibranch fauna. They state that variants grade from *Carbonicola* (?) *bellula* (Bolton) to short *Carbonicola* (?) virtually identical with forms shown in Eagar's Pl. xii, Fig. 3 "northern series"¹⁴. Both the pattern of growth of the shells and also the final form reached are particularly characteristic of a horizon below the *Gastrioceras subcrenatum* Marine Band and above that of *G. cumbriense*. Probably the horizon is only a short distance below the *G. subcrenatum* Marine Band.

If, therefore, the lower part of the Abbotsham Formation is Namurian it is important to look at the evidence for the foregoing succession, which places the Instow Beds with a *G. listeri* (Lower Westphalian) fauna 3,000 ft. below the Abbotsham Formation, the lower part of which is Namurian.

Let us first take the palaeontology. There is no doubt of the existence of a *G. listeri* fauna at the top of the Instow Beds as described by Prentice³. Simpson¹⁵ determined non-marine lamellibranchs found by Rogers at Abbotsham Cliff in the upper part of the Abbotsham Formation (at the top of cycle 4)¹² as belonging to the *lenisulcata* zone and those found in Roberts quarry (SS 470264), which Prentice⁴ considered lay just above the Abbotsham Formation, as belonging to the *ovalis* (= *communis*) zone. Eagar and Calver (personal communication) have confirmed that a high *lenisulcata* or low *communis* zone is likely for these faunas.

The only goniatites at present known from the Abbotsham Formation are 'minute goniatites' mentioned by Prentice⁴, which occur in shale near the base of Cycle 2 (ref. 12) immediately above the *C. (?) bellula* lamellibranchs. These goniatites could represent the *G. subcrenatum* Marine Band, one of the most widespread marine horizons in Europe. Support to this is given by correlating paralic cycles of the Bideford Group ranging from basinal mudstones to coastal plain sediments^{12,13} with the cycles (defined by marine bands) of the Namurian and *lenisulcata* zone of the South Crop of the South Wales coalfield^{16,17}. There, four major cycles occur in the Namurian and five to six occur in the *lenisulcata* zone¹⁷. In the Bideford Group there are nine, and *C. (?) bellula* occurs at the top of the fourth.

If we now turn to the field evidence, the coastal section and field mapping by myself and my colleagues between Bideford and the coast firmly establish the succession Westward Ho! → Northam → Abbotsham Formation^{12,13}. There is complete exposure on the coast, and though faults and folds exist, correlations of individual layers can be made across them. The succession generally youngs southwards and one might expect to find the Instow Beds at the northern end of the section. Recent sands of Bideford Bay, however, obscure the solid outcrop and the lowest exposed stratum is still in the Westward Ho! Formation. The evidence for a transition

between the Instow Beds and the Westward Ho! Formation is on field mapping and the recognition of lithologies in a single quarry. In this area facies differentiation inland is extremely difficult and mapping is inconclusive. Prentice⁴, however, considered that the base of his Northam Beds was to be seen at Hubbastone Quarry (SS 463297), where it lay abruptly on the Instow Beds. Although, as Walker¹³ pointed out, the upper part of Hubbastone Quarry is composed of grey silty mudstones similar to much of the Westward Ho! Formation rather than a 'coal measure' type of sedimentation as Prentice thought, there is no real disagreement here, since the Westward Ho! Formation equals the lower half of Prentice's Northam Beds. The question is whether the greywackes in the lower part of the quarry are the Instow Beds. While admitting that they look very similar to much of the Instow Beds, I consider that they may equally well be turbidites within the Westward Ho! Formation and that therefore the evidence for a formational junction in this quarry is not conclusive.

In the upper part of the succession, Prentice considered that the succession went from the 'coal measures' Abbotsham Beds through 'slump' Greencliff Beds, indicating steepening slope into the turbidites of the Cockington Beds. The upper part of the Greencliff Beds is infolded with the Cockington Beds and there is no reason to suspect this junction. The lower part, however, is faulted against the Abbotsham Formation. Structural disturbance in the Greencliff Beds is very considerable and exposure is by no means complete. Unlike the Westward Ho! to Abbotsham Formation part of the section, there is no clear field evidence that the Abbotsham Formation passes sequentially into the Greencliff Beds. An alternative explanation is that at the Abbotsham Formation to Greencliff junction there is a major tectonic dislocation.

The palaeontological position of the Cockington Beds is uncertain. Prentice⁴ claimed that it contained "identifiable gastrioceratids younger than the *lenisulcata* zone". On the other hand, Prentice and Thomas⁸ state that it contains *G. subcrenatum*. Lithologically, the Cockington Beds are indistinguishable from the Bude Sandstones and, like the latter, they occur adjacent to the Welcombe Beds. It is more likely, therefore, that the Cockington Beds also belong to the *lenisulcata* zone.

Thus an analysis of the present results, admittedly inconclusive, suggests that the stratigraphy may be more complex than the established succession allows. An alternative hypothesis which appears to fit the facts better is that there are at least four distinct successions in the Upper Carboniferous of south-west England which are partly time equivalents of each other.

Many problems remain. The position of the Welcombe Beds is still uncertain and will remain so until there is closer palaeontological control, and until field mapping by Mr. L. Moore, of King's College, London, around Hartland Point is complete. They may prove to be younger than the adjacent formations or to be their time equivalents, but they are unlikely to be older. The position of *communis* zone lamellibranchs recorded by Simpson¹⁵ was

Table 1. UPPER CARBONIFEROUS STRATIGRAPHY OF COASTAL REGIONS OF NORTH DEVON AND CORNWALL

	Westphalian <i>lenisulcata</i> - <i>communis</i>	Welcombe Beds <i>G.</i> (c. 2,000')				Bideford Group	Instow Beds <i>G. listeri</i> (1,500')
		<i>G.</i> <i>listeri</i>	Bude Sandstones <i>G. listeri</i> (4,000')	Cockington Beds <i>G. subcrenatum</i> (700')	Abbotsham Formation <i>lenisulcata</i> near top <i>C. (?) bellula</i> near base		
		<i>G. subcrenatum</i>			(1,200')		
Namurian	<i>G.</i> ₁				Northam Formation no fossils (1,450')		Lamekin Beds <i>R.</i> ₁ , <i>R.</i> _{2a} (100')
	<i>R.</i> ₁		Crackington Measures <i>E.</i> ₁ - <i>R.</i> _{1a} (c. 3,000')	Greencliff Beds no fossils (500')	Westward Ho! Formation no fossils (1,660' +)		
	<i>H.</i> ₁						
	<i>E.</i> ₁						

thought by Prentice⁴ to be above the Cornborough Sandstone which forms the top of the Abbotsham Formation. The location is inland, east of Bideford, and the formation may there continue up into the *communis* zone.

There is, on the foregoing hypothesis, a series of laterally equivalent successions, none of which contains a great thickness of Upper Carboniferous strata. What remains of the fill of the Culm geosyncline neither extends very far into the Lower Westphalian nor is large in volume.

To bring together such sedimentologically distinct formations lateral translations of considerable magnitude are required. Dearman¹⁸ has emphasized the importance of north-north-westerly dextral wrench faults in Cornwall and south Devon. Although wrench faults undoubtedly complicate the picture, their displacement is seldom more than a mile and is insufficient to give the required movement. It is suggested, therefore, that there are major thrust faults, not easily detectable in the very low topography, which separate a number of structural units. These faults are most probably very low angle thrust zones of broken and contorted strata along which individual blocks have ridden, rucking up the strata within each block to give the tight fold pattern so characteristic of the Carboniferous in south-west England. It is even possible that some blocks originally deposited to the south have overridden the more northerly ones, so that to-day the original sedimentological pattern is reversed, as in the Alps. There seems to be no other explanation, if the suggested stratigraphy proves correct, for the turbidites of the Limekiln Beds and Instow Beds lying to the north of the northerly derived Westward Ho! Formation and paralic Bideford Group.

H. G. READING

Department of Geology and Mineralogy,
University of Oxford.

¹ Owen, D. E., *Proc Geol Assoc.*, 45, 451 (1934).

² Owen, D. E., *Trans. Roy. Geol. Soc., Cornwall*, 18, 65 (1950).

³ Prentice, J. E., *Quart. J. Geol. Soc.*, 115, 261 (1960).

⁴ Prentice, J. E., *Quart. J. Geol. Soc.*, 116, 397 (1960).

⁵ Prentice, J. E., in *Some Aspects of the Variscan Fold Belt*, edit by Coe, K., 93 (Manchester Univ. Press, 1962).

⁶ Mackintosh, D. M., *Proc Ussher Soc.*, 1, 88 (1964).

⁷ Butcher, N. E., and Hodson, F., *Palaeontology*, 3, 75 (1960).

⁸ Prentice, J. E., and Thomas, J. M., *Abs. Proc Third Conf. Geol. and Geomorphologists working in South-West England*, 6 (1960).

⁹ Ashwin, D. P., *Abs. Proc. Conf. Geol. and Geomorphologists in South-West England*, 2 (1958).

¹⁰ Crookall, R., *Proc Cotswold Naturalists Field Club*, 24, 27 (1930).

¹¹ Lovell, J. P. B., *Proc Ussher Soc.* (in the press).

¹² De Raaf, J. F. M., Reading, H. G., and Walker, R. G., *Sedimentology*, 4, 1 (1965).

¹³ Walker, R. G., *Proc. Ussher Soc.*, 1, 85 (1964).

¹⁴ Eagar, R. M. C., *Liverpool and Manch. Geol. J.*, 1, 190 (1953).

¹⁵ Simpson, B., *Geol. Mag.*, 70, 433 (1933).

¹⁶ Woodland, A. W., Archer, A. A., and Evans, W. B., *Bull. Geol. Survey Gt. Britain*, 13, 39 (1957).

¹⁷ Woodland, A. W., and Evans, W. B., *Mem. Geol. Survey Gt. Britain*, 248 (1964).

¹⁸ Dearman, W. R., *Proc Geol. Assoc.*, 74, 265 (1963).

THE positive identification as *Gastrioceras subrenatum* (by W. H. C. Ramsbottom) of the goniatites from Rowden Cliff (SS 391252), in beds formerly attributed by me to the Cockington Beds (ref. 4, 402), shows that these are really the equivalent of the Instow Beds. This marine band, and one with *G. listeri*, also recurs repeatedly in the cliffs between Clovelly and Hartland (Moore, L. R., personal communication). Dr. H. G. Reading's hypothesis, which he has kindly allowed me to study, is, however, only one of several possible alternatives. His equation of Greencliff Beds here, with the Limekiln Beds of the Fremington region, contradicts my assertion that the former follow in stratigraphic order above the Abbotsham Beds. The evidence for this had to be omitted from the *Geological Society Journal* and it is worth while stating it now in detail. The coast section at Greencliff (Fig. 1) is not as clear as could be wished, but my interpretation is as follows. The coarse white sandstone (Cornborough Sandstone; Fig. 1, a) is followed by a few feet of black shales with *Planolites*, and then by the 'culm' bed (b), here strongly shattered and quartz-veined. This, in turn, is followed by laminated black silty shale with ironstone nodules (c). The section is then faulted; south of the second fault are 'turbidite' grits (d) which young to the north, and which rest on the laminated slumped siltstones (e) of the Greencliff Beds. These continue, still younging northwards, to south of the Limekiln, where they rest on black cleaved mudstone with ironstone nodules (f). It seemed reasonable to equate these with the lithologically similar beds (c) above the Cornborough Sandstone, and thus to recognize no major structural translocation at this point. Although there is undoubtedly faulting in this section (at d) the faults are high angle, and can be traced across the foreshore as north-westerly and north-easterly faults with a wrench component. Half a kilometre to the south, however, undoubted low-angle thrust faults appear (ref. 4, 400), and it is south of these that the beds contain *G. subrenatum*. It seems likely that it is these faults which are the most significant, bringing up the Instow Beds in tectonic superimposition with the lithologically similar Cockington Beds.

Similar relationships are seen inland. On the left bank of the River Torridge above Bideford, for example, black cleaved mudstone appears to overlie the Cornborough Sandstone, and to occupy the whole river bank from Bideford Bridge to Ford Rock (SS 453258); here they are overlain by grey siltstones (Greencliff Beds?) which are now well exposed in the new road cutting. South of these a group of greywacke sandstones is much shattered, and *Gastrioceras* occurs north and south of the confluence of the stream (SS 453252). The outcrop of the 'culm' and the underlying Cornborough Sandstone has been mapped eastwards from the Torridge to Gammaton (SS 490262), and regularly has a broad outcrop of laminated siltstones to the south, which is in turn followed by the outcrop of

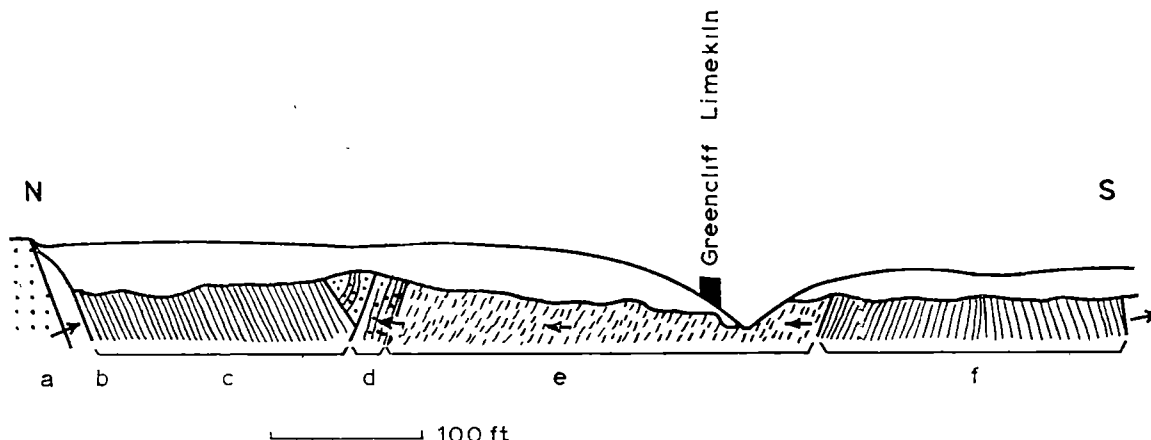


Fig. 1 Arrows indicate observed younging

greywacke sandstones. Old mining records show that the 'culm' outcrop continues eastwards to beyond the River Taw, and further east still E. E. Swarbrick has found a group of greywacke sandstones, the Alswear Formation apparently overlying the paralic Mole Valley Formation (= Northam and Abbotsham Beds) south of South Molton.

The interpretation of relationships between the base of the Westward Ho! Formation and the Instow Beds depends on the investigation of Hubbastone Quarry, Appledore. Since the Westward Ho! Formation can be mapped widely over the area north-east of Northam, and it is admitted that the beds at this quarry are lithologically similar to them, there seems no reason why they should not be regarded as part of this Formation. The lower horizons in this quarry, however, have abundant turbidite structures, and the shales are strongly pyritic—features which characterize the Instow Beds. It should be noted that Inkerman Rogers (1910) recorded 'marine nodules' from this quarry, though no specimens are extant.

The argument that it is implausible that the *lenisulcata* zone should be more than 4,000 ft. thick I find difficult

to accept; it is even more difficult to believe in facies changes from the 100 ft. of the Limekiln Beds southwards into 3,000 ft. of paralic sediments, and in giant tectonic translocations bringing these facies into juxtaposition. It is, moreover, difficult to see, without supposing major tectonic translocation, how deltaic sediments believed to have come from the north could have accumulated south of the zone of thin 'distal turbidites' represented by the Limekiln Beds: or how the paralic Abbotsham Beds could lie south of turbidites derived from the south, and north of turbidites derived from the north. Dr. Reading's theories then introduce more problems than they solve.

This is not to say that Dr. Reading's interpretation is impossible, nor can either of our successions be fully proved. The answer lies in the accumulation of more data, and it is hoped that our controversy may stimulate its collection.

J. E. PRENTICE

Department of Geology,
King's College,
University of London.

APPLICATION OF AN AGAR-AGAR DIFFUSION PROCEDURE TO PESTICIDE RESIDUE ANALYSIS AND TO THE CHOLINESTERASE SCREENING OF CANDIDATE PESTICIDES

By DR. K. I. BEYNON and G. STOYDIN

'Shell' Research, Ltd., Woodstock Agricultural Research Centre, Sittingbourne, Kent

SANDI *et al.*¹⁻³ have described an agar-agar diffusion procedure which can be used for the analysis of crops, soils, and animal tissues for residues of organophosphorus and carbamate insecticides. In their procedure, a layer of agar-agar 5 mm thick containing cholinesterase was prepared from human crassamentum and bromothymol blue, at pH 7.8-7.9. An isopropanol solution (0.1 ml.) of the insecticide was applied to a hole in the agar-agar. Diffusion was allowed to take place for 18 h at room temperature. The plate was then sprayed with acetylcholine chloride solution and the blue layer turned yellow within 30 min, apart from those areas where the enzyme had been inhibited, which remained as blue circles.

A rectilinear relationship was found to exist between log (amount insecticide) and the diameter of the ring. When the same amount of different compounds was applied to the plate, the strongest inhibitor produced the largest blue circle.

Sandi *et al.*^{2,3} reported that 0.003 µg of parathion could be detected in standard solutions after oxidation and described³ the application of the method to the determination of residues of parathion in edible oils down to a level of 0.75 p.p.m.

The procedure is of interest since it will detect very small amounts of some insecticides using the simplest of apparatus. However, the precision of the method as described by Sandi *et al.* does not appear to be good since the increase in diameter of the rings with increasing amounts of compound is not very great.

We have varied the experimental conditions using human plasma in an attempt to improve the precision of the method for residue analysis. We have also investigated the use of fly-head cholinesterase particularly as a method for the screening of the cholinesterase inhibition produced by candidate insecticides.

The experimental procedures differed from those described by Sandi² only in the following points. In some experiments human plasma was used at concentrations of

1-8 per cent (vol./vol.) relative to the agar-agar gel. In other experiments cholinesterase from fly-heads was used and the pH of the gel was adjusted to 7.5, instead of 7.8-7.9 as recommended by Sandi for plasma. The lower pH gave more consistent results. The supernatant solution from the maceration of fly-heads with 0.9 per cent saline (1 ml.-0.08 g of fly-heads) was used at a concentration of 5 ml. for every 60 ml. of agar-agar gel. In the preparation of gel mixtures containing fly-head cholinesterase the gel temperature was not allowed to exceed 40° C after the fly-head concentrate had been added.

The sizes of the inhibition rings produced by different concentrations of organophosphorus compounds are summarized in Figs. 1-3. It is evident that very small quantities of organophosphorus compounds produce measurable inhibition rings and 0.001 µg of 'Birlane' (1-chloro-2-(2,4-dichlorophenyl)-vinyl diethyl phosphate) and dichlorvos and 0.01 µg of mevinphos can be detected using fly-head cholinesterase. The precision of the procedure was not good, in that an increase of only 5 mm in ring diameter corresponded to a 2-5-fold increase in the amount of compound. Variation of the gel thickness and plasma concentration did not affect the precision of the procedure (Fig. 2).

Mevinphos was added to isopropanol extracts of lettuce, carrots, onions, potatoes, sugar beet and tomatoes to correspond to 0.05 p.p.m. in the original sample. The extracts were neutralized, if necessary, by the addition of N/10 NaOH. Aliquots of the extracts (50 ml. = 25 g crop) were concentrated to 5 ml. by blowing a jet of clean air over the surface of the solution. The mevinphos content of each solution was measured by the agar-agar diffusion procedure at a plasma concentration of 3 per cent v and gel thickness of 5 mm. Standard solutions of mevinphos and crop extracts containing no mevinphos were run on the same plate as the treated extracts. The neutralized, untreated extracts produced no inhibition rings. The treated samples produced inhibition rings of 26-28 mm and corresponded to recoveries of 115 per cent

from carrots, lettuce, potatoes and sugar beet, and 130 per cent from onions and tomatoes. While the recoveries were high the ring diameter corresponding to 100 per cent recovery was 25 mm, which was close to the values observed with the treated samples. The 1-3 mm difference, however, corresponds to a 15-30 per cent difference between the actual and theoretical recovery and this arises from the poor precision of the method.

While the agar-agar diffusion procedure is not precise for most residue analysis it is useful for the approximate determination of residues of organophosphorus and carbamate insecticides when sophisticated equipment is not available.

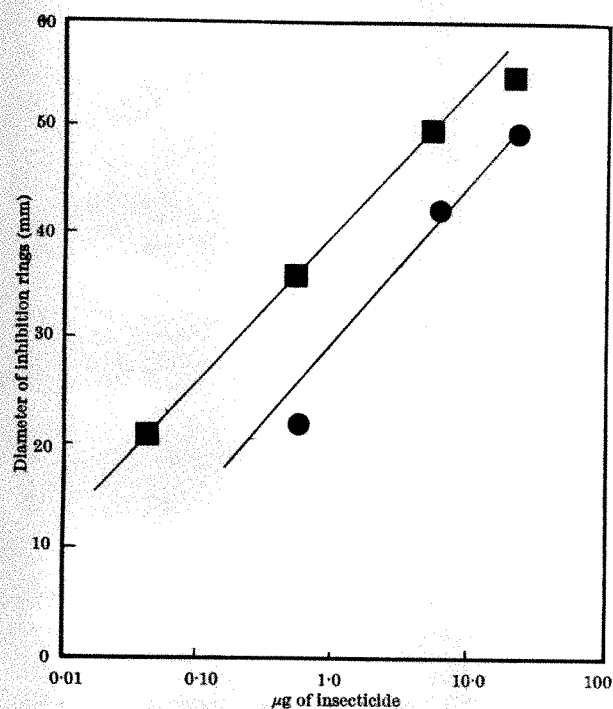


Fig. 1. Inhibition rings produced by mevinphos and dichlorvos using 4.5 mm layers of gel and 3 per cent concentration of human plasma. ■, Mevinphos, dimethyl 1-methoxycarbonyl-1-propen-2-yl phosphate; ●, dichlorvos, 2,2-dichlorovinyl dimethyl phosphate

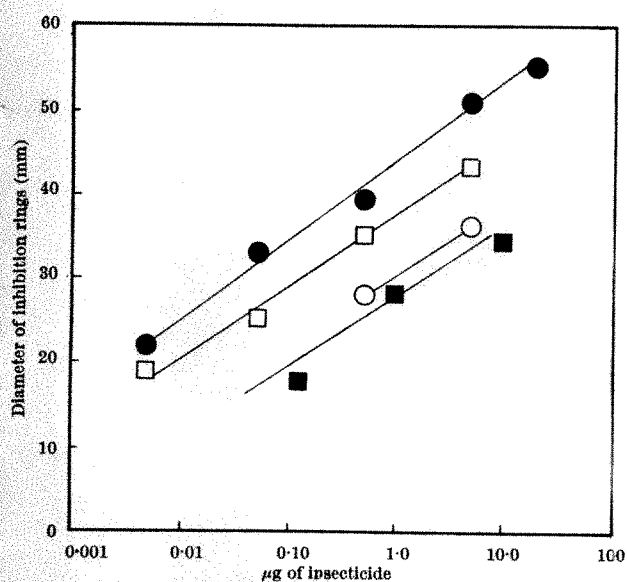


Fig. 2. Inhibition rings produced by 'Birlane' 1-chloro-2-(2,4-dichlorophenyl) vinyl dimethyl phosphate. ●, 4.5 mm gel layer 1 per cent plasma concentration; □, 4.5 mm gel layer 3 per cent plasma concentration; ○, 4.5 mm gel layer 5 per cent plasma concentration; ■, 2.0 mm gel layer 3 per cent plasma concentration

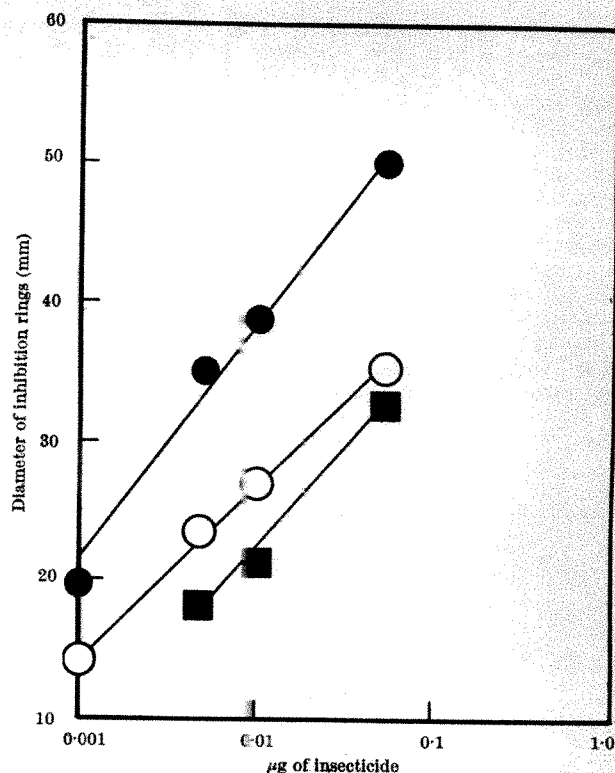


Fig. 3. Inhibition rings produced by organophosphorus compounds after agar-agar diffusion (5 mm layer) using fly-head cholinesterase. ●, Dichlorvos; ○, 'Birlane'; ■, mevinphos

In spite of the poor precision the method is useful for the routine screening of candidate insecticides for their *in vitro* inhibition of insect enzymes. While many precise procedures are available⁴ for measuring the inhibition of cholinesterase by organophosphorus compounds

Table 1. APPLICATION OF AGAR-AGAR DIFFUSION TO CHOLINESTERASE SCREENING

Structure of insecticide	Diameter of inhibition ring (mm) produced by 1.5×10^{-4} moles of the insecticide	pI_{50} *
$(C_2H_5O)_2P(O)OC(=CHCl)-C_6H_3Cl_2-CH_3$ ('Birlane')	49	8.7
$(CH_3O)_2P(O)OC(=CHCl)-C_6H_3Cl_3$	46	7.9
$CH_3NHC(O)O-C_6H_3(CH_3)_2$	38	7.0
$(CH_3O)_2P(S)O-C_6H_3(CH_3)_2-CO-N-CH_2-C_6H_5$	36	7.2
$(CH_3O)_2P(O)OC(CH_3)=CHC(O)HCH_3$	31	6.6
$(CH_3O)_2P(S)O-C_6H_3S-N=N$	<1	5.9
$(C_2H_5O)_2P(S)O-C_6H_3S-N=N$	<1	<1

* - \log_{10} (molar concentration of compound to produce 50 per cent inhibition of fly-head cholinesterase).

and carbamates, such methods are time-consuming when many dozens of compounds are to be examined each week.

Several candidate insecticides (1.5×10^{-8} moles in 0.1 ml. IPA) were applied to an agar-agar plate (5 mm thickness containing fly-head cholinesterase) and the layer was sprayed with acetylcholine chloride after 18 h. The inhibition rings were then measured. In addition the pl_{50} values for each compound were measured by a precise procedure by plotting the percentage inhibition of fly-head cholinesterase produced by different concentrations of each compound. The enzyme activity for such measurements was determined by the method described by Michel⁵, and the organophosphorus compounds and enzyme were incubated at 25° C for 30 min before the determination of the activity.

The ring diameters are compared with the pl_{50} values in Table 1 and the results suggest that the agar-agar diffusion method can provide an indication of the inhibitory powers of the compounds examined. The inhibition of up to 36 compounds can be determined at the same time on one 33 cm \times 33 cm agar plate.

While screening by the diffusion method gives far less precise information than pl_{50} values, the procedure permits the rapid examination of a large number of compounds and is useful in preliminary work.

¹ Sandi, E., and Wight, J., *Chem. and Indust.*, 1161 (1961).

² Sandi, E., *Die Nahrung*, 6, 57 (1962).

³ Lipparini, L., and Sandi, E., *Rassegna Chimica*, 129 (1964).

⁴ Archer, T. E., in *Analytical Methods for Pesticides, Plant Growth Regulators and Food Additives*, 1, 373, edit. by Zwieg, G. (Academic Press, New York, 1963).

⁵ Michel, H. O., *J. Lab. Clin. Med.*, 34, 1564 (1949).

EVALUATION OF ION EXCHANGE CARTRIDGES FOR FIELD SAMPLING OF IODINE-131 IN MILK

By R. H. JOHNSON, jun., and T. C. REAVEY

Northeastern Radiological Health Laboratory*, Winchester, Massachusetts

IODINE-131 may be introduced into the environment through the normal discharges from nuclear reactors and nuclear fuel processing plants, through industrial accidents and through the detonation of nuclear weapons or other nuclear devices. The volatility of iodine and many of its compounds makes it one of the radio-elements most likely to escape to the environment in accidents at nuclear reactor plants. In unseparated fission products, this radionuclide is the critical element in terms of radiation dose to the population for early periods from radionuclides reaching man through his diet¹. For these reasons and because the major route of entry of radioiodine into the human diet is through milk, the rapid determination of iodine-131 in this foodstuff is important.

A number of authors have described procedures for the rapid determination of iodine-131 in milk. One of the most common approaches is to assay this radionuclide directly in fluid milk by γ -spectrometry². A second method consists of adsorbing the iodine-131 on anion exchange resin followed by γ -counting³⁻⁵. Although only ionic forms of iodine are adsorbed by anion exchange resin, approximately 95 per cent of the iodine-131 content of milk is present as iodide, so that only a small error is involved. A third method has also been described in which the radioiodine, once adsorbed on anion exchange resin, is eluted from the resin and prepared for β -counting⁶.

On the basis of the second of these methods, a field technique has been developed for the rapid collection of iodine-131 from milk⁷. In this procedure, a small cartridge, containing anion exchange resin, is used for concentrating and separating this radionuclide from the other fission products in milk. Experience⁸ has shown that such resin has a very high capacity for adsorbing this radionuclide. For example, approximately 160-200 resin bed volumes may be passed through a given volume at a flow rate of up to 1 resin-bed volume per min and still yield a removal efficiency of 95 per cent. With the use of the cartridge, the method is simple, inexpensive, and applicable to both emergency and routine situations. The purpose of this article is to describe the recent application of this technique in the field on a trial basis.

The opportunity for the investigation was provided by the October 1964 nuclear test by the People's Republic of China which introduced fresh fission products (including iodine-131) into the atmosphere. This material, carried by winds and deposited on open pasture, led to contamina-

tion of milk supplies in several areas within the United States. The investigation reported here was conducted in the vicinity of Burlington, Vermont, where the cartridges were used to sample milk from the major dairy plants in that city. Samples from bulk-tank trucks arriving at milk-processing plants and from silo tanks in the plants were first taken and analysed as a test of the applicability of the cartridge technique to routine environmental monitoring. In a second phase, the application to more detailed surveillance, or research investigations, was evaluated by a more intensive sampling programme in which samples from individual cows on a farm with relatively high activity of iodine-131 were processed.

The type of ion exchange cartridge used (see Fig. 1) was a modification of a model developed at the Southeastern Radiological Health Laboratory⁷. The unit consists of a polyethylene vial containing approximately 40 ml. of strongly basic quaternary amine-type anion exchange resin, 20-50 mesh, chloride form, suspended in distilled water. Milk is introduced through a separatory funnel attached to the top of the cartridge, passed through the resin bed, and then out through ten 2-mm diameter holes drilled in the bottom of the vial (see Fig. 2). Tests have shown that a litre (the quantity used in this investigation) of fresh milk will pass through a cartridge in about 20-30 min, the rate of flow being mainly determined by the choice of resin size.

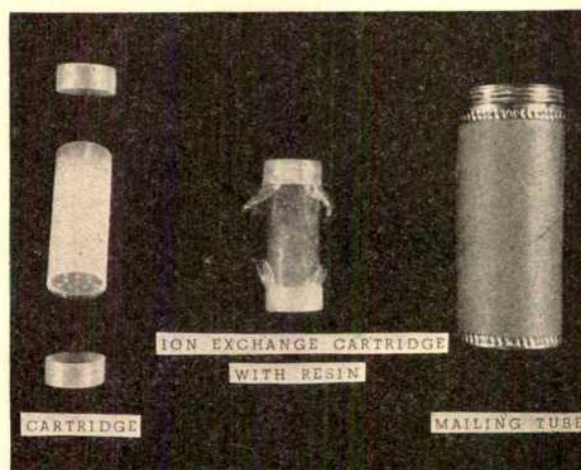


Fig. 1. Cartridge and mailing tube

* U.S. Department of Health, Education and Welfare, Public Health Service, Division of Radiological Health.

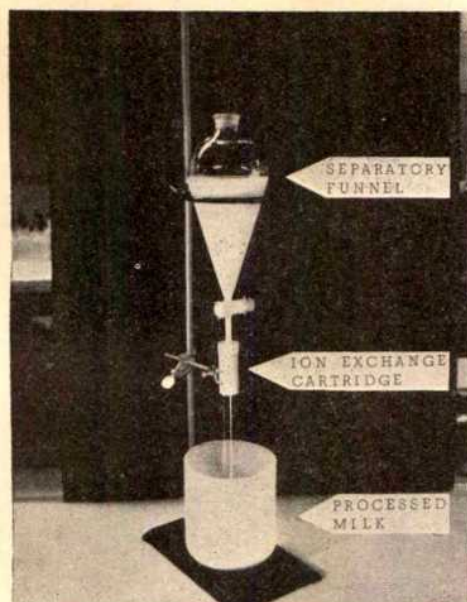


Fig. 2. Cartridge set up for field use

Since some fluid milk samples were brought back to the laboratory for duplicate processing, the study permitted an investigation of the suitability of thimerosal⁹ as a preservative for samples awaiting analysis by this technique. Previous experience had shown that formaldehyde, the preservative at present used in samples collected under the Public Health Service's Pasteurized Milk Network¹⁰, binds radioiodine to the milk protein and thereby prevents its adsorption by anion-exchange resin¹¹.

Although 'spot' counting of some samples was performed in the field using a portable multichannel γ -spectrometer, all the data reported in this article were based on radioactivity determinations made on the resins in the laboratory. For this purpose, the resin containing the adsorbed iodine-131 was emptied into cottage cheese containers which were then counted on a solid cylindrical 4 in. \times 4 in. NaI(Tl) crystal, coupled to a multichannel γ -spectrometer. Subsequent to this work, a cartridge of smaller diameter has been developed at the Northeastern Radiological Health Laboratory which can be directly inserted into a well-type crystal for counting. It is believed that this approach will have two advantages, one being that the well-type crystal will yield a greater counting efficiency, and the second being that such samples can be readily adapted to counting procedures utilizing automatic sample changers.

As previously mentioned, initial application of the ion-exchange cartridge technique was to samples of fluid milk arriving at milk processing plants. These samples represented supplies from the various dairies in the area surrounding Burlington, Vermont, and a summary of the observed data on levels of iodine-131 is shown in Table 1. As will be noted, the average concentration of this nuclide in the ten 1-l. samples, weighted according to the produc-

Table 1. IODINE-131 IN MILK FROM PROCESSING PLANTS*

Plant	Concentration of ¹³¹ I (pc./l.) Ion exchange technique (1 l.)
A ₁ †	57
A ₂	39
A ₃	30
B ₁	33
B ₂	29
C	18
D	21
E	48
F	14
G	19
Weighted average	38 \pm 10
Pasteurized Milk Network sample	31 \pm 10

* All samples were collected at Burlington, Vermont, on November 3, 1964.

† Samples A₁, A₂, and A₃ all came from a single plant but were drawn from three different silo tanks. The same is true for samples B₁ and B₂.

tion of the given plant, was 38 pc./l. On this same date, the iodine-131 concentration in the composite Pasteurized Milk Network sample, collected at this same location and analysed in liquid form (3.5 l.) by γ -spectrometry³, was 31 pc./l. Since the counting error for each of these measurements was \pm 10 pc./l., the two results are in agreement.

To evaluate the acceptability of thimerosal as a preservative, additional samples were collected from three different tank trucks arriving at one of the larger plants serving this area. The preservative (3 ml. of a 10 per cent aqueous solution of thimerosal per litre of milk) was added to the samples in the field and they were then returned to the laboratory where 3.5 l. portions were analysed for iodine-131 by γ -spectrometry and 1-l. volumes were passed through the cartridges for analysis by the ion-exchange technique. Data on the concentrations of iodine-131 determined by the two methods are shown in Table 2. Again, considering the counting error in each of the reported values, the agreement in the data is acceptable.

Subsequent to these two investigations, a number of milk samples were obtained from individual cows at a dairy farm in Williston, Vermont. One-litre portions of most of these were passed through ion-exchange cartridges in the field, and additional 1-l. portions (some preserved with thimerosal and some not) were brought back to the laboratory where they, in turn, were also passed through similar cartridges 1-5 days later. All resins were then counted under identical conditions and a summary of the data for the three sets of samples is shown in Table 3.

Data for the eleven samples which were processed with and without preservative are presented in Fig. 3. The correlation coefficient for these data was 0.90. The close agreement in the values confirms the acceptability of thimerosal as a preservative and indicates that the processing of samples can be handled in the field with results

Table 2. COMPARISON OF ANALYTICAL METHODS FOR IODINE-131 IN MILK

Date and time of sample collection	Concentration of ¹³¹ I (pc./l.) Ion exchange technique (1 l.)	Fluid γ -spectrometry (3.5 l.)
Evening, Nov. 3, 1964	43 \pm 10	32 \pm 10
Morning, Nov. 4, 1964	36 \pm 10	35 \pm 10
Evening, Nov. 4, 1964	50 \pm 10	37 \pm 10

Note: Samples were collected from tank-trucks serving a milk processing plant in Burlington, Vermont; analytical work was performed at the Northeastern Radiological Health Laboratory during November 6-10, 1964.

Table 3. IODINE-131 IN MILK FROM INDIVIDUAL HOLSTEIN COWS*

Sample No.†	Concentration of ¹³¹ I (pc./l.) Field determination (without preservative)	Laboratory determination (without preservative)	Laboratory determination (with preservative)
1	42	73	—
2	34	—	—
3	—	—	14
4	35	49	—
5	43	39	—
6	44	47	—
7	—	—	42
8	—	—	21
9	44	78	—
10	—	—	24
11	47	—	—
12	—	—	48
13	35	—	29
14	—	58	57
15	13	—	—
16	6	—	—
17	33	—	27
18	19	—	34
19	—	83	80
20	—	—	61
21	49	—	63
22	49	—	44
23	—	—	56
24	11	—	—
25	11	—	—
26	32	—	—
27	—	—	10
28	74	—	—
29	27	30	—
30	32	—	—
31	—	69	—
32	—	26	24
33	—	—	36
34	62	70	—
35	16	—	29
36	42	—	48
Composite	—	—	44

* Samples collected on a farm in Williston, Vermont, on November 5, 1964.

† Each sample was from a different cow.

comparable with the more favourable conditions of the laboratory. The data also show that no chemical changes sufficient to affect the results occurred during the 1-4 days that the samples stood while preserved with thimerosal. Data for the fourteen samples processed both in the field and in the laboratory are shown in Fig. 4. Here the data had a correlation coefficient of 0.68. Except for three samples, not containing thimerosal, the data conformed to the expected relationship within the counting errors. Although not confirmed, it is possible that the lack of agreement in the data for these three samples was largely due to insufficient control of the milk flow-rate in the field. It is also possible that the cartridges were allowed to drain dry prior to initiation of the flow of milk, thus permitting short-circuiting of the fluid through the column. As will be noted, the samples processed in the laboratory yielded much greater concentrations of iodine-131 which tends to confirm one or both of these possibilities.

Of interest in Table 3 is the range of values of iodine-131 in the milk from the individual cows at the dairy. All

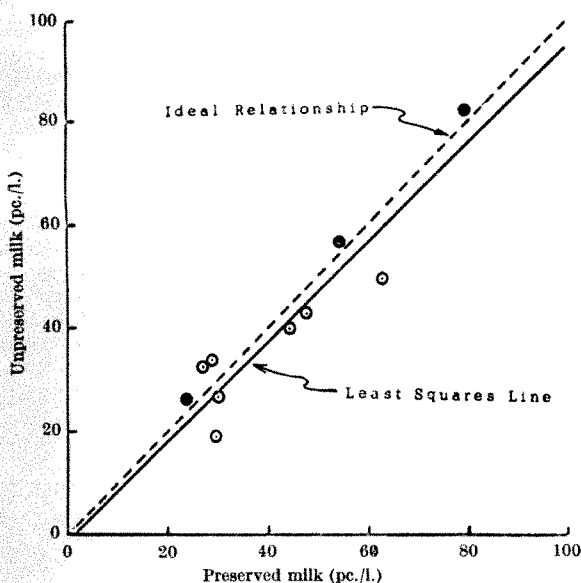


Fig. 3. Relationship between the observed iodine-131 concentrations in preserved and unpreserved milk. ●, Both samples processed in the laboratory; ○, one sample processed in the laboratory and one in the field.

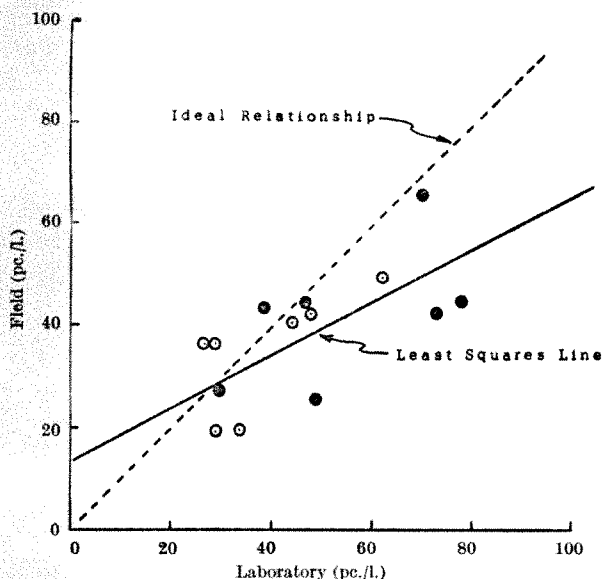


Fig. 4. Relationship between observed iodine-131 concentrations as determined in the field versus the laboratory. ○, Field samples unpreserved, laboratory samples preserved; ●, both field and laboratory samples unpreserved.

cows in the herd were of the same breed (Holstein) and had been on open pasture until 3 days before samples of milk were taken. Even so, the data show that the levels of iodine-131 in the milk samples varied by a factor of ten. Two conditions possibly contributing to this wide variation were: (a) the proportion of supplemental (stored) feed consumed by each cow while still on open pasture; (b) the fact that the biological half-life of iodine-131 is shorter than the 3 days the cows had been off pasture. For this reason, the precise length of time the individual cows had been indoors before the milk samples were collected could be important. Attempts to correlate the individual radionuclide-levels with the age of the cows or the time of lactation were unsuccessful.

The major advantages of the use of ion-exchange cartridges are their simplicity and low cost. One complete field model, as shown in Fig. 1, costs approximately 3.00 dollars and all components can be used many times. The iodine-131 can be stripped from the resin with 2 N hydrochloric acid and the resin reconditioned with 2 M sodium chloride⁸. If the resin is washed clean with water after use, it can be used five or ten times (without exceeding the exchange capacity), provided the previously adsorbed iodine is allowed to decay. Where samples have to be sent to a centralized or regional laboratory for counting, there is a considerable saving in shipping cartridges rather than the 1-gal. milk samples normally required for determination of iodine-131. Because 1 l. (~1 quart) of milk is adequate for most determinations using the cartridges, there is also a saving in the amount of milk required for analysis.

In contrast to the procedure followed in this investigation, it is possible that the cartridges could be shipped in advance to a field location for use and then returned to a laboratory for counting. Since one funnel could be used to pass samples through a number of cartridges, once several funnels had been provided at a given sampling location, they could be retained there for use as required. Since iodine is the only radio-element separated from milk by anion resin, it would also be feasible to conduct entire studies of this type in the field. Under such conditions, counting could be carried out using a well-crystal scintillation detector and a suitable scaler. At higher levels, however, such as those contemplated under emergency conditions¹, measurement of the iodine-131 activity on the cartridges could be undertaken in the field with a simple Geiger-Müller survey meter.

On the basis of this study, it can be concluded that the ion exchange cartridge is an effective means for field sampling of iodine-131 in fluid milk. Because of the simplicity of the method, it appears especially advantageous wherever it is necessary to collect large numbers of samples.

We thank Dr. H. L. Wildasin and F. Norton and M. Lamson of the H.P. Hood Milk Co. for their co-operation in arranging the collection of the samples, W. I. Carr and B. Barrows of the Dairy Division, Vermont State Department of Agriculture, for their assistance in arranging the investigation, and E. J. Baratta, K. C. Lamson, and P. R. Groulx of the Northeastern Radiological Health Laboratory for assistance in collecting and analysing the samples and resulting data.

¹ Federal Radiation Council, *Background Material for the Development of Radiation Protection Standards*, Rep. No. 5 (U.S. Government Printing Office, Washington, D.C., 1964).

² Hagee, G. K., Karches, G. J., and Goldin, A. S., *Talanta*, **5**, 36 (1950).

³ Bone, A. L., *Analyst*, **88**, 64 (1963).

⁴ Murthy, G. K., in *Rapid Methods for Estimating Fission Product Concentrations in Milk*, U.S. Publ. Health Serv. Publ. No. 999-R-2 (U.S. Government Printing Office, Washington, D.C., 1963).

⁵ Smith, H., and Whitehead, E. L., *Nature*, **199**, 503 (1963).

⁶ Kahn, B. J., *Agric. Food Chem.*, **13**, No. 1, 21 (1965).

⁷ Porter, C. R., and Carter, M. W., *Public Health Reports*, **80**, No. 5, 453 (1965).

⁸ Murthy, G. K. (personal communication).

⁹ Lamanna, A., Holmes, J., Abbot, T. P., and Yancik, V., *Health Physics*, **11**, No. 6, 586 (1965).

¹⁰ *Radiological Health Data*, **5**, No. 11, 535 (November 1964).

¹¹ Murthy, G. K., and Campbell, J. E., *J. Dairy Sci.*, **43**, 1042 (1960).

EXAMINATION OF A POLYSACCHARIDE FLOCCULANT AND FLOCCULATED KAOLINITE BY ELECTRON MICROSCOPY

By DR. ARNOLD AUDSLEY and ANITA FURSEY

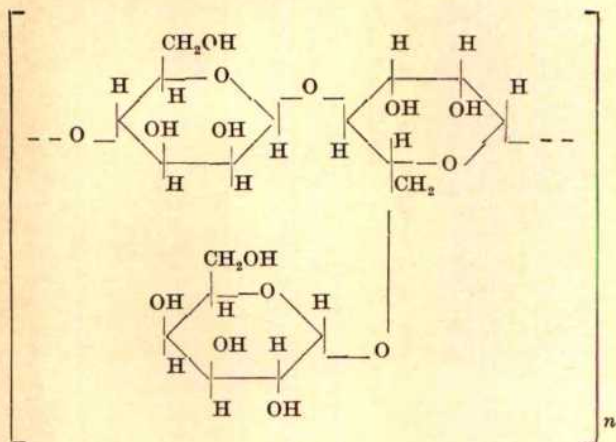
National Physical Laboratory, Teddington, Middlesex

THE scope of the electron microscope as a tool in polymer science has recently been extended by Richardson¹, who has shown that individual molecules of amorphous polymers may be isolated by spraying a dilute solution of the polymer, in the form of fine droplets, on to a suitable supporting film. Polyacrylamide sprayed from an aqueous solution showed a unique, highly fibrous structure; the finest filaments were believed to be individual molecular chains. It is reasonable to assume that this structure is a replicate of the molecular configuration in aqueous solution. When controlled quantities of precipitant were added to the solution the molecules were isolated in the closely-coiled configuration and this enabled molecular weights to be determined by direct measurement.

Richardson's methods¹ have been applied in an investigation of the flocculation of suspensions of solids by organic polymers. In this Laboratory the electron microscope has been used both to examine a polysaccharide flocculant and to study the structure of polymer adsorbed on the surface of kaolinite particles.

Suspensions of ground minerals such as are encountered in hydrometallurgy may be flocculated by the addition of trace quantities of certain organic polymers, notably polyacrylamide and naturally occurring polysaccharide-type materials, of which guar, the ground endosperm of seeds from the legume *Cyamopsis tetragonolobus* shows the most strongly developed properties. A limited amount of evidence is available² to show that when suspensions are flocculated the particles are bonded together by adsorbed polymer molecules. If this is correct it may be assumed that rigid, linear polymers of high molecular weight will be the most efficient flocculants.

The major polysaccharide constituent of guar is a galactomannan of the structure³:



In the course of the present work the pure galactomannan was extracted from samples of commercial guar flocculant, fractionated from ethanol/water mixtures and isolated in the form of long white fibres. A middle fraction was used for all the experimental work (found: C—44.07 per cent, H—6.16 per cent, N < 0.1 per cent, P—0.011 per cent, $[\alpha]_D^{25} = 61.9^\circ$. Calc. for $C_{18}H_{30}O_{15}$, C—44.45 per cent, H—6.22 per cent).

The pure galactomannan contained 14.5 μg boron/g and it is probable that a trace of borate had been added

by the manufacturer to improve the flocculant properties of the polymer since it is known that when other polymeric flocculants are lightly cross-linked by suitable reagents a flocculant of greater efficiency is obtained^{4,5}. Borate ions are known cross-linking agents for polysaccharides which contain adjacent hydroxyl groups in the *cis* position. A pure galactomannan prepared from an untreated guar seed contained only 4.1 μg boron/g. It showed a lower value of the limiting viscosity number ($[\eta]$) than the borated sample (typical values of $[\eta]$ were 10.2 and 19.0 dl./g respectively) and by comparison it was an inferior flocculant of kaolinite suspensions. The borated polymer was used in the subsequent work because of its superior flocculant properties.

Solutions of the galactomannan were prepared by shaking the finely-ground solid with water in a nitrogen-filled ampoule for 16 h. A suitable quantity of solution was placed on an evaporated carbon film using both droplet and spray techniques and the polymer molecules shadowed with gold/palladium alloy at a height-to-shadow ratio of 1:4 before examination by electron microscopy. A very clean carbon film was important for this work as some particles produced during carbon evaporation were similar in size and shape to the polymer droplets under examination. To distinguish between the polymer and extraneous particles produced during the film preparation, the evaporated carbon was shadowed with gold palladium before any polymer was added and then turned at right angles before reshadowing. This meant that polymer particles had only one shadow on the final electron micrograph and any impurities present on the original evaporated carbon had two shadows perpendicular to one another.

Galactomannan deposited from an aqueous solution showed a similar fibrous structure to that of polyacrylamide¹. The structure was demonstrated when a droplet of a 0.1 per cent solution of the polymer was evaporated on a carbon film at normal temperature and pressure and shadowed, as described, before examination. Fig. 1 shows

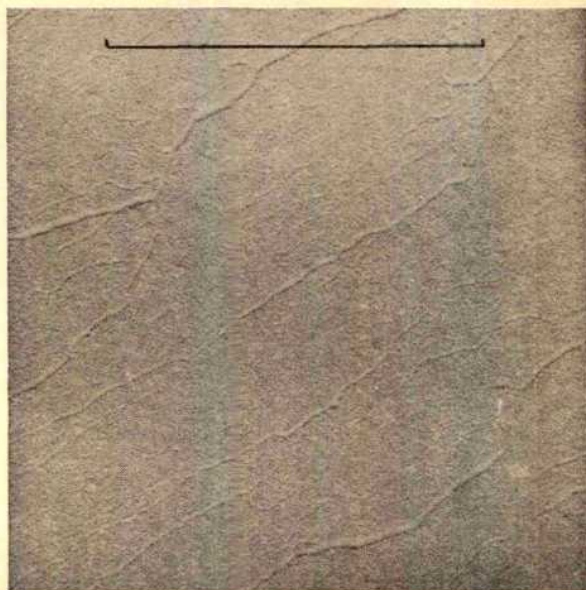


Fig. 1. Fibres of polysaccharide from evaporated aqueous solution. Scale, 1 μ

a typical electron micrograph. The smallest diameter of fibre seen on a number of pictures was 0.004μ . These fibres are probably aggregates containing several molecular chains.

It is noteworthy that organic polymers of such widely differing chemical types, namely, polyacrylamide and a polysaccharide such as galactomannan, should show such close similarity in their molecular configuration in aqueous solution. It is logical to associate the fibrous structure of these polymers with their high limiting viscosity numbers (typical values of $[\eta]$ for aqueous solutions of polyacrylamide and galactomannan were 20.0 and 19.0 dl./g respectively) and their strong flocculant properties.

When a suitable precipitant (ethylene glycol) was added to a dilute aqueous solution of galactomannan and the mixture sprayed on to a carbon film, the appearance of the polymer changed and under appropriate conditions individual molecules were isolated in a partially collapsed oblate spherical configuration. A very dilute solution (10^{-2} per cent) of the polymer was necessary and it was applied to the carbon film in the form of a very fine spray with a 'Vaponefrin' nebulizer. Ideally this method may be used to determine the molecular weight distribution of the polymer¹, but in the limited time available it was not possible to find reproducible conditions in which all the molecules were in the fully collapsed spherical configuration which is necessary if the method is to be applied. An electron micrograph is shown in Fig. 2. It is apparent that the sample is still highly polydisperse and the original fractionation from aqueous solution was not efficient. In a system of this degree of polydispersity, it may be impossible to ensure that all the polymer molecules exist in the fully collapsed spherical configuration irrespective of polymer size.

A sample of kaolinite which had been flocculated with galactomannan was then examined by electron microscopy. If flocculation is due to polymer bridging, the orientation of the polymer chains in proximity to the particle surface might be observable by electron microscopy, assuming that the polymer structure is not completely destroyed when the sample is dried.

The flocculated solid was freeze-dried, dispersed on a supporting film and shadowed with gold/palladium alloy. The electron micrograph (Fig. 3) shows clearly the structure of the polymer in proximity to the kaolinite surface. A diagrammatic representation of the most prominent features is shown in Fig. 4. At 120,000 magnification some

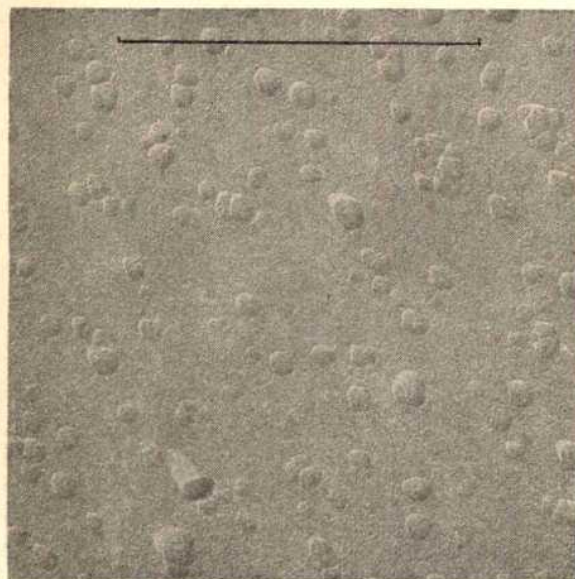


Fig. 2. Particles of polysaccharide from sprayed water/ethylene glycol solution. Scale, 1μ .

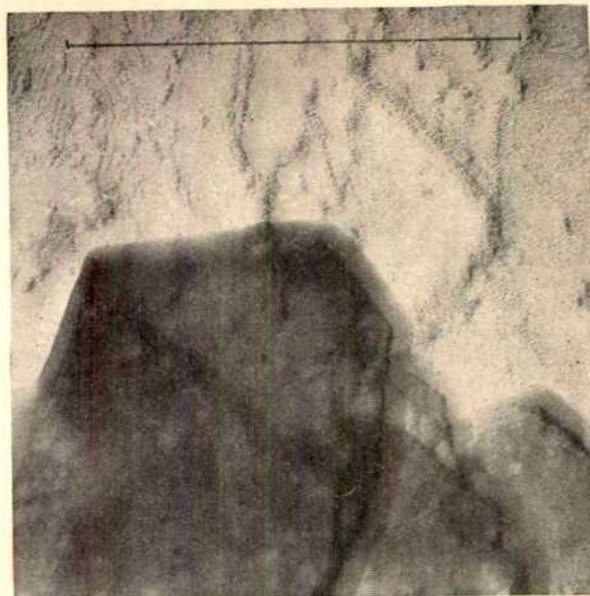


Fig. 3. Kaolinite flocculated with polysaccharide. Scale, 0.5μ .

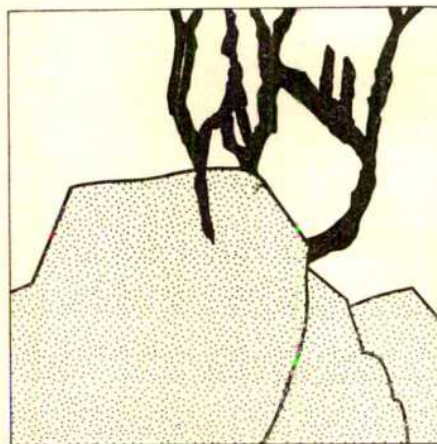


Fig. 4. Flocculant polysaccharide fibres shown diagrammatically

of the shadowed fibres attached to the kaolinite particles had a parallel stranded structure along their surface suggesting that they were composed of an aggregate of polymer chains. It was not possible to measure the diameter of the polymer fibres attached to the kaolinite surfaces with accuracy, but in general these appeared to be approximately 0.01μ diameter, which corresponds to a cross-sectional area about one order of magnitude larger than that of the smallest fibres seen on evaporation of an aqueous solution of the polymer.

This work provides strong evidence in favour of the polymer bridging theory of flocculation. The potential flocculant properties of both polyacrylamide and galactomannan may be correlated with their high limiting viscosity numbers and with their highly fibrous structure in solution, a structure which is retained to some extent after the polymer has been adsorbed on the surface of the solid.

We thank Dr. M. J. Richardson of this Laboratory for his advice.

¹ Richardson, M. J., *Proc. Roy. Soc., A*, **279**, 50 (1964).

² La Mer, V. K., *J. Coll. Sci.*, **19**, 291 (1964).

³ Whistler, R. L., and Smart, C. L., *Polysaccharide Chemistry* (Academic Press, 1953).

⁴ La Mer, V. K., *et al.*, NYO-7403.

⁵ Goren, M. B., U.S. Patent, 2,937,143 (1960).

EFFECTS OF HYDROCORTISONE SUCCINATE ON THE COMPLEMENT SYSTEM

By DR. HENRY GEWURZ*, PAUL R. WERNICK, DR. PAUL G. QUIE† and PROF. ROBERT A. GOOD‡

Pediatric Research Laboratories of the Variety Club Heart Hospital, University of Minnesota, Minneapolis

AMONG immunosuppressive agents, few have been as useful clinically as the adrenal steroids and few have been so little understood pharmacologically. Perhaps the most provocative of the recent findings on the activity of steroids have been the demonstrations that these drugs are capable of 'stabilizing' lysosomes *in vivo* and *in vitro*¹⁻⁸ and of protecting red cells against both immune and non-immune lysis *in vitro*, usually interpreted as an effect on the cell membrane⁹⁻¹³.

The findings reported here indicate that the capacity of hydrocortisone to inhibit immune haemolysis is largely dependent on interaction with the fluid phase constituents of complement-containing sera rather than with cell membranes directly.

In these investigations hydrocortisone succinate powder (kindly supplied as 'Solu-cortef' by the Upjohn Co.), free of contaminating reagents, was dissolved in an isotonic gelatine-veronal-saline buffer (GVB) with supplemental Ca^{++} and Mg^{++} (ref. 14) rather than in the accompanying anticomplementary diluting fluid, and the salt concentration adjusted to isotonicity; pH was 7.4. Sodium succinate, in the concentration present in the steroid powder, was not anti-complementary.

The activity of guinea-pig C' was tested by observing the rate and extent of lysis of sheep red cells sensitized with rabbit antibody (Sh EA). 0.5 ml. of a C' dilution was pre-incubated with 0.5 ml. of a hydrocortisone dilution at 37° or 0° C for various time-intervals. 1 ml. GVB and 0.5 ml. Sh EA (1×10^8 /ml.) were added and the mixture incubated for 60 min at 37° C. Time for complete haemolysis was recorded; if complete haemolysis did not occur within 60 min, the tubes were centrifuged and the 50 per cent lysis end point estimated visually. Hydrocortisone concentrations are expressed in terms of amounts present in the 1.0 ml. pre-incubation mixtures; final concentrations in the lytic step are two-fifths of this amount. The pool of guinea-pig serum used had a titre of 228 $C'H_{50}$ /ml. in the assay described by Osler *et al.*¹⁵, the reference units ('standard units') used in this report, and a titre of 1,792 $C'H_{50}$ /ml. in the assay system already described here, which is based on methods described by Nelson¹⁶.

Hydrocortisone was found to be an inhibitor of guinea-pig C' if certain conditions were met. The concentration of hydrocortisone, the time of pre-incubation, and the temperature during pre-incubation all played a part in the inhibition of a given amount of C' . The effect of varying concentrations of hydrocortisone on a fixed amount of C' during a fixed incubation period is shown in Fig. 1: concentration and degree of inhibition were directly correlated. Pre-incubation of low concentrations of hydrocortisone with C' was necessary to achieve inhibition; thus, while hydrocortisone in the range of 25 mg/ml. gave complete inhibition of 22.8 $C'H_{50}$ without pre-incubation, a pre-incubation period was required for lower concentrations.

As the pre-incubation period with a given hydrocortisone concentration (less than 25 mg/ml.) was increased, we noted a proportionate increase in the resultant inhibition of haemolysis (Fig. 2). Pre-incubation at 0° C was also

effective, but much greater intervals were required to achieve similar levels of inhibition.

There were other indications that the steroid effects were directed against the fluid phase constituents rather than against the cell membrane. Pre-incubation of hydrocortisone and sensitized cells before addition of complement, or pre-incubation of hydrocortisone before addition of sensitized cells and complement, gave minimal and no augmentation of inhibition, respectively. When a fixed amount of hydrocortisone was pre-incubated under standardized conditions with varying amounts of complement, the degree of inhibition varied with the amount of complement, again suggesting that fluid phase constituents rather than the size of the sensitized cell population determined the degree of haemolysis (Table 1).

6-Methyl prednisolone ('Solu-medrol', kindly supplied by the Upjohn Co.) was found to be significantly more effective than hydrocortisone in exerting this inhibition

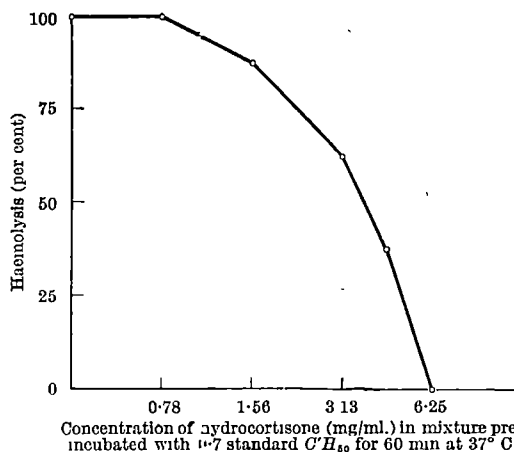


Fig. 1 Effect of hydrocortisone concentration on the inhibition of guinea-pig complement *in vitro*

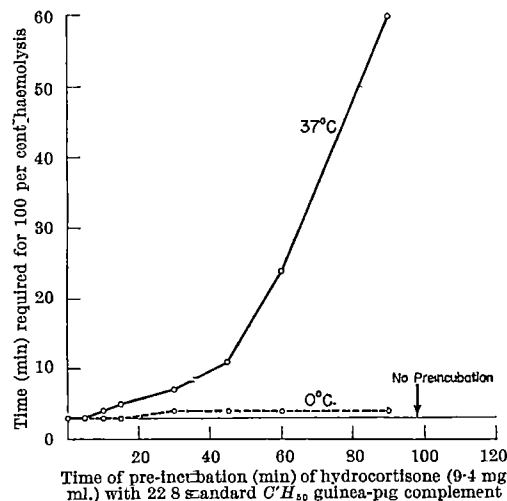


Fig. 2. Effect of length of pre-incubation period at 37° C and 0° C on hydrocortisone-induced inhibition of guinea-pig complement *in vitro*

*Fellow, U.S. Public Health Service, cardiovascular graduate training grant 577-HE-5222

† Markle scholar

‡ American Legion Memorial heart research professor of pediatrics and microbiology.

Table 1. EFFECT OF PRE-INCUBATION OF A FIXED CONCENTRATION OF HYDROCORTISONE (0.25 mg/ml.) WITH VARYING AMOUNTS OF C' FOR 60 MIN AT 37° C

No. of EA	No. of $C'H_{50}$ (standard units)	Inhibition (%)
5×10^7	5.7	0
5×10^7	2.9	50
5×10^7	0.7	100

when equimolar quantities were tested in identical assays (Fig. 3), indicating that more than simply the steroid structure of this molecule is responsible for the inhibitory effect on C' .

From these experiments we conclude that hydrocortisone exerts a concentration-, time- and temperature-dependent inhibitory effect on guinea-pig C' in the fluid phase. In another series of experiments, a similar effect was seen on human C' .

In the next set of experiments we sought to determine which complement components were inhibited by the steroids. Thus, 22.8 $C'H_{50}$ was pre-incubated in 12.5 mg/ml. hydrocortisone for a 2-h interval at 37° C (sufficient to achieve complete inhibition). This mixture, together with parallel control mixtures of guinea-pig C' and of hydrocortisone with normal saline solution with appropriate metals added, was dialysed against isotonic saline supplemented with calcium and magnesium, for a 24-h period. Effectiveness of the dialysis was assessed by determination of optical density in a Beckman DU spectrophotometer at 240 and 280 m μ ; on this basis, more than 98 per cent of the hydrocortisone initially present had been removed from the mixtures. This afforded opportunity to assay for each of the four classical C' components in a system containing amounts of hydrocortisone insufficient to have caused inhibition in the original pre-incubation mixture. $C'1$, $C'4$ and $C'2$ activities were tested using the assays described by Nelson¹⁸, and each was found to be markedly decreased in the hydrocortisone-treated sera; overall $C'3$ activity was also markedly decreased, using the assay described by Osler *et al.*¹⁷. Further, hydrocortisone introduced at any step in the interaction of the classical four C' components, that is, after formation of the intermediates $EAC'1$, $EAC'1,4$ or $EAC'1,4,2$ had been allowed, still was inhibitory. Thus, hydrocortisone seemed to inhibit each of the four classical complement components.

In order to test the inhibitory power of hydrocortisone on non-lytic C' -dependent functions, immune adherence activity was tested using the assay system described by Nishioka¹⁸. 12.5 mg/ml. hydrocortisone inhibited completely the immune adherence potential of a pool of guinea-pig C' which otherwise showed titres greater than 1/5,000.

The striking inhibition which corticosteroids exert on C' in the fluid phase, though suggested by various *in vivo* observations in experimental animals¹⁹⁻²² and man^{23,24}, had not to our knowledge been realized *in vitro*. Simonsen¹⁹ studied the effect of cortisone acetate on guinea-pig complement *in vitro*, but under his experimental conditions observed no effect. Fischer *et al.*^{10,11} clearly showed that prednisolone had a dose-dependent inhibitory effect on the immune haemolysis of red cells, but attributed this to effects on the red cell membrane. Jennings and Taylor¹³ found that hydrocortisone had a profound inhibitory effect on the immune haemolysis of sheep red cells, and addressed themselves to identification of the site of this inhibition. They found that pre-incubation of C' and hydrocortisone did not augment the inhibition; dialysis of an initially inhibited mixture of sensitized cells, C' and hydrocortisone against saline eventually resulted in lysis, presumably after the hydrocortisone had dialysed away; and washing hydrocortisone away from a suspension of sensitized cells left them susceptible to subsequent lysis by additional C' . These experimental results, together with the evidence for the capacity of steroids to protect cells against non-immune lysis, led these investigators to conclude that hydrocortisone was not

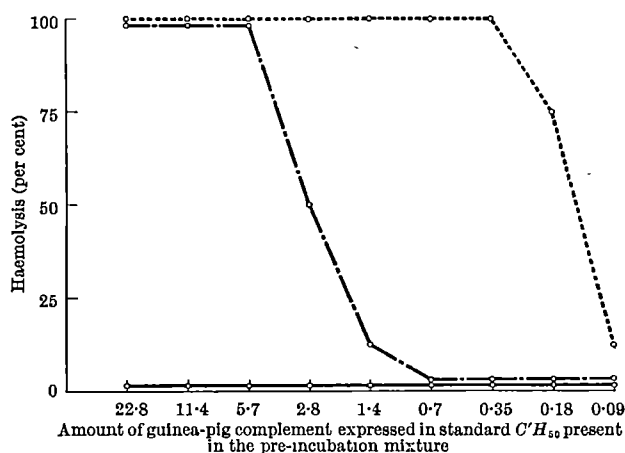


Fig. 3. Comparison of the effect of equal concentrations (6.25 mg/ml.) of hydrocortisone and 6-methyl prednisolone on varying amounts of guinea-pig complement in the pre-incubation mixture
---, Control; ···, hydrocortisone; —, 6-methyl prednisolone

exerting its anti-haemolytic effect directly on C' , but rather on the cell membrane, presumably by preventing attachment of one or more components of C' .

Our own results indicate that hydrocortisone and 6-methyl prednisolone can interact directly with the guinea-pig serum, with consequent inhibition of the C' components. Osler, in a subsequent personal communication, had observed a similar effect. The use of prolonged pre-incubation periods of the steroid with complement at physiological temperatures, with appropriately selected concentrations of steroids and amounts of complement, makes this interaction more apparent, and, further, suggests that it may occur *in vivo* with therapeutic concentrations of steroid by selective accumulation at appropriate sites, though at present the amounts of steroid used in our experiments make us cautious about extrapolating these data to immunological phenomena in health and disease. Since each of the classical C' components (known to be or to include α - and β -globulins) seems to be inhibited, and certain globulins are known to bind cortisone preparations, as reviewed by Gray *et al.*²⁵ and Doe *et al.*²⁶, we assume that a direct interaction with the C' globulins is occurring; but indirect effects are also a possibility. We believe that activation with consequent consumption of the C' components is unlikely because hydrocortisone exerts its inhibitory effects even when added late in the kinetic reaction sequence, that is, even after the $EAC'1,4,2$ complex had been formed. Further, preliminary results indicate that, paradoxically, concentrations of hydrocortisone not inhibitory to the C' in the test system inhibit the anti-complementary effects of aggregated globulins when sensitive assay systems are used. Since hydrocortisone serves to protect against, rather than to augment, the activation and subsequent consumption of C' by aggregated γ -globulin, we doubt whether it provides a surface on which the globulins can be aggregated as chylomicrons did in the studies of Quie and Hirsch²⁷. The characteristics of these interactions of hydrocortisone with antibody and complement globulins are being further investigated.

Immune adherence was tested in the presence of hydrocortisone to see if the steroid was effective in inhibiting C' -dependent functions in which lysis was not the end-point, functions in which potential 'membrane stabilization' would not be as prominent a factor. The inhibition noted was in accord with the conclusions of Packer *et al.*²⁸, who had found various corticosteroid preparations to inhibit immune erythrophagocytosis by human polymorphonuclear leucocytes *in vitro*. The mechanism of this inhibition was not studied by these investigators, but since immune erythrophagocytosis has been shown by Nelson¹⁸ and Gerlings-Petersen and Pondman²⁹ to be C' -dependent, and Nelson¹⁸ has shown the same C' com-

ponents to be involved in both erythrophagocytosis and immune adherence (his $C'1$, $C'4$, $C'2$ and $C'3c$ of the nine C' components he found to be necessary in order to obtain immune haemolysis) we would postulate that Packer *et al.* were observing the effects of C' inhibition by corticosteroids.

Many other of the effects of steroids, both *in vivo* and *in vitro*, may find partial or total explanation in the direct (on the C' components) and indirect (on aggregated γ -globulins) effects of these compounds on the C' system, including those ranging from the anti-inflammatory and anti-phagocytic to the still unexplained beneficial effects of steroids in haemolytic anaemias, systemic lupus erythematosus, plasma cell hepatitis, rheumatoid arthritis and transplantation rejections.

In summary, hydrocortisone was found to exert an inhibitory effect on the C' activity of guinea-pig serum in the fluid phase, an effect which was dependent on time, temperature and concentration. Each of the classical four C' component activities was inhibited, as was immune adherence activity.

This work was supported by grants from the U.S. Public Health Service (AI-00798, HE-02085 and HE-05662), the American Heart Association, and the National Foundation.

¹ Beaufay, H., van Campenhout, E., and de Duve, C., *Biochem. J.*, **73**, 617 (1959).

² Weissmann, G., and Dingle, J., *Exp. Cell. Res.*, **25**, 207 (1961).

³ Weissmann, G., and Thomas, L., *J. Exp. Med.*, **116**, 433 (1962).

⁴ Weissmann, G., Keiser, H., and Bernheimer, A. W., *J. Exp. Med.*, **118**, 205 (1963).

⁵ Weissmann, G., and Thomas, L., *J. Clin. Invest.*, **42**, 661 (1963).

⁶ Weissmann, G., *Biochem. Pharmacol.*, **14**, 525 (1965).

⁷ de Duve, C., Wattiaux, R., and Wibo, M., *Biochem. Pharmacol.*, **9**, 97 (1962).

⁸ Weiss, L., and Dingle, T., *Ann. Rheum. Dis.*, **23**, 57 (1964).

⁹ Berliner, F., and Schoenmelmer, R., *J. Biol. Chem.*, **124**, 525 (1938).

¹⁰ Fischer, H., Argenton, H., and Fritzsche, W., in *Siebentes Freiburger Symposium der Gesellschaft Deutscher Hämatalogen über Hämolyse und Hämolytische Erkrankungen*, edit. by Schuboth, H. (Springer-Verlag, Berlin, 1961).

¹¹ Fischer, H., Ferber, E., Fritzsche, W., Woseggen, F., and Spielmann, W., *Bibliotheca Haematol.* (Basle), **12**, 76 (1961).

¹² Agarwal, K. N., and Garay, L., *Acta Endocrinol.*, **46** (Suppl. 93), 3 (1964).

¹³ Jennings, J. F., and Taylor, G., *Nature*, **203**, 661 (1964).

¹⁴ Mayer, M. M., in *Experimental Immunochimistry*, edit. by Kabat, E. A., 133 (Charles C. Thomas, Springfield, Illinois, 1961).

¹⁵ Osler, A. G., Strauss, J. H., and Mayer, M. M., *Amer. J. Syph. Gon., Ven. Dis.*, **36**, 140 (1952).

¹⁶ Nelson, R. A., jun., in *The Inflammatory Process*, 2, edit. by Zweifach, B. W., Grant, L., and McCluskey, R. T. (Academic Press, New York, 1965).

¹⁷ Osler, A. G., Randall, H. G., Hill, B. M., and Ovary, Z., *J. Exp. Med.*, **110**, 811 (1959).

¹⁸ Nishioka, K., *J. Immunol.*, **90**, 86 (1963).

¹⁹ Simonsen, M., *Scand. J. Clin. Lab. Med.*, **2**, 287 (1950).

²⁰ Muschel, L., in *Mechanism of Cell and Tissue Damage Produced by Immune Reactions*, Second Intern. Symp. Immunopathology, edit. by Grabar, P., and Meischer, P., 324 (Benno Schwabe and Co., Switzerland, 1962).

²¹ Guthrie, R., Renshaw, L., and Elliott, A., *Canad. J. Comp. Med.*, **24**, 122 (1960).

²² Gordon, P., and Benditt, E. P., *Proc. Soc. Exp. Biol. and Med.*, **89**, 286 (1955).

²³ Vaughan, J. H., Bayles, T. B., and Favour, C. B., *J. Clin. Invest.*, **29**, 850 (1950).

²⁴ Gardner, F. H., McElfre, A. E., Harris, J. W., and Diamond, J. K., *J. Lab. Clin. Med.*, **37**, 444 (1951).

²⁵ Gray, C. H., Greenaway, M., and Holness, N. J., in *Hormones in Blood*, edit. by Gray, C. H., and Bacharach, A. L., 440 (Academic Press, New York, 1961).

²⁶ Doe, R. P., Fernandez, K., and Seal, U. S., *J. Clin. Endocrinol. Metab.*, **24**, 1029 (1964).

²⁷ Quile, P. G., and Hirsch, J. G., *Yale J. Biol. Med.*, **36**, 224 (1963).

²⁸ Packer, J. T., Greendyke, R. M., and Swisher, S. N., *Trans. Assoc. Amer. Phys.*, **73**, 93 (1960).

²⁹ Gerlings-Petersen, B. T., and Pondman, K. W., *Vox Sang*, **7**, 655 (1962).

CORRELATION BETWEEN UNIT ACTIVITY AND SLOW POTENTIAL CHANGES IN THE UNANAESTHETIZED CEREBRAL CORTEX OF THE CAT

By A. D. J. ROBERTSON

Autonomics Division, National Physical Laboratory, Teddington, Middlesex

MANY authors have described more or less consistent relationships between sensory evoked potentials and unit firing in the cerebral cortex¹, and Fox and O'Brien have recently shown that: "Computer compilation of the probability of firing of a single cell in cat cortex following a physiological sensory stimulus (somatic or light flash) indicates that the frequency distribution of the firing of a single cell closely corresponds to the average waveform of the evoked potential recorded from the same microelectrode"². Good correlations are also found between cell firing and synchronized slow waves such as spindles³. In general, surface positive potential shifts correspond to an increased probability of unit firing, as would be predicted from reasonable assumptions about cell shape and orientation. Rusinov demonstrated that surface positive polarization could make large areas of cortex excitable to hitherto indifferent stimuli⁴, and Morrell has repeated this and investigated the effect at the unit level. Polarization may change the rate of learning, as shown in conditioning experiments⁵. The great interest in potential fields and their effects on cortical activity (see John *et al.*⁶ for a recent discussion) makes it even more important to discover the relation between unit firing and slow potential changes in the unanaesthetized, spontaneously active brain. No clear-cut correlations have been reported and there has been some controversy as to whether any correlation exists at all⁷. At first sight the situation is more complex than in refs. 1-3, as the electrocorticogram (ECG) is desynchronized and must have many unrelated components from different sources within the central nervous system. The results in this article show that correlations may be found in the un-

anaesthetized cortex when averaging techniques are used in analysis.

Twelve cats were used in these experiments. After the mid-brain had been sectioned at the mid-collicular level under ether anaesthesia the animals were allowed to recover from the anaesthetic, but were paralysed with 'Flaxedil' (gallamine triethiodide) to prevent muscular movements which might interfere with the recording. A large area of cortex was exposed on one side and covered with warm mineral oil. Extra-cellular recordings of unit activity were made using glass micro-pipette electrodes with internal tip diameters of 1-5 μ and resistances between 0.2 and 0.8 M ohms, filled with 9/10 saturated sodium chloride solution. The micro-electrode was referred to an electrode on the cortical surface immediately above. This consisted of a cotton wick impregnated with saline-agar protruding from a glass tube filled with the same medium. Contact with the conducting medium of both electrodes was made with Ag-AgCl wires. The preparation was grounded through the mouth-piece of its head-holder. Two recording channels were used, one a.c.-coupled, the other d.c.-coupled. The d.c. signal was led to a modulator the output frequency of which varied between 2 and 10 kc/s and was proportional to the applied voltage. Both signals were stored on magnetic tape for later analysis of the results.

The experiments simply involved the recording of the spike activity of cortical cells and the simultaneous slow potential changes between the cells and the cortical surface. Several authors have made similar recordings referring the surface electrode to a distant point⁸; but in the experiments reported here it was felt that the potential

developed across the cortex would be more significant, particularly as most of the cells recorded from it were in the layer of large deep pyramids 0.8–1.5 mm from the cortical surface⁹. No attempt was made to select cells from particular areas of the cortex, although most cells were in the median suprasylvian or lateral gyri.

Fig. 1 shows the result of an analysis, from a 4-min length of record containing 962 spikes, to find the relation between surface potential and spike frequency. On a semi-logarithmic plot this gives a fairly straight line. All cells so far examined (fourteen) show similar relations, but it is significant that even for cells so close together that their activity may be recorded from the same micro-electrode the relationship is not identical. This result supports the idea that each cortical cell has a different threshold to incoming excitation, perhaps as a result of its past 'experience'.

Smith and Smith have shown that the spontaneous activity of cortical cells may be considered as consisting of two components, one being 'bursts' of spikes separated by relatively short intervals, the other representing the longer intervals between these bursts¹⁰. The distributions of both types of interval approximate to randomness, but the short interval distribution is much the more stable. The long interval distribution can be changed by physiological or electrical stimulation and by polarization. These results led to the hypothesis that the long interval distribution represented a gating process, switching the cell on into its standard activity. As surface positive polarization (Bindmann *et al.*⁸) is known to increase the activity of underlying cells it seemed profitable to examine the relation between bursts of spike activity and surface positive potential shifts. Fig. 2 shows the results of such an analysis. The spike record is divided into bursts and inter-burst intervals using the criterion described in ref. 10. Similarly, the slow wave record is divided into periods spent above and below an arbitrary threshold. The degree of overlap of the two processes, that is, the correlation between them, is very much better than would be expected were they unrelated, but it is not perfect. (Perfect correlations can be seen in records from preparations anaesthetized with both barbiturates and volatile anaesthetics. In these cases there is good evidence that activity within the cortex, depending on transmission across many

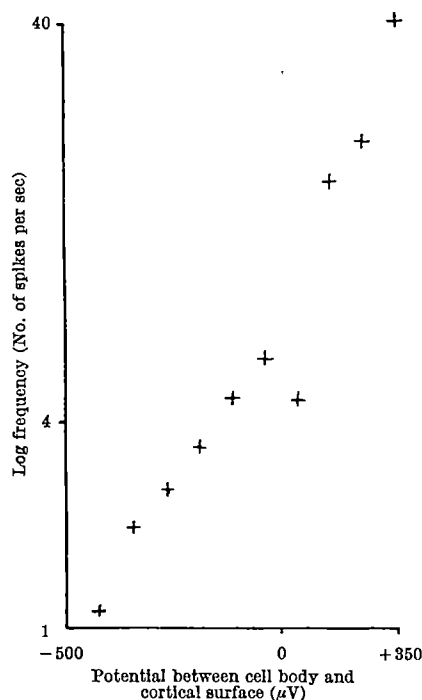


Fig. 1. The relationship between spike frequency and the potential difference between the cell body and the cortical surface

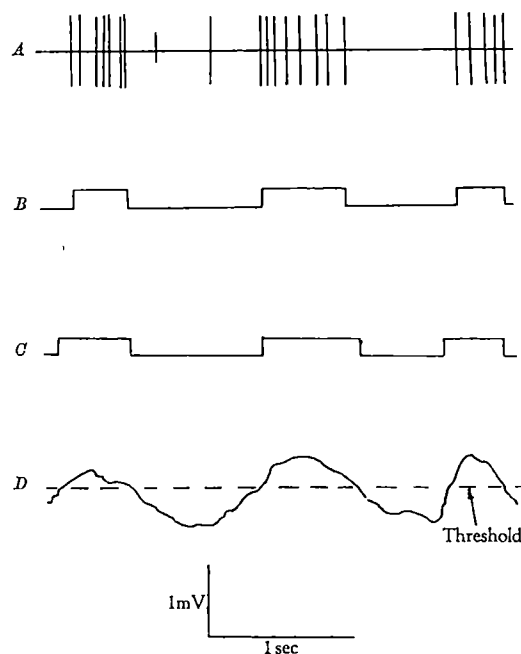


Fig. 2. A is an idealized spike record divided in B into bursts and inter-burst intervals. C shows the periods during which the slow potential variations D between the cell body and cortical surface cross a threshold. The overlap between B and C gives the correlation between bursts and surface positive potential shifts. In one example bursts occupied 16.4 per cent of the record and potential excursions above 50 μ V surface positive occupied 33.4 per cent of the record. If the two were unrelated a 5.5 per cent overlap would be expected. In fact there was a 13.4 per cent overlap. That is, whereas 30.2 per cent of the bursts would be expected to overlap, 81.2 per cent of the bursts overlapped with surface positive potential shifts above the threshold.

synapses, is much reduced, giving rise to a simplified ECG with few high-frequency components—so that one would indeed expect a better correlation¹¹.)

Much recent work has provided a basis for the probabilistic relationship between single unit activity and slow waves described in this article¹². It seems likely that slow waves are more than the mere expression of complex unit activity below the cortical surface and may have important functions in controlling the excitability of cortical neurones. There are abundant widely distributed fibre systems which could provide an anatomical basis for such a function (Sholl, *op. cit.*). Furthermore, there are situations in which considerable slow wave activity is present without accompanying spike activity, for example in young animals before the maturation of cortical synapses and in anaesthetized animals¹³. A fuller discussion of these ideas and a more detailed presentation of results is in preparation.

- ¹ Chang, H. T., in *Handbook of Physiology*, 1, Sect. 1, 299 (Williams and Wilkins, Baltimore, 1959). Li, C.-L., and Chou, S. N., *J. Cell. Comp. Physiol.*, **60**, 1 (1962). Stohr, P. E., Goldring, S., and O'Leary, J. L., *Electroenceph. Clin. Neurophysiol.*, **15**, 882 (1963). Li, C.-L., Cullen, C., and Jasper, H. H., *J. Neurophysiol.*, **19**, 111 (1956).
- ² Fox, S. S., and O'Brien, J. H., *Science*, **147**, 888 (1965).
- ³ Verzeano, M., and Negishi, K., *J. Gen. Physiol.*, **43**, 177 (1960). Jasper, H. H., and Stefanis, C., *Electroenceph. Clin. Neurophysiol.*, **18**, 541 (1965).
- ⁴ Rustinov, V. S., *Comm. Nineteenth Intern. Physiol. Congr.*, Montreal (1953).
- ⁵ Morrell, F., *Ann. N.Y. Acad. Sci.*, **92**, 860 (1961). Morrell, F., and Naitoh, P., *Exp. Neurol.*, **8**, 507 (1962).
- ⁶ John, E. E., Ruchkin, D. S., and Villegas, J., *Ann. N.Y. Acad. Sci.*, **112**, 302 (1964).
- ⁷ Li, C.-L., and Jasper, H. H., *J. Physiol.*, **121**, 117 (1953). Calvet, J., and Scherrer, J., *C.R. Acad. Sci., Paris*, **252**, 2297 (1961). Li, C.-L., and Salmoraghi, G. C., *Nature*, **198**, 858 (1963).
- ⁸ Fromm, G. H., and Bond, H. W., *Electroenceph. Clin. Neurophysiol.*, **17**, 520 (1964). Bindmann, L. J., Lippold, O. C. J., and Redfearn, J. W. T., *J. Physiol.*, **172**, 369 (1964).
- ⁹ Sholl, D. A., *The Organization of the Cerebral Cortex* (Methuen, 1965).
- ¹⁰ Smith, G. K., and Smith, D. R., *Nature*, **202**, 253 (1964). Smith, D. R., and Smith, G. K., *Biophys. J.*, **5**, 47 (1965).
- ¹¹ Burns, B. D., *The Mammalian Cerebral Cortex* (London, 1958). Butler, J. C., *Pharmacol. Rev.*, **2** (1950). Robertson, A. D. J., *Nature*, **205**, 80 (1965).
- ¹² Calvet, J., Calvet, M. C., and Scherrer, J., *Electroenceph. Clin. Neurophysiol.*, **17**, 109 (1964). Kandel, E. R., Spencer, W. A., and Brimley, Jun., F. R., *J. Neurophysiol.*, **24**, 225 (1961). Klee, M. R., Offenloch, K., and Tigges, J., *Science*, **147**, 514 (1965).
- ¹³ Purpura, D. P., *Intern. Rev. Neurobiol.*, **1**, 47 (1959). Anokhin, P. K., *Electroenceph. Clin. Neurophysiol.*, **16**, 27 (1964).

BLOOD FLOW IN TURTLES

By FRED N. WHITE and GORDON ROSS

Departments of Physiology and Biophysics and Nuclear Medicine, University of California, Los Angeles

ALTHOUGH of great interest to the comparative physiologist, the circulation of the blood in reptiles has received little study. There is no information on blood flow in the great vessels, cardiac output or the factors affecting its distribution between systemic and pulmonary circuits in intact animals. This article reports the electromagnetic determination of blood flow in the aorta, subclavian and pulmonary arteries of intact unanaesthetized turtles and illustrates the instantaneous flow patterns in these arteries and also the changes resulting from temperature variation, respiration, and painful stimuli.

Turtles (*Pseudemys scripta*) weighing 2-3 kg were refrigerated for several hours at 5° C and then transferred to a tray of ice. A portion of the plastron (8 cm × 8 cm) was removed and 1-cm lengths of the arteries to be studied were separated from adjacent connective tissue. Flow was measured with the gated sine-wave electromagnetic flowmeter using non-cannulating probes^{1,2}. In some animals indwelling polyethylene catheters were inserted into an aorta and pulmonary artery for pressure determination. The excised plastron was then replaced and secured with stainless steel sutures and sealed with acrylic denture material. The flow probe cables and pressure leads were strapped to the carapace after removing the turtle from the ice tray. The investigations reported here were performed during the first 4-7 post-operative days, during which time the animal's behaviour was identical to that before operation. The turtles usually died within 10 days of operation and autopsy invariably showed a large blood clot at the operation site but no evidence of damage or obstruction to the arteries carrying the probes. Calibration was performed by allowing saline to flow from a gravity feed through the flow probes into a graduated cylinder. In some experiments, for example during forced diving, very low heart rates of 1-2 per min were observed. The portion of the flow trace immediately preceding one of these infrequent beats was considered to

represent zero flow and was used as the reference level for flow measurements.

Simultaneous pressures and flows in the pulmonary and subclavian arteries of a 3-kg turtle are shown in Fig. 1.

Fig. 2 shows the simultaneously recorded instantaneous flow contours of the left aortic arch, right aortic arch (distal to brachiocephalic artery) and left pulmonary artery at a heart rate of 26/min. The left aortic flow contour strikingly resembled the aortic flow pattern of mammals in showing a steep upstroke, a rounded summit, a less steep initial downstroke, and a prominent back flow phase. Right aortic arch flow, recorded distal to the brachiocephalic artery, was similar to that of the left arch except for the absence of a prominent back flow component. The subclavian flow pattern also resembled that of the left aorta. The initial back flow phase seen in Fig. 1 was an inconstant feature. Pulmonary flow commenced approximately 0.15 sec before aortic and the flow contour resembled that of the pressure. No pulmonary back flow was recorded. Flow in the pulmonary artery continued throughout the cardiac cycle whereas flow in the aorta occupied approximately 40 per cent of the cardiac cycle.

The effect of temperature on pulmonary flow is shown in Fig. 3. At 20° C and a heart rate of 30/min the combined stroke flow in the pulmonary arteries was 2.8 ml., giving a

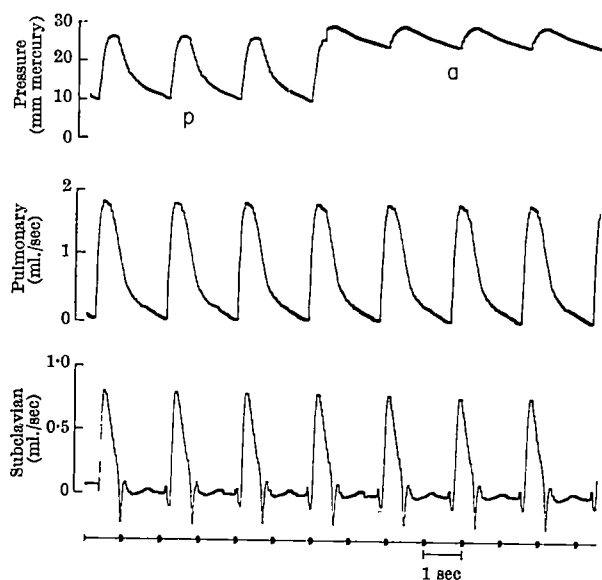


Fig. 1. 3-kg unanaesthetized turtle. Upper trace, pressures in a pulmonary artery (p) and aorta (a) obtained from the same pressure transducer; middle trace, pulmonary flow, lower trace, subclavian flow. Heart rate, 32/min; aortic pressure, 30/25 mm mercury; pulmonary pressure, 27/10 mm mercury, stroke pulmonary flow, 1.2 ml; stroke subclavian flow, 0.12 ml.

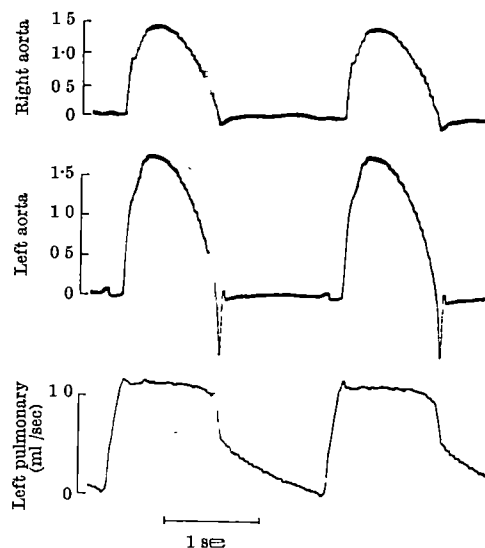


Fig. 2. 2.5-kg unanaesthetized turtle. Simultaneously recorded instantaneous flow contours. Upper trace, right aorta (distal to brachiocephalic); middle trace, left aorta, lower trace, left pulmonary artery

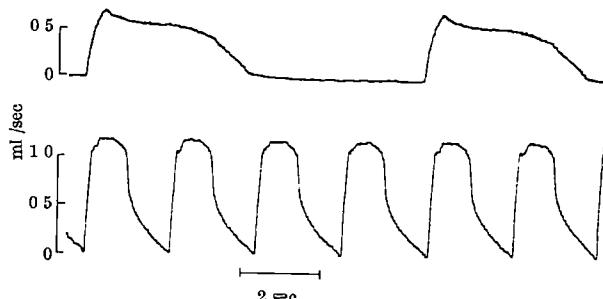


Fig. 3. 3-kg unanaesthetized turtle. Effect of temperature on instantaneous pulmonary flow. Upper trace, 7° C, heart rate 7/min, stroke flow 2 ml., lower trace, 20° C heart rate 30/min, stroke flow 1.4 ml.

minute flow of 84 ml. At 7° C and a heart rate of 7/min, total stroke pulmonary flow was 4 ml., giving a minute flow of 28 ml. The increased stroke volume of 7° C occurs despite a reduction in average flow velocity because of prolonged duration of flow.

The respiration of turtles is irregular with long periods of apnoea interrupted by variable numbers of respiratory cycles. All turtles showed an increased pulmonary blood flow during periods of respiratory activity. In some animals the heart rate increased with respiration and bradycardia occurred during the apnoeic periods. Systemic flow changed little with respiration but was usually slightly reduced. In the example shown in Fig. 4, at the end of a period of apnoea lasting 2 min, the heart rate was 22/min. At the peak of the succeeding respiratory activity the rate had increased to 29/min, stroke pulmonary flow had increased 28 per cent while stroke subclavian flow had diminished by 6 per cent.

The effect of a painful stimulus is shown in Fig. 5. Pinching the tail caused a simultaneous reduction in subclavian flow and increase in pulmonary flow. Both aortic and pulmonary pressures increased. These

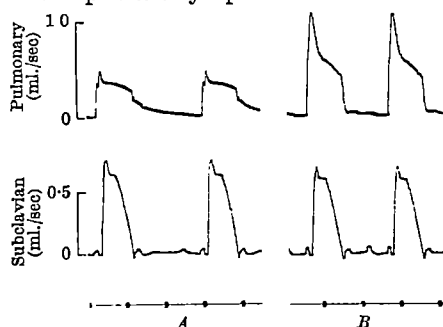


Fig. 4. 3-kg unanaesthetized turtle. Effect of respiration on subclavian and pulmonary blood flow. A, After 2 min apnoea; B, at peak respiratory activity

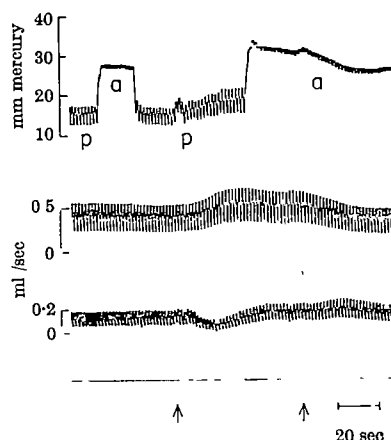


Fig. 5. 3-kg unanaesthetized turtle. Effect of painful stimulus. Upper trace, mean pressures obtained from the same pressure transducer: aortic (a); pulmonary (p); middle trace, mean pulmonary flow; lower trace, mean subclavian flow. Painful stimulus applied between arrows

changes are compatible with an increased systemic resistance causing more of the output of the incompletely divided ventricle to be diverted into the pulmonary circulation with corresponding reduction of systemic flow.

In addition to the work reported here the electromagnetic method of blood flow determination has proved valuable in an investigation of the circulatory changes produced by prolonged diving.

We thank Dr. Alexander Kolin for his interest in and support of the project and much helpful advice concerning electromagnetic flow measurement. This work was aided by grants from the U.S. National Science Foundation (GB 923) and the U.S. Office of Naval Research (233-38).

- ¹ Kolin, A., and Kado, R. T., *Proc. U.S. Nat. Acad. Sci.*, **45**, 1312 (1959)
² Kolin, A., Austin, S., and Cox, P., *Nature*, **201**, 573 (1964)

BINDING OF XENON TO HORSE HAEMOGLOBIN

By DR. B. P. SCHOENBORN

Medical Research Council Laboratory of Molecular Biology, Hill's Road, Cambridge

THIS article is a sequel to the recent report on the binding of xenon to sperm whale myoglobin¹. There it was shown by an X-ray diffraction analysis that a single atom of xenon binds to a specific site buried in the interior of myoglobin, in contact with one of the pyrrole

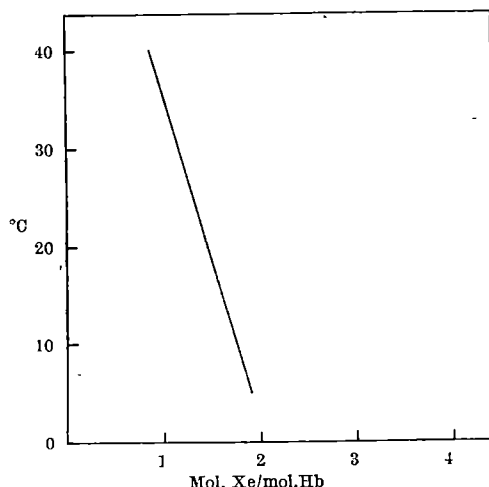


Fig. 1. Binding of xenon to haemoglobin as calculated from solubility measurements in 5 per cent haemoglobin solutions. The ratio of its solubility in water and in haemoglobin solutions is independent of temperature to within 5 per cent

rings of the haem group, with the haem-linked histidine, and with several other groups.

It has been found that during xenon anaesthesia² about 45 per cent of the xenon in blood is carried by haemoglobin³. Fig. 1 shows the affinity of xenon for horse methaemoglobin measured in aqueous solutions with a constant volume Barcroft type manometer.

Henry's law is obeyed in the range of pressure used (0.5–1.5 atm.). The fact that no saturation occurs suggests that there must be several xenon binding sites. The amount of xenon bound by reduced and oxyhaemoglobin was the same within 5 per cent as that bound by methaemoglobin.

Horse methaemoglobin crystals in 2 M ammonium sulphate solution at pH 7.0 were mounted in thin-walled quartz capillaries. The crystals were flushed with xenon before introducing some xenon-saturated mother liquid at one end, which was then sealed. To ensure high xenon occupancy, the gas pressure was increased to 2 atm. before sealing the other end. At higher pressures the capillaries tended to explode. The intensities of the *h0l*, *hk0* and *0kl* reflexions were collected to a resolution of 2.7 Å. After the usual corrections, difference-Fourier projections were calculated using the appropriate version of the general equation:

$$\Delta\rho(x,y,z) = \frac{1}{V} \sum_h \sum_k \sum_l (|F_{(hkl)}| - |F_p|) \exp(-2\pi i(hx + ky + lz) + i\alpha)$$

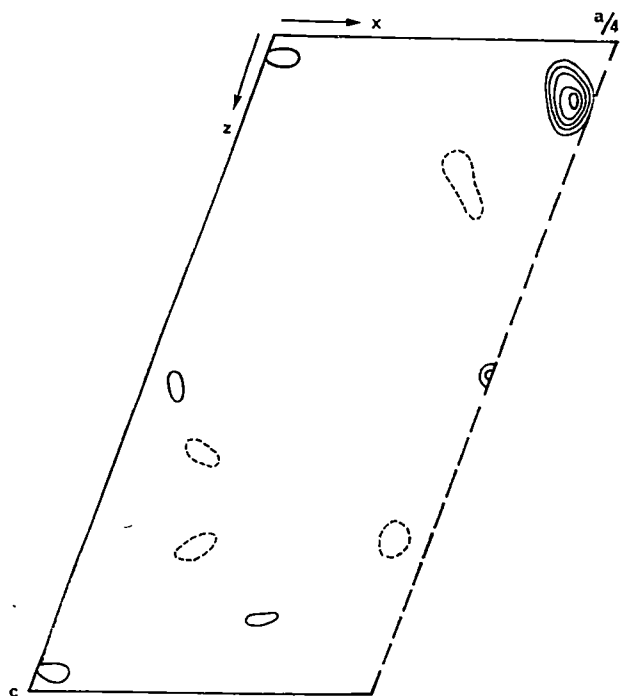


Fig. 2a. $\Delta\rho(x,z)$. Difference in electron density in projection on (010), 2.7 Å resolution

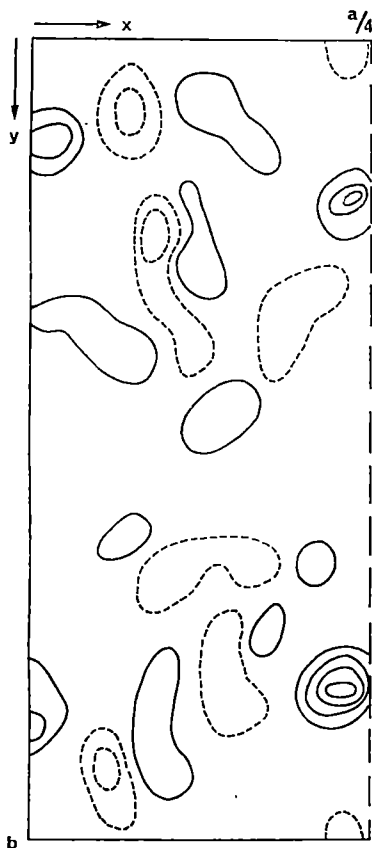


Fig. 2b. $\Delta\rho(x,y)$. Difference in electron density in projection on (001), 5.5 Å resolution

$|F_p|$ and $|F_{(x,y,z)}|$ represent the moduli of the structure amplitudes of the native haemoglobin and its xenon derivative respectively; $\Delta\rho(x,y,z)$ is the difference in electron density between the two compounds. The phase angles used were those for native horse haemoglobin which have been determined out to spacings of 2.7 Å for the centrosymmetric $h0l$ terms and of 5.5 Å for the non-

centrosymmetric $h\bar{k}0$ and $0kl$ terms, using isomorphous replacement⁴.

The difference electron density map of the centrosymmetric projection on (010) shows one slightly elongated peak per asymmetric unit. The projection on (001) shows two circular peaks per asymmetric unit at approximately the same x -co-ordinate as the single peak in the centrosymmetric projection, indicating that the elongated peak in the latter is made up of two superimposed peaks. The projections (Figs. 2a and b) provide the following fractional xenon co-ordinates:

$$x_1 = 0.23 \quad y_1 = 0.81 \quad z_1 = 0.10 \quad \text{occupancy} = 0.8$$

$$x_2 = 0.22 \quad y_2 = 0.20 \quad z_2 = 0.62 \quad \text{occupancy} = 0.5$$

In this space group (space group C2, unit cell dimensions: $a = 108.95$ Å, $b = 63.51$ Å, $c = 54.92$ Å, $\beta = 110^\circ 50'$) the asymmetric unit contains only half the haemoglobin molecule, that is, one α - and one β -chain, and by comparing the co-ordinates of the two peaks with the model of haemoglobin, it can be seen that one xenon atom must lie within the α sub-unit and the other within the β sub-unit. The location of the sites is quite different from that in myoglobin (Fig. 2b) and slightly different in the two chains (Figs. 3a and b). The xenon atoms are located inside the sub-units, but close (6 Å) to their external

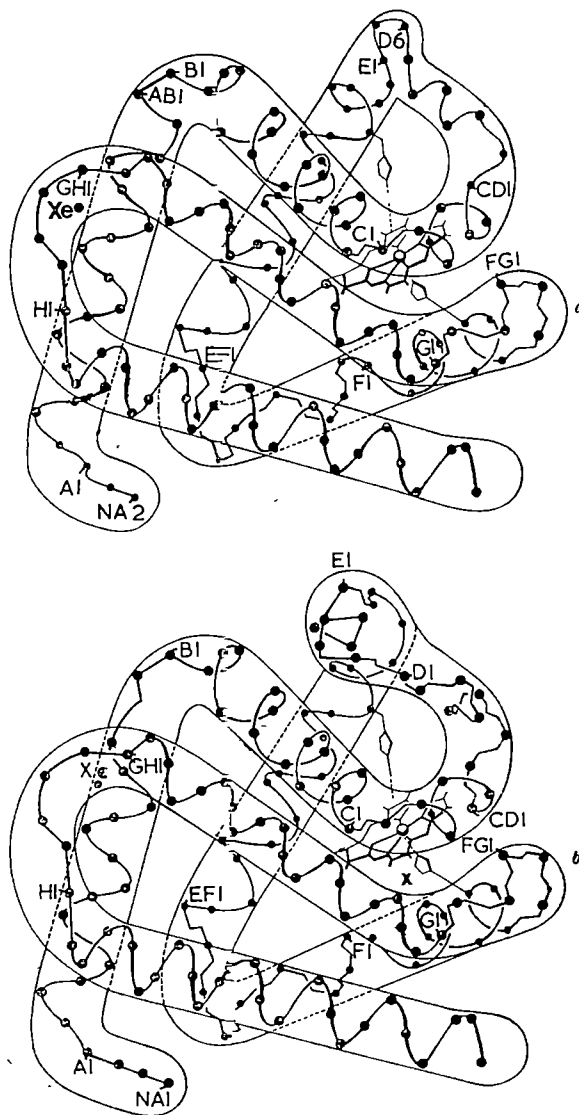


Fig. 3. Location of xenon in (a) the α - and (b) the β -chain. The xenon atom lies buried underneath the GH corner which faces the reader, and nearer the AB corner than this view of the chains suggests. The xenon site in myoglobin is indicated by an x. (Drawing of the chain adapted by permission, from M. F. Eder, *Sci. Amer.*, 211, 5, November 1964)

surface between the *AB* corner and the *GH* corner and are approximately related by the pseudo-dyad axis of symmetry named dyad 1 by Cullis *et al.*⁴. However, the xenon atom in the α -chain lies nearer the *GH* corner and that in the β -chain closer to the *AB* corner. At first it might appear surprising that the xenon positions in the two chains are different from one another, and not the same as in myoglobin. The *AB* corners of the α - and β -chains differ in both the sequence and the number of residues they contain, giving rise to structurally different environments. The amino-acid sequences in the *GH* corners are also different, though here the two chains are of equal length. It should also be noted that the amino-acid sequences of myoglobin⁶ and haemoglobin are quite different. Any change in atomic distribution near a cavity could easily change the electronic interaction with xenon towards an energetically unfavourable state.

The exact analysis of the xenon sites will have to await determination of the haemoglobin structure at high resolution. On the basis of Perutz's tentative atomic model of haemoglobin⁷, the nearest neighbours of both xenon atoms are valine, leucine and phenylalanine. This complex is presumably stabilized, as in myoglobin, by dipole- and quadrupole-induced dipole and quadrupole moments and London interactions. A theoretical investigation by Kittel and Shore⁸ of xenon polarizability has shown that the quadrupolar (as well as the dipolar) polarizability is particularly high, thus favouring binding

in situations like this where one might not otherwise expect it. An analysis of the change in protein-bound water⁹ between haemoglobin and the haemoglobin-xenon complex by a microwave technique showed an increase of protein-bound water, due to the presence of xenon. Any attempts to demonstrate this directly by X-ray methods must also await the final analysis of haemoglobin at high resolution, but changes in the charge distribution caused by xenon atoms located close to the surface of the molecule could account for the increase in bound water.

I thank Dr. M. F. Perutz for his advice and use of his structural data of haemoglobin.

This work was supported in part by the U.S. Public Health Service grant NB 03625 to R. M. Featherstone, chairman, Department of Pharmacology, University of California Medical Center, San Francisco.

¹ Schoenborn, B. P., Watson, H. C., and Kendrew, J. C., *Nature*, **207**, 28 (1965).

² Cullen, S., and Cross, E., *Science*, **113**, 580 (1951).

³ Muehlbacher, C., De Bon, F. L., and Featherstone, R. M., *Intern. Anesthesiol. Clinics*, **1**, 937 (1963).

⁴ Cullis, A. F., Muirhead, H., Perutz, M. F., Rossmann, M. G., and North, A. C. T., *Proc. Roy. Soc., A*, **285**, 161 (1962).

⁵ Matsuda, G., Gehring-Muller, R., and Braunitzer, G., *Biochemistry*, **2**, 338, 669 (1963).

⁶ Edmundson, A. B., *Nature*, **205**, 883 (1965).

⁷ Perutz, M. F., *J. Mol. Biol.*, **13** (in the press).

⁸ Kittel, C., and Shore, H. (private communication).

⁹ Schoenborn, B. P., Featherstone, R. M., Vogelhut, P. O., and Süsskind, C., *Nature*, **202**, 695 (1964).

POLYMORPHISM AND MITOCHONDRIAL ACTIVITY IN SLEEPING SICKNESS TRYPANOSOMES

By DR. KEITH VICKERMAN

Department of Zoology, University College, London

THE trypanosomes of human sleeping sickness, *Trypanosoma gambiense* and *T. rhodesiense*, are at all times morphologically indistinguishable from *T. brucei* of wild game and domestic animals in Africa, and can be regarded as genetic variants of *T. brucei* with the ability to infect man. These flagellates, previously designated the *T. brucei* sub-group, and now assigned to the sub-genus *Trypanozoon* by Hoare¹, differ from other tsetse-borne trypanosomes in two important features: (1) They undergo a complex life-cycle, involving changes in form (Fig. 1), in the tsetse fly (*Glossina* spp.), developing first in the midgut and later in the salivary glands. (2) In the bloodstream of the natural mammalian host they show a wide variation in form, ranging from long slender flagellates with a free flagellum at the anterior end, to short stumpy forms with no free flagellum (Fig. 1).

Because of this second character these trypanosomes are often referred to as the 'polymorphic' trypanosomes, but this description is misleading. The morphological variation is continuous, biometrical investigations² giving no support to the idea that several distinct forms co-exist in the bloodstream, and any implication of a genetic basis to this multiplicity of forms was ruled out by Oehler³, who showed that polymorphism can persist in clone infections. Strictly speaking, the flagellates are pleomorphic, but the change in form appears to follow a regular pattern in the course of infection, for the bulk of available evidence shows that slender forms are most abundant when the parasitaemia is rising, whereas short, stumpy forms preponderate when the number of flagellates in the blood is falling³⁻⁵; slender forms appear to transform into stumpy forms^{7,8}.

The cause and adaptive significance of polymorphism in *Trypanosoma brucei*-like trypanosomes are obscure. It has been suggested that the host's immune response

stimulates the transformation from slender to stumpy forms^{7,8}. In chronic infections the number of trypanosomes in the blood fluctuates, and it appears that each peak of parasitaemia represents a different antigenic variant⁹, the trypanosomes changing their antigens to avoid the host's immune response. If host antibodies do induce the

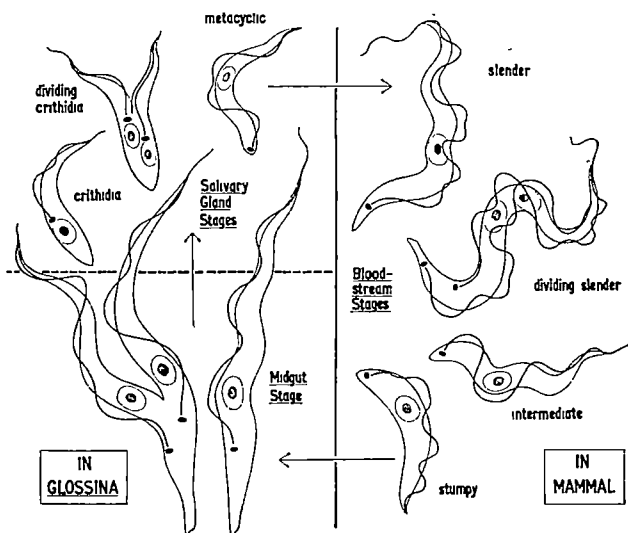


Fig. 1. Diagram of life-cycle in *Trypanosoma brucei*-like trypanosomes. In the mammalian bloodstream slender forms divide or transform into stumpy forms via intermediate forms. Stumpy forms, on entering the tsetse fly, transform into elongate midgut flagellates which, after multiplication and migration in the fly, multiply as crithidial forms in the salivary glands. The metacyclic forms which arise from crithidias are infective to the mammalian host when injected with the fly's saliva.

morphological transformation, then the stumpy forms may represent the forms which are changing their antigens; but as yet there is no direct evidence that this is so. Slender forms may be present at all stages in the infection, and Ashcroft has suggested that these forms might undergo antigenic change to give rise to the relapse strain⁷.

Robertson⁸, and later Reichenow¹⁰ and Wijers and Willett¹¹, observed that, should infected blood be ingested by a tsetse fly, it is the stumpy, rather than the slender, trypanosome which develops in the midgut to initiate the cycle in the fly. Corroborating evidence for this comes from observations on the behaviour of polymorphic trypanosomes maintained by syringe passage in laboratory animals. After several passages the trypanosomes become monomorphic, slender-like forms only being found in the blood^{12,13}. This loss of polymorphism is accompanied by loss of transmissibility by tsetse flies¹³. *Trypanosoma evansi* represents the natural counterpart of these 'non-transmissible' strains. It is morphologically indistinguishable from monomorphic *T. brucei*, and Hoare¹⁴ has suggested that it arises from *T. brucei* when this flagellate is carried outside the tsetse belt by camels, and transferred from host to host by biting flies (for example, *Tabanus*) acting like syringes.

The behaviour of bloodstream trypanosomes on entering the fly is paralleled by their behaviour when inoculated into suitable culture media. Only strains which will infect *Glossina* will establish themselves in culture on blood agar media¹⁵, and the forms assumed in culture are morphologically and physiologically identical with those found in the fly midgut¹⁶.

At present, then, polymorphism in *Trypanosoma brucei*-like trypanosomes appears to be associated with cyclical transmissibility, in that its loss is accompanied by failure to develop in the midgut of *Glossina* and reluctance to be cultivated *in vitro*, but the reason why these characters should be linked is not understood.

Morphogenesis in *Trypanosoma brucei*. In developmental terms, the life-cycle in Protozoa can be envisaged as a series of morphogenetic responses on the part of competent cells to certain environmental changes. The cellular basis of this morphological transformation from one stage to the next in the trypanosome life-cycle is not known, but ultrastructural and biochemical investigations are beginning to provide some clues. Seen with the light microscope, the morphological changes in *Trypanosoma brucei* are extremely simple. Apart from the relative changes in length and width of the body, and length of the flagellum with respect to body-length, the most noticeable changes are seen in the spatial relationships of the kinetoplast, at the base of the flagellum, to the nucleus and to either end of the flagellate's body. In bloodstream forms the kinetoplast lies close to the posterior end of the flagellate; in the fly midgut forms it lies posterior to the nucleus but some distance from the posterior extremity of the flagellate. In crithidial forms attached to the walls of the salivary gland the kinetoplast lies immediately anterior to the nucleus, while in the metacyclics which develop from them the kinetoplast is once again at the posterior end of the flagellate (Fig. 1).

I have suggested that adaptive changes in the chondriome of *Trypanosoma brucei* and allied species might be at least partly responsible for cyclical transformation¹⁷. Investigations with the electron microscope have shown that the kinetoplast in trypanosomes is the DNA-containing part of the chondriome. The structure at the base of the flagellum which is stained by Romanowsky techniques and gives a positive Feulgen reaction¹⁸ is seen with the electron microscope as a fibrous disk embedded in a mitochondrion¹⁹⁻²¹ (Fig. 2). The kinetoplast has been known to stain with Janus green B for some time²². There is mounting evidence that DNA is a widespread component of mitochondria²³; the large amount detectable in the trypanosome kinetoplast may indicate the presence of multiple chondriome DNA units²⁴.

An ultrastructural comparison of monomorphic bloodstream forms of *T. brucei* with fly midgut forms (as obtained in culture) showed that the appearance of the chondriome is very different in the two¹⁷. In bloodstream forms a mitochondrial tube, with scarcely any cristae, pursues a sinuous course from the kinetoplast to the anterior end of the flagellate, and a similar, but very short, tube may extend to the posterior end. The midgut forms, on the other hand have an extensive network of mitochondrial tubes at the anterior end and a prominent post-kinetoplastic chondriome, the kinetoplast in these forms lying some distance from the posterior extremity of the flagellates. Comparative respiratory investigations of these forms give meaning to this structural difference. In monomorphic bloodstream forms, glucose is respired only as far as pyruvate which is excreted, as a functional Krebs cycle appears to be wanting in these forms^{25,26}; the flagellates are almost unique in that their energy is derived from aerobic glycolysis. Although large amounts of oxygen are consumed by the flagellates, terminal respiration is independent of cytochrome pigments, which are absent, and is effected by an L- α -glycerophosphate-oxidase-L- α -glycerophosphate dehydrogenase system²⁷⁻²⁹; Ryley³⁰ has recently produced evidence that this alternative terminal respiration system is housed in distinct cytoplasmic bodies outside the chondriome. The fly midgut forms, however, respire their pyruvate completely and appear to have conventional oxidative phosphorylation linked to a Krebs cycle and cytochrome carrier system^{26,27,31,32}.

From comparative ultrastructural and biochemical investigations it would appear that the chondriome of the bloodstream trypanosomes is inactive, as Krebs cycle enzymes and cytochrome pigments, which are mitochondrial components, are absent. In the fly midgut, on the other hand, the trypanosome chondriome appears to be very active. On the basis of such investigations I suggested that the bloodstream trypanosome, on entering the fly, produced mitochondrial enzymes and a more extensive and active chondriome to enable it to switch its pattern of respiration to a more economical one in keeping with its new surroundings. I also suggested that adaptive proliferation of the posterior chondriome in particular resulted in the change in form, the new chondriome growing out from the kinetoplast, extending the posterior end of the trypanosome. In *Trypanosoma brucei*, then, adaptive mitochondriogenesis might explain the different positions adopted by the kinetoplast, and changes in size, which are an integral part of morphogenesis. A similar conclusion was reached for the transformations observed in the kinetoplastic flagellate *Leishmania donovani* by Rudzinska, d'Alessandro and Trager³³. It may be noted here that the microtubules, which spiral in a longitudinal direction beneath the entire pellicle of the trypanosome (Fig. 2), probably serve as a corset directing growth along the longitudinal axis of the flagellate. Microtubules are now known to be commonly associated with elongating cells³⁴.

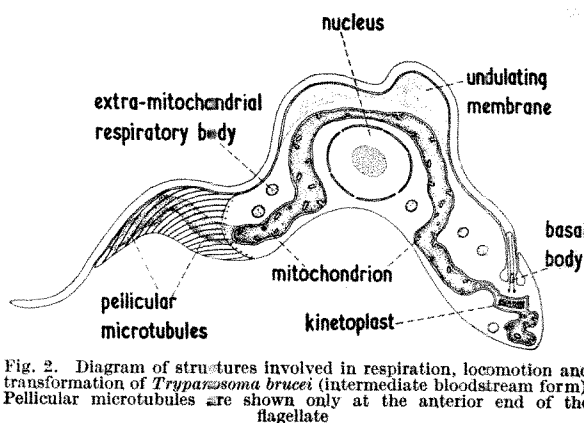


Fig. 2. Diagram of structures involved in respiration, locomotion and transformation of *Trypanosoma brucei* (intermediate bloodstream form). Pellicular microtubules are shown only at the anterior end of the flagellate.

These ideas on the transformation of bloodstream forms entering the tsetse fly, however, neglected the fact that monomorphic bloodstream forms rarely, if ever, infect the fly, whereas the stumpy forms of polymorphic strains do; an important metabolic difference between slender (or monomorphic) and stumpy forms is implied, which confers a selective advantage on the latter, enabling them to develop into midgut forms in *Glossina*.

Mitochondrial activity in bloodstream trypanosomes. Most trypanosomes do not appear to show the extremes of respiratory behaviour encountered in the monomorphic bloodstream and fly midgut forms of *Trypanosoma brucei* as recounted here. Steinert³⁵, using NAD diaphorase as a mitochondrial marker, has reported mitochondrial activity in both amphibian and leech (vector) forms of *Trypanosoma mega* as obtained in culture, though differences in mitochondrial morphology were evident between these stages.

After brief fixation (5 min at 4°C) in 0.1 M cacodylate-buffered 5 per cent glutaraldehyde (pH 7.2), I have examined the distribution of NAD diaphorase activity in several African trypanosomes and found that it agrees with the picture of the chondriome obtained from electron-microscope investigations. In bloodstream *Trypanosoma vivax* and *T. congolense* (which are not polymorphic) the single anterior mitochondrion, kinetoplast and short posterior mitochondrion are filled with blue-black formazan deposit when fixed smears are incubated for 40 min with NADH₂ as substrate and nitro-blue tetrazolium salt as acceptor^{36,37}, indicating diaphorase activity. Stages in division of the chondriome can be seen in such smears. The anterior mitochondrion appears to cleave first in the region of the nucleus, the slit extending backward to the kinetoplast and posterior mitochondrion, and forward to the extremity of the anterior mitochondrion. All the trypanosomes in a smear appear to show diaphorase activity, whereas controls incubated without substrate do not. Electron micrographs of both these bloodstream trypanosomes show well-developed cristae in the chondriome^{17,38}, and there is evidence³⁹ that pyruvate is catabolized, so that, unlike *T. brucei*, the chondriome of *T. vivax* and *T. congolense* appears to be active in the bloodstream phase.

When smears of *Trypanosoma brucei* and allied trypanosomes were tested for NAD diaphorase activity, surprising results were obtained. Old monomorphic laboratory strains showed no diaphorase activity in the mitochondria and similar results were obtained for *T. evansi*. In recently isolated polymorphic strains, however, intense enzyme activity was evident in the chondriome of intermediate and stumpy forms (Fig. 3), while slender forms resembled monomorphic forms in having virtually no diaphorase activity in the mitochondrion. It appears, therefore, that the transition from slender to stumpy forms is accompanied by the acquisition of mitochondrial activity as shown by the intramitochondrial oxidation of NADH₂ (Fig. 4). Controls incubated without the substrate again indicated no enzyme activity.

In slender and monomorphic *Trypanosoma brucei*, and in *T. evansi*, NADH₂ oxidation, as registered by formazan deposits, was occasionally witnessed in extra-mitochondrial bodies scattered throughout the cytoplasm of the bloodstream forms. These granules probably represent the location of the L- α -glycerophosphate (L- α -GP) oxidase cycle, which, as previously mentioned, mediates terminal respiration in bloodstream forms. L- α -GP dehydrogenase is NAD linked, catalysing the reduction of dihydroxyacetone phosphate to L- α -GP by NADH₂; L- α -GP oxidase transfers hydrogen directly from L- α -GP to atmospheric oxygen, yielding dihydroxyacetone phosphate once more.

Further evidence for activity of the chondriome in polymorphic infections comes from the ability of some of these trypanosomes to utilize Krebs cycle intermediates, particularly α -ketoglutaric acid (α -KGA) as shown by a simple motility test⁴⁰. Ryley³⁰ noted that cultured midgut

forms of *Trypanosoma rhodesiense* could maintain their motility if supplied with α -KGA while monomorphic bloodstream forms could not. In the work recorded here, it was found that, when bloodstream forms are washed free of exogenous respirable substrate and suspended in buffered Ringer's solution alone and with added M/10 glucose or M/10 α -KGA, motility is retained in the glucose and α -KGA long after it has ceased in the saline control (Fig. 5). Both slender and stumpy forms are active in the glucose, but in α -KGA only late intermediate and stumpy forms are active.

Morphologically, the respiratory switch is characterized by more than just a change in outward form. In many of the intermediate forms stained for NAD diaphorase activity, the mitochondrion appears to be in a state of division (Fig. 3). Steinert³⁹ has noted in *Trypanosoma mega* that transformation and division appear to be mutually exclusive. Of the different bloodstream forms, only slender trypanosomes are known to divide; it is possible that forms with incipient mitochondrial fission are most sensitive to the transforming stimulus. A further change noted in the transformation from slender to stumpy forms is the increase in diameter of the mitochondrial tube and the appearance of well-defined cristae within its lumen as seen with the electron microscope.

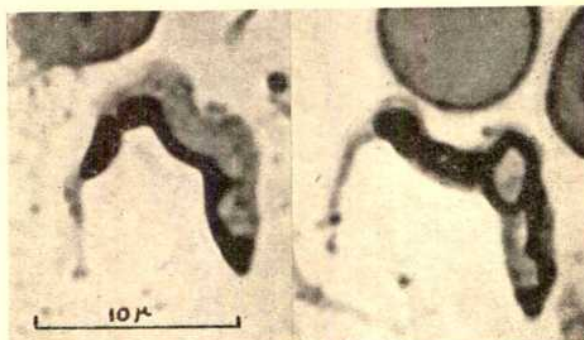


Fig. 3. Distribution of NAD diaphorase activity in late intermediate forms of *Trypanosoma brucei*. Blue-black formazan deposits indicate localization of enzyme in the mitochondrion. The mitochondrion is splitting in the trypanosome on the right.

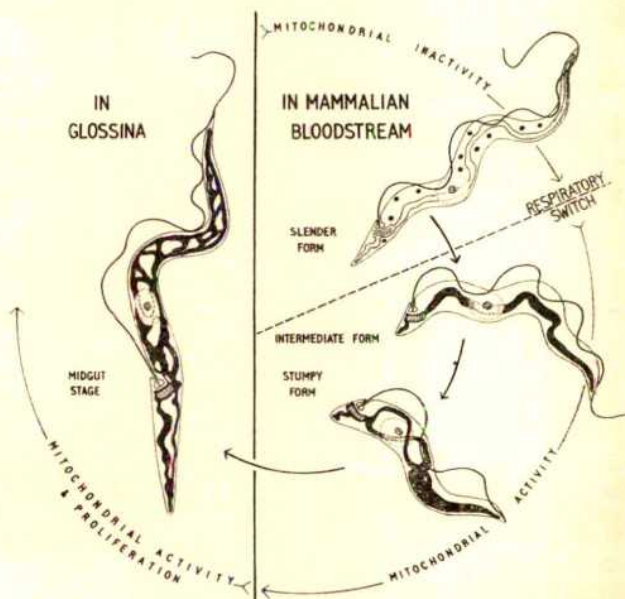


Fig. 4. NADH₂ oxidation by different stages in the life-cycle of *Trypanosoma brucei*. Sites of substrate oxidation are shown in black (representing formazan deposits derived from reduction of nitro-blue tetrazolium). When slender forms change into intermediate forms the mitochondrion becomes active in NADH₂ oxidation. When stumpy forms enter the fly, proliferation of the active mitochondrion occurs. Salivary gland stages are not shown.

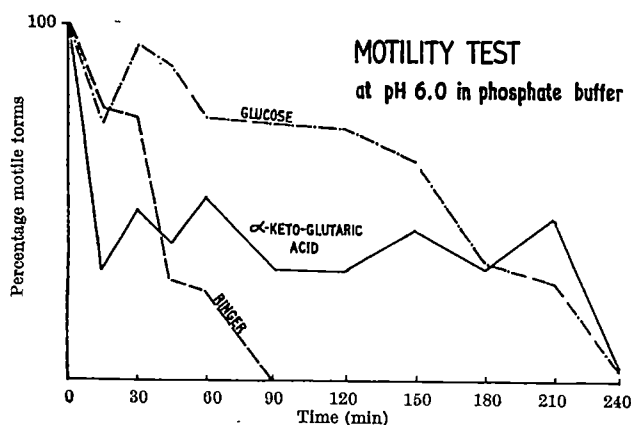


Fig. 5. Graphs to show motility of *Trypanosoma brucei* (*T. rhodesiense*) in Ringer's saline, M/10 glucose and M/10 α -ketoglutaric acid. All forms can utilize glucose to maintain motility. A fraction can also utilize α -KGA indicating mitochondrial activity. This fraction corresponds to the intermediate and stumpy forms.

The nature of the stimulus which causes the transformation bringing about polymorphism and activating the mitochondrion is not known. The most likely instigator of the respiratory switch is a change in permeability of the trypanosome surface. For example, if surface permeability changed to decreased ability to take up glucose, continuation of life would be favoured by a more efficient utilization of what glucose was taken in. Host antibodies might well produce such a surface change. The shortening of body-length might be effected by a contraction of the pellicular microtubules accompanying surface change.

The assumption of mitochondrial activity by the intermediate and stumpy forms may explain why these forms survive in the fly more readily than slender trypanosomes—they are pre-adapted to the mode of respiration necessary in the midgut of the vector. The possibility that the switch might occur when slender forms enter the fly midgut cannot be precluded, however, for Wijers and Willett¹¹ noted transformation of slender forms to stumpy forms in the midgut of *Glossina*.

Hollingshead *et al.*⁴⁰ have noted that monomorphic bloodstream and fly midgut (culture) forms of *Trypanosoma rhodesiense* differ in their electrophoretic behaviour. It would be interesting to see if the change in surface character accompanies the slender-stumpy transformation or whether it takes place on entering the fly midgut or culture medium.

Mitochondrial activity: the genetic basis. The kinetoplast of flagellates has long been known to be a self-replicating structure (see Mühlipfordt⁴¹ for a comprehensive review of its history). The more recent finding that it represents the DNA component of the trypanosome chondriome has brought us nearer to an understanding of its function. Steinert²⁰ regarded the kinetoplast as a genetic system transmitting the information necessary for the synthesis of mitochondrial enzymes. Some evidence for this is forthcoming from a study of trypanosomes which have lost the kinetoplast⁴².

Monomorphic *Trypanosoma brucei*, *T. evansi* and forms closely related to them can spontaneously lose the kinetoplast as a result of faulty division and survive in the bloodstream as akinetoplastic strains⁴³. The akinetoplastic condition can be induced in most trypanosomatids by treatment with certain dyes (for example, acriflavine, pararosaniline)⁴⁴, but the resulting akinetoplastic individuals are only viable in the case of bloodstream *T. brucei* and *T. evansi*, that is, those forms which do not need mitochondrial respiration in the bloodstream. The akinetoplastic trypanosomes appear to be incapable of synthesizing mitochondrial enzymes: in the work recorded here, NAD diaphorase could not be detected in akinetoplastic forms. Reichenow⁴⁵ noted the inability of akinetoplastic *T. gambiense* to infect *Glossina*. The electron microscope reveals, however, that akinetoplastic trypano-

somes retain a membranous ghost of a mitochondrion ('pro-mitochondrion'), and this is found in all their progeny^{42,46}. The kinetoplast may be essential for the synthesis of mitochondrial enzymes; but it is not necessary for the continuous replication of the mitochondrial membrane system.

Once lost, the kinetoplast cannot be regenerated, and this fact has enhanced its reputation of autonomy. But, as yet, there is no evidence that any cytoplasmic organelle with genetic continuity is independent of nuclear control. In the case of chloroplasts and the mitochondria of yeasts, there is ample evidence that the function of these structures is subject to the nuclear genes²⁴. Reports that fusion of the kinetoplast envelope with the nuclear envelope can be observed with the electron microscope^{41,42} have not yet been satisfactorily confirmed; I have never seen such fusion in examining hundreds of sections of juxtannuclear kinetoplasts from different species.

Using yeast as a model, Gibor and Granick²⁴ suggest that mitochondrial DNA represents a multigenic hereditary system. They envisage that, in addition to constitutive genes governing replication, other mitochondrial genes might control the differentiation of enzyme systems through operon-regulator mechanisms⁴⁷ subject to external inducers as well as nuclear influences. In anaerobically grown yeast, oxygen appears to act as an inducer to the synthesis of components of the mitochondrial respiratory system, the cytochrome chain and Krebs cycle enzymes being formed simultaneously⁴⁸. Similar co-ordinate synthesis of respiratory enzymes can be induced by oxygen in an anaerobic plague bacillus, *Pasteurella pestis*⁴⁹. In *Trypanosoma brucei* co-ordinate synthesis of mitochondrial components appears to occur in the transition from slender to intermediate bloodstream forms, but here it is unlikely that oxygen is the inducer; the respiratory switch is not from anaerobic to aerobic respiration, but from aerobic glycolysis to complete oxidation.

The reverse respiratory switch in *Trypanosoma brucei* (that is, back to mitochondrial inactivity) probably takes place in the salivary glands of the tsetse fly, possibly during the genesis of metacyclic forms, but it could equally well occur on entering the bloodstream. The agents inducing regression of mitochondrial activity are not known.

Loss of polymorphism: evolutionary aspects. When a polymorphic strain of *Trypanosoma brucei* becomes monomorphic and non-transmissible, it presumably loses the ability to produce certain mitochondrial enzymes, for it can no longer perform the respiratory switch. The absence of selection for the respiratory switch over several generations in the mammalian bloodstream (possibly provided by rapid passage through laboratory animals) would allow the accumulation of mutations affecting the mitochondrial enzyme systems: a high rate of spontaneous mutation appears to be characteristic of cytoplasmic genetic systems²⁴. A deeper analysis of the mechanisms involved in the evolution of monomorphism is scarcely possible in our present state of uncertainty about the relationship of the host's immune response to polymorphism. If we accept that, in untreated animals, antibodies produced by the host elicit both antigenic change and transformation from slender to stumpy forms in the trypanosomes⁵¹, then we are faced with the question of whether parallel effects are registered in all flagellates or in different fractions of the parasite population. At least two possibilities present themselves.

The simplest possibility is that, under the influence of antibodies, slender forms switch their respiratory pathway and become stumpy; the antigenic change then occurs and the stumpy forms revert to slender forms, as suggested by Wijers⁸; antigenic change is closely linked with polymorphism. But this pattern of behaviour is not in keeping with the observation that syringe-passaged monomorphic strains can change their antigens without changing into stumpy forms⁵². If antigenic change is

linked to polymorphism it is difficult to see why selection maintaining antigenic change has not also maintained polymorphism in old laboratory strains.

The elimination of polymorphism is perhaps more readily explained if antigenic change and transformation are unlinked and regarded as alternative responses on the part of the trypanosome population. Under the influence of antibodies certain slender trypanosomes might switch their respiration and transform into stumpy forms while others might change their antigens and so produce the next variant. As both transformation and antigenic variation can occur within a clone strain, the difference in response must depend on differences in competence (in the embryological sense) rather than genetical heterogeneity within the population. If antigenic change is not bound to transformation, as in this model, then it is conceivable that the ability to transform might be lost in the absence of selection, whereas the ability to undergo antigenic change might be retained, as it is in monomorphic strains. This being so, the slender forms would serve to continue the infection in the mammalian host, the stumpy forms serve to infect the tsetse fly vector. Should the stumpy forms not be ingested by a fly they are eliminated by the host.

I thank Dr. J. F. Ryley for his advice, Mr. T. M. Leach for facilities at the Nigerian Institute for Trypanosomiasis Research, and the Royal Society, the Medical Research Council and the Wellcome Trust for grants supporting this work.

¹ Hoare, C. A., *J. Protozool.*, **11**, 200 (1964).

² Swellengrebel, N. H., *Zbl. Bakt.*, **61**, 193 (1911).

³ Oehler, R., *Z. Hyg. InfektKr.*, **77**, 356 (1914).

⁴ Fantham, H. B., and Thomson, J. G., *Ann. Trop. Med. Parasit.*, **4**, 417 (1911).

⁵ Robertson, M., *Proc. Roy. Soc.*, **B**, **85**, 527 (1912).

⁶ Ogawa, M., *Zbl. Bakt.*, **68**, 332 (1913).

⁷ Ashcroft, M. T., *Ann. Trop. Med. Parasit.*, **51**, 301 (1957).

⁸ Wijers, D. J. B., thesis, Univ. Amsterdam (1960).

⁹ Massaglia, A., *C.R. Acad. Sci., Paris*, **145**, 687 (1907).

¹⁰ Reichenow, E., *Z. Hyg. InfektKr.*, **94**, 266 (1921).

¹¹ Wijers, D. J. B., and Willett, K. C., *Ann. Trop. Med. Parasit.*, **54**, 341 (1960).

¹² Fairbairn, H., and Culwick, A. T., *Ann. Trop. Med. Parasit.*, **41**, 28 (1947).

¹³ Ashcroft, M. T., *Ann. Trop. Med. Parasit.*, **54**, 44 (1960).

¹⁴ Hoare, C. A., *Parasitology*, **32**, 105 (1940).

¹⁵ Reichenow, E., *Z. Parasitenk.* (Berlin), **4**, 784 (1932).

¹⁶ Thomson, J. G., and Sinton, J. A., *Ann. Trop. Med. Parasit.*, **6**, 331 (1912).

¹⁷ Vickerman, K., *Trans. Roy. Soc. Trop. Med. Hyg.*, **56**, 487 (1962).

¹⁸ Bresslau, E. L., and Scremin, L., *Arch. Protistenk.*, **48**, 509 (1924).

¹⁹ Clark, T. B., and Wallace, F. G., *J. Protozool.*, **7**, 115 (1960).

²⁰ Steinert, M., *J. Biophys. Biochem. Cytol.*, **8**, 542 (1960).

²¹ Schulz, H., and MacClure, E., *Z. Zellforsch.*, **58**, 389 (1961).

²² Shipley, P. G., *Anat. Rec.*, **10**, 439 (1916).

²³ Nass, M. M. K., Nass, S., and Afzelius, B. A., *Exp. Cell Res.*, **37**, 516 (1965).

²⁴ Gbor, A., and Granick, S., *Science*, **145**, 890 (1964).

²⁵ Marshall, P. B., *Brit. J. Pharmacol.*, **3**, 8 (1948).

²⁶ Ryley, J. F., *Biochem. J.*, **62**, 215 (1956).

²⁷ Fulton, J. D., and Spooner, D. F., *Exp. Parasit.*, **8**, 137 (1959).

²⁸ Grant, P. T., and Sargent, J. R., *Biochem. J.*, **76**, 229 (1960); **81**, 206 (1961).

²⁹ Grant, P. T., Sargent, J. R., and Ryley, J. F., *Biochem. J.*, **81**, 200 (1961).

³⁰ Ryley, J. F., *Proc. First Intern. Congr. Parasitol.*, Rome, 1964 (in the press).

³¹ von Brand, T., and Johnson, E. M., *J. Cell. Comp. Physiol.*, **29**, 33 (1947).

³² Ryley, J. F., *Ann. Trop. Med. Parasit.*, **55**, 149 (1961).

³³ Rudzinska, M. A., d'Alesandro, P. A., and Trager, W., *J. Protozool.*, **11**, 166 (1964).

³⁴ Porter, K. R., Ledbetter, M. C., and Badenhausen, S., *Proc. Third Europ. Conf. Electron Microscopy*, Prague, 119 (1964).

³⁵ Steinert, M., *J. Cell. Biol.*, **20**, 192 (1964).

³⁶ Nachlas, M. M., Walker, D. G., and Seligman, A. M., *J. Biophys. Biochem. Cytol.*, **4**, 29 (1958).

³⁷ Burstone, M. S., *Enzyme Histochemistry* (Academic Press, N.Y., 1962).

³⁸ Vickerman, K., *Trans. Roy. Soc. Trop. Med. Hyg.*, **56**, 270 (1962).

³⁹ Steinert, M., *J. Protozool.*, **12**, 291 (1965).

⁴⁰ Hollingshead, S., Pethica, B. A., and Ryley, J. F., *Biochem. J.*, **89**, 123 (1963).

⁴¹ Mühlpfordt, H., *Z. Tropenmed. Parasit.*, **15**, 289 (1964).

⁴² Mühlpfordt, H., *Z. Tropenmed. Parasit.*, **14**, 357 and 475 (1963).

⁴³ Hoare, C. A., *J. Protozool.*, **1**, 28 (1954).

⁴⁴ Mühlpfordt, H., *Z. Tropenmed. Parasit.*, **10**, 19 (1959).

⁴⁵ Reichenow, E., *Arch. Inst. Biol., S. Paulo*, **11**, 433 (1940).

⁴⁶ Vickerman, K., *J. Protozool.*, **10** (Suppl.), 15 (1963).

⁴⁷ Jacob, F., and Monod, J., in *Cytodifferentiation and Macromolecular Synthesis*, edit. by Locke, M. (Academic Press, New York, 1963).

⁴⁸ Slonimski, P. P., *La Formation des Enzymes Respiratoires chez la Levure*.

⁴⁹ Englesberg, G., Levy, J. B., and Gbor, A., *J. Bact.*, **68**, 178 (1954).

⁵⁰ Balls, J., *Rev. Elev. Med. Vet. Pays Trop.*, **17**, 361 (1964).

⁵¹ Gray, A. R., thesis, Univ. London (1963).

⁵² Gray, A. R., *Ann. Trop. Med. Parasit.*, **56**, 4 (1962).

ANCESTRY OF SEA-STARS

IN a recent series of articles, Prof. H. B. Fell¹⁻³ attempted to link the morphology of the skeletal elements of living sea-stars with somasteroids. This early Palaeozoic echinoderm group, originally described from the Ordovician of Europe⁴, is generally held to be ancestral to the asteroids and ophiuroids of to-day. Prof. Fell's researches on living sea-stars have permitted recognition of the close comparison between the skeleton of living forms, in particular the genus *Platasterias*, and archaic somasteroids. Indeed, he refers this genus to the Somasteroidea.

This comparison of living sea-stars with the somasteroids will not be discussed here. It is a broader conclusion which invites the present comment. Prof. Fell³ argues persuasively that somasteroids evolved from the pelmatozoan class Crinoidea, and that they are phylogenetically intermediate between later sea-stars and crinoids. Because other eleutherozoans have a shape different from sea-stars ('meridional growth gradients' in contrast to 'radial growth gradients'), seemingly these echinoderms did not evolve from crinoids. Prof. Fell therefore rejects the sub-phylum Eleutherozoa as polyphyletic, and advocates Haeckel's groupings Asterozoa and Echinozoa.

Two features are taken as indicating the kinship of somasteroids and crinoids. The first is a similarity of the apical plates of many living sea-stars and the pentacrinoid calyx. The second is an apparent relationship of ossicles of a pinnulate biserial crinoid arm and of a somasteroid ray. These are discussed in turn.

(A) *The homology of the apical system of sea-stars and the crinoid calyx.* Living sea-stars, particularly ophiuroids, possess regular circlets of plates around a central adapical ossicle in their early development. These can persist to maturity so that ophiuroids may show a well-defined apical system during all growth stages (Fig. 1c). A similar situation may obtain in living asteroids, although the pattern is more often lost during growth, and radial elements may be absent (Fig. 1d). The pentacrinoid stage of development of living comatulids is very similar. There is a central plate (the dorso-central) surrounded by a circlet of five basal plates (interradial in position) and succeeded by a circlet of five radials (Fig. 1e). During development the basals are lost, as are small infrabasals present in some comatulids.

The various circlets of plates of these apical systems are directly homologized by Prof. Fell³. Now this idea is far from new. It is a partial revival of the so-called 'calycinal theory', which held sway in the latter part of the nineteenth century. As Fell neglects to discuss the facts which led to a rejection of this theory, it is pertinent to trace briefly its rise and fall.

Löwen⁵ appears to have first propounded the theory in detail. He stated that the suranal plate (the central plate of the apical system of calycinal echinoids) was homologous with the centrale of the Cretaceous crinoid *Marsupites* (Fig. 1a, b) and, further, that the echinoid apical system was the homologue of the pentacrinoid calyx.

Similar homologies were suggested for sea-stars. The idea became widely accepted⁸⁻⁹. Main discussion centred on details of homologies between major echinoderm groups. An attempt was made to homologize the circumoral ring of holothurians with oral plates of crinoids⁸; Studer even suggested that echinoid and asteroid apical systems corresponded with monocyclic crinoids, and ophiuroids with dicyclic crinoids⁶. There was general agreement on the homology of the apical systems in living echinoderms, and the consequent view of their close phyletic relationship.

The theory, however, slowly fell from favour. First Neumayr¹⁰ rejected homologies relating to echinoids, for the suranal plate is demonstrably a recent evolutionary development. Sasarin and Sasarin¹¹ also rejected the theory for this reason and, as Mortensen¹² expresses it, "therewith the whole Crinoid phantom". Stürtz¹³, from a study of Palaeozoic ophiuroids, maintained that the theory had no bearing on the ancestry of this group, for he considered that no Palaeozoic ophiuroid shows an apical system. This was later reaffirmed by Spencer¹⁴, and extended in a more general way to Asteroidea. Because it was clear by the turn of the century that the calycinal systems of different echinoderm groups were independent developments late in their evolution, the theory was no longer seriously entertained¹⁵, and, in the ensuing sixty-five years, no new facts have been adduced which call for its revival. In relation to Fell's present views on homologies the fossil record shows that: (1) The patina of living crinoids is highly modified compared with Palaeozoic forbears of the group. The centrodorsal of comatuloids, and the absence of an anal plate associated with the right posterior radial are post-Palaeozoic developments. (2) No pre-Carboniferous ophiuroid shows an apical system comparable with that of living ophiuroids. (3) Lower Palaeozoic asteroids such as *Hudsonaster* may show regular series of apical plates, but these defy close comparison with the later calycinal systems, and are fundamentally different from those of contemporary crinoids.

(B) *The homology of the crinoid arm and the somasteroid ray.* The comparison between the ossicles of the arm of a living biserial crinoid and those of a somasteroid ray is striking (Fig. 2). In the former the brachials support the pinnules; in the latter the ambulacral ossicles support the virgalia. These are directly homologized by Prof. Fell⁸.

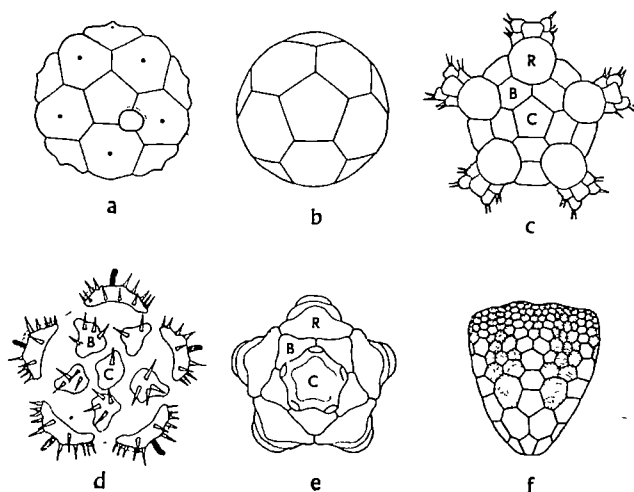


Fig. 1. Calycinal systems of various echinoderms. Plates homologized by Prof. Fell similarly labelled (C="centrodorsal"; B="basal"; R="radial"). a, Apical system of calycineid stirodont echinoid; b, ventral view of the calyx of the Cretaceous articulate crinoid *Marsupites*; c, adapical view of the disk of an immature specimen of the living ophiuroid *Ophiosteira*; d, adapical view of a juvenile specimen of the living asteroid *Ophiopyrgus*; e, ventral view of the calyx of the pentacrinite stage of development of the living articulate crinoid *Promachocrinus*; f, anterior view of a typical Ordovician camerate crinoid *Archaeocrinus*. Note uniserial brachia rigidly incorporated in the polyplacate theca; inter-radials stippled. (a-b after Lovén (ref. 5); c-e after Fell (ref. 3))

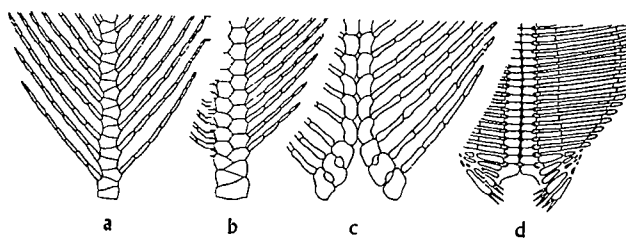


Fig. 2. Supposed morphological transition from a uniserial pinnulate crinoid arm through to the ray of a living sea-star *Platasterias*. a, Uniserial crinoid arm; b, biserial crinoid arm; c, chelonicasteroid somasteroid ray; d, *Platasterias* ray. (Modified from Fell (ref. 3))

Now, are these truly homologous structures? If *Platasterias* can be considered a somasteroid, and if we are entitled to compare it with a living crinoid, then differences are sufficient to suggest that the arrangements are only analogous. Pinnules of crinoids are arms in miniature; they bear food grooves and branches of the water vascular system; in *Platasterias* the food grooves are located between the virgalia and particles are passed to the radial groove by ciliary action². In sea-stars the aboral body-wall and perivisceral coelom extend into the rays; this is not so in crinoids.

No solution to the question of homology (or, indeed, to the whole question of sea-star ancestry) can be found without reference to the fossil record. It is possible, in terms of the evolution of Palaeozoic crinoids, for a crinoid with biserial pinnulate arms to have given rise to somasteroids.

Somasteroids appear in the Tremadocian (earliest Ordovician). The oldest described crinoid is apparently *Iocrinus cambriensis* (Hicks) of Lower Arenigian (upper Lower Ordovician age)¹⁶. This inadunate crinoid, in accordance with primitive members of the sub-class, possesses uniserial, non-pinnulate arms. Through a process of heterotomous branching, pinnules were developed by inadunates in the Silurian, but it was not until the Carboniferous that biserial pinnulate arms evolved. This group could not have given rise to somasteroids in the late Cambrian.

The Flexibilia must also be discounted as a possible ancestral group for simple, non-pinnulate arms are retained throughout its entire history. Therefore, if the somasteroid ray evolved from a crinoid arm it must have evolved from a camerate crinoid. Camerate crinoids appear first in Middle Ordovician times and, although all camerates have pinnules, early representatives possess uniserial arms. The distal ends of rami may become biserial in Upper Ordovician forms, but it is not until Middle Silurian times that biserial pinnulate arms are common. The evolution of the camerate arm therefore parallels that of the Inadunata.

Now camerate crinoids are very different from other crinoids. Early representatives possess a polyplacate theca in which the proximal brachials are incorporated (Fig. 1f). The posterior is modified by anal plates different from those of other crinoids both in origin and modification. A rigid tegmen covers the mouth and food grooves which therefore pass into the theca at the bases of the free brachia. The first camerates therefore contrast strongly with the contemporary pauciplacate Inadunata. Indeed, Jaekel^{17,18} separated the Camerata as his Cladoidea, considering that they arose independently from polyplacate cystoids. That camerates are phylogenetically distinct from other crinoids cannot be doubted.

For the camerate arm to produce a somasteroid ray, a biserial condition must have existed in the most proximal brachials to give the mouth frame (Fig. 2). Because of the inclusion of fixed 'brachials' in the cup, it can be asserted that this condition does not obtain in any camerate crinoid. Even in later Palaeozoic camerates in which brachials may become free above the radials, the proximal brachials remain uniserial.

It is therefore concluded that:

(1) The seeming homology of the apical systems of living sea-stars and crinoids, and the suggested homology of the pinnulate biserial crinoid arm and the somasteroid ray are plausible only if living forms are studied. If Palaeozoic echinoderms are considered neither interpretation can be sustained.

(2) The similarity of the apical systems of living sea-stars and crinoids is due to convergence, and no strict homology of the plates represented therein is possible. Arrangements of plates seen in living representatives of each group are demonstrably recent evolutionary developments and have no bearing on phyletic relationships.

(3) Present understanding of the evolution of Palaeozoic crinoids prohibits the conclusion that somasteroids hold a phyletic position intermediate between crinoids and later sea-stars. Not only do somasteroids appear before crinoids in the fossil record, but early crinoids show a progressive evolution of the arm structure toward biserial pinnulate arms (necessary in a crinoid ancestor of the Somasteroidea) which were developed by the Carboniferous in the Inadunata, and by late Ordovician times in the Camerata (if they be properly regarded as crinoids). No camerate crinoid, however, shows biserial brachials immediately above the radials, a condition demanded by the hypothesis of the homology of the crinoid arm and the somasteroid ray. The supposed evolution of somasteroids from crinoids is opposed to the evidence of the fossil record, as is any other hypothesis involving their close phyletic relationship.

What positive conclusion can be reached as to the origin of sea-stars and their relationship to other echinoderm classes? The older view that asteroids were derived from edrioasteroids was based on the inferred homology of the plates of an open ambulacral groove in these classes. Spencer's work on somasteroids⁴, the first of the sea-stars, led him to reject this hypothesis. At present no worthwhile alternative can be offered. Indeed, as our knowledge of early Palaeozoic echinoderms increases so any apparent relationship of the various eleutherozoan classes diminishes (except, perhaps, the echinoids and holothurians). The mutual relations of the free echinoderm groups are as obscure one to another as they are to the Pelmatozoa.

G. M. PHILIP

Department of Geology,
University of New England,
Armidale, New South Wales.

¹ Fell, H. B., *Science*, 136, 633 (1962).

² Fell, H. B., *Pal. Contrib. Univ. Kansas*, 6 (1962).

³ Fell, H. B., *Phil. Trans. Roy. Soc.*, B, 246, 381 (1963).

⁴ Spencer, W. K., *Phil. Trans. Roy. Soc.*, B 235, 87 (1951).

⁵ Lovén, S., *K. Svensk. Vet-Akad. Handl.*, 11 (7) (1874).

⁶ Agassiz, A., *Mem. Mus. Comp. Zool. Harvard* 5 (1877).

⁷ Ludwig, H., *Z. Wiss. Zool.*, 30, 99; 31, 59, 216, 346; 32, 672, 34, 333, 36, 181; 37, 1 (1877-1882).

⁸ Sladen, W. P., *Quart. J. Micro. Sci.*, 24, 24 (1884).

⁹ Carpenter, P. H., *Quart. J. Micro. Sci.*, 18, 351, 19, 176; 20, 322; 21, 169, 22, 371; 23, 597, 24, 1, 319, 25, Supp. 139; 27, 379, 28, 303 (1878-1887).

¹⁰ Neumayr, M., *Sitz. Acad. Wissen. Wien*, 84, 143 (1879).

¹¹ Sarsin, P. B., and Sarsin, C. F., *Erg. Naturw. Forsch. Ceylon*, 1 (3) (1888).

¹² Mortensen, T., *A Monograph of the Echinoidea II* (C. A. Reitzel, Copenhagen, 1935).

¹³ Stüdtz, B., *Jahrg. Verh. Naturh. Ver. Preuss. Rheinl.*, 50, 1 (1893).

¹⁴ Spencer, W. K., *Palaeontogr. Soc. Monogr.* (1914).

¹⁵ Bather, F. A., and Lankester, E. R., in *A Treatise on Zoology*, 3, 1 (Adam and Charles Black, London, 1900).

¹⁶ Ramsbottom, W. H. C., *Palaeontogr. Soc. Monogr.* (1961).

¹⁷ Jaekel, O., *Jahrg. Stzber. Ges. Naturf. Freunde Berlin*, 101 (1894).

¹⁸ Jaekel, O., *Palaont. Z.*, 3 (1921).

THE gist of Dr. G. M. Philip's article is that, in advocating the homology of the asterozoan and crinozoan calyx, I have neglected the historical development of the calycinal theory; have inferred the homology of the echinoid apical system with the calyx of crinoids in spite of the fact that such homology was disproved long ago by authors he cites; have similarly included the calcareous ring of holothurians in the inferred homology; have failed to account for the absence of a calyx from pre-Carboniferous ophiuroids, and from the Palaeozoic Hudsonasteridae; have failed to indicate the serious difficulty of deriving a somasteroid

from a crinozoan when no suitable crinoid is yet known from pre-Ordovician horizons; and have in general paid insufficient attention to fossils, the conclusions reached being plausible only when considered in the context of recent forms.

I fear that Dr. Philip has not had access to all the literature included in his reference list, for he has unfortunately misconceived my views.

The inference that "there is no real calyx at all in echinoids and holothurians", which is what Dr. Philip appears to be saying, is expressed in precisely those words in one of the papers he cites (ref. 1, p. 421); I cannot imagine why he omits my name from his list of authors who reached this view.

His inclusion of the holothurian calcareous ring in his discussion of the inadequacies of the calycinal theory seems pointless, since I have never advocated such a hypothesis as to its homology. He appears to be unaware of the recent publications in which the homology of this structure with the ambulacral oral skeleton of edrioasteroids is deduced², and its relationship to the subsequent evolutionary trends of holothurians utilized in a taxonomic context⁴.

In taxing me with failure to account for the absence of a calyx from pre-Carboniferous ophiuroids, he ignores my lengthy discussion of this topic (ref. 1, pp. 416-421), where the conclusion is reached that "the late Palaeozoic Aganasteridae . . . have been wrongly interpreted as giving evidence of the late appearance of the calyx in phylogeny in ophiuroids"; and in particular he omits all reference to the published finding that calyx plates are transitory features in the embryonic stages in surviving members of archaic ophiuroid groups, the succeeding adult stages having a naked disk, and the consequential inference that this fact "gives the explanation of why we do not find a calyx in Palaeozoic fossil ophiuroids" (ref. 1, p. 420). Similarly, Dr. Philip's remarks on *Hudsonaster* imply that he is producing this criticism *de novo*, completely omitting to state that the Hudsonasteridae have been found to fall in a late (that is, post-astropectinid) grade of asteroid morphological differentiation (ref. 1, pp. 389, 391). He likewise ignores my published conclusion (ref. 1, p. 392) that "The fossil Hudsonasteridae . . . are too specialised to have much relevance to the origin of asteroids".

The differences between somasteroids and crinoids listed by Dr. Philip are offered without any acknowledgment that these same differences, expressed in much greater detail, have already been set out in the literature (ref. 1, pp. 411-422), and that a hypothesis was also deduced to account for the differences.

Dr. Philip especially stresses the fact that no crinoid with suitable structural features is provided by the fossil record at a sufficiently low horizon for it to be considered ancestral to somasteroids. I was, in fact, the first to stress this very difficulty (ref. 1, pp. 423-424); neither does he mention my plea that final judgment be withheld pending further investigation of Cambrian sediments, especially in the Indo-Pacific, nor does he indicate the cases to which I directed attention where subsequent finds have demonstrated the dangers of relying on the known geological time-ranges of groups in the face of morphological evidence to the contrary. The repeated recognition of so-called 'living fossils' demonstrates how incomplete the geological record is at present.

Dr. Philip's claim that the suggested homologies are plausible only if living forms are considered is surely refuted by the mass of data, including much new data, on fossils included in the papers he cites. Except for new specimens not yet reported on, my paper was based on an examination of all known specimens of fossil somasteroid, not to mention many other fossils; and before publishing my conclusions I circulated them, and the data on which they rest, among palaeontologists in Europe and America known to be interested in the topic, and their valuable

opinions were individually acknowledged (ref. 1, p. 434); surely it cannot be said that relevant fossil evidence was ignored.

Questions relating to the phylogeny of echinoderms, the homologies of the calcareous ring, the calyx and the apical system of echinoids, and the origin and affinities of the asterozoans, are of broad interest to both zoologist and palaeontologist. They have prompted much international co-operation and co-ordination of research and publication over the past few years, as witness contributions now on point of issue from the Press⁴⁻⁷. In addition, work is now actively in progress on Palaeozoic asterozoans and echinozoans at this University and, far from ignoring palaeontological evidence, our aim is to co-ordinate it with that yielded by the morphology of extant forms.

Finally, I must protest at the incorrect use of diagrams taken from my paper¹ in Dr. Philip's Fig. 1, which he captions "Calyceinal systems of various echinoderms".

The juxtaposition of echinoid apical systems with crinoid and asterozoan calyces serves only to discount the alleged homology of all these structures, a homology which I have never supported, and have in fact opposed, as stated here.

H. BARRACLOUGH FELL

Museum of Comparative Zoology,
Harvard University.

¹ Fell, H. B., *Phil. Trans. Roy. Soc.*, B, 246, 381 (1963).

² Fell, H. B., *Breviora, Mus. Comp. Zool.*, 219, 1 (1965).

³ Pawson, D. L., and Fell, H. B., *Breviora, Mus. Comp. Zool.*, 214, 1 (1965).

⁴ Wright, C. W., in *Treatise on Invertebrate Paleontology*, edit. by Spencer, W. K., and Wright, C. W., Vol. U, *Asterozoans* (in the press).

⁵ Pawson, D. L., in *Treatise on Invertebrate Paleontology*, edit. by Spencer, W. K., and Wright, C. W., Vol. U, *Holothuroidea* (in the press).

⁶ Moore, R. C., and Fell, H. B., in *Treatise on Invertebrate Paleontology*, edit. by Spencer, W. K., and Wright, C. W., Vol. U, *Echinozoans, Orientation and Ray Homologies* (in the press).

⁷ Fell, H. B., and Moore, R. C., in *Treatise on Invertebrate Paleontology*, edit. by Spencer, W. K., and Wright, C. W., Vol. U, *Echinozoans, Introduction* (in the press).

NATURE OF THE NON-HAEM IRON IN FERREDOXIN AND RUBREDOXIN

By DR R. D. GILLARD, DR. E. D. MCKENZIE, PROF. R. MASON, S. G. MAYHEW*,
DR. J. L. PEEL* and J. E. STANGROOM

Department of Chemistry and Agricultural Research Council Unit for Microbiology, Department of Microbiology, University of Sheffield Id

THE name 'ferredoxin' was first introduced by Mortenson, Valentine and Carnahan¹ for a brown, non-haem, iron-containing protein of low redox potential which they isolated from *Clostridium pasteurianum* and which was shown to function as an electron carrier in that organism. Closely similar proteins have since been isolated from several other anaerobic bacteria.

Tagawa and Arnon² showed that this bacterial ferredoxin has several properties in common with a brown iron protein from plants (previously identified as haemoglobin-reducing factor³ and "photosynthetic pyridine nucleotide reductase"⁴) although there are marked differences in their electronic absorption spectra. They extended the use of the term 'ferredoxin' to include this plant substance. A brown pigment recently isolated from the photosynthetic bacterium *Rhodospseudomonas palustris* has an absorption spectrum close to that of plant ferredoxin⁵.

A red protein, observed to separate during the purification of ferredoxin from *C. pasteurianum*^{6,7}, has recently been crystallized by Lovenberg and Sobel⁸ and shown to contain non-haem iron. It was found to act as an electron carrier, but the absorption spectrum was different from those of bacterial and plant ferredoxins. The name 'rubredoxin' was proposed. A protein which is identical in all properties so far examined has been isolated from *Peptostreptococcus elsdenii* by one of us (S. G. M.), while Wilder, Valentine and Akagi⁹ prepared a protein with a similar absorption spectrum and electron carrier function from *Clostridium thermosaccharolyticum*. These last-mentioned authors described their product as a 'ferredoxin'.

In accordance with the predominant present practice, we shall, for convenience, refer to these three types of protein as 'bacterial ferredoxin', 'plant ferredoxin' and 'rubredoxin', respectively. This nomenclature is, however, recognized as having certain shortcomings.

We now report the results of optical rotatory dispersion and other work on bacterial ferredoxin and a rubredoxin, which allow us to make some general comments on the nature of the non-haem iron atoms in these proteins.

The ferredoxins from *C. pasteurianum* and *P. elsdenii* were prepared, with minor modifications, by the method of Mortenson⁷ from cell material provided by the Microbiological Research Establishment, Porton.

Rubredoxin was prepared from *P. elsdenii* by first following the procedure for ferredoxin and then eluting the rubredoxin from diethylaminoethylcellulose (DEAE) with 0.4 M *tris*-HCl buffer of pH 8.0. Further purification was by chromatography on two successive columns of DEAE developed with 0.15 M *tris*-HCl buffer of pH 7.6, containing sodium chloride (0.2 M). Ferredoxin from *C. acidi-urici* was given to us by J. C. Rabinowitz, and the plant ferredoxin, prepared from parsley, was a gift from H. E. Davenport. All measurements used aqueous solutions of the proteins in 0.05 M *tris*-HCl buffer of pH 7.3-7.4.

The electronic spectra. The rotatory dispersion spectra of the bacterial ferredoxins from *C. acidi-urici*, *C. pasteurianum*, and *P. elsdenii* are all extremely similar in the visible and near ultra-violet regions (Fig. 1). The absorption spectra of these ferredoxins show a band centred at 390 mμ with a long structureless tail extending into the visible region where no distinct absorption bands can be discerned⁶, but the optical rotatory dispersion data indicate the presence of two optically active transitions centred at 490 mμ and 570 mμ, underlying this diffuse absorption tail. The signs of the Cotton effects of these bands are negative (490 mμ) and positive (570 mμ), respectively, in all three ferredoxins. Attempts to measure the circular dichroism of these bands have been unsuccessful as yet because of the high optical densities of the protein solutions. The rotatory dispersion spectrum of rubredoxin from *P. elsdenii* is very similar to that of the bacterial ferredoxins; the electronic absorption spectrum of rubredoxin actually shows resolution of the absorption bands at 490 mμ and 570 mμ, the presence of which in the bacterial ferredoxin could only be deduced from their dispersion curves. The Cotton effects are again negative (490 mμ) and positive (570 mμ) (Fig. 2). The optical rotatory dispersion spectrum of parsley ferredoxin is shown in Fig. 3; it is closely related to that reported by Ulmer and Vallee¹⁰ for spinach ferredoxin but different from that of the bacterial ferredoxins and rubredoxin.

These results suggest:

(1) That the chromophores responsible for the visible and near ultra-violet absorption spectra of rubredoxin and bacterial ferredoxin are in similar asymmetric environments.

(2) That the bonding of the iron in plant ferredoxin is entirely different from that in bacterial ferredoxin and

* Present address: Earlham Food Research Laboratory, Recreation Road, Norwich.

rubredoxin. If the nature of the iron atom be regarded as critical in deciding the scope of the term 'ferredoxin', it should clearly include rubredoxin but exclude plant ferredoxin.

(3) That, since the absorption and rotatory dispersion data for the bacterial ferredoxin and rubredoxin are unrelated to those which have been previously reported for other non-haem iron proteins (for example, ref. 10), the nature of the metal-ligand bonds in the bacterial ferredoxin and rubredoxin systems is so far unique.

A quantitative consideration of the Cotton effects in bacterial ferredoxin and rubredoxin provides further useful information. The relative magnitudes of the Cotton effects allow the characterization of the various electronic transitions. The most strongly optically active bands in the spectrum are centred at 570 m μ and 490–500 m μ , which suggests that they are magnetic-dipole-allowed transitions of the ligand-field type; the absorption band at 390 m μ shows a much weaker Cotton effect, and may well arise from a charge-transfer type of transition, presumably involving the iron and the sulphur ligands. The amplitude of the Cotton effect from the trough at 530 m μ to the peak at 470 m μ for a given concentration of

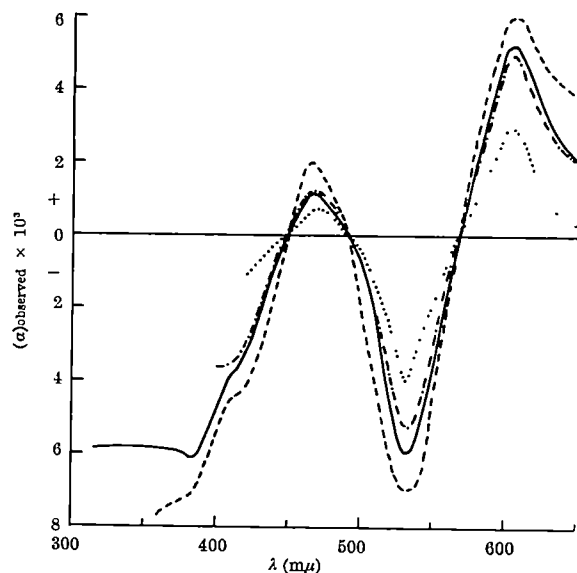


Fig. 1. Optical rotatory dispersion spectra of bacterial ferredoxins (solutions of optical density 0.2 at 500 m μ in 1-cm cells were measured in 1-cm polarimetric cells). —, *C. acidithiobacillus*; ---, *C. pasteurianum*; ····, *P. elsdentii*; - · - · - ·, *C. acidithiobacillus* in 8 M urea

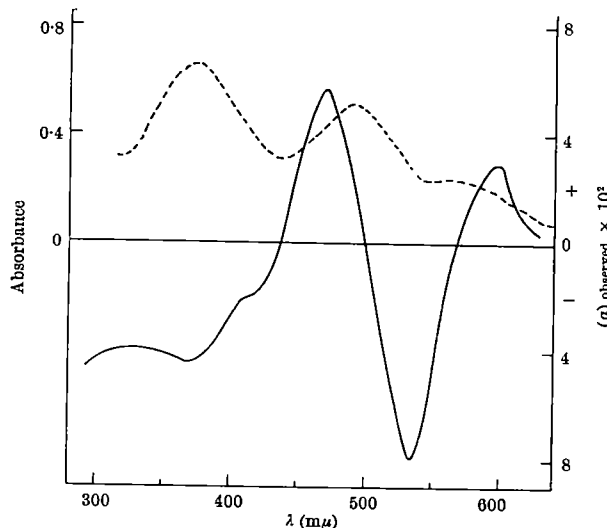


Fig. 2. Optical rotatory dispersion and absorption spectra of rubredoxin from *P. elsdentii*; —, rotatory dispersion (1-cm cell); ---, absorption of same solution (1-cm cell)

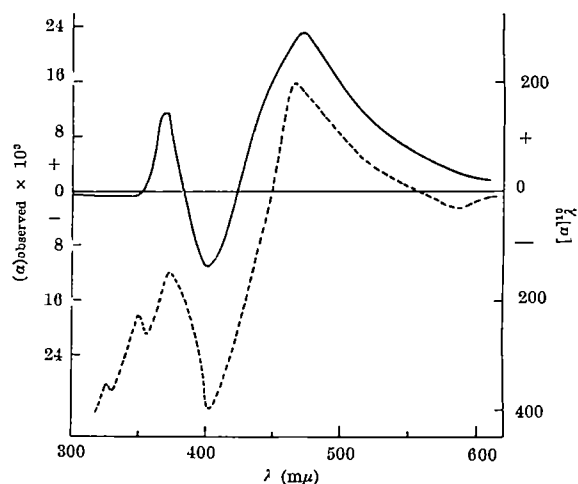


Fig. 3. Optical rotatory dispersion spectra of plant ferredoxins. —, Parsley ferredoxin (left-hand scale, this work). The solution used had an optical density of 0.3 at 500 m μ in a 1-cm cell; ---, spinach ferredoxin (right-hand scale, drawn from the data of Ulmer and Vallee (ref. 10))

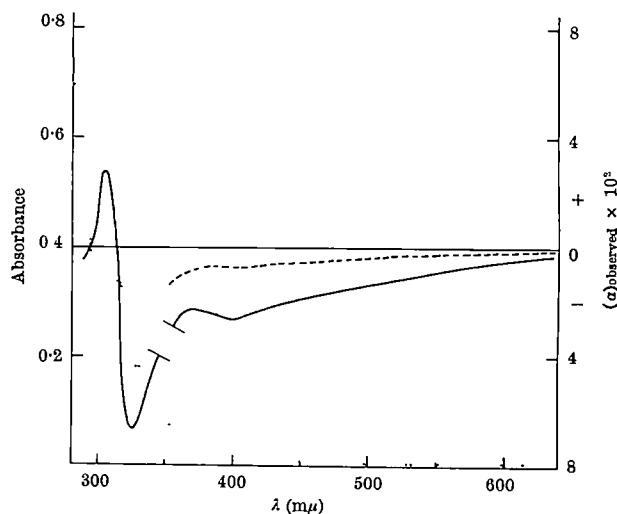


Fig. 4. Reduction of rubredoxin and ferredoxin. —, Rotatory dispersion of enzymatically reduced rubredoxin (the curve for chemically reduced protein coincides). (At wave-lengths below 350 m μ , the scale denoting rotation is five times reduced). ---, rotatory dispersion of chemically reduced ferredoxin from *C. acidithiobacillus* (the oxidized curve was the unbroken line of Fig. 1); ····, absorption spectrum of reduced rubredoxin

material provides a quantitative physical characteristic which may be useful in characterizing and assaying bacterial ferredoxins and rubredoxin.

Reduction of ferredoxin and rubredoxin. Support for the assignment of the transitions at 565, 490 and 390 m μ to iron-sulphur chromophores is provided by observations on the behaviour of both rubredoxin and bacterial ferredoxins on reduction. Reduction by dithionite ions leads to the loss of the absorption bands and of the Cotton effects in the visible and near ultra-violet region. A new Cotton effect appears at 320 m μ . Identical changes were observed when the reduction was carried out enzymatically, using hydrogen with a small amount of a ferredoxin-free preparation from *P. elsdentii* as a source of hydrogenase. Illustrative data are given in Fig. 4. On shaking the reduced preparations in air, the optical rotatory dispersion spectra of the original oxidized species were regenerated. These observations indicate that the changes in the chromophores resulting from chemical and enzymatic reduction are identical and are the same for all three bacterial ferredoxins and rubredoxin. They also add strong support to previous evidence that the iron atoms in these proteins are intimately involved in their enzymatic activity.

The optical rotatory dispersion spectrum of the oxidized ferredoxin from *C. acidi-urici* is unchanged following the addition of the metal-chelating agents, α, α' -bipyridyl and o-phenanthroline, no characteristic absorption at approximately 520 m μ of the trischelateferrous complexes appearing within 30 min. It is therefore clear that the iron is non-labile. On reduction, however, with dithionite ions, in the presence of α, α' -bipyridyl, the rotatory dispersion spectrum, in the visible region, of ferredoxin disappears and is replaced by that of reduced ferredoxin. These changes are accompanied by the appearance, within a few minutes, of the characteristic visible absorption band of the tris α, α' -bipyridyliron(II) cation. It seems clear that reduction of the protein renders the iron labile, it being then accessible for bipyridyl substitution. It is noteworthy that apoferredoxin may be prepared from reduced ferredoxin by complexing the iron as a tris (α, α' -bipyridyl)iron(II) salt, which is later removed by dialysis, and that on treatment of the apoferredoxin with ferrous iron, ferredoxin is reconstituted¹². We find, from dialysis experiments with the ferredoxin of *C. pasteurianum*, in the absence of bipyridyl, that a considerable part of the iron of the reduced ferredoxin may be removed by dialysis; this is not the case for the oxidized ferredoxin, where only a very small loss of iron was observed in dialysis.

The tris (α, α' -bipyridyl)iron(II) cation produced when bacterial ferredoxin or rubredoxin is reduced by dithionite ions in the presence of bipyridyl is optically inactive. In general, the substitution of a transition metal ion in an asymmetric site leads to the production of one enantiomer of any resolvable product in marked excess over the other. The lack of optical activity in the trisbipyridyl iron(II) cation formed from reduced bacterial ferredoxin and rubredoxin implies that the complexable iron in the reduced species is in a site of low asymmetry, and that the highly asymmetric environment of the iron in oxidized ferredoxin, evidenced by its rotatory dispersion spectrum, is changed on reduction.

This alteration in the environment of the iron atom when bacterial ferredoxin is reduced is also clear from the changes in rotatory dispersion which occur. In other redox systems involving asymmetric iron chromophores, if the asymmetry about the iron atom remains unchanged, the Cotton effects of the oxidized and reduced states are of the same order of magnitude. For example, the resolved tris α, α' -bipyridyl iron(II) cation shows a series of Cotton effects in the visible region; the tris α, α' -bipyridyl iron(III) cation formed from it by oxidation shows different Cotton effects in the visible spectrum. Since only one electron transfer is involved in this oxidation, the helical environments about the iron atoms are the same in the reduced and oxidized states, the asymmetry of the iron chromophores is similar, and the order of magnitude of the Cotton effects is the same for the complexes of iron(II) and iron(III).

Behaviour in urea solutions. The asymmetry in the environment of the iron atoms in oxidized bacterial ferredoxin does not appear to depend on the secondary or tertiary structure of the protein. In the presence of 8 M urea, the absorption spectrum and optical rotatory dispersion in the visible region of oxidized ferredoxin from *C. acidi-urici* are initially no different from those in water (Fig. 1). However, in solutions of urea and α, α' -bipyridyl, the complex tris(α, α' -bipyridyl)iron(II) cation is formed quite readily from oxidized ferredoxin of *C. acidi-urici* and *C. pasteurianum*. The rate of formation of trisbipyridyl complex from *C. acidi-urici* ferredoxin has been followed spectrophotometrically, a first-order law being observed with $k = 2.2 \times 10^{-3}$ sec⁻¹. Clearly, the iron is not completely labilized in the presence of urea, since if it were, the formation of the trisbipyridyl complex would be instantaneous. Moreover, the trisbipyridyl iron(II) cation produced by the urea treatment is the optically active (-) [Fe(bipy)₃]²⁺, which must be produced through

a stereoselective stepwise reaction with the iron in a highly asymmetric site in the oxidized protein. The rotatory dispersion curve of the (-) [Fe(bipy)₃]²⁺ observed in this work is enantiomorphous with that of the less-soluble diastereoisomer (+) [Fe phen₃] (+) antimonyl tartrate.

There is no discontinuity in the rate of formation of the trisbipyridyl complex in the experiments with urea, the electronic spectra and rotatory dispersion measurements showing the disappearance of the original visible absorptions in ferredoxin after approximately 2 h. Thus the iron atoms, which gave the ferredoxins their characteristic chromophore, appear to be removed completely by this treatment. This suggests that either the iron atoms are all in kinetically equivalent sites or that in a polynuclear model¹¹ a slow rate-determining reaction of the bipyridyl with the terminal iron atoms is followed by a fast reaction with the remaining ones.

Blomstrom, Knight, Phillips and Weiher have proposed a model for the active site in ferredoxin, based on magnetic and Mossbauer effect measurements, which involves a linear array of seven iron atoms with bridging sulphur atoms¹¹. One essential feature of this model—the presence of 'inorganic' sulphide in the molecule—has recently been questioned by chemical evidence¹². (We have also found that metal complexes of cysteine and its peptides evolve H₂S on acidification of their solutions and that it is therefore likely that the 'labile inorganic sulphur' reported previously is derived from cysteine residues.) Since the rubredoxin from *C. pasteurianum* probably contains two iron atoms per molecule (mol. wt. ~6,000), and will carry out some of the biological functions of ferredoxin⁸, there is no compelling reason to assume that all seven iron atoms in ferredoxin lie very close to one another. Indeed, X-ray crystallographic data for ferredoxin from *C. acidi-urici* at a resolution of ~3.0 Å appear to rule out such an arrangement¹³. The amino-acid sequence in ferredoxin (*C. pasteurianum*)¹⁴ shows that there are two sets of cysteine residues in groups of four. A possible explanation for our work is that the two sets are brought together by the iron atoms in oxidized ferredoxin and are separated in the reduced molecule. Structure factor amplitudes calculated for a set of four irons in oxidized ferredoxin (*C. acidi-urici*) show a promising agreement with the observed X-ray data.

The present work also emphasizes the difficulty in assigning a formal oxidation state to the iron atom since it is evident that the iron may appear in either valence state in a way which depends critically on experimental conditions. There exist intrasystem reduction and oxidation mechanisms that have yet to be clarified.

We thank the Agricultural Research Council for a maintenance grant (S. G. M.) and the Science Research Council for the award of a fellowship (J. E. S.), for financial support (E. D. McK.), and also acknowledge other research grants. We thank Dr. H. E. Davenport, Dr. J. C. Rabinowitz and the Microbiological Research Establishment for samples of the several ferredoxins and cell materials.

¹ Mortenson, L. E., Valentine, R. C., and Carnahan, J. E., *Biochem. Biophys. Res. Comm.*, **7**, 448 (1962).

² Tagawa, K., and Arnon, D. I., *Nature*, **195**, 537 (1962).

³ Davenport, H. E., and Hill, R., *Biochem. J.*, **74**, 493 (1960).

⁴ San Pietro, A., and Lang, H. M., *J. Biol. Chem.*, **231**, 211 (1958).

⁵ Yamanaka, T., and Kamen, M. D., *Biochem. Biophys. Res. Comm.*, **18**, 611 (1965).

⁶ Lovenberg, W., Buchanan, B. B., and Rabinowitz, J. C., *J. Biol. Chem.*, **238**, 3899 (1963).

⁷ Mortenson, L. E., *Biochem. Biophys. Acta*, **81**, 71 (1964).

⁸ Lovenberg, W., and Sobel, B., *Fed. Proc. Abst.*, **587**, 233 (1965).

⁹ Wilder, M., Valentine, R. C., and Akagi, J. M., *J. Bact.*, **86**, 861 (1963).

¹⁰ Ulmer, D. D., and Vallee, B. L., *Biochemistry*, **2**, 1335 (1963).

¹¹ Blomstrom, D. C., Knight, jun., E., Phillips, W. D., and Weiher, J. F., *Proc. U.S. Nat. Acad. Sci.*, **51**, 1085 (1964).

¹² Bayer, E., Parr, W., and Lazmaier, B., *Archiv. Pharmazie*, **298**, 196 (1965).

¹³ Bailey, N. A., Mason, R., Robertson, G. B., Russell, D. F., and Stangroom, J. E. (in preparation).

¹⁴ Tanaka, M., Nakashima, T., Benson, A., Mower, H. R., and Yasonobu, K. T., *Biochem. Biophys. Res. Comm.*, **16**, 422 (1964).

LETTERS TO THE EDITOR

GEOPHYSICS

Antimatter and Tree Rings

RECENTLY, Cowan *et al.*¹ discussed the interesting case of the Tunguska meteor—the event and its origin mainly in the context of release of a rather high energy of $\sim 10^{24}$ ergs on its impact. Various theories concerning its origin and the nature of the energy source were discussed (for example, asteroidal origin and energy from impact or nuclear reactions). They have shown that none of the theories can satisfactorily explain the amount of energy released during the impact. The authors have invoked the antimatter hypothesis and, as an experimental verification to this, have calculated the expected increase in the carbon-14/carbon-12 ratios in the atmosphere subsequent to the fall of the meteorite. Considering the total energy release, they obtained a value of 7 per cent for the expected increase in activity. Their measurements of the atmospheric carbon-14/carbon-12 ratios, based on annual rings of a 300-yr.-old tree, show a possible increase of 1 per cent in the year 1909, leading them to the conclusion that probably 1/7th of the energy release in the Tunguska meteorite impact came from antimatter annihilation.

It is the purpose of this communication to point out that the probability of such an interesting conclusion is unfortunately very much reduced if one considers the nature of secular variations of carbon-14/carbon-12 ratios in the atmosphere. It was Stuiver² who first pointed out that there existed a good inverse correlation, for the past 1,300 yr. of record, between the solar activity and carbon-14/carbon-12 ratios in the atmosphere. (In what manner sunspot activity brings about this correlation is, however, not well understood as yet.) If we compare the solar activity and the observed carbon-14/carbon-12 ratio during 1870–1933, within the errors of measurements, we do find a fair anticorrelation between the sunspot activity

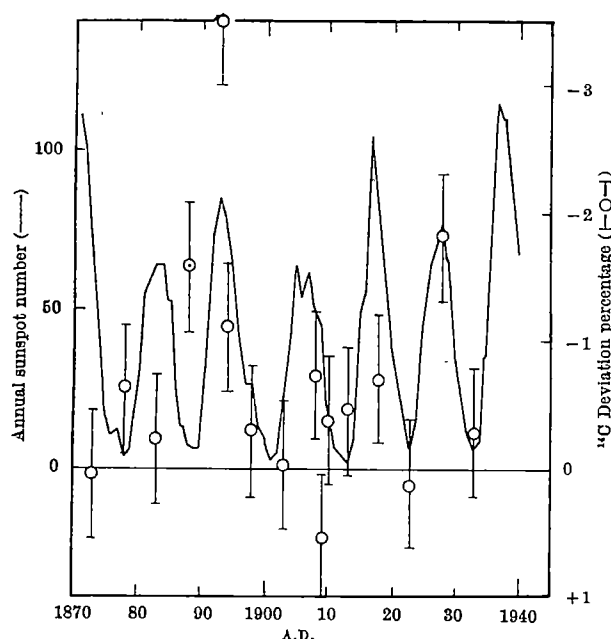


Fig. 1. Measured carbon-14 activity in tree rings (ref 1) and sunspot activity during the same period

and the carbon-14 deviations (see Fig. 1), there being some phase differences which are not unexpected because of time delays in interactions of relevance to the carbon-14/carbon-12 ratios, for example, air-biosphere and air-sea exchange.

Thus, it is clear that if one takes into account the nature of secular variations of carbon-14/carbon-12 ratios in the atmosphere, it becomes difficult to reach any conclusions which may be of significance to a possible antimatter content of the Tunguska meteorite.

V. S. VENKATAVARADAN

Tata Institute of Fundamental Research,
Colaba, Bombay-5.

¹ Cowan, C., Atluri, C. R., and Libby, W. F., *Nature*, 206, 861 (1965).

² Stuiver, M., *J. Geophys. Res.*, 66, 273 (1961).

PHYSICS

Delayed Fluorescence from Organic Crystals

DELAYED fluorescence lifetimes and spectra have been observed from crystals of naphthalene, anthracene, pyrene, 3,4-benzpyrene, *t*-stilbene and *p*-terphenyl, using conventional techniques¹. In each case a delayed fluorescence spectrum was observed with a resolution of 8 nm, the spectrum being identical with the prompt fluorescence spectrum. Precautions reported necessary for the observation of delayed fluorescence from micro-crystalline samples² were not found important in the present work, as the crystals used were at least 1 mm thick.

A description of phosphorescence and delayed fluorescence in pure and mixed crystals has been obtained, based on a triplet-triplet annihilation process and assuming a uniform distribution of impurity traps throughout the host crystal^{3,4}. This description predicts an extremely rapid triplet-triplet annihilation rate in pure crystals, or crystals containing a high concentration of impurity traps. Associated with such a rapid annihilation rate is a very short lifetime of delayed fluorescence, and, consequently, a vanishingly small efficiency of phosphorescence and delayed fluorescence when observed using normal msec time resolution techniques.

In the present work, the triplet-triplet annihilation rate constant (γ) has been calculated from delayed fluorescence measurements made on commercially available anthracene crystals. For times short compared with the triplet lifetime, the fluorescence signal (F) is given by⁵:

$$F = \frac{1}{2} A \gamma [\eta_{T0} / (1 + \gamma \tau_{T0})]^2$$

where A is an instrumental factor and η_{T0} the initial density of triplet states. From a plot of $1/\sqrt{F}$ against t , the ratio of slope/intercept gives the quantity $\gamma \tau_{T0}$. Linear plots were obtained for each anthracene crystal, the results being similar to work reported previously for anthracene crystals⁶. For excitation times (t_x) short compared with the triplet lifetime, the initial density of triplets is given by:

$$\eta_{T0} \doteq Q_T t_x \varepsilon I_0$$

Q_T represents the efficiency of triplet formation, equal to one minus the prompt fluorescence efficiency⁷. This gives $Q_T = 0.35$ for thick anthracene crystals⁸. ε is the absorption coefficient for the exciting light⁵. I_0 is the exciting light intensity measured using a thermopile.

Table 1

Crystal thickness	Prompt fluorescence decay time	Delayed fluorescence decay time			$\gamma\eta\tau_0$	γ
		(1) 365 nm	(2) 434 nm	(3) 550 nm		
1 mm	23×10^{-9} sec	1.4×10^{-3} sec	1.6×10^{-3} sec	—	$1 \cdot 10^3$	1.7×10^{-11} cm ² /sec
2 mm	23 " "	2.0 " "	1.3 " "	—	0.45 "	0.75 " " "
5 mm	26 " "	5.6 " "	6.6 " "	8.2×10^{-3} sec	0.40 "	0.89 " " "
20 mm	25 " "	5.7 " "	3.9 " "	5.8 " "	1.5 "	2.5 " " "

Table 1 shows values of the exponential decay times for delayed fluorescence from anthracene: (1) for surface excitation of the crystal using light of wave-length 365 nm; (2) for volume excitation using weakly absorbed light of 434-nm wave-length; (3) for excitation directly into the triplet (3L_a) state using light of wave-length longer than 500 nm. Graphically estimated values of $\gamma\eta\tau_0$ and the values thus calculated for γ are also shown. These results were derived from the volume excitation data (column (ii)) for each crystal.

An additional value of γ was obtained using an extremely pure zone refined sample of anthracene 4 mm thick. In this case, the value for γ of $2.1 \cdot 10^{-11}$ cm²/sec was similar to the values obtained from the unpurified samples.

The mean of the values obtained for the triplet-triplet annihilation rate constant (γ) of $1.6 \cdot 10^{-11}$ cm²/sec is in good agreement with previously reported values of from $1.0 \cdot 10^{-11}$ to $5.5 \cdot 10^{-11}$ cm²/sec (ref. 9), but is lower than a minimum value of about 10^{-9} cm²/sec, calculated from recent data on the diffusion coefficient for anthracene crystals¹⁰.

Observations have also been made of the delayed fluorescence from pure and impure samples of anthracene and naphthalene as a function of temperature. The relative intensity of delayed fluorescence (I_D) was measured as a function of the exciting light intensity (I_0) for each crystal sample, I_0 being altered using a series of wire mesh light attenuators.

Commercially available (impure) samples of naphthalene and anthracene at room temperature, showed $I_D \propto I_0^2$ when excited with strongly absorbed light. The same result was obtained with excitation directly into the naphthalene and anthracene triplet levels.

After prolonged zone refining under vacuum, however, pure crystals of anthracene and naphthalene exhibited a linear dependence ($I_D \propto I_0$) of delayed fluorescence with exciting light intensity (Table 2). Excitation of the pure crystals directly into the triplet state showed $I_D \propto I_0^2$ as found using the impure crystals. None of the pure crystals used in these measurements showed any phosphorescence at 77° K.

Table 2

Temperature	Naphthalene Lifetime		Anthracene Lifetime	
293° K	$5.2 \cdot 10^{-3}$ sec	$I_D \propto I_0^{1.0}$	$2.1 \cdot 10^{-3}$ sec	$I_D \propto I_0^{1.0}$
196° K	6.1 " "	$I_D \propto I_0^{1.3}$	3.9 " "	$I_D \propto I_0^{1.2}$
77° K	50 " "	$I_D \propto I_0^{1.4}$	30.2 " "	$I_D \propto I_0^{1.4}$

Both naphthalene and anthracene showed an increase in delayed fluorescence lifetime as the crystal temperature was reduced, and showed a change in the intensity dependence towards the impure crystal behaviour (where $I_D \propto I_0^2$).

Observations of crystals of pyrene, 3,4-benzpyrene, *t*-stilbene and *p*-terphenyl as a function of crystal purity showed behaviour similar to naphthalene and anthracene. The lifetimes of delayed fluorescence increased as the crystal temperature was reduced. Attempts to increase the purity of the samples (by further zone refining) decreased the lifetime of delayed fluorescence in some cases to <0.1 msec, and produced a corresponding decrease in efficiency of emission.

These results show an increase in triplet lifetime and decrease in triplet-triplet annihilation rate associated with either a decrease in temperature or with the addition of impurity to a pure crystal. Nevertheless, a description of

delayed emission from organic crystals in terms of triplet-triplet annihilation alone does not appear to be sufficient, in view of both a linear and square intensity dependence being observed from a singlet and triplet excited pure crystal respectively. A previous report has shown also a linear dependence for delayed excimer fluorescence from pyrene crystal and solid solution¹¹. It seems likely, therefore, that the storing of energy in singlet trapping states may act simultaneously with the process of triplet annihilation, both these processes contributing to delayed emission from the crystal.

We thank Dr. J. B. Birks for his advice. One of us (G. F. M.) also thanks the Science Research Council for the provision of a maintenance grant.

G. F. MOORE
I. H. MUNRO

Physical Laboratories,
University of Manchester.

- Birks, J. B., Moore, G. F., and Munro, I. H., *Spectrochimica Acta* (in the press).
- Stevens, B., and Hutton, E., *Proc. Phys. Soc.*, **81**, 893 (1963).
- Sternlicht, H., Nieman, C. C., and Robinson, G. W., *J. Chem. Phys.*, **38**, 1326 (1963).
- Azumi, T., and McGlynn, S. P., *J. Chem. Phys.*, **39**, 1186 (1963).
- Weisz, S. Z., Zahlan, A. B., Silver, M., and Jarnagin, R. C., *Phys. Rev. Letters*, **12**, 71 (1964).
- Weisz, S. Z., Zahlan, A. B., Gilreath, J., Jarnagin, R. C., and Silver, M., *J. Chem. Phys.*, **41**, 3451 (1964).
- Medinger, T., and Wilkinson, F., *Trans. Far. Soc.*, **61**, 620 (1965).
- Birks, J. B., *The Theory and Practice of Scintillation Counting*, 218 (Pergamon Press, 1964).
- King, T. A., and Voltz, R., *Proc. Roy. Soc.* (in the press).
- Kepler, R. G., and Switendick, A. C., *Phys. Rev. Letters*, **15**, 56 (1966).
- Smith, F. J., and McGlynn, S. P., *J. Chem. Phys.*, **42**, 4308 (1965).

Transmission through a Tapered Quartz Tube in the Laser Near Field

THE concentration of laser light by the use of tapered dielectric tubes and rods has been discussed by Vogel¹. The purpose of this communication is to report the dependence of the transmission on the position of the tube in the laser near field. The laser near field pattern for circular apertures is a function of $a^2/\lambda X$, where a is the aperture radius, λ is the wave-length of the light (6328 Å for the He-Ne laser used in this experiment), and X is the distance from the aperture². In this case the laser beam was expanded, collimated, and passed through apertures with various known values of a . The quartz tube is shown in Fig. 1.

Fig. 2 shows the per cent energy transmission through the tube as a function of $a^2/\lambda X$ for various values of a . As expected, the transmission depended on a . Of more interest for the work recorded here is the fact that the

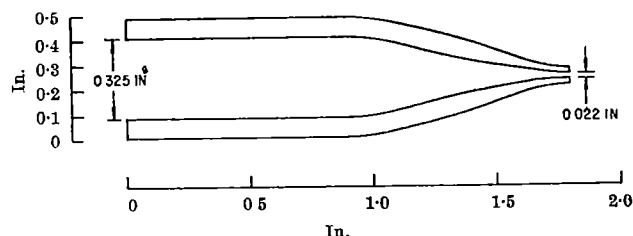


Fig. 1

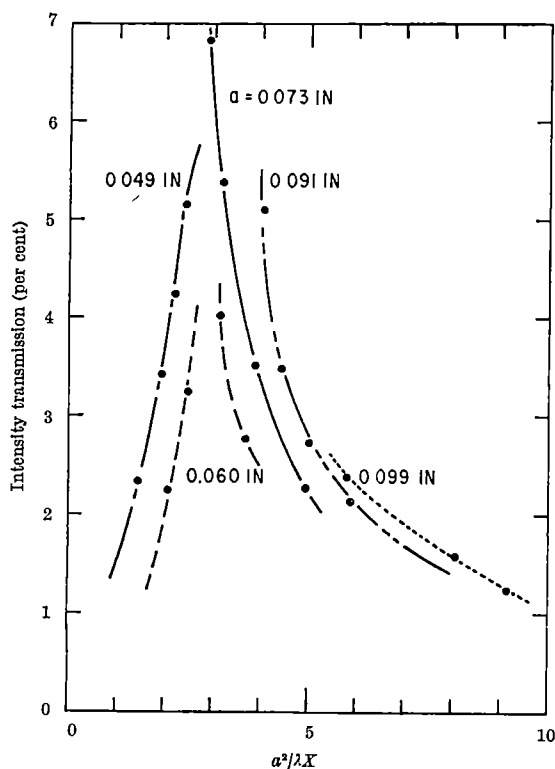


Fig. 2

transmission appears to peak somewhere in the range $2.5 < a^2/\lambda X < 3.0$. Similar behaviour has been noted for focusing with a mirror in the near field². The transmission percentage could be increased by better choice of α , optimum taper design, appropriate optical coating, etc. It appears to be true, however, that the percentage transmission for a given tube depends on its position in the laser near field.

I thank H. D. Jester, R. E. Carter and J. G. Cobb for their assistance.

H. J. CAULFIELD

Texas Instruments Incorporated,
Dallas, Texas.

¹ Vogel, K., *Nature*, 207, 281 (1965).

² Caulfield, H. J., and Eden, D. D. (to be published).

Surface Charges on Arc Track Oxide Layers

In experiments by us and by others¹, with arcs driven by magnetic fields along non-refractory metal electrodes, it has been found that the arc velocity increases when the arc travels over the electrode surfaces a second and subsequent times, within a short time interval after the first run. Other experiments with a short arc rotating around annular electrodes² have shown that, above a certain gas pressure, the forward velocity was no longer limited by the time taken for the transfer of emitting sites along the cathode surface, as occurred with a single passage of the arc. In each case the time needed to set up new emitting sites has apparently been considerably reduced by the previous passage of the arc. If cold-cathode arc emission is due to the charging of oxide layers by positive ions³, then a possible cause of these results would be that some positive ions remain un-neutralized on cathode oxide layers for appreciable times.

In order to investigate this possibility, cathode and anode tracks on various non-refractory metals have been examined by electrometer methods. Two methods were used in order to check whether their results were in agreement, as in fact they have proved to be. In the first,

the arc track to be examined was formed on a rotating disk by arcing to a radial electrode, and driving the arc outwards across the disk and electrode by a magnetic field. The arc track thus formed passed periodically under a probe connected to the grid of an electrometer pentode, and the voltage across the valve was displayed on a cathode-ray oscilloscope. By this method it was possible to measure pulses occurring at each passage of the track under the probe within 8 msec of its formation, and then at any time thereafter. The Kelvin vibrating electrode method with the same electrometer and probe, and a thin strip electrode, was used to confirm the results.

It has been found that on both cathode and anode tracks of all metals tested (aluminium, copper, brass and platinum) the polarity of the pulses has been that corresponding to negative charges on the tracks. After a rapid initial decay, the pulse height decreased relatively slowly and after 6 weeks was still about 25 per cent of the initial value. The p.d. between that of the arc track and that of the surrounding metal surface was mainly in the range 0.1–0.5 V within a few msec of the passage of the arc, and was generally somewhat lower on the anode than on the cathode, an exception being a high-current high-velocity arc on brass where the reverse was found.

The pulses in the rotating disk method could arise from: (1) variation of capacitance between the probe tip and disk; (2) collection at the probe of electrons emitted from the track; (3) differences between the distribution of charge or the oxide thickness on the track and that on the surrounding surface. The first possibility may be eliminated since surface measurements showed a small depression in the region of the arc track, which would tend to give rise to an extremely small positive pulse.

Although there is the possibility of significant electron emission within a very short time of the establishment of the tracks, for various reasons it appears to be more likely that the voltages measured are differences in surface p.d. between the track and the rest of the surface, due to dipole layers on the oxide caused by adsorbed gas. They could arise because the oxide layer is thicker on the track than on the rest of the surface with both having the same negative-charge density, or because the oxide thicknesses are the same but the track offers more adsorption sites and the charge density there is greater, or there could be a combination of the two effects.

No evidence has therefore been found of positive ions remaining un-neutralized on the surface oxide layers, although if they were responsible for emission from the cathode for a short time, their presence could be masked by emitted electrons and the negative dipole layer. The anode track p.d. suggests that oxide growth effects rather than positive ions are largely responsible for the results.

Any association between the higher arc velocities on second and subsequent runs and the negative dipole layers of the oxide will need further investigation. Suggestions that electron emission can occur from negative ions on oxide layers⁴ have referred to oxide layers of about 1000 Å, and there is no evidence that if oxide thickness is increased on arc tracks it can reach this value.

We thank the Science Research Council for a special grant supporting one of us (K. A. N.). We also thank colleagues for advice.

W. P. JAYASEKARA *
A. E. GUILLE
K. A. NAYLOR

Department of Electrical and
Electronic Engineering,
University of Leeds.

* Now at the University of Ceylon.

¹ Bronfman, A. I., *Elektrichestvo*, 8, 56 (1963).

² Bliz, E. D., and Guile, A. E., *Brit. J. App. Phys.*, 16, 857 (1965).

³ Guile, A. E., Lewis, T. J., and Secker, P. E., *Proc. Sixth Int. Conf. Ionisation Phenomena in Gases* (Paris S.E.R.M.A.), 2, 223 (1963).

⁴ Llewellyn Jones, F., and Nicholas, D. J., *Brit. J. App. Phys.*, 13, 514 (1962).

METEOROLOGY

Determination of Cloud Altitudes from Gemini-Titan-5

DURING the GT-5 manned spacecraft flight mission of August 21-29, 1965, an experiment to determine the cloud top altitude was performed. The instrument used in this experiment has been described by Saiedy *et al.*¹. It is a compact grating spectrograph-camera which records simultaneously a photograph of the cloud and its spectrum from 7500 Å to 7800 Å. Fig. 1 shows a reproduction of one of the 26 observations obtained during the mission. This photograph-spectrogram was taken over a cloudy area in the inter-tropical convergence zone (ITC) at 12° 37' N., 99° 10' W., at 1944:02 G.C.T., August 25.

The principle of deducing cloud-top heights from the absorption by the oxygen 'A' band has been discussed in detail by Wark and Mercer². Briefly, it requires a measurement of the fractional transmittance at a suitable wave-length in this band; the transmittance is determined by the height of the cloud and the local zenith angles of the Sun and the observer. That is, the transmittance depends on the effective amount of oxygen in the optical path above the cloud.

Fig. 2 shows microdensitometer traces of three spectra. The top trace is that of low stratus off Baja, California, at 27° 35' N., 119° 8' W., at 1743:00 G.C.T., August 27. The second trace is taken from the spectrogram shown in Fig. 1. The third trace is that of an observation of tropical storm "Doreen", taken at 20° 8' N., 129° 55' W., at 2120:08 G.C.T., August 24. From the calibration of the film, the transmittances given in Table 1 at selected wave-lengths were deduced. The heights of the cloud tops, calculated from these transmittances and from the solar and observer angles, are also shown in Table 1.

The results were compared with estimated heights for the stratus and with aircraft observations of "Doreen"

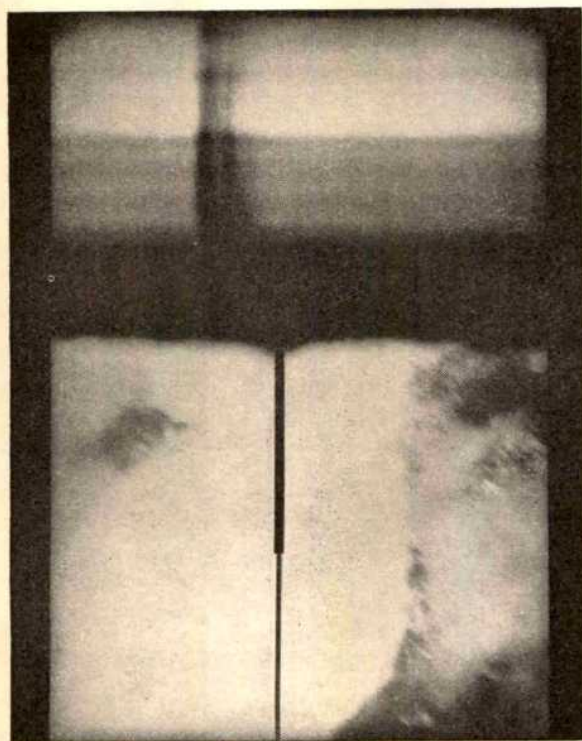


Fig. 1. Photograph and spectrogram taken from the GT-5 spacecraft. The spectrogram in the upper part shows the oxygen 'A' band near 7600 Å. The position of the spectrograph slit is outlined in the photograph below. It may be noted that half of the slit is wider than the other in order that a proper exposure could be assured; the effect is apparent in the spectrogram.

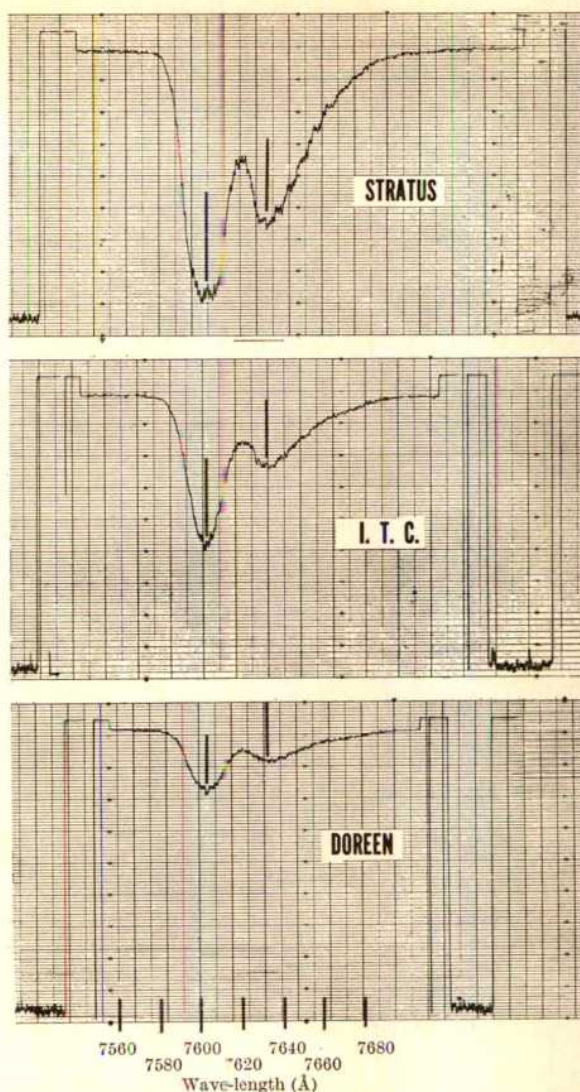


Fig. 2. Microdensitometer traces of three spectra obtained by the GT-5 astronauts. The vertical dashed lines are at 7607 Å and 7631 Å, the wave-lengths at which the oxygen transmittances given in Table 1 were calculated.

two days later; no verification is available as yet for the other observation. These preliminary results seem to confirm the most optimistic view of this technique for routine observations from unmanned spacecraft.

Table 1. PRELIMINARY RESULTS OF THREE GT-5 OBSERVATIONS OF CLOUD HEIGHT, DEDUCED FROM TRANSMITTANCES IN THE OXYGEN 'A' BAND. These represent clouds with low, medium and high tops.

Date (August 1965)	Time (G.C.T.)	Cloud identi- fication	Transmittance 7607 Å	7631 Å	Estimated pressure altitude (mbar)	Actual pressure altitude (mbar)
27	1743:00	Stratus	—	0.31	980	960
25	1944:02	I.T.C.	0.39	—	440	—
24	2120:08	"Doreen"	0.51	—	320	(350-220)

We thank the many personnel of the National Aeronautics and Space Administration, and particularly astronauts L. Gordon Cooper and Charles Conrad, jun., for their assistance. We also thank D. M. Mercer and other personnel of the National Weather Satellite Center.

F. SAIEDY

University of Maryland,
College Park, Maryland.

W. A. MORGAN
D. Q. WARK

Environmental Sciences Services Administration,
Weather Bureau, Washington, D.C.

¹ Saiedy, F., Hilleary, D. T., and Morgan, W. A., *App. Optics*, **4**, 495 (1965).
² Wark, D. Q., and Mercer, D. M., *App. Optics*, **4**, 839 (1965).

Water Balance and Evaporation Studies

PENMAN¹ has put forward an elegant expression for evaporation E for various types of surfaces with a wide range of climatic regions taking into consideration the energy balance and the aerodynamic factors. The equation is:

$$E = \frac{H + Ea \frac{\gamma}{\Delta}}{1 + \frac{\gamma}{\Delta}}, \quad H = R_c(1 - r) - R_B$$

where R_c is incoming radiation, r is the reflexion coefficient for a vegetative surface, R_B is out-going long-wave radiation calculated from:

$$R_B = \sigma T_a^4 \{0.56 - 0.09\sqrt{e_a}\} (1 - \alpha m)$$

σ is the Stefan Boltzmann constant, e_a is the actual vapour pressure and αm is the fraction of sky covered by clouds in tenths.

Ea = aerodynamic term $0.0105(0.5 + 0.01 U) (e_a - e_d)$
 U = miles per day at 2 m height, e_a is the saturation vapour pressure of air at temperature T_a . γ is the psychrometric constant; Δ is the gradient of saturation vapour pressure against temperature curve at air temperature.

The first and last terms in the equation are positive, and contribute positively to evaporation. The term R_B contributes negatively, which means there is condensation taking place due to long-wave radiation. This term assumes a significant role during the night when the incoming radiation is cut off. The evaporation term then consists of two terms, one of which is negative and hence there will in effect be a condensation process taking place. If the aerodynamic term remains unchanged during the night, it contributes to the evaporation a magnitude which may result in the total being negative or positive.

During the winter months on clear-sky days systematic measurements have been made to find the surface soil moisture over Poona. It is found that there is a daily variation of soil moisture, reaching a minimum at the maximum epoch of temperature and a maximum at the minimum temperature epoch. Various explanations have been offered for this variation of moisture. One such explanation is based on the fact that near the surface soil there is a reversal of the vapour pressure gradient at night resulting in invisible condensation.

It appears from Penman's equation that both during day and night there is a process of condensation taking place near the surface of the Earth which in the day is completely outbalanced by the incoming short-wave radiation, resulting in a net evaporation. In the absence of sunlight long-wave radiation plays a predominant part in condensation of water vapour on the soil. This explains also the different moisture-holding capacity of soils under identical meteorological conditions.

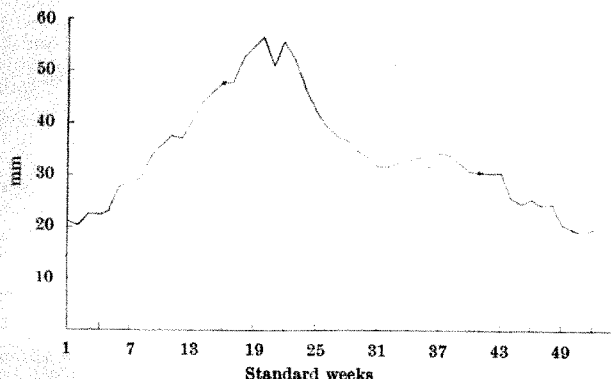


Fig. 1. Evaporation from an open water surface at Poona, estimated according to Penman (ref. 1)

In arid and semi-arid zones this factor is important for the study of water balance in relation to crop development in winter and may explain the large vegetation growth during these months when there is no rainfall. A study of the various meteorological factors in contributing to evaporation over Poona using Penman's equation reveals that on an average the annual evaporation is mainly contributed by the term containing the incoming radiation, the sum of other two terms over 52 weeks having equal positive and negative values. The study of evaporation over Poona week by week using Penman's equation shows that about the 16th week and the 40th week, reckoned from January 1 (corresponding to the middle of April and the end of September), the net contribution for evaporation by the aerodynamic term and long-wave radiation term is practically zero. The variation of evaporation with time week by week as calculated by Penman's equation is shown in Fig. 1 and agrees reasonably well with observed open pan evaporimeter values. This investigation will be pursued for all the agrometeorological and crop weather observatories in India where all the parameters required for the calculation of evaporation by Penman's equation are available for more than 10 or 15 years.

M. RAMA RAO

Observatory,
Poona, 5,
India.

¹ Penman, H. L., *Proc. Roy. Soc., A*, **193**, 120 (1948).

CHEMISTRY

Diperoxodiethylenetriamminechromium(IV) 1-hydrate: a New Chromium(IV) Peroxo Compound

DIPEROXOTRIAMMINECHROMIUM(IV), $\text{Cr}(\text{NH}_3)_3(\text{O}_2)_2$, one of the more stable chromium compounds containing peroxo groups¹, has been known for many years², and recently the X-ray crystal structures of this and other chromium peroxo compounds have been reported^{1,3-8}.

We have now prepared an analogous triamine containing the tridentate ligand diethylenetriamine ($\text{NH}_2(\text{CH}_2)_2\text{NH}(\text{CH}_2)_2\text{NH}_2 = \text{dien}$) which crystallizes from water as the 1-hydrate, $[\text{Cr}(\text{dien})(\text{O}_2)_2] \cdot \text{H}_2\text{O}$. The compound was prepared by the dropwise addition of 15 ml. of 30 per cent hydrogen peroxide to a stirred solution of 5 g of sodium dichromate 2-hydrate, 10 ml. of water and 6 ml. of dien at 5°–10°. Effervescence occurred and an orange-brown coloured solution developed due to the formation of the $\text{Cr}(\text{O}_2)_4^{3-}$ anion. The solution was kept at 15° for about two days, the colour slowly changing to lime green and olive-green crystals of $[\text{Cr}(\text{dien})(\text{O}_2)_2] \cdot \text{H}_2\text{O}$ (calc.: Cr, 21.92; C, 20.26; H, 6.37; N, 17.72. Found: Cr, 21.95; C, 20.51; H, 6.34; N, 17.80) were formed. These were collected by filtration and washed with methanol.

The oxidizing power was measured by determining the amount of iodine liberated by the solid from acidified potassium iodide and also by treating the solid with excess permanganate solution, adding excess iron(II) ion and back-titrating with permanganate. The mean of three determinations gave a value of 2.48 moles of iodine per mole of diperoxo complex (theory = 2.50) and 2.95 oxidation equivalents of permanganate per mole of complex (theory = 3.00), supporting the chromium(IV) diperoxo assignment.

The diperoxo complex is soluble in water to give a lime-green solution (λ_{max} 610 m μ ($\epsilon = 38.6 \text{ M}^{-1} \text{ cm}^{-1}$), 520 (41.5), 382 (460), 250 (5,400); λ_{min} 570 (38.5), 485 (38.8), 355 (415), 235 (4,880)) and both the aqueous solution and the solid appear to be stable for at least 24 h at room temperature in laboratory light, although both decompose when warmed. The solid explodes when heated to 109°–110° (heating rate = 2°/min). We have found that

the corresponding water-insoluble aquoethylenediamine ($\text{NH}_2(\text{CH}_2)_2\text{NH}_2 = \text{en}$) analogue^{8,9}, $[\text{Cr}(\text{en})(\text{O}_2)_2(\text{OH}_2)] \cdot \text{H}_2\text{O}$, explodes at 96–97°.

The infra-red spectrum (Nujol mull; Perkin-Elmer model 421) of the new solid shows a strong sharp doublet at 885 and 870 cm^{-1} which is assigned to the —O—O— stretching frequency^{10–12}, and the absence of a strong band in the 930 cm^{-1} region indicates the absence of Cr=O bonding.

Thus there seems little doubt that this stable chromium peroxo compound belongs to Type I of Stomberg's classification¹ (with a geometry probably that of a pentagonal bipyramid, the peroxo oxygen atoms probably being coplanar with the central nitrogen atom of the dien ligand and with the central chromium atom), and that the formal oxidation state of the chromium is +4.

This work was supported by the U.S. Atomic Energy Commission under contract AT(11-1)-34, project 12.

D. A. HOUSE
CLIFFORD S. GARNER

Department of Chemistry,
University of California, Los Angeles.

Stomberg, R., *Arkiv Kemi*, **24**, 282 (1965).

Wiede, O. F., *Ber.*, **30**, 2178 (1897).

Stomberg, R., *Arkiv Kemi*, **22**, 20 (1964).

Stomberg, R., *Arkiv Kemi*, **22**, 49 (1964).

Stomberg, R., *Arkiv Kemi*, **23**, 401 (1965).

Stomberg, R., *Arkiv Kemi*, **24**, 47 (1965).

Stomberg, R., *Arkiv Kemi*, **24**, 111 (1965).

Stomberg, R., *Acta Chem. Scand.*, **17**, 1563 (1963).

Hoffman, K. A., *Ber.*, **39**, 3181 (1906).

Griffith, W. P., *J. Chem. Soc.*, 3948 (1962).

Griffith, W. P., *J. Chem. Soc.*, 5345 (1963).

Griffith, W. P., *J. Chem. Soc.*, 5248 (1964).

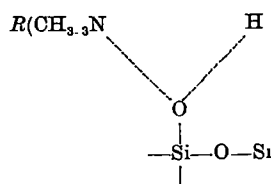
Infra-red Spectra of Quaternary Ammonium Compounds adsorbed on Silica-gel 'Aerosil'

We have examined by infra-red spectrophotometry the reaction of alkyl trimethyl ammonium bromides of chain-length 4–20 with silica surfaces. For these investigations 10^{-2} M solutions of the ammonium compounds were added to 'Aerosil' powder, which had been conditioned by hydration in water at 100° C for 4 h, dried and ignited at 700° C for 72 h, rehydrated as before and finally dried at 120° C. Hockey¹ has shown that this treatment gives a surface containing 4–6 surface silanol groups per 100 Å². Since optimum adsorption was found to occur within the pH range 6.6–8.5, the suspension was adjusted to pH 7 by the addition of 10^{-2} M sodium hydroxide solution. After 2 h the silica plus adsorbate was centrifuged out, dried at 120° C, then pressed into a coherent wafer containing 10–15 mg sample per cm² in a die at 12,000 lb/in.² for 5 min. The wafer was placed in a gas cell, evacuated at a pressure of 10^{-5} mm of mercury for 4 h, and the infra-red spectrum recorded on a Perkin-Elmer 237 double-beam instrument.

Curve B for hexadecyl trimethyl ammonium bromide ($\text{CH}_3(\text{CH}_2)_{15}\text{N}^+(\text{CH}_3)_3\text{Br}^-$) in Fig. 1 is typical of the spectrum obtained for a whole range of compounds. On washing the samples with water, the spectra of the compounds of chain-length <6 revert to that of the original 'Aerosil' (curve A, Fig. 1). For compounds of chain-length 7–20 the spectra are not greatly affected by washing, showing that the compounds are strongly bonded to the silica surface. The particular features of the spectra of the treated silica are the absence of the band at 3,750 cm^{-1} , which in untreated silica is ascribed to free surface silanol groups, and the formation of a new broad band at 3,240 cm^{-1} . (The band at about 2,900 cm^{-1} is from the normal methylene stretching vibrations of the adsorbed molecules.)

Previous workers^{3,4} have postulated a mechanism of attachment for dodecyl trimethyl ammonium bromide from aqueous solutions on silica via cation exchange with surface silanol groups. The present findings do not accord with this since the replacement of the sharp 3,750 cm^{-1}

band by one at 3,210 cm^{-1} is indicative of hydroxyl stretching vibrations in which the normal O—H band has been weakened. On this basis we postulate as a working model the structure:



The complete disappearance of the 3,750 cm^{-1} band suggests that all the surface silanol groups are influenced similarly; but it is not clear from the present evidence whether one quaternary ammonium group is able to react with more than one surface silanol group. Also the possible role of the bromine anion is not elucidated and studies are in hand to check both these points.

General support for this hypothesis is obtained by deuterium oxide exchange. Curve A of Fig. 2 shows the infra-red spectrum of hydrated 'Aerosil' after exposure to deuterium oxide vapour. The 3,750 cm^{-1} band ascribed to —OH stretching is replaced by a similar shaped —OD stretching band at 2,780 cm^{-1} . Likewise curve B for deuterated 'Aerosil' treated with hexadecyl trimethyl ammonium bromide shows that the 'modified' OH band at 3,240 cm^{-1} (see curve B, Fig. 1) is shifted to 2,410 cm^{-1} as expected from simple mass considerations—while the methylene stretching band at about 2,900 cm^{-1} is unaltered as no exchange has occurred.

Finally, we wish to comment on the absorption bands observed at 1,640 cm^{-1} , which has been a matter of contention among many workers, and at 3,660 cm^{-1} . Thompson⁵ has examined the effect of increase in temperature on the

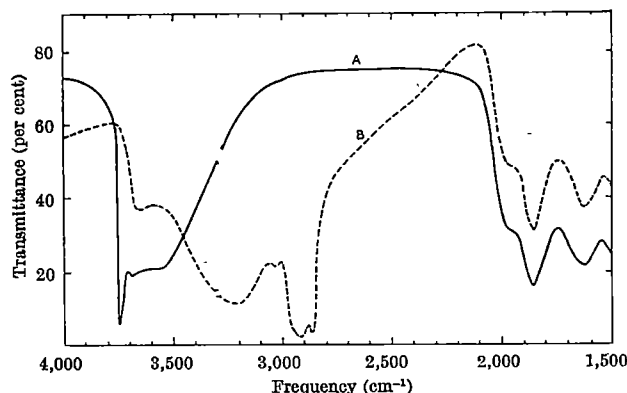


Fig. 1. Comparison of infra-red spectra of 'Aerosil' silica. A, Hydrated to a surface concentration of 4–6 silanol groups per 100 Å; B, as before but treated with 10^{-2} M hexadecyl trimethyl ammonium bromide at pH 7.

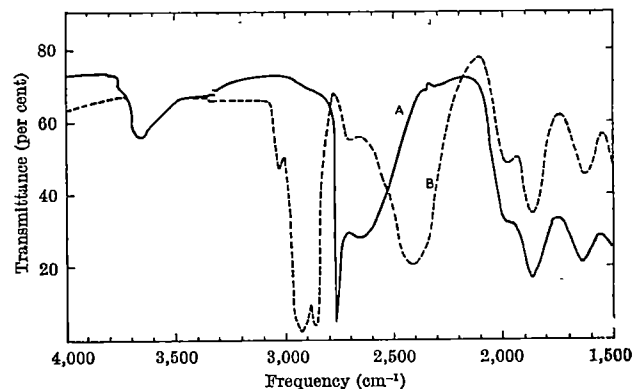


Fig. 2. Comparison of infra-red spectra of 'Aerosil' silica. A, Hydrated to a surface concentration of 4–6 silanol groups per 100 Å followed by reaction with deuterium oxide vapour; B, as before but treated with 10^{-2} M hexadecyl trimethyl ammonium bromide at pH 7.

1,640 cm^{-1} band and showed that at 150°C, and at a pressure of 3×10^{-5} mm of mercury, the spectrum showed an absorption apparently equivalent to the background absorption for annealed silica; similar high-temperature treatment has also been found necessary completely to remove the band at 3,660 cm^{-1} . We have compared the spectra of untreated, treated and deuterated silica with ordinate expansion in some cases, and have observed that both the small residual band at 3,660 cm^{-1} (seen clearly in Fig. 2) and the band at 1,640 cm^{-1} (sharply defined in all spectra) are completely unaffected by the deuterium exchange. Although some contribution to the 1,640 cm^{-1} band is undoubtedly due to the harmonic of the SiO vibration, we propose from this evidence that both bands are associated with hydroxyl groups 'trapped' within capillaries or cracks and, therefore, unable to react either with quaternary ammonium compounds or with deuterium oxide.

We thank Dr. J. A. Hockey for his advice.

L. C. F. BLACKMAN*
R. HARROP

Chemical Research Division,
British Railways Research Department,
The Avenue, Muswell Hill,
London, N.10.

* Now at the British Coal Utilization Research Association, Leatherhead, Surrey.

¹ Hockey, J. A., *Chem. and Indust.* No. 2, 57 (1965).

² Lygin, V. I., and Kiselev, A. V., *Kolloid Zhur.* 23, 252 (1961).

³ Ter-Minassian-Seraga, L., *Advances in Chemistry Series*, No. 43, 232, *J. Chem. Physics*, 57, 10 (1960).

⁴ Sexmith, F. H., and White, jun., H. E., *J. Colloid Sci.*, 14, 598 (1959).

⁵ Thompson, W. K. (personal communication).

Inhibition of Corrosion of Copper in Chromic Acid

CORROSION of copper is inhibited in 0.1 N chromic acid of pH 1.6, a loss of 10 mg/dm^2 and no change in appearance is observed after four weeks' immersion. On the other hand, corrosion is significant in sulphuric acid of this pH, and a loss of 150 mg/dm^2 is recorded after an identical period of immersion. This communication describes experiments designed to obtain a fuller understanding of the mechanism by which chromate inhibits the corrosion of copper.

(1) Measurements of electrode potential and film thickness were made on copper chemically polished and left for 3 days in a desiccator over calcium chloride. As shown by the electrometric reduction technique devised earlier¹, specimens treated in this way are covered by a film made up of 14 Å of cuprous oxide and an unidentifiable mixture of copper salts requiring 0.25 mC/cm^2 for reduction.

The results in Table 1 were obtained. These show that the protective power of the oxide film in copper is less in sulphuric acid than in chromic acid.

Table 1

(a) Electrode potential measurements (hydrogen scale)

(i) Copper in 0.1 N sulphuric acid	
On immersion	+0.18 V
After 43 h	+0.20 V
(ii) Copper in 0.1 N chromic acid	
On immersion	+0.65 V
After 43 h	+0.68 V

(b) Oxide film thickness (calculated from a-c bridge measurements at 2,000 c/s using a dielectric constant of 10.5 for cuprous oxide (ref. 2),

(i) In 0.1 N sulphuric acid the apparent film thickness (or approximate thickness of the coherent section of the oxide film adjacent to the metal surface) decreased from 80 Å on immersion to ≤ 50 Å after 43 h.

(ii) In 0.1 N chromic acid the apparent film thickness increased from 22 Å on immersion to 32 Å after 43 h.

(2) An electrometric technique¹ was used to measure film thicknesses on copper which had been stored three days in a desiccator after a treatment comprising chemical polishing³ followed by electrochemical reduction of the

oxide. The cuprous oxide film on the copper surface was ≤ 3.0 Å immediately after this treatment, which increased to 6.0 Å after three days in the desiccator and to 16.0 Å after a further 43 h. Specimens were immersed in test solutions after three days in a desiccator, and left in the solution for 43 h. On removal from the test solution, these specimens were washed with distilled water, rinsed in acetone, and placed in the electrometric cell. The specimens were afterwards immersed in deaerated solution and the nature and thickness of the oxide film were determined (Table 2).

Table 2

- (a) In 0.1 N sulphuric acid, the cuprous oxide film after 43 h immersion was 38 Å thick.
(b) In 0.1 N chromic acid, the film after 43 h immersion was 17 Å thick and no evidence of irreversible adsorption of chromate was obtained.

The results showed the total film thickness to be greater after immersion in sulphuric acid than in chromic acid; however, a.c. bridge measurements indicated that the film thickness decreased in the former solution. Thus it is evident that the film formed in sulphuric acid is porous, and the a.-c. bridge measurements give an indication of the thickness of the coherent or non-porous section of the oxide film.

(3) Measurements of rates of solution of copper from bulk oxide, and oxide-covered copper, were compared and the results are shown in Table 3. Although the anion did not have any significant effect on the rate of solution of bulk oxide, the rate of solution of oxide from the metal surface was markedly decreased in chromic acid.

Table 3

	1 min	1 h	24 h
Concentration of copper, g./ion/l. dissolved from bulk cuprous oxide in 0.1 N H_2SO_4	—	4×10^{-2}	7×10^{-2}
Concentration of copper, g./ion/l. dissolved from bulk cuprous oxide in 0.1 N H_2CrO_4	—	10^{-2}	6×10^{-2}
Concentration of copper, g./ion dissolved from 90 cm^2 in 0.1 N H_2SO_4	3.1×10^{-5}	1.3×10^{-4}	10^{-3}
Concentration of copper, g./ion dissolved from 90 cm^2 copper in 0.1 N H_2CrO_4	3.5×10^{-5}	4.3×10^{-5}	6.4×10^{-5}

These three sets of experiments show that the effect of chromate on copper is to lower the chemical reactivity of the oxide film. The electrometric investigations of copper which had been immersed in chromate solution revealed no evidence of significant irreversible adsorption of chromate. However, when copper was immersed in chromic acid with a ratio of 2.2 ml. solution to 1 cm^2 of metal surface for 1 h, the oxide film stripped from the metal in a mixture of 10 ml. concentrated hydrochloric acid and 10 ml. 1:10 hydrogen peroxide made up to 100 ml. with washings from the specimen and the solution analysed, 2.5×10^{-5} g./ion of chromium was present. These results indicate that chromic ions formed by local electrochemical action had entered the oxide lattice to form a mixed cuprous chromic oxide having a lower chemical activity than the bulk cuprous oxide. It is considered that lowering the chemical activity of the oxide film is the major factor in inhibiting the corrosion of copper in chromic acid.

K. F. LORRING

Australian Defence Scientific Service,
Aeronautical Research Laboratories,
Melbourne.

¹ Mills, T., and Evans, U. R., *J. Chem. Soc.*, 2182 (1956).

² Hoey, G. R., *J. Electrochem. Soc.*, 108, 387 (1961).

³ de Jong, J. J., *Metalen*, 9, 2 (1954).

Effect of Environment on the Orientation of the Nitrate Ions in Crystals

A ROTATIONAL or orientational disorder at room temperature in the arrangement of the nitrate ions has been observed in the crystalline lattices of a number of co-ordination compounds¹⁻⁴, although no one has so far offered any explanation for this phenomenon. The factors

which are known to influence the arrangement of any ionic group in a crystal are the symmetry, the size, and the moment of inertia of the group in question, as well as the nature of packing of various cations and anions. In some cases the charge on the complex cation is completely shielded by neutral groups which surround it, and so the electrostatic interaction with the anionic group is very weak. The net result is that the anion exhibits free rotation. An example of this is provided by the crystal structure of silver perchlorate dioxane complex⁵ in which the perchlorate ion exhibits free rotation.

The factor which has not been considered so far is the influence the nature of the environment has on the orientation of the nitrate ion. The orientation of the nitrate ion (or any other ionic group) in a particular position is effected by those atoms of the surrounding cationic groups, which have a close approach to this group. In all compounds in which the orientation of the nitrate ion is ordered, the symmetry of the environment matches with the symmetry of the nitrate ion. For example, in *tris*-glycine nickel nitrate⁶, every oxygen of the nitrate ion has two hydrogen atoms as neighbours and they are arranged in such a manner that the symmetries of the nitrate ion and its environs match with each other (see Fig. 1). If the nitrate ion in this compound is forced

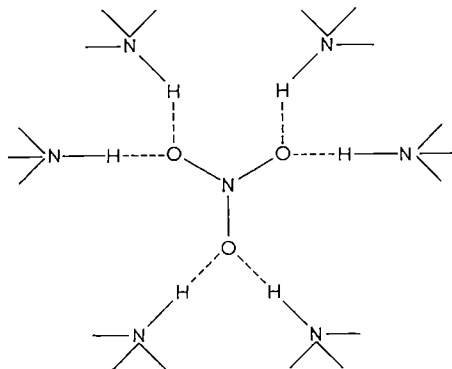


Fig. 1. Nitrate ion and its environs in the crystal structure of *tris*-glycine nickel nitrate

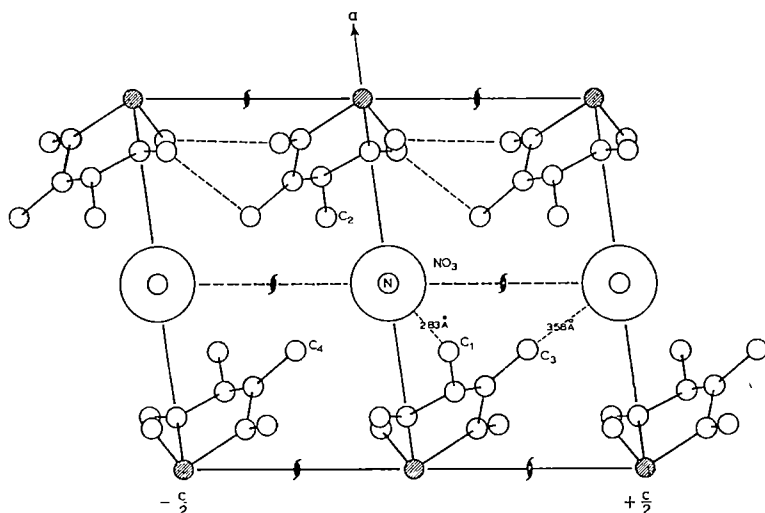


Fig. 2. 3D projection of the crystal structure of *bis*-dimethylglyoximinodiammine cobalt(III) nitrate, showing the freely rotating nitrate ion and its environs

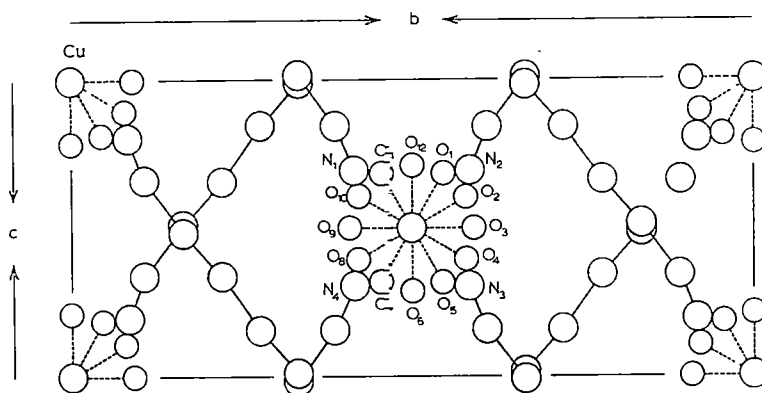


Fig. 3. Nitrate ion and its environs in the crystal structure of *bis*-adiponitrilo copper nitrate

to rotate, the neighbouring atoms of the environs, which exert an attractive force on the oxygens of nitrate ion, keep this ion in fixed position. All such forces act in the same direction and try to prevent the nitrate ion from exhibiting free rotation.

In all compounds which are known to have disordered arrangement of the nitrate ions, the symmetry of the environment does not match with the symmetry of the nitrate group. In dimethylglyoximinodiammine cobalt nitrate¹, the nitrate ion exhibits free rotation. The environment of this ion has approximately a four-fold symmetry (see Fig. 2). In an arrangement of this type, there are several possible positions for this arrangement of the nitrate ions which correspond to a minimum of the potential energy. Free rotation of this ion then becomes possible since the potential barrier it has to overcome in order to rotate is small under these conditions.

In the crystals of *bis*-adiponitrilo copper(I) nitrate², there is an infinite network of the complex ion $[\text{Cu}(\text{NC}-\text{CH}_2-\text{CN}_2-\text{CN}_2-\text{CH}_2-\text{CN}_2)]_n^{2+}$ and the nitrate ions are located in the gaps of this network (see Fig. 3). The symmetry of the environment of the nitrate ion is four-fold. In this structure, there are four possible arrangements of the nitrate ion which correspond to a minimum of the potential energy. All the four possibilities are present in equal proportions in the crystal structure, which then has an orientational disorder in the arrangement of the nitrate ions.

The crystals of *bis*-glutaronitrilo copper(I) nitrate³ consist of a network of $[\text{Cu}(\text{NC}-\text{CH}_2\text{CH}_2-\text{CH}_2-\text{CN})_2]_n^+$ and the nitrate ion is located in the gaps of this network.

The environment of the nitrate ion has a four-fold symmetry and so the nitrate ion exhibits orientational disorder (see Fig. 4).

The crystal structure of *trans*-dichloro-*bis*-ethylenediammine cobalt(III) nitrate⁴ provides a striking example of the influence which the environment has in producing disorder in the arrangement of the nitrate ions (see Fig. 5). The symmetry of the environment is two-fold, so that in 50 per cent of the unit cells the nitrate ions are nearer to the two carbon atoms of one complex cation and in the remaining cells they are closer to the two carbon atoms of the complex cation. Each of the two arrangements corresponds to a minimum of the potential energy.

A three-dimensional refinement of some of these structures is in progress so that precise information regarding the positions of the nitrate ions and their environs may be obtained in order to discuss the problem in a quantitative manner. In conclusion, it can be stated that by choosing cations and anions of appropriate size, shape and sym-

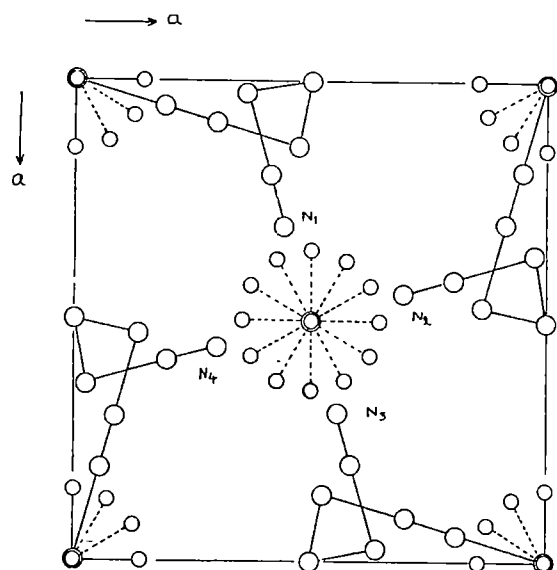


Fig. 4. Nitrate ion and its environs in the crystal structure of *bis*-glutaronitrilo copper nitrate

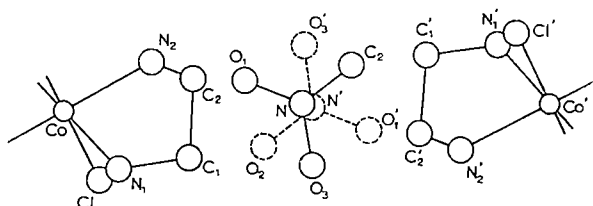


Fig. 5. Nitrate ion and its environs in the crystal structure of *trans*-dichloro *bis*-ethylenediamine cobalt nitrate

metry, compounds having either rotational or orientational disorder can be made to order.

N. R. KUNCHUR*

Department of Chemistry,
University of Western Ontario,
London, Ontario.

* Present address: Center for Crystallographic Research, Roswell Park Memorial Institute, Buffalo, N.Y., U.S.A.

¹ Viswanathan, K. S., and Kunchur, N. R., *Acta Cryst.*, **14**, 675 (1961).

² Kinoshita, Y., Matsubara, I., Higuchi, T., and Saito, Y., *Bull. Chem. Soc. Jap.*, **32**, 1221 (1959).

³ Kinoshita, Y., Matsubara, I., and Saito, Y., *Bull. Chem. Soc. Jap.*, **32**, 1216 (1959).

⁴ Ooi, S., and Kuroya, H., *Bull. Chem. Soc. Jap.*, **36**, 1083 (1963).

⁵ Prosen, R. J., and Trueblood, K. N., *Acta Cryst.*, **8**, 741 (1956).

⁶ Swink, L. N., and Ajoti, M., *Acta Cryst.*, **13**, 639 (1960).

Ultrasonic Method of Determining the Critical Micelle Concentration of Surfactants

SEVERAL methods¹ are available for measuring the critical micelle concentration in surfactant solutions. An examination of the models of the micelle structure shows that the major concept is that several micellar structures are possible and do exist. The formation of a micelle and each different type of its structure is looked on as that of a new phase. Hence we expected that an examination of ultrasonic velocity at different concentrations in the region of the critical micelle concentration should exhibit a discontinuity in the plot of velocity versus concentration. Sodium dodecyl sulphate at 40° C, cetyl trimethyl ammonium bromide at 35° C, potassium laurate at 35° C and tetrahydroanacardol ammonium monosulphonate at 50° C have been examined. Results obtained are plotted in the graphs (Figs. 1-4).

The solutions were made in conductivity water. The temperature was maintained using a circulating thermostat. Ultrasonic velocity measurements were made at

5 Mc/s using the Debye-Sears² method. The distances between successive diffraction fringes were measured using Hilger's X-ray microphotometer L-486.

From the graphs it is clear that each surfactant shows a reversal in the slope of the velocity versus concentration curve in the region of the critical micelle concentration—the values of the latter given in the literature agree with those shown in the graphs.

In the region in which the ultrasonic velocity falls, the grouping of the surfactant dipoles seems to be occurring

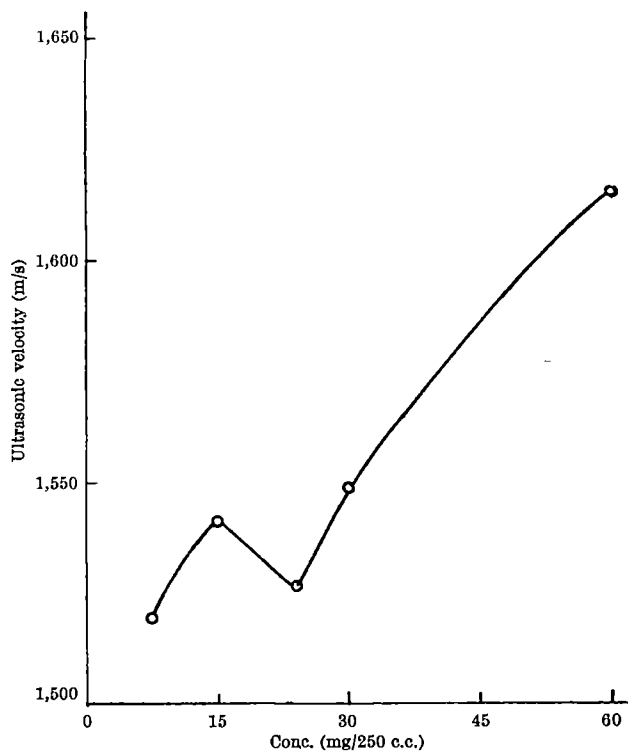


Fig. 1. Tetrahydroanacardol ammonium monosulphonate (50° C)

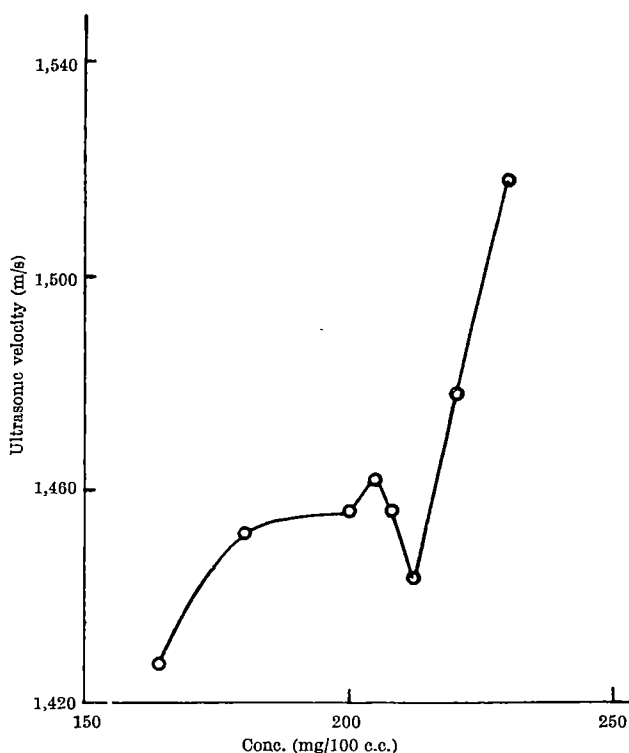


Fig. 2 Sodium dodecyl (lauryl) sulphate (40° C)

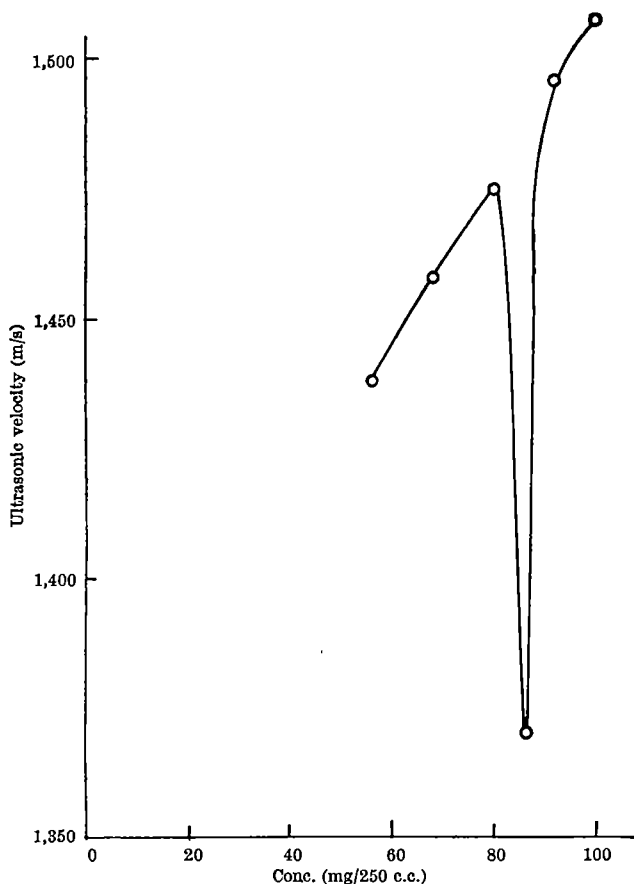


Fig. 3. Cetyl trimethyl ammonium bromide (35°C)

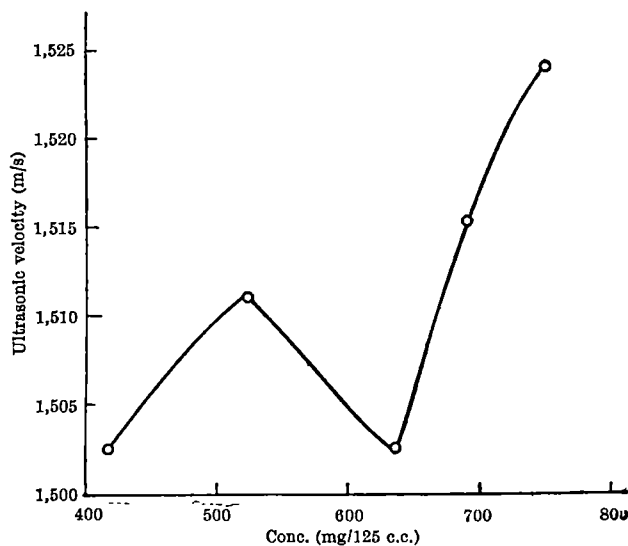


Fig. 4. Potassium laurate (35°C)

progressively through intermediate stages of association. The fall in ultrasonic velocity in the region of micelle formation may be explained on the basis of internal pressure given by the relation:

$$\pi = \frac{T\alpha}{\beta_T} \quad (1)$$

where T is absolute temperature, α and β_T are coefficients of thermal expansion and volume compressibility respectively. At constant temperature, if α is assumed to vary little, the internal pressure π is proportional to $\frac{1}{\beta_T}$:

$$\text{where } \beta_T = \frac{\gamma}{u^2 \rho} \quad (2)$$

where γ is the ratio of specific heats, u the ultrasonic velocity and ρ the density. Assuming ρ to vary very little in this region and γ to be constant, the internal pressure varies as the square of the ultrasonic velocity. Hence in the region of micelle formation there is a heavy fall in internal pressure which perhaps points to either an increase in the free volume⁷ of the medium or a heavy fall in the electrostatic attractive forces or both. Subsequent increase in the ultrasonic velocity after the critical micelle concentration region may have to be explained as due to progressive increase in the electrostatic attractions, or by some mechanism by which the free volume in the medium decreases.

Table 1

No.	Substance	Critical micelle concentration Observed	Literature	Ref.
(1)	Tetrahydroanacardyl ammonium monosulphonate	60-96 mg/l. at 50°C	75-100 mg/l. at 50°C	3
(2)	Sodium dodecyl sulphate	7.11-7.36 mM at 40°C	7 mM at 40°C	4
(3)	Potassium laurate	1.75-2.13 x 10 ⁻² M at 35°C	2.3-2.4 x 10 ⁻² M at 25-8°C	5
(4)	Cetyl trimethyl ammonium bromide	0.87-0.94 mM at 35°C	0.9 mM at 25°C	6

We thank Prof. K. S. G. Doss, director of this Institute, for his advice. We also thank Dr. A. B. Biswas, National Chemical Laboratory, Poona, for supplying the surfactants investigated here.

J. KUPPUSAMI
C. V. SURYANARAYANA

Central Electrochemical Research Institute,
Karaikudi, India.

¹ Schwartz, A. M., Parry, J. W., and Berck, J., *Surface Active Agents and Detergents*, 2 (Interscience Publishers Inc., N.Y., 1949).

² Debye, P., and Sears, F. W., *Proc. U.S. Nat. Acad. Sci.*, **18**, 409 (1932).

³ Katti, S. S., and Kulkarni, S. B., *Indian J. Tech.*, **2**, No. 3 96 (1964).

⁴ Kashiwagi, Maresuke, *Bull. Chem. Soc. (Japan)*, **30**, No. 2, 193 (1957).

⁵ Corrin, M. L., Kleven, H. B., and Harkins, W. D., *J. Chem. Phys.*, **14**, 480 (1946).

⁶ Nash, T., *J. App. Chem.* (London), **8**, 440 (1958).

⁷ Hildebrand, J. H., and Scott, R. L., *Solubility of Non-electrolytes*, third ed., 74 (Reinhold Publishing Corp., N.Y.).

Radiolysis of Sodium Tetrathionate Dihydrate

As part of a study of the radiation chemistry of inorganic sulphur compounds some preliminary results have been obtained for the radiolysis of solid $\text{Na}_2\text{S}_4\text{O}_6 \cdot 2\text{H}_2\text{O}$.

Sodium tetrathionate dihydrate was prepared by making iodine to react with sodium thiosulphate¹. It was purified by repeated precipitation from a warm aqueous solution by the addition of 95 per cent ethanol. Tests indicated that the desired salt had been obtained. The irradiation source was a γ -cell 220 cobalt-60 irradiation unit supplied by Atomic Energy of Canada, Ltd. The dose rates were of the order of 1,200 rads/min. The samples, which were at a temperature of $25^\circ \pm 5^\circ \text{C}$, were irradiated in the presence of air. Samples of the solid salt were irradiated for varying lengths of time and then dissolved in de-aerated triple-distilled water. The resulting solutions were analysed for SO_4^{2-} , $\text{S}_2\text{O}_3^{2-}$, H_2S and acid. Sulphite was found to be absent. The presence of colloidal sulphur was observed. The analytical methods used have been described elsewhere^{2,3}. The amount of substances oxidized by iodine was found by dissolving the samples in an excess of an aqueous solution of iodine and then back-titrating with a standard thiosulphate solution. In Table 1 are given the yields, in μmoles of product/g of irradiated $\text{Na}_2\text{S}_4\text{O}_6 \cdot 2\text{H}_2\text{O}$, of the products for various absorbed doses. Electron spin resonance spectra were used to estimate the number of spins present in the irradiated samples. Electron spin concentrations were measured by graphical double integrations of the recorded spectra and comparison, through a standard of γ -irradiated barium dichloroacetate, with solutions of

Table 1

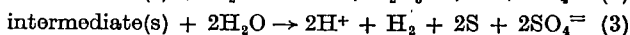
Yield of products ($\mu\text{moles/g}$, $\text{Na}_2\text{S}_4\text{O}_6 \cdot 2\text{H}_2\text{O}$)

Absorbed dose (eV/g $\times 10^{-10}$)	Spins (No./g $\times 10^{-17}$)	Oxidizable substances ($\mu\text{equiv. of I}_2$)	$\text{SO}_4^{=}$	H^+	H_2S	$\text{S}_2\text{O}_3^{=}$
0.95	1.8	5.4	4.0	3.0	0.39	2.0
1.90	3.5	9.9	6.4	5.3	0.50	—
2.85	4.8	14.8	8.2	6.0	0.82	3.6
3.80	5.2	17.8	10.8	6.8	1.02	5.5
4.75	6.7	20.9	11.2	8.4	1.26	5.5
5.70	6.8	24.2	12.2	8.4	1.78	5.7

diphenylpicrylhydrazyl in benzene. The results are included in Table 1.

Experiments using tetrathionate labelled with sulphur-35 were also made to determine the source of the sulphur which appeared in the H_2S . The radioactivity of the H_2S was determined by absorbing it in 'hydramine hydroxide' and then using a liquid scintillation counter^{2,3}. With $\text{Na}_2\text{S}_4\text{O}_6 \cdot 2\text{H}_2\text{O}$ prepared from $\text{Na}_2\text{S}_2\text{O}_3$ labelled in the 'outer' sulphur position (the sulphur linked only to the central sulphur and not to oxygen), the results indicated that 98 per cent of the sulphur in the H_2S originated from a labelled position. Presumably the labelled positions were the two central sulphur atoms of the chain of four sulphur atoms in the $\text{S}_4\text{O}_6^{=}$ ion. The colloidal sulphur formed from the radiolysis of $\text{Na}_2\text{S}_4\text{O}_6 \cdot 2\text{H}_2\text{O}$ labelled as already described here was found to be radioactive. Sodium thiosulphate labelled in the inner sulphur position (the central, oxygen-bonded sulphur atom) was also used to prepare $\text{Na}_2\text{S}_4\text{O}_6 \cdot 2\text{H}_2\text{O}$, the labelled sulphur atoms were presumably the terminal ones of the chain of four sulphur atoms of the $\text{S}_4\text{O}_6^{=}$ ion. The results indicated that 25 per cent of the H_2S sulphur came from the labelled positions, that is, from the oxygen-bonded sulphur atoms of the $\text{S}_4\text{O}_6^{=}$ ion. The reason for the discrepancy of the two sets of results is not known. Considering both sets of results, it appears that at least 75 per cent of the H_2S sulphur comes from the two sulphur atoms in the centre of the chain of four sulphur atoms.

The foregoing results are not sufficient to deduce a very complete mechanism for the radiolysis of $\text{Na}_2\text{S}_4\text{O}_6 \cdot 2\text{H}_2\text{O}$. However, it does seem possible to form some tentative conclusions concerning the mechanism. It appears likely that the two central sulphur atoms of the tetrathionate ion gave rise to the H_2S and the colloidal sulphur produced, whereas the two outer, oxygen bonded, sulphur atoms gave rise to the thiosulphate and sulphate. The results indicate that approximately twice as many $\text{SO}_4^{=}$ ions are produced as are $\text{S}_2\text{O}_3^{=}$ ions. Also the yield of $\text{SO}_4^{=}$ ions is approximately equal to twice the yield of H_2S plus the yield of acid, suggesting that the yield of H^+ is approximately equal to the yield of $\text{SO}_4^{=}$. The following set of reactions is consistent with the foregoing facts:



The water in reactions (2) and (3) would include that present in the solid as water of crystallization as well as that used for dissolving the sample. The water of crystallization would also undergo radiolysis, the products of which could react either with $\text{S}_4\text{O}_6^{=}$ ions or with the postulated intermediates.

No very definite conclusions can be drawn as to the identification of the postulated intermediates. The number of spins observed is only approximately 10 per cent of the number of ions found, hence a large fraction of the intermediates must be other than free radicals. One possibility would be an excited $\text{S}_4\text{O}_6^{=}$ ion which could react with water according to reactions (2) or (3). Since reaction (2) requires the breaking of fewer sulphur-sulphur bonds than does reaction (3), it might be anticipated that the former reaction would be more favoured than the latter. If reaction (2) occurred twice as fast as reaction (3), the observed yield of two $\text{SO}_4^{=}$ ions for each $\text{S}_2\text{O}_3^{=}$ ion would be explained.

It should be noted that reaction (2) results in a ratio of H^+ to $\text{SO}_4^{=}$ which is twice that actually found. It may be, however, that not all the H^+ produced is found since $\text{S}_2\text{O}_3^{=}$ is the anion of a weak acid and the phenolphthalein end-point may correspond to the formation of HS_2O_3^- . If this were so, then the observed yield of H^+ would be in agreement with reactions (1), (2) and (3).

The foregoing tentative mechanism does not account for the formation of any H_2S . The yield of H_2S is less than 10 per cent of the yield of $\text{SO}_4^{=}$ plus $\text{S}_2\text{O}_3^{=}$. It may be that in a small fraction of the reactions of the intermediates with water the central sulphur atoms acquire electrons and appear as $\text{S}^{=}$ ions instead of neutral S atoms as in reactions (2) and (3).

This work was supported by the National Research Council of Canada.

R. L. EAGER

D. S. MAHADEVAPPA *

Department of Chemistry and Chemical Engineering,
University of Saskatchewan,
Saskatoon,
Saskatchewan, Canada.

* Present address: Department of Chemistry, Manasagangothri University of Mysore, India.

¹ *Abegg's Handbuch der Anorganischen Chemie*, 4, 554 (Verlag Von S. Hirzel, Leipzig, 1927).

² Eager, R. L., and Mahadevappa, D. S., *Canad. J. Chem.*, 43, 614 (1965).

³ Mahadevappa, D. S., and Eager, R. L., *Curr. Sci. (India)*, 34, 15 (1965).

BIOCHEMISTRY

Denaturation of Lactic Dehydrogenase Isozymes and its Clinical Application

It is known that lactic dehydrogenase (LDH) can be inactivated by treatment with various protein denaturants, for example, urea and guanidine hydrochloride^{1,2}. In the denaturing process the tertiary structure of the enzyme is destroyed with the concomitant loss of enzymatic activity. There is evidence that fast-migrating isozyme (LDH_1), a predominant fraction in heart tissue, is less sensitive to these denaturing processes than the slower migrating isozymes^{3,4}. The various isozymes are known to differ from each other in their amino-acid composition and this may play a central part in susceptibility to denaturation.

We have compared the denaturing effect of urea and β -mercaptoethanol on pig heart LDH_1 and rabbit muscle LDH_5 . The effects of urea on human electrophoretically separated LDH_1 and LDH_5 isozymes as well as on the LDH activity of human heart and liver homogenates were determined. The purpose of the work recorded here was to clarify the basis for developing a simple test which would be suitable for clinical detection of the LDH_1 isozyme.

By means of a Beckman DB spectrophotometer at 340 m μ (lightpath 10 mm), the LDH activity was measured in Sørensen phosphate buffer (0.067 M) at pH 7.4 and in a water bath at a temperature of $25 \pm 0.1^\circ\text{C}$. The final concentrations were 2.5×10^{-4} M for pyruvate, 3.3×10^{-3} M for α -ketobutyrate, and 1.3×10^{-4} M for NADH_2 . All determinations were made in duplicate.

Denaturation of LDH_1 and LDH_5 isozymes was measured in various urea and β -mercaptoethanol concentrations following the incubation of buffered enzyme-substrate solution for 10 min at 25°C ; the reaction initiated by means of co-enzyme. Human LDH_1 and LDH_5 isozymes from pooled heart and liver homogenates were prepared by starch-gel electrophoresis modified on the basis of previous reports^{5,6} using phosphate buffer at pH 7.0, from two autopsies about 24 h after death. Individual tissues were homogenized in Sørensen phosphate buffer, pH 7.4, using a Bühler homogenizer. To

minimize the inactivation of the enzyme activity, these procedures were carried out immediately after the tissues were received, and with cooling during the treatment. After electrophoresis the plate was stored overnight at -20°C . Separation of isozyme fractions (1 in. between each fraction) occurred within 6 h. To control the individual content of the various isozyme proportions, agar-gel electrophoresis was performed simultaneously with heart and liver homogenates. Crystalline pig heart LDH_1 and rabbit muscle LDH_5 were purchased from Boehringer and Soehne, Mannheim.

As shown in Fig. 1, urea denaturates electrophoretically separated human LDH_1 to the extent of only about 25 per cent in the concentration used (1.5 M), but brings about denaturation of LDH_5 isozyme to the extent of 90 per cent when the test substrate was pyruvate. The denaturation of the LDE activity of human heart and liver homogenates is slightly greater when concentrations of urea of up to 1.5 M are used; 35 per cent for heart homogenate and 90 per cent for liver at 1.5 M urea. At higher urea concentrations, the denaturation of heart homogenate exceeds that of LDH_1 isozyme, as can be seen by comparing Figs. 1 and 2.

Using a urea concentration of 1.5 M, crystalline LDH_1 (pig heart) was found to bring about a 55 per cent denaturation, while the greatest difference in denaturation between crystalline LDH_1 and LDH_5 from various animals

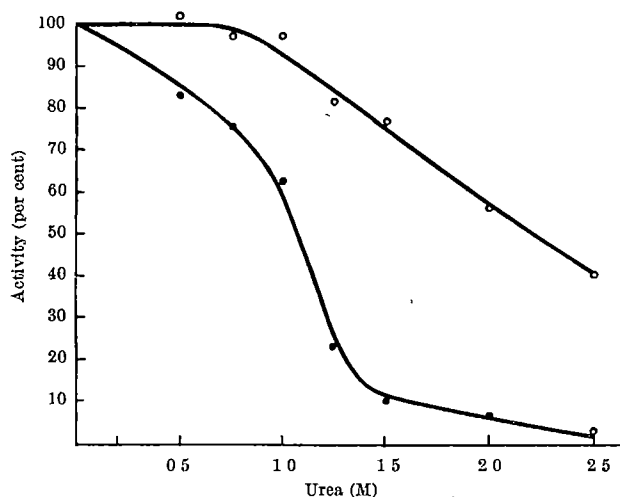


Fig. 1. Percentage enzymatic activity of human LDH_1 (O) and LDH_5 (●) isozymes separated by starch gel electrophoresis after inhibition with urea at the indicated concentrations. Pyruvate was used as substrate.

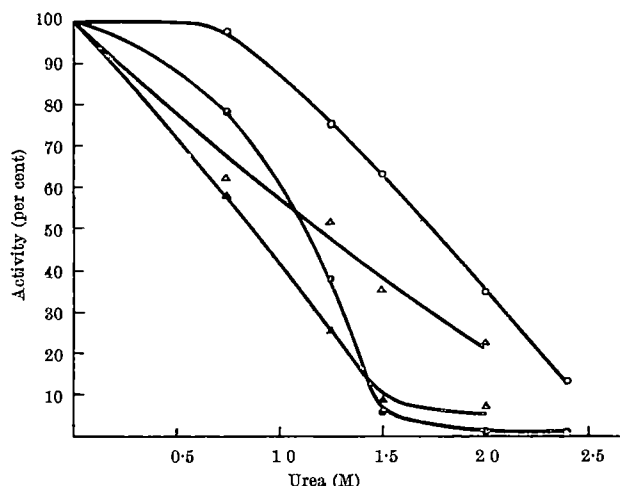


Fig. 2. Percentage LDH activity of human heart and liver homogenates after inhibition with urea, pyruvate or α -ketobutyrate as substrate. Heart and pyruvate (O), liver and pyruvate (●), heart and α -ketobutyrate (Δ), and liver and α -ketobutyrate (\blacktriangle).

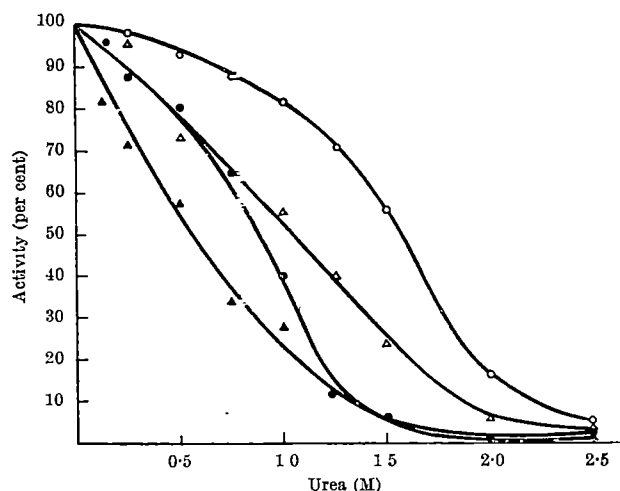


Fig. 3. The percentage enzymatic activity of crystalline pig heart LDH_1 and rabbit muscle LDH_5 isozymes after inhibition with urea, pyruvate or α -ketobutyrate as substrate. LDH_1 and pyruvate (O), LDH_5 and pyruvate (●), LDH_1 and α -ketobutyrate (Δ), and LDH_5 and α -ketobutyrate (\blacktriangle).

was seen when a urea concentration of 1.25 M was used (Fig. 3).

Human tissue homogenates tested against α -ketobutyrate showed a higher degree of denaturation than when pyruvate was used as substrate (Fig. 2). The same was also observed with crystalline isozymes (Fig. 3).

The electrophoretically separated human isozyme LDH_1 , consisting of monomers B^1 (or H^1), showed much greater resistance to urea denaturation than LDH_5 , consisting of monomers A (or M). The slightly greater denaturation of human heart homogenate compared with the separated human LDH_1 is most probably due to the presence of a small amount of the slower migrating isozymes in the heart tissue. To ensure that electrophoretically separated isozymes were not contaminated with other fractions they were checked by means of agar electrophoresis.

The denaturation of crystalline pig heart LDH_1 with 1.5 M urea was found to be greater (45 per cent) than that of the non-crystalline human heart LDH_1 . This difference might depend on the different degree of purification or on the difference in denaturation susceptibility in various species.

The denaturation of LDH activity of the tissue homogenates as well as of the isozymes tested showed a greater denaturation against α -ketobutyrate than against pyruvate. So it would seem that pyruvate has a stabilizing effect on the enzyme molecule. This is probably because the active centre of the enzyme takes part in the maintenance of the tertiary structure of enzyme molecule. It has also been observed that there is a greater competitive inhibition of LDH fractions by oxamate when α -ketobutyrate was used as substrate than when pyruvate was used⁸. Furthermore, the use of α -ketobutyrate as substrate does not make possible such a clear differentiation between LDH_1 and LDH_5 as does pyruvate, and does not seem so suitable for clinical application. An even more similar denaturation was noted when β -mercaptoethanol was used, with pyruvate as substrate, on crystalline LDH_1 and LDH_5 tested (Fig. 4).

It should be noted that the enzymatic reaction was initiated by means of co-enzyme in order to avoid the formation of enzyme-co-enzyme complex during the incubation. It has been suggested that the formation of this complex would have some effect on the denaturation with various denaturants⁹ and with various co-enzymes^{9,10}. In our preliminary investigations we also noted that NADH₂ may have some effect on the urea denaturation.

We have also tested the denaturing effect of urea (1.5 M) on the LDH activity of added heart and liver

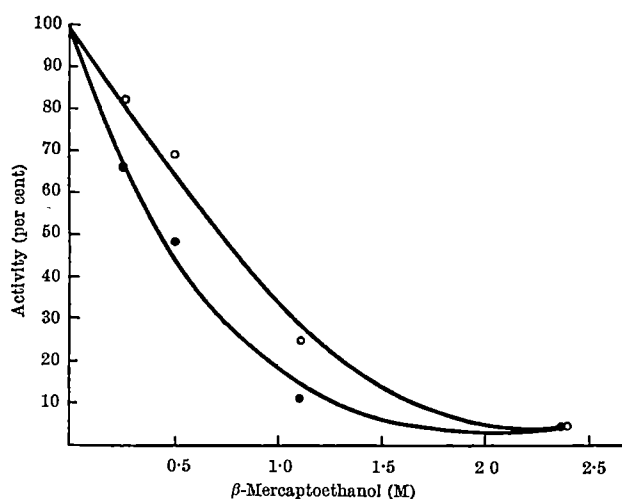


Fig. 4. Percentage enzymatic activity of crystalline pig heart LDH, (O) and rabbit muscle LDH, (●) isozymes after inhibition with β -mercaptoethanol at the concentrations indicated. Pyruvate was used as substrate.

homogenates in normal human serum in order to simulate more closely the situation which arises in myocardial infarction. In these experiments it was verified, by calculation on the base of data observed with homogenates alone, that the degree of denaturation by urea in the serum corresponded with that received. Our observations of denaturation with 1.5 M urea in the conditions described indicate that this technique would be useful for separating the monomers occurring preponderantly in the heart tissue from others for clinical purposes; certainly the test described is simple to carry out.

After we had prepared our communication, Hardy (*Nature*, 206, 933; 1965) published work concerning the denaturation of LDH of human and liver homogenates by urea. The concentration used by him was higher (2.6 M). At this concentration our curves show nearly complete denaturation of all isozymes studied. This difference might well be due to the shorter incubation period used by him. Also the fact that the co-enzyme was added to the incubation solution may contribute to differences in the results.

This work was supported by the Sigrid Jusélius Foundation, Helsinki, Finland.

AARNE KONTTINEN
SEPPO LINDY

Wihuri Research Institute,
Helsinki, Finland.

- ¹ Appella, E., and Markert, C. J., *Biochem. Biophys. Res. Comm.*, **6**, 171 (1961).
- ² McKav, R. H., and Kaplan, N. O., *Biochim. Biophys. Acta*, **52**, 156 (1961).
- ³ Plummer, D. T., Wilkinson, J. H., and Withycombe, W. A., *Biochem. J.*, **89**, 48P (1963).
- ⁴ Brody, I. A., *Nature*, **201**, 685 (1964).
- ⁵ Plagemann, P. G. W., Gregory, K. F., and Wróblewski, F., *J. Biol. Chem.*, **235**, 2282 (1960).
- ⁶ Fine, I. H., and Costello, L. A., in *Methods in Enzymology*, edit. by Colowick, S. P., and Kaplan, N. O., **6**, 958 (Academic Press, New York, London, 1963).
- ⁷ Cahn, R. D., Kaplan, N. O., Levine, L., and Zwilling, E., *Science*, **136**, 962 (1962).
- ⁸ Plummer, D. T., and Wilkinson, J. H., *Biochem. J.*, **87**, 423 (1963).
- ⁹ Di Sabato, G., and Kaplan, N. O., *J. Biol. Chem.*, **239**, 438 (1964).
- ¹⁰ Shifrin, S., Kaplan, N. O., and Clott, M. M., *J. Biol. Chem.*, **234**, 1555 (1959).

Ultra-violet Spectroscopy in the Analysis of Chromatograms

THE sensitivity of the normal human eye to light does not extend far below 400 m μ . Nevertheless, ultra-violet-absorbing compounds appear as dark spots on paper chromatograms illuminated with ultra-violet light. This is due to absorption of the light before it can excite the fluorescence of the paper, and constitutes one variety of quenching. Compounds which fluoresce in ultra-violet light will be detected on paper chromatograms if their

fluorescence exceeds that of the surrounding paper. The light sources commonly employed emit the mercury lines at 254 m μ or 366 m μ . In chromatograms of biological extracts the short-wave lamp detects principally the absorbing compounds and the long-wave lamp fluorescing compounds. Beyond this the method possesses little inherent specificity. Thus compounds such as cortisone, nicotinic acid, caffeine and uric acid (λ_{max} 240, 260, 273 and 292 m μ respectively) all appear indistinguishable as dark spots under the short-wave lamp. Furthermore, for substances such as oestrogens, the absorption maximum (280 m μ) of which is very different from the 254 m μ emission of the handlamp, the method is relatively insensitive. A need therefore exists for an instrument with which absorption and activation maxima can be rapidly determined on the paper. I have accordingly designed an ultra-violet spectroscopie in which the ultra-violet light is shifted to the visible region by the fluorescence of either the paper itself or a superimposed screen.

The light source, an 'Osram' 450-W xenon arc, provides an ultra-violet continuum. After passing through a quartz prism monochromator (Schoeffel Instrument Co., Westwood, N.J.) with variable exit slit, the light emerges through a $1\frac{1}{8} \times \frac{3}{4}$ in. rectangular aperture as a slightly divergent beam which illuminates the chromatogram. The latter is attached by spring clips to a frame, which can be moved manually in three planes, or by a motor in the direction of solvent flow only. A screen coated with a suitable phosphor can be placed in position over the illuminated portion of the chromatogram if desired. Among several phosphors examined, $ZnSiO_4:Mn$ and $(Sr, Mg)_3(PO_4)_2:Sn$ were found to be the most generally useful. The former is slightly phosphorescent, but not excessively so. It provides a bright illumination over the range 230–295 m μ and 330–400 m μ . The latter exhibits a continuous though less intense fluorescence over the range 230–370 m μ , and is therefore suitable for rapid, routine searching. To obtain linear wave-length drive the prism is rotated by means of a cam, which is attached to a digital counter. The optical arrangement is shown in Fig. 1, and the assembled apparatus in Fig. 2.

In order to make a visual estimate of the absorption maximum of a compound on a chromatogram, the wave-length knob is rotated until the spot is darkest. The fluorescent screen may be needed to provide adequate background illumination. With certain compounds the maximum is best approached from higher wave-lengths, as the absorption does not always fall off as sharply below the maximum. It is desirable that the brightness of the paper or screen should remain constant while the wave-length of the incident light is varied. To accomplish this a second cam can be used to vary the slit width at different wave-lengths, so as to compensate for changes in the output of the xenon arc, the dispersion of the monochromator, and the spectral response of the paper or screen phosphor.

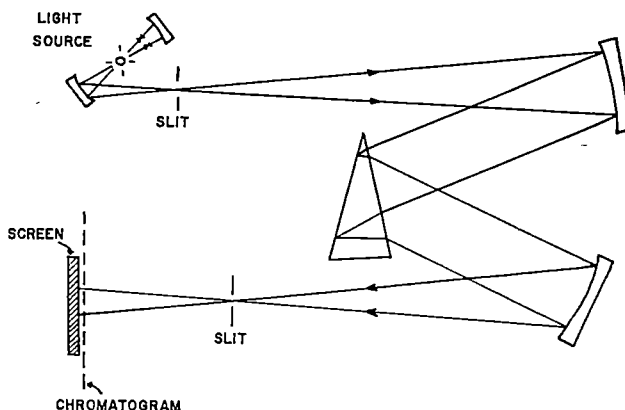


Fig. 1. Optical arrangement of the ultra-violet spectroscopie

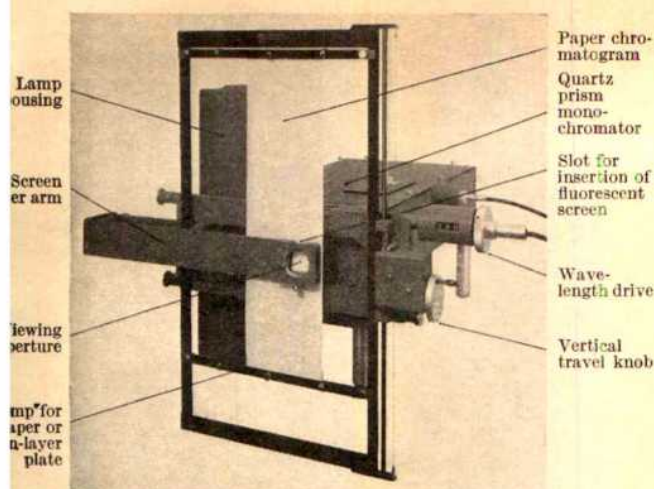


Fig. 2. The assembled spectroscope

If 'Kodabromide F5' or other suitable photographic paper¹ is placed in contact with the chromatogram, a record of absorbing or fluorescing² compounds can be obtained at any desired wave-length within the operating range of the instrument. If the entire strip is to be photographed the rectangular aperture is replaced by a narrow slit, past which the paper is drawn either by hand or by means of a motor. Arnold *et al.*³ described a similar arrangement for photographing chromatograms, but they used a mercury source, which would only be suitable for observations at certain wave-lengths.

For the determination of the activation spectrum of a fluorescent compound the screen is best avoided, as its fluorescence may mask that of the compound under study. The wave-length knob is rotated until maximal fluorescence is produced. The point at which this occurs will differ slightly from the true activation maximum of the compound, because the intensity of the light issuing from the monochromator increases with wave-length (unless the slit is automatically adjusted by means of a cam). However, a reasonable approximation can be made.

Another application lies in the analysis of thin-layer chromatograms. For this purpose the addition of 10 per cent $\text{ZnSiO}_4 \cdot \text{Mn}$ to the solid phase converts the plate itself into a fluorescent screen. Better contrast is obtained with this technique than with the screen superimposed on a paper chromatogram, which absorbs a significant proportion of the incident light. It was found that quantities of oestrogens which were barely detectable under the 254 m μ handlamp could easily be seen with the spectroscope set at 280 m μ .

The foregoing description has chiefly been concerned with the analysis of compounds on chromatograms. Analogous results can be obtained with compounds in solution in quartz cuvettes. To facilitate the observation of small changes in optical density a reference cell containing the solvent alone is placed alongside the unknown solution. With the aid of the fluorescent screen, absorption maxima can be estimated in exactly the same way as on paper. For example, as little as 2 μg cortisol in 1 ml. absolute ethanol could be spectrally analysed; and the end-absorption of absolute ethanol was discernible below 220 m μ in comparison with a reference cell containing water.

The apparatus can be adapted for automatic scanning of chromatographic strips at a given wave-length, or for recording the absorption spectra of compounds on chromatograms. Modifications of existing spectrophotometers for such purposes have been described by others⁴⁻⁶. The principal advantage of the present technique is that it provides a tool of high sensitivity, permitting the

spectral characteristics of compounds to be studied visually. Furthermore, areas which appear only as streaks on conventional ultra-violet illumination can often be shown by spectroscopy to contain several discrete spots with different absorption or activation maxima. In this way, full advantage can be taken of the high resolving power of the paper or thin-layer chromatogram. These developments have been made possible by the availability of sufficiently intense sources emitting a continuous spectrum in the ultra-violet region.

This work was supported by grants AM-5912 and 2A-5370 from the National Institutes of Health.

DENIS M. ABELSON

Department of Medicine,
The Graduate Hospital,
University of Pennsylvania.

¹ Abelson, D. M., and Rosenfeld, L., *J. Chromatog.*, **9**, 522 (1962).

² Abelson, D. M., *Nature*, **188**, 850 (1960).

³ Arnold, W., Bührer, R., Euse, J. v., Lüscher, E., Schindler, O., Stich, K., Zoller, P., and Reichstein, T., *Helv. Chim. Acta*, **46**, 178 (1963).

⁴ Tennent, D. M., Whitt, J. E., and Florey, K., *Anal. Chem.*, **23**, 1748 (1951).

⁵ Brown, J. A., *Anal. Chem.*, **25**, 774 (1953).

⁶ Brown, J. A., and Marsh, M. M., *Anal. Chem.*, **25**, 1865 (1953).

Accumulation and Incorporation of Amino-acid in Rat Intestine *in vitro*

INVESTIGATIONS of the absorption of amino-acids from the intestinal tract have usually been concerned with a single amino-acid or, in some cases, simple mixtures of a few amino-acids¹⁻³. Under physiological conditions, on the other hand, an initial stage in the assimilation of dietary proteins is the enzymatic liberation during digestion of a mixture composed of peptides and 18-20 free amino-acids⁴. Because of the interactions between the amino-acids in competing for several relatively specific transfer mechanisms, the total rate of absorption of amino-acids from a mixture need not be closely related to the absorption rates of the same amino-acids when measured singly, but will depend on the composition of the mixture present in the intestinal lumen⁴. There is also evidence that during absorption for 1 h of glycine *in vitro*⁵ and of protein hydrolysate *in vivo*⁶ there is an incorporation of the absorbed amino-acids into the protein of rat intestinal mucosal cells. This communication records an investigation of amino-acid transport and protein synthesis in rat intestine during absorption of an amino-acid mixture *in vitro*.

Amino-acid transport was measured over 8 min as the entry of ¹⁴C-labelled amino-acids into the tissue water and proteins of rings of jejunum prepared from male albino rats ranging in weight from 260 to 300 g. The rings⁷ (2-5 mg dry weight) were incubated in 2.5 ml. of medium containing a mixture of 18 amino-acids giving a total amino-acid concentration of 1.7 mM, and containing 0.4 μC of a ¹⁴C-labelled protein hydrolysate ('CFB 25', Radiochemical Centre, Amersham). The incubations were terminated by the addition of an excess of an unlabelled casein hydrolysate and the rings rapidly removed from the medium, blotted, and transferred to 3 ml. 0.5 M perchloric acid at 0-5° C. The tissue was dispersed with a glass rod and the precipitated protein removed by centrifugation. An aliquot of the supernatant was counted to give a measure of the amount of labelled material in the perchloric acid extract. A correction was made for the carry-over of label from the medium by counting similar aliquots of perchloric acid extracts of rings incubated for 15 sec in a medium identical except for the presence of the excess of unlabelled casein hydrolysate. Apart from this correction no attempt was made to allow for the extracellular space or for any non-absorbing tissue present in the rings. If such corrections were made, the ratio of the concentration of amino-acids in the tissue fluid to that in the medium (T/M) would tend to increase when the ratio was greater than 1.

To measure the amount of amino-acid incorporation, the precipitated protein was washed in perchloric acid, dissolved in N sodium hydroxide, and then reprecipitated. This process was repeated several times according to the method of Truman and Korner⁸. Both the acid and the base used in this procedure contained an excess of unlabelled casein hydrolysate. The precipitated protein was then extracted with lipid solvents, dried with ether, dissolved in formic acid and finally transferred to a previously weighed planchet. After the sample was dried, the planchet was re-weighed and counted.

In Table 1 the figures for transport show the total amount of amino-acid entering the rings during incubation and, therefore, includes both amino-acids incorporated into protein, as well as 'free' amino-acids. The T/M values show the ratio of 'free' amino-acid (that is, the radioactivity in perchloric acid soluble form) in the tissue water to that in the medium at the end of the incubation. It is evident that mucosal tissue *in vitro* can accumulate amino-acids from a protein hydrolysate to give a value of T/M which is significantly greater than 1. However, the substitution of K⁺ for Na⁺ in the incubation medium reduced the rate of transport to a low level and prevented accumulation. It seems significant to us that the only condition in which we failed to observe an accumulation of amino-acids was when the cation gradient between the cells and medium was abolished. In other similar experiments in which Na⁺ was replaced by Li⁺ or choline, T/M values significantly greater than 1 were obtained. Table 1 also indicates that in the presence of glucose neither anoxic conditions, 2,4-dinitrophenol (5×10^{-4} M) nor oligomycin ($10 \mu\text{g ml}^{-1}$) prevented accumulation from taking place, and oligomycin actually increased the rate of transport. These findings suggest that the energy for amino-acid transport is provided by the movement of ions, and that these movements can be sustained by energy derived from glycolysis rather than from oxidative phosphorylation. Thus it has previously been found that in the presence of glucose, the mucosal tissue K⁺ is maintained under anaerobic conditions⁹.

Table 1. NET ACCUMULATION OF A MIXTURE OF AMINO-ACIDS IN RINGS OF RAT INTESTINE AFTER 8 MIN ABSORPTION. INCUBATION MEDIUM: HCO₃-RINGER, 500 MG/100 ML. GLUCOSE

Values are of means \pm S.E.M. (No. of observations)			
Predominant cation incubation medium	Additions	Net amino-acid transport 10^{-4} M/mg tissue dry weight	T/M
Na ⁺	None	20.4 \pm 1.4 (10)	2.57 \pm 0.25 (10)
K ⁺	None	8.1 \pm 1.3 (6)	0.86 \pm 0.14 (6)
Na ⁺	5×10^{-4} M DNP	22.2 \pm 2.9 (9)	2.50 \pm 0.30 (9)
Na ⁺	10 $\mu\text{g/ml}$ oligomycin	32.7 \pm 2.7 (9)	3.72 \pm 0.31 (9)
Na ⁺	'Anaerobic' (average pO_2 7.5 torr)	19.9 \pm 1.9 (6)	2.26 \pm 0.23 (6)

Table 2. NET INCORPORATION OF A MIXTURE OF AMINO-ACIDS INTO THE PROTEIN OF RINGS OF RAT INTESTINE AFTER 8 MIN INCUBATION

Values are of means \pm S.E.M. (No. of observations)			
Predominant cation incubation medium	Additions	Amino-acid incorporated into protein 10^{-4} M/mg. tissue dry weight	% incorporation
Na ⁺	None	2.31 \pm 0.34 (10)	11.2 \pm 1.3 (10)
K ⁺	None	0.64 \pm 0.028 (6)	9.0 \pm 1.5 (6)
Na ⁺	5×10^{-4} M DNP	0.26 \pm 0.04 (9)	1.4 \pm 0.27 (9)
Na ⁺	10 $\mu\text{g/ml}$ oligomycin	2.32 \pm 0.45 (9)	6.9 \pm 0.96 (9)
Na ⁺	'Anaerobic' (average pO_2 7.5 torr)	0.13 \pm 0.03 (6)	0.7 \pm 0.2 (6)

Table 2 shows, for the same experiments as those in Table 1, the amount of amino-acid incorporated in protein together with the percentage of the total amount of amino-acid entering the jejunal rings which is incorporated in the protein. It is seen that of the amino-acids taken up by the tissue, a remarkably high proportion was incorporated into protein during the 8 min incubation period. Although the substitution of K⁺ for Na⁺ reduced the absolute extent of the incorporation, it

did not change the percentage incorporation. The same rough correspondence between changes in transport rate and changes in the extent of incorporation has been noted over a range of different conditions. However, unlike transport, the incorporation of amino-acids into protein requires energy supplied by oxidative phosphorylation, since dinitrophenol and anoxia virtually abolished incorporation. Oligomycin, at a concentration sufficient to slow the rate of oxygen uptake significantly, did not inhibit the amino-acid incorporation.

We conclude that our findings are consistent with the view that the existence of a cation gradient between the mucosal fluid and the mucosal cells appears to be a necessary condition for the 'active' transport of amino-acids into these cells and that, in the presence of glucose, such a gradient can be maintained without the participation of energy from aerobic sources. The processes underlying the incorporation of amino-acids into the proteins of the absorbing cells, although dependent on the rate of transport of the amino-acids into the cells, have, in contrast, an absolute requirement for energy derived from oxidative reactions. The fact that the incorporation of amino-acid is independent of the presence of oligomycin indicates that, as is the case for both ion accumulation^{10,11} and amino-acid incorporation¹²⁻¹⁴ in mitochondria, ATP may not be an obligatory intermediary in supply of energy for these processes.

J. R. BRONK*
D. S. PARSONS

Department of Biochemistry,
University of Oxford.

* Fellow of the J. S. Guggenheim Memorial Foundation.

¹ Wiseman, G., *J. Physiol.*, **120**, 63 (1963).

² Wilson, T. H., *Intestinal Absorption* (W. B. Saunders Co., Philadelphia, 1962).

³ Wiseman, G., *Absorption from the Intestine* (Academic Press, London, 1964).

⁴ Gitler, C., in *Mammalian Protein Metabolism*, edit. by Munro, H. N., and Allison, J. B., 1 (Academic Press, London, 1964).

⁵ Winnick, T., Friedberg, F., and Greenberg, D. M., *Arch. Biochem.*, **15**, 160 (1947).

⁶ Dawson, R., and Holdsworth, E. S., *Brit. J. Nutr.*, **16**, 13 (1962).

⁷ Agar, W. T., Hird, F. J. R., and Sidhu, G. S., *Biochim. Biophys. Acta*, **14**, 80 (1954).

⁸ Truman, D. E. S., and Korner, A., *Biochem. J.*, **83**, 588 (1962).

⁹ Brown, M. M., and Parsons, D. S., *Biochim. Biophys. Acta*, **59**, 249 (1962).

¹⁰ Brierley, G. P., Murer, E., and Green, D. E., *Science*, **140**, 60 (1963).

¹¹ Chappell, J. B., Grenville, G. D., and Bicknell, K. D., *Biochem. J.*, **84**, 61P (1962).

¹² Kroon, A. M., *Biochim. Biophys. Acta*, **72**, 391 (1963).

¹³ Truman, D. E. S., and Löw, H., *Exp. Cell Res.*, **31**, 230 (1963).

¹⁴ Bronk, J. R., *Proc. U.S. Nat. Acad. Sci.*, **50**, 524 (1963).

Changes in Amino-nitrogen Content of Solutions of γ -Keratose from Wool Keratin

NUMEROUS workers¹⁻³ have fractionated wool keratin after oxidation of disulphide bonds with performic or peracetic acids into three fractions, α , β and γ -keratose (β -keratose is insoluble in water, α -keratose is that fraction precipitating at pH 4 and γ -keratose the remaining fraction). While these fractions vary somewhat in the amounts of terminal amino-acids⁴, no comments have yet been made on any changes in constitution of the isolated fraction of γ -keratose on re-dissolution in water. As an introduction to some work on the hydrolysis of γ -keratose (prepared by the method of Corfield, Robson and Skinner²), the free amino-nitrogen value was determined by reaction with nitrous acid, the method of Van Slyke^{5,6}, and it was found that reproducible results were not obtained on samples until the solution had been standing for some time. Accordingly the determination of amino-nitrogen of γ -keratose (10 mg/ml.) at various periods of time after dissolution was carried out and plotted as a function of time in acid (0.2 M potassium hydrogen phthalate buffer, pH 4), alkaline (0.2 M boric acid in 0.2 M potassium chloride buffer, pH 10), salt (0.2 M potassium chloride) and simple aqueous solution. The results are shown in Fig. 1.

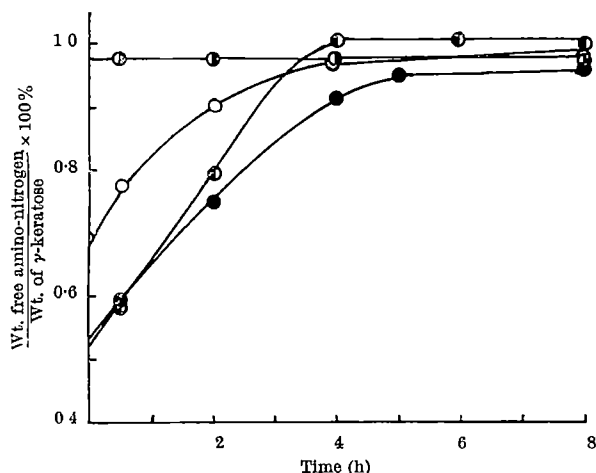


Fig. 1. Amino-nitrogen of γ -keratose. \bullet , in water; \circ , in 0.2 M potassium chloride; \bullet , in buffer, pH 10, \bullet , in buffer, pH 4

With the exception of γ -keratose under acid conditions, the amino-nitrogen value rose to a steady value over a period of time, the time depending on the pH of the solution. Although apparently instantaneous in acid solution, the equilibration time is longer in simple aqueous solution than in salt or alkaline solution.

This effect was definitely not bacteriological decomposition, as deliberate introduction of non-sterile air on particular runs showed increased amino-nitrogen figures after a lag of about two days.

Use of the biuret reaction⁷ indicated no peptide bond breakdown, as readings taken 20 min and 24 h after dissolution showed no statistical difference. $[\alpha]_D$ is very sensitive to the configuration of the peptide chain⁸, but on redissolution of γ -keratose no significant changes in $[\alpha]_D$ were observed.

The reason for this change therefore remains unexplained, but for further structural studies it would appear safer either to equilibrate the solution by standing or treating with acid at pH 4 before use.

R. S. ASQUITH
P. A. WATSON

Department of Chemical Technology,
Bradford Institute of Technology.

Alexander, P., and Hudson, R. F., *Wool: Its Chemistry and Physics* (Chapman and Hall, Ltd., London, 1954).

² Corfield, M. C., Robson, A., and Skinner, B., *Biochem. J.*, **68**, 348 (1958).

³ O'Donnell, I. J., and Thompson, E. O. P., *Austral. J. Biol. Sci.*, **12**, 294 (1959).

⁴ Alexander, P., and Smith, L. F., *Proc. Intern. Wool Res. Conf., Austral.*, Vol. B, B-56 (1955).

⁵ Milton, R. F., and Waters, W. A., *Methods of Quantitative Micro-analysis*, 576 (Edward Arnold Ltd., London, 1955).

⁶ Van Slyke, D. D., *J. Biol. Chem.*, **83**, 425 (1929).

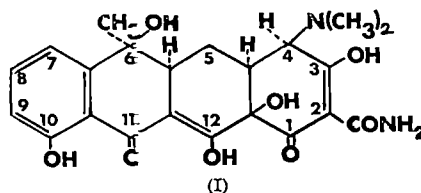
⁷ Gornall, A. G., Bardawill, C. J., and David, M. M., *J. Biol. Chem.*, **177**, 755 (1949).

⁸ Imahori, K., *Biochim. Biophys. Acta*, **37**, 366 (1958).

Binding of Tetracyclines to Bone

TETRACYCLINE (I) (ref. 1) and its derivatives oxytetracycline, chlortetracycline and (1-pyrrolidinylmethyl)-tetracycline² are taken up in newly-formed bone after injection into the living organism, to form a zone that is intensely fluorescent under ultra-violet light. This reaction, which occurs wherever there is active deposition of new bone³, can be used for the detection of calcification. Because mineralized dead bone also takes up tetracycline on every surface (including, although to a lesser extent, the non-growing surfaces)⁴, the reaction does not depend on the activity of living bone cells or on the integrity of the physical properties of the intercellular components. Instead, there appears to be a direct reaction between tetracycline and one or more inorganic components of the mineralized tissue, the most likely species being

hydroxyapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, which is the major constituent of all such tissue⁵. A plausible suggestion as to the nature of the bonding involved is now offered.



Tetracyclines have well-defined metal-complexing abilities⁶, and spectra evidence indicates that the phenolic β -diketone group attached to carbons 10, 11, and 12 is concerned in complex formation with calcium and other metal ions⁷. However, if the attachment of tetracyclines to bone surfaces is through the calcium ions of the latter, the binding is much stronger than would be expected from the stability of the metal complexes in aqueous solution. For example, the stability constant of the 1:1 magnesium-tetracycline complex⁸ is only about 10^4 , and for electrostatic reasons the corresponding calcium complex should have a comparable, or even smaller, stability constant. Present observations suggest that this relative weakness is more than offset by a particularly favourable stereochemistry of the hydroxyapatite crystal.

Using the published^{9,10} crystal structure data, models of several unit cells of hydroxyapatite have been constructed to a scale of 0.8 in. \equiv 1 Å. For this purpose, pointed 1/8-in. diameter brass rods, carrying short pieces of plastic tubing to denote the positions of atoms, were inserted into the appropriate positions of a grid drawn on a polyurethane baseboard. This scale was chosen to be the same as for the Courtauld atomic models used in constructing a molecule of tetracycline of the correct¹⁰ stereochemistry.

When the tetracycline molecule was placed edge-on to the hydroxyapatite structure (that is, perpendicular to the a, b plane), there was a close superposition of three oxygen atoms of the former on to three calcium ions of the latter, provided the tetracycline replaced two phosphate ions. The relevant calcium and phosphate ions are indicated in Fig. 1. (For the complete structure of hydroxyapatite, see ref. 8.) These calcium ions fit, within about 0.1 Å, the oxygen atoms (at least one of which is present as an anion) on C_{10} and C_{12} , and the oxygen of the carbamoyl group on C_2 while, at the same time, the structure remains sufficiently open to enable the insertion of any hydroxyl ions that may be needed to maintain electrical neutrality. I suggest that it is the ability to form bonds through all three positions concurrently and without steric strain that is responsible for the observed strong adhesion of tetracyclines to bone surfaces.

In the resulting complex the tetracycline molecule presents an essentially hydrophobic surface to its environment, so that there would be little tendency for the complex to dissociate in aqueous media. Also, the portion of the tetracycline molecule that is substituted in forming 5-hydroxy- (terramycin), 7-chloro- (aureomycin), 7-bromo-, and similar derivatives is remote from the binding

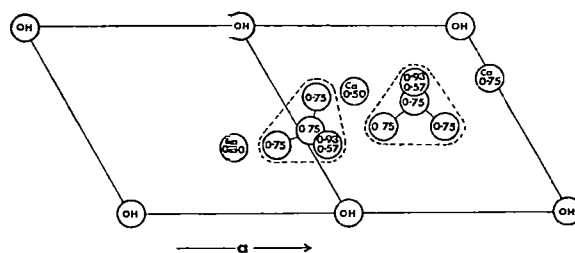


Fig. 1. Projection of two unit cells of hydroxyapatite on the a, b plane, to show the locations of the calcium ions on which tetracycline can be fitted. Dimensions are (ref. 3) $a = b = 9.432$ Å, c (perpendicular to a and b) = 6.881 Å. The broken lines enclose the phosphate groups that are displaced by the tetracycline. Numbers indicate heights in terms of c .

sites and hence exerts no direct steric effects. Fluorapatite is isomorphous with hydroxyapatite, and crystal dimensions¹¹ are similar, so that the same type of tetracycline binding probably also occurs in fluorapatite.

D. D. PERRIN

Department of Medical Chemistry,
John Curtin School of Medical Research,
Australian National University,
Canberra, Australia.

- ¹ Milch, R. A., Rall, D. P., and Tobie, J. E., *J. Nat. Cancer Inst.*, **19**, 87 (1957), *J. Bone Joint Surg.*, **40**, A, 897 (1958). Rall, D. P., Loo, T. L., Lane, M., and Kelly, M. G., *J. Nat. Cancer Inst.*, **19**, 79 (1957).
- ² Branceni, D., and Levy, J., *C. R. Soc. Biol.*, **155**, 1489 (1961).
- ³ Harris, W. H., Jackson, R. H., and Jowsey, J., *J. Bone Joint Surg.*, **44**, A, 1308 (1962).
- ⁴ Deleu, J., and Bohr, H., *Nature*, **204**, 1103 (1964).
- ⁵ De Jong, W. F., *Rec. Trav. Chim.*, **45**, 445 (1926).
- ⁶ Albert, A., *Nature*, **177**, 433 (1956).
- ⁷ Conover, L. H., *Chem. Soc. Spec. Publ.*, No. 5, 48 (1956).
- ⁸ Kay, M. I., Young, R. A., and Posner, A. S., *Nature*, **204**, 1050 (1964).
- ⁹ Posner, A. S., Perloff, A., and Diorio, A. F., *Acta Cryst.*, **11**, 308 (1958).
- ¹⁰ Hochstein, F. A., Stephens, C. R., Conover, L. H., Regma, P. P., Pasternack, R., Gorda, P. N., Pilgrim, F. J., Brunings, K. J., and Woodward, R. B., *J. Amer. Chem. Soc.*, **75**, 5455 (1953). Fields, T. L., Kende, A. S., and Boothe, J. H., *J. Amer. Chem. Soc.*, **82**, 1250 (1960).
- ¹¹ Náráyzabó, S., *Z. Krist.*, **75**, 387 (1930).

PHYSIOLOGY

Determination of Nicotine in Smokers' Urine by Gas Chromatography

THE highly controversial subject of the effects of smoking on health has been extensively investigated and reviewed in recent years^{1,2}. It has been reported that nicotine has an effect on the cardiovascular system. However, its relation to coronary disease is not fully understood. The absorption of nicotine as the result of smoking is dependent on a number of factors³. The quantitation of nicotine and its metabolites in the body fluids of smokers would be helpful for the investigation of the relation between nicotine exposure and heart disease. It should also give a more reliable estimate of the quantities of other tobacco smoke ingredients to which the body is exposed. This communication describes such a method.

Corcoran has demonstrated⁴ that nicotine is present in smokers' urine. McKennis *et al.*⁵ have found by isolation from urine that cotinine is the main metabolite of nicotine in human.

Gas chromatography has been used by Quin⁶ to detect nicotine and related compounds in tobacco and in cigarette smoke. The presence of at least sixteen basic compounds was demonstrated. This technique has been used to quantitate nicotine in tobacco⁷.

The procedure used in the work recorded here is as follows: 5-ml. portions of 24-h urine collections (smokers or non-smokers) were adjusted to pH 1 with 6 M sulphuric acid and exhaustively extracted with methylene chloride. This removed neutral, phenolic and other acidic materials. The aqueous layer was adjusted to pH 11 with 50 per cent aqueous sodium hydroxide. The liberated nitrogen bases were then extracted immediately with methylene chloride and the aqueous layer discarded. The extract was then acidified with hydrochloric acid and evaporated to dryness with nitrogen. The residue was made alkaline with 3 μ l. of 2 N aqueous sodium hydroxide to liberate the nitrogen bases and diluted with 200 μ l. of acetonitrile. Aliquots of this solution (2–3 μ l.) were injected into the gas chromatograph. To determine recoveries during the extraction procedure, nicotine randomly tritiated (specific activity 40 mc./mmole) was added to the urine. Recoveries ranged from 60 to 95 per cent.

A Glowall Corp. 'Chromalab' gas chromatograph equipped with a strontium-90/argon ionization detector was used. The column was a glass coil 12 ft. long and 3.4 mm internal diameter arranged for on column liquid injection. It was packed with 14.5 per cent SE-30

silicone gum rubber on 'Anakrom ABS', 100–110 mesh. The column was kept at 200° and argon flow rates of 10–20 c.c./min were used. The detector was operated at 1,350 V. An internal standard (3-methyl, 3-phenyl piperidine) was added to the acetonitrile solution as a retention time reference. It also served for the purpose of quantitation using the peak height ratio method. (–) Nicotine was obtained commercially. (–) Cotinine was obtained from Dr. H. McKennis. (We thank him for this, and Unimed, Inc., for gifts of other metabolites.) Both substances showed single peaks on gas chromatography.

Fig. 1 shows the separation of (–) nicotine, (–) cotinine and the internal standard. A calibration curve

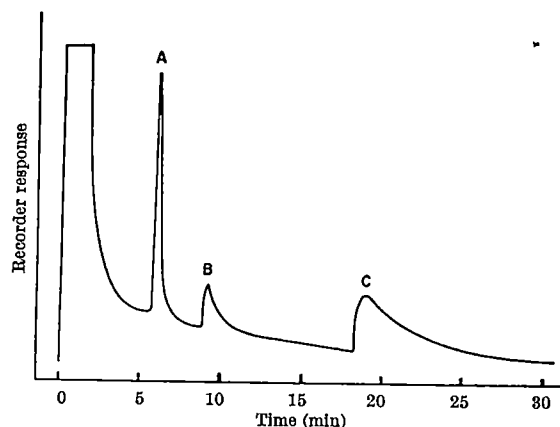


Fig. 1. Gas liquid chromatography of authentic (–) nicotine (A); 3-methyl 3-phenyl piperidine (B); and (–) cotinine (C)

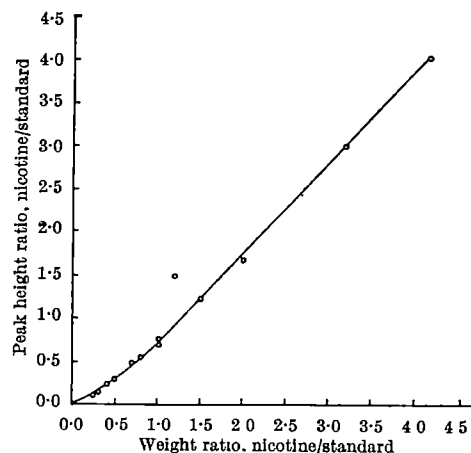


Fig. 2. Calibration curve for gas liquid chromatography of nicotine

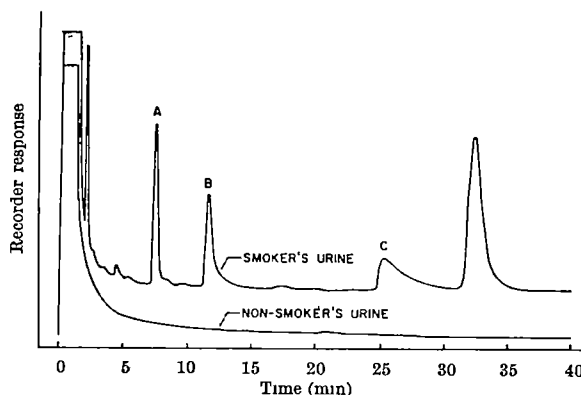


Fig. 3. Gas chromatography of extract from smoker's and non-smoker's urine. A, Nicotine; B, added standard 3-methyl 3-phenyl piperidine; C, cotinine

relating peak height ratio to weight ratio of nicotine to standard is shown in Fig. 2. The extract of a cigarette smoker's urine gave the curve shown in Fig. 3. The nicotine and cotinine peaks were identified by their retention times and by their augmentation on addition of authentic nicotine and cotinine. This curve shows that the method is selective for nicotine and cotinine since no other compounds in the urine interfere. Comparison with a non-smoker's urine confirms this (Fig. 3). The overall reproducibility of the method for nicotine was found to be ± 14 per cent $n = 4$, $P = 0.99$. Analysis of non-smokers' urine to which had been added known quantities of nicotine in the range 5–10 mg/l. showed overall recoveries of 80 and 92 per cent. Some preliminary results are shown in Table 1.

Table 1. NICOTINE IN SMOKERS' URINE

Subject	Tobacco	No. or amount	Nicotine excretion mg/day
1	Cigarettes	4	<0.1
2	"	10	0.27
3	"	25	0.74
4	"	27	1.37
3	"	30	10.5
3	"	31	5.46
5	"	31	9.06
6	"	43	5.81
7	Cigars	20 g	<0.1
8	"	55 g	8.50
9	Pipe	20 g	0.11

The variation in nicotine excretion between individuals, tobacco forms and amount of tobacco consumed is probably a reflexion of the smoking habits of the individual. Other factors which have been discussed in ref. 3 are probably important.

This work was supported by a grant from the U.S. Council for Tobacco Research.

NEAL L. MCNIVEN
KANAYA H. RAISINGHANI
SANFORD PATASHNIK
RALPH I. DORFMAN

Worcester Foundation for Experimental Biology,
Shrewsbury, Massachusetts.

¹ Rep. to the Surgeon General of the U.S. Public Health Service *Smoking and Health* (1964).

² Little, C. C., Rep. of the Scientific Director, Council for Tobacco Research, U.S.A. (1964).

³ Ref. 1, 74.

⁴ Coreoran, A. C., Halmer, O. M., and Page, I., *J. Biol. Chem.*, **128**, 89 (1939).

⁵ Bowman, E. R., Turnbull, L. B., and McKennis, jun., H., *J. Pharm. and Exp. Therapeutics*, **127**, 92 (1959).

⁶ Quin, L. D., *J. Org. Chem.*, **24**, 911 (1959).

⁷ Quin, L. D., and Pappas, N. A., *Agric. and Food Chem.*, **10**, 79 (1962).

Diurnal Variations in Ovarian Ascorbic Acid Content of Pseudopregnant Immature Rats used in Parlow's Test

DEPLETION of ascorbic acid from the ovary of the immature rat rendered pseudopregnant by treatment with pregnant mare's serum (PMS) and human chorionic gonadotrophin (HCG) is used as an assay for luteinizing hormone (LH). A significant diurnal variation in the ovarian ascorbic acid (OAA) content was observed by Stevens¹ in such rats. These variations may be important in the assay of LH by Parlow's method.

We considered it to be of interest to study the variations in the OAA content of our rats by comparing the values obtained in the morning (8–12 a.m.) with those obtained in the afternoon (4–8 p.m.) during a period February 1963–August 1964.

The values that we determined correspond to the concentration of ascorbic acid in the left ovary which acts as a control with respect to the right one in experiments

intended to study the LH-releasing substance of human brain². The concentrations are expressed in mg/100 g gland. Immature albino rats of our own breeding were injected at the age of 26 days with 50 i.u. of PMS (Gormon-Laboratorio Richter, Chile) and, 48–60 h later, with 25 i.u. of HCG (API-Ayeris Laboratories Inc.). Five to eight days later the animals were investigated, but until then the rats were housed in a special room illuminated for 14 h each day (7 a.m. until 9 p.m.) and allowed permanent access to food in the form of pellets of constant composition. The ascorbic acid content of the left ovary was measured by means of the method of Roe and Kuether³. The rats were anaesthetized by an intraperitoneal injection of 'Avertin' (Winthrop Laboratories), and the ovaries carefully dissected out and weighed on a Roller Smith torsion balance to the nearest 0.1 mg. The experiments were performed from 8 to 12 a.m. and from 4 to 8 p.m.

A total of 571 rats were used during 1963, 384 in the morning and 187 in the afternoon. During 1964, 542 rats were used, 355 in the morning and 187 in the afternoon.

The means of the morning and afternoon values (Table 1) were compared, assuming that the standard deviations were not necessarily equal⁴.

Table 1. OVARIAN ASCORBIC ACID CONCENTRATION IN THE MORNING AND IN THE AFTERNOON DURING 1963 AND 1964

	8–12 a.m.	4–8 p.m.
1963	85.34 \pm 18.23 (384)	77.28 \pm 19.67 (187)
1964	112.39 \pm 23.46 (355)	110.87 \pm 24.12 (187)

Values are expressed in mg/100 g \pm S.E. The number of test animals is shown in brackets.

The t' test of significance is given by:

$$t' = \frac{\bar{x} - \bar{y}}{\sqrt{s_x^2/n_x + s_y^2/n_y}}$$

$$v = \frac{(s_x^2/n_x + s_y^2/n_y)^2}{(s_x^2/n_x)^2 + (s_y^2/n_y)^2} - 2$$

$$(n_x + 1) + (n_y + 1)$$

All the data were processed in an IBM 1620 computer, at the Centro de Computación Universidad Católica, Santiago.

In Figs. 1 and 2 and in Table 1 it can be seen that the OAA showed higher values during 1964 than during 1963. During the low level period, morning values were significantly higher than the afternoon values ($P < 0.005$). During the 1964 period, with values permanently higher than 90 mg/100 g, the difference was not significant. The results obtained during 1963 are in keeping with those reported by V. C. Stevens¹, who found most ascorbic acid values coincident with our range of that period. These results suggest that rats with high levels of OAA may be more convenient for use in Parlow's test because they do not show such great diurnal variations as do those with low levels.

Ovarian ascorbic acid level and its sensitivity to LH depends on the luteotrophin secretion as shown by the

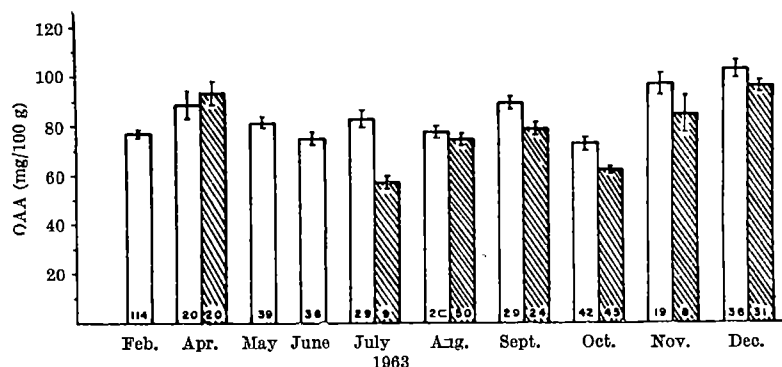


Fig. 1. Ovarian ascorbic acid concentration S.D. from February to December 1963. The number of set animals used is shown at the base of each bar. White, 8–12 a.m.; hatched, 4–8 p.m.

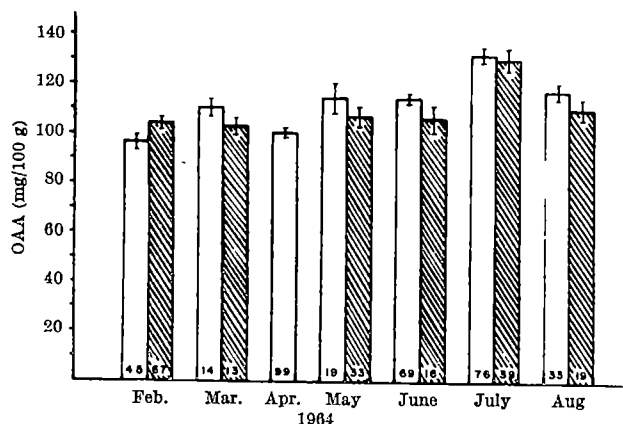


Fig. 2. Ovarian ascorbic acid concentration from February to August 1964. White, 8-12 a.m., hatched, 4-8 p.m.

rapid loss of ascorbic acid after hypophysectomy⁵⁻⁷ and the prevention of such effect by luteotrophin injection or pituitary autografts in the kidney⁸ which produce only luteotrophin.

Rats made pseudopregnant by treatment with exogenous gonadotrophins (as used for Parlow's test) show signs of luteal function, which is promoted by pituitary luteotrophin secretion during a period of 10-12 days. We may suppose that variations in luteotrophin secretion may influence the OAA concentration. Recently, R. H. Clark and B. L. Baker⁹ have shown a circadian periodicity in the concentration of luteotrophin in the rat hypophysis with highest values at 4 p.m. and no significant changes at other times of day. These changes in hormone content are most probably the consequence of changes in secretion rate, which is lowest at the time coincident with the highest pituitary concentration. This assumption enables us to explain the lower levels of ascorbic acid in afternoon determinations, because during this period of the day the delivery of luteotrophin is low.

We can offer no explanation for the difference in OAA observed between 1963 and 1964; the method of handling the animals was the same throughout the whole period.

We thank E. Bollo, Centro de Computación Universidad Católica, Santiago, for undertaking the statistical analysis of the results, and Miss Gladys Suarez for her help.

This work was supported by the U.S. Air Force under grant AF-AFOSR-447-63, monitored by the Air Force Office of Scientific Research, Office of Aerospace Research.

M. DE LA LASTRA
H. CROXATTO

Laboratorio de Fisiología,
Escuela de Medicina,
Universidad Católica,
Santiago de Chile.

¹ Stevens, V. C., Owen, L., Fukushima, M., and Vorys, N., *Endocrinology*, **74**, 493 (1964).

² de la Lastra, M., and Croxatto, H., *Nature*, **204**, 583 (1964).

³ Roe, J. H., and Kuether, C. A., *J. Biol. Chem.*, **147**, 399 (1943).

⁴ Bowker, A. H., and Lieberman, G. J., in *Engineering Statistics* (Prentice Hall, Inc., Englewood Cliffs, N.J., 1960).

⁵ McCann, S. M., Taleisnik, S., and Friedman, H. M., *Proc. Soc. Exp. Biol. and Med.*, **104**, 432 (1960).

⁶ Courrier, R., Guillemain, R., Jutisz, M., Sakiz, E., and Aschheim, P., *C.R. Acad. Sci., Paris*, **253**, 922 (1961).

⁷ Guillemain, R., and Sakiz, E., *Endocrinology*, **72**, 813 (1963).

⁸ Baldr, J. M., Wolf, R. O., and Rennels, E. G., *Proc. Soc. Exp. Biol. and Med.*, **106**, 362 (1961).

⁹ Clark, R. H., and Baker, B. L., *Science*, **143**, 375 (1964).

Intracellular Chloride Activity of Crayfish Giant Axons

RECENT measurements of chloride concentrations in the squid giant nerve axon have indicated that intracellular chloride ion is of higher concentration than would be expected if chloride ions were distributed across the cell

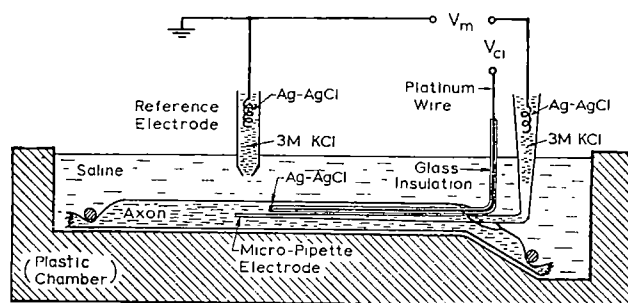


Fig. 1

membrane in equilibrium with the transmembrane potential¹. Measurements of intracellular chloride activity of squid giant axons with an Ag-AgCl electrode have also indicated higher intracellular chloride activities than could be accounted for from equilibrium considerations². Similarly, it has also been found in smooth muscle that intracellular chloride is of higher concentration than expected^{3,4}. In this communication, experiments are described which indicate that the intracellular chloride activity of crayfish giant axons (*Procambarus clarkii*) is higher than that expected from considerations based on chloride being distributed in equilibrium with the membrane potential. These experimental results are based on chloride activity measurements made with an intracellular Ag-AgCl electrode.

The experiments consisted of dissecting free from crayfish the ventral nerve chord, which contains several giant axons, and mounting the isolated nerve chord in a chamber (Fig. 1) containing van Harreveld's saline of the following composition: NaCl, 205 mM; KCl, 5.4 mM; CaCl₂, 13.5 mM; MgCl₂, 2.6 mM; NaHCO₃, 2.3 mM; pH adjusted to 7.4 by adding small amounts of HCl.

In the chamber holding the nerve was also placed a long KCl-filled micro-pipette cemented with insulating lacquer to an Ag-AgCl electrode. These electrodes were inserted length-wise into a giant axon from one end of the nerve. Connecting the physiological saline to earth was a reference KCl-AgCl electrode. The micro-pipette served to record transmembrane potential when inserted into the nerve axon and also acted as a reference electrode for the Ag-AgCl electrode for measuring chloride activity. The Ag-AgCl electrodes were made by pulling out 1-2 mm-diam. glass tubing inside of which was coiled 25 micron diameter wire of either silver or platinum. Long glass-covered lengths of wire were obtained with diameters initially about 50μ which increased to 100μ after 5-10 mm. Silver was plated on to the exposed wire end from an AgNO₃ and KCN plating solution. Silver chloride was deposited on the silver plate by chloriding in a 0.1 N HCl solution until 15-25 per cent of the silver plate was chlorodized. In some instances, the electrodes made with silver wire were chlorided directly without the additional silver plating being deposited. In general, the electrodes with silver plate on platinum wire were more stable. Erratic behaviour and incorrect potential changes with chloride shifts occurred occasionally with the electrodes, most often with the electrodes utilizing silver wire. This erratic behaviour was found to result in part from poor sealing of the wire to the glass insulation which exposed unchlorided metal to the solutions. For this reason, the electrodes were always calibrated before use.

Both the external bath reference electrode and cannulating micro-pipette electrode were filled with 3 M KCl to minimize liquid junction potentials and connected to Ag-AgCl electrodes. Potentials were recorded with an electrometer amplifier. The micro-pipette electrodes had tip diameters of less than 1μ to minimize the effect which chloride diffusion from the pipette tip would have on intracellular activities. The magnitude and importance of this chloride diffusion were investigated by measuring the

chloride activity gradient near the tip of a 3 M KCl-filled micro-pipette placed inside a giant axon. To accomplish this, a 3 M KCl-filled micro-pipette was inserted into one end of the axon, and from the opposite end of the axon the Ag-AgCl electrode was inserted and advanced toward the micro-pipette, which was held stationary. No change in measured chloride activity was detected until the Ag-AgCl electrode touched the micro-pipette tip, and even then the potential change in the chloride electrode was less than one millivolt. The intracellular chloride was therefore considered unaffected by chloride diffusion from the tip of the micro-pipette electrode.

Before the electrodes were inserted into one of the giant axons to measure membrane potential and chloride activity, calibration of the Ag-AgCl electrode was performed to determine if the electrodes functioned satisfactorily for measuring chloride activity. Calibration of the Ag-AgCl electrode was accomplished by changing the chloride activity of the van Harreveld's solution and recording the potential change of the Ag-AgCl electrode with respect to the micro-pipette electrode. Chloride was changed in the van Harreveld's solution by a one-to-one substitution with propionate or glucuronate ions in order to maintain constant ionic strength and activity coefficients. The possibility that activity coefficients might change when chloride was replaced with a weaker univalent ion was investigated by comparing the sodium activity of the different solutions with a sodium-sensitive glass electrode. These measurements showed no change in sodium activity for the different chloride solutions in which sodium and total ion concentrations were kept constant. In addition, the chloride electrodes gave the thermodynamically expected potential changes with chloride shifts, which indicated no change in activity coefficients. This indicated that activity coefficients remained constant in the different calibrating solutions. In the calibration the chloride activity changes were therefore directly proportional to the chloride concentration changes. In the external van Harreveld's solution, the activity coefficient for both the sodium and chloride ion was taken as 0.72. These activity coefficients were calculated from potential measurements obtained, with a sodium glass electrode and an Ag-AgCl electrode measured against a saturated KCl calomel cell reference, first in 0.001 M NaCl and then in van Harreveld's solution. The activity coefficients of sodium and chloride in 0.001 M NaCl were assumed equal and taken as 0.966 (ref. 5) from which the activity coefficients in van Harreveld's solution were calculated.

After determining the adequacy of the Ag-AgCl electrode for measuring chloride activities, an opening was made at one end of one of the giant axons lying in the ventral nerve chord. Into this opening was inserted the micro-pipette and Ag-AgCl electrode for distances of 5–10 mm. The measured potential of the micro-pipette with respect to the external bath electrode gave the membrane potential while the potential of the Ag-AgCl electrode with respect to the intracellular micro-pipette as a reference gave the intracellular chloride activity.

These simultaneous measurements of intracellular chloride activity and membrane potential were performed successfully on 29 different giant axons of crayfish (*Procambarus clarkii*) over a period of one year. The axon diameters varied from 100 to 150 μ , with dissected lengths of more than 3 cm. The results at average room temperature of 20° C gave a transmembrane potential of 80.3 ± 0.8 mV (mean \pm S.E.). The change in the potential of the chloride electrode corresponded to an intracellular chloride activity of 25.2 ± 2.9 mM/l. (mean \pm S.E.). If the activity coefficient of intracellular chloride is taken to be approximately that of the external physiological saline^{1,6,7}, the corresponding intracellular chloride concentration is 35 ± 4 mM/l. (mean \pm S.E.). If intracellular chloride ions are distributed in equilibrium with the transmembrane potential, the expected intracellular chloride activity should have been 7.3 mM/l. These results, which give an

intracellular chloride activity three and a half times higher than expected from equilibrium considerations, may be explained on the basis of chloride ion being actively transported in addition to sodium and possibly potassium ions. These experiments suggest, therefore, that in the crayfish nerve, and as has been suggested for the squid axon¹, none of the dominant ions is passively distributed in equilibrium in accordance with its electrochemical potentials, but instead active transport is probably more general and involves all these ions.

This work was supported in part by a grant from the National Institutes of Health, U.S. Public Health Service.

A. STRICKHOLM
B. G. WALLIN

Department of Physiology,
University of California School of Medicine,
San Francisco, California,
and
Physiological Institute,
University of Uppsala.

¹ Keynes, R. D., *J. Physiol.*, **169**, 690 (1963).

² Mauro, A., *Fed. Proc.*, **13**, 63 (1954).

³ Durbin, R. P., and Monsen, R. R., *Fed. Proc.*, **20**, No. 1 (1961).

⁴ Goodford, P. J., *J. Physiol.*, **170**, 227 (1964).

⁵ Robinson, R. A., and Stokes, R. H., *Electrolyte Solutions* (Butterworth and Co., Ltd., London, 1959).

⁶ Mullins, L. J., and Noda, K., *J. Gen. Physiol.*, **47**, 117 (1963).

⁷ Hinkle, J. A. M., *J. Physiol.*, **156**, 314 (1961).

PHARMACOLOGY

Effects of Propylthiouracil and Thyroid Feeding on the Response of Mice to Injected Convulsant Barbiturate

In 1961, Carrier and Buday¹ enumerated the substances the toxicity of which is increased in hyperthyroid animals. Among these was hexobarbital². Prange *et al.*³ showed that mice fed desiccated thyroid slept longer than controls after injection of pentobarbital, while mice fed propylthiouracil (PTU) slept less.

Ellinwood and Prange⁴ explored these relationships in connexion with epinephrine pretreatment. Both epinephrine and thyroid feeding increased sleeping time to pentobarbital, but there was no additive effect when both pretreatments were used. PTU-fed mice slept a shorter time than controls. Like controls, however, their sleeping time could be increased by epinephrine pretreatment. The duration of these effects after the cessation of the various pretreatments was delineated.

A thyroid-induced enhancement of mortality and a PTU diminution has been described also for the anti-depressant drug imipramine^{5,6}. Prange *et al.*⁷ suggest that in the case of pentobarbital, at least, the effect of PTU is not due to hypothyroidism *per se* since thyroidectomized rats sleep longer, and maintain tissue levels of pentobarbital longer, than controls. Probably PTU induces activity of liver enzymes that degrade certain substances including pentobarbital.

In light of the foregoing, it was considered of interest to study the effects of altered thyroid function on a drug that is a structural barbiturate but a pharmacologic stimulant, 5-(1,3-dimethylbutyl)-5-ethyl barbituric acid (DMBEB).

Young male Swiss-Webster white mice were given 0.2 per cent PTU in their water for 30 days, or fed 2 per cent desiccated thyroid in their food for 4 days, or given tap water and standard lab chow. DMBEB was prepared in isotonic saline, 1 mg per ml. Animals were injected intraperitoneally with various doses; 30 PTU-fed mice; 40 control mice; 30 thyroid-fed mice. All animals showed signs of gross stimulation, and those that died did so within half an hour. Terminal convulsions were usually observed. The dose-mortality data were analysed according to the method of Litchfield and Wilcoxon⁸. The following LD_{50} s were estimated with upper and lower

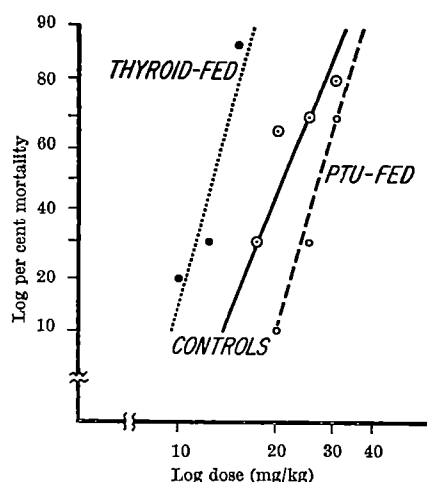


Fig. 1. Half-hour mortality of adult male Swiss Webster white mice after intraperitoneal injection of 5-(1,3-dimethylbutyl)-5-ethyl barbituric acid

confidence limits: PTU-fed, 27.0 mg/kg (31.1–23.5); controls, 21.0 mg/kg (24.8–18.0); thyroid-fed, 12.2 mg/kg (13.9–11.1). These data are shown in Fig. 1. It was found that the LD_{50} for PTU-fed mice was statistically significantly greater than for controls ($P < 0.05$); the LD_{50} for thyroid-fed mice was significantly less than for controls ($P < 0.05$).

We considered it desirable to study another parameter of drug effect. Sleep time, of course, could not be measured, as in previous experiments; we elected to measure activity. A device for this purpose was obtained from Woodard Research Corporation, Herndon, Virginia. It consists of a doughnut-shaped cage (outer diameter 14½ in.) with a wire mesh floor. A centre core emits six infra-red beams directed toward photocells in the perimeter. When a light beam is broken, the impulse is recorded on a counter where such events are shown cumulatively. An animal must purposefully move about, as in exploring behaviour, to acquire a high score.

Animals with the various pre-treatments were injected intraperitoneally every ten seconds with saline in groups of 5 (cage mates) and placed in the cage, which was covered to exclude light. After brief initial excitement, all groups showed decreasing activity reaching a stable low level after about one hour. Thyroid-fed mice showed a pattern identical to the other two groups but at a lower level of activity throughout the time span. Observation of the mice revealed that thyroid-fed mice cringed, appeared frightened and tended to huddle when left alone. Only when externally stimulated did they dart about. After saline injection, PTU-fed and control mice showed similar patterns of spontaneous activity.

Other mice from the various pretreatment groups were injected intraperitoneally with DMBEB, 5 mg/kg, and placed in the cage. All groups now showed decreased spontaneous activity as compared with respective controls. That is, they resembled and behaved like (saline-injected) thyroid-fed mice. This experiment was carried out twice, with different mice. Data were plotted for activity per minute against time. Inspection of the data showed that the DMBEB inhibition of spontaneous activity was most pronounced in thyroid-fed mice and least pronounced in PTU-fed mice. In another experiment, activity after DMBEB injection was recorded every 10 sec for 10 min and no difference in onset of drug action between groups could be shown. Here, as in other work⁷, we suggest that possible differences in absorption between groups can scarcely be a sufficient explanation of the phenomena reported.

On the basis of the foregoing findings we concluded that in mice thyroid feeding potentiates the action of DMBEB while PTU feeding diminishes it. The problem of differen-

tiating thyroid-fed mice from others on the basis of activity deserves brief comment. In such mice, spontaneous activity as measured by our technique was decreased. This finding allowed differentiation but in a direction that seems paradoxical. Again, a stimulating drug, paradoxically, reduced spontaneous activity in all groups, but had its greatest effect in thyroid-fed mice. The use of evoked activity and the measurements of fine movements might yield group differentiation in the expected directions.

This work was supported by National Institute of Mental Health grants MH-04939 (A. J. P.) and AM-05062-04 (M. A. L.). We thank Dr. K. K. Chen of Lilly Laboratories, who kindly furnished a supply of 5-(1,3-dimethylbutyl)-5-ethyl barbituric acid, and Dr. Carl M. Cochran of the University of North Carolina, who performed the statistical tests.

ARTHUR J. PRANGE, JUN.
MORRIS A. LIPTON

School of Medicine,
University of North Carolina,
Chapel Hill.

¹ Carrier, R. N., and Buday, P. V., *Nature*, **191**, 1107 (1961).

² Conney, A. H., and Garren, L., *Pharmacologist*, **2**, 82 (1960).

³ Fouts, J. R., *Fed. Proc.*, **12**, 1107 (1962).

⁴ Ellinwood, jun., E. H., and Prange, jun., A. J., *Nature*, **201**, 305 (1964).

⁵ Prange, jun., A. J., and Lipton, M. A., *Nature*, **196**, 588 (1962).

⁶ Prange, jun., A. J., Lipton, M. A., and Love, G. N., *Nature*, **197**, 1212 (1963).

⁷ Prange, jun., A. J., Lipton, M. A., Love, G. N., and Shearin, R. B., *Biochem. Pharmacol.* (in the press)

⁸ Litchfield, jun., J. T., and Wilcoxon, F., *J. Pharmacol. Exp. Therap.*, **96**, 99 (1949).

Epileptogenic Effect of Extradural and Extracranial Cobalt

CHRONIC epileptogenic foci have been produced in rats and mice by metallic cobalt powder placed in the brain or on its surface^{1,2}. The lesions were destructive; necrosis and abscess formation were common. We have studied the epileptogenic effects of extradural and extracranial cobalt.

Fifty female Holtzman rats with initial body-weight of 250–300 g were divided into five groups of ten each. Trepanation of right frontal bone, 1–3 mm wide, was performed in four groups. One of these groups received no further treatment. Cobalt powder of 325 mesh was applied topically to the intact dura (extradural), or to the surface of the brain (subdural), or a pellet of the powder in the lumen of a 20-gauge needle was implanted into the brain (intracerebral). In the fifth group, the skull bone was drilled only part way through, and cobalt powder was applied over the remaining layer of intact bone (extracranial).

Twenty days later, the epileptogenic effect of cobalt was determined with injectable 'Indoklon'³ (a 10 per cent solution of the volatile convulsant, hexafluorodithyl ether, in polyethylene glycol 55 per cent, U.S.P., alcohol 20 per cent, and water 15 per cent). A single intraperitoneal dose of 0.1 ml./100 g body-weight caused convulsions in most of the cobalt-treated rats but not in the normal controls (Table 1). After a ten-day rest, 'Metrazol' (penta-methylentrazol) thresholds were determined. 'Metrazol', 15 mg/kg, was injected repeatedly, intraperitoneally, at 15-min intervals until convulsions occurred. The average number of injections was less in cobalt-treated rats than in normal controls (Table 1), that is, the 'Metrazol' threshold was reduced. Extradural cobalt was as effective

Table 1. RESPONSE OF EXPERIMENTAL EPILEPTIC RATS TO 'INDOKLON' AND 'METRAZOL'

Location of cobalt	'Indoklon'* convulsion incidence (%)	'Metrazol'† threshold	Brain necrosis and abscesses (%)
Sham operation	0	3	0
Extracranial	40	2.2	50
Extradural	70	1.8	40
Subdural	70	1.8	90
Intracerebral	70	2	100

* 0.10 ml. per 100 g body-weight.

† Average number of 'Metrazol' injections required to produce convulsion.

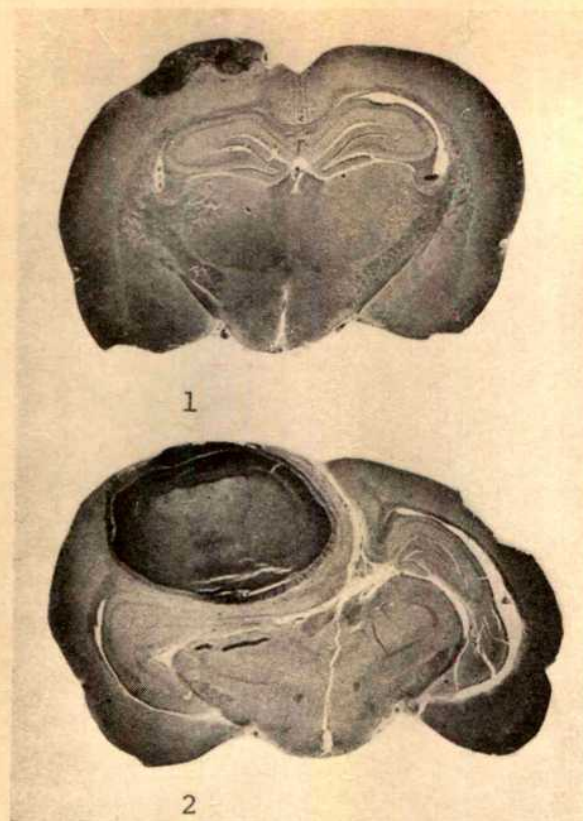


Fig. 1. Extradural cobalt application caused focal granulation tissue

Fig. 2. Intracerebral cobalt application caused extensive necrosis

as subdural or intracerebral cobalt in producing experimental epilepsy. Even extracranial cobalt was epileptogenic, although less effective than the other routes. Extradural and intracerebral cobalt (Fig. 1) caused less necrosis and fewer abscesses than subdural or intracerebral cobalt (Fig. 2). About half the brains from the former groups exhibited nothing more than meningeal adhesions, focal depression of the cortical surface, minimal subjacent necrosis and granulation tissue. Therefore, cobalt epilepsy can be produced without concomitant severe necrosis.

HUSHANG PAYAN
RALPH STREBEL
SEYMOUR LEVINE

Department of Pathology,
New York Medical College Center for Chronic Disease,
Welfare Island, New York.

¹ Kopeloff, L., *Proc. Soc. Exp. Biol. and Med.*, **104**, 500 (1960).

² Dow, R., Guardiola, A., and Manni, E., *Electroenceph. Clin. Neurophysiol.*, **14**, 399 (1962).

³ Impastato, D., Buckman, C., Milici, P., Pinsley, I., Krell, A., Impastato, A., and Padula, L., *Dis. Nerv. Syst.*, **21**, 1 (1960).

Formation of Amines in the Gastric Mucosa of the Rat

HISTAMINE may have some role, as yet unknown, in the chemical regulation of gastric secretion¹. This view is based on the effectiveness of histamine in producing acid secretion, on the presence of histamine in the gastric juice, and on the high concentration of histamine in the gastric mucosa of several species. The rich supply of histamine in this tissue in the rat is coincident with a high histidine decarboxylase activity², probably indicating a local synthesis of the amine. Several animal tissues contain L-3,4-dihydroxyphenylalanine (DOPA) decarboxylase, which attacks all aromatic amino-acids, including 5-hydroxytryptophan (5-HTP) and histidine³⁻⁵. This enzyme, however, decarboxylates histidine only inefficiently and the physiological significance of this aspect of

its function remains uncertain^{3,6}. Another more specific histidine decarboxylase has been demonstrated in the foetal rat^{7,8} and in the bone marrow of the adult rat⁹. In a recent investigation DOPA decarboxylase, as well as the specific histidine decarboxylase (cf. Weissbach *et al.*³), were demonstrated in the gastric mucosa of the rat¹⁰. While the presence of specific histidine decarboxylase seems to account for the formation of gastric histamine and ultimately for the postulated control of gastric secretion, the physiological significance of a high gastric DOPA decarboxylase activity is unknown.

The present study is a preliminary attempt to elucidate the possible significance of gastric DOPA decarboxylase. Saline suspensions of DL-DOPA or DL-5-HTP were injected intraperitoneally into rats in doses of 300 mg/kg body-weight and the concentration of amines in the entire stomach wall was determined at different times after injection (Fig. 1). The stomach was taken out, cut open along curvature minor, washed several times in saline and dried on filter paper to remove excess fluid. After weighing, the tissue was homogenized in acid according to routine techniques^{11,12}. Dopamine was extracted and isolated by ion exchange chromatography as described by Bertler *et al.*¹¹ and determined fluorometrically according to Carlsson and Waldeck¹². 5-Hydroxytryptamine was extracted and determined as described by Bogdanski *et al.*¹³.

While the stomach walls of untreated animals contained no dopamine and only moderate amounts of 5-hydroxytryptamine (cf. Ersparner¹⁴), large amounts of both amines could be demonstrated 1 or 2 h after injection of the precursors. The major part of the amines was found in the mucosa; the muscular layer contained very little. The concentration of amines in the pyloric part was found to be more than twice as high as in the fundus, a distribution which is similar to the distribution of DOPA decarboxylase¹⁰. In some animals, haemorrhagic erosions developed in the glandular portion of the gastric mucosa 2 or 3 h after administration of 5-HTP. This finding is in agreement with earlier observations by Haverback and Bogdanski¹⁵. Treatment with α -methyl DOPA or α -methyl-m-tyrosine (500 mg/kg i.p.) was found to produce marked inhibition of gastric DOPA decarboxylase as evidenced by direct estimation of the enzyme activity according to the radiometric method of Håkanson⁶. Injection of DL-5-HTP 2 h after administration of an enzyme inhibitor

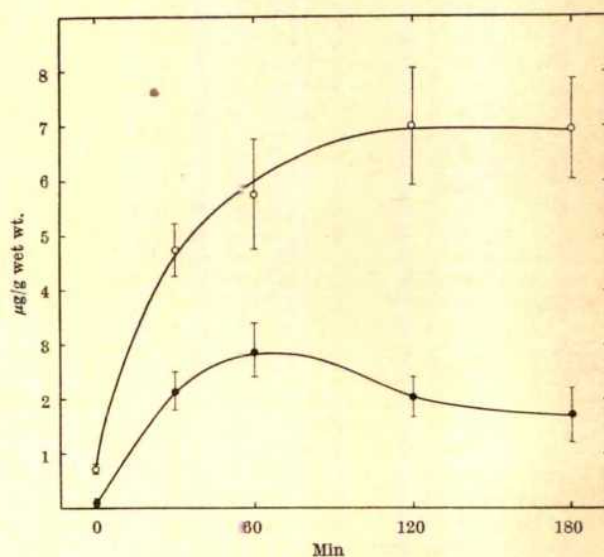


Fig. 1. Concentration of dopamine and 5-hydroxytryptamine in the entire stomach wall of the rat at different time intervals after intraperitoneal injections of the respective precursor. Each point represents the mean value from at least four experiments. The vertical lines indicate standard error of the mean. The curves are approximated from the points. Open circles, 5-hydroxytryptamine; solid circles, dopamine

produced only a slight increase in gastric 5-hydroxytryptamine.

Preliminary evidence indicates that DOPA decarboxylase is located in a specific, enterochromaffin-like cell system in the gastric mucosa¹⁰. From the data available there seems to be little doubt that the gastric DOPA decarboxylase is physiologically active. The argument that the rather low concentration of dopamine and 5-hydroxytryptamine in the stomachs of untreated animals indicates inefficiency of the enzyme is not necessarily valid since a rapid turn-over would make accumulation of the amines impossible under normal conditions. It should be noted that while the gastric mucosa of several species seems to lack the specific histidine decarboxylase, high DOPA decarboxylase activity has been demonstrated in all species studied¹⁰, possibly indicating a general role for this enzyme in gastric function.

ROLF HÅKANSON

Department of Pharmacology,
University of Lund, Sweden.

Code, C. F., in *CIBA Foundation Symposium on Histamine*, edit. by Volstenholme, G. E., and O'Connor, C. M., 189 (Churchill, London, 1956).

² Schayer, R. W., *Amer. J. Physiol.*, **187**, 63 (1956).

³ Weissbach, H., Lovenberg, W., and Udenfriend, S., *Biochim. Biophys. Acta*, **50**, 177 (1961).

⁴ Lovenberg, W., Weissbach, H., and Udenfriend, S., *J. Biol. Chem.*, **237**, 89 (1962).

⁵ Rosengren, E., *Acta Physiol. Scand.*, **49**, 384 (1960).

⁶ Ganrot, P. O., Rosengren, A. M., and Rosengren, E., *Experientia*, **17**, 263 (1961).

⁷ Burkhalter, A., *Biochem. Pharmacol.*, **11**, 315 (1962).

⁸ Håkanson, R., *Biochem. Pharmacol.*, **12**, 1289 (1963).

⁹ Håkanson, R., *Experientia*, **20**, 205 (1964).

¹⁰ Håkanson, R., and Öwman, Ch., *Biochem. Pharmacol.* (in the press).

¹¹ Bertler, A., Carlsson, A., and Rosengren, E., *Acta Physiol. Scand.*, **44**, 273 (1958).

¹² Carlsson, A., and Waldeck, B., *Acta Physiol. Scand.*, **44**, 293 (1958).

¹³ Bogdanski, D. F., Pletscher, A., Brodie, B. B., and Udenfriend, S., *J. Pharmacol. Exp. Therap.*, **117**, 82 (1956).

¹⁴ Ersparmer, V., in *CIBA Foundation Symposium on Hypertension*, 436 (Churchill, London, 1954).

¹⁵ Haverback, B. J., and Bogdanski, D. F., *Proc. Soc. Exp. Biol. and Med.*, **95**, 392 (1957).

HAEMATOLOGY

A Factor stimulating DNA Synthesis derived from the Medium of Leucocyte Cultures

BAIN *et al.*^{1,2} have shown that when human peripheral blood leucocytes from two individuals are mixed and cultured, large immature cells appear and the incorporation of tritiated thymidine into DNA is stimulated. It has been suggested that variations in this response may reflect differences in the number of histocompatibility antigens shared by the donors of the cells¹⁻³. It has also been shown that transplantation antigens can be detected in the medium from tissue cultures of rabbit⁴ and dog⁵ spleen cells. We, therefore, decided to determine whether factors released into the medium from cultured peripheral blood leucocytes might stimulate tritiated thymidine uptake (radioactive content) in cultures of leucocytes from another individual.

The subjects were normal volunteers, unrelated to one another. The culture technique was as previously described^{1,2}. The cell cultures were prepared with sterile precautions. The leucocytes were suspended in TC-199 medium (Microbiological Associates, Inc., Bethesda, Maryland, U.S.A.) containing 20 per cent autologous plasma and penicillin (100 units/ml). In every instance the leucocyte count was approximately 2,000/mm³. For each culture, 4 ml. of cell suspension was placed in a 17 mm × 100 mm disposable plastic culture tube and incubated at 37° C.

After incubation for 24, 72 or 120 h at 37° C, the culture medium was collected from approximately 20

tubes containing mixed or unmixed cultures (total volume 60–80 ml.), and centrifuged at 1,500g for 15 min to remove cells and gross cellular debris. Leucocyte counts and smears were done on this supernatant; intact leucocytes were not found in any of these preparations. A portion of the medium was then spun in a Spinco model L preparative ultracentrifuge at 100,000g for 1 h. All but about 0.2 ml. of supernatant was removed from each centrifuge tube. The precipitate was resuspended by vigorous shaking and stirring with a Pasteur pipette, and the resuspended sediments were pooled (total volume about 2 ml.). The volume of the precipitate added to each culture tube represented that derived from 30 to 40 ml. of culture medium. There were then three culture medium preparations: (a) cell-free medium (*M*); (b) supernatant after centrifugation for 1 h at 100,000g (*S*); (c) precipitate after centrifugation for 1 h at 100,000g (*P*). Various volumes of the whole medium from the previous cultures, or fractions of this medium prepared as already described here, were added to freshly prepared leucocyte cultures from one of the original donors. At the same time, cultures of mixtures of intact cells from the same donors were again set up. All these cultures were incubated for five days at 37° C. Each culture was prepared in triplicate.

At the end of the incubation period, smears were made from one of the culture tubes and tritiated thymidine (specific activity 7.2 c./mmole) was added to give a concentration of 1 µc./ml. After 1 h at 37° C, radioactive content of cells was measured in the other two tubes as described previously².

When cell-free medium, or its fractions, from previously cultured leucocytes was added to fresh unmixed homologous leucocytes, it was found to induce the appearance of large basophilic cells and to increase the uptake of tritiated thymidine. The stimulating activity appeared to be concentrated in the precipitate obtained on ultracentrifugation of the culture medium from both mixed (Table 1) and unmixed leucocytes (Table 2). The activity of the precipitate was significantly higher than that noted in the cell-free medium before ultracentrifugation or in the supernatant after ultracentrifugation.

The activities of 1.0 ml. of the cell-free culture medium, *M*, and the supernatant after culture centrifugation, *S*, were approximately equal (Table 1). However, when the activities of 4.0 ml. of these materials from 5-day-old mixed cultures were tested, *M* was consistently more active than *S*. These findings indicate that only a fraction of the original activity can be found in the precipitate, *P*. The relatively high degree of activities of *P* was due to the fact that each ml. of *P* was derived from 30 to 40 ml. of *M*. The uptake of tritiated thymidine by leucocyte cultures from single donors to which fraction *P* had been

Table 1. STIMULATORY EFFECT OF CULTURE MEDIUM PREPARATIONS

Exp. No.	Control cultures of unmixed leucocytes	C.p.m./4 ml. (*†) Leucocyte cultures from single subjects containing 1 ml. of medium preparation from 5-day cultures of mixed cells			Cultures of mixed intact leucocytes
		<i>M</i>	<i>S</i>	<i>P</i>	
1	590	3,424	1,917	34,075	41,486
2	594	3,321	4,013	26,591	76,433
3	339	2,716	1,908	5,044	28,624
4	763	4,031	3,891	32,483	70,361
5	603	3,859	2,643	13,594	49,193

* Count in a liquid scintillation counter (Packard, model 3002).

† Average of duplicate 5-day cultures.

M Cell-free culture medium before ultracentrifugation.

S Supernatant medium after ultracentrifugation of *M*.

P Precipitate obtained by ultracentrifugation of *M*.

Table 2. STIMULATORY EFFECT OF PRECIPITATE DERIVED FROM AUTOLOGOUS OR HOMOLOGOUS UNMIXED LEUCOCYTE CULTURES

Exp. No.	Control cultures of unmixed leucocytes	C.p.m./4 ml. (*†) Leucocytes from single subjects with added precipitate from unmixed or mixed 3-day cell cultures		
		Autologous	Homologous	Mixed
6	422	520	4,446	6,256
7	1,042	190	2,074	—
8	409	492	4,151	—
9	509	516	1,855	5,196

(*†) See legend for Table 1.

Table 3. RELATION OF DURATION OF LEUCOCYTE CULTURES TO STIMULATORY EFFECT OF PRECIPITATE

Exp. No.	Length of initial cultures	Control cultures of unmixed leucocytes	C.p.m./4 ml. (*†)	
			Homologous leucocyte cultures from single subjects precipitate	Homologous leucocyte cultures from mixed cell cultures
10	1 day	914	2,442	
11	1 "	738	1,896	
12	1 "	535	3,907	
13	3 days	666	3,575	
14	3 "	435	5,226	
15	3 "	308	5,482	
1	5 "	590	34,075	
2	5 "	594	26,591	
4	5 "	763	32,483	

(*†) See legend for Table 1.

added was in no instance as great as that found in cultures of mixtures of intact leucocytes (Tables 1 and 2). The precipitate from mixed leucocyte cultures was more active than that from homologous unmixed leucocyte cultures. The precipitate from cultures of leucocytes from a single donor produced no blastogenesis when added to freshly prepared cultures from the same individual. The precipitate from 5-day cultures was more active than that from 3-day cultures, and the precipitate from a 24-h culture medium showed slight but still significant activity (Table 3). The stimulatory activity was increased by increasing the volume of the added medium. When the volume was doubled (2 ml.), a definite increase of stimulatory activity was observed in some, but not all, instances. When the volume was quadrupled (4 ml.), the activity almost uniformly was sharply increased.

These experiments have shown that when human blood leucocytes are cultured, a material is released into the culture medium which is concentrated by ultracentrifugation and which stimulates blastogenesis when added to cultures of homologous leucocytes. The amount of this material released into the culture medium seemed to be related to the numbers of large basophilic cells which appeared in the cultures. The results presented here permit no conclusions as to whether the presence of this factor precedes and stimulates the production of these blast cells or is produced by these cells. It is not known whether the stimulatory factor is secreted by these or other cells or is a sub-cellular fraction released into the medium as a result of damage or death of cells. At present, the nature of this factor is unknown. The absence of blastogenic effect when this material is added to cultures of autologous leucocytes demonstrates its antigenic specificity. It is possible that this factor may contain transplantation antigen⁴ or complexes between RNA and antigens.

In summary: (1) The medium from human leucocytes cultivated *in vitro* stimulated blastogenesis when added to cultures of homologous leucocytes. (2) Medium from mixed leucocyte cultures was more active than that from homologous unmixed leucocyte cultures, but was less active than mixtures of intact leucocytes from the same donors. (3) Medium from unmixed leucocyte cultures was inactive when added to cultures of autologous leucocytes. (4) Some activity was found in the precipitate obtained after ultracentrifugation.

This work was supported by U.S. Army Medical Research and Development Command research contract DA-49-193-MD-2579 and Medical Research Council of Canada grant MBA-1664.

SHINPEI KASAKURA
LOUIS LOWENSTEIN

Division of Haematology,
Department of Medicine,
Royal Victoria Hospital and
McGill University Clinic,
Montreal, Canada.

¹ Bain, B., Vas, M. R., and Lowenstein, L., *Blood*, **23**, 108 (1964).

² Bain, B., and Lowenstein, L., *Science*, **145**, 1315 (1964).

³ Hirschhorn, K., Bach, F., Kolodny, R. L., Firschein, I. L., and Hashem, N., *Science*, **142**, 1185 (1963).

⁴ Mannick, J. A., Graziani, J. T., and Egdahl, R. H., *Surg. Forum*, **14**, 148 (1963).

⁵ Mannick, J. A., and Southworth, J. G., *Proc. Soc. Exp. Biol. and Med.*, **116**, 445 (1964).

A Lymphocyte-stimulating Factor produced *in vitro*

Bain, Vas and Lowenstein¹ have recently reported that when peripheral leucocytes of two individuals are cultured together, some of the cells enlarge and undergo mitosis. Bach and Hirschhorn² have further shown that not only intact cells, but also extracts of leucocytes, disrupted by freezing and thawing, could stimulate cells to divide. Experiments reported in this communication demonstrate that cell-free media obtained from leucocytes in culture contain mitogenic factor(s), and suggest that these factors might be produced by the cells *in vitro*.

Cells of normal human volunteers were cultured according to the method of Bain *et al.*¹ with the exception that no antibiotics were used. Single cultures contained 4×10^6 cells derived from one donor in a total volume of 4 ml. TC199 containing 10–20 per cent autologous plasma. Mixed cultures contained a total of 4×10^6 cells derived from two donors in the same volume of medium.

To test preparations for blastogenic activity the following procedure was used: Single or mixed cultures from cells of two donors were prepared; leucocytes in single, or in most experiments, in mixed cultures were treated after different periods of incubation by either one of the following procedures: centrifugation, three cycles of freezing and thawing, disruption by ultrasonic vibration, lysis in distilled water or heating at 50° C for 5 min. Cells, cell fragments, or cell-free culture media derived from these cultures were transferred for testing to single cultures initiated at the beginning of the experiment. The cultures were examined for blastogenesis 5 days after transfer.

Cell-free culture media used for assays were collected 1–5 days after initiation of the cultures. The tubes containing the cultures were centrifuged at 540g for 10 min. The medium was removed using Pasteur pipettes and was either tested at once or was filtered on a column of 'Sephadex G-25'. The material appearing in the effluent immediately after the void volume was collected and lyophilized. For testing, the powder was dissolved in fresh culture medium and passed through a Millipore filter (pore size 0.22 μ). In some experiments the supernatant was frozen and was kept at -20° C until used.

Experiments were carried out to test the effect of 5-fluorodeoxyuridine (5FUdr) and puromycin on mixed leucocyte cultures. (5FUdr was a gift from Hoffman-La Roche, Ltd., Montreal, while puromycin dihydrochloride was purchased from Nutritional Biochemicals Corp.). These substances were added at the beginning of the incubation period, in concentrations of 10 and 2.5 μ g/ml., respectively. Media obtained from these cultures and from others to which 5FUdr or puromycin was added at the end of the culture period were filtered on a column of 'Sephadex G-25' lyophilized, and assayed as already described here.

The activity of any given fraction was assessed in terms of its capacity to induce cell transformation in single cultures. Smears prepared from cultures after 5 days of incubation were graded using an arbitrary scale of 0 to + + +. In each experiment every preparation was tested in duplicate or triplicate cultures and smears from some experiments were also scored independently. (We thank Dr. B. Bain, of the Department of Haematology of this Hospital, for examining these slides.)

In six experiments, the extracts of $2-40 \times 10^6$ freshly drawn leucocytes, disrupted by either of the three methods used, gave consistently negative results, whereas strong reactions were obtained using as few as 2×10^6 intact cells (Table 1). Cells heated at 50° C for 5 min also failed to stimulate cells to transform. In contrast to the negative results obtained with freshly drawn disrupted cells, leucocyte cultures (cells and medium) subjected to freezing and thawing after 24 h of incubation gave consistently positive results. Cell-free culture media

removed from leucocyte cultures after 1-5 days of incubation also possessed stimulating capacity.

Media from single cultures were active but were less potent than those obtained from mixed cultures. Media from single cultures added to autologous cells displayed no activity.

Mitogenic activity appeared to accumulate in the culture media; the longer the incubation period, the more activity was recovered. Furthermore, aliquots of fresh culture media put in contact with the same cells on two or three consecutive days each possessed activity.

Since media removed from cultures after incubation possessed activity, experiments where both medium and disrupted cells were assayed together (Table 1) did not disclose whether disrupted cells alone could give rise to blastogenesis. In the following experiment (Table 2) culture media and washed disrupted cells obtained from the same cultures after 4 days of incubation were tested separately. Whereas the media were found to be highly active, the disrupted cells failed to stimulate the target cells to transform. Single cultures from cells of the same donors incubated for 4 days, then washed and mixed, were still able to mount just as strong a reaction as cells mixed at the beginning of the experiment, that is, 4 days previously.

Table 1. STIMULATION OF CELLS IN SINGLE CULTURE BY FRACTIONS OBTAINED FROM CULTURES AFTER DIFFERENT PERIODS OF INCUBATION

Source of mitogenic factor	Incubation before transfer, days	Material transferred	Mitogenic* activity
Single cultures	Nil	2×10^6 intact cells	61/63
Single and mixed cultures	Nil	$2 - 40 \times 10^6$ disrupted cells	0/6
Single culture	Nil	Heated cells 50°C , 5 min	0/2
Mixed cultures	1	Disrupted cells and medium	3/3
Mixed cultures	1-5	Cell-free medium	16/16
Single cultures	4-5	Cell-free medium	3/3† 0/3‡

* The number of positive experiments is given in the numerator; the denominator denotes the total number of experiments performed.

† Transferred to homologous cells.

‡ Transferred to autologous cells.

Table 2. STIMULATION OF CELLS IN SINGLE CULTURE BY CELL-FREE MEDIUM AND DISRUPTED CELLS DERIVED FROM THE SAME CULTURES

Cells incubated	Incubation before transfer, days	Fractions tested*	Reaction	
			A	B
AB	4	Cell-free medium, washed disrupted cells	+++	+++
A	4	Intact cells	0	0
			0	+++

* The cell-free media and disrupted cells tested were derived from the same cultures.

5FUdr and puromycin were each found to inhibit completely the reaction in mixed leucocyte cultures (Table 3) and media from these cultures were also devoid of mitogenic activity. Culture media from cells of the same donors, grown in the absence of inhibitors, tested against the same target cells were fully active. Puromycin or 5FUdr added to these supernatants prior to gel filtration did not abolish the activity, indicating that the lack of demonstrable activity of the media from cultures grown in the presence of the inhibitors was probably not due to a transfer of free inhibitor to the target cells.

The experiments described demonstrate that the factor or factors capable of inducing mitosis of homologous lymphocytes is released by leucocytes in culture. Evidence

Table 3. STIMULATION OF CELLS IN SINGLE CULTURE BY HOMOLOGOUS CELLS OR CELL-FREE MEDIA: THE EFFECT OF INHIBITORS

Material used for stimulation	Inhibitor	Results*
Intact cells	5FUdr†	0/7
Intact cells	Puromycin‡	0/4
Media from cultures inhibited by 5FUdr or puromycin	—	0/2
Media plus 5FUdr or puromycin	—	2/2

* The number of positive experiments is given in the numerator; the denominator denotes the total number of experiments performed.

† 5-Fluorodeoxyuridine, $10 \mu\text{g/ml}$.

‡ Puromycin, $2.5 \mu\text{g/ml}$.

§ Control experiments: the inhibitors were added at the end of the culture period prior to gel filtration.

presented indicates that mitogenic activity accumulates in the culture medium; media collected after 3 days of culture possess more activity than that obtained at 2 days which, in turn, is more potent than that collected after incubation for 1 day. Furthermore, aliquots of fresh culture medium put in contact with the same cells on two or three consecutive days each contained activity, indicating that mitogenic activity is released to the medium continuously for at least 4 days.

The mitogenic activity could be due to a preformed factor present in cells and released either continuously or on cell death, or it could be synthesized by the cells during incubation. Failure to find activity after cell disruption by three different methods would make release after cell death unlikely. Indeed, activity could not be recovered from cells disrupted when drawn or after 4 days of incubation. This latter is especially interesting in view of the high levels of activity found in the culture media derived from the same tubes (Table 2). The ability of the cells to elaborate mitogenic activity remained after 4 days of incubation since leucocytes mixed after having been kept in single cultures for 4 days exhibited strong blastogenesis. Finally, freezing and thawing itself would not be expected to deactivate the factor since whole cultures, comprising cells and media subjected to this treatment, yielded activity.

Thus, the experiments described do not support the view that preformed mitogenic factor is present in the cells in concentrations detectable by the methods used. The results presented can be more readily interpreted by assuming that the mitogenic factor is synthesized *in vitro*. This assumption is supported by the results obtained using inhibitors of protein and nucleic acid synthesis; both puromycin and 5FUdr inhibited blastogenesis and media obtained from these cultures were devoid of mitogenic activity. If mitogenic factors were preformed and released from cells during the culture period, then one would have expected to recover activity from these media.

This work was supported by the John A. Hartford Foundation, Inc., and the Medical Research Council of Canada.

We thank Dr. L. Lowenstein and Dr. B. Bain, of the Division of Haematology, Department of Medicine, Royal Victoria Hospital, for their advice.

J. GORDON

L. D. MACLEAN

Department of Experimental Surgery,
Royal Victoria Hospital and
McGill University,
Montreal, Canada.

¹ Bain, B., Vas, M. R., and Lowenstein, L., *Blood*, **23**, 108 (1964).

² Bach, F., and Hirschhorn, K., *Science*, **143**, 813 (1964).

HISTOCHEMISTRY

Fluorochrome Stains for Histological Diagnosis of Visceral Mycoses

FLUORESCENCE microscopy facilitates the demonstration of various fungi in tissues, because it is possible to examine larger parts of the tissue-sections in a short time. Simple stains with fluorochrome dyes—acridine orange, for example—are inadequate for diagnostic use. Two methods which have hitherto been used for the demonstration of polysaccharides proved useful in staining fungi.

(1) Among the fluorescent Schiff-type reagents (Kasten¹) acridine yellow after oxidation with periodic acid gives the brightest fluorescence. The strength of reaction in the different tissue-forms of fungi of importance in Europe is registered in Table 1. A fluorescence not specifically weak appears in the tissue, unless the fraction of the dye is separated in glacial acetic acid. In practice

Table 1

Species and tissue-forms	Periodic acid-acridine yellow	Sulphation-acridine orange ²	Gomori's method
1. <i>Candida</i>			
(a) Budding cells	+++	+++	+++
(b) Pseudohypha	++	+++	+++
(c) Degenerating forms	φ to +	(+) to ++	+ to ++
2. <i>Geotrichum</i>	+ to ++	++	++
3. <i>Cryptococcus</i>			
(a) Capsule	(+) to +	+++	++
(b) Cell-wall	++ to +++	+++	+++
(c) Degenerating forms (without capsule)	φ to +	(+) to ++	++
4. <i>Aspergillus</i>			
(a) Hypha	+++	+++	+++
(b) Degenerating hypha	(+) to +	+ to ++	+++
(c) Conidiophores	+	+++	+++
(d) Spores	φ to (+)	++	++
5. <i>Mucorales</i>			
(a) Hypha	φ	(+)	+ to ++
(b) Spores (only in culture-phase)	φ to +	+++	

Note: (+) = yellow-green to green (colour of the neighbouring tissue).
 +; ++; +++ = Different intensities of yellow or red fluorescence and different intensities of greyish to deep black for Gomori's method.

the tissue-fluorescence disappears if the sections are previously stained with Weigert's iron haematoxylin (cf. Fig. 1).

(2) Fluorochromes, especially acridine orange, are added after sulphation (Moore and Schoenberg²). The fungi show an excellent red-fluorescence (cf. Table 1). In consequence of the low pH, between 2.6 and 2.9, the tissue-fluorescence attains only a green colour (cf. Fig. 2). The fluorescence of the fungi is quickly extinguished on slides that are rich in alkalis; contrary to this, the binding of acridine yellow in the first method is stable.

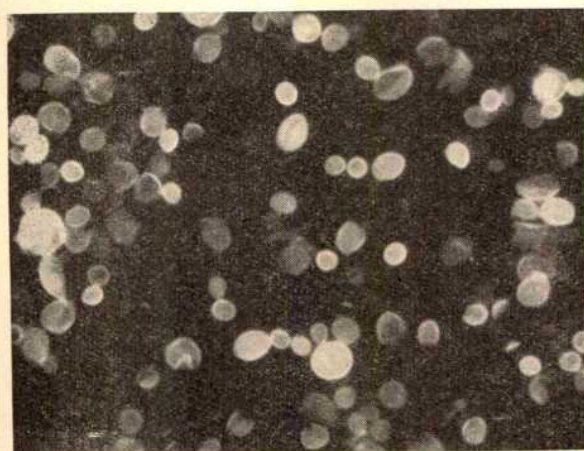


Fig. 1. Subcutaneous granuloma of candidose in animal-experiment. Periodic acid-acridine yellow technique ($\times c. 850$)



Fig. 2. Human aspergillus-pneumonia. Sulphation-acridine orange technique ($\times c. 300$)

These two methods, especially the sulphation-acridine orange technique, enable a quick diagnosis to be made in frozen sections of fresh tissue. The various tissue forms demonstrated by the morphological methods also provide a rapid guide to the identity of the fungal species³. But other optical methods are by no means superseded, for example, Gram's stain and Ziehl-Neelsen's stain for *Actinomyces* and *Nocardia*, the periodic acid-Schiff technique and Gomori's methenamine silver nitrate. The latter is the most sensitive method for demonstrating details. It impregnates the cell-wall of fungi in progressive degeneration, when other stains fail, and *Mucorales* are best demonstrated by this method. The examination of the sections previously stained according to Gomori is facilitated in polarized light.

We thank the Section of Dermatology in the Society of Experimental Medicine—Academy of Sciences, Berlin, for supporting this work.

G. BADER
D. STILLER
K. RUFFERT

Institute of Pathology,
Friedrich-Schiller-University,
Jena, Germany.

¹ Kasten, F. H., *Histochemie*, 1, 466 (1956); *Acta Histochem.* (Jena) Supp., 3, 240 (1963).

² Moore, R. D., and Schoenberg, M. D., *Stain Technol.*, 32, 245 (1957).

³ Bader, G., *Die viszerale Mykosen*, 423 (G. Fischer, Jena, 1965).

IMMUNOLOGY

Antithymic Antibody Localization in the Mouse

ONE of the present-day beliefs is that the thymus is the original source of precursors of the small lymphocyte¹. If this were true, antibody developed against thymus cell antigen might react with cells of the lymphoid series. The purpose of this investigation was to determine whether such antibodies could be developed. The ultimate aim was to explore the usefulness of these antibodies for the depression of the immune response to permit homograft survival either by their use as carriers of large doses of radioactive isotope or by their inherent cytotoxic effect.

Subtotal thymectomy was performed on live mice of the A/J line. The mice were anaesthetized with pentobarbital. The sternum was incised to the level of the xiphoid and a preparatory suture placed to permit rapid closure of the chest. The upper portion of the gland was resected and immediately the suture was closed to prevent pneumothorax. 'Dicrystin' was used to prevent infection.

The microsomal portion of the cell has been reported to contain the most organospecific antigens². The glands were processed so as to isolate this portion as has been described by Nairn³. The nitrogen content was found to be about 50 $\mu\text{g}/\text{ml}$, using the Nessler's reagent method¹.

Male white Dutch rabbits were used for antibody production investigations. The control animal received no injections. The sensitized animal received subcutaneous and intramuscular injections of the microsome preparation accompanied by simultaneous injections of incomplete Freund's adjuvant. Three weeks later, intravenous injections of the same antigen were given with no Freund's adjuvant. At the end of 4 weeks a titre of 1:160 was noted. The microsomal fraction was diluted 1:5 for the microscopic agglutination reaction. Serum-saline and antigen controls were used after incubation in a water bath for 2 h, the tubes were stored at 4° C for 18 h, centrifuged, then viewed.

The crude globulin was purified after the method of Sober⁴. This consisted basically of ammonium sulphate precipitation and chromatographic separation on a column of diethylamino ethyl cellulose. The purified

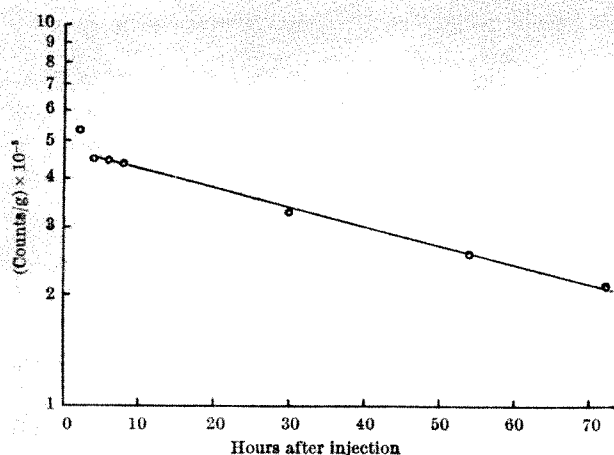


Fig. 1. Blood clearance of ^{125}I -antithymic antibodies. Slope is 0.004g/h

globulins were labelled with microcurie amounts of iodine-131 (ref. 5). 0.5 ml. of the purified, labelled, antithymic globulin containing 1.87 μc . iodine-131 was injected intravenously into a mouse by way of the eye sinus. The clearance from the blood was determined by means of serial samples from a tail vein into weighed, heparinized capillary tubes. The activity per gram of blood versus time is plotted in Fig. 1.

It was questionable as to whether the counts were localized to any extent on the formed elements of the blood as well as on the serum proteins. To answer this, several samples of blood in heparinized capillary tubes were centrifuged. Each tube was divided into three sections: serum, red blood cells (RBC), and buffy coat (WBC). A comparison was made with a mouse receiving rabbit globulin prepared in the same manner but not immunized against mouse thymus. Using samples collected at 4 h, the results are:

	Immunized globulin Ratio of counts to RBC	Control globulin Ratio of counts to RBC
Serum	26.2	43.0
WBC	19.2	4.4
RBC	1	1

It was also questionable as to whether the high WBC might be due to serum trapped in the buffy coat. Therefore the buffy coat was resuspended in 1.0 ml. physiological saline and allowed to stand overnight at 40° C. Approximately 29 per cent of the counts remained on the WBC. However, much of the count in the serum could have been due to disintegrated white cells or to the release of antibodies previously bound to the lymphocytes. Even with only 29 per cent of the counts definitely associated with the buffy coat, this amounts to approximately 300,000 counts/gram—five times greater than the count for RBC.

72 h following intravenous injection of ^{125}I -labelled globulin, the activity of various organs of the killed mouse was determined. These values are reported as a fraction of the activity in the peripheral blood.

Because iodinated serum albumin (human) (RISA) does not leave the blood stream significantly in 10 min, values obtained by using RISA and otherwise performing the experiment in the same manner represent activity due to blood supply alone. The ratio formed by the uptake of ^{125}I -labelled globulin and uptake of RISA indicates the specificity of the various body tissues for the antibody. The results appear in Table 1.

The differential localization of tagged γ -globulin indicates that the prepared antibody is most specific for the thymus gland. However, lymph nodes, bone, striated muscle, and the GI tract have significant localization. The lymphatic tissue in the lymph nodes and in the GI tract is probably the area where the activity is centralized in these two organs. The relatively high concentration

	Table 1				Ratio Column (2) Column (4)
	^{125}I -labelled globulin (1) Counts/g/ min	Fraction of the counts/g/min for blood (2)	(3) Counts/g/ min	RISA (4) Fraction of the counts/g/min for blood	
Blood	104,460	1.00	88,130	1.000	1.00
Thymus	34,220	0.328	8,200	0.093	3.52
Striated muscle	7,690	0.074	2,430	0.028	2.67
GI	10,530	0.101	1,720	0.053	1.89
Bone (sternum)	13,600	0.130	6,156	0.070	1.86
Lymph node	30,580	0.293	14,250	0.162	1.81
Lung	35,810	0.343	26,760	0.304	1.13
Kidney	27,140	0.260	24,454	0.278	0.94
Liver	22,230	0.213	22,790	0.258	0.82
Spleen	19,995	0.124	13,770	0.156	0.80

of radioactivity in the bone may be due to common antigenicity between the reticulum cells in bone and lymphoid tissue. The relative specificity for muscle is difficult to understand. However, the simultaneous presence of thymoma and myasthenia gravis and the suspicion that this latter disease has an antigen-antibody aetiology make one wonder if this muscle specificity might not be meaningful. The counts/g of muscle are very low for labelled globulin and RISA, but the ratio of these activities indicates uptake in excess of that attributable to blood supply alone. The failure of specific uptake of antibody by the spleen is most puzzling. The presence of considerable activity in the buffy coat of centrifuged blood indicates that the antibody does have some affinity for circulating lymphocytes—as was previously postulated.

The slow clearance of antibody from the blood makes it undesirable to depress the antibody-producing system by means of radioactivity tagging, since this becomes tantamount to utilizing external, whole-body radiation. Another approach would be to inject a relatively large amount of unlabelled antibody. The idea behind this scheme is that cells to which antibody adheres might be rendered non-functional. Thus, with preferential localization of the antibody or lymphatic cells the rejection response of the host to a homologous graft might be suppressed.

BRACE HINTZ
MILO M. WEBBER

Department of Radiology,
University of California,
Center for the Health Sciences,
Los Angeles, California.

¹ Lanni, F., and Campbell, D., *Stanford Medical Bulletin*, 6, 97 (1948)

² Nairn, R. C., Ghose, T., Fothergill, J. E., and McEntegart, M. G., *Nature*, 196, 385 (1962).

³ Nairn, R. C., Richmond, H. G., McEntegart, M. G., and Fothergill, J. E., *Brit. Med. J.*, ii, 1335 (1960).

⁴ Sober et al., *J. Amer. Chem. Soc.*, 78, 756 (1956).

⁵ Helmkamp et al., Univ. of Rochester U.S.A.C.C. Project report No. UR-568.

⁶ Arnason et al., *Blood J. Hematol.*, 20 (5), 617 (1962).

Isoprecipitin in the Sera of Rats resistant to Tumour Transplantation

REJECTION of transplanted tumour cells is one of the most important phenomena for understanding the immunology of cancer, but the appearance of precipitin in the sera of recipient animals has not yet been clearly demonstrated. I have recently detected explicit isoprecipitin in the sera of rats with acquired resistance to transplantation of Yoshida ascites hepatoma cells, AH49.

A high percentage of rats accept the transplanted Yoshida ascites hepatoma when inoculated with a sufficient number of the hepatoma cells. For example, in the case of AH49 strain of the ascites hepatoma, 10^7 cells are sufficient to produce more than 90 per cent successful transplantation. However, for an unknown reason, some rats reject the transplantation. These resistant rats, usually, do not take the same cells again by repeated transplantation.

The sera of these resistant rats were examined for the precipitin reaction to an antigen isolated from the cancer cells. A fraction referred to as S_5 -protein was extracted from homogenates of the ascites hepatoma cells at pH 4.8 with acetate buffer, 0.1 M, as previously reported^{1,2}. This fraction contains cancer specific antigenic protein(s) as examined by precipitin reaction with rabbit antisera against S_5 -protein. The specific protein(s) was partially purified by ion exchange cellulose chromatography³. The purification was also carried out by combination of ammonium sulphate fractionation and a zinc ion precipitation reaction. This procedure gave a purified protein material referred to as 6P-fraction (unpublished data).

A strain of Yoshida ascites hepatoma⁴, AH49, which was originally developed in the livers of Japanese hybrid rats by feeding with *p*-dimethylamino-azo-benzene, was used in this investigation. Average survival time of the rats transplanted with these cancer cells is 7–9 days. The tumour cells of this strain were transplanted into Japanese hybrid rats. The rats which rejected the transplants were inoculated with the same cells repeatedly at intervals of two weeks.

The precipitin reactions of the sera of resistant rats were examined either by the ordinary ring test or by the agar diffusion technique.

Some experimental results are presented in the accompanying tables and photographs. As seen in Table 1, resistant rats which were inoculated with AH49 cells twice or more (at two-week intervals) showed, without exception, positive precipitin reactions. A negative reaction was seen in 75 per cent of rats inoculated only once.

Table 1. PRECIPITIN REACTION OF RESISTANT RAT SERA

No. of inoculation*	No. of rats tested	Titre of precipitin† (two-fold dilution)	Positive reaction‡
1	12	0–32	3/12 (25%)
2	8	16–64	8/8 (100%)
3	8	16–64	8/8 (100%)
4	4	16–128	4/4 (100%)
5	6	32–64	6/6 (100%)
6	6	16–128	6/6 (100%)
7	14	16–256	14/14 (100%)
9	12	32–128	12/12 (100%)
11	10	32–128	10/10 (100%)

S_5 -protein or a purified material, 6P-protein, of AH49 cells was used as antigen of the precipitin reaction.

* Approximately 2×10^7 cells of AH49 hepatoma were inoculated intraperitoneally every two weeks.

† Titration by the ring test.

‡ Positive precipitin reaction tested by the agar diffusion technique.

The reaction was never demonstrated in the sera either of normal rats or of tumour-bearing rats as shown in Table 2.

The antisera reacted specifically with the proteins (S_5 -protein or 6P-protein) of AH49 hepatoma cells only, but not with the protein fractions of various normal

Table 2. PRECIPITIN REACTION OF THE SERA OF RATS WITH OR WITHOUT TUMOUR

	No. of rats	No. of rats of positive precipitin reaction*	Percentage
Normal rats	50	0	0
Tumour-bearing rats†	49	0	0
Resistant rats‡	80	71	90

* By the agar diffusion technique.

† AH49 ascites hepatoma-bearing rats.

‡ Resistant to AH49 hepatoma cell transplantation.

rat tissues or with the proteins of other strains of Yoshida ascites hepatoma, for example, AH66, AH127, AH41B, AH131B, etc. The reaction was highly specific.

Antibody titres examined by the two-fold dilution method of the antisera were 0–256 times dilution depending on the number of inoculations.

Immunoelectrophoretic analyses revealed that the precipitin is located in the region of γ -globulin fraction of the antisera. Electrophoresis of the rat sera indicated that the γ -globulin fraction of the resistant rats increased remarkably while that of tumour-bearing rats decreased drastically.

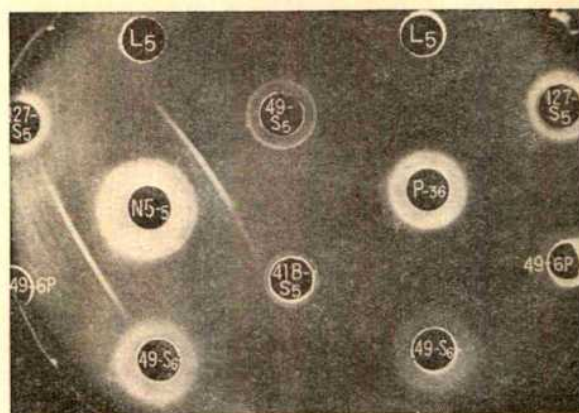


Fig. 2. Precipitin reaction of rat sera: N5, serum of resistant rat No. 5 inoculated with AH49 cells five times; P-36, serum of an AH49 ascites hepatoma-bearing rat; L5, as in Fig. 1; 49-S5, as in Fig. 1; 49-6P, purified antigenic protein from S_5 -protein of AH49 cells; 41B-S5, S_5 -protein fraction isolated from AH41B cells; 127-S5, S_5 -protein fraction isolated from AH127 cells.

Thus, the isoprecipitin reaction in the sera of resistant rats suggests an intimate connexion with the antitransplantation phenomenon. However, the essential role of isoprecipitin in development of the antitransplantation mechanism is still obscure. AH49 cells were originally developed in the Japanese hybrid rats as previously mentioned. In the present study this strain of hepatoma was transplanted to the hybrid rats. Results obtained in isologous and autologous system may differ. The immunogenetic relation between host and transplant is under investigation.

I thank Dr. Kotaro Warabioka, Cancer Institute, Japanese Foundation for Cancer Research, Tokyo, Drs. Hidehiko Isaka, Hiroshi Satoh and Motoi Ishidate, Sasaki Foundation for Medical Research, Tokyo, and Miss Hiroko Taga and Hiroko Inai, Department of Biochemistry, Faculty of Medicine, University of Tokyo, for their co-operation.

HIDEMATSU HIRAI

Department of Biochemistry,
University of Hokkaido School of Medicine,
Sapporo, Hokkaido, Japan.

¹ Hirai, H., Sekine, K., Iijima, A., and Warabioka, K., *J. Biochem.*, Tokyo, **49**, 682 (1961).

² Hirai, H., Taga, H., Isaka, E., Satoh, H., and Warabioka, K., *Gann*, **54**, 177 (1963).

³ Hirai, H., Taga, H., and Warabioka, K., *J. Biochem.* (Tokyo), **55**, 178 (1964).

⁴ Yoshida, T., *Ann. N.Y. Acad. Sci.*, **63**, 852 (1956); **76**, 610 (1956).

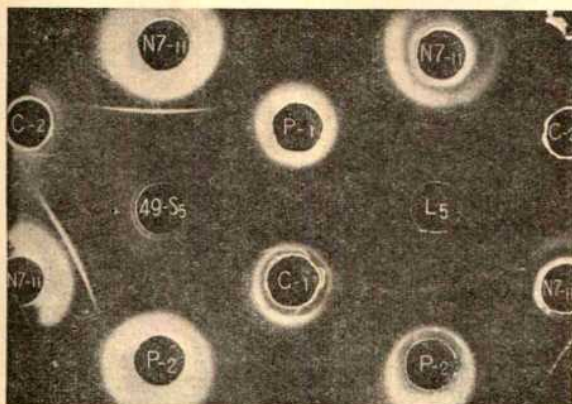


Fig. 1. Precipitin reaction of rat sera: 49-S5, S_5 -protein isolated from AH49 ascites hepatoma cells; L5, protein fraction of normal rat liver corresponding to S_5 -protein of the cancer cells; C1, C2, sera of normal rats; P1, P2, sera of AH49 ascites hepatoma bearing rats; N7-11, sera of resistant rat No. 11 inoculated with AH49 cells seven times.

PATHOLOGY

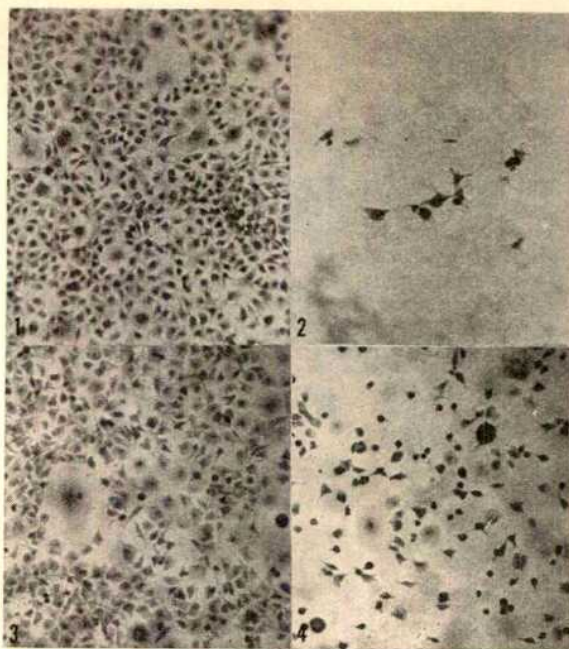
Inhibition of Growth of Malignant Cells *in vitro* by a Component of Normal Adult Connective Tissue

NUMEROUS reports during the past 30 years support the thesis that biologically active factors present in normal adult tissue, growth-stimulating and growth-inhibiting substances, form a complex which regulates the growth of normal adult tissue. Upset of this balance may result in the unrestrained growth of cancer¹⁻⁷. We have described the extraction by mild tryptic digestion of constituents of normal adult connective tissue (aorta, tendon, and skeletal muscle) which were growth-stimulating or growth-inhibitory to normal fibroblasts in tissue culture⁸. The effect on growth *in vitro* varied according to the conditions of extraction. Inhibitory extracts and crude precipitates from them further inhibited the growth in primary tissue culture of a series of human tumours from 75 to 100 per cent (refs. 9, 10).

It seemed of interest to determine whether results obtained with established strains of malignant cells would correlate with those from tests of fresh tumour tissues. An attempt was also made to separate, by DEAE-cellulose chromatography, material from the crude preparations which would inhibit specifically different types of tumour cells. During the past two years we have tested the effect of extracts, fractions, and DEAE-cellulose sub-fractions on the following strains of normal and malignant tissue in culture: short-term strains of two tumours carried for many years in animals, the Rous chicken sarcoma, and a human sarcoma, *H.S.1* of Toolan-Fjelde^{11,12}; three strains of normal human fibroblasts and two strains of highly malignant epithelium, a 12-year-old strain of human epidermoid carcinoma of the larynx, *H.Ep.2* of Toolan-Fjelde^{11,12}, and a 5-year-old strain of human grade III serous cystadenocarcinoma of the ovary isolated at this institution, *J.M.*¹³. Fractions were obtained which were 100 per cent inhibitory to the cells of the Rous sarcoma and to *H.S.1*, but had little or no effect on the normal fibroblasts¹⁴. Many fractions were isolated which were markedly inhibitory to the young strain, *J.M.*, but had no effect on the older epithelial strain, *H.Ep.2*¹⁴. This report deals with the effect of DEAE-cellulose chromatography sub-fractions on the growth *in vitro* of the *J.M.* strain of ovarian tumour cells.

Crude inhibitory extracts were prepared from chicken skeletal muscle or beef tendon by mild tryptic digestion under conditions which had previously been found to be optimal for the extraction of inhibitors⁸⁻¹⁰. The inhibitory factors were precipitated by ethyl alcohol and CaCl_2 as described previously¹⁰. These precipitates were fractionated on 2×80 cm columns of DEAE-cellulose equilibrated with phosphate buffer 0.005 M, pH 8.4. In one typical run, 140 mg of the ethanol precipitate was applied to the column, in another, 220 mg of the CaCl_2 precipitate. A pH and ionic strength gradient was established to pH 6, 0.1 M and 2 M NaCl. 6-ml. fractions were collected and combined according to the ultra-violet patterns.

The cell strains were maintained as a monolayer on glass in milk dilution bottles in a medium of 25 per cent mixed horse and human placental serum in Parker's medium 199 at pH 7.2. For tests for effect on growth of cells in tissue culture, dense bottle growths of cells were brought into suspension by trypsinization, the cells were counted in a haemocytometer, suspended in fresh medium and distributed to Carrel flasks or Leighton tubes in 1-ml. lots containing two hundred thousand cells. In two to three days, when the cells had formed uniform sheets, the medium was replaced with 10 per cent serum in the inhibitory extracts. In each experiment, there were two controls of 10 per cent serum in Moscona solution. After two days' incubation the cells in one flask were



Figs. 1-4. Photomicrographs of cultures of serous cystadenocarcinoma of the ovary (*J.M.*). Figs. 1 and 2, sister cultures; Fig. 1, in control medium, 10 per cent serum in balanced salt solution; Fig. 2, in 10 per cent serum in crude inhibitory extract from skeletal muscle; Figs. 3 and 4, sister cultures; Fig. 3, in control medium, 10 per cent serum in balanced salt solution; Fig. 4, in 10 per cent serum in inhibitory fraction separated by DEAE-cellulose chromatography from the crude extract in Fig. 2. (Giemsa $\times 50$)

counted and duplicate flasks were stained for microscopic observation.

Crude extracts, alcohol and CaCl_2 precipitates and DEAE-cellulose fractions eluted at high ionic strength all had a markedly inhibitory effect on the cells of the highly malignant cystadenocarcinoma of the ovary. Figs. 1 and 2 show the destructive effect on these cells of the crude muscle preparation described above. DEAE-cellulose chromatography of precipitates derived from either skeletal muscle or tendon yielded ultra-violet absorbing peaks at about pH 6 and relatively low ionic strength with no effect on growth or mildly stimulating to these cells. On the other hand, material more strongly adsorbed on the column and therefore eluted at later stages at a lower pH, 4.5, and high ionic strength, 1-2 M NaCl, inhibited growth. These sub-fractions have repeatedly reduced the number of cells countable in tissue culture by 75-100 per cent. Such cells as remain in the preparations appear to be completely degenerate in the histological preparations. Figs. 3 and 4 show the destructive effect of the inhibitory DEAE-cellulose fraction prepared from the crude extract on the same cystadenocarcinoma cells. The factors had relatively little effect against the cells of the human epidermoid carcinoma of the larynx, *H.Ep.2*.

These growth inhibitors, which are components of normal adult connective tissue, are highly active biologically, since very small amounts by weight, 0.05 mg/ml. or less, clearly show the effects described. The chemical nature of the inhibitors has not as yet been determined. We have strong indications that several chemically distinct factors are involved, differing in resistance to heat and dialysis. The major component appears to be non-dialysable and resistant to 58°C. Nitrogen values indicate about 50 per cent protein. There is no DNA present, but approximately 10 per cent RNA. Organically bound sulphate is detectable, but sensitive tests failed to reveal significant amounts of hexoses, hexosamines or hexuronic acids¹⁵. In addition, there is at least one dialysable component which contributes to the observed activity. It appears to be a peptide or conjugated peptide associated with RNA but free of hexose or hexosamine.

We thank Mrs. Lillian Adams, who developed the strain of ovarian cancer cells for our original stock, and Mrs. Grace Lien Lew and Miss Tess Kourkouvelis for their help. This investigation was supported by Public Health research grant CA-04870 from the National Cancer Institute, and institutional grants from the American Cancer Society.

MARY STEARNS PARSHLEY
INES MANDEL

Department of Surgery, and
Department of Obstetrics and Gynecology,
College of Physicians and Surgeons,
Columbia University,
New York City.

area heavily infested with pigeons. One of the three samples originated from a thick layer of dry undisturbed pigeon droppings. The other two positive samples were taken from a layer of dark dust on the wall ledges of the church.

This finding of virulent strains of *C. neoformans* from pigeon manure confirms the view of Emmons⁵ that the association between virulent *C. neoformans* strains and pigeon excreta is not an unusual or geographically restricted phenomenon.

H. S. RANDHAWA
Y. M. CLAYTON
R. W. RIDDELL

Department of Medical Mycology,
Institute of Dermatology, London.

¹ Rook, A., and Woods, W., *Brit. J. Derm.*, **74**, 43 (1962).

² Lodder, J., and Kreger van Rij, N. J. W., *The Yeasts* (North-Holland Publishing Co., Amsterdam, 1952).

³ Seeliger, H., *J. Bact.*, **72**, 127 (1956).

⁴ Staib, F., *Zbl. Bakt.*, **130**, 274 (1962).

⁵ Emmons, C. W., *Publ. Health Rep., Wash.*, **75**, 362 (1960).

BIOLOGY

Activation of Spores of *Penicillium roqueforti*

GERMINATION of fungal spores is characterized physiologically by transformation of the spore from a dormant state of low activity to a state of high metabolic activity, and morphologically at a later stage by swelling of the spore and production of a germ tube. Since activation, that is, the termination of the dormancy of the spore, is the initial stage of germination, an examination of the activation process itself makes a logical starting point to the study of the biochemical aspects of germination. This communication describes the effect of different physiological agents on the ability of spores of *Penicillium roqueforti* to oxidize octanoic acid and hence provides a method of assessing the influence of these agents on activation. The method may also be applicable for studies of the effects of these agents on the subsequent stages of the germination process.

Spores of *P. roqueforti*, strain 6989, obtained from the American Type Culture Collection, Washington, were grown on slopes of Czapek-Dox agar (Oxoid) at 22°. After 6-10 days' incubation the spores were collected by adding distilled water and carefully scraping them from the surface of the slope with a sterile needle. They were separated from small pieces of agar and mycelium by filtering the suspension through two layers of cheesecloth. The spores were then collected on a 'Millipore' filter, washed with distilled water and transferred to M/10 sodium phosphate buffer. The suspension so obtained was standardized in a haemocytometer to give a final concentration of 0.5×10^8 spores per ml. For every 15 ml. spore suspension 10 mg streptomycin were added in order to minimize growth of contaminating bacteria¹. The progress of oxidation of fatty acids (3 μ moles) was followed manometrically at 27° in a standard Warburg apparatus, 0.2 ml. 20 per cent KOH being used in the inner cell. The methyl ketone concentration was determined at intervals during the experiments by pipetting 1 ml. of the contents of the Warburg flask into 1 ml. of 2,4-dinitrophenyl hydrazine (2 g/litre 2 N HCl) in a stoppered test-tube and the 2,4-dinitrophenyl hydrazones estimated by the procedure outlined by Lawrence².

Preliminary work showed that although washed spores of *P. roqueforti* could oxidize fatty acids only extremely slowly, the addition of glucose or 'Bacto' casamino acid solution (Difco) dramatically increased both oxygen uptake (Fig. 1) and methyl ketone formation after an initial lag period of 1-2 h (Fig. 2). A subsequent decrease in rate of oxygen uptake and methyl ketone formation indicated the completion of fatty acid utilization. The striking preferential utilization of a small amount of fatty acid (3 μ moles) in the presence of a large excess of sugars

Isolation of *Cryptococcus neoformans* from Pigeon Habitats in London

FROM the several cases of human cryptococcosis (torulosis or European blastomycosis) reported from Great Britain it is apparent that this is not an uncommon mycosis in this part of the world. Recently, Rook and Woods¹ listed twenty-two cases of the disease recorded in Britain. There does not appear to have been any published information, however, on the saprophytic habits of the causative fungus (*Cryptococcus neoformans*) in Britain. The following is a report on the isolation of *C. neoformans* from pigeon habitats in London, a preliminary notification of which was made at the International Academy of Pathology Symposium on Mycotic Diseases (June 1964) by one of us (R. W. R.).

Samples of pigeon manure from the concrete floors, walls and window-ledges of buildings inhabited by pigeons, and of soil from localities frequented by pigeons, were collected from four areas in west-central and one area in south-west London. These were examined for the presence of *C. neoformans* by suspending the samples in sterile normal saline, the supernatant being cultured on Sabouraud's dextrose agar containing chloramphenicol. Incubation was at 37° C, a temperature which inhibits many of the saprophytic moulds and yeasts. Identification of the yeasts isolated was based on methods described by Lodder and Kreger van Rij². In addition, biochemical properties included a positive urease test³ and the ability to assimilate creatinine⁴. Pathogenicity of the *C. neoformans* strains isolated was shown by animal inoculation tests using white mice. The results of 49 samples of pigeon manure and soil examined are given in Table 1. All the three positive manure samples came from the yard of a well-known west-central London church sited in an

Table 1. RECOVERY OF *C. neoformans* FROM PIGEON HABITATS

Sample	No. examined	No. positive
Pigeon manure	18	1
Pigeon manure + dust	11	2
Pigeon manure + soil	5	0
Pigeon nest	5	0
Soil	10	0
Total	49	3 (6%)

(250 μ moles) and of amino-acids (between 100 and 1,000 μ moles) is consistent with the finding of Farkas and Ledingham³ that uredospores of wheat stem rust preferentially use fatty acids as respiratory substrates.

The formation of 2-heptanone from octanoic acid was almost independent of pH between pH 4.5 and 7 (unpublished results), the limits of pH for optimum fungal spore germination⁴. Between 60 and 70 per cent of the acid was ultimately oxidized to 2-heptanone after 3–6 h incubation with the stimulating agents. The activated spores, on microscopic examination, were found to be apparently unchanged morphologically. Similarly, with spores of *Penicillium chrysogenum*, increases in total nitrogen, protein, RNA and the rate of oxygen uptake were noted about 7 h before the appearance of germ tubes⁵.

To confirm that the stimulatory effect of casamino acids resulted from the influence of one or more amino-acids, the effect of individual amino-acids on the ability of the spores to oxidize octanoic acid was tested. An increasingly pronounced stimulatory effect was associated with the following amino-acids, used at a concentration of 100 μ moles/3 ml.: L-leucine, L-arginine, β -alanine, α -aminobutyric acid, γ -aminobutyric acid, L-glutamine, L-proline, L-serine, D-alanine, L-alanine. No individual amino-acid was as effective as either casamino acids (0.1 g/3 ml.) or glucose (250 μ moles/3 ml.) (Fig. 2). Aspartic acid, asparagine, glycine, threonine, isoleucine, tryptophan, and tyrosine had no effect, while cysteine, histidine, methionine, valine and glutamic acid retarded formation of 2-heptanone from octanoic acid.

Of the monosaccharides tested for their ability to stimulate the oxidation of octanoic acid by spores, D-xylose at a concentration of 250 μ moles/3 ml. was the most active, although D-glucose and D-galactose were

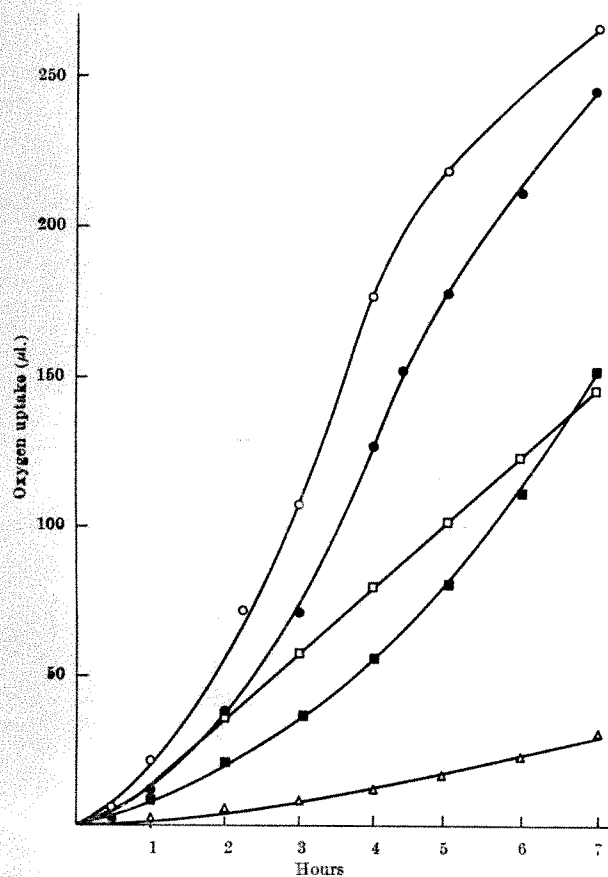


Fig. 1. The effect of octanoic acid (Δ - Δ), glucose (\square - \square), glucose plus octanoic acid (\circ - \circ), casamino acids (\blacksquare - \blacksquare), and casamino acids plus octanoic acid (\bullet - \bullet) on the oxygen uptake of washed 7-day-old spores (1.5×10^8) in phosphate buffer, pH 6.5 (100 μ moles). 250 μ moles glucose, 0.05 g casamino acids and 3 μ moles octanoic acid were used. Total volume 3 ml.

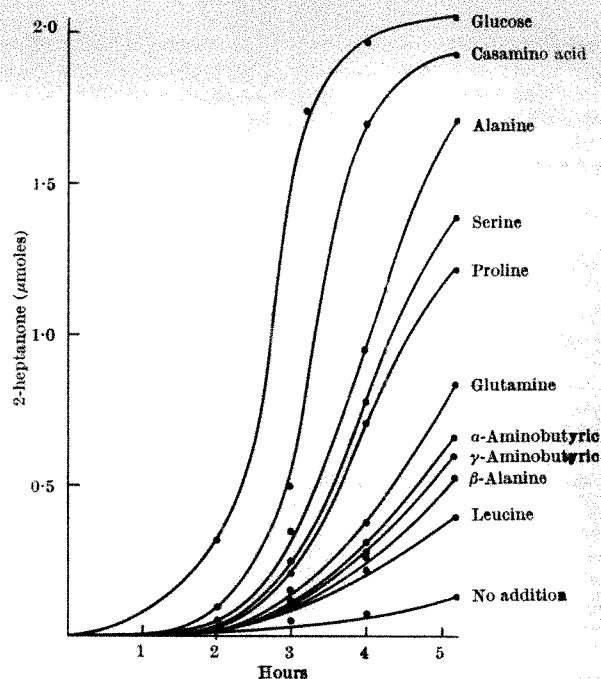


Fig. 2. The effect of amino-acids (100 μ moles), casamino acids (0.05 g) and glucose (250 μ moles) on the formation of 2-heptanone from octanoic acid (3 μ moles) by washed 7-day-old spores (1.5×10^8) in pH 6.5 phosphate buffer (100 μ moles). Total volume 3 ml.

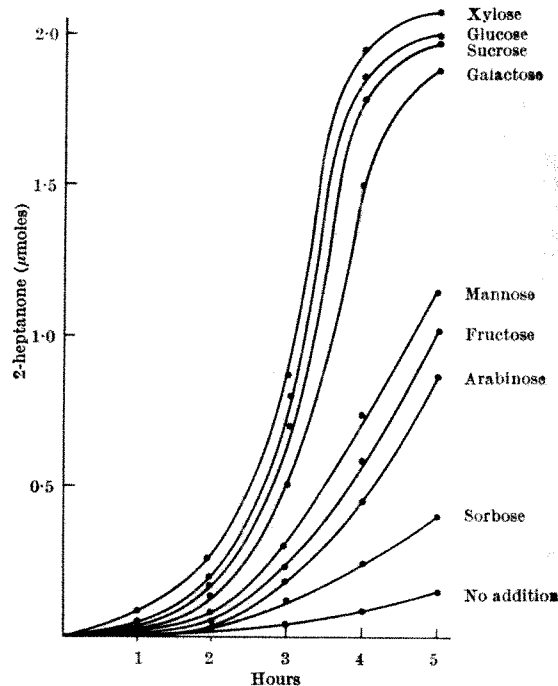


Fig. 3. The effect of sugars (250 μ moles) on the formation of 2-heptanone from octanoic acid (3 μ moles) by washed 7-day-old spores (1.5×10^8) in pH 6.5 phosphate buffer (100 μ moles). Total volume 3 ml.

almost as efficient. The spatial configuration around carbon atoms 1, 2 and 3 is the same in these 3 sugars. D-mannose, D-fructose, L-sorbose, D-arabinose, D-sorbitol and L-rhamnose were less stimulatory (Fig. 3). Galactitol, D-mannitol, D-ribose, D-lyxose, D-tagatose, D-glucuronic acid and D-glucosamine were almost without effect. It would appear therefore that some sugars are metabolized more readily than others to yield either energy or metabolic products essential for the activation process, and that the spatial arrangement of H atoms and —OH groups around the carbon atoms in the sugars may have special significance.

Of the oligosaccharides examined, only sucrose was as stimulatory as glucose. Maltose, trehalose, cellobiose and melezitose had a slight stimulative effect, whereas lactose and melibiose had no effect. Other compounds found to have no stimulatory effect were adenosine, adenosine triphosphate, inosine, adenine, glyceraldehyde, glycerol, pyruvate, citrate, succinate, malate, lactate, ethanol and propionate.

The specificity of the stimulatory agents suggests that their metabolism may provide an insight into the overall termination of dormancy. Results to be published in detail elsewhere suggest that their role is connected with the transport of fatty acid across the cell wall and that the effect being measured in these experiments is, in part, the stimulation of transport of octanoic acid into the cell. Suitable biosynthetic conditions are needed for the development of transport mechanisms which in turn regulate the entry into the cell of specific substances⁶. The lag period observed with washed spores could also be a result of the need for induced enzyme synthesis (for example, of β -keto acid decarboxylase) or a change in permeability. Levinson and Hyatt⁷ have suggested that the prime event in germination is a breach of a permeability barrier. Whatever the actual mechanism, however, the formation of methyl ketone would still provide a reliable measure of whether or not the spores had been activated.

In the comparatively few studies that have been carried out to determine the effect of different physiological agents on fungal spore germination, various criteria have been used to distinguish germinated from non-germinated spores; for example, the swelling of spores¹ and the proportion of spores that form a germ tube in a given time⁸. Such criteria, however, are somewhat subjective in nature and difficult to measure in practice. The marked similarity between the agents that stimulate the oxidation of octanoic acid by spores and those, in particular glucose, *l*-proline and *l*-alanine, that have been reported to stimulate the germination of fungal spores^{1,9,10} suggests that some reactions are common to both. Since Franke and Heinen¹¹ have shown that 30 out of 38 fungi tested were able to form methyl ketones from fatty acids, the utilization of this property to indicate which compounds are likely to stimulate germination of non-activated spores might have wide application.

R. C. LAWRENCE

Dairy Research Institute (N.Z.),
Palmerston North,
New Zealand.

¹Yanagita, T., *Arch. Mikrobiol.*, **26**, 329 (1957).

²Lawrence, R. C., *Nature*, **205**, 1313 (1965).

³Farkas, G. L., and Ledingham, G. A., *Canad. J. Microbiol.*, **5**, 141 (1959).

⁴Cochrane, V. W., in *Physiology of Fungi*, 388 (John Wiley and Sons, Inc., New York, 1958).

⁵Kornfeld, J., in *Spores II*, edit. by Halvorson, H. O. (Burgess Publishing Company, Minneapolis, 1961).

⁶Quastel, J. H., *Canad. J. Biochem.*, **42**, 907 (1964).

Levinson, H. S., and Hyatt, M. T., *Ann. N.Y. Acad. Sci.*, **102**, 773 (1963).

⁸Bonner, J. T., *Mycologia*, **40**, 728 (1948).

⁹Gottlieb, D., *Endavour*, **23**, 85 (1964).

¹⁰Cochrane, J. C., Cochrane, V. W., Simon, F. G., and Spaeth, J., *Phytopathology*, **53**, 115 (1963).

¹¹Franke, W., and Heinen, W., *Arch. Mikrobiol.*, **31**, 50 (1958).

Warted Pollen Grains of the Epacridaceae

WATSON¹ has directed attention to the ektexine ornamentation of pollen grains in the genus *Styphelia* Sm. Ten species were available for examination and their pollen grains bore curious warts.

An examination of the pollen of another genus of the Epacridaceae has shown that some species of *Epacris* Frost. et. f. emend. Cav. also have grains with warts or papillae.

Australian botanists generally agree that the classification of *Epacris* requires revision. A list of species at present acceptable has been drawn up on the advice of

Table 1. SPECIES OF *Epacris* EXAMINED. MEASUREMENTS TAKEN FROM ONLY ONE PREPARATION FOR EACH SPECIES

	Mean maximum tetrad dimension (μ) N = 9	Warts
<i>E. acuminata</i> Benth.	43.2 \pm 0.32	—
<i>E. apiculata</i> A. Cunn.	35.2 \pm 0.29	—
<i>E. breviflora</i> Stapf.	30.1 \pm 0.35	—
<i>E. calvertiana</i> F. Muell.	49.4 \pm 0.19	—
<i>E. coriacea</i> A. Cunn.	35.2 \pm 0.17	—
<i>E. crassifolia</i> R. Br.	65.0 \pm 0.29	Present
<i>E. exserta</i> R. Br.	49.4 \pm 0.51	Present
<i>E. hamiltoni</i> Maiden et Bettle	43.3 \pm 0.47	—
<i>E. heteronema</i> Labill.	61.9 \pm 0.71	Present
<i>E. impressa</i> Labill.	50.3 \pm 0.55	—
<i>E. lanuginosa</i> Labill.	50.8 \pm 0.30	—
<i>E. longiflora</i> Cav.	63.9 \pm 0.38	—
<i>E. marginata</i> Melville	50.6 \pm 0.66	Present
<i>E. microphylla</i> R. Br.	30.7 \pm 0.21	—
<i>E. mucronulata</i> R. Br.	46.7 \pm 0.48	—
<i>E. myrtifolia</i> Labill.	61.4 \pm 0.36	Present
<i>E. obtusifolia</i> Sm.	67.5 \pm 0.39	Present
<i>E. paludosa</i> R. Br.	44.7 \pm 0.43	—
<i>E. pulchella</i> Cav.	39.3 \pm 0.23	—
<i>E. purpurascens</i> R. Br.	49.7 \pm 0.34	—
<i>E. reclinata</i> A. Cunn. ex Benth.	53.4 \pm 0.42	—
<i>E. rigida</i> Sieber. ex Spreng.	48.9 \pm 0.29	Present
<i>E. robusta</i> Benth.	56.7 \pm 0.51	Present
<i>E. serpyllifolia</i> R. Br.	48.9 \pm 0.25	Present
<i>E. stuartii</i> Stapf.	57.9 \pm 0.35	Present

taxonomists in Australia. The species are given in Table 1, with the exception of *Epacris barbata* Melville, *E. petrophila* Hook. and *E. sparsa* R. Br. It has not yet been possible to examine the pollen of the three latter species.

The *Epacris* pollen was examined after acetolysis. The papillae appear in general to be more closely placed on the grains than those noted for *Styphelia*². The warts tend to become detached from the pollen grains (cf. *Styphelia*) and it is possible that acetolysis has removed these features from some grains.

The warts vary from 1 to 10 μ in diameter between the species. There is also variation within the species. The

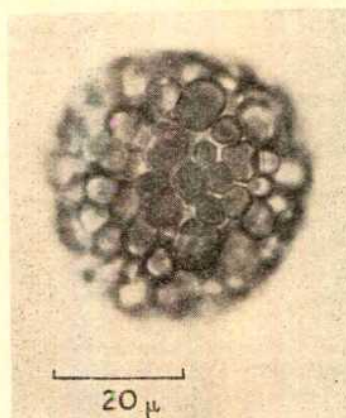


Fig. 1. *Epacris rigida* tangential section

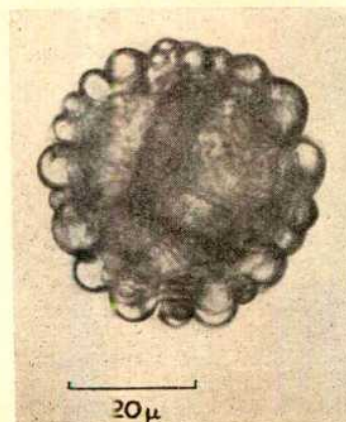


Fig. 2. *Epacris rigida* optical section

diameter of warts on one grain of *Epacris rigida* was observed to vary between 2 and 10 μ .

It would be premature to assess the significance of warts or papillae for the classification of *Epacris*, but there can be no doubt that the presence or absence of these structures will be an aid to further systematic investigation.

The assistance of Dr. N. T. Burbidge, C.S.I.R.O. Division of Plant Industry, Canberra; Mr. J. Willis, the National Herbarium, Melbourne; and Mrs. P. Walker, the Department of Botany, the Australian National University, Canberra, in the selection of acceptable species of *Epacris* is gratefully acknowledged.

J. M. MATTHEWS

Research School of Pacific Studies,
Australian National University,
Canberra.

¹ Watson, L., *Nature*, **194**, 889 (1962).

² Watson, L., *Pollen et Spores*, **5**, 1 (1963).

Relationships between Constituents of Cones and Male Flowers of the Hop (*Humulus lupulus*, L.)

Hop breeding is complicated by the fact that the flowers of male plants are useless for breeding, so that their genotype in this respect cannot be indicated phenotypically. Hence male parents for breeding work have hitherto had to be selected by progeny testing. Brewing quality of hop cones (the female inflorescences) depends mainly on the amounts of soft resins present (the α -acid fraction being the most important) and the composition of the essential oil. The resins produce the bitter character of beer, while the oils contribute the characteristic flavour.

Brooks and Likens¹ have analysed the soft resins in the flowers of 20 male clones and demonstrated significant genotypic differences between them. They have not yet demonstrated that the resin characteristics of these males are transmitted to their progeny.

Using gas chromatographic methods, Roberts² analysed the essential oils in the cones of female, and flowers of male, plants and found that in a seedling family the characteristics of both parents reappeared among the progeny. Tetraploid hop plants with XXXY sex chromosomes are monoecious and both sexes are fertile, so such plants can be selected for the brewing quality of their cones and then used as pollen parents in a breeding programme³. Such plants also offer a means of determining whether cones and male flowers on the same plant and, therefore, genotypically identical give comparable results when analysed for resins and essential oils, or whether there are fundamental differences between them.

During the 1964 season, resin and essential oil analyses were carried out on cones and male flowers of 16 tetraploid monoecious plants, the resin determinations being of α -acid (a mixture of humulone and analogues) and of total soft resin (the resin fraction soluble in paraffinic hydrocarbons, ether and methanol and determined by solvent extraction). The difference between these values is referred to as the β -resin. The β -acid contents of both sexes were determined by similar polarimetric methods using ether extraction⁴. The mean value for α -acid content in the cones was 13.0 times that for the male flowers, but there was a significant correlation between the two sets of values ($P < 0.01$). The β -resin content in the cones was only 5.9 times that for the male flowers and there was no significant correlation between these values. As shown in Table 1, the α -acid β -resin ratio was higher in the cones than in the male flowers; very similar results were obtained from analyses on male and female plants from two normal seedling families. As β -resin is an imprecisely defined mixture, it is possible there is a component that is abundant in male flowers which is absent or present only in small amounts in cones.

Table 1. MEAN VALUES FOR α -ACID AND β -RESIN CONTENTS IN CONES AND MALE FLOWERS OF TETRAPLOID MONOECIOUS PLANTS AND DIPLOID SEEDLING FAMILIES

	Sex	α -Acid content %	β -Resin content %	Ratio α -acid/ β -resin
Monoecious plants	Female	4.03	8.17	0.50
	Male	0.31	1.38	0.22
Dioecious family 17/62	Female	8.47	6.99	0.50
	Male	0.19	1.05	0.18
Dioecious family 21/62	Female	4.08	9.31	0.44
	Male	0.14	0.90	0.15

The essential oil compositions of both cones and male flowers of the monoecious plants were determined by ether extraction (room temperature) of the dried flowers (0.5 g) and evaporation of the dried solution on to 'Celite' at room temperature, followed by gas chromatography of the 'Celite' sample². Myrcene, caryophyllene, humulene and sometimes farnesene (depending on variety) are the major oil components in cones, but myrcene does not show up by this technique. In all cases the cones and male flowers from the same plant gave similar proportions of caryophyllene and humulene. Two plants contained farnesene in the cones, but no male flowers contained measurable quantities of this compound. All the male flowers contained at least one extra component that was absent from the cones (Fig. 1).

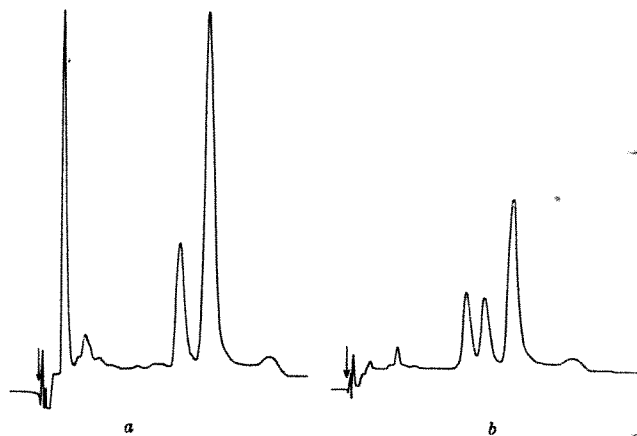


Fig. 1. Chromatograms of (a) male flowers and (b) cones from the monoecious plant 25/57/84. (Pye Argon chromatograph, 4 ft. column of 10 per cent 'Apiezon L' on 60-80 mesh 'Celite', 175° C, rate of flow 60 ml/min)

The differences noted between cones and male flowers of tetraploid monoecious plants have also been found between male and female diploid plants. In one seedling family, large farnesene peaks were present in all 15 female seedlings examined. In other families farnesene was present in some females but absent from others. None of the male seedlings examined showed definite farnesene peaks, nor did the male parents, although some transmitted this character to their female progeny. The ratios obtained suggest that the presence of farnesene is a sex-limited character controlled by a single pair of genes with presence of farnesene dominant to absence. The unidentified peak found only in the male flowers of monoecious plants was characteristic of all diploid male plants examined, but was absent from diploid females. Preliminary evidence indicates that another component (probably selinene) which is abundant in some females may be much less abundant or absent in males.

This work has clearly indicated that there are fundamental differences between the essential oils and resins of cones and male flowers, and the same differences are found whether these are on separate dioecious plants or occur together on monoecious plants. Since there was a correlation between the α -acid contents of cones and male flowers on monoecious plants, it is probable that α -acid determina-

tions on male plants will be a valid method of selecting them for breeding purposes.

R. D. HARTLEY
R. A. NEVE

Department of Hop Research,
Wye College (University of London),
Nr. Ashford, Kent.

¹ Brooks, S. N., and Likens, S. T., *Crop Sci.*, **2**, 189 (1962).

² Roberts, J. B., Ph.D. thesis (Univ. London), 1963.

³ Neve, R. A., *J. Inst. Brew.*, **69**, 129 (1963).

⁴ Institute of Brewing: Hops Analysis Sub-Committee, *J. Inst. Brew.*, **70**, 156 (1964).

Radiation-induced Sex Reversal in *Ecballium elaterium*

Galán^{1,2}, whose work was reviewed by Mather³, showed that the cucurbitous plant *Ecballium elaterium* has been shown to exhibit the simplest sex-determining mechanism ever found in higher plants. A dioecious variety growing in the south of Spain and in North Africa maintains a 50 per cent pro-sex ratio in Nature by means of a pair of alleles $a^D > a^d$. Male plants are heterozygous while female ones are recessive. On the other hand, the well-known monoecious variety which grows in Mediterranean Europe is homozygous in a third allele a^+ . Through reciprocal hybridization between both varieties, Galán was able to demonstrate that allele a^+ dominates a^d , but behaves as a recessive factor in relation to a^D .

During the past three years we have attempted to induce mutations in the allelic series $a^D > a^+ > a^d$, especially in the two alleles which are involved in the dioecious variety. Gamma rays, neutrons and diethyl sulphate (DES) have been used as mutagenic agents. Our tests in the first year recommended a dose of approximately 20,000 rads of ionizing radiation for treatment of dry seeds. DES was used at a concentration of 1/500 during 24 h. Chronic gamma irradiation of growing plants was also carried out with dose rates ranging from 10 to 120 rads per day. Neutron exposures were delivered at the J.E.N. nuclear reactor of La Moncloa (Madrid) while gamma rays were provided by our own gamma facility ('El Encin', Alcalá de Henares) equipped with a 2,250-c. Cs-¹³⁷Ba source.

However chronic or acute was the treatment, notes were only taken of the actually irradiated generation (R_1), since it can easily be learned that mutations $a^D \rightleftharpoons a^d$ in any direction would show up readily from genotypes as $a^D a^d$ or $a^+ a^d$, with no need for back-crossing. Nearly 2,000 mature individuals were observed in each of their branches, for in irradiated seeds an induced mutation would normally affect a single cell in the embryo, and it would only appear partially in the adult plant.

In the course of three seasons we have been able to observe a number of sex aberrations which were clearly not mutations but probably originated from disturbances in the mechanisms concerned in sex phenotypic expression. Some new characters not affecting sex also appeared, a few of them probably genetic changes. These are now being observed for a future tentative list of mutable genes in this interesting plant species.

Late in summer 1964, one instance of a sex reversal with a high probability of being of genetic origin, was detected in a masculine plant of the dioecious variety, arising from a neutron-irradiated seed. It consisted of a complete reversal to morphologically quite normal female flowers, affecting one of the lower branches of the plant; moreover, it did not involve the whole branch but certain nodes of it. After a detailed phyllotactic map was built, the new character appeared to be distributed clearly in a sector of approximately 180 degrees. Sectoriality strongly suggests that we are dealing with a mutation, at least in the wider sense of this word that includes chromosome deletions or re-arrangements.

No other character was observed to change in association with the flower sex. Leaf and stem colour, leaf shape, hair pattern, etc., were unaltered. Curiously, it was the fact that while flower morphogenesis reached a quite reversed expression, the aspect of the inflorescence remained essentially masculine. In the leaf axil of a male plant of *E. elaterium* var. *dioicum*, the inflorescence is elongated and bears up to 15-20 flowers successively during the season (see Fig. 1, upper left); the first of these flowers originates from a different primordium and bears no bract, though its peduncle is largely connated to the inflorescence axis⁴. This first flower is the only one which develops in the axil of female plants, of course with a female expression⁴. In this case, after fecundation, the stalk thickens and elongates rapidly, with a marked negative geotropism (upper right); the fruit at its top explodes later by the well-known hydraulic seed dispersal mechanism of this species. On our mutated branch, female flowers were found not only in the axil, but also rather clustering along non-thickened, non-geotropic axes (lower figure, on the right).

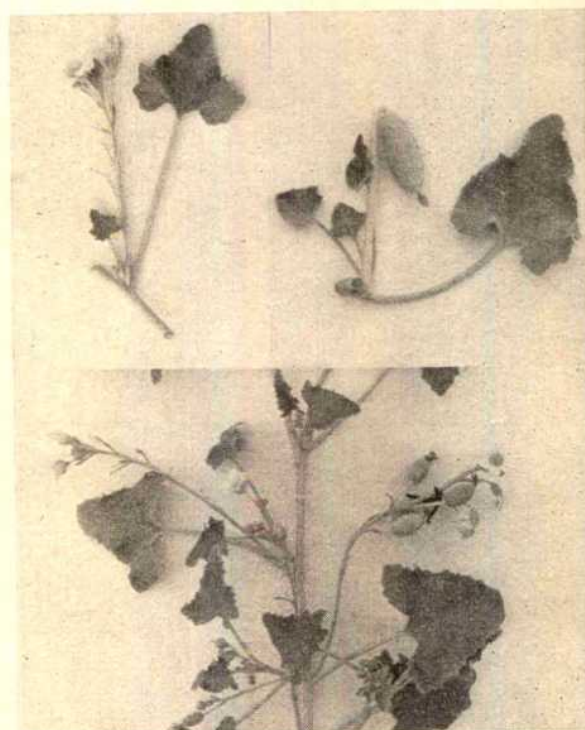


Fig. 1

Unfortunately, female flowers of the reversed branch yielded only three or four poor-looking seeds which would probably be unable to germinate, and in any case would not provide enough information on sex proportions in the R_2 . Many of these flowers gave rise to well-developed though seedless fruits, or in other words, to parthenocarpic fruits. The observed sterility may be the consequence of an induced chromosome aberration, perhaps a deletion involving gene a^D . Lack of apparent side effects on the phenotype would seem to indicate that the hypothetical chromosomal damage is not too drastic. The possibility still remains of a mutation $a^D \rightarrow a^d$ where the female genotype loses most of its fertility within a 'male environment'.

CÉSAR GÓMEZ-CAMPO
MANUEL CASAS-BUILLA

Instituto Nacional de Investigaciones Agrícolas,
Madrid, 3.

¹ Galán, F., *C.R. Acad. Sci., Paris*, **222**, 1130 (1946).

² Galán, F., *Acta Salmantica*, **1**, 7 (1951).

³ Mather, K., *Nature*, **163**, 926 (1949).

⁴ Galán, F., unpublished.

Ingestion of Soil by Sheep in New Zealand in Relation to Wear of Teeth

In certain parts of New Zealand, excessive wear occurs in the incisor teeth of sheep, with the result that sheep must be culled from flocks at a comparatively early age, because they are no longer able to graze efficiently.

It has been proposed that constituents of actively metabolizing herbage which predispose sheep's teeth to excessive wear appear to be: (1) enzymes (proteinases), which attack the organic bonding material of the dentine; (2) acids which chelate calcium of the apatite. By these agencies tooth structure may become weakened and susceptible to attrition by the tougher constituents of the herbage such as fibre and plant phytoliths¹. Wear has been attributed by Australian workers to abrasion by plant phytoliths^{2,3}.

The investigation reported in part here arose from a survey into wear by J. Wellington, Department of Agriculture, and was undertaken to assess the relative importance of physical and chemical factors that might be responsible for differences in wear noted in sheep on selected farms on different soils in the Wairarapa area of the North Island.

Farm A. High level of wear in sheep's teeth. High production ryegrass clover pasture. Yellow-grey earth soil (Wharekaka silt loam).

Farm B. Medium level of wear in sheep's teeth. High production ryegrass clover pasture. Yellow-brown loam soil (Tauherenikau stony loam).

Farm C. Low level of wear in sheep's teeth. Low production, unimproved pasture with high proportion of native grasses and moss. Steepland soil (Ruahine steep-land soil).

As part of the investigation into physical and chemical factors, analysis of sheep faeces was carried out to measure the amounts of soil taken in with pasture by the grazing animal, since soil could be an important factor in any abrasive process. The data for soil ingested over the winter months are of special interest.

Faeces samples were collected at approximately three-weekly intervals from July to December on the three farms. Only fresh faeces were collected and particular care was taken to collect pellets or stool material not in contact with pasture or soil. For each sample on each farm approximately 5 g of fresh faeces was collected from each of 40 separate piles of faeces, to give a composite sample of about 200 g. The whole sample was dried, ashed at 550° C and extracted with successive portions of 3 N hydrochloric acid to yield an acid-insoluble residue. Comparison of faeces samples collected in this way with faeces samples taken direct from the rectum of sheep grazing the same pasture shows close agreement in acid-insoluble residue figures. In Table 1 the acid-insoluble residue levels for the period July–December are expressed as a percentage of faeces on a dry matter basis (100° C). For the purpose of calculating weight of soil ingested per day it has been assumed that dry matter intake is at a level of 1 kg per day and that the ratio of dry matter faeces : dry matter pasture is 1 : 6. If dry matter intake of pasture per day is less than 1 kg per day, soil ingestion figures will be correspondingly reduced; if the ratio of dry matter faeces : dry matter pasture is greater than 1 : 6, soil ingestion figures will be increased. It is estimated that at a dry matter intake of 1 kg per day approximately 15 g of the acid-insoluble residue per day will come from the pasture, probably mostly in the form of plant phytoliths. This figure has been subtracted from the daily total of acid-insoluble residue to give a net figure which is taken to be ingested soil—this has been confirmed by the presence of quartz peaks in X-ray diffraction studies of the acid-insoluble residue. The results are presented in Fig. 1 on this basis.

It can be seen that soil ingestion is high over the wet winter period on the 'high-wear' farm, Farm A, when

Table 1. ACID-INSOLUBLE RESIDUE IN FAECES (Per cent dry matter)

Period of collection	Farm A (high wear)	Farm B (medium wear)	Farm C (low wear)
July 14, 1964	45.9	34.2	9.5
July 24–30, 1964	44.4	25.3	9.4
Aug. 18–19, 1964	59.6	34.7	12.7
Sept. 4–9, 1964	51.4	36.2	11.2
Sept. 29–30, 1964	34.3	17.1	11.7
Oct. 13, 1964	19.4	9.3	10.4
Nov. 4, 1964	17.7	3.0	7.0
Nov. 24–30, 1964	11.3	4.9	6.3
Dec. 15–16, 1964	10.4	1.3	5.4

pastures were short and muddled, and earthworm casts abundant. Farm B, 'medium wear', although as heavily stocked as Farm A, has a lower level of soil ingestion, which agrees well with field observations that, even when pastures on this farm were short, the high proportion of stones in this soil results in a firm pavement and less muddled pasture. Farm C, 'low wear', although carrying poorer low-production pastures of native grasses and moss, has a close mat of turf, with little possibility of soil contamination. It can be seen that on this farm ingestion of soil is low.

It is intended to follow soil ingestion for a full year to obtain annual figures for soil intake. The figures gained so far suggest soil intakes of the order of 25 lb., 10 lb., and 1 lb., respectively, over the winter period on high-, medium- and low-wear farms. At peak intake ewes may be ingesting about 3 lb. soil/week on Farm A. Ingestion falls over the spring period when pastures are longer and competition for feed is reduced. Some increase in ingestion may occur over the dry summer months, but it would seem unlikely that this will approach the winter peak. It is possible that a figure approaching 50 lb./year might be obtained on high-wear farms in wet years.

It would seem, then, that there is an obvious correlation between the amount of soil ingested and the degree of wear in sheep's teeth on the three farms. While chemical agents in pasture that might contribute to wear are being investigated, at this stage ingested soil would appear to be a

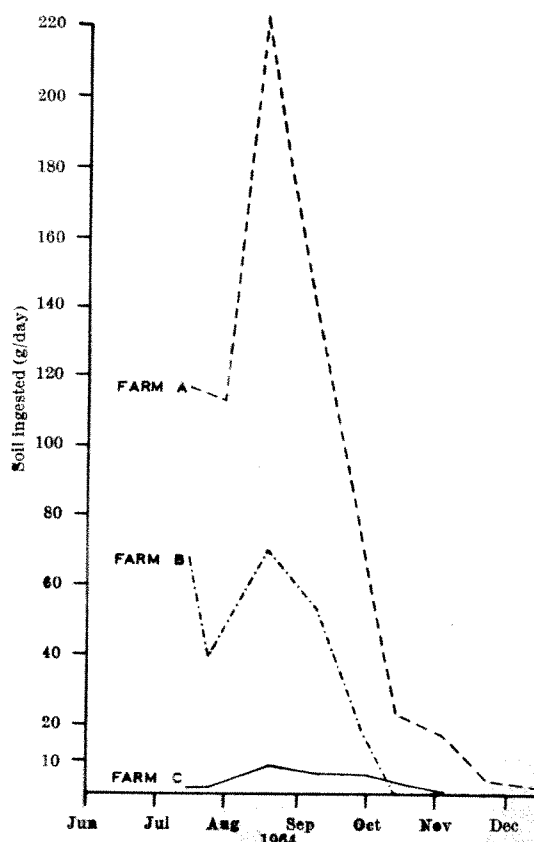


Fig. 1

prime factor in wear, at least under New Zealand conditions. Although Baker *et al.*^{2,3} suggest, in a number of interesting papers, that plant phytoliths could be responsible for wear, it seems unlikely that plant phytoliths would exceed 15 g/day at the 1 kg/day dry-matter intake-level, a relatively small figure as compared with peak intakes of soil of more than 200 g/day. Further, the percentages of phytoliths in pasture samples from the three farms are not sufficiently different to explain the wear differences. This would suggest that, under New Zealand conditions, plant phytoliths are not a primary cause of wear.

After this investigation was under way it was found that another New Zealand worker, E. Suckling⁴, who was studying the effect of stocking rates on pasture composition, body-weights, wool weights, footrot, and internal parasites, had noted excessive wear in sheep at high stocking rates, which he has attributed to abrasion by soil. Co-operative studies between us now under way should enable us to establish if wear is optimal at peak soil ingestion periods.

The surprisingly high amounts of soil ingested on the high-wear farm may have other implications in the soil-plant-animal field. Ingested soil may be a significant source of microelements and of insecticides, and these aspects are also being investigated.

W. B. HEALY

Soil Bureau, D.S.I.R.,
Private Bag, Lower Hutt, New Zealand.

T. G. LUDWIG

Dental Research Unit,
New Zealand Medical Research Council,
P.O. Box 3155, Wellington.

¹ Barnicoat, C. R., *Mechanisms of Hard Tissue Destruction*, Pub. No. 75, Amer. Assoc. Adv. Sci., 155 (1963).

² Baker, G., Jones, L. H. P., and Wardrop, I. D., *Nature*, **184**, 1583 (1959).

³ Baker, G., Jones, L. H. P., and Wardrop, I. D., *Austral. J. Agric. Res.*, **12**, 462 (1961).

⁴ Suckling, E., *N.Z. J. Agric.*, **109**, 153 (1964).

ENTOMOLOGY

Changes in Size of the Corpus Allatum in a Polymorphic Insect

In order to gain some insight into the part played by the endocrine system in determining form in a polymorphic insect, an investigation was undertaken of changes in volume of the corpus allatum in virginoparous alate and apterous forms of the cabbage aphid, *Brevicoryne brassicae* (L.).

Aphids of known ages were fixed in alcoholic Bouin containing 0.5 per cent trichloroacetic acid, sectioned frontally at 8 μ , and stained with iron-haematoxylin. The length and width of the corpus allatum were measured using a screw-type eyepiece micrometer and the approximate volume of the gland was calculated assuming it to be an ellipsoid having equal minor axes.

It was found that during the third and particularly the fourth nymphal instar apteriform aphids had larger corpora allata than did alatiform aphids. At the imaginal moult the corpus allatum of the apterous aphid was twice as large as that of the alate. The corpus allatum of the alate did not increase in volume between the beginning of the third instar and the imaginal moult, but by 24 h after the imaginal moult it had doubled in size. In contrast, the corpus allatum of the adult aptera decreased sharply in volume soon after the imaginal moult.

The observations on the nymphal stages support the theory that apterous characteristics may be brought about by a relatively high concentration of juvenile hormone^{1,2}.

The relative sizes of the corpora allata in adult apterous and alate aphids were particularly interesting. It has been observed that alate parents give birth almost exclusively to apterous young, while the progeny of

apterous parents may be apterous or alate³. There is also evidence which suggests that the young of alate parents are determined as apterae before birth, while the progeny of apterae may not be irreversibly determined as apterous or alate until as late as the second instar⁴. This could be explained by supposing that the form of the embryos is influenced by the hormones of the mother. An active corpus allatum in the mother would thus increase the amount of juvenile hormone passing into the embryos and direct them to the apterous pathway of development. A small or inactive corpus allatum such as occurs in apterous adult aphids would have less effect on the form of the young, and its influence could be modified postnatally by various environmental factors.

DINAH WHITE

School of Biological Sciences,
University of Sydney.

¹ Johnson, B., *Entomol. Exp. Appl.*, **2**, 82 (1959).

² Lees, A. D., *Symp. Roy. Entomol. Soc., London*, **1**, 68 (1961).

³ Bonnemaison, L. E., thesis, University of Paris (1951).

⁴ Johnson, B., and Birks, P. R., *Entomol. Exp. Appl.*, **3**, 327 (1960).

Periodic Aggregating and Take-off in Anthophorid Bees, *Anthophora acraensis* Fab.

It is well known that a number of insects refrain from work at dusk, congregate to 'sleep' (or at least to rest in a comatose state), and then resume activity as soon as it becomes bright again¹. However, little detailed work² seems to have been done on the pattern of the alighting and take-off phases of these 'sleeping' aggregations. The present observations have established both the periodicity and temporal pattern of the two phases in *Anthophora acraensis* Fab.

The observations were made between October 10 and November 14, 1964, on small groups of the species aggregating daily in the same tree in a garden at Achimota, Ghana. The number of bees in each day's group varied between 33 and 17 in October; but thereafter fell steadily until there were only 6 individuals coming to the tree on November 14. These groups consisted apparently of males only. On two separate days a single male specimen of *A. albigena* Lep. was observed among the *A. acraensis*. In analysing the results 'William's mean'³ has been used as the measure of central tendency.

The bees alighted in the evening between 1720 and 1800 h, with a peak in the period 1745–1750; spent the night on the tree, using more or less the same branches each time; and took off in the morning between 0525 and 0605 h, with a peak in the period 0535–0540 (Fig. 1—based on detailed observations made on twenty evenings and twenty mornings). This pattern was repetitive from day to day. The evening peak occurred during the first 5-min period after sunset (1745 h); whereas the morning peak occurred during the second 5-min period before sunrise (0545 h). In these circumstances, the light intensities during the two peak periods could not obviously have been the same; and records (on four evenings and five mornings) made with an 'Eel' photometer indicated that the peak and cessation of alighting occurred under greater illumination than the onset and peak of take-off (Fig. 1).

There was a general tendency for the bees to settle very close to each other on the branches, usually about half an inch apart. It was also observed frequently that attempts by oncoming bees to alight between those that had thus settled were actively repulsed, the latter raising their hind legs at the approach of the former. This behaviour towards an intruder somewhat resembles that noted by Young⁴ in the beetle, *Altica bimarginata*, aggregating to 'sleep'.

On alighting, the bees instantaneously clasped the selected branch of the tree with their mandibles and apparently spent the night like this, without using their legs for holding on. Prior to take-off they stretched their hind legs vigorously for a few minutes and became very

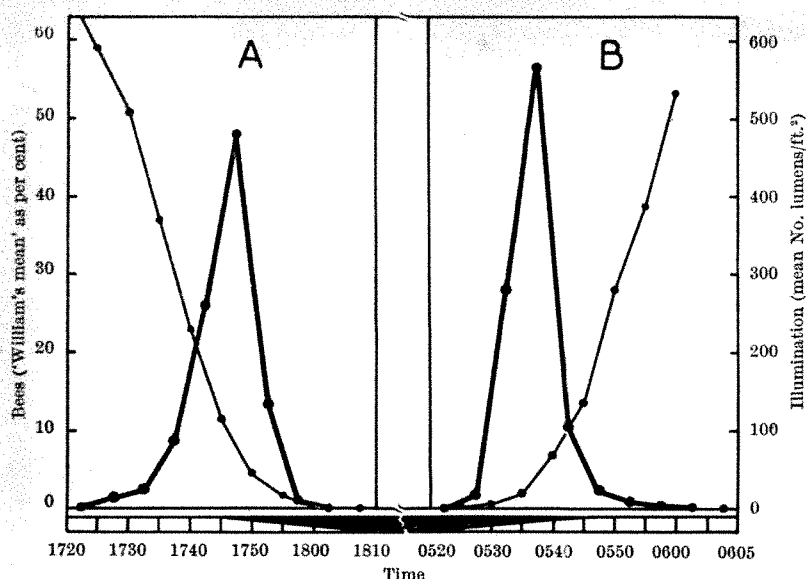


Fig. 1. A, Alighting; B, take-off (thick line, bees; thin line, illumination)

active. This was probably not just a simple warming-up performance, since the bees sometimes did the same thing immediately after alighting in the evening, and the wings remained folded all the time this was going on.

Early in January 1965 one or two bees were still coming to spend the night on the tree. If and when the numbers build up again, or if other similar groups of *A. acraensis* could be discovered elsewhere in the neighbourhood, it is hoped to investigate more fully the possible influence of illumination and other factors on the alighting and take-off phases and also the regularity of individual bees in these activities.

Reports published by Matthewson and Daly⁵ and Evans and Linsley⁶ confirm my observations of sleeping clusters (males only⁵), and of the clasping of branches with the mandibles.

I thank Dr. I. H. H. Yarrow for identifying the bees for me.

L. K. H. GOMA *

Department of Zoology,
University of Ghana,
Legon, Accra.

* Present address: School of Natural Sciences, University of Zambia, Lusaka, Zambia.

¹ Frost, S. W., *Insect Life and Insect Natural History* (Dover Publications, Inc., New York, 1959).

² Evans, H. E., and Gillaspay, J. E., *Amer. Midl. Nat.*, **72**, 257 (1964).

³ Haddow, A. J., *Bull. Entomol. Res.*, **50**, 759 (1960).

⁴ Young, R. T., *Science*, **81**, 435 (1935).

⁵ Matthewson, J. A., and Daly, V. D., *J. Kansas Entomol. Soc.*, **28**, No. 3 (1955).

⁶ Evans, H. E., and Linsley, E. G., *Bull. Soc. Calif. Acad. Sci.*, **59**, Part 1 (1960).

Relationship between the Removal of the Frontal Ganglion and Protein Starvation in *Locusta migratoria* L.

It has been shown¹ that the removal of the frontal ganglion in *Locusta migratoria* L. stopped any further growth in weight of the insect. Based on co-related changes in the endocrine system, it was suggested that failure to grow was basically a failure of protein metabolism. The possibility that cessation of growth was brought about by gross or partial starvation due to the operation interfering with the insect's ability to eat or pass food through its gut was rejected for the following reasons:

(1) The operated locusts were observed to eat frequently and to defaecate often; no obvious differences between these and normal locusts were observed in this matter.

(2) Dissections of the operated locusts revealed the presence of grass throughout the length of the gut.

(3) The operated locusts live four or five times as long as starved ones and are fully active throughout their lives. In adults, flight is not impaired in any way.

(4) The operated locusts remain at a constant weight for the rest of their lives. It is extremely improbable that just that amount of damage is done by the operation which would allow the locust to take in just that amount of food which would (a) make good the loss of weight due to post-operative treatment, (b) precisely make good the daily energy expenditure of the animal.

(5) The oxygen consumption of operated locusts was not significantly different from normal locusts, while that of starved animals is always significantly lower.

(6) The effects of starvation could be reversed by simply giving the locusts food, but no way of correcting the results of the removal of the frontal ganglion was found.

Since the publication of this work, the possibility has been raised a number of times that starvation, that is the failure of the animal to take into its gut sufficient food for growth, was responsible for the observed results. This theory has been put forward sufficiently often to warrant re-iteration of the original reasons together with

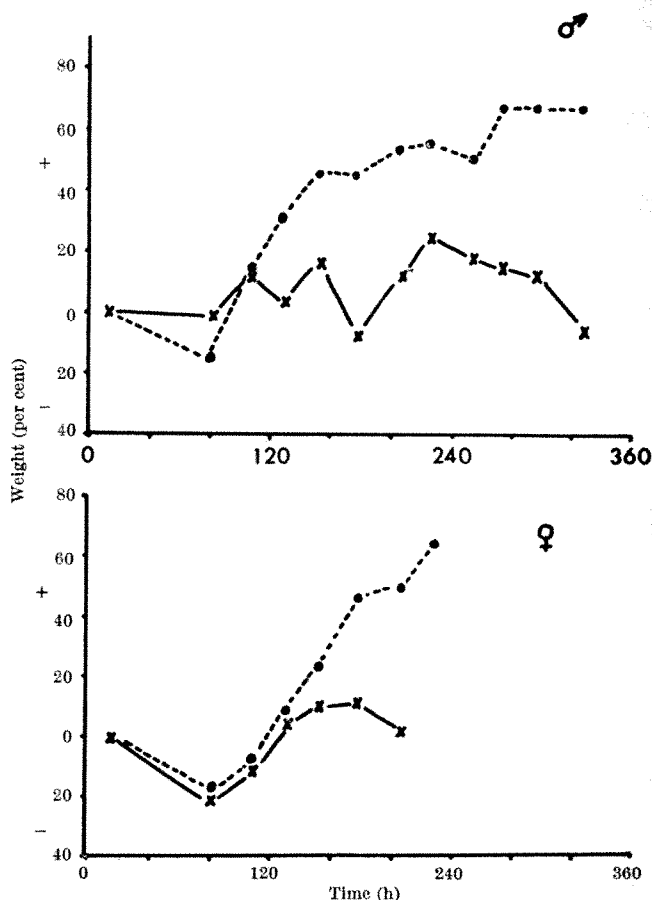


Fig. 1. The percentage change in weight of fourth instar *Locusta migratoria* from which the frontal ganglion has been removed within 24 h of ecdysis (hour 0) and daily injections commenced at 80 h. ●—●, Locusts which were injected with corpora cardiaca extract; ×—×, locusts which received control injections. Each curve represents the responses of one individual

a brief statement of the extra facts (taken from our continuing programme of research into the physiological effects of the removal of the frontal ganglion):

(a) Active proteinases are present in the gut of operated locusts and in operated controls. In the operated controls the amount present increases as the animal grows; in the operated locusts the amount remains the same as it was at the time of the operation.

(b) Studies on ^{14}C -glycine injected into the haemocoel (thus by-passing any barrier the gut may present) of operated locusts and operated controls show that it is incorporated into the tissues of the former to a much lesser extent than in the latter.

(c) Daily injections of an extract of corpora cardiaca taken from normal locusts caused near normal growth in operated locusts (Fig. 1). Control injections are without effect.

From the evidence given here the following relationship is deduced between forcibly starved locusts and those from which the frontal ganglion has been removed but are in the presence of abundant food and water.

The effect of the operation is to stop, or reduce to a very low level, protein synthesis in the insect's body. This effect can also be achieved by forcibly starving locusts, either completely by withholding all food, or specifically by withholding all protein. The gross effect of these two procedures is similar, but they are fundamentally different for the following reason. In operated locusts it is the machinery of protein synthesis that has been interfered with, while in starved locusts it is the fuel which is being withheld.

Further work has shown that the removal of the frontal ganglion affects protein metabolism by way of the action of the corpora cardiaca hormones and the synthesis of mRNA in the tissue cells. We are affecting, therefore, the growth of the locust at a far more fundamental level than is achieved by starvation and, by the proper exploitation of the effects of the removal of the frontal ganglion, learning much about the control of growth and metabolism in the insect.

K. U. CLARKE
C. GILLOTT

Department of Zoology,
University of Nottingham.

¹ Clarke, K. U., and Langley, P. A., *J. Insect. Physiol.*, **9**, 287 (1963); **9**, 363 (1963); **9**, 411 (1963); **9**, 423 (1963).

MICROBIOLOGY

Isolation of Bacteria able to metabolize Fluoroacetate or Fluoroacetamide

FLUOROACETATE or fluoroacetamide, after conversion to fluorocitrate, inhibit aconitase¹ and therefore block the tricarboxylic acid cycle; consequently these chemicals are toxic to animals and are used as pesticides. There have been at least two occasions when accidental contamination of soil and water by fluoroacetamide has occurred². This communication reports the isolation of bacteria able to use these compounds for growth.

Agar plates containing mineral salts medium³ with 25 mM fluoroacetate as carbon source and 25 mM ammonium chloride as nitrogen source, pH 7.2, were inoculated with aqueous extract from garden soil or water from Dartford Creek (Kent) and incubated at 30°. Those inoculated with water produced small yellowish colonies after incubating for about fourteen days; the others were still negative after three weeks. A single colony was sub-cultured on fresh fluoroacetate agar; satisfactory growth was obtained in five days and the colony isolation procedure was repeated. The bacteria were short, non-motile, Gram-negative rods and occurred singly, in pairs or, very rarely, in short chains. Growth on fluoroacetate agar plates was slow; colonies

were homogeneous, yellowish in colour, small, flat and circular. On nutrient agar, growth was more rapid and colonies were larger than on fluoroacetate but otherwise very similar in appearance. The bacteria grew more slowly at 37° than at 30° on both nutrient agar and fluoroacetate. Fluoroacetamide, glucose, fructose, mannose, lactose, mannitol and glycerol could serve as carbon sources for growth; citrate was not used. A culture has been deposited with the National Collection of Industrial Bacteria and assigned the number 9562.

Fig. 1 illustrates growth of the isolate in an aerated liquid medium containing fluoroacetamide; an uninoculated flask served as control. The pH values and optical densities were measured at intervals and, after removal of bacteria by centrifugation, inorganic fluoride and fluoroacetamide were determined (Snell and Snell^{4,5}). In the control flask the level of fluoroacetamide remained constant. A blank reading equivalent to 2 $\mu\text{moles/ml}$. of fluoride was obtained at the start of the experiment and did not change. In the inoculated flask the level of fluoroacetamide slowly decreased until, after 9 days, none could be detected. There was a corresponding increase in the level of fluoride. Growth was linear, not exponential, throughout most of the growth curve. The pH of the control remained constant whereas that of the inoculated culture dropped to 6.8. In corresponding experiments using fluoroacetate medium, approximately 40 mM fluoride was present in the growth medium supernatant after 6 days' incubation at 30°.

For experiments with resting cells, bacteria were grown under forced aeration in 250 ml. amounts of mineral salt medium containing 40 mM succinate, acetate, fluoroacetate or fluoroacetamide as carbon source. Fluoroacetamide also served as nitrogen source and was sterilized by filtration; other media had 40 mM ammonium chloride as nitrogen source and were sterilized by autoclaving. Succinate or acetate medium supported yields of about 0.7 mg dry weight of bacteria/ml. in 24–36 h, whereas comparable growth in fluoroacetate or fluoroacetamide took 5–10 days. Cells were collected by centrifugation, washed once in 0.05 M phosphate, pH 7.4, and re-suspended in more of the same buffer.

Cells grown with acetate oxidized acetate or succinate immediately; those grown with succinate oxidized succinate at once but showed a slight lag with acetate. Neither type metabolized fluoro-compounds during 90 min. Fig. 2 illustrates that those grown on fluoroacetate oxidized this substrate or acetate but not fluoroacetamide. Those grown on fluoroacetamide oxidized this compound, fluoroacetate or acetate.

Thus these bacteria grow with fluoroacetate or fluoroacetamide adaptively but very slowly; the non-exponen-

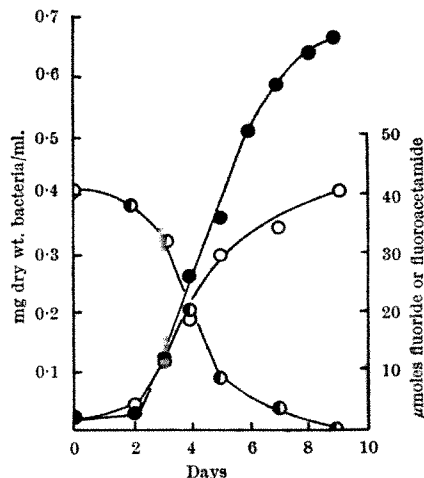


Fig. 1. Levels of inorganic fluoride \circ and of fluoroacetamide \bullet during growth of N.C.I.B. 9562 with an initial concentration of 40 mM fluoroacetamide, pH 7.2, temperature 30°.

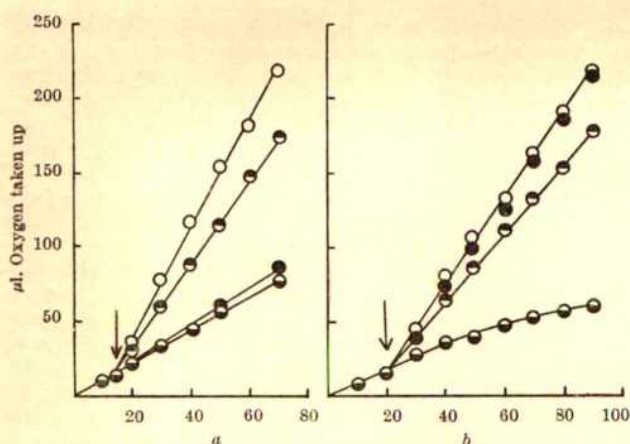


Fig. 2. *a*, Oxidation of \circ fluoroacetate; \bullet , fluoroacetamide and \square , acetate by resting cells grown with 40 mM fluoroacetate; \circ signifies control. Each Warburg flask contained the equivalent of 8.5 mg dry wt. of cells; 100 μ moles potassium phosphate buffer, pH 7.4, temperature 30°. At the point marked \downarrow 10 μ moles of substrate were added. *b*, Oxidation of substrates by the equivalent of 8 mg dry wt. of cells grown with 40 mM fluoroacetamide. Conditions otherwise as for *a*.

tial character of growth suggests that product inhibition occurs. The amide is probably converted to the acid by an adaptive amidase. Since permease formation is unlikely to provide a complete explanation of the adaptive nature of fluoro-compound metabolism, further investigations of this organism would be of particular interest because it could contain either an unusual aconitase or an enzyme specific to fluorine-carbon linkages. Suitably adapted strains might be of value for the decontamination of soils and water.

M. KELLY

Agricultural Research Council,
Unit of Nitrogen Fixation,
Chemical Laboratory,
University of Sussex,
Brighton.

¹ Peters, R. A., *Adv. Enzymol.*, **17**, 113 (1957).

² Hansard, 688-9 (612), 333 (1964).

³ Kelly, M., and Clarke, P. H., *J. Gen. Microbiol.*, **27**, 315 (1962).

⁴ Snell, F. D., and Snell, C. T., *Colorimetric Methods of Analysis*, 2, 750 (D. Van Nostrand Company Inc., New York, 1949).

⁵ Snell, F. D., and Snell, C. T., *Colorimetric Methods of Analysis*, 4, 33 (D. Van Nostrand Company Inc., New York, 1949).

CYTOLOGY

Functional Similarities between the Cytoplasmic Organelles of Melanocytes and the Mitochondria of Hepatocytes

In an earlier communication¹, it was reported that the cytoplasm of melanocytes, located in the liver of the amphibian *Amphiuma*, is packed with large organelles containing melanin granules in varying numbers. These melanocytes have few mitochondria and no visible ribosomes, in contrast to the hepatocytes which present the familiar organelles in large numbers. The sharp structural differences between the respective liver cells evoked our interest in comparing the functions of the organelles which abound in each. The insight to be gained from such investigations seemed to transcend the particular melanocytes under discussion: normal cells with similar structures are being seen in other organs²⁻⁴ and in other vertebrates^{5,6}, while abnormal metabolism of melanins is being encountered in human diseases ranging from neoplasia⁷ to extrapyramidal disorders of the brain^{8,9}.

For reasons which will become evident, the investigations recorded here were restricted to comparisons between mitochondria and melanin-containing organelles. Both had to be isolated from their respective cells in useful

yields and without intercontamination. Centrifugal fractionations of 10 per cent homogenates in 0.25 M sucrose showed that the technique of Schneider and Hogeboom¹⁰ was applicable to the collection of pure *Amphiuma* liver mitochondria. This technique had to be modified for the isolation of the melanin bodies: although these organelles were larger and heavier than the mitochondria, their separation in pure form necessitated the use of a sharp gradient. Since these organelles disintegrated in 30 per cent sucrose (0.88 M), 30 per cent dextran (0.002 M) was used successfully instead. These bodies also disintegrated at low pH and with routine homogenization. Hence the suspending media contained 0.02 M phosphate buffer, pH 7.4, and instead of a Potter-Elvehjem homogenizer a Latapie mincer was used.

The following technique is used at present: the mince is suspended in 10 volumes of 0.25 M sucrose at 4° C. The suspension is filtered through paper (Schleicher and Schull, No. 588) to remove tissue fragments, red cells and isolated nuclei. The filtrate is spun at 1,000g for 15 min. To obtain melanin-containing organelles the sediment is re-suspended in 2 ml. of 0.25 M sucrose; this suspension is layered over 15 ml. of 30 per cent neutralized dextran and the system is centrifuged for 35 min at 1,000g. The ensuing pellet consists of melanin-containing organelles and few free melanin granules but no visible mitochondria. The latter are gathered from the supernatant of the initial filtrate, by centrifuging at 5,000g. These are comparable to the mitochondria derived from liver homogenates according to the unmodified method of Schneider and Hogeboom¹⁰. The purity of both fractions was ascertained by light and electron microscopy (Fig. 1). Isolated melanocytes, obtained by incubation of liver slices with collagenase, yielded inferior preparations.

Metal-transport into each of these organelles was measured by assaying their uptake of carrier-free ⁵⁴MnCl₂ which was injected intraperitoneally. This isotope was selected because it accumulates preferentially in mammalian liver mitochondria both *in vivo*¹¹ and *in vitro*¹². Twenty-four hours after injection of 50 μ c., the whole liver contained 1.99×10^5 c.p.m./mmole nitrogen. By comparison, the washed mitochondria contained 2.22×10^5 c.p.m./mmole nitrogen, and the washed melanin organelles contained 2.02×10^5 c.p.m./mmole nitrogen. Repetition of this experiment yielded similar results.

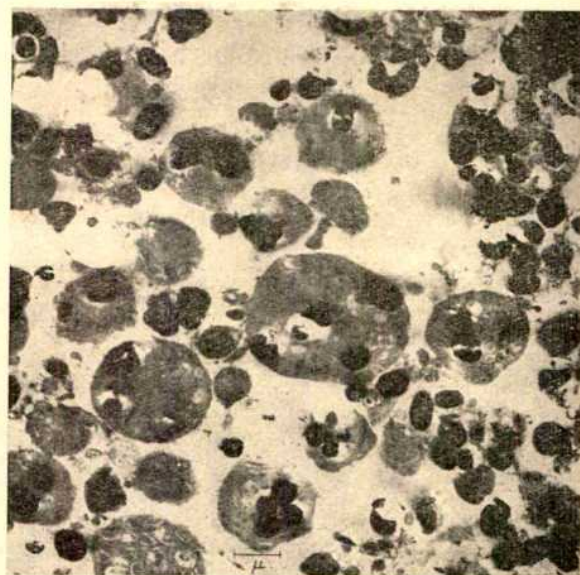


Fig. 1. Electronmicrograph of osmium-fixed, uranyl-acetate-stained cytoplasmic organelles of melanocytes isolated from *Amphiuma* liver by 30 per cent dextran gradient centrifugation. Some free melanin granules are also present. ($\times c. 5,904$)

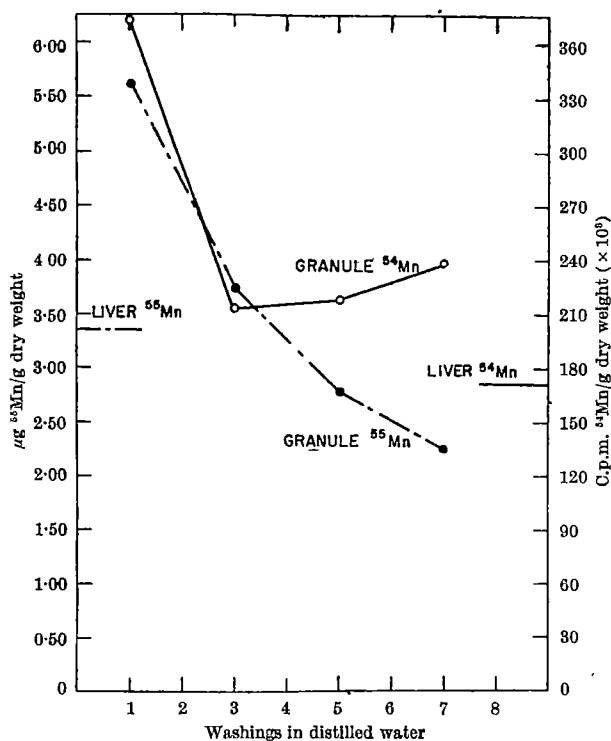


Fig. 2. Comparison of the loss of both the radioisotope ^{54}Mn and the natural isotope ^{55}Mn from the melanin granules of the *Amphiuma* liver. The melanin granules were isolated and washed by differential centrifugation in neutral distilled water. The amounts of ^{54}Mn and ^{55}Mn in the whole liver, prior to the isolation of the melanin granules, are shown at each vertical margin of the graph.

On a previous occasion, melanins from other sources were observed to contain high concentrations of natural, non-radioactive manganese¹³. Hence it was of interest to analyse the melanin granules located within the matrix of the organelles under discussion. Such analyses would show whether manganese crosses the membranes of the melanin granules: or only those of the melanocytes and of the organelles which contain these granules.

The melanin granules were isolated according to Claude¹⁴ after one initial wash with triple-distilled demineralized water. The concentrations of both the radioisotope ^{54}Mn and the natural isotope ^{55}Mn were determined as follows: The radioactive one was determined first, the natural one was rendered radioactive by neutron bombardment¹⁵ and the ensuing ^{56}Mn was quantified after subtraction of the long-lived radioactivity of ^{54}Mn . The results are shown in Fig. 2. The initial high degree of accumulation shown on the figure constitutes an underestimate of the native state, since subsequent washings decreased the concentration of both isotopes in these granules.

These results showed that the melanin-containing organelles concentrate manganese in a manner similar to mammalian mitochondria. They indicated the desirability of further comparisons between the novel organelles and the mitochondria. Among the enzymes known to be mitochondrial ones in the mammal, the following were selected: (1) succinic oxidase¹⁶ because of its importance in energy metabolism¹⁷; (2) monoamine oxidase¹⁸ because of the production of melanins which accompanies its reaction *in vitro*^{19,20}. These considerations prevailed in spite of the known lability of these enzymes, particularly in the amphibian²¹.

The lability of these enzymes reflected itself in the fact that the sum of fractions obtained according to Schneider and Hogeboom¹⁰ contained less activity than did the initial homogenate. The losses did not exceed 30 per cent and were not reflected in the recoveries of either ^{54}Mn or of nitrogen. Still, it was necessary to ascertain whether

the particular fractions of interest to us were not primarily affected. These fractions were therefore tested before and after incubation at 4°C overnight. No losses of either enzymatic activity were found in the isolated mitochondria. The whole liver homogenate lost only about 15 per cent of its succinic oxidase but none of its monoamine oxidase activity. In contrast, the melanin-containing organelles lost about 45 per cent of their succinic oxidase and only 10 per cent of their monoamine oxidase activity. Hence it was evident that the enzymatic activities recovered with the melanin-containing bodies would constitute underestimates of the native state.

The relative specific activities of these two enzymes are shown in Table 1 as the mean and range for each. It is evident that both enzymes were localized to about the same degree in the melanin-containing bodies and the mitochondria. The melanin granules isolated from the organelles which contain them had contributed significant amounts of nitrogen to these computations, but they contained no measurable enzymatic activity.

Table 1. SUCCINIC OXIDASE AND MONOAMINE OXIDASE IN *Amphiuma* LIVER FRACTIONS

	Succinic oxidase ($\mu\text{l. O}_2/\text{h/mM N}$)	Monoamine oxidase ($\mu\text{M NH}_3/\text{h/mM N}$)
Homogenate (5)	762 (512-908)	28 (22-34)
Mitochondria (8)	954 (232-1,703)	57 (30-120)
Melanin bodies (8)	758 (305-1,297)	38 (12-83)

The sum of this evidence indicates that some mitochondrial functions are localized also in the recently described organelles of the melanocytes in *Amphiuma* liver¹. This is indeed in agreement with the conclusions of du Buy *et al.*²² in spite of the fact that we have investigated large, complex organelles from a normal tissue, whereas their material contained melanin granules and small melanosomes from mammalian tumours. It should be recalled that the melanocytes examined here contained no visible ribosomes or lysosomes. Hence the melanin-containing organelles are worth comparing with ribosomes and with lysosomes in a manner similar to that reported here for mitochondria.

We thank Dr. Horton A. Johnson for his preparation and interpretation of the electronmicrographs.

This work was supported by the U.S. Atomic Energy Commission.

MELVIN H. VAN WOERT
ALEXANDER R. NICHOLSON
GEORGE C. COTZIAS

Medical Research Center,
Brookhaven National Laboratory,
Upton, Long Island, New York.

- ¹ Prasad, K. N., Johnson, H. A., and Cotzias, G. C., *Nature*, **205**, 525 (1965).
- ² Drochmans, P., *Intern. Rev. Exp. Pathol.*, **2**, 357 (1963).
- ³ D'Agostino, A. J., and Luse, S. A., *Neurology*, **14**, 529 (1964).
- ⁴ Vogel, F. S., and McGregor, D. H., *Lab. Invest.*, **13**, 787 (1964).
- ⁵ Weissensfels, N., *Z. Zellforsch.*, **45**, 60 (1956).
- ⁶ Balogh, K., *Fed. Proc.*, **24**, 432 (1965).
- ⁷ Miner, R. W., editor, *The Biology of Melanomas* (New York Acad. Sci., 1948).
- ⁸ Denny-Brown, D., *Lancet*, 1099, 1155 (1960).
- ⁹ Fellman, J. H., *J. Neuro. Neurosurg., Psychiat.*, **21**, 68 (1958).
- ¹⁰ Schneider, W. C., and Hogeboom, G. H., *J. Biol. Chem.*, **183**, 123 (1950).
- ¹¹ Maynard, L. S., and Cotzias, G. C., *J. Biol. Chem.*, **214**, 489 (1955).
- ¹² Chappell, J. B., Greville, G. D., and Bicknell, K. E., *Biochem. J.*, **84**, 618 (1962).
- ¹³ Cotzias, G. C., Papavasiliou, P. S., and Miller, S. T., *Nature*, **201**, 1228 (1964).
- ¹⁴ Claude, A., *Trans. N.Y. Acad. Sci.*, **4**, 70, Ser. II (1942).
- ¹⁵ Papavasiliou, P. S., and Cotzias, G. C., *J. Biol. Chem.*, **236**, 2365 (1961).
- ¹⁶ Schneider, W. C., Claude, A., and Hogeboom, G. H., *J. Biol. Chem.*, **172**, 451 (1948).
- ¹⁷ Lehninger, A. L., *The Mitochondrion* (W. A. Benjamin, Inc., New York, 1964).
- ¹⁸ Cotzias, G. C., and Dole, V. P., *Proc. Soc. Exp. Biol. and Med.*, **78**, 157 (1951).
- ¹⁹ Pugh, C. E. M., and Quaszel, J. H., *Biochem. J.*, **81**, 2306 (1937).
- ²⁰ Van Woert, M. H., and Cotzias, G. C., *Parkinson Symp. Mono.* (Grune and Stratton, N.Y., in the press).
- ²¹ Smith, C. L., *Comp. Biochem. Physiol.*, **1**, 305 (1960).
- ²² du Buy, H. G., Showaere, J. L., and Hesselbach, M. L., *Ann. N.Y. Acad. Sci.*, **100**, 569 (1963).

VIROLOGY

Growth and Cytopathogenicity of *H*-viruses in Human and Simian Cell Cultures

THE *H*-viruses are a new group of small agents, 180–250 Å in diameter, distinguished by their ability to produce a 'mongoloid-type' deformity in new-born hamsters^{1,2} and, in the case of *H*-1, to cross the placenta and cause malformation of the hamster embryo^{3–5}. These viruses have been isolated from two host sources, man and rat. Two members of the group, *H*-1 and *H*-3, which differ from one another serologically, were first isolated from human tumours transplanted in conditioned animals; afterwards they, as well as *HT* and *HB*, were obtained directly from human embryos, placentas, and neoplasms⁶. Recently it was demonstrated that two human subjects injected with *H*-1 virus developed haemagglutination-inhibiting (HA-I) and neutralizing antibodies to *H*-1 (ref. 7). This indication that man is susceptible to infection by *H*-1 virus is supported by the observation that HA-I and neutralizing antibodies to *H*-1 virus have been found in the sera of several cancer patients, laboratory personnel, and pregnant women^{6,8}. It has also been learned that rhesus monkeys are susceptible to infection with *H*-1 (ref. 9).

Other members of the *H*-group, the Kilham virus, *RV* (ref. 10), and the related virus *X*-14 (ref. 11), both of which are serologically similar to *H*-3 though they have different types of haemagglutination patterns with red blood cells from various animals, have been isolated from rat tumours. So far only rat embryo cells, and occasionally hamster embryo cells, have been used to grow *H*-1 or *RV* (refs. 11–15). This communication reports that *H*-1 and *H*-3 can proliferate and be passaged in a number of human and simian cell culture lines.

The cells tested for growth of the *H*-viruses are listed in Table 1. The human continuous cell lines of HeLa (Gey), HeLa S-3 and FL amnion were obtained from Difco Laboratories, Detroit, Michigan; Chang liver, Chang conjunctiva and Henle intestine from Baltimore Biological Laboratories, Baltimore, Maryland; and Hep-2 from Flow Laboratories, Rockville, Maryland. Primary cultures of human amnion were prepared in the laboratory from fresh placentas by the method of Wilt *et al.*¹⁶. The permanent simian cultures of rhesus kidney (*LLC-MK* 2), Salk heart and chimpanzee liver (Douglas *CL* I) came from Flow Laboratories. Other cells tested were the permanent hamster kidney culture, *HaK*, from Flow Laboratories, and secondary hamster embryo cultures

derived from trypsinized primary cultures of minced hamster embryos, approximately 14 days of age. Cell sheets were prepared by growing trypsinized cells in 30 ml. Falcon tissue culture flasks containing 5.0 ml. of Eagle's minimum essential medium (MEM) with 10 per cent calf serum. When an almost confluent layer of cells formed, the test cultures were washed twice with tris-buffered saline (TBS) (ref. 17) and infected with 1.0 ml. of virus prepared from infected new-born hamster livers¹⁸ and diluted with TBS to contain 10–20 haemagglutinin (HA) units/ml. Parallel titrations of *H*-1 and *H*-3 strains were done. After incubation for 2 h at 37° C, the control and infected cultures were washed twice with TBS and covered with 5.0 ml. of MEM containing 2 per cent foetal calf serum. The cultures were examined daily or at suitable intervals for haemagglutinin production and for the development of cytopathic changes (CPE). For HA titrations guinea-pig red cells were used¹⁸. End-points were read as that dilution of virus which completely agglutinated all the red cells.

Growth of *H*-1 and *H*-3 was obtained in all the permanent human cell lines tested (Table 1). Although both viruses appeared to multiply to approximately the same extent, producing maximal HA titres at about the same time, the cells differed in their response to the two viruses. The CPE induced by *H*-1 appeared earlier, concomitant with virus multiplication, and progressed considerably more rapidly than that induced by *H*-3. Early CPE consisted of rounding of cells and granularity, followed by a disruption of the cell sheet, and finally detachment of the necrotic and shrunken cells. This CPE was very similar to that found in infected rat embryo cultures by Moore¹². The difference in the timing and progression of cellular pathogenicity induced by *H*-1 and *H*-3 in human cells was also observed in the permanent simian cell lines of Salk monkey heart and Douglas chimpanzee liver, the permanent hamster kidney line, *HaK*, and the secondary hamster embryo cultures. Only minimal CPE by *H*-3 was seen at the time when maximal or nearly maximal haemagglutinin production occurred. However, although the CPE induced by *H*-3 was delayed, a slow progression to fairly complete CPE usually occurred. It is noteworthy that it took considerably longer for maximal HA production and CPE to occur in the hamster cells than in the human and simian cultures. Whether this finding represents a genuine difference in cell susceptibility or could be attributed to other factors, such as variations in cell concentration, is unknown.

Several successful passages of *H*-1 and *H*-3 were carried out in all the cell lines tested. After three to five passages

Table 1. SUSCEPTIBILITY OF CELLS OF DIFFERENT ORIGIN TO *H*-VIRUSES

Source	Cell	Maximal haemagglutinin (HA) production*		Days after inoculation maximal HA obtained		CPE at time maximal HA obtained†		
		H-1 virus	H-3 virus	H-1 virus	H-3 virus	H-1 virus	H-3 virus	
Human	Permanent	HeLa (Gey)	4	not done	5	not done	4	not done
		HeLa S-3	3	2	4	3	3	1
		Liver (Chang)	4	4	4	3	4	1
		Conjunctiva (Chang)	3	3	5	3	3	1
		Intestine (Henle)	3	3	4	4	4	1
		HEp-2	3	not done	6	not done	3	not done
		FL amnion	3	4	4	4	3	1
	Primary	Embryo kidney	0	0	21 days observation	—	—	—
		Amnion	0	0	23 days observation	—	—	—
	Simian	Permanent	Rhesus kidney, LLC-MK 2 (Hull)	0	0	23 days observation	—	—
Monkey heart (Salk)			4	4	5	8	3	2
Chimpanzee liver (Douglas)			4	4	4	6	4	1
Other	Permanent	HaK (kidney, Syrian hamster) (Spence)	4	3	12	15	3	1
		Hamster embryo	3	3	9	7	3	1

* Haemagglutinin production was assayed by haemagglutination of guinea-pig red cells and reported in haemagglutination units as the reciprocal of the highest dilution that completely agglutinated all the red cells as follows: 1, 10–20 units; 2, 40–160 units; 3, 320–640 units; 4, 1,280+ units.

† Cytopathic changes (CPE) were graded as follows: 1, rounding and granularity of scattered cells; 2, beginning of disruption of cell sheet; 3, extensive cellular granularity and disruption of sheet; 4, considerable detachment of degenerated cells.

of H-3, extensive CPE occurred when haemagglutinin production became maximal. H-1 is at present in its sixth passage in Chang liver and Salk monkey heart and so far has retained its full virulence for new-born hamsters. The finding that H-1 and H-3 failed to produce HA or CPE in the primary human embryonic kidney or amnion tissue cultures is of interest and is being investigated.

The work was supported by grant E-343 from the American Cancer Society and grant CA-07826-02 from the National Cancer Institute, U.S. National Institutes of Health.

H. TOOLAN
N. LEDINKO

Putnam Memorial Hospital,
Institute for Medical Research,
Bennington, Vermont.

- ¹ Toolan, H. W., *Science*, **131**, 1446 (1960).
- ² Toolan, H. W., Daldorf, G., Barclay, M., Chandra, S., and Moore, A. E., *Proc. Soc. Exp. Biol. and Med.*, **46**, 1266 (1960).
- ³ Toolan, H. W., *Fed. Proc.*, **19**, 208 (1964).
- ⁴ Fern, V. H., and Kilham, L., *Science*, **145**, 510 (1964).
- ⁵ Fern, V. H., and Kilham, L., *J. Embryol. Exp. Morph.*, **13**, 151 (1965).
- ⁶ Toolan, H. W., *Proc. Amer. Assoc. Cancer Res.*, **5**, 64 (1964).
- ⁷ Toolan, H. W., Saunders, E. L., Southam, M. C., Moore, A. E., and Levin, A. G., *Proc. Soc. Exp. Biol. and Med.*, **119**, 711 (1965).
- ⁸ Monif, G. R., Sever, J. L., and Cochran, W. D., *J. Pediatrics*, **67**, 253 (1965).
- ⁹ Toolan, H. W. (unpublished results).
- ¹⁰ Kilham, L., *Virology*, **13**, 141 (1961).
- ¹¹ Payne, F. E., Beals, T. F., and Preston, R. E., *Virology*, **23**, 109 (1964).
- ¹² Moore, A. E., *Virology*, **18**, 182 (1962).
- ¹³ Kilham, L., *Virology*, **7**, 428 (1959).
- ¹⁴ Portella, O., *Archiv. Virusforschung*, **14**, 19 (1963).
- ¹⁵ Bernhard, W., Kasten, F. H., and Chaney, C., *C.R. Acad. Sci., Paris*, **257**, 1566 (1963).
- ¹⁶ Wilt, J. C., Miller, D., and Butler, J., *Canad. J. Microbiol.*, **10**, 169 (1964).
- ¹⁷ Dulbecco, R., Hartwell, L. H., and Vogt, M., *Proc. U.S. Nat. Acad. Sci.*, **53**, 408 (1965).
- ¹⁸ Greene, E. L., *Proc. Soc. Exp. Biol. and Med.*, **118**, 978 (1965).

Stimulation of Sendai Virus Multiplication by Puromycin and Actinomycin D

THE rate of virus multiplication may ultimately be limited by the number of unprogrammed ribosomes available to viral nucleic acid. There is presumably only a finite probability in unit time that an infecting viral nucleic acid molecule can successfully compete with a vast excess of cellular messenger RNA molecules for an unoccupied ribosome. Even after attachment has been achieved, the subsequent rate of synthesis of viral constituents may be expected to depend on the availability of free ribosomes. This hypothesis was tested experimentally in cells pre-treated with two antibiotics, puromycin and actinomycin D, each capable of increasing the number of unprogrammed ribosomes.

Actinomycin D inhibits the synthesis of messenger RNA by binding to DNA and interfering by steric hindrance with the function of DNA-dependent RNA polymerase^{1,2}. This leads to the gradual disappearance of polyribosomes with a consequent increase in the number of unprogrammed ribosomes³ as existing messenger RNA is slowly destroyed during the ensuing few hours^{1,3,4}. Sendai is one of several RNA viruses capable of normal multiplication in the presence of actinomycin D^{1,5}. It commends itself for the proposed experiment because, by analogy with other myxoviruses^{6,7}, it probably does not bring about the early inhibition of host cell protein or messenger RNA synthesis so evident, for example, with mengovirus⁸ and poliovirus⁴.

The parameters of Sendai virus multiplication were examined in suspended allantois-on-shell⁹. Dilutions of virus were added to pieces of allantois-on-shell (AOS) cut from a single egg and suspended in a simple maintenance medium in a plastic tray. The tray was shaken at 36.5° for 30 min, then 100 units of receptor-destroying enzyme (RDE) added to remove residual inoculum and to restrict virus multiplication to a single cycle. After rinsing, the tissues were transferred to a fresh tray every 2 h and the

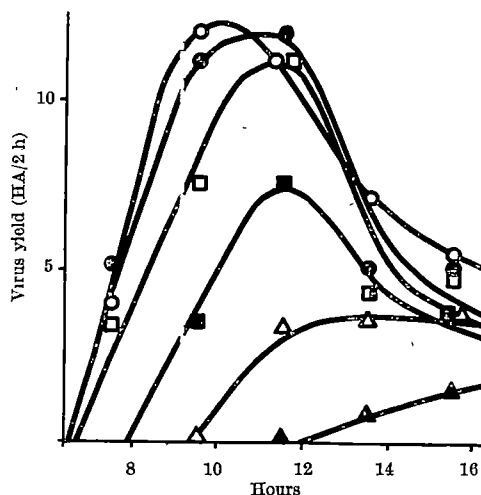


Fig. 1. 'Delay'¹⁰ in the multiplication of Sendai virus. AOS was inoculated with Sendai at m.o.i. of 100 (open circles), 30 (closed circles), 10 (open squares), 3 (closed squares), 1 (open triangles) or 0.3 (closed triangles). The graph shows the amount of haemagglutinin newly produced in each 2-h interval.

remaining supernatant fluids titrated for their content of haemagglutinin (Fig. 1).

At high multiplicity of infection (m.o.i.) virus begins to appear after an eclipse period of 6½–7 h and the maximum rate of virus production (given by the peak of the differential curve) is seen during the tenth or eleventh hour post-infection (p.i.). At low m.o.i., on the other hand, the eclipse period is longer and the rate of virus production is still increasing at 16 h. This clearly establishes the occurrence of multiplicity-dependent 'delay'¹⁰ in the multiplication of Sendai virus.

Actinomycin D (0.03, 0.1, 0.3, 1, 3, 10 µg/ml. or nil) was added to AOS 2 h before Sendai virus at various m.o.i., and cumulative yields of haemagglutinin were titrated. Regardless of m.o.i. and at concentrations of actinomycin of 0.3 µg/ml. to 10 µg/ml. inclusive, the antibiotic markedly increased the rate of virus production. Fig. 2 shows the results of a typical experiment using actinomycin at 2 µg/ml. There is good reason to suppose that the increase would be even greater if the cells could be maintained in actinomycin for a longer period before infection to allow more adequate time for decay of existing messenger RNA.

Puromycin inhibits protein synthesis by removing the developing peptide chains from ribosomes¹¹. As a result

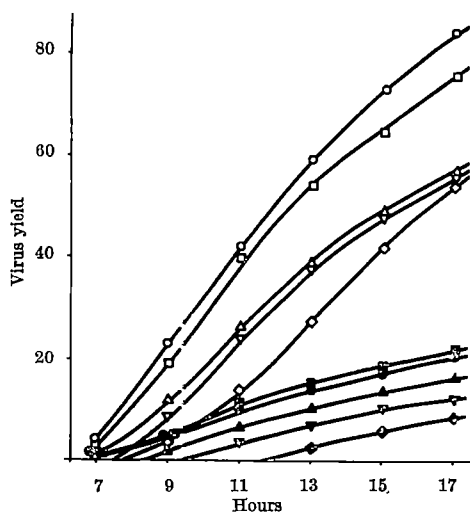


Fig. 2. Stimulation of Sendai virus multiplication by actinomycin D. AOS was inoculated with actinomycin D, then 2 h later with various m.o.i. of Sendai virus. Cumulative yields of haemagglutinin were titrated at 2-h intervals. Closed symbols, controls; open symbols, actinomycin-treated. Circles, m.o.i. 60; squares, m.o.i. 20; triangles, m.o.i. 6; inverted triangles, m.o.i. 2; diamonds, m.o.i. 0.6.

messenger RNA is released and polyribosomes are disaggregated¹². AOS was treated with puromycin (1, 3, 10, 30, 100, 300 µg/ml. or nil) for 2 h before infection with Sendai virus (m.o.i. = 1). The drug was removed and the membranes were rinsed immediately before infection, then again on removal of RDE and residual inoculum 30 min later. Cumulative yields of virus were titrated as before. To serve as controls, comparable membranes were treated with *p*-fluorophenylalanine (600 µg/ml.), which inhibits protein synthesis just as effectively but in a completely different way¹³. At a concentration of 300 µg/ml. puromycin actually retarded multiplication because it could not be removed completely by rinsing. However at concentrations of 30 µg/ml. and 100 µg/ml., puromycin consistently stimulated the rate of virus production. Results from two such experiments are given in Table 1.

Table 1. EFFECT OF PUROMYCIN, ACTINOMYCIN D AND FPA ON THE MULTIPLICATION OF SENDAI VIRUS

Treatment	Cumulative yield of virus (haemagglutinin)				
	9 h	11 h	13 h	15 h	17 h
Experiment 1 (m.o.i. = 0.5)					
Control	—	—	3.0	6.2	9.4
FPA (600 µg/ml.)	—	—	2.3	6.5	9.8
Puromycin (30 µg/ml.)	—	1.2	6.0	12.4	17.6
Actinomycin D (2 µg/ml.)	—	3.8	15.0	29.4	37.4
Actinomycin D + puromycin	—	3.4	13.0	25.0	31.8
Experiment 2 (m.o.i. = 1)					
Control	—	2.8	6.8	10.0	13.2
FPA (600 µg/ml.)	—	1.8	5.8	9.0	12.7
Puromycin (30 µg/ml.)	—	3.4	9.4	15.0	18.6
Actinomycin D (2 µg/ml.)	2.4	15.2	29.6	43.2	53.6
Actinomycin D + puromycin	0.8	7.2	16.0	23.6	30.0

Doubtless the degree of stimulation would have been greater had it been possible to remove the puromycin more effectively. That residual traces of the drug actually inhibit virus multiplication can be seen from the fact that pretreatment with puromycin plus actinomycin D gives substantially less stimulation than actinomycin alone. Nevertheless pretreatment with puromycin alone does enhance the rate of virus production. The fact that *p*-fluorophenylalanine (FPA) is ineffective shows that the stimulating activity of puromycin is attributable not to the inhibition of protein synthesis *per se*, but to some more specific action of the drug. Had it proved experimentally practicable to use larger concentrations of puromycin or to leave it in until immediately before viral protein synthesis began, the degree of stimulation by puromycin may well have been comparable with that obtained with actinomycin.

A recent report by Heller¹⁴ indicates that actinomycin can increase yields of Chikungunya virus by inhibiting the synthesis of interferon and so preventing the late decline in virus production that otherwise occurs following the first cycle of multiplication in cell cultures inoculated with virus at low m.o.i. It seems unlikely that our own results have anything to do with interferon, because all experiments were confined to a single cycle of infection, and stimulation of the rate of virus production is apparent from the moment new virus becomes detectable at the end of the eclipse period. Bukrinskaya and Zhdanov⁵ have recently reported a shortening of the Sendai eclipse period by actinomycin D without any increase in viral multiplication rate or total yield. By contrast, Fig. 1 in a paper by Wheelock¹⁵ reveals a slight (25–30 per cent) stimulation of final yield of NDV in the presence of actinomycin without any increase in multiplication rate or shortening of the eclipse period.

Our experiments demonstrate that both actinomycin D and puromycin stimulate the rate of virus production. As a result the yield is also increased (the relative increase being greater the later the yield is titrated) and the eclipse period appears to be shortened because the yield reaches the lower limit of detection earlier. Both antibiotics may act by making available to viral RNA greater numbers of unprogrammed ribosomes.

This work was supported by a grant from the National Health and Medical Research Council of Australia, and one of us (I. M. C.) was supported by the Commonwealth

Serum Laboratories Commission. The actinomycin D was a gift from Merck, Sharpe and Dohme Research Laboratories, New Jersey.

DAVID O. WHITE
IAN M. CHEYNE

School of Microbiology,
University of Melbourne,
Parkville, Victoria, Australia.

- ¹ Reich, E., Franklin, R. M., Shatkin, A. J., and Tatum, E. L., *Proc. U.S. Nat. Acad. Sci.*, **48**, 1238 (1962).
- ² Reich, E., *Science*, **143**, 684 (1964).
- ³ Darnell, J. E., Penman, S., Scherrer, K., and Becker, Y., *Cold Spring Harbor Symp. Quant. Biol.*, **28**, 211 (1963).
- ⁴ Holland, J. J., *Bact. Rev.*, **28**, 3 (1964).
- ⁵ Bukrinskaya, A. G., and Zhdanov, V. M., *Nature*, **200**, 920 (1963).
- ⁶ Hausen, P., Hausen, H., Rott, R., Scholtissek, C., and Schäfer, W., *17th Symp. Fundamental Cancer Res., Houston, Texas* (1963).
- ⁷ Wheelock, E. F., *Proc. U.S. Nat. Acad. Sci.*, **48**, 1358 (1962).
- ⁸ Franklin, R. M., and Baltimore, D., *17th Symp. Fundamental Cancer Res., Houston, Texas* (1963).
- ⁹ Fazekas de St. Groth, S., and White, D. O., *J. Hyg., Camb.*, **56**, 151 (1958).
- ¹⁰ Cairns, H. J. F., *Virology*, **3**, 1 (1957).
- ¹¹ Nathans, D., *Proc. U.S. Nat. Acad. Sci.*, **51**, 585 (1964).
- ¹² Williamson, A. R., and Schweet, R., *Nature*, **202**, 435 (1964).
- ¹³ Muner, R., and Cohen, G. N., *Biochim. Biophys. Acta*, **31**, 378 (1959).
- ¹⁴ Heller, E., *Virology*, **21**, 652 (1963).
- ¹⁵ Wheelock, E. F., *Proc. Soc. Exp. Biol. and Med.*, **114**, 56 (1963).

GENETICS

A Lactate Dehydrogenase Variant in the Mouse

A GENETICALLY determined lactate dehydrogenase (LDH) variant has been demonstrated in the mouse, using starch-gel electrophoresis. After electrophoresis, the sliced surface of the gel is incubated with lactate substrate.

Table 1. RESULTS OF TEST-CROSSES

Cross	C57BL pattern	F ₁ pattern	CBA pattern	Total
C57BL♂ × F ₁ ♀	22	19	0	41
F ₁ ♂ × C57BL♀	15	11	0	26
	37	30	0	67
CBA♂ × F ₁ ♀	0	9	5	14
F ₁ ♂ × CBA♀	0	9	6	15
	0	18	11	29

The work recorded here was carried out on two inbred mouse strains, CBA and C57BL. All haemolysates contain the LDH isozyme band which moves most slowly towards the anode in tissue homogenates. Adult CBA mice have a second, faster-moving band, which is roughly equal in intensity to the slow band. This band is either absent or very faint in adult C57BL mice. A band of intermediate intensity is present in hybrids from both reciprocal crosses (Fig. 1). The variable band is not present in new-born CBA mice and appears to increase with age of the mouse (Fig. 2). Animals were screened only after they were at least 90 days old, by which time the pattern had become stable. Controls of the same age were always used. Test-cross data indicate that the variant is determined by a single gene (Table 1). Further work on red cells and on

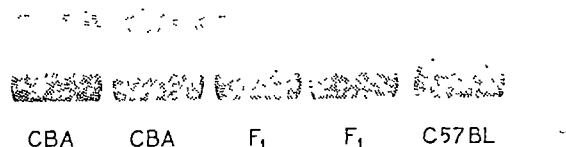


Fig. 1. Starch-gel electrophoresis of LDH in mouse haemolysates

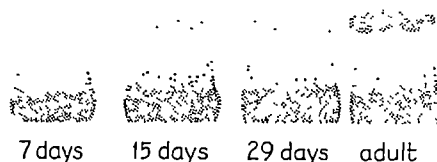


Fig. 2. Starch-gel electrophoresis of LDH in CBA mice of various ages

tissue homogenates is in progress to determine more about the nature of the variant LDH.

Department of Animal Genetics,
University College, London.

¹ Smithies, O., *Adv. Protein Chem.*, 14, 65 (1959)

LINDA RILES

PSYCHOLOGY

A General Theory for the Evolution of Intelligent Behaviour

THE problem of the evolution of intelligent behaviour has recently achieved publicity¹ in relation to work on the learning abilities of various vertebrates². Over the past three years I have developed a general theory of the evolution of intelligence from, and within the framework of, an instinct system of Lorenz-Tinbergen type³. This theory is of wider scope than any which could be advanced on the basis of the present theories of learning; it can, it is felt, accommodate most of the 'learning theory' views; and it appears to provide a context for the understanding of a considerable variety of other phenomena. An extended account and discussion of this general theory of the evolution of intelligence is being offered for publication elsewhere—but a very brief preliminary account appears desirable, to stimulate interdisciplinary appraisal and discussion.

If a generalized and non-committal view of intelligent behaviour be adopted, namely, that its salient characteristic is adaptive variability, the evolutionary problem can be stated in terms of the generation and selection of this characteristic from out of the relative fixity and rigidity of a system of instinctive behaviour. Detailed analysis of what is involved brings to light three features or 'factors' which must be represented in the finally-produced 'intelligence'. There must develop: (1) a capacity not to respond (or to delay the response) to the 'releasers'⁴ of responses which were previously normal, so as to allow the substitution of new kinds of response; (2) a dynamic 'memory store', 'knowledge' of the environ-

ment in relation to the individual animal, on the basis of which new responses can be elaborated; (3) an ability to abstract and generalize, to perceive similarities and differences, so that in the light of the past experience of the individual (2), the new variants of behaviour can be made adaptive as quickly as possible.

These three features correspond remarkably with three factors isolated by Halstead⁵ within 'biological intelligence'. The foregoing three features mentioned correspond to Halstead's *P*, *C*, and *A* factors respectively. Halstead's fourth, the *D* factor, is one which measures the efficiency of the individual's sensorimotor intercourse with the environment—and this must be under positive selection alike in 'intelligent' and in 'instinctive' animals. Halstead's approach was through the analysis of the behaviour-impairment of brain-injured humans; and the agreement between conclusions arrived at by very different routes is felt to constitute strong support for the view of intelligence which has emerged.

The Russells⁶ have shown the relevance of Halstead's 'biological intelligence' to the differentiation between normal and pathological behaviour in humans. The concept of 4-factor biological intelligence developed severally by Halstead and myself is also illuminating with regard to present researches into personality differences among scientists⁷, and into 'creativity' in humans⁸; and it provides a theoretical framework for the interpretation of some neurological experiments⁹ and various other phenomena. Exploratory activity, and play, assume an enhanced significance; further discussions will be offered for publication elsewhere.

I am greatly indebted for encouragement and criticism in the development of this work to Dr. N. Tinbergen, and to my friends and colleagues, G. F. K. Naylor, M. C. Bleakly, M. J. Thorne, and D. Judge.

DAVID STENHOUSE

Department of Zoology,
University of Queensland, Brisbane, Australia.

¹ Bitterman, M. E., *Scientific American*, 212, 92 (1965).

² See, for example, Bitterman, M. E., *American Psychologist*, 15, 704 (1960).

³ Tinbergen, N., *A Study of Instinct* (Clarendon Press, Oxford, 1951).

⁴ Used in the broad sense of 'total releaser situation', cf. Tinbergen, ref. 3 above.

⁵ Halstead, W. C., *Brain and Intelligence* (Chicago University Press, 1947).

⁶ Russell, Claire, and Russell, W. M. S., *Human Behaviour* (Deutsch, London, 1961).

⁷ Hudson, L., *Nature*, 196, 601 (1962), *Nature*, 198, 913 (1963).

⁸ *Contemporary Approaches to Creative Thinking*, edit. by Gruber, H. E., Terrell, G., and Wertheimer, M. (Atherton Press, N.Y., 1962).

⁹ See, for example, Ellen, P., and Wilson, A. S., *Experimental Neurology*, 8, 310 (1963).

Effect of Foster-mothers' Strain and Pre-natal Experience on Adult Behaviour in Rats

THE examination of the effects of the experiences of one generation on the behaviour of their offspring is of intrinsic interest. It is also of importance in relation to experiments in both psychogenetics and the study of the effects of pre-natal environments. Experiments with rodents have shown that both the strain and the early experiences of foster-parents can affect the later behaviour of the offspring they rear. Thus Ressler¹ showed that the strain of the foster-parents of mice can affect the later exploratory behaviour of the fostered offspring. Such effects may be mediated by differences in parental behaviour towards the pups², and, since the behaviour of parent animals can be affected by their own experiences, the behaviour of an organism can thus be affected by the previous experience of the parents which rear it.

Denenberg and Whimbey³ have shown that the weaning weights of rats and later their emotional defaecation scores in the open field were significantly influenced by the experiences their mothers had had as infants, and Ressler⁴ that the visual exploration scores of offspring of mice, which had been reared by either one of two strains of foster-parents or by their biological parents,

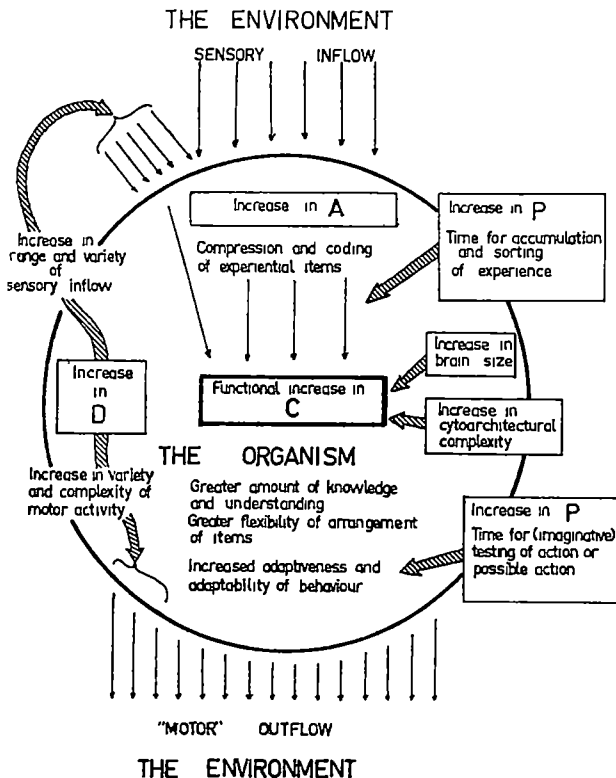


Fig. 1. Four-factor biological intelligence in evolution

were affected by the strain of their foster-grandparents, as well as by the fostering procedure *per se* carried out on their parents.

The experiment recorded here was carried out to explore further these aspects of parent-offspring interaction by using subjects from genetically uniform mothers, with the offspring reared to weaning by foster-mothers which differed in both experience and heredity. It also differs from previous experiments both in using two intensities of the experimental treatment administered to the foster-mothers and in applying them to the mothers in their maturity rather than in their infancy. In this way, the results may have some bearing on the adequacy of using biological mothers as rearing mothers in experiments on pre-natal stress.

Twelve litters of rats were fostered shortly after birth to a foster-mother of one of two strains, the Maudsley reactive (MR) or non-reactive (MNR) strains, selectively bred for 23 generations for high and low emotional defaecation respectively^{5,6}. Each foster-mother had previously received one of the following three treatments:

(1) Pre-mating avoidance training plus gestational stress: 14 days training to avoid shock of 0.3 m.amp (UCS) on presentation of a CS (illumination change) in a shuttle box, then mating, then 18 days of further trials in the shuttle box with no shock presented and the avoidance response physically blocked on 16 of the 24 daily trials. (2) Pre-mating avoidance training only: 14 days shuttle box training, mating, no further disturbance. (3) Controls: no disturbance other than mating.

The biological mothers of the subjects used were 12 primiparous, experimentally naïve females of the MNR strain, also at S_{23} ; the 12 litters were thus genetically homogeneous. Offspring were weaned at 21 days, ear-punched for identification at 50 days, and otherwise left undisturbed until tested at approximately 100 days of age.

At this stage 48 offspring (two males and two females randomly chosen from each of the 12 litters), equally divided among the two strains and the three pre-natal treatments of their foster-mothers, were tested in the standardized open-field test of emotionality (four daily 2-min trials)⁵ and on an avoidance conditioning task (one 50-trial session; UCS, shock of 0.25 m.amp; CS, a buzzer). Half were tested in the order stated and half in the reverse order.

In the analyses of variance applied to the resulting data, main effects and interactions based on between-litter comparisons were tested against between-litter error; if between-litter error was not significantly different from within-litter error, the two were pooled to provide an overall error variance.

There was a significant difference in the conditioning scores of subjects reared by mothers which had been stressed pre-natally. Both number of avoidances ($F = 5.63$, $P < 0.01$ with 2, 41, d.f.) and mean latency of response ($F = 5.74$, $P < 0.01$ with 2, 41 d.f.) differed significantly as a result of maternal experiences. The relevant means and standard deviations are shown in Table 1. Scheffe's test for multiple comparisons between means indicates that on both scores the group which had experienced pre-mating stress only is significantly inferior

Table 1 MEANS AND STANDARD DEVIATION FOR NUMBER OF AVOIDANCE SCORES AND AVOIDANCE LATENCIES IN CONDITIONING OF FOSTER-LITTERS REARED BY MOTHERS OF DIFFERENT EXPERIENCE

Experience of foster-mother	Avoidances (out of 50) (mean \pm S.D.)	Latencies (sec) (mean \pm S.D.)
Pre-mating plus gestational stress	32.7 \pm 12.9	4.4 \pm 2.01
Pre-mating stress only	29.4 \pm 11.9	5.1 \pm 1.91
Controls	38.1 \pm 11.5	3.5 \pm 1.63

Table 2. MEANS AND STANDARD DEVIATIONS FOR NUMBER OF AVOIDANCE SCORES AND AVOIDANCE LATENCIES IN CONDITIONING OF THE TWO SEXES TESTED IN TWO ORDERS

Test order	Avoidances (out of 50)		Latencies (sec)	
	Males (mean \pm S.D.)	Females (mean \pm S.D.)	Males (mean \pm S.D.)	Females (mean \pm S.D.)
Open field-avoidance conditioning	34.2 \pm 12.1	33.6 \pm 12.6	4.3 \pm 1.83	4.3 \pm 1.92
Avoidance conditioning—open field	39.1 \pm 12.7	26.8 \pm 14.5	3.2 \pm 1.27	5.5 \pm 2.47

to the controls but not to the gestational stress group; these two latter groups do not differ significantly. The analyses of variance of both scores also show an effect unrelated to pre-natal treatment. This was a significant sex \times test order interaction (avoidances: $F = 6.76$, $P < 0.025$ with 1, 27 d.f.; latencies: $F = 8.57$, $P < 0.01$ with 1, 27 d.f.). The relevant means, contained in Table 2, show that males perform better if tested in the shuttle box prior to the open field, whereas with females prior experience in the open field leads to improved avoidance conditioning.

Analysis of the open-field ambulation scores revealed no significant effects other than the usual sex differences, with females ambulating more than males. Defaecation scores were not analysed since, as might be expected from their constitutional background, only three of the 48 MNR subjects defaecated.

There were no significant differences in the weights of the foster-offspring at any age as a function of either maternal strain or experience.

It seems unlikely that the effects which were detected were mediated by changes in the emotionality of the foster-mothers resulting from the pre-natal treatments, since there were no differences in offspring behaviour as a function of the foster-mothers' strain although the strains differ markedly in emotionality, the characteristic for which they have been bidirectionally selected. While the treatments imposed did affect the foster-mothers' emotionality, as detected by testing in the open field prior to the experiment and again after the litters were weaned, the nature of the results does not especially correspond to the effects on the avoidance conditioning of the litters they reared. There were strain differences in the direction of the effects of the different treatments on ambulation scores (strain \times treatment interaction: $F = 10.63$, $P < 0.005$), with pre-mating stress resulting in the smallest increase in ambulation among MR females, all groups of which showed an increase, and gestational stress resulting in the smallest decrease among MNR females, all groups of which showed a decrease. Defaecation scores showed some increase on re-test but were not analysed, again because of zero MNR scores.

This investigation indicates that subjecting female rats to stress in maturity affected the adult avoidance conditioning but not the emotionality of the foster-offspring which they reared. The strain of the foster-mothers did not of itself affect the adult behaviour of the offspring. It confirms previous work^{3,4} in its general finding that conditions to which an organism is exposed can modify the behaviour of offspring which it rears. In thus showing that maternal experience in maturity can influence the behaviour of foster-offspring, it further indicates the necessity of using some method of controlling the post-natal maternal environment in experiments on pre-natal stress if the effects on offspring in such experiments are to be validly attributed to the pre-natal period; to do otherwise is to run the risk that the mothers' pre-natal experiences may affect offspring behaviour post-natally.

A full account of this and related work is being prepared for publication elsewhere⁷.

This work was supported by a grant from the Maudsley and Bethlem Royal Hospitals Research Fund. I thank Prof. P. L. Broadhurst and Prof. J. L. Jinks for their advice.

J. M. JOFFE

Department of Psychology,
University of Birmingham, Birmingham 15.

¹ Ressler, R. H., *J. Comp. Physiol. Psychol.*, **56**, 882 (1963).

² Ressler, R. H., *Science*, **137**, 129 (1962).

³ Denenberg, V. H., and Whimbey, A. E., *Science*, **142**, 1192 (1963).

⁴ Ressler, R. H., *Amer. Psychologist*, **19**, 505 (1964).

⁵ Broadhurst, P. L., in *Experiments in Personality*, **1**, *Psychogenetics and Psychopharmacology*, ed. by Eysenck, H. J. (Routledge and Kegan Paul, London, 1960).

⁶ Broadhurst, P. L., *Psychol. Rep.*, **10**, 65 (1962).

⁷ Joffe, J. M., *Prenatal Determinants of Behaviour* (Pergamon, Oxford, in preparation).

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, November 22

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 5 p.m.—Major N. J. D. Prescott, R.E.: "The Geodetic Satellite—SEGOR".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. H. Braunsteiner (Innsbruck): "Clinical Significance of Triglyceride Determinations, Especially in Relation to Age, Body Weight, Different Forms of Diabetes and Thyroid Disease".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Prof. Colin Cherry: "The Communication Explosion". (Second of three Cantor Lectures on "World Communication".)

SOCIETY FOR VISITING SCIENTISTS (at the English Speaking Union, Dartmouth House, 87 Charles Street, London, W.1), at 7.30 p.m.—Discussion Meeting on "The Retrieval of Scientific Information". Chairman: Dr. H. T. Hookway. Speakers: Prof. H. Kaiser, Dr. W. Batten and Mr. A. St. Johnston.

Monday, November 22—Tuesday, November 23

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2)—Conference on "U.H.F. Television".

Monday, November 22—Friday, November 26

INSTITUTE OF METALS (at the Royal Commonwealth Society, Craven Street, London, W.C.2)—Third International Conference on "Plutonium".

Tuesday, November 23

SOCIETY OF CHEMICAL INDUSTRY, AGRICULTURE GROUP (at 14 Belgrave Square, London, S.W.1), at 10.30 a.m.—Meeting on "Chemicals in Animal Nutrition".

UNIVERSITY OF LONDON (at Westfield College, Kidderpore Avenue, London, N.W.3), at 5.15 p.m.—Prof. D. H. Wilkinson, F.R.S.: "Elementary Particles".

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, Westminster, London, S.W.1), at 5.30 p.m.—Informal Discussion on "Use of Slag in Road Building", introduced by Mr. R. A. Kidd and Mr. R. T. Jackson.

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. W. G. Spector: "The Cytokinetics of Chronic Inflammation". (Eighth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation).*

INSTITUTION OF CHEMICAL ENGINEERS, SOUTH EASTERN BRANCH (at the Royal Aeronautical Society, Hamilton Place, London, W.1), at 6 p.m.—Mr. C. J. Stairmand: "Gas Cleaning".

ROYAL METEOROLOGICAL SOCIETY (in the Conference Hall, County Hall, London, S.E.1), at 6 p.m.—Dr. D. G. James: "The Use of Satellite Observations in Meteorology".

INSTITUTION OF MECHANICAL ENGINEERS, GRADUATES' AND STUDENTS' SECTION (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6.30 p.m.—Mr. D. Squires: "Blue Streak".

SOCIETY OF CHEMICAL INDUSTRY, PLASTICS AND POLYMER GROUP (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Prof. A. Hopff (Swiss Federal Institute of Technology, Zurich): "Studies on New Polymers and Monomers".

SOCIETY FOR ANALYTICAL CHEMISTRY, SPECIAL TECHNIQUES GROUP (at the Chemical Society, Burlington House, Piccadilly, London, W.1), at 7 p.m.—Annual General Meeting, followed by Dr. L. Brealey: "Some Analytical Problems in the Power Generating Industry".

Tuesday, November 23—Wednesday, November 24

INSTITUTION OF GAS ENGINEERS (at Church House, Westminster, London, S.W.1), at 9.15 a.m. on Tuesday and 9 a.m. on Wednesday—31st Autumn Research Meeting.

Wednesday, November 24

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 2 p.m.—Dr. G. W. Ashcroft: "Aspects of the Cerebral Metabolism of Tryptophan".*

GEOLOGICAL SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. G. A. Chinner: "The Distribution of Pressure and Temperature during Dalradian Metamorphism".

ROYAL STATISTICAL SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.15 p.m.—Discussion on "The National Plan" opened by Mr. W. Beckerman and Mr. K. J. Wigley.

INSTITUTE OF FUEL (at the Royal Institute of British Architects, 66 Portland Place, London, W.1), at 5.30 p.m.—Mr. W. G. Cummings, Mr. M. W. Redfern and Mr. W. Roberts Jones: "Air Pollution by Sulphur Dioxide".

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. P. Honey: "Electric Water-Heating Development".

UNIVERSITY OF LONDON (at Senate House, London, W.C.1), at 5.30 p.m.—Sir David Cuthbertson, C.B.E.: "The World Protein Problem".*

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, JOINT I.E.R.E./I.E.E. COMPUTER GROUPS (at 9 Bedford Square, London, W.C.1), at 6 p.m.—Mr. G. J. Crask and Mr. S. F. Miles: "Analogue Circuit Techniques Using Transistors".

INSTITUTION OF MECHANICAL ENGINEERS (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Mr. C. Seppel: "The Development of the Industrial Gas Turbine".

INSTITUTE OF INFORMATION SCIENTISTS (at the Whitehall Hotel, Bloomsbury Square, London, W.C.1), at 6.15 p.m.—Mr. C. W. Hanson *et al.*: "Report on the F.I.D. Conference at Washington".

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Mr. A. Lennard: "Odour in Packaging

Materials"; Mr. K. W. Taylor: "Hydraulic Conveying of Particulates in Narrow Bore Pipes"; Mr. E. L. Starkie: "Conversion of Malt Mash in a Plug Flow Converter".

SOCIETY FOR ANALYTICAL CHEMISTRY, MICROCHEMICAL METHODS GROUP (at "The Feathers", Tudor Street, London, E.C.4), at 6.30 p.m.—Discussion Meeting on "The Optimal Environment for Microanalysis", introduced by Mr. A. J. Cross.

Thursday, November 25

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Dr. F. B. O'Connor: "Soil Animals".*

INSTITUTE OF PETROLEUM, EXPLORATION AND PRODUCTION GROUP (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Prof. Irving Fatt (University of California): "Microscopic Heterogeneity in Reservoir Rock and its Influence on Reservoir Behaviour".

INSTITUTION OF ELECTRICAL ENGINEERS, CONTROL AND AUTOMATION DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Discrete versus Continuous Formulations in Control Theory" opened by Dr. A. T. Fuller, Dr. P. E. W. Grensted, Prof. E. I. Jury, and Prof. C. Storey.

ROYAL INSTITUTION, LIBRARY CIRCLE (at 21 Albemarle Street, London, W.1), at 5.30 p.m.—Mr. C. A. Cade: "Witchcraft—Delusion, Deception or Devilry?"

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. M. D. Milne: "Cystinuria 1810–1965". (Ninth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation).*

UNIVERSITY OF LONDON (at the Middlesex Hospital Medical School, Mortimer Street, London, W.1), at 5.30 p.m.—Prof. A. Rupert Hall: "Hooke's 'Micrographia', 1665–1965".

INSTITUTION OF MECHANICAL ENGINEERS, APPLIED MECHANICS GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Stress Analysis of Components Reinforced with Fibreglass".

CHEMICAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 6 p.m.—Discussion Meeting on "Far Infrared Spectroscopy".

BRITISH INDUSTRIAL BIOLOGICAL RESEARCH ASSOCIATION (at the School of Pharmacy, Brunswick Square, London, W.C.1), at 6.30 p.m.—Dr. J. M. Barnes: "Chemicals in Food".

Friday, November 26

INSTITUTE OF BIOLOGY (in the Jarvis Hall of the Royal Institute of British Architects, 66 Portland Place, London, W.1), at 10 a.m.—Meeting on "The Design of Biological Laboratories".

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 4 p.m.—Dr. C. F. Hawkins: "Megaloblastic Anemias—The Clinician's Approach".*

INSTITUTION OF ELECTRICAL ENGINEERS, CONTROL AND AUTOMATION DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. F. C. Widdis and Mr. K. Sharples: "The Teaching of Experimental Techniques".

TELEVISION SOCIETY (in the Conference Suite, I.T.A., 70 Brompton Road, London, S.W.3), at 7 p.m.—Mr. D. J. Wheeler: "Television Audience Measurement".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. R. J. Harrison: "Can Dolphins Talk?"

Saturday, November 27

BOTANICAL SOCIETY OF THE BRITISH ISLES (in the Botany Department, British Museum (Natural History), Cromwell Road, London, S.W.7), from 2 p.m. to 5.30 p.m.—Annual Exhibition Meeting.

INNER LONDON EDUCATION AUTHORITY (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Dr. D. A. Allan: "Volcanoes—The Caribbean Windward Islands".*

Monday, November 29

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2) at 10 a.m.—Colloquium on "Integrated Circuits".

INSTITUTION OF MECHANICAL ENGINEERS, RAILWAY ENGINEERING GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Specification and Inspection of Railway Materials".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2) at 6 p.m.—Prof. Colin Cherry: "The Future of World Communication" (Last of three Cantor Lectures on "World Communication".)

ROYAL SOCIETY OF MEDICINE (at 1 Wimpole Street, London, W.1), at 6 p.m.—Prof. R. H. S. Thompson: "A Biochemical Approach to the Problem of Multiple Sclerosis" (Jephcott Lecture).

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, W.C.2), at 6.30 p.m.—Symposium on "Control and its Applications".

PLASTICS INSTITUTE, LONDON SECTION REINFORCED PLASTICS SUB-GROUP (at the Wellcome Building, Euston Road, London, N.W.1), at 6.30 p.m.—Mr. B. A. Clark and Mr. D. F. Failey: "Some Aspects of Design and Construction of Reinforced Plastic Building Panels".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Mr. A. R. Hanbury-Tenison: "From the Orinoco to the Plate".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned.

LECTURER (with suitable academic qualifications and relevant experience) IN METALLURGY—The Principal, Lanchester College of Technology, Priory Street, Coventry (November 26).

JUNIOR RESEARCH ASSOCIATE (with a good honours degree in veterinary or agricultural science or biochemistry) IN AGRICULTURAL BIOCHEMISTRY,

for investigations into the mineral requirements of pigs—Head of the Department of Agricultural Biochemistry, School of Agriculture, University of Newcastle upon Tyne, Newcastle upon Tyne, 1 (December 1).

LECTURER or ASSISTANT LECTURER IN THE DEPARTMENT OF MATHEMATICS—The Secretary, The University, Edinburgh (December 1).

EXPERIMENTAL OFFICER IN THE DEPARTMENT OF CHEMISTRY IN THE FACULTY OF SCIENCE to work on an electron spin resonance instrument shortly to be installed in the Department and concerned with the study of inorganic complexes—The Registrar, The University, Manchester, 13 (December 4).

LECTURER or ASSISTANT LECTURER IN THE DEPARTMENT OF MATHEMATICS—The Secretary, The University, Aberdeen (December 4).

LECTURER or ASSISTANT LECTURER (Probationary) (man or woman graduate with special interests in the field of logic or the theory of sets) IN MATHEMATICS—The Secretary, Bedford College (University of London), Regent's Park, London, N.W.1 (December 4).

LIBRARIAN—The Secretary, University of Stirling, Academic Planning Board, Municipal Buildings, Stirling (December 4).

LECTURER (with high academic qualifications and the ability and desire to pursue research) IN INORGANIC CHEMISTRY—The Principal, Lanchester College of Technology, Priory Street, Coventry (December 6).

OFFICIAL FELLOW AND TUTOR IN PURE MATHEMATICS—The Master, St. Catherine's College, Oxford (December 6).

TEMPORARY LECTURER IN PHILOSOPHY—The Registrar and Secretary, University of Durham, Old Shire Hall, Durham (December 6).

CHIEF TECHNICIAN (with sound qualifications, a knowledge of modern techniques, and preferably some experience and interest in administration) IN THE DEPARTMENT OF ZOOLOGY—The Secretary, The University, Highfield, Southampton (December 7).

LECTURER IN THE DEPARTMENT OF ZOOLOGY, University of Ibadan, Nigeria—The Inter-University Council, 33 Bedford Place, London, W.C.1 (December 7).

SENIOR LECTURERS, LECTURERS or ASSISTANT LECTURERS (2) (preferably with a special interest in one or more of the following: vertebrate zoology, invertebrate zoology, parasitology or physiology) IN ZOOLOGY at the University of Zambia—The Inter-University Council, 33 Bedford Place, London, W.C.1 (December 10).

LECTURER (with qualifications in the teaching of geography) IN EDUCATION—The Registrar, The University, Manchester, 13, quoting Ref. 198/65 (December 11).

READER, SENIOR LECTURER, LECTURER and an ASSISTANT LECTURER (suitably qualified candidates with interests in mechanical, civil, electrical, electronic, production or chemical engineering, or in engineering design) IN THE SCHOOL OF ENGINEERING SCIENCE—The Registrar, University of Warwick, Coventry, Warwickshire (December 11).

RESEARCH FELLOW IN PHILOSOPHY—The Rector, Lincoln College, Oxford (December 11).

CHAIR OF BIOCHEMISTRY—The Secretary, The Queen's University, Belfast, Northern Ireland (December 15).

LECTURER/SENIOR LECTURER IN PURE MATHEMATICS at the University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, December 17).

PROFESSOR OF THE HISTORY OF PHILOSOPHY—The Registrar, University Registry, Oxford (December 23).

CHAIR OF PHYSICAL CHEMISTRY at Victoria University of Wellington, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, December 31).

LECTURER or ASSISTANT LECTURER (with interests in inorganic chemistry, physical chemistry, or theoretical chemistry) IN CHEMISTRY—The Registrar, University of East Anglia, Earlham Hall, Norwich, NOR 88C (December 31).

LECTURER (with a doctorate or equivalent research experience, and preferably specialist in the field of hard-rock petrology) IN GEOLOGY at the University of Newcastle, New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (January 1).

MARY MARSHALL AND ARTHUR WALTON CHAIR OF THE PHYSIOLOGY OF REPRODUCTION—The Registry, University Registry, The Old Schools, Cambridge, marking envelope "Confidential" (January 10).

HEAD (experienced soil scientist) OF THE SOIL SURVEY OF ENGLAND AND WALES—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (January 15).

KNIGHTBRIDGE CHAIR OF PHILOSOPHY—The Registry, University Registry, The Old Schools, Cambridge, marking envelope "Confidential" (January 17).

DIRECTOR OF THE UNIVERSITY COMPUTING CENTRE—The Assistant Registrar (Establishment), University of Sussex, Stanmer House, Stanmer, Brighton, Sussex.

LECTURER (with good academic qualifications and experience in systems programming and/or computing in industry) IN COMPUTING—The Registrar, Regional College of Technology, Leicester.

POST-DOCTORAL ASSISTANT IN INORGANIC CHEMISTRY—The Secretary, Department of Chemistry, St. Salvador's College (University of St. Andrews), St. Andrews, Fife, Scotland.

PRINCIPAL (with high academic and professional qualifications)—Clerk to the Governing Body, Borough Polytechnic, Borough Road, London, S.E.1.

TECHNICAL OFFICER (with a degree in agriculture or related sciences or equivalent qualifications, and preferably experience in field experiments) FOR ROOT AND FODDER CROP TRIALS—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge.

Research Council, 1965. Obtainable from H.M. Stationery Office.) 8s. 6d. [110]

Annual Report on the Meteorological Office for the year 1 January to 31 December 1964. (Met. O. 769.) Pp. vii + 75 + 10 plates. (London: H.M. Stationery Office, 1965.) 7s. 6d. net. [110]

The Royal Society. Report on Education in Cartography. (British National Committee for Geography.) Pp. 13. (London: The Royal Society, 1965.) [110]

British Productivity Council. Achieving Balance Between Capacity and Sales. (Proceedings of a Conference organized jointly by the British Productivity Council, the British Institute of Management and the Centre for Interim Comparison, in co-operation with the Birmingham and Coventry Productivity Associations.) Pp. 31. (London: British Productivity Council, 1965.) 15s. [110]

Mullard Educational Service. Educational Electronic Experiments. No. 14. Resistance and Capacitance. Pp. 4. No. 16: An Electronic Organ. Pp. 4. (London: Mullard Educational Service, Mullard, Ltd., 1965.) [110]

Freshwater Biological Association. Thirty-third Annual Report for the year ended 31 March 1965, including Reports of the Council and of the Director, and Accounts for 1964-65, with Lists of Officers, Council, Staff and Members. Pp. 112 + 4 plates. (Ambleside, Westmorland: Freshwater Biological Association, 1965.) 5s. [110]

Institute of Fuel. Proceedings at the Conference on Fuel Research and Development held at Eastbourne, 4th to 8th October, 1965. Vol. 1: Papers. Pp. 294. Conference Handbook. Pp. 14. (London: The Institute of Fuel, 1965.) [110]

Imperial College of Science and Technology (University of London). Shorter Postgraduate Courses, Autumn Term, Session 1965-66. Pp. 16. (London: Imperial College of Science and Technology, 1965.) [110]

Other Countries

Unesco. Scientific Institutions and Scientists in Latin America. Mexico (Cienciafics, A-G, Fascículo 1). Pp. 372. (Montevideo: Centro de Cooperación Científica para América Latina en colaboración con la Organización de los Estados Americanos, 1965.) [410]

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Economic Geology Report No. 22: Geology of Iron Deposits in Canada. Vol. 1: General Geology and Evaluation of Iron Deposits. By G. A. Gross. Pp. 181 (9 plates). (Ottawa: Queen's Printer, 1965.) 5 dollars. [410]

The Geological Society of America. Special Paper No. 80: Volcanism, Tectonism, and Plutonism in the Western United States. By James Gilluly. Pp. 69. (New York, N.Y.: The Geological Society of America, 1965.) 2 dollars. [410]

Délégation Générale à la Recherche Scientifique et Technique. La Recherche dans le Domaine de l'Eau: Rapports du Groupe de Travail EAU Concernant les Principales Recherches Effectuées ces Dernières Années et Répertoire des Laboratoires. Pp. 183. (Paris: La Documentation Française, 16, rue Lord Byron, 1964.) [410]

Zoology Publications from Victoria University of Wellington. No. 39 (18 August, 1965): The Bathyal Holothurians of the New Zealand Region. By David L. Pawson. Pp. 33. (Wellington: Victoria University of Wellington, 1965.) [410]

Annals of the New York Academy of Sciences. Vol. 119, Article 3: Chemistry and Metabolism of L- and D-Lactic Acids. By N. R. Alpert and 45 other authors. Pp. 851-1165. 6 dollars. Vol. 126, Article 2: Computation for Cardiovascular Research. By D. P. Asnes and 45 other authors. Pp. 681-940. 8 dollars. (New York: New York Academy of Sciences, 1965.) [410]

United States Department of the Interior: Fish and Wildlife Service. Bureau of Sport Fisheries and Wildlife. Circular No. 221: Bulrushes and Bulrushlike Plants of Eastern North America. By Neil Hotchkiss. Pp. ii + 19. 20 cents. Circular No. 224: Pesticide-Wildlife Studies by States, Provinces, and Universities—An Annotated List of Investigations Through 1964. Pp. i + 30. (Washington, D.C.: Government Printing Office, 1965.) [410]

Institut for Atomenergi, Kjeller, Norway. Kjeller Report No. 95: Determination of Selenium in Animal Tissue by Neutron Activation. By J. B. Dahl and E. Steinhed. Pp. 4. (Kjeller, Norway: Institut for Atomenergi, Kjeller Research Establishment, 1965.) [410]

Botanical Society of Japan. Address Book of Japanese Botanists, 1964. Edited by the National Committee of Botany. Pp. ii + 118. (Tokyo: Botanical Society of Japan, 1965.) [410]

Publications of the Observatory of the University of Michigan. Vol. 9, No. 5: The Spectrum of Nova Gemmorum 1912. By Dean B. McLaughlin. Pp. 113-179. Vol. 9, No. 6: First List of Stars Observed at the Lamont-Hussey Observatory of the University of Michigan. By Frank Holden. Pp. 181-184. (Ann Arbor: The University of Michigan, 1965.) [410]

Transactions of the American Philosophical Society. New Series, Vol. 55, Part 6: Enlightened Bureaucracy Versus Enlightened Despotism in Baden, 1750-1792. By Prof. Helen P. Liebel. Pp. 132. (Philadelphia: The American Philosophical Society, 1965.) 3.50 dollars. [410]

Proceedings of the United States National Museum, Smithsonian Institution. No. 3511, Vol. 117: Benthic Polychaetous Annelids from Bering, Chukchi, and Beaufort Seas. By Donald J. Reish. Pp. 131-158. No. 3512, Vol. 117: Haustoriidae of New England (Crustacea: Amphipoda). By E. L. Bousfield. Pp. 159-240. No. 3513, Vol. 117: Planktonic Copepods from Bahía Fosforescente, Puerto Rico, and Adjacent Waters. By Juan G. Gonzalez and Thomas E. Bowman. Pp. 241-304. No. 3514, Vol. 117: Revision of the Milliped Genera Boraria and Gyalostethus (Polydesmida: Xystodesmidae). By Richard L. Hoffman. Pp. 305-348. (Washington, D.C.: Government Printing Office, 1965.) [410]

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Ministry of Agriculture, Fisheries and Food. At the Farmer's Service (A handy reference to various services available to farmers in England and Wales.) Pp. 104. (Pinner, Middx.: Ministry of Agriculture, Fisheries and Food, 1965.) Gratis. [110]

Ministry of Technology. Forest Products Research Laboratory Leaflet No. 38: Termites and the Protection of Timber. Pp. 7. (London: H.M. Stationery Office, 1965.) 9d. net. [110]

Geology in Africa. By Prof. R. M. Shackleton. (Inaugural Lecture delivered before the University of Leeds on 27 April 1964.) Pp. 18. (Leeds: The University, 1965.) 2s. 6d. [110]

Agricultural Research Council. Radiobiological Laboratory Annual Report 1964-1965. (ARCL 14) Pp. viii + 90. (London: Agricultural

Editorial and Publishing Offices of "NATURE"

MACMILLAN (JOURNALS) LIMITED

4 LITTLE ESSEX STREET, LONDON, W.C.2

Telephone Number: Temple Bar 6633. Telegrams: Phusis London W.C.2

Annual subscription: Inland £14, Overseas £13 15s., payable in advance, postage paid to any part of the world.

Advertisements only should be addressed to

T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2.

Telephone Number: Holborn 4743

Registered as a newspaper at the General Post Office

Copyright © Macmillan (Journals) Limited, November 20, 1965

NATURE

No. 5013

SATURDAY, NOVEMBER 27, 1965

Vol. 208

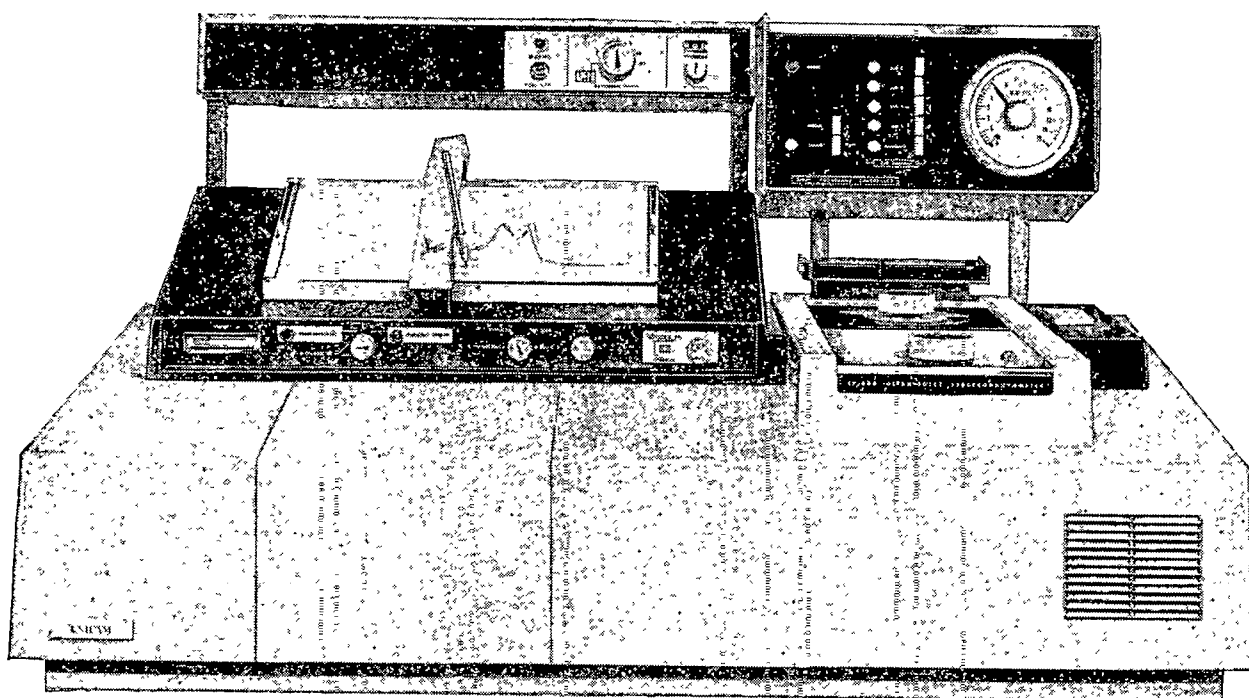
Published by
MACMILLAN (JOURNALS) LTD.

CONTENTS

	Page
JACK BRIMBLE	819
GRASSLAND AND FODDER IN INDIA. By Dr. William Davies	821
"THAT OLD COMMON ARBITRATOR, TIME". By Dr. W. Gooddy	822
DOCUMENTATION. By P. C. Williams	822
ENGINEERING APPLICATIONS OF RUBBERS. By Dr. L. R. G. Treloar	823
FAST BIOCHEMICAL REACTIONS. By Prof. S. Dagley	823
A WELL-BALANCED ACCOUNT OF THE ALGAE. By Dr. Mary Parke and G. T. Boalch	824
DRUG-DEPENDENCE. By the Right Hon. Lord Brain, F.R.S.	825
RESEARCH AND MANAGEMENT IN EAST AFRICAN WILDLIFE. By Dr. John Morton Boyd	828
OBITUARIES:	
SIR IAN RICHMOND By J. P. Gillam	831
K. R. BUTLIN. By Prof. J. R. Postgate	831
PROF. H. SCHARDIN. By G. H. Lunn.	832
NEWS AND VIEWS	832
ELECTROMAGNETIC DISTANCE MEASUREMENT	837
AUSTRALIAN BIOCHEMICAL SOCIETY MEETING. By Dr. F. J. R. Hird	837
CONTROL OF BIOLOGICAL DEVELOPMENT	838
SEAWEED RESEARCH. By Dr. E. Conway and Dr. E. G. Young	840
A SUGGESTED PHENOTYPIC CLASSIFICATION AND TERMINOLOGY FOR ENZYME MUTANTS IN MICRO-ORGANISMS. By Dr. J. F. Collins, Dr. J. Mandelstam, Prof. M. R. Pollock, F.R.S., Dr. M. H. Richmond and Dr. P. H. A. Sneath	841
CHARACTERIZATION OF A TRANSFER FACTOR ASSOCIATED WITH DRUG RESISTANCE IN <i>Salmonella typhimurium</i> . By Dr. E. S. Anderson and Dr. M. J. Lewis	843
DIRECT AND INDIRECT EFFECTS OF RADIATION ON PLANT CELLS: THEIR RELATION TO GROWTH AND GROWTH INDUCTION. By Dr. R. D. Holsten, Dr. M. Sugii and Prof. F. C. Steward, F.R.S.	850
NATURE OF THE HEPATOMEGALIC EFFECT PRODUCED BY ETHYL-CHLOROPHENOXY-ISOBUTYRATE IN THE RAT. By Dr. R. Hess, W. Stäubli and W. Riess	856
A LOCALIZED HAEMOLYSIS IN GEL METHOD FOR THE DETECTION OF CELLS PRODUCING 7S ANTIBODY. By Dr. J. Šterzl and Dr. I. Říha; Dr. D. W. Dresser and Dr. H. H. Wortis	858
A NEW MODEL FOR VIRUS RIBONUCLEIC ACID REPLICATION By Dr. F. Brown and Dr. S. J. MARTIN	861
CYTOPLASMIC ORGANELLES AND CELL GROWTH IN ROOT CAPS. By Dr. B. E. Juniper and Dr. F. A. L. Clowes	864
THE BURSA PHARYNGEA IN THE GIANT PANDA (<i>Ailuropoda melanoleuca</i>). By Prof. A. J. E. Cave	865
ELECTRON MICROSCOPE OBSERVATIONS ON THE FREE PROTEIN AND THE PROTEIN-POLYSACCHARIDE COMPLEX OF BOVINE SYNOVIAL FLUID. By Dr. James W. Smith and Dr. John Frame	867
POSSIBLE RELATIONSHIP BETWEEN TRACE METALS AND BLOAT IN RUMINANTS. By P. J. Harris and Prof. F. Sebba	869
2,6-DIAMINO-3-HYDROXYPIMELIC ACID IN MICROBIAL CELL WALL MUCOPEPTIDE. By H. R. Perkins	872
HETEROGENEITY OF WATER-SOLUBLE STRUCTURAL COMPONENTS OF HUMAN RED CELL MEMBRANE. By Prof. M. D. Poulik and Dr. P. K. Lauf	874
PECTIC POLYSACCHARIDES IN THE GROWTH OF PLANT CELLS: MOLECULAR STRUCTURAL FACTORS AND THEIR ROLE IN THE GERMINATION OF WHITE MUSTARD. By Sheila E. B. Gould, Dr. D. A. Rees, N. G. Richardson and I. W. Steele	876
QUANTITATIVE GAS CHROMATOGRAPHY OF AMINO-ACIDS AS TRIMETHYLSILYL DERIVATIVES By Prof. Edgar D. Smith and Howard Sheppard, jun.	878
CORRELATED ATOMIC COLLISIONS IN IRRADIATED CRYSTAL LATTICES. By Dr. Lewis T. Chadderton and Ian McC. Tortens	880
RECENT SCIENTIFIC AND TECHNICAL BOOKS	ccccxxx

(Continued overleaf)

Progress in Spectroscopy



Unicam SP800 Spectrophotometer with automation accessories

*Save operator time, increase speed
and efficiency with Unicam
automation for:*

ENZYME REACTION RATES

**LIQUID COLUMN
CHROMATOGRAPHY**

**ALL ROUTINE ULTRAVIOLET
& VISIBLE SPECTROSCOPY**

Send for descriptive brochure.

Among the many important applications for the Unicam SP800 Spectrophotometer none is a better example of its potential than the measurement of enzyme reaction rates. For this the Automation Accessories are vital components. Other widely acclaimed features are the Flat Bed Recorder, the Beam Balance Smoothing Cam and the unique Second Sample Position, all of which mark the SP800 as the outstanding U.V. and Visible Recording Spectrophotometer. The Automatic Equipment provides:

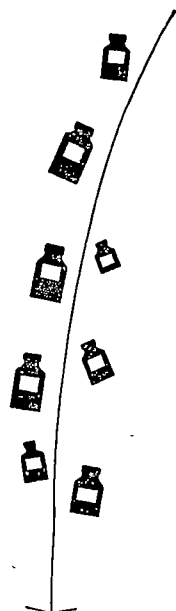
AUTOMATIC interchange of up to 4 sample and 4 reference cells,
AUTOMATIC re-cycling over any pre-selected wavelength range,
AUTOMATIC recording at predetermined time intervals.

UNICAM

Precision Spectrophotometers

LETTERS TO THE EDITOR

	Page		Page
PHYSICS		Primary Tissue Phase of <i>Plasmodium berghei</i> in Different Experimental Hosts.—Prof. Meir Yoeli, Dr. Jerome Vanderberg, Miss Rasma S. Upmanis and Prof. Harry Most	
Wave Mechanical Approach to the Plasma Interaction Problems.—Prof. V. C. Liu	883		903
rect Ultramicroscopic Observation of Thermal Scattering in Sodium Chloride.—Dr. Gerhard Heilmann	884	Virus Aetiology for Down's Syndrome (Mongolism).—Alan Stoller and R. D. Collmann	903
GEOLOGY		RADIOBIOLOGY	
rona Structures in the Basic Igneous Masses of East Aberdeenshire.—Dr. D. S. Weedon	885	Modification of Radiation 'Cerebral Death' by Hypoxia.—Dr. E. A. Wright and Jennifer Shewell	904
igocene in California ?—Jere H. Lipps	885	Acute Radio-sensitivity as a Function of Age in Mice.—Dr. J. F. Spalding, O. S. Johnson and R. F. Archuleta	905
GEOCHEMISTRY		Distribution and Effective Half-life of Cobalt-58 in Habrobracon.—Prof. Daniel S. Grosch	906
richment of Iron during Accretion in the Solar Nebula.—Prof. S. R. Taylor	886	BIOLOGY	
CHEMISTRY		Experimental Infections of Cattle with <i>Fasciola hepatica</i> : a Comparison of Low and High Infection Rates.—J. G. Ross	907
iffusion or Hydride Component of Hydrogen Overpotential at Platinized Platinum and Palladized Palladium Cathodes.—M. N. Hull and Dr. F. A. Lewis	887	Haemoglobin in <i>Ceriodaphnia quadrangula</i> (O. F. Müller).—B. Czezugala	907
lectron Spin Resonance Spectrum of Cu ⁺⁺ in Diamine Oxidase.—Prof. Alan van Heuvelen	888	Kinesis.—T. G. Amos	908
se of Dimethyl Sulphoxide in Liquid Scintillation Counting.—Evan Wright and K. R. Millar	889	Generation of Nutational Movements.—David G. Heathcote	909
ate of Micelle Formation of Sodium Alkyl Sulphates in Water.—P. F. Mijnlief and R. Ditmarsch	889	Thermal Contraction in Twigs.—I. J. McCracken and Prof. T. T. Kozlowski	910
olarization and Hybridization.—Dr. T. A. Claxton	891	Occurrence of Gnetalean Pollen Grains in the Katrol Formation (Upper Jurassic) of Kutch, India.—Y. K. Mathur and Mrs. K. Mathur	912
reakdown of Simple Hydrocarbons subjected to a Silent Electric Discharge.—P. E. Cross	892	Increase during Winter in Capacity for Root Regeneration in Detached Shoots of Fruit-tree Rootstocks.—Dr. B. H. Howard	912
BIOCHEMISTRY		VETERINARY SCIENCE	
Molecular Weight of Urokinase.—R. A. Burges, Dr. K. W. Brammer and J. D. Coombes	894	Carcinogenic Activity of Bracken.—Prof. I. Anticevans and J. Mason	913
ocalized Strand Separations within Deoxyribonucleic Acid during Selective Transcription.—Prof. John H. Frenster	894	MICROBIOLOGY	
roperties of Hyaluronic Acid from a Human Myxosarcoma.—Dr. Maria Luisa Marcante	896	Accumulation of Keto-acids in Tissues during Diphtheria Intoxication.—Dr. S. C. Agarwal	914
leic Acid labelled with Iodine-131, and Triolein Deiodination <i>in vitro</i> .—Leopoldo Jose Anghleri	896	VIROLOGY	
PHYSIOLOGY		Inhibition of Influenza Virus Haemagglutination by Polymerized Orosomucoid.—P. H. Whitehead, Dr. T. H. Flewett, J. R. Foster and Dr. H. G. Sammons	915
Heat Production in a Cardiac Contraction.—Dr. N. V. Ricchiuti and Dr. C. L. Gibbs	897	CYTOLOGY	
omesthetic and Visual Responses of Superior Colliculus Neurones.—Dr. Dora Jassik-Gerschenfeld	898	Demonstration of the Spiral Structure of Human Chromosomes.—Dr. Masashi Ohnuki	916
PHARMACOLOGY		GENETICS	
Thromboses in Large Veins and Pulmonary Embolisms induced by Catecholamines or Serotonin.—Prof. Hans Selye, Beatriz Tuchweber and Pavel Rohan	900	Chimerical Nature of the Entire-leaf Variant in the Potato Variety 'Majestic'.—Dr. H. W. Howard	917
Labelled Depolarizing Drugs in Normal and Denervated Muscle.—Prof. D. B. Taylor, R. Creese, O. A. Nedergaard and R. Case	901	Estimation of the Frequency of Functioning Gametes in Monosomics.—A. K. Roy Choudhury and J. G. Bhowal	917
PATHOLOGY		PSYCHOLOGY	
Conversion of the Inconvertible L-1 Lymphosarcoma to Ascitic Form.—Prof. Melvin R. Sikov	902	Memory Changes with Age.—Prof. David Schonfield	918
		MISCELLANEOUS	
		Ethology and the 'Beconian' Method.—Dr. William P. D. Wightman	918



DIFCO

*Microbiological
reagents
and media*

**delivered
to your bench quickly**

Hundreds of different products in the complete Difco range are kept in stock ready to be on your bench without delay. We shall always be pleased to obtain other items specially to order.

Speed, convenience, reliability . . . and remember that Difco offer the only *complete* line of culture media available in U.K. Please send for the latest literature concerning your special interests.



**complete
laboratory
service**



BAIRD & TATLOCK (LONDON) LTD., CHADWELL HEATH, ESSEX, ENGLAND
Branches in London, Manchester, Birmingham and Glasgow
Member of the Derbyshire Stone Group.

TAB/DO.13A

BCM 200 REFRACTORY CUTTING MACHINE

HIGH SPEED PRECISION CUTTING OF REFRACTORIES, ROCKS AND CERAMICS

Cutting Disc Diamond Impregnated 14" dia.
Max. depth of cut $4\frac{3}{4}$ ".

Table mounted on vee guide rollers fitted with
needle roller journal and thrust bearings.

Area 11" x 17", with tee slots for clamping.

Table traverse 16".

Mitre rest and work clamp fitted to table.

- Spindle fitted with taper roller bearings.

Fully protected from abrasive sludge.

Vibration free drive powered by $1\frac{1}{2}$ horse
power motor. All electrics totally enclosed
in pedestal.

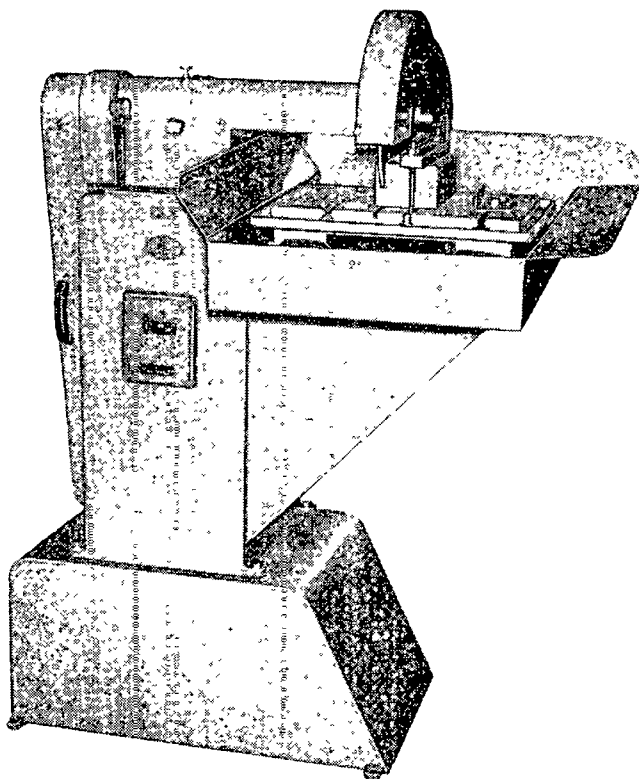
Coolant mainwater or soluble oil.

Finish Grey Stove Enamel.

- Adjustable Vise unit for controlled thickness
slicing.

- Reversible core vise for parallel facing of cores.

CUTROCK ENGINEERING CO. LTD.,
35 Ballards Lane, London, N.3
Telephone: FINCHLEY 5978



JACK BRIMBLE

A BOOK review in *Nature*, March 22, 1930, includes the following passage: "But these phenomena [growth and tropic movements of plants], like all others, demand an *ultimate exposition of the truth* [our italics]. This can never be cloaked, as an ideal, however enthusiastic one might become over a certain theory. One feels, therefore, to do this, we should strive for it in as simple a way as possible". This, in a nutshell, was what science meant to the reviewer, L. J. F. Brimble, and it remained so all his life—an ultimate exposition of the truth. These words of a new reviewer to *Nature* caught the eye of the then editor, Sir Richard Gregory. Thinking at the time that he required a second assistant, Sir Richard entered into correspondence with the reviewer and eventually invited him to London and to an assistant editorship of *Nature*.

Lionel John Farnham Brimble was born the son of a much-respected Somerset family on January 16, 1904. His early days in Radstock were happy ones, for he always cherished their memories. The family was an important unit to him—a view which he always maintained.

One of the factors which decided his later career was an innate curiosity of all that surrounded him. So strong was this that he rejected a scholarship to a Naval college and instead became a boarder at the nearby Sexey's School, Bruton. It was his great fortune that the headmaster of this school was far-sighted, and realized that science should be taught from a very early age. Not only were there science classes, but for those who wished, tuition was available in botany. In this respect Sexey's School was unique in the country. Botany was taught in a few girls' schools, but it was not, at that time, generally held to be a subject suitable for young men. Soon, with his natural abilities at drawing and sketching and his good knowledge of natural history gained from the surrounding countryside, his career was mapped out. He won a scholarship to the University College of Reading. There, where he read botany under Prof. W. Stiles, he proved himself an excellent field botanist and developed a particular interest in trees. Reading at that time was very much based on the Oxford College system, and for Brimble this was just the right kind of stimulation. His college friends remember how much he enjoyed the 'go' and gaiety of student life. He graduated at the time when Reading gained university status: prior to this, graduates had taken London degrees, but those who took their degrees at the time of the granting of the charter were awarded both London and Reading degrees. There being no science honours graduates whose names began with 'A'—or so far as the 'Bo's'—it was always Brimble's boast that he was the first to gain a B.Sc. (Reading). Brimble—or 'Jack' as he was now known to all his friends—was offered a research scholarship in Germany. Simultaneously a post of lecturer in plant physiology became available in the Department of Botany, University of Glasgow, under Prof. J. M. F. Drummond. By now something had clicked in his mind; pure research was not for him: he had a taste for gregarious living. Glasgow offered a post for both teaching and research. In 1926 perhaps the youngest science lecturer in the country arrived at Glasgow.

The prospect of having to lecture to eighty pre-clinical medical students, the majority of whom were much older than he, would have paled most, but not Jack Brimble. It was his personality that won through, and staff and students alike greatly appreciated the jollity of his character. He was an excellent, kind teacher who liked, and was liked by, his students. He had a fine sense of humour, an agreeable voice, and an infectious enthusiasm which made him bob up and down. He stayed but a year in Glasgow, being offered a lectureship in the University of Manchester, where he stayed from 1927 until the end of 1930. His research was very much in the Stiles idiom, namely, physiological research on ion exchange in tissues. However, his research ideas never underwent any great or imaginative developments. He was just not made that way, but he knew what he wanted. His days at Manchester were exciting, in that he could satisfy his deep love of music by listening to the Hallé Orchestra under Sir Hamilton Hartley. He had many literary friends and in his spare time was a theatre critic on a local paper. It was while he was at Manchester that he was approached by the Editor of *Nature* to review the book by Sir Jagadis Chunder Bose, *Growth and Tropic Movements of Plants*.

On reading the review, Sir Richard realized that here was someone who saw science as more than just his own speciality. Each man's work was only part of a greater all-encroaching pattern. Brimble showed that he firmly grasped the basic concept that lay behind the 'philosophy of science' and, at the same time, could put over his ideas. All this, coupled with his personality, fitted him for the work that lay ahead. Brimble, without really knowing why, and to the horror of his family, abandoned his academic life for an 'office life'—or so some thought—and joined *Nature* as an assistant editor to Sir Richard in 1931. Contrary to those who thought this move a mistake, his new role gave him the special opportunity to develop his innate needs and qualities—to write, to meet people and to have a wide awareness of what was going on, not only in the world of science, but also in the world of expanding education and educational beliefs. At heart Jack Brimble was an educator. While in many ways he and Sir Richard were unlike, in others they saw eye to eye very closely. He very soon became a disciple of Sir Richard, who encouraged his wide outlook and catalysed his exuberance. He remained throughout his life enthusiastic, but he never allowed enthusiasm to affect his judgment. *Nature* and belonging to the House of Macmillan gave him many outlets which he greatly enjoyed. For to know the activities of the changing world and what was going on in it was his *métier*. He enjoyed all this, partly as the fun of life, but also as an opportunity for exercising his developing flair for scientific news. However, behind all this there was a great fund of common sense and a surprising shrewdness in the business aspect of his profession. He was faithful 'to his salt', to the integrity of science, to the great and honourable traditions of *Nature*, and to the old and distinguished publishing House, for which he had deep respect.

Jack Brimble remained a true botanist, that is, a lover of plants as perceived by human kind, as an artistic entity

and as an inspiration of poetry and folk-lore. In such approaches to botany he was an unsurpassed contemporary exponent: hence the success of his better-known books, particularly *Flowers in Britain*, *Trees in Britain* and *The Floral Year*. His love of good illustrations and his personal delight in drawing were features of his work. Didactically, his *Intermediate Botany* had a very considerable success, partly because he understood so clearly the educational need for such a book, but in no small measure because the book was so well planned and comprehensive. Not all his books escaped critical review. He always delighted in relating his cruel experience of editing an adversely critical review of one of his own books, for publication in his own journal, and how he had to resist the temptation to 'blue-pencil' that which he thought unfair.

In the late 'thirties he was associated with the movement then afoot to develop the teaching of biology in schools (even at that time it was still restricted mainly to botany in girls' schools). Later he was involved with the organization of the British Social Hygiene Council, which eventually became the Central Council for Health Education. His associates included Sir Julian Huxley, Prof. Winfred Cullis, Prof. J. B. S. Haldane, the memorable Mrs. Neville Rolf and Sir Robert Fields. He, with many others, wrote articles, gave lectures, held courses at summer schools, organized brains-trusts and in general did much to promote these causes.

In 1938, on the retirement of Sir Richard, Jack Brimble and his colleague A. J. V. Gale were appointed joint editors of *Nature*. Thus began a new chapter in the history of the journal. The partnership was a fruitful one which was to last twenty-three years, to carry *Nature* through the Second World War into a realm of new science expanding exponentially on all frontiers. With this partnership the traditions which Sir Richard had built up were maintained and developed. This was especially so in encouraging the work of the British Association for the Advancement of Science. Brimble, like Gregory, was keen on focusing interest on social relations. He became involved in army education under Major-General Walter Beddall and Major-General Cyril Lloyd, and during the War lectured in many parts of the country to various Commands. His book on the social aspects of science, with its references to teaching, good Government, public and individual health, was a pioneer for much which has since been written. His visits abroad, both to collect information and to give it, were of lasting importance. His visit to Cairo was largely responsible for transforming the science facilities and teaching at the University (he was also responsible for quelling an anti-British student riot by threatening to cancel his lecture—such was the esteem in which he was held). Much the same may be said about his visit to India and later Malta where he was a member of the Royal University of Malta Commission. Certainly his liberal outlook was well ahead of his time.

It was always a great disappointment to Jack Brimble that he was not allowed to enter the Navy. He would dearly have liked to have served in submarines, but time after time he was rejected. Because he was unable to play an active part in the War, he made up for it by organizing the local fire-watch. It was for his generosity and cheerful personality in such trying times that he endeared himself to many in the London W.C.2 area and especially to the staff of Macmillans. He was appointed organizing warden of the local section. Besides the men who worked in the vicinity of his ward there were many

ladies (including some of renown). Although a general ruling had been given that men and women alike must undertake fire-watching, he broke this by never allowing the ladies to do so.

Jack Brimble quickly perceived that, because of the kind of gloom and misery that remained after the War, people were looking for something else. It was then that he strove forward with his *In Britain* series of books. He sensed that people were really recoiling from all the truth of war: he endeavoured to bring some light into life. He realized in 1944 that if we attained our objective in winning the Second World War, the Butler Education Act would need much more financial backing. He was instrumental in organizing and preparing leading articles which eventually led to the Government writing in an extra £8 million. Besides this, he did a great deal to direct the attention of men of science to the development of education during the Butler Act.

He had a deep respect for the dignity and integrity of the Press. With his usual enthusiasm he took it on himself to encourage the interpretation of science to the general public, and in many ways his own public lectures set the pace. A fund of anecdotes to suit any occasion (he never told 'jokes' in any of his public addresses, although he had, for private ears, an inexhaustible repertoire) ensured that his lectures were always lively: but there was a shrewd message in them, and he was never afraid to speak out against anything that he considered unprincipled. To him, an editor's ideas should not be opinionated, but reasoned and based on intuition instilled from experience.

Brimble always accepted the saying that 'a good editor wears out the soles of his shoes before the seat of his pants'. He developed through his visits a particular wealth of American and Australian friendships. He had perceived the great advances that American science was to make, and that to a large extent it would set the pace. He fruitfully strove to cultivate good relationships between the United Kingdom and the United States, and to steer them on a steady course. To do this, it had to be openly displayed that *Nature* made every effort to be dispassionate and objective; differences between race, colour, nationality, creed or political views were allowed no scope whatever.

Jack Brimble's health deteriorated in the late 'fifties, but he never outwardly allowed this to cloud his life. He suffered periodically from a wound received during the Blitz of London. But, by now, his dedication to the journal was so intense that he took little relaxation. Such dedication did not just stop at the material journal, but included all those associated with it, and it inspired a like dedication in all of them. No man could have treated his staff better than he did. Nothing was too much for him; if he had a problem he would play with it for hours, looking at it one way, then the other. He was a kindly, gentle human being, with great human sympathies which he did not parade overtly. To him the most important thing was 'people'. He compulsively involved himself with people, sharing their troubles and their elations.

At the end of 1961, A. J. V. Gale retired from the joint editorship, leaving Jack Brimble as sole editor. Even though the editorial staff had been depleted, he strove to increase the size of the journal. This meant more work for him, and typically he would say, "Oh well, have to make to-morrow a twenty-five hour day". But he had in very large measure the most vital attribute of a great editor—

an ability to select deftly (often with almost uncanny intuition) those items worthy of *Nature*. He never tired of discussing his plans and ambitions for the future, in which the tradition and the progress of *Nature* would be maintained without faltering.

In his inaugural address following his election to the Royal Society of Edinburgh in 1953—of which he was justly proud—he stated that no material for publication in *Nature* came from the U.S.S.R. and only a little from its satellites. The trend in the early 'sixties has been that material has flooded in for publication from the U.S.S.R. and its satellites. This was part and parcel of his deliberate policy to encourage the submission of material from the Eastern bloc. Similarly, he concentrated on the advancement of the emergent countries, offering all the encouragement and help possible in publication.

Jack Brimble lived long enough for the journal to reach its 5,000th issue on August 28, 1965. That he was not ostentatious and was still proud of his West Country lineage and accent was obvious to all who saw his B.B.C.2 interview at this time.

Jack Brimble's life, which came to a close on November 15, was dedicated to the editorship of *Nature*; but he will be remembered by those scientists who knew him as a man even more than as an editor. His immediate impact was one of outgoing gaiety and generosity of spirit. First there was a hearty greeting, puckishly provocative ("You don't seem to have published much lately!") and offered with a sincerity which made it evident that he revelled in talk and the company of friends. Then came the ebullient phase—his mind was always bubbling over with some question of the day—gossip about the scientific world—shrewd and always entirely free from malice—about who ought to get this chair or that; who ought to have his head examined; who ought to be put on this committee; what ought to be done to improve the universities or the Research Councils or the British Association. So then to the third phase: what should *Nature* say about this? Has *Nature* drawn attention forcibly enough to this or that? Just beneath the scientific-man-about-town was the keen editor, always on the alert for news, ideas, opinions; able even in his boisterous moments to catch hold of a point of view and to make sure (if he thought the view worth preserving) that it was included in that constant scanning of the scientific scene which was his life's work and which made *Nature* the most remarkable scientific journal of its class in the world.

He came to look on the journal as being his life and always insisted that it belonged to the world of science.

GRASSLAND AND FODDER IN INDIA

The Grassland and Fodder Resources of India by R. O. Whyte. Revised edition. (Scientific Monograph, No. 22.) Pp. x+553+58 plates. (New Delhi: Indian Council of Agricultural Research, 1964.) Rs. 20.00.

THIS revised edition of *The Grassland and Fodder Resources of India* follows that first printed in 1957 and deals exhaustively with fodder resource problems under Indian conditions. Recommendations are made on

a wide variety of production methods. The twenty chapters are devoted to fodder supplies in relation to the present-day needs of Indian livestock and to ways and means of attacking the almost insoluble problem of feeding so many cattle on too little grass and other feeds. Emphasis is on the need for better-quality fodders; those presently available are extremely poor by any standard. Plant breeding, herbage, seed production and an account

of Indian soils and their fertilizer requirements are dealt with in separate chapters. Problems of conservancy and the related ones of pasture management are considered fully, as also are details of the feeding régimes used on Government or corporation farms. These, however, are much above average and bear little relation to those used in the Indian villages. This is clearly reflected in the much higher levels of production attained on, say, the military farms of India. Although details of milk yields are not given, there is the clear suggestion that at their best, and when adequately fed, the Indian breeds of dairy cattle produce on a par with European cattle. Frequent mention is made of the potential output of a reshaped dairy industry in India, but it would have added to the value of the argument if precise figures of yield (per cow and per acre) could have been included.

Throughout the book is the continued theme which emphasizes the problem of fodder in India as one where the primary need is to reduce the number of cattle so as to bring it into line with fodder supplies (actual and potential). Cattle are the backbone of Indian agriculture, and efficiency must be raised if prosperity is to come to rural India. Clearly nutrition is the key, but at present the cattle are underfed and hence the rather distressing (but realistic) call for reducing their numbers. However, there are religious problems which ultimately can only be solved by the Indian people themselves and this depends on the education of the masses. It is part of the Hindu philosophy to prohibit the killing of cattle, however worthless and however competitive with the human for food. This point bears repetition, even though it has always been the major concern of agricultural developers in India and is frequently repeated by those from other countries who visit the sub-continent.

A plea is made for more research and also for making available nation-wide statistics of sufficient accuracy on which sound rural planning can be based. In India, man, cattle, sheep, pigs and poultry are all in competition for too little food. The farms are too small, and even if it were possible to intensify production the problem would still remain; namely, how the cultivator with less than five acres can grow bread, corn and fodders sufficient to provide for his family, let alone produce a surplus. Part of this problem concerns levels of crop yield; the average cereal and pulse yields in India are about 600 lb. and 400 lb. per acre respectively; whereas on the Government farms, including the experimental and seed farms, these figures are quadrupled. As with the animals, the key is partly nutritional; the usual story of a need for both organic and inorganic fertilizers, including, in some cases, trace elements. At the present level of yield in all crops, the supply of animal feed provides less than one-fourth of the protein and two-fifths of the energy requirements of the bovine population.

Natural grasslands are infrequent on the Indian scene, but man-made 'grassland' occupies extremely large areas and is basic for the nutrition of cattle and sheep. Many attempts have been made to classify these grazing lands and there is at present in progress the Grassland Survey of India, in which the author of the monograph has taken a prominent part. Descriptive notes concerning a number of contrasting sites dealt with in the survey are given in Chapter 7, while in the preceding chapter a valuable record is made of botanical features of grassland in several regions. Most of these 'grasslands' represent interim (seral) stages between forest and desert; few appear to be climax stages.

The book is well illustrated and has a useful bibliography. The glossary of Indian terms is indispensable for the reader outside India. The production is of reasonably good standard, but the text is not without its typographical errors. The volume should have the interest of a wide audience of pasture and animal scientists and is of particular importance to all who live in densely populated regions within the tropics.

WILLIAM DAVIES

"THAT OLD COMMON ARBITRATOR, TIME"

The Psychology of Time

By Prof. Paul Fraisse. Translated by Jennifer Leith. Pp. vi+343. (London: Eyre and Spottiswoode (Publishers), Ltd., 1964.) 30s. net.

THE author of this painstaking work on an important subject is professor of experimental psychology at the Sorbonne. He has contributed 27 papers on various aspects of time in the bibliography of this work, which is a valuable source book. Its very numerous references, some 550, have a French and German slant, and even though supposedly revised recently, several important works (for example, Bünning, Gooddy, Harker, Whitrow, and the 1960 symposia at Warm Springs and at Würzburg) are missing. The translation by Jennifer Leith seems too literal and American ("time-structured behaviors") for the subtlety of the ideas of the author.

The Psychology of Time is divided into three parts, each prefaced by a summary of the ideas of the next few chapters. The sections are on "Conditioning to Time", "Perception of Time" and "Control over Time". Prof. Fraisse believes that "under the influence of periodic changes, the organism becomes a physiological clock which provides cues for temporal orientation both in animals and man". It is this ability to reproduce regular sequences that permits us to appreciate durations. It is perhaps in this way that animals adapt to change through "temporally organised behaviour". The section on time perception reviews a great deal of work on the perception of duration, which is summed up as "a function of our attitude". The final section, on the "Control over Time", some 130 pages long, assesses the advantages of man over other animals in being able to consider changes outside the immediate present; though how animals achieve awareness of any form of time is uncertain. We are told: "Animals do not, however, refer explicitly to past events in their behaviour; nor do they have a purpose". We are all animals, and we ourselves may not know about the purposes except of our own species, since the concept of purpose can be discussed only in human language.

The author states: "We live in the present". His theme is to show man's superiority in being able to 'control' past and future time. He also states that periods in the recent past seem longer than similar periods years ago, a statement in great contrast with the almost universal feeling that the times (and spaces) of childhood were so much larger, when later reviewed, than the adult now remembers them to have been. We must disagree with the author here, for we live in the future. Every thought and action is a scheme for the future based on the data of memory, poised on the ever-vanishing springboard of the present. If we did live in the present, both past and future, immediate or remote, would always escape us, memory would serve no purpose, and prediction, our main advantage over other animals, would never be achieved. There could be no perception, no action, no sequence, no language, no process of living. The sign of life is, in Goldstein's fine phrase, an "attitude towards the possible". The present is an extrusion, even an excretion, of nervous activity; and we use it only to check our position on the chart of our world-lines.

If this book is lacking in lucidity and perhaps in clear aim, it may be that the author has attempted too formidable a task, in a field which is only now even beginning to be isolated and defined. He makes us aware of a new realm for scientific exploration, where the thinker and investigator must possess exceptional qualifications in several, at present separated, branches of science. To be successful in that synthesis of ideas expressed by the word 'time', the broad scientific education must be enhanced by an almost superhuman intelligence and technique. Present-day education, with its wasteful

separation into separate streams of sciences and arts, trains the thinker to suppose that precision of meaning and clarity of expression in understandable language are too trivial for the experimentalist to learn. Thus literary expression may falsely seem effete, while private scientific languages prevent the intrusion of the 'simple' man, who is our chief study, into the deliberate abstruseness of scientific study. If we are to advance into the study of the various aspects of human behaviour which are subsumed under the code-word, the algebraic symbol, 'time', we must have a method of defining our objects and explaining our methods of reaching them. 'Time' is of the common man, and must always be studied in direct relation to his form and to his language. W. GOODDY

DOCUMENTATION

Some Fundamentals of Information Retrieval

By John R. Sharp. Pp. 224. (London: Andre Deutsch, Ltd., 1965.) 35s. net.

THIS book, by a librarian, is chiefly concerned with the ways in which documents can be classified or indexed so that they may be stored. This, of course, is merely the reverse of the process by which they may be recovered for consultation. It does not deal at all with storage of fact or with deductions from stored facts, so that fashionable glances at symbolic logic are not very relevant.

Perhaps the chief value of *Some Fundamentals of Information Retrieval* to working scientists is that it serves a warning of what may be in store for them unless they take a more active interest in what the librarians are proposing on their behalf. For one thing, a nasty jargon is being developed and the book is fairly typical of the sort of prose that may be expected now that librarians have moved into the computer belt. A reviewer in *The Times Literary Supplement* has characterized Mr. Sharp's style as 'rebarbative'. Having consulted the dictionary, I would agree with the adjective, which is defined as: crabbed, unattractive, repellent. Librarians are seldom accorded the importance or the salary that the essential nature of their work in a scientific organization warrants possibly they are now having their revenge.

It is true that the classification and indexing of documents is the most important step if the information they contain is to be processed by computer so that it can be recovered. The book certainly gives a good idea of the difficulties involved in this process. Mr. Sharp, who was senior indexer on the Association of Scientific Libraries and Information Bureaux (ASLIB) Cranfield Research Project, has obviously given a good deal of thought to the matter, and his own particular system, Selective Listing in Combination (it has one of those chi-chi abbreviations SLIC), depends on the indexer selecting five key words from the title or content of the paper and feeding these into the computer in all possible combinations in alphabetical order. This system puts at a disadvantage those words that come late in the alphabet.

As for retrieval, there is only very cursory discussion of the various machine procedures, and one of the most fundamental aspects of the matter is not discussed at all—this is a question of cost in time, labour and machinery. The book makes it clear that the indexing job is a skilled one and the final chapter on auto-indexing and auto abstracting suggests that it would be a long time before the machine can cope with this task. As editors are often lax about ensuring fully informative titles to papers, the indexer may well have to read a paper to decide what information it contains and in what form it should be stored. Obviously a staff of indexers must be enormous to cope with all relevant scientific papers in any particular field—and is the exercise really worth while? To industrial and commercial scientists the answer may well be yes and it is to these that Mr. Sharp's suggestions may be

fruitful—and his examples are indeed chiefly drawn from aeronautics and textile technology. The problem is not so acute to the academic scientist, who can usually scan the relatively few top journals in his own subject and these contain most of the best work. If the sea of the world literature is to be dredged for all relevant papers, a great deal of rubbish will come to the surface—the indexer cannot be a judge as well. This sceptical view is, of course, an extreme one. But scientists should be made aware of the problems involved and be sure that present bibliographic aids (scarcely mentioned in this book) have failed before they allow librarians into the driving-seat of a computer.

P. C. WILLIAMS

ENGINEERING APPLICATIONS OF RUBBERS

Rubber in Engineering Practice

By A. B. Davey and A. R. Payne. Pp. x+501. (London: MacLaren and Sons, Ltd.; New York: Palmerton Publishing Co., Inc., 1964.) 90s.

PERHAPS the strongest impression gained on perusing *Rubber in Engineering Practice* is of the remarkable variety and range of what may be broadly called the 'engineering applications' of rubbers—a term which includes not only engineering in the narrower sense, typified by engine mountings, couplings for power transmission and spring suspensions, but also such widely diverse uses as sound insulation in buildings, anti-vibration mountings for instruments, and packaging devices in sponge or other form. All these applications are dealt with in considerable detail, and while, no doubt, some selection must have been unavoidable, it is difficult to believe that any really important application has been omitted. As a source of information on what devices are available, therefore, this book is likely to be eagerly sought by engineers interested in this general field.

The book, however, is far from being a mere compilation, for in addition to its clear presentation, with a very large number of detailed drawings, of the various engineering components utilizing rubber in one form or another, it includes a general scientific introduction to the physical (and where necessary chemical) properties of rubbers, and the way in which these are applied in the design of engineering components. Starting from the basic types of deformation, shear, torsion and compression, it shows how these may be applied, and in many cases combined, to give a wide variety of spring characteristics to meet the many types of complex loading encountered. In addition to this, the basic mechanics of suspension systems, of the transmission and absorption of vibrations, including resonance phenomena, and, in general, of the response of coupled systems to oscillatory disturbances, is discussed. Some of this material has already appeared in an earlier book on this subject (*Engineering Design with Rubber*, by Payne and Scott).

Not unnaturally, a large part of the book is taken up with engineering applications in the narrower sense, and in this Davey and Payne have drawn heavily on the industrial experience of particular firms who have specialized in the production of engineering components. Even so, the operation of the many components depicted—some of remarkable ingenuity and subtlety of design—is fully discussed in the light of the scientific principles expounded in the first section, and good and bad features of design are brought out.

While rubber components such as shock absorbers and vibration insulators, particularly in connexion with transport vehicles, have been with us for a long time, the growing field of application in civil engineering is of more recent development. Surprising as it may appear at first sight, the use of rubber as a support for bridges is an example of an application in which this material has a clear technical advantage over alternative systems.

It is interesting to learn that such bridges as the Tancarville bridge across the Seine (960 m) and the St. Lawrence Harbour bridge, Canada (3 km), are supported on rubber, and it is stated that not one of the many natural rubber bridge bearings which have been installed during the past 10 years, so far as is known, has failed or shown signs of failure.

The least satisfying part of the book is probably the introductory part, in which the physical properties of rubbers are discussed. The impression is given that too much ground is being covered, and the presentation is not always as clearly thought out, or as precise, as might be desired. For example, Fig. 3.2 gives the erroneous impression that the stress-strain relations for rubber are linear for compressions up to 40 per cent, and for extensions up to 25 per cent (though the text makes it clear that this is not so). Again, there is confusion over the stress-strain relations derived from the statistical theory; Kuhn did not derive equation (3.2), and in the reference to his paper dealing with the properties of a bulk rubber (dated 1936, not 1934) it is not true to say that he considered only a single molecule; though not explicitly defining a cross-linked network of molecules, he adopted a simplified model in which one-third of the molecules were oriented parallel to each of the three co-ordinate axes, which, as has since been shown, is basically equivalent to the more general network treatment. Readers who are concerned with the basic physical properties of rubbers will need to supplement this account with additional reading.

Apart from this, the book succeeds in its purpose of presenting a broad general survey of the whole subject of the engineering aspects of rubbers, and can be confidently recommended.

L. R. G. TRELOAR

FAST BIOCHEMICAL REACTIONS

Rapid Mixing and Sampling Techniques in Biochemistry

Proceedings of the First International Colloquium on Rapid Mixing and Sampling Techniques Applicable to the Study of Biochemical Reactions, Philadelphia, July 23–24, 1964. Edited by Britton Chance, Rudolf H. Eisenhardt, Quentin H. Gibson and K. Karl Longberg-Holm. Pp. xii+400. (New York and London: Academic Press, 1964.) 72s.

PHYSICAL chemists have long been interested in rapid reactions, and E. F. Caldin has recently written a book concerned with their study in solution¹. Biochemists who work with bacteria also know that these cells catalyse quite fast reactions and that Macbeth might well have been making reference to experiments with such systems when he said: "If it were done when 'tis done, then 'twere well it were done quickly"; for if it is desired to show that, for example, ¹⁴C-acetate is converted initially into malate by a growing culture of *Pseudomonas* then all the samples should be taken within the first half-minute of the start of the experiment². One of the contributors to this symposium is J. A. Bassham, whose work with M. Calvin and others revealed the pathway of carbon in photosynthesis by a technique that involved rapid sampling of alga cultures; and with M. Kirk this author describes an application of this technique to follow changes in the photosynthetic carbon reduction cycle that occur when inhibitors are added. However, most of the other authors deal with methods more suited to the study of rapid reactions catalysed by enzymes in isolation, or to the rapid arrest of metabolism in muscle or liver. In general the biochemical results obtained are not discussed: the emphasis throughout is placed on the design of the apparatus used. Theoretical principles involved in the construction of apparatus are given in appendixes: thus, H. K. Wiskind gives examples of the application of fluid dynamics to the development of rapid mixing techniques, since one problem in the study of rapidly reacting compounds is clearly that of mixing them fast enough.

The typescripts of contributors to this book are reproduced exactly as received, and at the end of each the discussion provoked by the contribution is recorded verbatim. This procedure is now familiar; and as one who usually fails to start his newspaper at the point the editor considers to be most important, namely at the headlines, I usually read the discussions of such symposia first. Sometimes they instruct and entertain, but occasionally they do neither, as when the dialogue takes the form: "Doctor A: Did you try 'Versene'? Doctor B: No". In this particular symposium, dealing as it does largely with the design and use of apparatus, the discussions have maximum value because few experiences sharpen a scientist's critical powers more keenly than those entailed by the use of an imperfect machine. In the discussion on p. 116, Q. Gibson shows impressive graphs for apparent changes in concentration of a reactant during a reaction time of 0.7 sec; however, the stopped flow apparatus he used contained only distilled water and the measured changes in extinction were due simply to temperature gradients. Such discussions of artefacts and limitations of apparatus add to the value of this book, but there is a lighter side to some of the discussions as the following excerpt shows, taken from p. 269. "Chance: Oh, I thought our chairman would have been content to let sleeping sarcosomes lie . . . Davies: It is not the sarcosomes that lie."

The symposium is divided into two main sections: the first, rapid flow methods, and the second, rapid stopping and sampling techniques. There is a charming introduction to the first section in which F. J. W. Roughton traces the origins of rapid flow methods, and this is followed by four papers on rapid mixing and fluid flow, another four concerned with injection into a fixed volume, two on stopped flow and three on continuous flow methods. The last four papers of the first section relate to non-equilibrium situations created by exposure to a jump in temperature or to a flash of light, including photolysis due to a laser beam. The second section contains fourteen papers dealing with such methods as rapid freezing for interrupting muscular contraction and for metabolite assay in frozen samples of liver; and various pieces of apparatus are described by which it is possible to sample rapidly various systems ranging from yeast cells which are in the process of assimilating phosphate to mitochondrial suspensions metabolizing adenine nucleotides.

All biochemists faced with problems arising from the difficulties of measuring rapidly changing concentrations of metabolites will find sections of interest in this unique collection of papers by acknowledged experts in the field.

S. DAGLEY

¹ Caldin, E. F., *Fast Reactions in Solution* (John Wiley and Sons, New York and London, 1964).

² *Macbeth*, Act 1, Scene vii.

³ Kornberg, H. L., *Biochem. J.*, **68**, 535 (1958).

A WELL-BALANCED ACCOUNT OF THE ALGAE

The Biology of the Algae

By Dr. F. E. Round. Pp. vii+269. (London: Edward Arnold (Publishers), Ltd., 1965.) 36s. net.

ALL workers on algae, whether phycologists or workers in other disciplines using algae as tools, will undoubtedly be pleased to see an up-to-date text-book on the algae in the English language, particularly when so much information is condensed to give a well-balanced account in such a small volume.

The book contains 13 chapters, copious references and an adequate index. The first two chapters survey morphology, reproduction and life-history and include 10 pages of clear line drawings (an average of 21 drawings to a page) which will help students to visualize the overall picture of the form range in all the algal classes except the Rhaphidophyceae. This is a most valuable contribu-

tion, since only too frequently quite a high proportion of the algal classes are completely ignored for teaching purposes and students are left with the impression that there are only four or five classes of algae. Chapter 3 deals with cytology and genetics including especially the results of the more recent work on ultrastructure; five plates, in addition to line drawings, illustrate this section. The coverage, in the following two chapters, of algal ecology, both freshwater and marine, is well balanced and these sections include, in an excellently condensed form, a great wealth of information.

Physiology is considered at length in Chapters 6-10. The section on culture and nutrition deals with both major and minor elements and organic factors, while the following chapter, on energy sources and pigments, includes up-to-date information on the distribution of pigments in the algal classes. Chapter 8, entitled "Energy Relationships", gives a clear account of recent information on this subject. In Chapters 9 and 10 four subjects—movement, rhythm, polarity and morphogenesis—are adequately covered.

Chapter 11, on fossil algae and the deposition of calcium carbonate and silica, outlines the part that algae have played in geological history. Important data have been admirably summarized in the next chapter on the economic aspects. Here, the importance of algae in everyday life is emphasized by the inclusion of information on the use of algae as food, in agriculture, industry, medicine, sewage disposal, water purification, pollution and land reclamation.

In Chapter 13 the author has endeavoured to present clearly a most difficult subject which is still in a state of flux—algal taxonomy. This chapter has undoubtedly been recast to embody new information on certain classes and therefore there is some lack of agreement between the system of classification presented in Chapter 13 and that used throughout the rest of the text. For example, on p. 9 the class Dinophyceae includes the genera *Exuviaella* and *Prorocentrum*, whereas in the taxonomy of the author follows Fott and puts them in a separate class (p. 239), the Desmophyceae. Four points in this section should be noted: (a) the ending for a sub-division—phytina should be included in the list of endings (p. 227), as sub-divisions are used afterwards (pp. 235, 238), (b) the ending for a sub-order should be -ineae, not -inales (p. 227); (c) under Eucaryota seven phyla and one class are indicated (p. 230), but only seven phyla are dealt with, so presumably the Rhaphidophyceae (chloromonadines) must be the class lost by the wayside; (d) in the division Chrysophyta the first paragraph (p. 231) has not been revised to cover the modern concept of this division, since the author speaks of three classes whereas in the following pages he includes four classes in this division.

In spite of some minor defects this book will be most helpful both to phycologists and to workers in other fields. Its users will appreciate the enormous amount of information on methods given in the various sections; they will also find many suggestions for research problems incorporated, since the author has also indicated very clearly the gaps in our knowledge of the algae. Some misprints occur, mainly among the generic and specific names, but these are not serious. There are a few mis-statements, but these are not likely to mislead. For example, certain references to Fig. 14 on pp. 46-47 should read Pl. 4B and on p. 138 the author speaks of *Ilea* and *Actinococcus* being present around the St. Lawrence and the Bay of Fundy but being absent from the British coast. The *Ilea* = *Petalonia fascia* and *P. zosterifolia* and the *Actinococcus* = the nemathecium of *Phyllophora brodiaei*, all of which are recorded for the British coast.

This book is exceptionally good value for money, the illustrations are attractive and instructive, and Dr. Round is to be sincerely congratulated on the production of his book which satisfies a long-felt need.

MARY PARKE
G. T. BOALCH

DRUG-DEPENDENCE

By THE RIGHT HON. LORD BRAIN, F.R.S.*

WHEN I chose the present subject, I hoped that before I presented it the new report of the Inter-departmental Committee on Drug Addiction would have been published. However, it is still in the printer's hands, so I can say nothing about that now. When the report appears, however, as I hope it will soon, I believe it will be self-explanatory and call for no comments from me. The report of the earlier Committee, of which also I was chairman, raised a general question which seems to me important enough to call for further discussion. I can best explain this by quoting from the report. We said: "Most of our witnesses affirmed that, to-day, drugs acting on the central nervous system are being used excessively, but they were unable to furnish records in support of this contention". We obtained some information about the quantity of barbiturates prescribed annually in England and Wales over the previous two years and said: "It is obvious that usage has expanded both progressively and substantially so that, in 1959, it was almost twice what it was in 1951". We also stated that analysis of National Health prescriptions showed that "barbiturates, other sedatives and hypnotics, together with analgesics and antipyretics (excluding dangerous drugs), account for no less than about 19 per cent of all the prescriptions issued". There was also reason to believe that the prescription of tranquillizers had also increased, and we noted that the amount spent on one particular tranquillizer by nine selected mental hospitals had increased ten-fold over a course of five years. After mentioning the dangers of addiction to amphetamine and phenmetrazine, we noted that an analysis of some 214 million National Health Service prescriptions in 1959 indicated that some 5,600,000 or approximately 2.5 per cent were for preparations of the amphetamines and phenmetrazines.

Reviewing these facts, we made the following observations. "To explain this trend in medication directed at the central nervous system, we have found no single answer. In part it must be due to the vigorous advertising of these drugs and their preparations by the pharmaceutical industry, both to the medical profession and to the public. To some extent the accelerated tempo and heightened anxieties of modern life have been held to blame; but this is an assertion based on assumption more than fact. Thirdly, and possibly of considerable consequence, there is the materialistic attitude adopted nowadays to therapeutics in general. This is one feature of an age which owes so much to science. For every deviation from health, great or small, a specific, chemical corrective is sought and, if possible, applied, and it is also widely believed that health may be positively enhanced by the use of drugs. When dealing with mental disease, psychotherapy may still be invoked. Often, however, a prescription is given for a drug when the patient's real need is a discussion of his psychological difficulties with the doctor. An obvious danger arises when the drugs so employed, far from being placebos, are undeniably potent, frequently toxic, and sometimes habit-forming as well. On the other hand, the newer drugs are proving of great value in psychiatry where they are to some extent replacing other methods of treatment.

This increasing use of sedatives, stimulants and tranquillizers raises issues on which we do not as a Committee feel confident to pronounce. In particular, it is not for us to decide whether their occasional or even regular use is justified if it enables that person to lead a happier and more useful life. In any case, if resort to potentially habit-forming drugs is sometimes to be regarded as a symptom of psychological maladjustment, it should be treated as a symptom, and its cause sought, perhaps as much in social conditions as in the mind of the individual. These are questions which should be considered not only by doctors, but by all concerned with social welfare."

These are certainly difficult questions, and I do not propose to try to answer them all now. What I shall try to do instead is to clarify them, to look at their implications, and to suggest ways in which we may hope to contribute towards answering them. Basic to the whole problem are the meaning and implications of drug-dependence.

This term was introduced by the World Health Organization Expert Committee on Addiction-producing Drugs in their thirteenth report, published in 1964. In the fourth section, with the significant title "Terminology in Regard to Drug Abuse", it directs attention to some confusion which had arisen in the use of the term 'drug addiction' and 'drug habituation'. Because, it said, "the list of drugs abused increased in number and diversity" it sought a term which could be applied to drug abuse generally, and since "the component in common appears to be dependence, whether psychic or physical or both", it adopted the term "drug dependence", which it defined as "a state arising from repeated administration of a drug, often on a periodic or continuous basis. . . . Its characteristics will vary with the agent involved and this must be made clear by designating the particular type of drug-dependence in each specific case—for example, drug-dependence of morphine-type, of cocaine-type, of cannabis-type, of barbiturate-type, of amphetamine-type, etc.". In using the term 'drug dependence', and relating it to the abuse of drugs, it may seem that the World Health Organization Committee was only emphasizing the obvious; but my object is to suggest that the term 'drug abuse' itself raises some important questions which are not only unanswered but which we have not at present the knowledge to answer.

At least one valuable thing has emerged from the philosophy of the present century—its emphasis on the importance of words. So let us begin by asking what the word 'drug' means, and why it means what it does. *Webster's Dictionary* defines 'drug' as "any substance used as a medicine, or in the composition of medicine, for internal or external use", and then it goes on, "whether or not a given substance should be included under the term drug depends upon the purpose for which it is sold (as regards the seller) or used (as regards the purchaser)". So it would seem that the same substance may sometimes be a drug and sometimes not. When we get to the verb 'to drug' a new meaning creeps in, for Webster gives three definitions: "(1) to affect or season with drugs or ingredients; especially to stupefy by a narcotic drug; (2) to tincture with something offensive or injurious; (3) to dose to excess with or as with drugs". So a sinister

* Substance of the Address at the Inaugural Ceremony of the School of Pharmacy, University of London, delivered on October 13

note has already appeared: though we begin with drugs as any ingredients of a medicine, the average person hearing that someone has been 'drugged' would not imagine that he had been given penicillin. This is sometimes reflected in what patients will say to a doctor, for example: "I don't mind taking medicine, but I hope you won't put me on any drugs, doctor!" Or: "I hope that this is not a habit-forming drug". So far as the public Press is concerned, if you see the word 'drugs' in a headline you may be fairly sure that what follows refers to some sinister aspect of pharmacy, either 'drug-addiction' or the supposed danger of taking some particular drug or group of drugs. In the annual report of the Chief Medical Officer of the Ministry of Health, though there is a chapter called "Therapeutic Agents: Control and Toxicology", when reference is made to the possible dangers of therapeutic agents the word 'drugs' appears, and the relevant committee is called not a Committee on the Safety of Therapeutic Agents, but a Committee on the Safety of Drugs.

This pejorative meaning of the word 'drug' is relevant to this article, for the subject of drug-dependence is a highly emotional one. 'Drug addiction' hits the headlines frequently in the Press, and is the subject of plays and broadcasts. As a social problem, it excites an interest out of all proportion to its magnitude. In Great Britain, the total number of addicts to heroin, cocaine and morphine is well short of a thousand, and we are fortunate compared with Canada, where there are at least four thousand, and the United States where there are a great many more. But the number of men and women who will die of lung cancer this year in Britain is 25,000. Most of these are men in middle life. Their sufferings, the distress of their families and the loss to the community are collectively enormously greater than the effects of what is normally called 'drug addiction', yet these deaths excite no similar emotional interest, although they, too, are the indirect result of a form of drug addiction, addiction to cigarettes.

I am not a psychologist, and perhaps the psychologists can tell us why drug addicts to morphine, heroin and cocaine arouse such a disproportionate amount of interest; even without going very deeply into the matter, however, one may surmise that it is partly because these drugs are potent, mysterious and potentially dangerous: and one of their most mysterious characteristics is their power of temporarily or permanently changing the personality. Add to that the association of 'drug addiction' with crime and violence and we reach the conception of "potions . . . drunk of Siren tears, distill'd from limbeck's foul as hell within".

This is no exaggeration. The drug addict in this narrower sense is indeed a pathetic figure and a potentially dangerous one, too, if he or she becomes a source of infection to others. But, as the World Health Organization Committee recognizes, these drug addicts, though creating special problems, are only part of the broader spectrum of drug dependence.

It is obvious that there is nothing wrong with drug dependence as such. If a diabetic requires regular dosage of a drug to maintain his blood sugar at a normal level, or if a patient suffering from a collagen disease requires regular amounts of steroids, such patients are drug-dependent as already defined, their dependence being a physical one. But no one would describe this kind of drug-dependence as drug abuse: exactly the contrary. So it would seem that something more than mere drug-dependence is necessary to create drug-abuse, and if we look at the list of such drugs of both abuse and dependence which appears in the report of the World Health Organization Committee, I think the basis of the distinction becomes clear, for they are all drugs which are taken for their psychological effects. This brings us to the next stage of our enquiry, which is the crucial one. Is dependence on a drug which is taken for its psychological effects

necessarily an abuse of that drug? Few people would, I suppose, quarrel with the view that this is true of morphine, heroin and cocaine, though someone recently wrote to the Press and complained that, since he found it necessary to take heroin, he did not see why his freedom to do so should be interfered with. But there is rather less unanimity about cannabis, and where do alcohol and tobacco stand? (I use the term tobacco here comprehensively to include whatever its pharmacologically potent constituents may be.)

Tobacco and alcohol may both be drugs of dependence. Their action is pharmacological, and in extreme cases the addict attempting to give them up suffers deprivation symptoms, which may be so severe that he is unable to do without them. The fact that they are both drugs which you prescribe and obtain for yourself, instead of through a doctor, and that there are social aspects of their use, is irrelevant to their pharmacological action. As in the case of other drugs of dependence, people vary very greatly in their liability to become addicted, and serious dependence is much more common in the case of tobacco than alcohol. I know of no evidence that tobacco-dependence has any bad psychological effects; but it undoubtedly may have bad physical effects, and it is here that the dependence becomes important, because it may make it extremely difficult for the addict to give up smoking. Alcohol-dependence, in its extreme forms, is recognized to be a manifestation of a psychological illness and calls for treatment accordingly, but as in Britain that cannot be carried out without the willing co-operation of the patient, which is usually difficult to obtain, the serious alcohol addict is a difficult medical and social problem.

I have mentioned both tobacco and alcohol addiction not only because of their intrinsic importance, though that is considerable, but in order to bring out a point of more general relevance. The World Health Organization Committee points out that the characteristic feature of drug-dependence is the strong desire or need to continue taking the drug. Leaving on one side the chronic alcoholic, using that term to describe people whose consumption of alcohol leads to such psychological or physical ill-effects that they are in need of medical treatment there are many others who habitually take alcohol in more moderate amounts and claim that they feel and are the better for it, and miss it, if for some reason they are unable to get it. They would agree with a patient of mine who once said: "Alcohol has been a very good friend to me". This is a point to which shall return.

What are we to think of this steadily rising consumption of barbiturates and tranquillizers? Before we can answer this question, which is a very complex one, we need a great deal of information which is not at present available. As the first Drug Addiction Committee said in its report: "It is clear that there is scope and a need for operation research into the prescribing pattern in this country with particular reference to habit-forming drugs". We need to know first how many patients are receiving prescriptions for barbiturates, and what is the average amount prescribed for these patients per annum. Then we must know what proportion of them are receiving barbiturates for treatment of epilepsy. An epileptic patient on barbiturates is drug-dependent because he cannot give up the drugs without serious ill-effects, but this type of drug-dependence is a use and not an abuse of the drug. Having eliminated those prescriptions, the next thing we need to know is what proportion of the tablets prescribed the patient actually consumes. Doctors, I am sure, would over-estimate their patients' faith in their prescriptions if they supposed that the tablets they prescribe are regularly and faithfully consumed by every patient. So we need to know from an appropriate sample of patients what proportion of the barbiturates prescribed for them they actually take.

and if they do not take them all, what do they do with the rest? This is a question with several practical implications. Are the superfluous tablets locked up, or thrown away, or left about, where perhaps a child can find them? Moreover, if the patient puts them safely away, does he himself know what they are when he turns them out again in a year's time? It has sometimes been suggested that the prescription of excessive amounts of barbiturates by some doctors may be a source of such drugs for the black market. In any event, I think it is clear that prescription figures are likely to be a very misleading guide to actual consumption.

Nevertheless, let us assume—and experience shows that this is a reasonable assumption—that there are patients who are drug-dependent on barbiturates, not in the sense that they are in a state of chronic barbiturate intoxication, but that they are more or less regular consumers of barbiturates, either as hypnotics or as general sedatives to blunt the sense of being stretched on 'the rack of this tough life'. If this is drug-dependence, is it a use or an abuse of the drug? In the passage I quoted from the first report of the Drug Addiction Committee, it will be remembered, occur the words "often, however, a prescription is given for a drug when the patient's real need is a discussion of his psychological difficulties with the doctor". No doubt, this is sometimes true, but we have to remember that the average doctor to-day is much too busy to be able to spare the time for that kind of psychotherapy except on rare occasions, and though it would undoubtedly sometimes be helpful, many people would doubt whether psychotherapy, even if it were available, is the most suitable form of treatment for many such patients. Underlying a critical attitude to the regular prescription of barbiturates or other sedatives for the purpose I have mentioned, I think I can detect a hidden assumption. Let us look for a moment at another instance, namely, the administration of tranquillizers, which amounts to drug-dependence, but is nevertheless universally regarded as a use and not an abuse: I refer to the modern pharmacological treatment of schizophrenia. The regular administration of chlorpromazine and similar drugs to schizophrenics has enabled many of them to leave a mental hospital and live in the community so long as they take the drug regularly. Comparable beneficial results have been achieved by the treatment of patients suffering from depression by the antidepressant drugs. The authors of a recent monograph on the subject summarized the results of these forms of treatment as follows: "In general, these psychopharmacological agents have facilitated the control of mood and behavioural disturbances. In the mental hospital setting, they have induced a more quiet and more orderly atmosphere. They have enabled the discharge of an increasing number of patients and the return of many, previously resistant to treatment, to their appropriate places in society. In addition, they have facilitated ambulatory and out-patient treatment and have lessened the need for shock therapy". Here, then, is a beneficent form of drug-dependence in the case of patients suffering from major psychological disorders. Why, then, or in what circumstances, should we question the use of similar drugs in the doctor's consulting room for the treatment of patients suffering from minor psychological disorders? Is it perhaps because we are inclined to draw a line between gross psychiatric illnesses, for which we feel the patient cannot in any way be held responsible, and which are due to the operation of unknown physical factors, which may turn out to be biochemical, and the minor psychological disorders for which we do not postulate any physical cause but tend to attribute to psychological causes, and to regard as reactions to stresses and strains to which, perhaps we think, the fortunate majority of us are too tough to succumb. We recognize that it is of no use to admonish the schizophrenic as a general rule, but would not many of our neurotic patients, we wonder, be better as a result

of some effort on their own part? This, of course, raises a difficult philosophical question involving the relationship between the mind and the brain. I shall not discuss this further now, but I doubt whether it is philosophically sound to make any such distinction between the major and the minor psychological disorders. We cannot interpret schizophrenia or depression purely in psychological terms, nor, for that matter, can we at present give a physiological explanation for them. It often appears easier to give a psychological explanation of the minor neurotic disorders from which many people suffer; but that does not exclude the probability that they, too, have physiological explanations which we shall one day discover. The mind and body constitute a complex unity, and when we are dealing with psychological disorders, we always need to take account of both psychological and physiological interpretations so far as we can achieve them. It may well be that for the kind of reasons I have already mentioned some patients receive prescriptions for sedatives indiscriminately; but that is a long way from saying their continuing use involves a form of drug-dependence which is an abuse. I quote once more from the report of the first Drug Addiction Committee: "it is not for us to decide whether their occasional or even regular use is justified if it enables a person to lead a happier and more useful life". If you are among those who regard alcohol as a friend, you may also think the same of the regular use of sedatives in suitable cases.

But, in my view, the real limitation to the best possible use of such drugs springs from our ignorance. Let me end by looking into the future. I foresee a day when we shall understand much more than we do now about the relationship between the brain and the mind. On one hand we shall have methods of making accurate psychological assessments of the personality, very possibly in terms of factors which find no place in our present psychological vocabulary. We shall then be able to interpret psychological disorders in terms of these functions, their mutual interplay, and their reactions to our experiences. Parallel with these developments, psychopharmacology will have been placed on a rational instead of an empirical basis. We shall think in terms of the normal biochemistry of nerve cells and synapses, their groupings and interactions, their disorders and the effect of drugs on them. As a result, correlating in this way psychology, physiology, biochemistry and pharmacology, we shall have not only a more comprehensive armamentarium of drugs, but much more precision and individualization in their use. All we shall need then will be enough doctors with time to use them. But it would be wrong to leave the matter there. If mind and body are a unity we must not concentrate on the physical treatment of stress to the exclusion of its psychological aspects. Here, however, we need to look beyond individual psychotherapy and take a broader view. As the first Drug Addiction Committee said in its report, the cause of psychological maladjustment should be sought perhaps as much in social conditions as in the mind of the individual. So far as we know, life has always been stressful, and looking back over history and prehistory, it becomes clear that, although we have our own peculiar stresses, we have eliminated a great many which our ancestors had to put up with. Indeed, stress seems inherent in the evolutionary principle. There will always be some who find life too much for them, and they will continue to need help from pharmacology and supportive psychotherapy. We are all aware of the challenge to international and social organization which some of our major current stresses present. Beyond that, there is still much to be said for the ideal of fighting our own battles if we can without the aid of pharmacy, but with the support of some philosophy which gives a meaning to life, and from which we can draw strength and support.

¹ Benson, W. M., and Schiele, B. C., *Tranquillizing and Antidepressive Drugs* (C. C. Thomas, Springfield, Illinois, 1962)

RESEARCH AND MANAGEMENT IN EAST AFRICAN WILDLIFE

By DR. JOHN MORTON BOYD*

Nature Conservancy, 12 Hope Terrace, Edinburgh

THE problems of management of national parks, game and forest reserves and pastoral lands have much in common, and there is an increasing awareness that many forms of use of land in these fields are wasteful, non-conservative and positively destructive. Almost without exception this applies to the pastoral and game lands of East Africa, involving so vast an area and so wide a range of problems, many of which are political and social as well as scientific, that the mind shirks the idea of reform. The solutions lie in wise management of the land, 'wise' meaning informed by long experience or research. There is thus established a close link between research and management in the planning of large natural areas. The ideal blueprint would appear to be a two-tier organization with a superstructure of management supported by a basement of research. Whether it is possible to achieve such an ideal in the national parks and game reserves of East Africa is doubtful, though it is already well established in forestry, and there is a semblance of such an arrangement in the Queen Elizabeth and Serengeti National Parks.

The Research Effort

The research being brought to bear on management in game, forestry and cattle falls far short of what is required, that on game lagging well behind the others. The national parks and game departments from time to time acquire the services of one or two biologists to undertake specific research projects (not all closely geared to management); with the exception of the very small and inadequate Fauna Research Section of the Kenya Game Department (FRSKGD), none has established a research division likely to cope with the future advisory needs of parks and reserves. The Tanzanian National Parks have set up the Serengeti Research Project (SRP) based on foreign aid; this may later develop into a permanent research division. The East African Common Services Organization (EACSO) provides advice on aspects of forestry and animal husbandry through the East African Agriculture and Forestry Research Organization (EAAFRO) and the East African Veterinary Research Organization (EAVRO); but the proposed Wildlife Research Division of EAAFRO is not yet in being. University departments maintain an independent approach to wildlife research: the work on mammalian ecology being done in the Department of Zoology, University College, Nairobi, includes ecology of the alpine zone of Mount Kenya, ecology of small mammals, biology and ecology of the rock hyrax, behaviour of wild ungulates, feeding habits of wild ungulates, temperature regulation and water balance in wild ungulates. All these investigations can be vaguely related to managerial problems in the national parks and game reserves, yet none is so orientated and will necessarily assist conservation. The Nuffield Unit of Tropical Animal Ecology (NUTAE), based in the Queen Elizabeth Park, is a research consortium between Cambridge and the East African university colleges; the work there concerns populations of large game species and their pastures within the managerial units of the Queen Elizabeth and Murchison Falls Parks.

* Nuffield Travelling Fellow, 1964-65.

Visiting research fellows are normally associated with one of the research institutions and usually their work has managerial overtones.

Terms of Reference

Terms of reference depend to a great extent on the way in which research workers are recruited. For the suitably qualified person the opportunities are almost unlimited, and it is a matter of making the necessary influential contacts and of choosing a project which will win financial support. This has resulted in a great deal of free-lancing by those intent on 'pure' rather than 'applied' work. With the exception of the Canadian biologists with the Uganda Game Department and those concerned with the immobilization techniques for big game, few have come forward to undertake projects in actual wildlife management. This dearth of applied workers probably stems from there being some grant-giving bodies strictly devoted to pure science, from grant-giving committees and the corps of research workers being drawn from the ranks of pure scientists, and from applied projects being usually more difficult to conceive and plan and requiring more money and time than pure ones. The situation has not developed into a 'free-for-all', however, and this has been largely due to the channeling of funds through the research institutes.

The Nuffield Unit of Tropical Animal Ecology, the Serengeti Research Project and the Fauna Research Section of the Kenya Game Department have been established in the field of wildlife research. The NUTAE terms of reference do not provide a linkage between research and management, while SRP and FRSKGD have as yet no terms of reference. Nevertheless, both NUTAE and SRP are situated in national parks, investigating populations of game which are subjects of management. The fact that the NUTAE programme began with the hippopotamus problem in the Queen Elizabeth Park and SRP with the game-migration/park boundary problem in the Serengeti leads one to believe that the intention of both was to assist in the solution of these problems. These research units fall between the stools of applied and pure research, and this has given rise to widely divergent views on what part they should play in providing a research arm to the national parks and game departments. Routines such as game-counts and cropping are, in the early stages of survey, matters of research; later, when the research has been completed, they become matters of management. This shift of routines from 'research' to 'management' has further confused the role of the research units, but clarification has lately been achieved at the Queen Elizabeth Park, where hippopotamus-counting and hippopotamus-cropping have been handed over by NUTAE (as expired research routines) to the park warden and his staff. Nevertheless, although NUTAE and SRP have as yet no planned connexion with management, they have provided advice for the management of the hippopotamus and the drawing of boundaries, respectively.

A trend is noticeable which may destroy the developing two-tier organization; this is the raising of investigations

from the simple to the complex, transforming what was originally immediately useful applied research into longer-term pure research. The irresistible desire of the research worker to probe deeper and deeper into his problem is the driving force to scientific progress and should not be blighted; but care must be taken that it does not violate the terms of reference of applied research. If no terms of reference are available, this trend to fundamentalism will go unchecked and research may become progressively detached from its managerial context; the terms of NUTAE are formulated to permit such trends in present research. The raising of what were originally ecological investigations of vegetation and game in fairly simple quantitative and qualitative terms to investigations of primary and secondary biological productivity could be one such trend. Measurements of this complex quantum may come to influence use of land in the long term; but in the meantime the pressing problems of the present and nearer future require to be tackled on simpler lines.

A much greater volume of applied research must be carried out with precise terms of reference stating clearly the limits to which the work can be taken and emphasizing that its object is application to wildlife management.

Impact of Research on Management

Park and game wardens often state that their managerial problems require research. This may be true; but it is questionable if the scientist can, in fact, provide the answers to them. The hippopotamus-cropping in Queen Elizabeth Park is an example of the scientist providing the answers. How far have these been applicable? In a classical study, R. M. Laws has described the population of hippopotami inhabiting the Queen Elizabeth Park, stating its size, spatial distribution, age structure, social structure, behaviour, reproductive rate and so on. Logically there should follow a detailed plan for the culling of the population showing the proportions of each sex and each age-group of each sex which should be taken. Yet there is little use of going to all this trouble, for the practical difficulties faced by management staff in recognizing the sexes and age groups in the water are almost insuperable. What, in fact, is resulting from this detailed investigation is a non-selective shooting of large hippopotami as they come into the sights of the park warden's rifle, with conservative culling levels set by the total numbers occupying the cropping sites. The links between this pure research and management of hippopotami are strong; but the managerial profit from it is disproportionately small. The same can be said for the plans produced from the cropping of elephant in the Tsavo Park in Kenya. Aerial counts of the elephant population were followed by J. Glover's statistical analysis of population size, age classes and class trends and schedule for the progressive reduction of numbers over ten years with minimum killing and damage to herd structure; but implementation proved unworkable.

Very often the rate of change of many of the forest and game habitats of East Africa outpaces the conservation machine set up to cope with it. One need only see the regrowth of the woody vegetation in the Ankole/Masaka Ranching Scheme, or the devastation by elephants to the savanna woodlands in the Tsavo Park, to appreciate this. Ecological research is a slow process more able to cope with relatively slow changes such as those caused by climatic variation, burning of vegetation or abstraction of water, the effects of which usually occur gradually over many years. Technical research in the wildlife field, on the other hand, can make rapid advances; for example, the development of capture techniques for large game, the prediction of live-weight of game animals from the weight of the dissected back leg, the elimination of the

tsetse fly, *Glossina morsitans*, with dieldrin and so on. But most of the management problems in East Africa are not simply scientific, they usually have political implications.

The findings of applied research take time to reach the ground for which they are intended. As they affect East African wildlife they require to be considered against a background of a sluggish system of consultations with senior advisers, usually from Europe or the United States, and committees in which financial and political considerations are dominant. Thus the impact of the research becomes progressively eroded: seldom can clear-cut scientific recommendations be applied, and the scale to which they are disregarded is of great importance to the morale of research workers. The resettlement scheme in the Serengeti provides an excellent example of a game-management problem in which the adjustment of boundary ecology is recommended as a result of applied research. The problem is, however, also a complex political and social one, and it remains to be seen whether the *raison d'être* of the research is not lost in political compromise between the interests of the Serengeti National Park and those of the neighbouring people.

To sum up, the impact which wildlife research in East Africa has on the management of the national parks and game reserves is greatly reduced by the following main factors: (1) much of the ecological research is 'pure', with no direct application to management; (2) research which started as 'applied' may later become more fundamental and 'pure'; (3) the recommendations which follow research may be impracticable on the ground; (4) the recommendations which follow research may be nullified by political and financial factors; (5) the recommendations of research are dealt with by inefficient consultative machinery.

Despite all this, there is no doubt that the scientist can make a significant contribution to the management of the wild habitats of East Africa. The great volume of inventory work on the flora and fauna, in which P. J. Greenway's on the flora is outstanding, and basic surveys such as H. F. Lamprey's on the ecological separation of game species, have laid a sure foundation. Nevertheless, positive conservation needs more than simple cataloguing; it needs ecological knowledge, insight and action. Despite the difficulties of research in organizing itself, making itself practically acceptable and making its voice heard in the political winds, the scientist has a major part to play. At present conservation is in the hands of the wardens, only one of whom, to my knowledge, has a university training in science. These men are driving their conservation very hard and calling for scientific advice which is seldom forthcoming. The warden has a major part to play, and few scientists could take the place of D. L. W. Sheldrick in his knowledgeable and intuitive handling of Tsavo (East); nevertheless, scientists are needed also. The complementary roles of scientist and warden are seen with E. M. Watson and M. I. M. Turner in the Serengeti, and it is salutary to think that this is the only fluent combination of research and management to be at present encountered in the national parks and game reserves.

Roles of Research Institutes

The existing research institutes dealing with wildlife and forestry in East Africa are the Government departments (including the national parks), EACSO and the university colleges (including NUTAE). All these are constituted differently and have a different attitude to research.

The game departments and the national parks are aiming at the conservation of the game habitat in perpetuity. This is a vast undertaking, involving management and research in water, soils, grasslands, forests, insects and game. The management side is already in

being in both, but as yet there is little development on the research side. SRP, FRSKGD and the two Canadian biologists with the Uganda Game Department are perhaps buds from which research divisions could grow, but they are not permanently engrafted and none is showing signs of growth.

The research divisions of national parks and game departments should be concerned almost entirely with applied research. Their primary aim should be the maintenance of the scientific record of the game lands by standard physical and biological measurements which will show ecological change. This would, in time, mean the establishment of a registry of scientific data and archives. The secondary aims of such a research division would be the establishment of permanent scientific staff to carry on very long-term projects of up to fifty years or more in the drawing-up of life-tables of the elephant, hippopotamus and other long-lived game and examining the vegetation and game successions. The scientific registry and archives would be available to short-term workers, a few of whom might work in the research division, but most of whom would be accommodated in the university colleges and elsewhere. Such research divisions can only be permanently established if the respective Governments make funds available; this has already been done with FRSKGD, but more is needed if the new divisions are to grow. The whole future of national park and game resources in East Africa may depend on such funds being made available immediately.

SRP is a national parks research unit in which a determined attempt is being made to bring inter-disciplinary research to bear on the management of the Serengeti. It has the makings of a research division to the Tanzanian National Parks, but is at present financed entirely from foreign sources and lacks permanency. Funds are provided by UNTAB, FAO, the Fritz Thyssen Stiftung, the Netherlands Foundations for Pure Research, the German Government, the Nuffield Foundation and the Deutsche Forschungsgemeinschaft. SRP work is likely to continue for some years as a group of related short-term projects of up to five years duration. There is no indication so far that really long-term work will be possible.

The research undertaken by the long-awaited Wildlife Research Division of EAAFRO will abide by the broad terms of reference of EACSO to provide research which has equal valency in Uganda, Kenya and Tanzania. It will follow the pattern of the work already in progress in forestry, agriculture and veterinary investigations. In a paper to the Wildlife Research Co-ordinating Committee (which has recently been reconvened after a year of inaction), E. W. Russell gives an outline of the functions of the Division. There would be two permanent scientists: the senior would maintain a bureau of East African wildlife research and act as a senior consultant and adviser; the junior would carry out specific research projects under the general direction of the senior, who would be head of Division. Short-term grant-aided workers might join the Division from time to time.

The research potential of the university colleges is provided by a few members of staff and postgraduate students working for M.Sc. or Ph.D. degrees. At University College, Nairobi, Prof. D. S. Kettle is giving emphasis to wildlife research in the constitution of his postgraduate school. As already stated, the choice of studies, however, is independent of the needs of management, and it is right that this independence be maintained by the universities. African wildlife is bristling with research opportunities in classical zoology (including ecology), and the university departments must not become too tied to wildlife management but be absolutely free to exploit these. The universities are therefore capable of providing short-term workers, mostly dealing with narrow, clear-cut studies in 'pure' science.

NUTAE is a university research unit; but its approach to research is coloured by its being based in the Queen Elizabeth National Park. It retains its independence from the Uganda National Parks, and although it is working in close contact with some of the important management problems both at the Queen Elizabeth and at Murchison Falls, it cannot in its present form be looked on as the beginnings of a research division of the Uganda Parks. NUTAE is endowed by both the Nuffield and the Ford Foundations and is linked with the International Biological Programme (IBP); workers are attached to the University of Cambridge, Makerere College and University College, Nairobi. The Unit is run by a committee headed by Prof. C. F. A. Pantin. Its work is likely to be pure research (some of which will be closely linked to management) and usually in short-term projects of up to five years.

The Uganda Forest Department has a thriving research division, and those who are planning the future of the national parks and game departments should note how this has been achieved. The silvicultural research in softwoods gives quick and simple answers to pressing questions on which depend the country's future timber resources. In hardwood silviculture, however, the task of finding the best methods of regenerating a high forest of desirable trees from the tattered remnants left after exploitation and refinement is a huge and complex one yielding slow results. Yet this is applied research with the stability and endurance which are needed in game management; some of the experimental plots in the Budongo Forest were established in the mid-1930's and are still yielding information. The division of effort in research between the forest departments and EAAFRO is also worth noting when coming to consider the relations between the Wildlife Division of EAAFRO and the national parks and game departments. The work of EAAFRO in forest genetics and pathology is more fundamental than the silviculture of the departments, and this sharing of load is a good working arrangement.

The existing research institutes cater almost entirely for short-term research, the span of which is set by a grant or by the number of years workers remain in university posts. EACSO may be an exception, but its Wildlife Division is not yet in existence. Nothing has yet been provided for long-term research with workers appointed to specific projects lasting twenty years or more. None of the present institutes can offer any opportunity for this; only Government departments and perhaps EACSO can provide the stability in finance and organization necessary to maintain such a long-term research facility; it has already been done in forestry.

The need is for properly constituted (joint) research divisions in the national parks and game departments of each territory, with a co-ordinating council in EACSO.

Conclusion

In his paper, "A Survey of the Past and Present Wildlife Research in East Africa", L. M. Talbot has shown what a vast effort has been invested by the 114 workers cited and the depth of their work as described in some 220 papers. Yet how much of this effort has contributed to the wise management of the East African game lands? I would venture to say very little, though the culling of hippopotamus and elephant in the Uganda Parks is a notable exception. The foggy terms of reference which research has to management, the lack of impact which research has on the course of events, and the lack of planning of long-term research and management cannot be allowed to continue while the wildlife resources are diminishing before our eyes. There can be no alternative to constructive management/research planning backed by efficient co-ordinating machinery for every national park and game reserve, just as there has already been for most forest reserves.

OBITUARIES

Sir Ian Richmond

I. A. RICHMOND, professor of the Archaeology of the Roman Empire in the University of Oxford, died on October 4 at the age of sixty-three. He was educated at Ruthin School and Corpus Christi College, Oxford. Studentships from his university and the British School at Rome enabled him to engage for two years after graduation in field research centred on Rome. In 1926 he was appointed lecturer in classical archaeology and ancient history at Queen's University, Belfast, whence he returned to Rome as director of the School. In 1935 he was appointed to a post in Roman-British history and archaeology in the Newcastle upon Tyne Division of the University of Durham, where he remained, as lecturer, as reader and as professor, until 1956, when he accepted the post in Oxford which he held until his death. He received a knighthood in 1964, the same year as he became president of the Society of Antiquaries of London.

Richmond had few equals either in investigation or in exposition. Almost all aspects of Roman provincial archaeology have been enriched or illumined by his activity, and he has been justly acknowledged for several years as the leading authority in a by no means narrow field.

The twin son of a Lancashire surgeon, Richmond sometimes compared his own work as an excavator with that of a surgeon. He had a flair for, and much experience in, the study of structural evidence. This enabled him to be economically selective in an excavation and still to obtain the full picture, as he did that of the legionary fortress at Inchtuthil in Perthshire, a little at a time over fourteen years. The method was only successful in his own hands, for less skilled excavators require a bolder approach. Richmond would excavate boldly when occasion demanded, and strip off a whole site level by level. Excavators the world over have benefited from working with him on the Corbridge training courses, where he taught techniques by example rather than precept.

On Hadrian's Wall, Richmond provided the sustained effort which brought to fruition programmes of research sometimes initiated by others and carried through in collaboration. In the decade before and the years immediately after the Second World War, problems which had remained obdurate for generations were tackled in a scientific spirit and solved finally and convincingly. The Museum of Antiquities in Newcastle upon Tyne, which he opened in 1960, was largely his creation, and is a permanent monument to him.

Richmond was a first-class teacher, but never surrounded himself with a large circle of undergraduates or recent graduates; for good or ill that was not his way. He was given honorary degrees by six universities, including that where he had spent half his working life. Learned societies and universities also honoured him by asking him to deliver endowed and named lectures. He will be remembered by a wider public for the hundreds of lectures, illustrated, delivered without a script out of the depth of his knowledge, sound, clear and gravely gay, which he gave, up and down the country, to any interested audience.

He held the highest office in national societies, and was a valued member of national boards and commissions in both England and Scotland; he also found time, when already a busy man, to hold office in local societies and to serve on committees and councils. He was no passenger on these bodies, and his cheerful common sense was much in demand. He used the time on the long train journeys to draft his reports.

Richmond's published work includes *The City Wall of Imperial Rome* (1930), a substantial contribution to the

Northumberland County History—*The Romans in Redesdale* (1940), the tenth (1947) and eleventh (1957) editions of the *Handbook to the Roman Wall, Roman Britain* (Collins, 1947), *Roman Britain* (Pelican, 1955); and *Roman and Native in North Britain* (1958). Excavation reports, illustrated by his own photographs and drawings, and substantial papers on many different facets of his main subject, are to be found in the journals of local and national societies.

He was not merely an able man, but a good man. He took an unconcealed delight in success, but was never ambitious at the expense of others. He would spend hours with the writer of a paper or report going through it with him sentence by sentence improving the language. On meeting an acquaintance he never failed to ask the right question about himself and his family. He could be stern, but was always open; his normal mood was of robust good humour and impish wit. He would help a friend or colleague when he needed it most and seemed to deserve it least. It was his explicit policy to do things for other people; he did so as if he were indefatigable, which unfortunately he was not. J. P. GILLAM

K. R. Butlin

KENNETH RUPERT BUTLIN died on October 1, 1965, at the age of sixty-eight. He was educated at Oundle School and, with an interruption for military service, at Trinity College, Cambridge. Shortly after the First World War he took a first in chemistry and almost immediately left for Argentina, where he worked on the fermentation of sugar. Thereafter he remained interested in the chemical activities of micro-organisms and, returning to the United Kingdom in 1929, he joined A. C. Thaysen's group of microbiologists, then working at the Royal Naval cordite factory at Holton Heath. This group was transferred as a unit to the Chemical Research Laboratory, Teddington, in 1933, and it was from Teddington that Butlin published the research for which he will be most remembered.

His earliest papers were concerned with the chemical activities of the acetic acid bacteria, and his review of these organisms is still required reading for those interested in the subject. During the Second World War, Butlin, with the rest of Thaysen's group, turned his attention to the problem of food yeast production, making use of molasses, a project which gave him valuable experience of large-scale production of microbes and which involved, incidentally, one of the earliest uses of continuous culture. At the end of the War, Thaysen and H. J. Bunker (a leading authority on the sulphur bacteria) left Teddington, and research on microbiology at the Chemical Research Laboratory was left in the hands of a small section of the Corrosion Group, headed by Butlin, studying bacterial corrosion. This process is largely the responsibility of the sulphate-reducing bacteria (*Desulfovibrio*), and Butlin's small group rapidly established themselves as leading authorities in this field: they were, for example, among the first to work with authentically pure bacterial cultures.

The then director of the Chemical Research Laboratory, R. P. (now Sir Patrick) Linstead, perceiving the broader ramification of chemical microbiology, initiated the expansion of Butlin's microbiology section until, in the early 1950's, it became an independent Microbiology Group, mainly concerned with the study of sulphate-reducing bacteria but also with a variety of bacteria of economic and industrial importance. In 1950, Butlin accepted responsibility for a few hundred bacterial cultures of industrial importance which the National Collection of Type Cultures proposed to discard, and thus

founded the National Collection of Industrial Bacteria, now located at the Torry Research Station, Aberdeen. The 1950's were the most productive period of Butlin's career: his Group worked on such strictly practical topics as the feasibility of producing sulphur on an industrial scale with the aid of bacteria, control of pollution in waterlogged gravel and clay pits, bacterial leaching of ores, bacterial contamination of petroleum; topics of less-direct applicability such as the mechanism of microbial oxidation of phenols (important in biological effluent treatment) and the continuous culture of anaerobes; relatively academic matters, though still having a practical background, such as the biochemistry of sulphate-reducing bacteria and the mechanism of the methane fermentation. By 1958, Butlin's Group was the major British research unit concerned with basic economic microbiology. The blow to British research, resulting from its disbandment by the Research Council of the Department of Scientific and Industrial Research, reverberated throughout the scientific world; it still evokes strong opinions among microbiologists, both here and abroad. Having staunchly resisted the disbandment of his Group, Butlin was compulsorily retired from the Scientific Civil Service in 1959, but, undismayed, he often emerged from retirement to undertake consultant work or to attend scientific meetings.

Butlin's conviction that microbes are of the greatest potential importance to industry, not only in production but also in the deterioration or disposal of industrial products, was fundamental both to his work and his scientific reputation. He was also a man of striking personality and culture. He was a great lover of opera; he enjoyed travel, good food, wine and companionship; he was firmly literate in the sense that he believed that, if work was worth writing up, it was worth writing well. The literary standard of his publications and, perforce, those of his Group, was high. But, most of all, he had an enormous capacity to enjoy life, an ability which he never failed to communicate to his companions, so that even quite solemn or troublesome occasions took on the character of a party or a convivial gathering. His personality inspired strong affection and he will be sadly missed, not only by his intimate friends, but by innumerable acquaintances and scientific colleagues all over the world on whom his sunny and friendly disposition made an unforgettable impact. His wife, *née* Helen Mary Fletcher, survives him; their son, Martin, is an assistant keeper at the Tate Gallery.

J. R. POSTGATE

Prof. H. Schardin

PROF. HUBERT SCHARDIN, ministerial director, Ministry of Defence, Bonn, honorary director of the Franco-German Research Institute at Saint-Louis, and director of the Ernst Mach Institute at Freiburg-im-Breisgau and Weil-am-Rhein, died on September 27, aged sixty-three.

Prof. Schardin was a good scientist and a fine man who inspired many to both scientific effort and to international understanding. The situations already mentioned do not include the capacities by which I best knew him. He was the German delegate to the International Congress on High Speed Photography, European pivot of this subject, one of the very few present at all such congresses so far and to many a legend in his own lifetime.

His whole career had been devoted to, first, ballistics, leading naturally to high-speed photography—Schlieren investigations in particular—and to philosophic interest in time. He joined Cranz in Berlin in the mid-1920's—and their association will always be remembered for their multiple spark, multiple camera technique of 1928 known simply as the Cranz-Schardin system and used in every laboratory which investigates projectiles in their many guises.

Cranz provides the link from to-day back to the scientific era of the nineteenth century and such names as Toepler and Mach, just as Schardin is a strong link to the earlier decades of this century.

When Cranz retired in 1935, Schardin took over as the German leader in ballistic photography and was in charge of the Ballistic Institute of the Technical Academy of the German Air Force from then until 1945. Later, the Franco-German Research Institute at Saint-Louis was founded with Schardin as its director. The works of this Institute, its director and his colleagues speak for themselves.

Prof. Schardin will be missed and remembered affectionately by many people in many countries, and especially by those interested in the same disciplines and philosophies. The British National Committee for High Speed Photography believes that a prize in his honour should be presented at International Congresses on High Speed Photography for worthy contributions to the subject. It could be known simply as the "Schardin Award", and the British Committee would be pleased to support such a commemoration.

G. H. LUNN

NEWS and VIEWS

The Royal Society of London : Award of Royal Medals

HER MAJESTY THE QUEEN has been graciously pleased to approve recommendations made by the Council of the Royal Society for the award of the three Royal Medals for the present year as follows: to Dr. R. A. Lyttleton, reader in theoretical astronomy in the University of Cambridge (presently at the Institute of Astrophysics, Brandeis University, Waltham, Massachusetts, U.S.A.), for his distinguished contributions to astronomy, particularly for his work on the dynamical stability of galaxies; to Dr. J. C. Kendrew, deputy chairman of the Medical Research Council Laboratory of Molecular Biology, Cambridge, for his distinguished contributions to the complete structural analysis of a protein molecule (myoglobin), particularly the biological aspects of this study; to Dr. H. C. Husband, chartered civil engineer (of Messrs. H. C. Husband and Co., Sheffield), for his distinguished work in many aspects of engineering, particularly for his design studies of large structures such as

those exemplified in the radio telescopes at Jodrell Bank and Goonhilly Downs.

Mathematics in the University of Edinburgh :

Prof. A. C. Aitken, F.R.S.

PROF. A. C. AITKEN has retired from the chair of mathematics in the University of Edinburgh, where he has been a lecturer and reader from 1925, and where he succeeded Sir Edmund Whittaker as professor in 1946. Born and educated in New Zealand, Aitken went to Edinburgh in 1923 at the age of twenty-eight as a research student, accompanied by his wife. There he received in 1925, not the degree of Ph.D. for which he was registered, but that of D.Sc., for a thesis recognized as being of quite outstanding originality and merit. No doubt his phenomenal powers of mental arithmetic provided the original impetus which led him, both in this thesis and later in many published papers, to devote so much of his life work to practical mathematics and numerical analysis. He has

also undertaken substantial and wide-ranging work in two other fields, namely, mathematical statistics and the theory of matrices and determinants. He collaborated with H. W. Turnbull in a text-book which has become a classic, *The Theory of Canonical Matrices* (1932). From 1939, together with D. E. Rutherford, he edited the series of University Mathematical Texts published by Oliver and Boyd, himself contributing the first two volumes. Between 1925 and 1962 he published some 70 mathematical papers, while his brilliancy as a lecturer inspired many generations of undergraduates and research students and his colleagues, with whom also he shared his profound knowledge of classical and modern literature and of music. Before coming to Britain, Aitken served with the New Zealand Expeditionary Force from 1915 until wounded in 1916. His experiences during the First World War were described with vivid realism in a manuscript drafted in 1917, which was rewritten and published in his book, *Gallipoli to the Somme* (1963).

Prof. Aitken has been succeeded jointly by Prof. A. Erdélyi and Prof. F. F. Bonsall (*Nature*, 208, 19; 1965).

Physics in the University of Sussex :

Prof. M. W. Thompson

DR. M. W. THOMPSON, who has been appointed to a chair in physics in the University of Sussex, was educated at Rydal School, Denbighshire, and at the University of Liverpool, graduating with first-class honours in physics in 1953. In 1963 he was elected to fellowship of the Institute of Physics and in 1964 was awarded a D.Sc. by the University of Liverpool. In 1953 he joined the Atomic Energy Research Establishment, Harwell, at first in the Reactor Physics Division and later in the Metallurgy Division, where he has been in charge of the Radiation Damage Processes Group from 1960 onwards. The first problems he worked on were the neutron economy of nuclear reactors and techniques for neutron detection. In 1954 he turned his attention to radiation damage in metals. By carrying out irradiations in a reactor at very low temperatures he was able to identify the temperature ranges in which point defects become mobile in the refractory metals. This work led to an interest in atomic collision processes in crystals, which are responsible for both radiation damage and sputtering; and, in a series of sputtering experiments, he was able to prove the existence of focused collision sequences and to study their characteristics. In pursuing his interest in the interaction of ion beams with crystals, he produced some of the first evidence for the channelling of ions through crystals. More recently, he has shown that, on account of channelling, nuclear reaction rates depend on crystal orientation; and, on the basis of the channelling phenomenon, he is at present developing a technique for investigating crystals and their defects.

Genetics in the University of Aberdeen :

Prof. H. J. Evans

THE University of Aberdeen has announced the appointment of Dr. H. J. Evans to a newly created chair of genetics. Dr. Evans has rapidly and deservedly earned an international reputation as a cell biologist. This stems from his work during the past ten years at the Medical Research Council Radiobiological Research Unit. Born in Llanelli nearly thirty-five years ago, he won a State scholarship to the University College of Wales, Aberystwyth, where he graduated B.Sc. in 1952 and went on to gain a Ph.D. for his work in genetics in the Department of Agricultural Botany under Prof. P. T. Thomas. From Aberystwyth and the cytogenetic study of mushrooms, Dr. Evans made the successful transition in 1955 to radiobiology at Harwell under Dr. G. J. Neary. The combination of physicist and cytogeneticist proved extremely fruitful, as witnessed by a series of much quoted joint investigations in mitotic delay, oxygen and other

environmental effects, relative biological effectiveness for various radiations including fast neutrons and ultra-soft X-rays. In 1960 Dr. Evans, after a profitable year at Brookhaven National Laboratory in Long Island, New York, adroitly escaped the blandishments of the brain drain. He returned to Harwell to continue the profitable association with his biophysical colleagues and to initiate work on radiomimetic agents, DNA synthesis and chromosomal breakage and repair. Within the past year he has led the newly formed Cell-Biology Group and increasingly utilized mammalian and human cells for his work. With his lively and engaging manner, Evans is much sought after as a speaker at scientific meetings in Britain and overseas. He is an active member of many societies and the secretary of the Association for Radiation Research.

British Hovercraft

IN a written answer in the House of Commons on November 11, the Minister of Technology, Mr. F. Cousins, stated that the development of the hovercraft had now reached an advanced stage. During the past year, undertakings both in the United Kingdom and abroad had been operating small hovercraft carrying up to 38 passengers on regular services, and in particular, more than 100,000 passengers had been carried across the Solent in little more than three months. The convenience and saving of time had led to the hovercraft becoming the preferred method of travel for many businessmen on this route. Licences had been acquired by American and Japanese companies to manufacture hovercraft to British designs and patents, and other countries were starting developments of their own. Britain had by far the greatest experience of design, manufacture and operation of hovercraft, and Mr. Cousins believed we still had a lead, thanks largely to the ingenuity and determination of the inventor and the faith shown by the National Research Development Corporation and the manufacturers and transport operators who had taken a stake in this field. A major step forward was being taken with the production of the 150-ton SRN4—four times the size of the largest hovercraft so far built. A Swedish consortium had ordered two SRN4 for a cross-Channel service and the Minister of Transport had approved the proposal of the Railways Board to negotiate for the first SRN4 to operate as a combined car and passenger ferry across the Solent, starting in 1968. Meanwhile, about 25 smaller passenger-carrying hovercraft had been delivered or were on order, and a new light-weight and relatively cheap craft would shortly be put into production. The Government intended to do everything it could to ensure the continuance of Britain's present pre-eminence, and to promote the growth of the industry and the development of exports.

East Midlands Regional Office of the Ministry of Technology

AN East Midlands regional office of the Ministry of Technology has been opened at Cranbrook House, 47 Cranbrook Street, Nottingham. Mr. J. K. L. Thompson has been appointed the senior regional officer in charge of the office, and will have four qualified technologists on his staff. Mr. Thompson has been a member of the Atomic Energy Authority at Aldermaston, specializing in explosive hydrodynamics, although for the past year he has been seconded to the Ministry of Defence. The regional office will help industry to make the best use of advanced technology, particularly by providing information of the latest technical developments, and will also collect information with the view of guiding the Ministry in planning future technical support. It will co-ordinate the work of industrial liaison officers and will co-operate with other Government departments in the Regional Planning Board of the Department of Economic Affairs. Another aspect of the work of the regional office will be

to strengthen the links between industry and the National Research Development Corporation, Government research establishments and those of the United Kingdom Atomic Energy Authority. Three existing regional offices at Cardiff, Edinburgh and Newcastle upon Tyne were taken over from the Department of Scientific and Industrial Research on April 1, 1965, by the Ministry of Technology. Other new offices are sited at Birmingham, Bristol, Leeds and Manchester.

Research and Economic Growth in Belgium

UNDER the title *Research and Economic Growth*, the National Council for Scientific Policy, Brussels, has issued a report on some aspects of industrial research in relation to economic growth, more especially dealing with scientific research and development in the metal industries (*Recherche et Croissance Économique: Rapport sur Certains Aspects de la Recherche Industrielle dans ses Relations avec la Croissance Économique*. Pp. 218. Bruxelles: Conseil National de la Politique Scientifique, 1965). The report is in two parts, each consisting of two chapters. The first chapter surveys the lines of development and demand in European and world trade, with particular reference to the metal industries in Europe and Belgium. The second chapter compares the scale and distribution of research in Belgium and in other countries. The third chapter, which opens the second part, and is based on an enquiry conducted by the University of Louvain, reviews the conditions of technical innovation in the metal industries of Belgium, while the fourth chapter, based on a study made by the Department of Applied Economics, University of Brussels, attempts to assess the development of demand for European metal manufacturers. The report concludes that a structural evolution of the Belgian economy is necessary, particularly in the way of diversification, and new, highly technical activities are most likely to succeed if they start from the strong points of the old technology. The comparatively low level of research in Belgium is attributed to the dominance of industries making intermediate products, and, in the metal industries, of sub-sectors with weak or old technology. The small size of Belgian firms, the majority of which employ and are likely to continue to employ only 100–500 persons, is another hindrance, and policy should be directed to stimulating research in the medium-sized firms. Progress depends on the stimulation of creativity and precedes growth, and the attitude of directors is a fundamental factor in determining the intensity of research. Firms need to become increasingly conscious of the importance of being receptive to change.

Industrial Research and Development in France

Le Progrès Scientifique, July–August, 1965, Nos. 86–87, issued by the General Delegation for Scientific and Technical Research, Paris, contains a survey of research and development in French industry, together with an analysis of the output of qualified engineers in 1964. In 1963 a total staff of 77,000 were engaged in research and development, of whom 16,700 were research workers or technologists, 31,700 technicians and 8,500 administrators. Expenditure is estimated at 3,080 million francs, the total national expenditure on research and development being estimated at 6,215 million francs. This effort is heavily concentrated in the Paris region (70.8 per cent), the Rhône-Alps region (9.44 per cent), the Marseilles region (4.4 per cent) and the Toulouse region (2.9 per cent). Of the personnel, 20.3 per cent are in aeronautics, 19.15 per cent in electronics, 11.3 per cent in the chemical industry, 6.3 per cent in the power industries, 6.25 per cent in electrical construction, 6.1 per cent in precision instrument industries, and 5.6 per cent in the pharmaceutical industry. Comparisons with the industrial effort of the United States are given in some detail.

The National Trust

THE seventeenth annual report of the National Trust for the year 1964–65 records the resignation of the Earl of Crawford and Balcarres, its chairman since 1945. In that period the membership of the Trust has grown from less than 8,000 to more than 155,000. The Trust now owns or protects more than 400,000 acres of land, and 189 historic buildings and gardens, compared with 112,000 acres and 95 buildings in 1945. Of its senior staff of 49, 30 work outside London; but the report emphasizes the need for a further increase in income and membership, which should reach at least 250,000. It is emphasized that there is full co-operation between the National Trust and the Nature Conservancy, and it is pointed out that the Trust now only accepts nature reserves if they are also places of 'outstanding natural beauty', or if it is thought that the Trust has special qualifications to protect the property concerned. Although the Trust owns some nature reserves of the highest quality and importance, its greatest contribution to the preservation of Nature is the fact that it protects all its properties against harmful development. Of the Coastal Preservation Campaign launched in May 1965, the report records that at the end of July, £461,000 had been given (or promised) to the appeal fund, and more than 200 acres at Llochtyn, Cardiganshire, had already been purchased. 31 acres of foreshore at Kearney in Northern Ireland, with covenants over a further 284 acres, and a stretch of more than 650 acres at Whiteford Burrows, in the Gower Peninsula, are being acquired. The Council's objection to the Manchester Corporation's proposals to take more water from the Lake District is also recorded, as well as the acquisition by the Trust of the freehold of the Stratford on Avon canal. An appeal for funds for improvement and maintenance of the canal is included with the report, which concludes with a moving appeal for wider and fuller support for the work of the Trust generally, taken from a speech by Sir Kenneth Clark, at a meeting in the Royal Festival Hall, in March.

Reconditioning Concrete Surfacing

A CASE has recently been reported in the United States of a large municipal car-park in which, following a severe winter in 1963–64, the concrete surfacing deteriorated badly, after only two years of service. "The four-inch layer of concrete on the upper level of the prestressed concrete structure had pitted and scaled, resulting in cracks, rough spots, and holes—some of them down to the reinforcing bars. The pavement was so rough in a number of places that women in high-heeled shoes found walking on it a risky proposition." This happened at a parking garage in Waterbury, Connecticut, and it was due to the repeated freezing and thawing of moisture which had penetrated the surface of the concrete, causing crumbling and pitting, these failures being aggravated by the tyre-chains used on the cars and by de-icing salt reactions on the surfacing, to be anticipated during a severe winter. The same thing has happened, of course, in similar circumstances in some concrete surfacings in Britain and elsewhere. What is of particular interest in the Waterbury example, however, is the method of reconditioning of this deteriorated pavement which, in some respects, is both novel and exemplary; it is certainly not without possibilities in its application, under similar conditions, to comparable problems in Britain and elsewhere. Picking up and repaving bad concrete surfacing with new is both a time-consuming and costly job, often involving many weeks, even months. In the case under review, time was paramount. The quick remedy lay in repairing the damaged surface by application of a cement mortar modified with a specific acrylic bonding agent. According to an article published in *Rohm and Haas Reporter* (Rohm and Haas, Philadelphia (Lennig Chemicals, Ltd., London, W.C.1), 23, No. 4; July–August 1965), procedure was as follows: the surface was scarified to remove loose and

scaling concrete; grease, oil, dirt and salt residue were cleaned off and cracks were filled with a sealant. After wetting with water, the 30,000 ft.² of the damaged surface was treated with a liquid acrylic mortar modifier ('Sonocrete') to "... assure a durable bond between the existing substrate and the fresh mortar subsequently applied". This was followed by a 0.25-in. layer of concrete mortar modified with 'Sonocrete'. The new surface was then swept with a stiff broom to create a serrated finish. After three days, a final coat of 'Sonocrete' was applied to afford additional protection from water and salt. Following a further winter of use, the pavement is still in excellent condition; adhesion to the old surface is described as outstanding; little damage has resulted from freeze-thaw cycles and the abrasion-resistant surface has withstood the punishing effect of tyre-chains and snow-removal plant. This article gives details of other 'Sonneborn' products for use with concrete and masonry, for example, sealants, caulking agents, waterproofing compounds, and decorative protective finishes; also described is a series of 'Rhoplex' acrylic emulsions manufactured by Rohm and Haas which, among other compounds, includes polymers developed specifically for use in modifying Portland cement.

New Zealand State Highways

THE Road Research Unit of the National Roads Board, New Zealand State Highways, publishes quarterly a *Newsletter* which is intended to portray a cross-section of the factors relating to New Zealand road and traffic research problems. In publicizing the activities of the Road Research Unit, this *Newsletter* is also designed to arouse interest and stimulate thinking about road problems, and to indicate periodically the tremendous and ever-growing fund of knowledge of road technology already available. A recent issue of this publication well illustrates its scope (*Road Research Unit Newsletter*, No. 8. Pp. 21. National Roads Board, Wellington, July 1965). The State Highways authorities attach great importance to traffic-volume records on their network of roads, especially for connecting links between towns and rural highways. Automatic counters are used for this purpose to record traffic flows at count stations at country-wide locations. At three-yearly intervals a complete analysis is made to establish the annual average daily traffic values, and a map of both North and South Islands produced to indicate the general form of traffic volumes; one such map, of a part of North Island prepared in 1964, is reproduced in this *Newsletter*; it is most instructive. Another feature is a summary of local road trials and research in progress throughout the country so that all interested parties may be kept up to date with the knowledge of 'who's doing what'; this is to encourage people with particular problems to consult others faced with a similar problem. Fifty-four separate activities are listed, under headings which include topic and scope (nature of the trials, problems involved, materials concerned); location; responsible authority; and state of the investigation to date. An article entitled "Why Full-Scale Road Tests?" lucidly explains, even to the uninitiated, the reasons underlying the practical use and value of trials of traffic-pavement relationship in aiding design of new road foundations and surfacings built to withstand the ever-increasing demands of traffic densities, vehicle speeds and wheel loads, particularly significant in the case of heavy commercial transport, as the Road Research Laboratory in Britain has emphasized for many years past, some of its work in this connexion being quoted in this issue of the *Newsletter*. This is a brief but admirable publication.

Australian Prehistory

In recent years, the prehistory of the southern part of Australia has been set on a firm basis. This is, in large measure, due to the influence of F. D. McCarthy, of the

Australian Museum at Sydney. Nowadays it is no longer a matter merely of collecting surface finds of stone tools and of vague descriptions of the painted rock-shelters, but sites are carefully located and excavated—care being taken to determine the stratigraphy, and to make complete lists of all the various types of tool found. Such an account appears in the June 5, 1964, issue of the *Records of the Australian Museum*, written by McCarthy himself, which deals with the investigation of a series of sites in the Capertee Valley in the Glen Davis area (26, No. 6. Pp. 197-246 + Plates 11-24. Sydney: The Australian Museum, 1964. 16s. 6d.). There are a number of excellent illustrations of the finds. The results show a definite development in the stone working during the occupation of the sites and the final assemblages are rich in types of tool. They include blades with notched edges, side and end scrapers, pointed blades, cores, microlithic tools of various types and pounders. Most of the article, of necessity, is given up to a description of the finds. It is an article not to be missed by students interested in the early prehistory of Australia.

Pyrethrum from New Guinea

As is well known, pyrethrum is a natural insecticide having the unique advantage of toxicity to almost all insect pests, but harmless to man and animals. No synthetic product has so far been discovered that is able to match, let alone to rival, its peculiarly rapid properties in problems of insect extermination. Yet we learn that at present there exists a world shortage of pyrethrum extract for this purpose, which has grown increasingly acute with a general rise in living standards, according to an article in a recent issue of *Albright Magazine* (Albright and Wilson, Ltd., London, October 11, 1965). The firm of Stafford Allen, Ltd., one of the Albright-Wilson group, with long-established experience of the pyrethrum business, in collaboration with the administration of the Territory of Papua and New Guinea, has set up a cultivation centre for production of pyrethrum insecticide on a large scale in the Western Highlands of New Guinea, complete with a modern extraction plant, at Mount Hagen, a few hundred miles north-west of Port Moresby, in what looks like most uninviting country; it is, in fact, quoted as "... a vast fertile plain ringed by mountain walls soaring to over 15,000 feet above sea-level and inhabited by natives still in the Stone Age". Pyrethrum will only flourish and produce economic quantities of its toxic ingredients under specific optimum conditions: a combination of altitude, climate and soil; this particular project in New Guinea is satisfied by all three. It is said that altitude is the most important single factor to successful cultivation, but such benefits as stable temperature, an equable balance of sunshine and rain, and equal hours of day and night at all seasons of the year, are material items. Such favourable environmental circumstances make it practicable to plant a selected strain of pyrethrum flowers in different areas, thereby ensuring that the harvest period is spread over the whole year, a vital factor to the continuous operation of the extraction plant and, incidentally, to the employment of a needy native population. This project is the first major industrial operation in this remote area of the Western Highlands of New Guinea. The capital investment involved in bringing it to fruition is in the region of £250,000, and to handle the scheme a new company—Stafford Allen (New Guinea) Pty., Ltd.—has been formed. The coloured illustrations to the article help to convey some idea of the territory concerned, including the pyrethrum flower involved (which resembles an ordinary marguerite) and of the natives who work in these somewhat unusual fields.

Hospital Lighting

DOCTORS examining patients and pathological specimens in hospitals frequently have to note accurately the colours

of the objects they examine, whether it be the skin of the patient, or lesions on it, the conjunctivae of the eyes, the tongue, or the mucous membranes of the mouth. A good example occurs in a slight case of jaundice. Daylight is best for this purpose, but it is not always available, and it is therefore important that artificial lighting used in hospitals should not distort the colour of anything examined by the doctor. For this reason, the Medical Research Council and the Building Research Station of the Ministry of Technology (formerly of the Department of Scientific and Industrial Research) have investigated this problem at Sheffield Royal Infirmary. Fifteen lamps, both fluorescent and tungsten filament, were tested, and the results are now published under the title *Spectral Requirements of Light Sources for Clinical Purposes* (Medical Research Council. Memorandum No. 43. Joint Committee on Lighting and Vision. Pp. vii + 56 + 2 plates. London: H.M.S.O., 1965). This clearly shows that the fluorescent lamps are better not only because they give the smoothest distribution of light and the colour appearances of warm sunlight, but also because they are more economical. Any reputation they may have gained for distorting colour is due to the use of lamps with a grossly uneven distribution of energy over the spectrum. The report proposes a detailed specification for a fluorescent lamp suitable for general use, and it also hopes that this will help manufacturers to supply lamps satisfactory for the hospital service.

Glove Damage as a Cause of Wound Infection

THE use of rubber gloves during surgical operations became general about 1900. In 1939, Devenish and Miles emphasized that damage to rubber gloves could lead to infection of operation wounds. In recent symposia concerning nosocomial infection, defects in rubber gloves were considered to be of significance in the development of wound infection. The object of an investigation by Palle Gad was to obtain an estimation of how frequently wound infection originates from bacteria on the hands of the operating staff (*Danish Medical Bulletin*, 12, No. 1; March 1965). Examination of the wounds following 433 'clean' operations, of the 3,125 rubber gloves used in these operations and of the bacterial flora of the hands which had worn 692 damaged gloves, revealed no connexion between the glove damage, the bacterial flora and the wound infections observed.

Aldosterone in Myocardial Infarction

THE urinary excretion of aldosterone in healthy adult Indian males was recently estimated in order to establish the normal standard. This value ranged from 6.5 to 19.2 $\mu\text{g}/24\text{ h}$ (*Ann N.Y. Acad. Sci.*, 118, Article 11: *Role of Aldosterone in Myocardial Infarction*. Pp. 537-554. New York: New York Academy of Sciences, 1965). The value was two to three times higher in acute uncomplicated myocardial infarction. Sodium-restricted diet and controlled posture in normal individuals caused a rise in the urinary excretion of aldosterone, but the rise was much less than that observed in myocardial infarction. R. B. Aroa of the All India Institute of Medical Sciences, New Delhi, has also shown that urinary aldosterone excretion in normal, healthy dogs fed a constant diet and later on in the same dogs, with experimental myocardial infarction, revealed a similar increase in the absence of liver damage. Animal experiments also preclude the possible influence of drugs used in myocardial infarction on the level of urinary excretion of aldosterone. Extrapolation of the findings from animal experiments permits the hypothesis that increased urinary excretion of aldosterone in patients with myocardial infarction is to be attributed mainly to the myocardial infarction, and not to the associated factors, that is, diet, drugs, and posture. The rise of sodium concentration in the infarcted myocardium appears to have a causal relationship with the aldosterone-induced arrhythmia in myocardial infarction.

Melville on Macromolecule Chemical Industry

IN an article in *Chemistry in Britain* (1, 404; 1965), based on his lecture at the Royal Institute of Chemistry conference earlier in the year, Sir Harry Melville compares three British industries which have to do with macromolecules. These are plastics, textiles and paper. In all of these there has been a tendency towards bigger enterprises. In plastics, for example, there were in 1958 six enterprises with more than 1,500 employees. The plastics industry has been one of the fastest growing sectors of British industry, with a volume production now greater than the total usage of all non-ferrous metals. There has been little tendency to diversification: nearly all the plastics now manufactured commercially have been known and manufactured for many years. Although there is plenty of evidence that research and development expenditure in an industry contributes directly to its profitability and growth, research and development are becoming relatively more expensive. Indeed, in the older and empirically developed paper and textile industries there is no certainty that increased research and development expenditure would be fruitful. The search for precise criteria for decisions on research and development expenditure should not obscure the value of innovators. These are rare people and should be encouraged in industry, even though they may not conform to the pattern which makes life easy for their administrators.

University News:

Belfast

THE following appointments have been made: *Professorships*, Dr. W. O. Brown (agricultural chemistry); Dr. J. C. Murdoch (crop and animal husbandry); Dr. R. K. McKee (mycology and plant pathology); *Lecture-ship*, Dr. W. C. Beattie (electrical engineering).

Birmingham

THE following appointments have been made: *Senior Lectureship*, Dr. C. E. Oxnard (anatomy); *Lectureships*, Dr. M. H. B. Hayes and Dr. A. J. Waring (chemistry); *Research Fellowships*, A. N. Bramley (mechanical engineering); J. L. Godman and B. H. Rees (chemistry); R. S. Lowery (microbiology).

Announcements

A MEETING on "The Degradation of Biological Macromolecules", organized by the Physical Biochemistry Group of the British Biophysical Society, will be held in Oxford on January 11. Further information can be obtained from Dr. A. R. Peacocke, St. Peter's College, Oxford.

A CONFERENCE on "Solid State Physics", organized by the Institute of Physics and the Physical Society, will be held in the Manchester College of Science and Technology during January 4-7. Further information can be obtained from the Meetings Officer, Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

A SYMPOSIUM on "Recent Advances in the Development, Production and Utilization of Medicinal and Aromatic Plants in India", under the auspices of the Central Indian Medicinal Plants Organization, will be held at Lucknow during January 12-14. Further information can be obtained from Dr. S. C. Datta, Central Indian Medicinal Plants Organization, 4 Sapru Marg, Lucknow.

ADDENDUM. In the communication entitled "Social Companions and the Mother-Infant Relationship in Rhesus Monkeys", which appeared on p. 301 of the October 16, 1965, issue of *Nature*, the following legend should have accompanied the illustration:

Fig. 1. Number of half-minutes in which the infants were recorded off their mothers, expressed as a percentage of number of half-minute periods for which they were watched. Shaded area shows the range for the isolate infants. Dotted area shows the range for group-reared infants. ○, Median for isolate infants; ●, median for group-reared infants

ELECTROMAGNETIC DISTANCE MEASUREMENT

THE International Association of Geodesy, on the invitation of the Royal Society of London, held a symposium on "Electromagnetic Distance Measurement" at Oxford during September 6-10, 1965. The precise measurement of long distances on the Earth's surface, and also to artificial satellites, has become an increasingly important technique of geodesy during the past twenty years. Numerous instruments are now available commercially for this purpose, using either modulated light or microwave beams, and much research is going on, including work on the application of lasers to distance measurement.

These instruments have created new problems for geodesists who seek accuracies of one or two parts per million. The speed of light is a vital parameter in the calculations, and the effect of atmospheric refraction is most important. The precise determination of the mean refractive index for long lines through the atmosphere at present constitutes the most intractable problem. A further complication with microwaves is that they are reflected from surfaces below or adjacent to the line measured, causing difficulties in observation and uncertainties in the final determination.

It was to investigate these and other associated problems, and to evaluate the available instruments, that Special Study Group No. 19 of the International Association of Geodesy was formed in 1958. The symposium was convened by Major-General R. C. A. Edge, president of the Study Group (and now director-general of the Ordnance Survey), to provide a forum in which the geodesists' problems could be discussed with physicists and meteorologists from all over the world whose work had an important bearing on the subject.

The working sessions covered microwave instruments, electro-optical instruments, propagation problems, laser applications, airborne systems, ranging to artificial Earth satellites, and finally the precise measurement of distances of the order of 1 km or less to accuracies better than one part per million. This last is a newcomer to the work of the Study Group, but one of increasing importance in precise engineering, as for example in the construction of particle accelerators. Plans are now being made to publish a full record of the proceedings, including discussions.

Further information concerning the symposium may be obtained from the Director-General of the Ordnance Survey, Leatherhead Road, Chessington, Surrey.

AUSTRALIAN BIOCHEMICAL SOCIETY MEETING

A MEETING of the Australian Biochemical Society was held at Monash University, Clayton, Victoria, during August 24-27. It took the form of general research papers and symposia on protein structure, function and synthesis—the latter in relation to the genetic apparatus—and the genesis and function of membrane systems. Speakers from overseas and Australia were invited.

S. Moore discussed the chemistry of the catalytic site of ribonuclease with particular reference to the histidine residues at positions 12 and 119 and the lysine residue at position 41. The proportion of each histidine alkylated at nitrogen-1 of histidine 119 and at nitrogen-3 of histidine 12 was found to vary with the chain-length and the particular isomer of the alkylating agent. Charged substitution (carboxymethyl) of lysine-41 lowered the rate of histidine alkylation, but an uncharged (carboxy-amido) derivative did not affect it. The spatial relationships of these three amino-acid side-chains as a part of the active centre can now be visualized.

The mechanism of action of certain hydrolytic enzymes as followed by kinetic analysis of the formation of acyl intermediates and their subsequent rate of decay was discussed by B. Zerner. In the case of chymotrypsin and trypsin the decay of the acyl derivative was found to be the rate-limiting step. The mechanism of hydrolysis by liver carboxylesterases, urease and certain other proteolytic enzymes seems to follow the same general chemistry of an acyl enzyme intermediate. He pointed out that the detailed chemistry underlying enzyme specificity is still unsolved.

E. O. P. Thompson gave an account of the accumulated information concerning keratin structure with particular emphasis on Australia's national product, wool. Urea and a reducing agent are required to disrupt the firmly bound structure. Subsequent alkylation of the thiol groups so formed gives protein fractions amenable to conventional separation methods. The large number of acetyl end

groups is of considerable interest. Three general heterogeneous classes of protein are present in wool—high sulphur, low sulphur and high glycine. The high sulphur proteins are found in the matrix between the fibrillar structures and may vary from 22 to 31 per cent with forced feeding. Serious attention is now being given to the bottom (growing) end of the wool fibres. There are suggestions that the high sulphur proteins are formed later than the rest and that consolidation of the protein system occurs higher up the fibre.

What was true and new about our oldest and best-known protein system—gluten—was amusingly discussed by M. V. Tracey. In latter years the separation methods successfully applied to other protein systems have been tested and shown to be not very useful with gluten. But the attempts have gone on. The protein as it occurs in flour has the original characteristics of the protein as synthesized, but with maturation of the grain, etc., subsequent alterations have been superimposed. Mr. Tracey, in opposition to the simplifiers, pointed out that, as isolated from the flour, the gluten complex has a range of molecular weights of 20,000 to many millions. Using common-sense arguments he pointed out that random polymerization through disulphide cross-linking would give rise to giant molecules. He argued further that an allo-polyploid with endosperm cells having three sets of chromosomes plus accumulated mutations would not be expected to synthesize storage proteins of simple variety.

V. Moses discussed the observation that proteins synthesized in *E. coli*, after depleted cells have been supplied with nutrients, appear at different rates. This discrimination was interpreted in terms of stability differences in the related molecules of *mRNA*. He raised the question of the control of protein synthesis when the related *mRNA* is of the stable kind. If it is exercised, control in such a situation must be at a later stage than transcription of DNA. B. W. Holloway reviewed the evidence relating to a general operon hypothesis for control

of genetic expression of clusters of related genes. He concluded that in *E. coli* and *Salmonella typhimurium* up to 70 per cent of the genes showed some clustering, but that in *Pseudomonas aeruginosa* it is quite rare. It was expensive, he thought, for each gene to have its own operator and so, without clustering, it was reasonable to expect regulation of enzyme synthesis at the translation level—a point arrived at by the previous speaker. W. H. Elliott also suggested control of ribonuclease synthesis in *Bacillus subtilis* at a stage later than transcription. He has found that actinomycin stimulates ribonuclease production—an observation which would seem to preclude control at the transcription stage. The presence in the cell of a firmly complexing inhibitor of the active ribonuclease secreted by this organism suggests that this enzyme is synthesized close to the cell membrane—from which it is released to the medium. Dr. Elliott also gave evidence for the synthesis of the exo-enzyme α -amylase close to the cell membrane.

The regulation of RNA biosynthesis in general was reviewed by L. R. Finch, who added the idea that the present concepts of the utilization of mRNA for protein synthesis, before its own transcription from DNA was completed, allow possible alternative methods of repression not immediately connected to the operator region. He saw combination of the repressor with the nascent peptide chain as offering more specificity than the simple operator region hypothesis.

G. L. Ada spoke of the structure of immune γ -globulin and the problems besetting the investigation of the mechanisms controlling the synthesis of antibody by cells from the spleen and lymph nodes. The heterogeneous nature of the lymph nodes makes conventional methods of following protein synthesis difficult to interpret in terms of antibody synthesis, but the use of radio-autography and specific fluorescent staining offers hope. Injection of powerful bacterial antigens labelled with radio-iodine into rats leads to the uptake of less than four molecules of antigen per plasma cell. It was inferred that intact antigen does not act as a template in the synthesis of antibody. The association of antigen with RNA as being involved as an intermediate in the antigenic response was discussed.

Attempts to define the number and function of proteins coded for by poliovirus RNA after infection were discussed by P. D. Cooper. Mutants resulting from treatment with 5-fluoro-uracil were selected for heat-defectiveness over the small range of temperature of 37°–39.5° C. Five to six genes affecting host cell metabolism have been detected, but the mechanism of their action is as yet little understood. He suggested that the proteins formed as gene

products in the mutants differed in perhaps one amino-acid only and that this alteration allowed easy temperature deformation.

J. K. Pollak discussed his work on the origin of the endoplasmic reticulum in developing and regenerating liver. He presented evidence supporting the formation of the reticulum from precursor granules (reticulosomes). These consist mainly of protein which combines with phospholipid to form stable complexes resembling a membranous reticulum. D. E. Green discussed his accumulated knowledge of the methods of separating and re-forming the structural protein phospholipid complexes of mitochondria. He also discussed the evidence for the association of the integrated enzyme systems with structural components of the mitochondria. The localization of the effect of chloramphenicol on the formation, in *S. cerevisiae*, of completed mitochondria with respect to a marked decrease in the number of cristae and of certain mitochondrial enzymes was discussed by A. W. Linnane. The organism grows extremely well on the readily fermentable glucose. His results suggest specificity in the effect of chloramphenicol on mitochondrial development. The synthesis of chloroplasts with respect to regulation, the greening process and the involvement of contained DNA, RNA was discussed by R. M. Smillie. Once again chloramphenicol was reported to show specificity in its effect on the synthesis of protein in the developing organelle.

Using mitochondrial swelling as a measure of substrate uptake, J. B. Chappell presented results which were consistent with the presence of carriers for malate and succinate dependent on phosphate and for citrate being dependent on the presence of L-malate in addition. He sees the process of uptake as being by exchange diffusion. Many in the audience came to sensible grips with mitochondrial swelling for the first time.

T. P. Singer summarized the existing knowledge on the complexities of NADH₂ dehydrogenase, with its flavin, non-haem iron and labile sulphur. Its functional position in the electron transport chain in relation to phospholipid and the modifications of its properties by heat and organic solvents and mercurials were discussed.

L. P. Vernon and N. K. Boardman discussed chloroplasts and their functional components which are concerned in electron movement following photochemical excitation. They considered these components with respect to the individual steps and pathways involved and with respect to sub-units having definable activities. Knowledge of chloroplast function now seems to be comparable in depth with that of mitochondria from animal tissues.

F. J. R. HIRD

CONTROL OF BIOLOGICAL DEVELOPMENT

THE seventh International Embryological Conference, sponsored by the Editorial Board of the *Journal of Embryology and Experimental Morphology*, was held in London during September 6–10. Three different forms of scientific communications were used: twelve main papers, five discussion groups and some eighty demonstrations were presented by scientists from twenty countries. The control of development formed the major topic of the meeting, since classical embryology is now being re-interpreted in terms of the mechanisms of control of protein synthesis by the genes. Development, of course, requires that a sequence of changes occurs in the types of synthesis carried out by a developing cell. Dr. S. Brenner (Cambridge) introduced the session on the genetics of embryogenesis by discussing the possible control mechanisms whereby a series of cistrons could bring about the sequential series of changes in synthesis required in differ-

entiation. Dr. H. MacGregor (St. Andrews) described recent work on the lamp-brush chromosomes found in newt eggs during their formation. These chromosomes are intensely concerned with the synthesis of materials which form the egg.

However, genetic control systems of embryogenesis tend to fail even if the chromosome number is haploid or polyploid rather than diploid. Prof. L. Gallien (Paris) described a number of instances of polyploidy in amphibia and correlated these abnormalities and examples of aneuploidy with the type of failure of development observed.

Dr. L. Hamilton (London) spoke on her work on the genetic factors which may be involved in the 'haploid syndrome' found in haploid amphibian embryos. This syndrome is characterized by oedema and a tendency to failure of development and is very variable in its expres-

sion. The question is whether the syndrome is due to a lack of hybrid vigour, which would normally be found frequently in a diploid wild-type population, to the presence of certain lethal genes, active only in the haploid state, or to other causes. If it were due to the absence of hybrid vigour the syndrome would be expected to appear at equal intensity in all haploids. If, however, it were due to certain specific genes, all animals obtained by nuclear grafting from the same clone of nuclei would show the syndrome to the same extent. Dr. Hamilton reported that on performing this experiment haploid animals were obtained which showed some variation in the extent of the haploid syndrome. This result suggests that the genetic explanations suggested here are incorrect.

Gene action in development is, of course, expressed in the control of protein synthesis, and another session was devoted to protein synthesis in embryos. Dr. P. N. Campbell (London) introduced the next session by outlining the biochemical background and problems in the investigation of this topic in embryos. He pointed out that embryos might present a fruitful field for the investigation of control mechanisms since a number of morphogenetic interactions are known to involve substances, some of which are proteins, which alter the general types of synthesis in target cells and which may act directly on the genetic system.

Prof. J. Brachet (Brussels) described histochemical and autoradiographic work on the localization of protein synthesis and production of messenger RNA in the early stages of amphibian development. He discussed the ways in which localization of certain substances in certain parts of the embryo might bring about locally different types of synthesis, and also considered the possibility that part of the protein synthesis might be under the control of extranuclear DNA. The amphibian egg is remarkable in that at the start of development there is roughly 1,000 times as much DNA outside the nucleus as within it.

Prof. A. Monroy (Palermo) reported his experiments on the initiation of protein synthesis in echinoderm embryos immediately following fertilization. There appear to be two separate waves of protein synthesis, one of which appears at gastrulation, as in many other embryos; but the other starts immediately after fertilization and appears to be connected with the activation of the egg.

Prof. Flickinger (Buffalo) described his experiments on the priming of RNA synthesis in different parts of the amphibian embryo at gastrulation. He found that priming required DNA or chromatin and suggested that protein from chromatin might tend to act as an inhibitor. Using this system he found that the endoderm appeared to be most active in synthesis of RNA. This result is rather surprising in view of the fact that simpler techniques had suggested that the dorsal lip cells were most active in RNA synthesis. Dr. B. Mintz (Philadelphia) chaired a discussion group on the initiation of synthesis (in particular protein synthesis) in the mammalian egg. The chief speakers were Dr. C. Austin (Tulane) and Dr. D. Szollosi (Philadelphia).

Another important topic at this meeting was the subject of developmental neurology. Prof. V. Hamburger (St. Louis) spoke on his work on the demonstration of spontaneous intrinsic rhythmic activity in the spinal cord of chick embryos. By separating parts of the spinal cord from the brain very early in development, it was nevertheless possible, later, to obtain motor activity in the isolated portions of the cord. This result suggests that these activities can surprisingly develop independently of control from higher centres of the central nervous system. Prof. M. Singer (Cleveland, U.S.A.) reported on his work on the control of limb regeneration in amphibia and reptiles, and demonstrated that whether or not regeneration took place depended in part on a certain threshold of nerve mass in the stump being exceeded. The discussion group on developmental neurology was chaired by Dr. A. F. Hughes (Bristol). It opened with a review by Dr. Rita

Levi-Montalcini (St. Louis and Rome) on the sequence of her researches on the nerve growth factor. The two remaining main papers dealt with the problem of how the precise pattern of the wiring of the nervous system is established.

Dr. R. M. Gaze (Edinburgh) gave an account of his work on optic nerve regeneration in fish and amphibians, in which the restoration of the retinotopic projection of fibres of the optic nerve on to the optic tectum is charted by means of systematic retinal stimulation, and electrical recording from the tectum. Among other experiments, Dr. Gaze showed that if half the eye rudiment is removed in early *Xenopus* tadpoles and a corresponding half eye grafted in its place, then fibres from the original half retina ultimately may project over the whole of the tectum. On the other hand, after hemisection of the optic nerve in the adult goldfish, there is no evidence that the projection of the remaining optic fibres spreads beyond its own half of the tectum.

The third main speaker was Dr. George Szekely (Pécs), who described recent experiments on limb movement in both urodeles and birds in which heterotopic transplantation of regions of the spinal cord were made at early stages of development. The final pattern of limb movement is greatly influenced by the source of the grafted cord. Thus, if lumbar cord is grafted at brachial levels in the chick, the wings of the young adult bird are unable to flutter when the animal is allowed to fall. Dr. Harkmark, Dr. Drachman and Prof. Singer also contributed to this discussion.

Other speakers in the main sessions were Dr. O. Vyasov (Moscow), who reported work on the immunology of embryos, Dr. D. Yaffe Rehovoth, Israel, who described his experiments on the factors affecting muscle differentiation, and Dr. T. Humphreys (Cambridge, U.S.A.), who spoke on his experiments on factors controlling the specificity of sponge re-aggregation. Dr. N. Verdonk (Utrecht) and Dr. T. Dettlaff (Moscow) spoke on their work on early stages of molluscan and fish development respectively. Prof. Pasteels (Brussels) read a paper by Prof. J. Mulnard (who was unable to attend) on the effect of ooplasmic segregation on the development of trophoblast or embryo in the mammalian embryo.

Three other discussion groups were held. Dr. Goss (Providence, U.S.A.) introduced a discussion on the control of organ size. Dr. A. Coulombre (Bethesda, U.S.A.) talked on his work on the control of lens and retina growth, in which he had showed by operative procedures that interactions took place between lens and retina to control their growth. Prof. W. Bullough (London) spoke of his work on the control of mitosis by chalcones. In the second group, J. Dingle (Cambridge) introduced a discussion on the role of lysosomes in development. He suggested that, though the concept of the lysosome as a discrete intracellular organelle containing a number of hydrolytic enzymes has greatly stimulated biochemical work on the isolated particle, the functions of the lysosome are only slowly beginning to emerge. The participants in the discussion group on the role of lysosomes in development clearly emphasized the importance of lysosomal enzymes in the dynamics of tissue synthesis and degradation during embryonic development.

Dr. Scheib (Paris) presented electron micrographs demonstrating the presence of numerous autolysosomes in the regressing Müllerian duct. These bodies, which are a form of lysosome concerned with digestion of other organelles, were large and complex and contained high acid phosphatase activity. Prof. Weber (Berne) and Dr. Eeckhout (Louvain) showed lysosomal particles to be present in the metamorphosing tadpole tail; continued synthesis of lysosomal hydrolases, particularly of protease, was found to be important in the process of regression. That changes in lysosomal activity or stability might affect other cellular functions, notably cell division, was suggested by Dr. A. C. Allison (Mill Hill). The final

paper, by Dr. M. Prestige and Dr. A. Hughes (Bristol), dealt with the possibility that lysosomes may play a part in cell degeneration in the central nervous system.

The very active discussion of the papers emphasized the present interest in the role of lysosomes in pathology as well as in normal physiology and embryology.

The third group was chaired by Prof. E. Wolff (Nogent-sur-Marne); in the introduction to a discussion on interpretation of epigenetic malformations he pointed out that numerous physical or chemical factors, when applied at the same stage of development, produce the same types of malformations, such as cleft palates. On the contrary, other drugs have a specific teratogenic effect. The action of thalidomide on human fetuses has dramatically confirmed this point of view. The question arises of how these two different mechanisms can be explained.

Dr. W. Landauer (London) described his work on some drugs such as insulin. Dr. J. Ebert and Dr. M. Reporter (Baltimore) reported their work on the action of actinomycin A on differentiation of muscles *in vivo* and *in vitro*. This substance specifically inhibits the differentiation of the heart of the chick embryo *in vivo* and of muscle fibres in *in vitro* cultures. The authors extracted from the mito-

chondrial fraction of tissues of the adult chick (liver and muscles) a protein that prevents the teratogenic effect of actinomycin A. As a consequence this substance may be considered as inhibiting the formation of a protein essential in muscular differentiation.

Finally, Dr. Kirrmann (Paris) showed that X-rays—a non-specific teratogenic factor—affect general respiratory metabolisms, which result in a decrease of oxygen consumption of the tissues. This metabolic effect as well as the teratogenic action of X-irradiation can be prevented by anoxia or by substances—such as cysteamine—which are able to combine with free radicals arising in the tissues as a result of the ionization of water. So we can assume that the two different mechanisms of activity of teratogenic substances may be related to whether they affect general or specific metabolisms.

In addition some eighty demonstrations were presented by members of the conference, ranging over all branches of embryology. The conference was made possible by the generosity of the International Union of Biological Sciences, the Royal Society and by a number of industrial organizations. The eighth International Embryological Conference will be held in Berne, Switzerland, in 1967.

SEAWEED RESEARCH

THE fifth International Seaweed Symposium was held at Dalhousie University in Halifax during August 25–28. The purpose of these conferences has been to foster original research, both fundamental and applied, in the field of marine algae and to facilitate the presentation of such investigations. Previous symposia have been held at Edinburgh, Trondheim, Galway, and Biarritz, on a triennial cycle. In a programme of four invited lectures and 55 original communications, the topics were evenly divided between botanical, chemical, and applied aspects. A symposium within a symposium developed on the subject of algal polysaccharides.

In discussing the seaweed industry of the future, F. N. Woodward (Scotland) emphasized the possibilities of mass culture as a source of protein, the cultivation of areas for selected species as in Japan, the great need for mechanization in harvesting and drying, and the possibilities of further applications of the unique algal polysaccharides in agriculture, industry, and medicine. The use of mustard, *Sinapis alba*, as a test plant with which to assess growth response to seaweed extracts was described by Challen and Hemingway (United Kingdom). Stephenson (United Kingdom) reported specific effects from the use of liquid seaweed fertilizer as contributing to enhanced resistance of some field crops to frost, to pathological fungi, as *Botrytis* on strawberries, and to animal parasites, such as aphids on beans and sugar beets.

In a masterly lecture on recent studies of the polysaccharides of Agarophytes, Araki (Japan) described his investigations of the chemical constitution of agarose and agaropectin. Sulphate groups as half-esters were established in agaropectin only. The remarkable finding of variable but appreciable amounts (1–20 per cent) of 6-O-methyl-D-galactose in agarose was reported. Pyruvic acid was confirmed as a constituent of only two agaropectins. In agarose the molar ratio of D-galactose + 6-O-methyl-D-galactose to 3,6-anhydro-L-galactose was always unity.

Anderson and Rees (Scotland) have found that carrabiose units account for 88–99 per cent of the molecule of κ - and λ -carrageenans based on the products after methanolysis of native and alkali-treated material. A new, widely distributed, sulphated heteroglycan, isolated first from *Ascophyllum*, has been discovered by Larsen and Haug (Norway) and named ascophyllan. It contains fucose, xylose and a uronic acid. The uneven distribution of

mannuronic and guluronic acids in the main chain of alginic acid was postulated by Haug and Larsen (Norway) from investigations of partial hydrolysis with oxalic acid. The isolation of specific κ - and λ -carrageenases from *Pseudomonas carrageenovora* was reported by Yaphe *et al.* (Canada).

The finding of pure poly-N-acetylglucosamine as the only constituent of the extracellular fibres of the diatom, *Thalassiosira fluviatilis*, by McLachlan, McInnes, Falk and Craigie (Canada) provides the only instance in Nature of the occurrence of this substance in pure form. It has been renamed chitan. Two other unusual compounds were reported as occurring in marine algae—dimethyl- β -propiethetin and 2,3-dibromobenzyl alcohol-4,5-disulphate.

Black *et al.* (Scotland) described a survey of many of the Rhodophyceae as possible sources of the carrageenans. Marked variations in yield, ratio, optical rotation, and chemical composition were recorded. Sharp fractionation into κ - and λ -components with potassium chloride was not always achieved, for example, in *Eucheuma spinosum* and *Polyides rotundus*. Fleming, Hirst and Manners (Scotland) have found notable similarity in the soluble and insoluble forms of laminarin except that the former exhibits a greater degree of branching. Painter (United Kingdom) presented evidence that the sulphate group in the galactose of κ -carrageenan and fucellaran is more or less randomly distributed over all available hydroxyl groups in the carbohydrate chains and not only attached at C-4 as previously believed.

The unexpected property of some seaweeds to “fix” radioactive ions was described by Czapke (Poland) and Skoryna *et al.* (Canada). The present explanation is based on ion-exchange with soluble or insoluble alginate. This property was applied as an index of the radioactivity of the aqueous environment and as a means of inhibiting absorption of strontium-89 in the gastro-intestinal tract of animals.

In a well-illustrated study of the tissues of *Fucus*, McCully (United States) pointed out differences between the walls of the outer epidermal cells with their amorphous outer layer, the underlying parenchyma and the central medulla with its reticulum of branched primary filaments and secondary intrusive fibres, all these being embedded in a mucilaginous matrix. Observations with the light microscope, using specific stains and extended by elector

microscopy of adjacent sections, suggest that fucoidin is confined almost entirely to the mucilaginous matrix, while cellulose and alginic acid are in well-defined bands in the fibrillar portions of all the walls. A similar topic was treated by Baardseth (Norway), who examined the localization and structure of alginate gels from *Ascophyllum*. He believes that alginates exist mainly as intercellular substances at all stages of development, and he finds no evidence for two walls in brown algae, a primary wall of alginate and a secondary wall of cellulose.

Chapman (New Zealand) discussed the physiological ecology of seaweeds in a general lecture. For a number of the macroscopic algae which are found on the coasts of Australia and New Zealand, Chapman showed how physical factors which affect their distribution can also effect morphological variations. He gave many experimental data suggesting that changes in light, temperature, depth, desiccation, etc., could influence the ability of the algae concerned to function at their optimum and hence could be responsible for the limits of distribution.

Kain (United Kingdom) showed that the lower level of *Laminaria hyperborea* in the Isle of Man may be determined partly by biotic factors: removal of colonies of *Echinus esculentus* from the lower limits of beds of *L. hyperborea* and *Saccorhiza polyschides* has resulted in new algal growth over rocks at a level which remained bare when *Echinus* was present. Leighton, Jones and North (United States) have developed a method of treatment with quicklime to control the infestation of beds of *Macrocystis* by such sea-urchins in the Pacific Ocean. Many phaeophyceean genera are known to produce tannins, and Conover and Sieburth (United States) gave evidence to show that these metabolites may influence the ability of certain organisms to grow in confined bodies of water.

In a session on specialized botanical topics, Anderson and North (United States) described ways in which estimates of the extent of sporing in *Macrocystis* have been made *in situ*. For isolated plants, sporlings have been found up to 5 m away, which suggests that the spores sink relatively quickly and do not wash about in the sea for any great length of time. Conway (Scotland) described the extensive sporing of species of *Porphyra* and the microscopic perennating phases into which the spores develop. Such phases may be of considerable ecological importance to this widely distributed genus. Aziz (Pakistan) reported,

for the first time, sexual reproduction in *Acrochaetium liagorae* Borg.

In one of two sessions devoted to the algal flora of the Antarctic, Lamb (United States) described the pattern of distribution seen in the supra-littoral, littoral and infra-littoral zones at the Argentine base at Melchior. Early in the southern summer almost no vegetation is visible on the littoral rocks. Once colonization begins in the gullies, development is very rapid and spreads across the zone. Examination of the infra-littoral by SCUBA diving showed marked zonation with some of the major algae, including *Desmarestia*, down to 30 m. Zaneveld (United States) described surveys carried out along the coasts of Ross Island and Victoria Land, Antarctica, often through enlarged seal holes. These showed that large algal beds were present at depths between 6 and 35 m, with red algae, such as *Phyllophora antarctica*, *Iridaea obovata* and *Phycodrys quercifolia*, as the dominant species. Light measurements emphasized the paucity of light in such a habitat (about 1.1 per cent of the surface values) and showed that benthic algae are capable of photosynthetic production under fast ice with a coverage of 9–10 months.

Friedmann (Israel) described the occurrence in Mediterranean waters of numerous gametophytes of *Padina pavonica* and of the Indo-Pacific species *P. gymnospora*. Much variation in the occurrence of bisexual and unisexual forms is found and there are indications that the variation in different populations is controlled by ecological factors. Moss (United Kingdom) reported experimental work which indicates the importance of the large apical cell in *Fucus vesiculosus*. Round (United Kingdom) has observed rhythms in migration of *Euglena obtusa* and of certain diatoms and the effect of varying the normal day-length pattern.

Most of the papers presented will appear in the official proceedings to be published as a separate volume by the Pergamon Press of London in 1966. There were 220 at the symposium who registered from 25 different countries. During the symposium a meeting of the International Phycological Society was held. The sixth symposium will be held in Spain in 1968, according to present plans. A permanent secretariat for the symposia has been set up in charge of Mr. E. Booth, Institute of Seaweed Research, Inveresk, Midlothian, Scotland.

E. CONWAY
E. G. YOUNG

A SUGGESTED PHENOTYPIC CLASSIFICATION AND TERMINOLOGY FOR ENZYME MUTANTS IN MICRO-ORGANISMS

By DR. J. F. COLLINS*, DR. J. MANDELSTAM, PROF. M. R. POLLOCK, F.R.S.*, DR. M. H. RICHMOND* and DR. P. H. A. SNEATH†

National Institute for Medical Research, Mill Hill, London

THERE is now a large and increasing number of different types of enzyme mutant being isolated among micro-organisms. The very wide range of activity levels and degrees of induction manifested emphasizes the need for some general phenotypic classification, based on enzymatic function, for indicating their physiological properties.

A convenient scheme can be obtained by classifying strains both according to their induction response and according to the maximum level of enzymatic activity expressed (Table 1). It is suggested here that four grades of performance with respect to each of these two independently variable functions (giving sixteen different

phenotypes) would, in this respect, cover the essential properties of most strains ever likely to be recognized. In this scheme, the various categories are classified, purely on the basis of their phenotypic characters, by reference to the basal and maximally induced enzyme activities of the parent wild-type strain of micro-organism. 'Non-inducible' thus corresponds to 'constitutive' in the sense originally used by Karstrom¹; the definition depends, therefore, on absence of induction response and not on level of enzyme activity.

Certain adjustments and additions to the symmetrical scheme that emerges from the foregoing considerations have inevitably arisen. It is scarcely practicable, for example, to abandon the term 'constitutive', which is well-established, and replace it by 'non-inducible'. But it is reasonable to suggest that it be used in its original

* Present address: Department of Molecular Biology, University of Edinburgh.

† Present address: Medical Research Council Microbial Systematics Research Unit, University of Leicester.

sense of non-inducible and not be restricted (as some workers would doubtless still insist) to strains where a certain 'high' level of enzymatic activity is reached without induction. For similar reasons, it is probably preferable to retain the term 'semi-constitutive' rather than change to 'semi-inducible'. But in that case it would be essential—in conformity with the definition of 'constitutive' used here—not to restrict its use (as previously²⁻⁴) to one particular type of 'semi-inducible'—namely, strains with raised basal activity and 'magnol' levels after induction^{3,4}. Moreover, to use the full terminology set out in Table 1 would be absurdly cumbersome.

The various categories finally suggested are therefore classified in a more practical and realistic form as follows.

(I) FULLY INDUCIBLE. Normal (that is, wild-type (WT)) induction ratio. (a) Magno-inducible: max. induced level similar to max. induced WT level. (b) Meso-inducible: max. induced level less than max. induced WT level but greater than uninduced (basal) WT. (c) Micro-inducible: max. induced level equal to, or less than, basal WT level. (d) Inducible 'Hypers': max. induced level above max. induced WT level.

(II) STRICTLY CONSTITUTIVE (= 'non-inducible' in Table 1). Induction ratio = 1.0. (a) Magno-constitutive: level formed by most constitutive strains (normally rather greater than max. level of maximally induced magno-inducible WT). (b) Meso-constitutive: less than magno-constitutive level but greater than basal WT level. (c) Micro-constitutive: equal to (= 'baso-constitutive'), or less than, basal WT level. (d) Constitutive 'Hypers': greater than level attained by the common ('magnol') constitutives.

(III) SEMI-CONSTITUTIVE (= 'semi-inducible' in Table 1). Induction ratio greater than unity but less than WT.

(IV) SUPER-INDUCIBLE. Induction ratio greater than WT.

(V) NEGATIVE. No detectable enzymatic activity, with or without induction.

Examples of all the classes mentioned in the foregoing list, with the exception of the 'super-inducibles' (included for logical symmetry), are known in one or more of the systems already being examined.

Within the 'micro-constitutive' group a sub-section ('baso-constitutives') is suggested for strains producing levels corresponding to normal wild-type uninduced activity. The reason for this is that in the penicillinase series so far examined in this laboratory there appears to be a possibly significant degree of 'clustering' around this level. It was considered that among micro-constitutives the complete inability to respond to induction from the basal WT level (six out of thirteen in a current series of penicillinase mutants of *Bacillus licheniformis*) might reflect some significant physiological and/or genetic property (cf. also the *i^s*-super-repressed mutants in the *E. coli* β -galactosidase system⁵).

All comparisons are intended to be with respect to corresponding WT inducible strains or 'usual' magno-constitutive mutants (as defined in II (a)). Induced levels are measured after treatment with one particular inducer under standard conditions. Margins for significant

differences permitting relegation to the micro- and meso-groups must depend on assay techniques and reproducibility of enzyme titres in the particular system concerned. 'Hyper' strains (both inducible and constitutive) have already been isolated and described for the *E. coli* β -galactosidase system⁶. A separate group comprising all strains with no detectable enzymatic activity, even after treatment with likely inducer ('negatives'), must also be included, since they cannot logically be classified in any of the other groups. The other categories are self-explanatory.

It is, of course, recognized that such a classification cannot possibly persist indefinitely and that individual strains may have to be re-classified as techniques change and information accumulates. For example, increased sensitivity of enzyme assay methods may obviously lead to 'promotion' of a negative into a micro-inducible or micro-constitutive. Transfer of a micro-constitutive to the inducible class may be demanded by the discovery of a permease system or a completely new type of inducer. The existence or emergence of a new or unsuspected type of induction specificity is always a particularly tricky problem, since the original choice of inducer and induction conditions must inevitably be partly arbitrary. Strains may also change their category according to conditions. For example, mutants with properties consistent with the presence of a thermolabile 'repressor' have been isolated in both the β -galactosidase⁷ and penicillinase⁸ systems, being semi-constitutive at 50° C and magno-inducible at 35° C. Some groups may eventually need further subdivision, particularly where multiple superimposed mutations may have occurred.

It might further be argued that yet another class should be created for mutants where formation of the enzyme is specifically inhibited, instead of stimulated, by the standard inducer, and which would therefore give 'induction' ratios of less than unity. Such mutations have indeed been reported^{4,5}, and might perhaps be predicted on the Jacob-Monod⁹ hypothesis. In one sense, however, these would fall into the class of repressible systems. A formally analogous classification and terminology could indeed also be logically applied to normal repressible enzyme systems; 'irrepressible' being substituted for 'constitutive', 'derepressible' for 'inducible' and 'repression ratio' for 'induction ratio'.

The foregoing main scheme is based on one outlined eight years ago⁸ for classification of the *B. cereus* penicillinase systems. This was subsequently expanded for categorization of penicillinase mutants in staphylococci⁹, and further elaborated for similar mutants in *B. licheniformis*⁸. It is only strictly applicable to a series of mutant strains derived from the normal wild-type or the 'usual' magno-constitutive mutant, which can serve as reference standards. But it would seem possible also to use it (provisionally) for closely related wild-type strains within a species, taking the most active enzyme producer as a standard, so long as the arbitrary nature of the comparison was properly appreciated.

There is barely need to emphasize that, being based purely on phenotypic characters, the scheme will cut right

Table 1. SCHEME FOR PHENOTYPIC CLASSIFICATION OF ENZYME MUTANTS ACCORDING TO ENZYMATIC ACTIVITIES AND INDUCTION RESPONSE

Maximum induced level of activity		Induction ratio:*			
		= WT	= 1.0	> 1.0 < WT	> WT
		Inducible	Non-inducible	Semi-inducible	Super-inducible
= MWT	Magno-	Magno-inducible	Magno-non-inducible	Magno-semi-inducible	Magno-super-inducible
> BWT	Meso-	Meso-inducible	Meso-non-inducible	Meso-semi-inducible	Meso-super-inducible
< BWT	Micro-	Micro-inducible	Micro-non-inducible	Micro-semi-inducible	Micro-super-inducible
> MWT	-hyper	Inducible-hyper	Non-inducible-hyper	Semi-inducible-hyper	Super-inducible-hyper

WT, wild-type value. MWT, maximally induced wild-type level. BWT, uninduced (basal) wild-type level.

* Induction ratio = $\frac{\text{Differential rate of enzyme formation under defined standard conditions for maximal induction}}{\text{Differential rate of enzyme formation without inducer}}$

across any genotypic classification. The definitions are operative in relation simply to enzymatic activities and the response to induction. Indeed, its main value should be that it is not prejudicial to any particular type of further physiological or genetic analysis. For example, a negative or micro-constitutive may obviously arise either from a structural gene mutation, yielding an altered enzyme of low specific activity or from a 'polarity' mutation¹⁰ in an unknown location, which decreases the spontaneous rate of formation of normal (WT) enzyme. After all, most enzyme systems already capable of being examined in some detail phenotypically are not yet amenable to genetical analysis. Even in the classical enzyme systems of *E. coli*, the genetics remains controversial or obscure at many points.

Mutant Proteins ('Muteins')

In many instances, however, further physiological characterization, which may help in determining the genotype, can be achieved relatively easily. For example, it has already been suggested¹¹ that in strains where the mutation has resulted in the formation of an altered protein, analogous to the normal wild-type version, this product be referred to as a 'mutein'. This term may also help to avoid present difficulties^{12,13} arising over the use of the term 'CRM' (= 'cross-reacting material') as originally defined¹⁴. 'Muteins' may be presumed usually to arise from a gene mutation resulting in one or more amino-acid substitutions in one or more of any of the

polypeptide chains which constitute the specific molecule. For the moment, however, it might be preferable to allow the term to refer to any stable structural alteration, of mutational origin, within the molecule, and not restrict it to the product of any specific type or locus of mutation. Muteins may or may not possess any of the characters—enzymological, immunological or physico-chemical—of the corresponding wild-type protein. Their existence and recognition do not, therefore, depend on retention of immunological reactivity with anti-wild-type protein antiserum. Thus they need not necessarily be 'CRMs'.

¹ Karstrom, H., *Ergeb. Enzymforsch.*, **7**, 350 (1957).

² Pollock, M. R., in *Drug Resistance in Microorganisms*, 78, Ciba Found. Symp. (Churchill, London, 1957).

³ Dubnau, D. A., and Pollock, M. R., *J. Gen. Microbiol.*, **39**, 195 (1965).

⁴ Richmond, M. H., *Brit. Med. Bull.*, **21**, 260 (1965).

⁵ Willson, C., Perrin, D., Cohn, M., Jacob, F., and Monod, J., *J. Mol. Biol.*, **8**, 582 (1964).

⁶ Novick, A., and Horuchi, T., *Cold Spring Harb. Symp. Quant. Biol.*, **26**, 239 (1961).

⁷ Horuchi, T., and Novick, A., *Cold Spring Harb. Symp. Quant. Biol.*, **26**, 247 (1961).

⁸ Jacob, F., and Monod, J., *J. Mol. Biol.*, **3**, 318 (1961).

⁹ Novick, R. P., *J. Gen. Microbiol.*, **33**, 121 (1963).

¹⁰ Ames, B. N., and Hartman, P. E., *Cold Spring Harb. Symp. Quant. Biol.*, **28**, 349 (1963).

¹¹ Pollock, M. R., Fleming, C., and Petrie, S., Proc. in Coll. *Antibodies to Biologically Active Molecules*, second meeting European Fed. Biochem. Soc., Vienna, 1965 (in the press).

¹² Kaplan, S., Ensign, S., Bonner, D. M., and Mills, S. E., *Proc. U.S. Nat. Acad. Sci.*, **51**, 872 (1964).

¹³ Suskind, S. R., Wickham, M. L., and Carsotlis, M., *Ann. N.Y. Acad. Sci.*, **108**, 1108 (1963).

¹⁴ Suskind, S. R., Yanofsky, J., and Bonner, D. M., *Proc. U.S. Nat. Acad. Sci.*, **41**, 577 (1955).

CHARACTERIZATION OF A TRANSFER FACTOR ASSOCIATED WITH DRUG RESISTANCE IN *Salmonella typhimurium*

By DR. E. S. ANDERSON and DR. M. J. LEWIS

Enteric Reference Laboratory, Public Health Laboratory Service, Colindale Avenue, London, N.W.9

IN a previous article¹ we reported the results of an investigation of multiple drug resistance in phage-type 29 of *Salmonella typhimurium*. This type has become predominant in bovine infection in Britain, and has caused many human infections which are almost certainly bovine in origin. We believe that its prevalence in cattle is due to a combination of two major factors: low standards of hygiene in intensive farming, in cattle markets, and in the transport of animals on the one hand; and on the other the widespread use in animal husbandry of antibiotics and synthetic anti-bacterial drugs. The first factor predisposes to the spread of infection in calves, a spread amplified by the distribution of infected animals by dealers; the second ensures the emergence and maintenance of drug-resistant strains of *S. typhimurium*. This article presents a more detailed analysis of drug-resistance in phage-type 29 and discusses the significance of our findings both in relation to the systems we have examined, and to drug resistance transfer in general in the Enterobacteriaceae.

We shall use the same notation for resistance to the various drugs as in our earlier report¹. This is: *A*, ampicillin; *S*, streptomycin; *Su*, sulphonamides; and *T*, tetracycline.

Our previous observations may be summarized as follows: phage-type 29 had apparently acquired its drug resistance in the following order: streptomycin and sulphonamides (these seem to be linked); tetracycline; ampicillin. Attention was concentrated principally on strains showing this complete pattern of resistance, which was originally designated *A S T Su*. The level of resistance to all these drugs was higher than that found earlier in phage-type 1a of *S. typhimurium*², which had an identical spectrum of resistance. Moreover, it was found

that each component of the spectrum in type 29 (assuming *S Su* to be single) was transferred independently in crosses with *Escherichia coli* K12F⁻, in contrast to type 1a, in which the complete resistance pattern was transferred simultaneously. When ampicillin resistance was lost spontaneously in type 29, resistance to streptomycin, sulphonamides and tetracycline was retained. This again differed from the behaviour of type 1a, where loss of ampicillin resistance was accompanied by loss of resistance to all the other drugs. The transferable resistance factors (*R*-factors) of type 29 were designated *A'*, *S'* and *T'*. Their frequencies of transfer from type 29 to K12 in mating mixtures incubated for 24 h were approximately: *A'* 2×10^{-2} , *S'* 10^{-2} , *T'* 2×10^{-6} . Joint transfer of *A'* and *S'* took place at a frequency of 2×10^{-3} , which is a log unit higher than would be expected if these factors were completely independent. *T'* was not transferred jointly with *A'* or *S'*, nor was joint transfer of all three factors encountered. *S'*, *T'* and *A'* were sequentially transferred into a drug-sensitive strain of type 29 from K12 clones to which the single factors had originally been transferred from the wild resistant strain of type 29. In this way the *A' S' T'* resistance spectrum of type 29 was 'synthesized' artificially, and the possibility of step-wise acquisition of resistance in the wild state was confirmed.

R-factors are postulated to consist of two components: the genetic fragment determining the specific drug resistance, designated the resistance determinant (*R*-determinant); and the resistance transfer factor (*RTF*), the episome which confers autonomy on the complex and mediates the conjugation resulting in transfer. During transfer the *R*-determinant and the *RTF* move jointly, and infectivity of the resistance is maintained. The *R*-factor replicates independently of, and faster than, the

bacterial chromosome and it is believed that several copies of it exist in each infected cell.

In extending our investigation we considered the possibility that in nature, in the animal intestine, for example, *R*-factors might be passing continuously between Enterobacteriaceae. Thus, *Salmonellae* might acquire *R*-factors from *E. coli* and might transfer them to other *E. coli*, which might then pass them to other *Salmonellae* or to other *E. coli*, and so on. In other words, we thought of the possibility of 'cycles' of transfer of resistance, and we decided, using *S. typhimurium* of several phage-types and *E. coli* K12^F— as the bacterial hosts, to examine the results of repeated 'cycling' of the *R*-factors *A'*, *S'* and *T'* between these hosts, in order to determine what information could be gained about the operation of *R*-factors by this procedure.

The following notation has been devised to describe the characters of the strains. The description starts with the designation of the strain, that is, the phage-type in the case of *S. typhimurium*, K12 in the case of *E. coli* K12^F—. This is followed by the symbol of the drug resistance, for example, *A'* for high resistance to ampicillin. Cycles of transfer are designated in steps of 0.5 and the respective stage is marked as a subscript to the resistance symbol. The wild donor strain is regarded as zero in the series of transfer cycles, so that, for example, ampicillin resistance transferred from wild resistant type 29 to K12 appears in the K12 progeny as *A'*_{0.5}. Thus, K12*A'*_{0.5} is K12 which has received *A'* from the wild type 29*A'S'T'*; and type 29*A'*_{1.0} is wild sensitive type 29 that has received *A'* from K12*A'*_{0.5}. K12*A'*_{1.5} has received *A'* from type 29*A'*_{1.0}; and type 29*A'*_{2.0} has in turn received *A'* from K12*A'*_{1.5}; and so on. In some instances individual *R*-factors in a single clone represent different transfer cycles, and this is indicated. For instance, K12*A'S'*_{0.5} would be K12 that had received *A'* direct from the wild resistant type 29 and *S'* from type 29*S'*_{1.0}. When *R*-factors are transferred jointly in a cross, the cycle number is placed at the end of their designation only. Thus, K12*A'S'*_{0.5} indicates the joint transfer of ampicillin and streptomycin-sulphonamide resistance to K12 from wild resistant type 29.

Liquid medium (Difco broth) for the growth of cultures and mating mixtures contained 20 g of Bacto dehydrated nutrient broth (Difco Laboratories) and 8.5 g of sodium chloride/l. Solid medium for the routine growth of cultures contained 13 g of New Zealand powdered agar per litre of Difco broth. 'Bacto' MacConkey agar (Difco Laboratories) was used throughout to differentiate between *S. typhimurium* and K12 in mating mixtures.

We effected great technical economy in examining the results of crosses by streaking successive dilutions of mating mixtures with a standard loop delivering a volume close to 0.01 ml. This enabled us to inoculate the complete series of dilutions, 10⁰ to 10⁻⁷, in parallel streaks on two plates, the first with 10⁰ to 10⁻³, the second with 10⁻⁴ to 10⁻⁷. Each plate was streaked in duplicate and the results used were the mean of the two plates. This method is not so statistically accurate as using separate spread plates and larger volumes for each dilution, but it indicates orders of magnitude of transfer frequencies with acceptable precision to about 10⁻⁷, is reproducible, and the results were found to agree closely with those in which separate spread plates were used for each dilution.

When the donor strain was *S. typhimurium* it was eliminated from the mating mixture with phage 01 (refs. 1 and 4), and this phage was used for the same purpose in interrupted mating experiments. When the donor was K12 it was eliminated with colicine *E*2, which reduces K12 to about 10⁻⁵ to 10⁻⁶ of its original count. For this elimination of donor cells, 0.3 ml. of phage 01 (titre > 10¹¹ particles/ml.) or of colicine *E*2 (titre 1:32), as required, was spread on the MacConkey plates before they were streaked with the mating mixtures.

With the exception of interrupted mating experiments, crosses were carried out by mixing donor and recipient

cultures grown for 6–8 h at 37° C with agitation, incubating overnight, and streaking decimal dilutions of the mixture on MacConkey medium containing the respective anti-bacterial drugs in the concentrations previously specified¹ on the following day. Controls were always included of the separate cultures tested on the anti-bacterial drugs and inoculated with phage 01 or colicine *E*2, as appropriate. The separate donor and recipient cultures and the mating mixtures were also plated on MacConkey medium devoid of drugs; these plates were designated 'unselected' because of the absence of drugs, and were of great value in later experiments.

Transfer frequencies were calculated in terms of the final total count of the recipient strain in the mating mixture.

The controls of sensitive recipient strains on medium containing drugs gave the same result throughout, that is, no resistant colonies were found.

The first cross in the present series was type 29*A'*_{1.0}*S'*_{1.0}*T'*_{1.0} × K12. The results of this cross are shown in Table 1, together with those of the cross of wild type 29*A'S'T'* × K12 (see ref. 1).

Table 1. COMPARISON OF CROSSES OF WILD-TYPE 29*A'S'T'* × K12 AND TYPE 29*A'*_{1.0}*S'*_{1.0}*T'*_{1.0} × K12

Donor × recipient	Progeny	Frequency
29 <i>A'S'T'</i> × K12	K12 <i>A'</i> _{0.5}	2.5 × 10 ⁻³
	K12 <i>S'</i> _{0.5}	1.0 × 10 ⁻³
	K12 <i>A'S'</i> _{0.5}	2.0 × 10 ⁻³
	K12 <i>T'</i> _{0.5}	2.0 × 10 ⁻⁴
	K12 <i>A'S'T'</i> _{0.5}	2.0 × 10 ⁻³
29 <i>A'</i> _{1.0} <i>S'</i> _{1.0} <i>T'</i> _{1.0} × K12	K12 <i>A'</i> _{1.5}	5.0 × 10 ⁻³
	K12 <i>A'S'</i> _{1.5}	7.0 × 10 ⁻⁴
	K12 <i>T'</i> _{1.5}	4.0 × 10 ⁻⁴
	K12 <i>A'S'T'</i> _{1.5}	4.0 × 10 ⁻⁴

K12, *Escherichia coli* K12^F—

Type 29, *Salmonella typhimurium* phage-type 29.

29*A'S'T'*, wild resistant type 29.

29*A'*_{1.0}*S'*_{1.0}*T'*_{1.0}, type 29 into which the *R*-factors were introduced in three separate steps from K12*A'*_{0.5}, K12*S'*_{0.5} and K12*T'*_{0.5}, respectively.

Several interesting features are apparent in Table 1. The most striking is the increase in the frequency of transfer of *T'* from 2.0 × 10⁻⁶ in the 0.5 cycle to 4.0 × 10⁻⁴ in the 1.5 cycle. The second is the high degree of association apparent in the 1.5 cycle between *A'* and *T'* on one hand and *S'* and *T'* on the other, an association absent in the 0.5 cycle. The third is the frequency of 7.0 × 10⁻⁴ of joint transfer of all three resistances in the 1.5 cycle, in contrast to the complete absence of this class in the 0.5 cycle. However, if we ignore *T'* transfer, it is apparent that the transfer frequency of *A'* and *S'* alone or together is about the same in the 1.5 cycle as it was in the 0.5 cycle.

In order to distinguish between the efficiently moving tetracycline *R*-factor demonstrated in the 1.5 cycle and the much slower-moving tetracycline *R*-factor in the wild type 29*A'S'T'*, we designated the more rapidly moving factor *T''*, in contrast to *T'* found in the wild strain. The two factors conferred the same degree of tetracycline resistance, that is, the minimal inhibitory concentration of the drug in liquid medium was 250 µg/ml. with *S. typhimurium*, and 125 µg/ml. with K12. The symbol *T''* will now be used from K12*T''*_{0.5} onwards.

Bearing in mind the high rate of transfer of *T''* alone (4.0 × 10⁻⁴), it might be thought that the explanation for its presence in most cells receiving *A'* or *S'* or *A'S'* is that a high proportion of cells finally containing *A'* or *S'* would receive *T''*, even if the latter were unlinked to *A'* or *S'*. This hypothesis was tested by carrying out a cross of type 29*A'*_{1.0}*S'*_{1.0}*T''*_{1.0} × K12, interrupted from zero time onwards. The results of this experiment, which will be given in detail later, showed that the total frequency of transfer of *A'* at zero time was 10⁻⁴, that the majority of colonies selected on penicillin were of the type *A'T''*, but that about 10 per cent were pure *A'* colonies. Similarly, *S'*, which usually transfers more slowly than *A'* (ref. 1), appeared alone in about 10 per cent of colonies selected on streptomycin at 60 min. Pure *A'* and *S'* colonies were isolated up to 4 h after mixing donor and recipient cultures, but were no longer found among colonies selected (46 in each case) on penicillin and streptomycin plates after mating was continued for a further 20 h. The classes

then identified on penicillin selection were: $A'T''$ (46/46); and on streptomycin selection $S'T''$ and $A'S'T''$ (39/46 and 7/46 respectively).

It is thus apparent that the association between T'' on the one hand and A' or S' on the other is present from zero time, which leads to the conclusion that it is due to linkage between T'' and A' , or S' , in the 1.5 cycle, a linkage absent in the 0.5 cycle. Because of the transfer of the three R -factors alone, however, this experiment also indicates either that each factor segregates frequently from linkage with the other two, or that each exists independently as well as in combination in the donor strain $29A'_{1.0}S'_{1.0}T''_{1.0}$. If the latter suggestion is correct, the possible independent R -factor groupings available for transfer in this donor strain then become: A' ; S' ; T'' ; $A'S'$; $A'T''$; $S'T''$ and $A'S'T''$. Of these, only the class $A'S'$ was not identified in the recipient strain of K12 in this experiment, although it has been found in the progeny of later crosses.

The transfer of A' , including classes in which it was transferred jointly with T'' , or with S' and T'' , was 10^{-4} at zero time and reached 2.0×10^{-3} in 2 h; it remained unchanged between 2 and 4 h, and did not greatly increase after overnight contact. Pure T'' transfer reached a frequency similar to that of A' after 2 h, but greatly increased overnight to attain a figure close to unity. Although $A'T''$ transfer predominated over that of A' at zero time, its relative frequency increased between 4 and 24 h. Similar observations to those with A' and $A'T''$ were made with S' and $S'T''$, where a relative increase in frequency of the $S'T''$ class was also apparent between 4 and 24 h.

The next cross was $K12A'S'T''_{1.5} \times$ wild sensitive type 29, which is shown in Table 2.

Table 2. CROSS OF $K12A'S'T''_{1.5} \times$ WILD SENSITIVE TYPE 29

Selected on	No. tested	Progeny (all type 29) (2.0 cycle)	Frequency
P (penicillin)	20	19 $A'T''$ 1 $A'S'T''$	10^{-2}
S (streptomycin)	20	15 $S'T''$ 5 $A'S'T''$	7.0×10^{-3}
T (tetracycline)	20	All T''	7.6×10^{-1}
PS	20	All $A'S'T''$	1.1×10^{-2}
PT	20	19 $A'T''$ 1 $A'S'T''$	1.2×10^{-2}
ST	20	14 $S'T''$ 6 $A'S'T''$	8.0×10^{-3}
PST	20	All $A'S'T''$	1.3×10^{-2}
Unselected	50	31 T'' 1 $A'T''$ 18 sensitive	6.2×10^{-1} 2×10^{-2}

It can be seen from Table 2 that the results of this cross (2.0 cycle) are qualitatively similar to those of the 1.5 cycle of type 29 \times K12 (Table 1) that is, that there is a high degree of linkage between T'' on the one hand and A' or S' on the other, and the T'' transfer frequency is very high at c. 7.0×10^{-1} .

An interrupted mating experiment with the same donor and recipient strains established that recipients of the classes $29A'_{2.0}$ and $29S'_{2.0}$ were detectable at early stages of the cross, but that linkage of A' and S' with T'' again predominated. An unexpected finding was that, in the progeny of this cross, the class $29A'_{2.0}$ would not transfer A' to K12, although four lines of $29A'_{2.0}$ were tested for this transfer. Superficially, then, $29A'_{2.0}$ is indistinguishable from a drug-resistant mutant. It retains its high ampicillin resistance and produces abundant β -lactamase. The natural conclusion is that $29A'_{2.0}$ cells do not carry the RTF , but the question then to be solved is whether the A' determinant has become integrated into the bacterial chromosome or whether it is cytoplasmic but autonomous. In exploring this phenomenon further, we tested $K12A'_{1.5}$ (Table 1) to find that this was also unable to transfer to type 29. Bearing in mind that the cross $K12A'S'T''_{1.5} \times$ type 29 yielded the class $29A'_{2.0}$, and also that all cycles after 1.0 showed a high degree of association between T'' and A' , it seemed probable that if we transferred T'' into the 'infertile' $29A'_{2.0}$, the A' R -determinant would be

re-mobilized. This expectation was fulfilled, as Table 3 shows.

Table 3. MOBILIZATION OF $A'_{1.0}$ IN TYPE 29 WITH T'' FROM $K12T''$

Cross Donor \times recipient	Selected on	Progeny	Frequency
$29A'_{1.0} \times K12$	P	—	$< 10^{-7}$
$K12T''_{0.5} \times 29A'_{1.0}$	T	$29A'_{1.0}T''_{1.0}$	2×10^{-1}
$29A'_{1.0}T''_{1.0} \times K12$	P	$K12A'T''_{1.0}$ $K12T''$	10^{-2} $\sim 10^0$

It is evident that, once mobilized by T'' , the A' determinant was transferred to K12 with the frequency it has maintained from our first experiments, that is, about 10^{-2} . In transfer of the mobilized A' into K12, only the class $A'T''$ was identified, but preceding experiments show that this is to be expected since, in overnight crosses with $A'T''$ donors, the A' class is easily detectable only at an early stage of conjugation. It may be concluded from this experiment that, although the introduction of T'' results in the restoration of the transferability of A' , the rate of A' transfer is independent of T'' and is probably a function of the complex formed by A' and the RTF . The mobilizing activity of T'' may thus be due to a close association with the RTF , and the high frequency of coincidental transfer of T'' with A' would be an expression of this association. When T'' and A' are transferred simultaneously, therefore, it is unnecessary to postulate a linkage between them other than their attachment to the same RTF unit.

An examination of S' yielded similar results, although it was found that the loss of the RTF by S' occurred earlier than with A' , since three out of four lines of $29S'_{1.0}$ tested would not transfer S' to K12. Table 4 shows the mobilization of S' by T'' in type $29S'_{1.0}$.

Table 4. MOBILIZATION OF $S'_{1.0}$ IN TYPE 29 WITH T'' FROM $K12T''_{0.5}$

Cross Donor \times recipient	Selected on	Progeny	Frequency
$29S'_{1.0} \times K12$	S	—	$< 10^{-7}$
$K12T''_{0.5} \times 29S'_{1.0}$	T	$29S'_{1.0}T''_{1.0}$	5×10^{-1}
$29S'_{1.0}T''_{1.0} \times K12$	S	$K12S'T''_{1.0}$ $K12S'T''_{1.0}$	10^{-3}
	T	$K12T''_{1.0}$	7.0×10^{-1}

Although the $S'T''$ class predominated in recipient colonies selected on streptomycin, S' alone was detected in a small minority of colonies. The total transfer frequency of S' (including lines in which it was jointly transferred with T'') to K12 was 10^{-2} , slightly lower than that observed in transfer from wild type $29A'S'T''$ to K12. As with A' transfer, it can be concluded that the transfer rate of S' is characteristic of the $S'-RTF$ attachment and is independent of T'' , which appears with S' only because of its coincidental attachment to the same RTF unit.

At this point we introduced type 14 of *S. typhimurium* into the investigation. This type is commonly associated with avian infection, while type 29 is at present predominantly bovine. As drug resistance in *S. typhimurium* is apparently less of a problem in fowls than in cattle, we thought it worthwhile to determine whether type 14 was less able to pick up and to transfer resistance than was type 29. $K12A'S'T''_{1.5}$ (Table 1) was chosen as the donor in the first cross with type 14. The resulting *S. typhimurium* progeny were tested for drug resistance and transfer, and were phage-typed. An analysis of our findings in these experiments is given in Table 5.

Several conclusions may be drawn from a study of Table 5. First, it is evident that transfer of resistance takes place into and out of type 14, so that complete 'infertility' is not the reason for the low incidence of drug resistance in this type. Second, the transfer of T'' to type 14 shows a much lower frequency than that encountered when the same donor is crossed with type 29, indicating that different strains may vary in their efficiency as recipients of R -factors. Third, the patterns of transfer conform to those already found in type 29 (the absence of the class $14S'_{2.0}$ is due to the examination of insufficient colonies from the selection on streptomycin). Fourth, in most instances the acquisition of resistance was accompanied by a change of phage-type; the resulting type

Table 5. TRANSFER OF DRUG RESISTANCE INTO AND FROM *S. typhimurium* PHAGE-TYPE 14

Cross	Donor × recipient	Progeny	Frequency	Phage-type
K12 A'ST _{1.5} × 14		14 A' _{0.5}	5 × 10 ⁻⁴	14*
		14 A'T _{0.5}		29
		14 A'ST _{0.5}		29
		14 S'T _{0.5}		29
K12 A' _{0.5} × 14		14 A' _{1.0}	3 × 10 ⁻³	29
		14 T _{0.5}		29
K12 T _{0.5} × 14		14 A' _{1.0}	3 × 10 ⁻³	29
		14 T _{1.0}		29
14 A'T _{0.5} × K12		Unselected (50 colonies)	All sensitive	1 = 29
		K12 A'T _{0.5}	to T	49 = 14
		K12 T _{0.5}	6 × 10 ⁻¹	—

* One colony only, unable to transfer A'.

is designated '29' because, although its phage-typing reaction is closer to 29 than to any other type, it is nevertheless distinguishable from 29. Fifth, this change of phage-type was the same, irrespective of the resistance acquired. Sixth, the frequency of transfer of A' from K12A'_{0.5} to 14 was effectively the same as the total frequency of transfer of the A' factor (including the classes in which it was transferred simultaneously with T'' and S') in the cross K12A'S'T''_{1.5} × 14. Seventh, one line presented ampicillin resistance without change of phage-type. Eighth, a colony, picked without selection, from the cross K12T''_{0.5} × 14 showed a change of phage-type without the acquisition of tetracycline resistance.

Type 14 is unable to produce the type 1 or type 2 fimbriae found in salmonellae⁶ and the strain we used in these experiments is predominantly non-flagellate. The absence of fimbriae and flagella should facilitate the detection of new surface structures, and we are currently investigating the possibility that type 14 may provide a means of detecting the structures responsible for conjugation in resistance transfer.

The transfer investigations on type 14 showed the need for a notation which would indicate when necessary whether a strain of *S. typhimurium* had changed its phage-type on being crossed with a drug-resistant donor, and whether the change of phage-type was accompanied by transfer of resistance. Using type 14 as our model, the system adopted was to indicate, from left to right, the original phage-type, the R-determinant and its cycle number, followed by the final phage-type in brackets. Thus, 14A'T''_{2.0}(29) is type 14 that has acquired resistance to ampicillin and tetracycline jointly in a second cycle cross and has changed its phage-type to '29'. In contrast, 14A'_{2.0}(14) has received ampicillin resistance but has not changed its phage-type. And 14(29) has changed its phage-type without acquiring resistance.

Changes in phage-type can have only a limited expression in type 14, because its sensitivity to the *S. typhimurium* typing phages is already restricted. A better host from this point of view is type 36, which is sensitive to all of the 30 typing phages and in which changes in phage sensitivity are thus easy to detect. Mating experiments were, therefore, carried out with this type and K12. These are summarized in Table 6.

As Table 6 shows, all the R-factors can be transferred to type 36. The frequency of transfer of A' from K12A'_{0.5} to 36 is closely similar to that of the sum of A'T'' and A'S'T'' from K12A'S'T''_{1.5} to 36, and can be taken in both cases as a measure of the transfer of A', as we have already suggested. The class A'S'T''_{2.0} measures the joint transfer of A' and S'. This is confirmed by the results of the cross of K12A'S'_{0.5} × 36 (not included in Table 6), in which A' was transferred at 2.5 × 10⁻⁴, S' at 1.5 × 10⁻⁴ and A'S' at 2.5 × 10⁻⁵. T'' alone, as Table 6 shows, was transferred with high frequency to type 36, both from K12T''_{0.5} and from K12A'S'T''_{1.5}, that is, 3.0 × 10⁻¹ and 2 × 10⁻¹ respectively. This is close to the frequency of transfer of the same factor to type 29 and K12, and much higher than that to type 14, which is 3.0 × 10⁻³.

In most cases transfer of resistance caused the recipient strain of *S. typhimurium* to change its phage-type from 36, which is sensitive to all 30 of the typing phages, to type 6, which is sensitive to only 9 of the phages. This change

was irrespective of the R-factor received. However, the classes 36A'(36) and 36S'(36) were also isolated. In addition, tests of unselected colonies in the crosses K12A'_{0.5} × 36 and K12S'_{0.5} × 36 resulted in the isolation of a number of colonies of *S. typhimurium* that had changed their phage-type to 6 without receiving drug resistance.

Table 6. TRANSFER OF DRUG RESISTANCE INTO AND FROM *S. typhimurium* PHAGE-TYPE 36

Donor × recipient	Selected on	Progeny	Frequency	No. phage-typed	Phage-type
K12 A' _{0.5} × 36	P	A' _{1.0}	4 × 10 ⁻⁴	6	5 = type 6 1 = type 36
	Unselected	All sensitive to P	—	4	2 = type 6 2 = type 36
K12 S' _{0.5} × 36	S	S' _{1.0}	3 × 10 ⁻⁴	9	All type 6
	Unselected	All sensitive to S	—	10	3 = type 6 7 = type 36
K12 T'' _{0.5} × 36	T	T'' _{1.0}	3 × 10 ⁻¹	10	All type 6
	Unselected (10 colonies picked)	9 sensitive to T''	—	10	9 = type 36 1 = type 6
K12 A'S'T'' _{1.5} × 36	P (50 picked)	47 A'T'' _{0.5}	2 × 10 ⁻⁴	50	All type 6
		3 A'S'T'' _{0.5}		50	1 = type 36
	S (50 picked)	42 S'T'' _{0.5}	5 × 10 ⁻⁵	42	7 = type 6
		7 A'S'T'' _{0.5}		—	—
	T (50 picked)	All T'' _{0.5}	2 × 10 ⁻¹	50	All type 6
		12 T'' _{0.5}		—	12 = type 6
K12 × 36	Unselected	38 sensitive to P, S and T	—	50	38 = type 36
	Unselected	—	—	200	All type 36
				100	All type 36

It was found that those lines of 36 into which the transfer of A' and S' had caused a change of phage-type would transfer their drug resistance to K12 with the usual frequencies, 2.4 × 10⁻² for A' and 2.0 × 10⁻² for S'. In contrast, lines that had acquired A' or S' without a change of phage-type were unable to transfer their resistance to K12. We also had a line of type 14 that had acquired ampicillin resistance without changing its phage-type (see Table 5) and this could not transfer its resistance to K12, while type 14 that had received this resistance accompanied by a change of phage-type to '29' could do so. Moreover, there was a line of type 14 that had changed to '29' but had not acquired drug resistance (Table 5). It seemed probable that lines which had received drug-resistance and had changed in phage-type and would transfer resistance had acquired both the R-determinant and the RTF; that those which had received resistance but had not changed in phage-type and would not transfer, had received the R-determinant but not the RTF; and that those which had changed in phage-type but had not received resistance had received the RTF but not the R-determinant, that is, that the RTF was responsible for the characteristic change of phage-type in the host cells of *S. typhimurium*. Furthermore, because of the uniformity of the change of type, only one RTF seemed to be implicated in the transfer system we were studying. It was also evident that the RTF could move independently of the R-determinant-RTF complexes and it was probable that the lines of *S. typhimurium* in which we believed the RTF alone to be present would transfer it to 'uninfected' strains and to strains carrying R-determinants only, in the latter case restoring the integrity of the R-factors and the ability to transfer. We have provisionally assigned the Greek letter Δ to the RTF present in this system.

A complete R-factor should be designated by the symbol of the drug resistance plus that of the RTF, and this will now be included where necessary. Thus, 36S'Δ+(6) is a line of type 36 that has received the R-determinant S' and the RTF, has changed its phage-type to 6, and will transfer S'; 36S'Δ-(36) has received the R-determinant but not the RTF, has not changed its phage-type, and will not transfer S'; and 36Δ+(6) has received the RTF but not the R-determinant, has changed its phage-type, and will transfer Δ.

Table 7 summarizes experiments aimed at demonstrating the transferability of Δ and its mobilization of

R-determinants believed to have lost the transfer property because of the absence of Δ . When Δ alone is transferred into a recipient strain an asterisk is added.

Table 7. TESTS OF INDEPENDENT MOBILITY OF THE *RTF Δ* AND OF ITS ACTIVATION OF RESISTANCE TRANSFER

Cross No.	Donor \times recipient	Progeny	Frequency
I	36 <i>S'</i> Δ + (6) \times K12	K12 <i>S'</i>	3×10^{-3}
II	36 <i>S'</i> Δ - (36) \times K12	—	$< 10^{-7}$
III	36 Δ + (6) \times 36 <i>S'</i> Δ - (36)	36 <i>S'</i> Δ * (6)	3.6×10^{-1}
IV	36 <i>S'</i> Δ * (6) \times K12	K12 <i>S'</i>	10^{-2}
V	14 <i>A'</i> Δ + (20) \times K12	K12 <i>A'</i>	1.3×10^{-3}
VI	14 <i>A'</i> Δ - (14) \times K12	—	$< 10^{-7}$
VII	36 Δ + (6) \times 36 <i>S'</i> Δ - (36) \times K12	K12 <i>S'</i>	4×10^{-5}
VIII	36 Δ + (6) \times 14 <i>A'</i> Δ - (14) \times K12	K12 <i>A'</i>	10^{-4}

* Indicates the transfer of Δ alone.

Crosses I and V demonstrated that *S. typhimurium* that had initially received a complete *R*-factor (that is, the *R*-determinant plus Δ), and had changed in phage-type, would transfer resistance to K12; crosses II and VI confirmed that *S. typhimurium* strains which had received the *R*-determinant but not Δ and had not changed in phage type, would not transfer; cross III showed the transfer of Δ from 36 Δ + (6) into 36 *S'* Δ - (36), with resultant change of phage-type; and cross IV confirmed that the transfer of Δ into the Δ - strain of type 36 had, in addition to changing its phage-type, endowed it with the ability to transfer *S'* to K12. Cross VII, in which 36 *S'* Δ - (36) was used as the intermediate and K12 as the final recipient, was carried out to determine whether the two-stage transfers incorporated in crosses III and IV could be combined in a single experiment, and this proved to be so, although the frequency of transfer of *S'* to K12 was much lower than when the experiment was performed in separate stages. Having established that this type of cross was successful, the method was applied to the mobilization of *A'* in type 14 *A'* Δ - (14), as cross VIII shows.

A striking feature of cross III is the high frequency of transfer of Δ , 3.6×10^{-1} , into *S. typhimurium* type 36 lacking it. This frequency is the same as that of the transfer of *T''* from K12 into sensitive type 29 (Table 3) and into type 36. The significance of this will be discussed later.

These experiments confirm that Δ , or a factor associated with Δ , is responsible for the characteristic change in phage-type in *S. typhimurium*; that drug-resistant strains lacking Δ will not transfer their resistance; and that when Δ is introduced into such strains it unites with the *R*-determinant to form the *R*-factor, which can then be transferred.

It is thus clear that Δ alone may exist in Enterobacteriaceae, and that it is highly communicable in its 'free' state. Although we have so far identified only the properties of communicability, ability to change the phage-type of *S. typhimurium* in a defined fashion, and mobilization of non-transferring *R*-determinants, it may have other genetic properties that we have not yet recognized.

We have prepared lines of K12 containing Δ alone, that is, K12 Δ +, by crossing 36 Δ + with K12 *F* -. A number of K12 colonies were picked without selection from this cross and ten of these were tested individually by incorporation in mating mixtures containing both type 36 *S'* Δ - and K12. Transfer of streptomycin resistance to K12 in these crosses could occur only by the infection of 36 *S'* Δ - with Δ from K12 Δ + to produce 36 *S'* Δ +, which could then transfer *S'* to K12. Nine of the ten colonies tested proved to be K12 Δ +. The mobilization of *R*-determinants in this way may be a useful method of detecting the presence of transfer factors in drug-sensitive strains of Enterobacteriaceae.

One of the K12 Δ + colonies was crossed with type 36, and 50 colonies of the recipient strain picked without selection and phage-typed. Sixteen belonged to phage-type 6, representing a Δ transfer frequency of 3.2×10^{-1} .

Having established the role of Δ in the transfer of drug resistance in the systems we have studied, the possibility

that it might be able to mediate the transfer of other properties was investigated. It is known that the *E2* colicinogenic factor (col *E2*) cannot be transferred from strains carrying it unless the I colicinogenic factor (col I) is first introduced into them⁶. It seemed possible that we were dealing with an analogous situation in the mobilization of the *A'* and *S'* resistance-determinants by the *T''* *R*-factor, and that the I colicinogenic determinant was active in col *E2* transfer only because of its attachment to a transfer factor, in the same way as the mobilizing activity of *T''* is due to the presence of Δ . We therefore investigated the possibility that Δ might mediate col *E2* transfer. The *T''* *R*-factor was used as a marker of Δ transfer in this experiment. It was transferred from K12 *T''* -₅ into a strain of *S. typhimurium* LT2 colicinogenic for *E2* but unable to transfer *E2* colicinogeny to K12. A tetracycline-resistant colony of LT2 (col *E2*) resulting from this cross was then mated with K12. Colicinogeny was detected by inoculating selected colonies in a premarked pattern on a Difco agar plate already flooded with the indicator strain, which was K12 *F* -. Zones of colicinogeny were clearly apparent after 8 h incubation at 37° C.

These experiments are summarized in Table 8.

Table 8. MOBILIZATION OF COLICINOGENIC FACTOR *E2* WITH *R*-FACTOR *T''*

Cross	Selected on	Progeny	Frequency
K12 <i>T''</i> \times LT2 (col <i>E2</i>)	<i>T</i>	LT2 <i>T''</i> (col <i>E2</i>)	5×10^{-1}
LT2 <i>T''</i> (col <i>E2</i>) \times K12	<i>T</i>	K12 <i>T''</i> (col <i>E2</i>)	$\sim 10^{-4}$
LT2 (col <i>E2</i>) \times K12	Unselected	—	$< 10^{-6}$

The second cross in Table 8 shows the transfer of col *E2* into K12, using the *R*-factor *T''* as the vector. The frequency of col *E2* transfer was about 10^{-4} per LT2 donor cell. The third cross is a control which shows that without the mediation of *T''* this transfer will not take place.

One of the K12 *T''* (col *E2*) progeny from this experiment was then crossed with type 36. The result is given in Table 9.

Table 9. TRANSFER OF *T''* AND *E2* COLICINOGENY FROM K12 TO TYPE 36

Cross	Selected on	No. picked	Progeny	Frequency
K12 <i>T''</i> (col <i>E2</i>) \times 36	<i>T</i>	50	5 <i>T''</i> (col <i>E2</i>)	7×10^{-2}
			45 <i>T''</i>	6.3×10^{-1}
	Unselected	50	3 <i>T''</i> (col <i>E2</i>)	6×10^{-2}
			23 <i>T''</i>	4.6×10^{-1}
			24 sensitive and col -	

All *S. typhimurium* progeny carrying *T''* or *T''* (col *E2*) had changed from phage-type 36 into phage-type 3.

The results of this cross are analogous to those with donors carrying *A'* and *T''*, or *S'* and *T''*, that is, col *E2* shows a high degree of association with *T''* in transfer, but *T''* alone transfers with much higher frequency. This suggests that in strains carrying *T''* and col *E2*, the *R*-determinant and the colicinogenic determinant are independently attached to Δ , but that, because of the close association of *T''* with Δ , the great majority of col *E2* determinants are attached to a Δ particle already carrying *T''* (the term particle is used for convenience only). This makes the detection of the class 36 (col *E2*) Δ + difficult. However, it can be predicted that if *A'* or *S'* were used to mobilize col *E2* in LT2 (col *E2*), transfer experiments into K12, and thence into type 36, would reveal the following *S. typhimurium* classes (in this example the *A'* resistance factor is used as the vector of Δ): 36 *A'* Δ +; 36 (col *E2*) Δ +; and 36 *A'* (col *E2*) Δ +. It should also be possible to demonstrate lines of both 36 *A'* and 36 (col *E2*) that were Δ -. Finally, about 59 per cent of the total *S. typhimurium* progeny would receive Δ alone.

Having demonstrated the mobilization of col *E2* by *T''*, it was obvious that the reverse test should be applied, that is, the mobilization of a non-transferring *R*-determinant by colicinogenic factor I. The experiments in which this was demonstrated are summarized in Table 10.

The appearance of K12 *S'* (col I) colonies in the first cross in Table 10 could result only from the transfer of the col I factor into 36 *S'* Δ -, which would then act as a donor of

Table 10. MOBILIZATION OF NON-TRANSFERRING S' BY COL I

Cross Donor \times recipient	Selected on	No. of colonies tested	Progeny	Frequency
LT2 (col I) \times 36 S' Δ - \times K12	S'	25	All K12 S' (col I)	10^{-3}
LT2 (col I) \times K12	Unselected	12	All K12 (col I)	$\sim 10^0$
K12 (col I) \times 36	Unselected	50	45 = 36 5 = 36 (col I)	10^{-1}
LT2 (col I)	Unselected	50	9 = 36 S' (col I) Δ - 41 = 36 S' Δ -	2×10^{-1}
\times 36 S' Δ -	S'	50	All K12 S' (col I)	10^{-2}
36 S' (col I) Δ -	Unselected	40	1 K12 S' (col I) 39 K12 (col I)	$\sim 2.0 \times 10^{-2}$ 9.8×10^{-1}
\times K12				

the S' (col I) complex to K12. It is apparent from Table 10 that col I is as effective in mobilizing S' as is T'' (Table 5), and its activity in this respect must be due to its association with a transfer factor. The high frequency of transfer of col I to both K12 and *S. typhimurium*, and the fact that an obvious majority of K12 cells receiving S' from 36 S' (col I) Δ - also received col I, suggest that the colicinogenic determinant is firmly attached to its transfer factor in the same way as the T'' determinant is attached to Δ , and that the col I transfer factor is highly communicable. Although this factor produces a detectable change of phage-type in type 36 of *S. typhimurium*, this is neither so clear-cut nor so extensive as that produced by Δ . Thus, the col I transfer factor is different from Δ . We have not so far isolated it in cells devoid of the determinant for colicine I synthesis but are endeavouring to do so.

In our earlier report we suggested that the A' , S' and T'' R -factors were independent of each other, with separate transfer frequencies from type 29 to K12 of about 2.0×10^{-2} , 10^{-2} and 2×10^{-6} respectively. The only finding inconsistent with this hypothesis was that the joint transfer of A' and S' was about a log unit higher than would be expected if these two R -factors were truly independent. Watanabe *et al.*⁷ observed a similar phenomenon in the joint transfer of f_1+ and f_1- R -factors, but we have so far no evidence of the existence of an RTF other than Δ , which seems to be f_1- , in the system on which we have concentrated. A further problem arose with the observation that T'' was transferred with frequencies approaching unity, whereas the transfer frequency of T' in the cross of wild type 29A' $S'T'' \times$ K12 was of the order of 10^{-6} . It was observed, moreover, that once T'' had appeared it showed a high degree of 'linkage' to A' and S' in all subsequent crosses, a feature absent from our initial crosses. The characteristic change of phage-type in *S. typhimurium* types 14 and 36 on receipt of R -factors, the absence of such changes in the same phage-types receiving non-transferring A' and S' , and the demonstration of a transferable factor which changed the phage-type of *S. typhimurium* without conferring resistance and which would mobilize non-transferring R -determinants, also required explanation.

We offer the following interpretations of these phenomena. In the original strain of *S. typhimurium* type 29A' $S'T''$ there were, in fact, only two R -factors, A' and S' . The tetracycline resistance was probably mutational, chromosomally located, and not associated with an RTF . The cross in which we observed the transfer of T' to K12 was thus a rare demonstration of the pick-up of the tetracycline-resistance gene from the chromosome by the RTF which we have designated Δ . T'' was transferred alone in this cross, which could be interpreted as an indication that there were free Δ particles in the host cell, or that T' was substituted for the A' or S' determinants in complete R -factors. Later experiments have inclined us to the view that a free Δ particle was concerned. Once effected, the attachment of T' to Δ has shown itself to be very stable; the R -factor, now T'' , thus shows a mobility closely similar to that of Δ , and the tetracycline R -determinant is rarely found in the absence of Δ , although, because of the persistence of free Δ particles, Δ may exceptionally be transferred without T'' (Table 6). The transferable agent changing the phage-type of *S. typhimurium* is Δ ,

the RTF ; crosses into *S. typhimurium* in which the R -determinant is transferred but Δ is lost, produce drug-resistant lines not showing the characteristic change of phage-type and unable to transfer their resistance. Such R -determinants may be integrated into the chromosome, but it seems more probable, because of the ease with which they are mobilized by Δ , that they are located in the cytoplasm. The transfer of T'' into lines of this type introduces Δ , to which the R -determinant, A' for example, becomes attached. This attachment is independent of that of T'' but, because of the close association of T'' with Δ , most copies of Δ will carry T'' , so that most Δ particles acquiring A' will already possess T'' and the resulting progeny will be of the type $A'T''$. When these cells are mated with a sensitive recipient they will transfer A' , $A'T''$ and T'' , but the A' class will be easily detectable early in the cross only.

Watanabe and Lyang⁸ also observed the mobilization of non-transferable R -determinants by a tetracycline R -factor, but offered in explanation the hypothesis that the initial loss of transferability was due to the RTF becoming 'defective', a fault repaired by the newly introduced tetracycline R -factor. We believe our explanation of this phenomenon to be more acceptable, and the identification of Δ as an infective agent producing a characteristic change of phage-type in *S. typhimurium*, apparently without transmitting drug-resistance, and independently capable of mobilizing non-transferring R -determinants, supports our hypothesis.

So far as we can judge, Δ seems to be the only RTF in this system. As R -determinants will transfer only when associated with Δ , the more frequent their association with it, the more frequently will they be transferred; that is, the frequency of transfer of a given R -determinant is governed by the number of R -determinant- Δ complexes available during the period of conjugation. A' transfers from *S. typhimurium* to K12 with slightly higher frequency than does S' , because A' is more frequently attached to Δ than is S' . The complexes $A'\Delta$ and $S'\Delta$ are independent and are dissociable; a high proportion of cells contain them in the dissociated state. Cells of this type transfer Δ only, and this has been shown to occur with a frequency approaching unity. (There are obvious similarities between this system and that of the F' factor in *E. coli* K12 (refs. 9, 10) that demand further exploration.) Cells containing the complete R -factors, $A'\Delta$ for example, probably transfer it alone. The tetracycline R -determinant is closely associated with Δ , which explains why its frequency of transfer approximates to that of Δ alone, why transfer of Δ only from $T''\Delta$ cells is unusual, and why non-transferring T'' is a rarity (we have not so far succeeded in identifying it).

The frequency of joint transfer of A' and S' might be due to independent transfer of the two R -factors to the same cells. However, on this hypothesis, the frequency of recipients showing joint transfer of A' and S' in our original experiments¹ should be about 2×10^{-4} , that is, the product of the individual frequencies of the two R -factors, whereas it was uniformly a log unit higher than expected. On the other hand, a small number of cells will contain A' and S' simultaneously attached to Δ , and these will therefore transfer both determinants together in the same way as A' and T'' are jointly transferred. This could raise the frequency of joint transfer of A' and S' beyond the level expected from independent transfers of the two R -factors, and it seems to us to be the most probable explanation of the discrepancy.

We have mentioned that, in crosses in which the classes A' and $A'T''$ or S' and $S'T''$ are transferred, the single R -factor classes A' and S' are usually detectable early in the cross only, and that there is a relative increase in the frequency of the double R -factor classes $A'T''$ or $S'T''$ late in the cross. We suggest that this is due to the fact that A' and S' tend to lose Δ during transfer, whereas T'' does not. The classes A' and S' , therefore, will spread to

only a limited extent between recipient cells once they have been transferred from the donor, while the classes $A'T''$ and $S'T''$ will continue to spread in the recipient culture because of the presence of T'' which acts as a vector of Δ . Moreover, recipient cells containing A' or S' only, without Δ , will be super-infected with T'' , which will produce a further increase in the $A'T''$ or $S'T''$ class.

It can be predicted that if the R -factor A' is used to introduce Δ into non-transferring S' in type 36 of *S. typhimurium*, and the resulting progeny, that is, $36A'S'\Delta+$, is then crossed with K12, the following classes of K12 should appear with approximately the following frequencies: A' , 2.0×10^{-2} ; S' , 10^{-2} ; $A'S'$, 10^{-3} . Table 11 shows the actual results of this experiment.

Table 11. MOBILIZATION OF $S'A-$ WITH $A'\Delta+$

Cross Donor \times recipient	Selected on	Progeny	Frequency
*36 $A'S'\Delta+$ \times K12	P	K12 A'	2.4×10^{-2}
	S	K12 S'	1.3×10^{-2}
	PS	K12 $A'S'$	3.0×10^{-3}

* A' and Δ had been introduced into type 36 $S'A-$ from K12 $A'\Delta+$.

Table 11 establishes the accuracy of the predictions. The experiment demonstrates that, once Δ is introduced into a cell carrying a non-transferring R -determinant, the frequency with which the resulting R -factor will transfer to other strains is that characteristic of the R -determinant- Δ complex, irrespective of whether the two elements are introduced together or separately. It also shows that, if Δ is introduced into such a cell in association with another R -determinant, the two resistances will manifest their usual frequencies of separate and joint transfer.

The frequency of transfer of R -factors may be postulated to depend on:

(1) *The recipient cell.* It is to be expected that different members of the Enterobacteriaceae will vary in their susceptibility to RTF infection. For example, type 14 of *S. typhimurium* is a poor recipient of T'' compared with types 29 and 36. A variability of transfer efficiency may also be found in different donor strains.

(2) *The rate of transmissibility of the RTF alone.* Under defined conditions this is evidently constant for each RTF .

(3) *The frequency or firmness of attachment of individual R -determinants to the RTF .* This is apparently constant for each R -determinant.

When a particular R -determinant has a firm attachment to the RTF , it will be transferred with a frequency approaching that of the RTF alone. It will also be transferred with high frequency simultaneously with other R -determinants which are attached to the same RTF . This will give the appearance of linkage between the two R -determinants concerned, but findings in the systems we have described suggest that this impression is probably illusory. Other R -determinants than T'' may exist which have a high frequency of attachment to an RTF . If a number of these are simultaneously attached to the same RTF they will usually be transferred together and this will suggest that the determinants themselves are closely linked. If the determinants were linked, however, the construction of the linkage group would demand that the respective drug-resistance genes, presumably occupying different chromosomal sites, should be picked up independently by the RTF and assembled into a linkage group, or that a cytoplasmic genetic element should exist which had mutational potentialities to confer resistance to a number of drugs. The hypothesis that the R -determinants themselves, whatever their origin, are independent of each other and attach independently to the RTF , seems to us a simpler conception. This argument is without prejudice to the possibility that some R -determinants may be small linkage groups conferring resistance to more than one drug. An example of this may be S' in type 29, which confers resistance to both streptomycin and sulphonamides. We have not so far been able to separate these two resistances.

The mediation of col $E2$ transfer by Δ , and of non-mobile S' transfer by the transfer factor of col I, establish that the transfer of genetic information other than that of drug resistance can be carried out by the resistance transfer factors, and of information other than that of colicinogeny by colicinogenic transfer factors. There seems to be no reason for supposing that such transfer does not take place in the wild state. The transfer of a single resistance with loss of the RTF , which we have demonstrated, results in the production of a line which, if isolated in the field, would be regarded as a drug-resistant mutant. This would apply to any character identified which could not be shown to be transmissible, and it raises the question of how many characters in the Enterobacteriaceae may owe their origin to transfers in which the transferring agent has been lost.

Iijima¹¹ reported mobilization of colicinogenic factor $E1$ with R factors. We have demonstrated the reciprocal transfer activities of Δ and the col I factor in relation to colicinogeny and drug resistance. It has also been shown that the F factor will mediate the transfer of tetracycline resistance¹² and that an RTF will potentiate recombination in $F-$ strains of K12 (ref. 13).

Watanabe³ pointed out operational analogies between the $RTFs$, the F factors and colicinogenic factor I (we have suggested that the transmissibility of the latter is due to a transfer factor and is probably independent of the colicinogeny determinant). The work we have described gives further support to the idea that the agents responsible for the transmission of genetic information in the Enterobacteriaceae by conjugation constitute a group of unknown size, showing remarkable catholicity in relation to the hosts they will "infect" and to the characters they will transfer.

We thank Mrs. B. E. Burford, Miss M. Burton, Mrs. L. Ward and Mr. F. J. Flynn for their assistance.

Note added in proof. Since this article was written, the technique of mobilization of a non-transferring R -determinant for the detection of transfer factors (TF) in drug-sensitive strains has been successfully applied to wild drug-sensitive strains of *S. typhimurium*. The wild strains were each incorporated in a mixture of type 36 $S'A-$ (intermediate recipient of the TF) and K12 (recipient of the transferable $S'-TF$ complex). Of 12 sensitive strains tested, 5 proved to be carrying transfer factors. Colicinogeny tests of the donor strains were negative. These transfer factors are now under investigation.

Yet another transfer factor has been identified in a strain of type 29 resistant to ampicillin, neomycin, streptomycin(-sulphonamides) and tetracycline. All resistances except that for tetracycline were easily transferable to K12; ampicillin and neomycin resistance at a frequency of about 5×10^{-1} , and streptomycin resistance at about 10^{-2} . Preliminary experiments suggest that the ampicillin R -determinant in this strain is the same as that in the type 29 strain in which the TF is Δ . If this is so, the high transfer frequency of ampicillin resistance in the new strain (5×10^{-1}) as compared with type 29 $A'S'T''$ (2×10^{-2}) indicates that the frequency of transfer of a given R -determinant depends on the transfer factor with which it is associated.

¹ Anderson, E. S., and Lewis, M. J., *Nature*, **206**, 570 (1965).

² Anderson, E. S., and Datta, R., *Lancet*, **i**, 407 (1965).

³ Watanabe, T., *Bact. Rev.*, **27**, 37 (1963).

⁴ Felix, A., and Callow, B. C., *Brit. Med. J.*, **ii**, 127 (1943).

⁵ Duguld, J. P., Anderson, E. E., and Campbell, I., *J. Path. Bact.* (in the press).

⁶ Ozeki, H., Stocker, B. A. D., and Smith, S. M., *J. Gen. Microbiol.*, **28**, 671 (1962).

⁷ Watanabe, T., Nishida, H., Ogata, C., Aral, T., and Sato, S., *J. Bact.*, **88**, 716 (1964).

⁸ Watanabe, T., and Lyang, K. W., *J. Bact.*, **84**, 422 (1962).

⁹ Adelberg, E. A., and Burns, S. N., *Genetics*, **44**, 497 (1959).

¹⁰ Adelberg, E. A., and Burns, S. N., *J. Bact.*, **79**, 321 (1960).

¹¹ Iijima, T., *Abstr. Thirty-third Meeting, Genet. Soc. Japan*, **7** (1961).

¹² Harada, K., Kameda, M., Suzuki, M., and Mitsuhashi, S., *J. Bact.*, **88**, 1257 (1964).

¹³ Sugino, Y., and Hirota, Y., *Abstr. Thirty-third Meeting, Genet. Soc., Japan*, **22** (1961).

DIRECT AND INDIRECT EFFECTS OF RADIATION ON PLANT CELLS: THEIR RELATION TO GROWTH AND GROWTH INDUCTION

By DR. R. D. HOLSTEN, DR. M. SUGII and PROF. F. C. STEWARD, F.R.S.

Laboratory of Cell Physiology and Growth, Cornell University, Ithaca, New York

CONVENTIONAL interpretations of the biological effects of radiation are heavily committed to the direct consequences of the radiation on cells, particularly on their chromosomes. The genetic consequences of the radiation damage are interpreted in terms of the ability of the radiation to penetrate to the nucleus, to inactivate some particular part of the genetic apparatus and, by causing one or other of a variety of chromosomal aberrations, to affect not only that cell but also its lineal descendants.

Following this line of thought, one of us (R. D. H.) embarked on an investigation of the radiation-induced mutations which could be produced by exposing free-cell cultures of carrot to a cobalt-60 source¹. The basic idea was a simple one. Since carrot tissue can now be grown in free-cell culture² and the free cells spread on agar plates and grown into colonies³, and since the free cells have, under suitable conditions, been grown with the intermediate formation of embryos⁴ into whole plants with flowers, there was every reason to believe that the effects of radiation could be examined using this system. Free cells spread on agar plates should permit one to detect the consequences of a radiation-induced effect in all the cells of the progeny raised from one cell. Thus the exposure of free cells to radiation should be a more effective means of examining the biological effects than by radiating tissues, organs or whole plants (for example, seeds), since any cells so affected by the radiation would transmit the effects only to those cell lines to which they give rise. If the affected cell is isolated and the precursor of a colony, an organ or an organism, the radiation-induced effect should be apparent in the behaviour of the whole.

Prior to this investigation there was some evidence on the effects to be expected⁵. Carrot-tissue explants had

been exposed to cobalt-60 radiation before the onset of growth induction by coconut milk and, by contrast, after the rapid growth rate (for example, 7-8 days of culture) had already taken effect in the coconut milk-supplemented medium. Briefly, the tissue was very sensitive to radiation prior to growth induction—the ultimate site of the coconut-milk growth induction effect being very vulnerable to radiation damage. Similar effects on the same growth response, and presumably at the same sites, which were due to cyanide and to a light-reversible carbon monoxide inhibition, indicate that a cytochrome mediated response was involved. To all these inhibitory treatments (that is, with radiation and cyanide or carbon monoxide) the growing tissue was less sensitive than when it was in the quiescent state. Freshly isolated tissue explants treated with cobalt-60 failed, therefore, to respond to coconut milk by cell division; although at non-lethal doses (up to 0.05 Mrads) the living cells which failed to divide could still expand. Comparable tissue radiated some 7 days later, while it was still in the coconut milk-containing medium, responded quite differently. The already rapidly dividing cells continued to grow and to divide even after exposures to radiation (2 Mrads) which would have completely inactivated the initial site of growth induction by coconut milk. Similarly, the actively growing tissue explants proved to be less sensitive to cyanide, and even the smaller inhibition by carbon monoxide, which could still be observed, differed with respect to its reversibility by light from that obtained with the initial carrot explants⁶.

The first investigations of radiation damage to free cells, exposed on agar plates under such conditions that the cells were so separated that there was no direct contact between them, produced the expected evidence to confirm

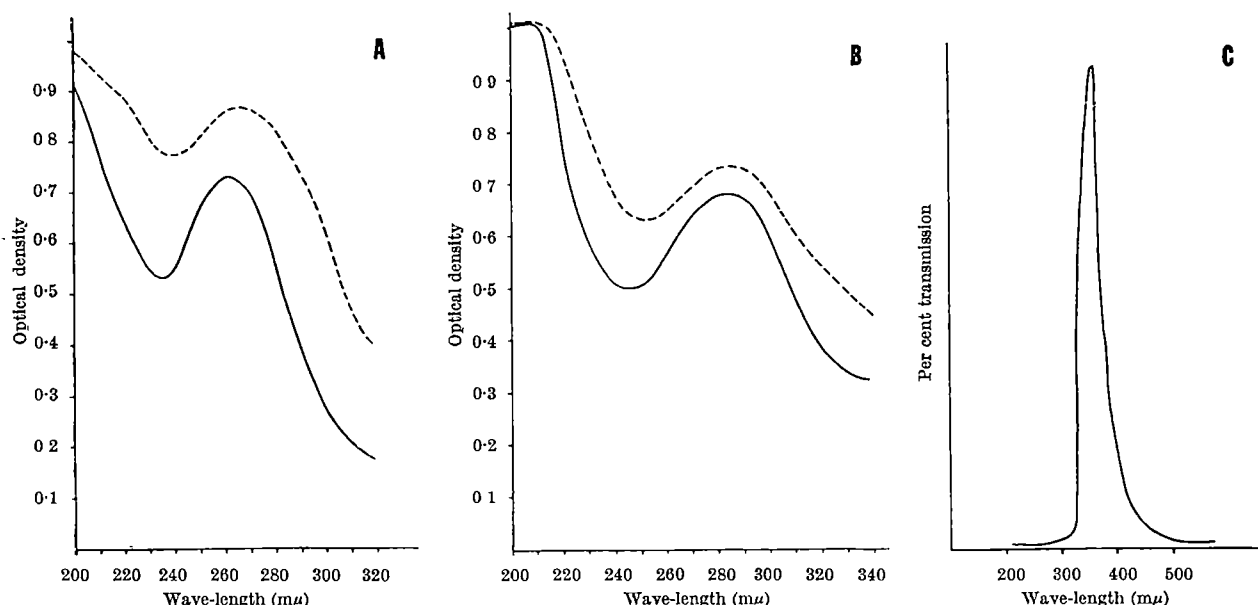


Fig. 1. The ultra-violet absorption and fluorescence spectra of irradiated (2 Mrad) sucrose solutions. *A*, Absorption spectra at pH 4.5, with and without autoclaving, *B*, absorption spectra at pH 11.0, with and without autoclaving, *C*, fluorescence spectrum at an activation wave-length of 290 mμ, at a pH of 4.5. --- Irradiated and autoclaved; —, irradiated

Interpretation: The absorption spectra were determined at pH 4.5 (*A*) and at pH 11.0 (*B*) both with and without autoclaving the irradiated solution. In *C* the fluorescence spectrum of irradiated sucrose is recorded as determined with the Aminco Bowman spectrophotofluorometer at an activation wave-length of 290 mμ at a pH of 4.5

Table 1. EFFECT OF GAMMA-RADIATION ON THE GROWTH OF VIABLE COLONIES FROM FREE CELLS OF CARROT

Radiation dose (Mrad)	Colonies per plate	
	30	35
2.0	0	0
1.0	0	0
0.5	0	0
0.05	10	12
Control	12	16

Interpretation: The cells were irradiated at the dosages indicated, in the basal liquid medium containing coconut milk, and were then diffusely spread on agar plates. The basal medium was that of White¹⁴, slightly modified with respect to calcium and iron, but supplemented with casein hydrolysate as a general source of reduced nitrogen to give greater growth. The plates, sealed against desiccation, were incubated for the periods shown at 70° F in continuous light. The controls passed through all these operations but received no cobalt-60 radiation. Colonies visible to the naked eye were counted and are reported as the mean number/plate in four separate determinations. Colonies still grew virtually unaffected by the radiation at 0.05 Mrad although, at this dosage, the initial tissue would not have grown.

what had been observed previously with the tissue explants (Table 1). However, in making experimental controls, it was observed that the effects in question could be equally as great if one irradiated the culture medium alone prior to placing on it the suspended, viable, non-irradiated free cells or whole explants. It was this observation that prompted the work which will be described in the remainder of this article.

First, it is convenient to state in broad outline the principal outcome of this investigation. As already mentioned, the prior exposure of the culture medium to γ -radiation was found to be as biologically effective as the simultaneous exposure of free cells or tissue explants in the culture medium. Experiments, made to implicate (by methods of elimination) some particular component of the culture medium, clearly pointed to sucrose as the substance by which the indirect effects in question were mediated. At this point, and to obtain better evidence of the nature of the radiolysis products from sugar which inhibit growth as it is induced by coconut milk, samples of ^{14}C -sucrose were radiated and, by autoradiographic means, the range of products so produced was demonstrated. One could also utilize the quite strong ultra-violet absorption (at 260–290 $\text{m}\mu$) of the irradiated sucrose solution to measure its conversion to potentially inhibitory substances.

Only the meagre reports in the literature served as a guide to the kind of substances that might emerge from the γ -radiolysis of sugar and become, thereby, biologically deleterious. Such radiolysis products of sugar, which strongly absorb ultra-violet radiation (260–290 $\text{m}\mu$), comprise several conspicuous compounds which are separable into basic, acidic and neutral fractions on columns of appropriate ion exchange resins. In the outcome, the neutral fraction produced the most outstanding biological effects (it was inhibitory at higher concentration, but somewhat stimulatory when more dilute), and its nature was, therefore, investigated. A simple, three-carbon substance, reductone, reported by Laurent⁶ to be one of the reducing substances produced from glucose by ultra-violet radiation was prepared by one of us (M. S.) according to the directions previously published⁷.

At the time of writing, the balance of evidence is that the synthetic product thus obtained does not nullify the growth induction effect of a coconut milk-supplemented medium, and therefore its identity as reductone, or as a closely related sub-

stance, need not concern us here. Nevertheless, there are, among the products of sucrose radiolysis, other strongly ultra-violet absorbing substances, which are separable on a one-directional chromatogram and are demonstrable by their ultra-violet absorbance, their fluorescence, and by carbon-14 autoradiography. These substances interact strongly with the carrot tissue to produce the equivalent effect of direct radiation on the cells. Furthermore, it was shown that products derived from sucrose by γ -irradiation can also produce chromosomal aberrations in *Vicia faba* and *Tradescantia paludosa* of the kind which would normally be ascribed to the direct effects of radiation. The evidence will be presented here.

Experiments which Demonstrate the Indirect Effects of Radiation on Carrot Cells. In the investigation of the effects of γ -radiation on the growth of carrot phloem tissue in liquid culture, a control experiment was set up in which only the coconut milk growth supplement was exposed to γ -radiation; the results are shown in Tables 2 and 3. Surprisingly, the higher doses of γ -radiation, when applied to the coconut milk supplement alone, produced growth responses in the tissue which were qualitatively similar (though quantitatively somewhat less) to those produced by direct irradiation of the tissue. There were almost no cell divisions observed after the 2- and 4-Mrad treatments and a definite stimulation of cell division followed the treatment at 0.05 and 0.2 Mrad (Table 2).

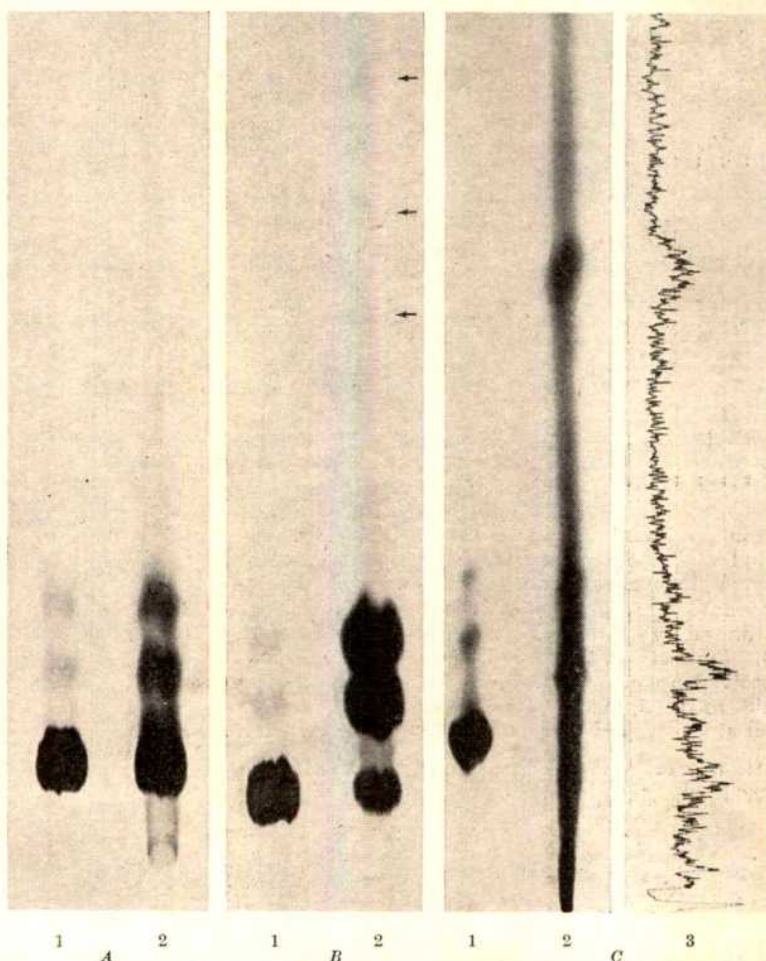


Fig. 2. Autoradiographic demonstration of the radiolysis products from ^{14}C -sucrose. **Interpretation:** In Fig. A the non-irradiated sucrose (A1) is compared with the irradiated (2 Mrad over 24 h) sucrose (A2). Accentuated breakdown of sucrose to glucose and fructose is evident. In B the same treatments of sucrose were followed by autoclaving and the degradation products are consequently more obvious. In C the dosage of 2 Mrad was delivered over 2 h, with some resultant polymerization, that is, to relatively immobile carbon-14 compounds, and much greater degradation of ^{14}C (C2) compared to its control (C1). C3 shows a strip-scan analysis of the radiolysis products in C2 and several zones of activity are obvious.

Table 2. EFFECT OF IRRADIATED COCONUT MILK ON THE GROWTH OF FRESH-CUT CARROT PHLOEM EXPLANTS IN A BASAL MEDIUM

Initial explants	Radiation dose (Mrad)	Wt./explant (mg)	Cells/explant (in thousands)	Cells/ μ g	μ g/cell
20th day	4.0	3.4	23.10	6.80	0.148
	2.0	5.8	42.36	7.23	0.138
	1.0	15.8	191.53	12.14	0.082
	0.5	41.4	722.26	17.50	0.157
	0.2	61.9	1,080.04	17.38	0.057
	0.05	63.4	1,263.03	19.94	0.050
	none	52.2	959.73	18.34	0.054

Interpretation: The coconut milk, irradiated at the dosages indicated, was incorporated into the basal medium, dispensed into the culture tubes and these were then autoclaved. The fresh-cut explants were removed aseptically from the carrot root and inoculated into the tubes. Growth in weight and number of cells was observed after 20 days under standard conditions. All recorded data are per explant and the average for ten explants. Both the inhibition of cell division at 0.5 Mrad, or greater dosage, and the stimulus observed at 0.2 Mrad, or lesser dosage, were statistically significant; where cell division is active, cell sizes tend to be small; where cell division is less active, cell sizes may increase.

When the carrot phloem explants were caused to grow prior to their exposure to irradiated coconut milk in basal medium (Table 3), they acquired a resistance to the inhibitory effects of the irradiated coconut milk supplement; but there was no sign of stimulated cell division over the controls even at the lowest dosage of γ -radiation used. It is noteworthy that, at the higher dosages of radiation (2 and 4 Mrad), although cell division was noticeably inhibited, cell expansion (expressed as μ g/cell) nevertheless continued (Table 3).

Table 3. EFFECT OF IRRADIATED COCONUT MILK ON THE CONTINUED GROWTH OF CARROT PHLOEM EXPLANTS PRE-GROWN (7 DAYS) ON A BASAL MEDIUM SUPPLEMENTED WITH NON-IRRADIATED COCONUT MILK

Initial explants	Radiation dose (Mrad)	Wt./explant (mg)	Cells/explant (in thousands)	Cells/ μ g	μ g/cell
7th day		3.1	20.75	6.67	0.151
20th day	4.0	11.4	290.16	25.44	0.039
	2.0	131.3	883.19	6.72	0.148
	1.0	159.5	1,185.74	7.41	0.135
	0.5	161.3	1,274.29	7.85	0.129
	0.2	186.5	1,813.33	9.71	0.103
	0.05	199.6	1,961.12	9.80	0.098
	none	183.4	2,096.86	11.43	0.087
		192.1	2,146.74	11.17	0.089

Interpretation: After the prior growth period of 7 days, the cultured explants were transferred aseptically to a medium containing irradiated coconut milk prepared as in the experiment of Table 2. After 20 days the growth was again measured and the data expressed in the same manner as before and as the mean of ten replicates. Even at dosages of 0.5 or more Mrad, substantial cell division continued in these cultures but, since divisions were somewhat suppressed, cells expanded more than in the controls. At the lower radiation dosages (0.2 Mrad, or less) no significant stimulatory effects were observed in this tissue, which had already passed through the growth induction phase.

Having recognized the indirect effects of radiation which could be elicited by irradiation of the coconut milk supplement, the effects of radiating the basal medium were next investigated. The basal medium alone was exposed to γ -radiation and it was then supplemented with non-irradiated coconut milk and inoculated with fresh-cut carrot phloem explants. Again, as with the irradiated coconut milk, growth of the explants was markedly inhibited by irradiating the external basal medium (Table 4). Similar radiation treatments of the medium which received pre-grown (10-day cultured explants in coconut milk medium) explants did not completely suppress their cell division and their continued cell enlargement was

Table 4. EFFECT OF IRRADIATED BASAL MEDIUM ON THE GROWTH OF FRESH-CUT CARROT EXPLANTS AS STIMULATED BY NON-IRRADIATED COCONUT MILK

Initial explants	Radiation dose (Mrad)	Wt./explant (mg)	Cells/explant (in thousands)	Cells/ μ g	μ g/cell
20th day	4.0	3.2	33.68	10.52	0.095
	2.0	3.3	30.62	9.18	0.109
	1.0	2.9	29.78	10.27	0.098
	0.5	3.4	32.57	9.58	0.104
	0.2	33.5	287.12	8.57	0.167
	0.1	85.6	937.64	10.98	0.091
	0.05	97.5	1,291.38	13.37	0.075
	none	115.7	943.57	8.17	0.123

Interpretation: The basal medium, irradiated at the dosages indicated, was supplemented with non-irradiated coconut milk and the subsequent procedures followed those of the experiment of Table 2. At dosages of 0.2 Mrad or more the coconut milk stimulus was unable to exert its normal effect and, at the lowest radiation dosage used (0.05 Mrad), a significant stimulus of cell division was observed.

Table 5. EFFECT OF IRRADIATED BASAL MEDIUM ON THE CONTINUED GROWTH OF CARROT PHLOEM EXPLANTS PRE-GROWN (10 DAYS) ON A NON-IRRADIATED BASAL MEDIUM SUPPLEMENTED WITH COCONUT MILK

Initial explants	Radiation dose (Mrad)	Wt./explant (mg)	Cells/explant (in thousands)	Cells/ μ g	μ g/cell
10th day		4.5	40.36	8.97	0.111
18th day	1.0	25.1	532.95	21.26	0.047
	0.5	45.7	797.08	17.58	0.058
	0.2	60.7	677.57	11.19	0.090
	0.05	86.7	1,187.78	13.78	0.073
	none	88.9	1,172.51	13.25	0.076

Interpretation: The procedure was patterned after the experiment of Table 3 except that the basal medium, not the coconut milk, was irradiated. As will be seen, the effects of radiation which were transmitted to the growing cells were also similar to those described in Table 3.

Table 6. EFFECT OF IRRADIATED SUCROSE ON THE GROWTH OF FRESH-CUT CARROT PHLOEM EXPLANTS IN AN OTHERWISE NON-IRRADIATED BASAL LIQUID CULTURE MEDIUM

Culture medium	Wt./explant (mg)	Cells/explant (in thousands)	Cells/ μ g	μ g/cell
Basal	4.4	20.29	4.5	0.219
Basal + coconut milk + irradiated sucrose	1.4	10.91	8.0	0.128
Basal + coconut milk + non-irradiated sucrose	25.8	452.94	17.8	0.057
Basal + coconut milk	78.5	621.30	7.9	0.127

Interpretation: Randomly sampled batches of fresh-cut carrot explants were inoculated concurrently into (a) the basal medium alone, (b) the un-irradiated basal medium, containing 2 per cent sucrose, plus coconut milk, plus the irradiated sucrose (2 per cent); (c) the same constituents as in b except that non-irradiated sucrose was substituted for the irradiated material; (d) the basal medium, plus coconut milk without any further addition of sucrose. After 18 days the dramatic effect on growth by cell division occurred whenever irradiated sucrose (at 2.0 Mrad) was present in the medium.

consequently less conspicuous (Table 5). In other words, irradiation of the basal medium gave the same type of response as radiating the coconut milk or the complete system including the tissue.

Substance which Mediates the Indirect Effects of Radiation on the Cells. Systematically, and by elimination, the effects of γ -radiation on the various components of the basal medium were tested and it was revealed that the inhibitory effect described was due to an alteration of the sucrose moiety of the culture medium (Table 6). Not only were the various components of the basal medium and of the coconut milk tested separately, but some known products of radiolysis of water (for example, hydrogen peroxide) were also eliminated as causal agents of the effect in question. Since all the culture media used in these investigations were autoclaved, and since autoclaving might be considered to be necessary for the indirect effects of irradiation to become effective, the inhibitory properties of

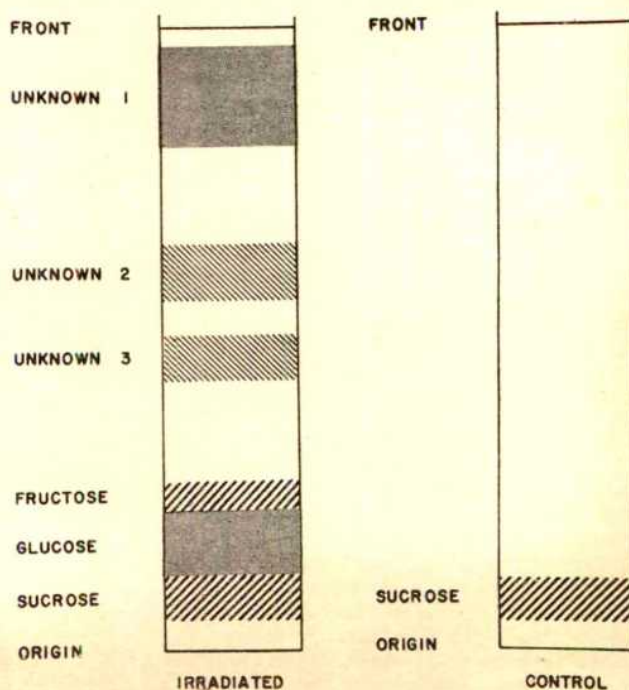


Fig. 3. Diagram to show chromatographic separation of radiolysis products from sucrose

Table 7. EFFECTS OF AUTOCLAVED AND SEITZ-FILTERED IRRADIATED SUCROSE ON THE GROWTH OF FRESH-CUT CARROT PHLOEM EXPLANTS IN A NON-IRRADIATED BASAL MEDIUM SUPPLEMENTED WITH COCONUT MILK

Medium	Wt./explant \pm S.D.
Basal medium (control)	17.3 \pm 2.2
Basal (sucrose irradiated and autoclaved) + coconut milk	2.6 \pm 0.8
Basal (sucrose irradiated and Seitz-filtered) + coconut milk	4.4 \pm 1.2
Basal + coconut milk (control)	154.1 \pm 17.4

Interpretation: The procedure in this experiment was patterned after the experiment of Table 6. The only difference was that the effects of the irradiated sucrose were determined after 18 days by the weight of the explants. These results confirm those in Table 6 and show that the effect in question was not elicited by autoclaving.

irradiated sucrose, both autoclaved and Seitz-filtered (Table 7), were compared. These results show that the biological effects of irradiated sucrose, though somewhat accentuated by autoclaving, do not require this treatment.

Knowing that irradiated sucrose mediates the biological effects which have been observed in the cultured carrot tissue, the physicochemical properties of the irradiated carbohydrate were investigated. The first change to be noted was the appearance of a prominent reducing power in the irradiated sucrose. This reducing power was markedly increased by autoclaving the irradiated sucrose solution, indicating a further breakdown of the irradiated material under these conditions (15 lb./in.²; 121° C, 15–20 min).

It was also noted that the originally neutral solution of sucrose (pH 7.0) became acid (pH 3.3) when it was irradiated. If the irradiated solution was readjusted to neutrality with base (0.1 N sodium hydroxide) and then autoclaved, the solution again became acid (pH 3.5). The ability of the irradiated sucrose solution to absorb ultra-violet radiation was also noted. As reported for glucose by Laurent⁶ and Phillips *et al.*⁸, and for other carbohydrates by Phillips⁹, irradiated sucrose solutions absorb strongly in the ultra-violet region (Table 8 and Fig. 1).

Using carbon-14 labelled as well as unlabelled sucrose, the products of γ -radiolysis were examined using paper chromatography and autoradiography. At a radiation dose of 2 Mrad, delivered over a 24-h period, there were at least three zones containing unidentified radiolysis products derived from the original sucrose, along with glucose and fructose (Fig. 2). The zone attributed to glucose also contained fluorescent materials (see Fig. 6) and, although the data are not here given, the chromatographic, fluorescence and colour reaction characteristics of at least six products of radiated sucrose have been determined. There was also some generalized streaking of the radioactivity along the length of the chromatogram. The composite diagram of Fig. 3 shows the radiolysis products revealed by autoradiography and by standard colour reactions.

Other Biological Effects of Radiation which are Mediated Indirectly by Radiolysis Products of Sugar. This section will record evidence to suggest that there are other biological effects which may be induced by irradiated sucrose; these comprise effects on meiotic and mitotic cell divisions (observed in *Vicia* roots and *Tradescantia* microspores respectively) and possible mutations in *Drosophila*. The test of the effects of irradiated sucrose on chromosomes was made in buds of *Tradescantia paludosa* which were exposed to a basal medium that contained 2 per cent irradiated sucrose; normal non-irradiated sucrose was used in control

Table 8. ULTRA-VIOLET ABSORPTION OF GAMMA-IRRADIATED SUCROSE SOLUTIONS AS AFFECTED BY pH

	Maximum (m μ)	Minimum (m μ)
Irradiated (pH 4.5)	263	233
Irradiated; autoclaved (pH 4.5), diluted 1:5	268	240
Irradiated (pH 11.0)	285	246
Irradiated; autoclaved (pH 11.0), diluted 1:5	286	252

Interpretation: After irradiation (2 Mrad) the sucrose solution (2 per cent) became acid (pH 4.5) and has a strong ultra-violet absorption with maxima and minima that were affected by pH and greatly so by autoclaving.

cultures employed. The buds were removed daily from these cultures and the dissected anthers stained with Gomori's haematoxylin¹⁰ and their chromosomes examined; Fig. 4 shows the cytological abnormalities which were observed. It is evident from Fig. 4 that irradiated sucrose mediates chromosomal abnormalities which are similar in kind and degree to those attributable to direct radiation of the tissue.

Another experiment was designed to examine the effects of irradiated sucrose on mitoses in roots of young *Vicia faba* seedlings. The results in Table 9 show that the products from irradiated sucrose act as strong antimutagenic agents and they also cause abnormal mitoses and chromosomal aberrations.

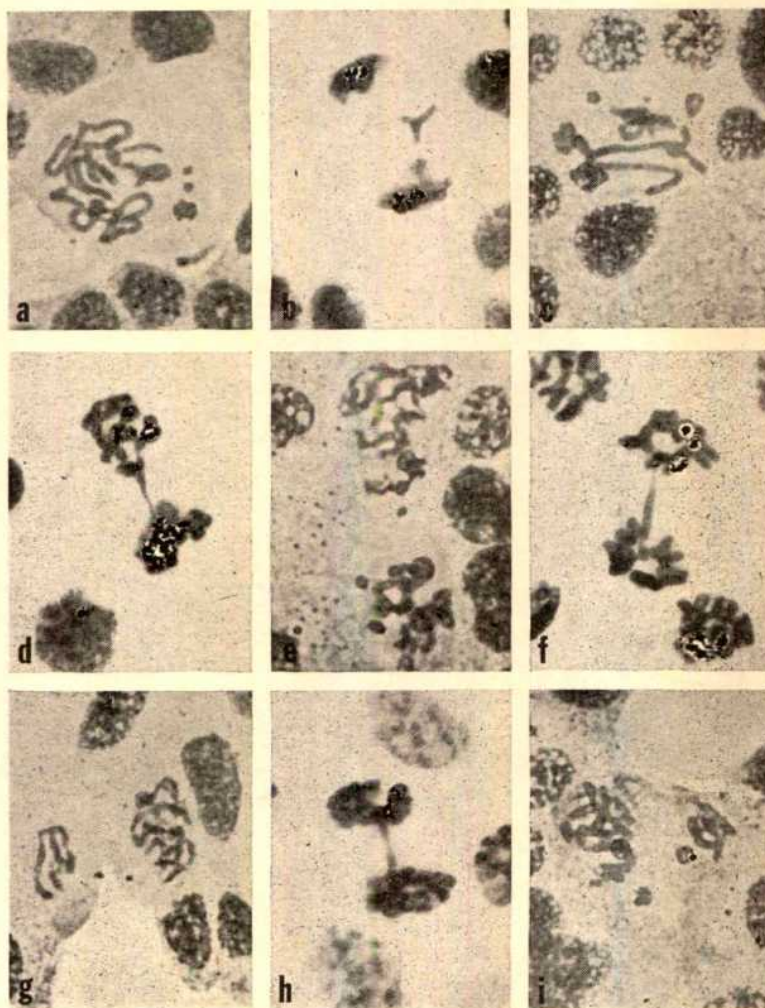


Fig. 4. Flower buds of *Tradescantia paludosa* were placed aseptically on a basal medium which contained either 2 per cent irradiated sucrose or, in the controls, the non-irradiated sugar. Buds were removed at three 24-h intervals, the anthers dissected and smears of microspores made and stained with Gomori's haematoxylin as described by Melander and Wingstrand (ref. 10). Obvious chromosomal abnormalities in this meiotic situation are evident in the form of fragmentations (cf. a, b, c); apparent chromosomal bridges (cf. d, e, f and h); unequal divisions and formation of micronuclei (cf. e, f, g and i).

Table 9. EFFECTS OF IRRADIATED SUCROSE ON MITOSIS IN ROOTS OF *Vicia faba* SEEDLINGS

	% prophase	% metaphase	% anaphase	% telophase
2 h	75.3	9.5	9.3	6.3
4 h	69.2	22.0	1.0	7.8
12 h	86.0	7.0	2.0	5.0
24 h	53.0 (17)	17.0 (84)	21.0 (89)	9.0 (18)
48 h	97.6	1.7	0.8 (50)	0
Control	56.5	16.5	17.5	9.5

Interpretation: The roots of young seedlings were immersed in a 2 per cent solution of sucrose which had been irradiated (2 Mrads) and, in controls, in 2 per cent normal sucrose. At the intervals specified, smears were made of root tips, and in the stained preparations the percentage of cells in the various stages of mitosis was determined. The percentages of abnormal nuclei are indicated in brackets in those instances where these were noted. After 48 h treatment with irradiated sucrose only very few divisions still occurred. Through the aid of Prof. C. H. Uhl these results were assembled from many root tips because they were obtained under supervision by a class in cytological technique.

A first test (being subject to confirmation) of the production of sex-linked lethal mutations in *Drosophila melanogaster* when reared on a medium which contained irradiated sucrose was carried out by Miss Carol Sachs in the laboratory of Prof. Bruce Wallace of this University. The results of this experiment are shown in Table 10. When analysed statistically, the effect attributable to irradiated sucrose failed to reach the level of full statistical significance; even so the data are suggestive of an increased mutation rate in the flies reared on the medium containing irradiated sucrose. These results are in substantial agreement with those reported for the effect of ultra-violet radiated glucose on the frequency of mutation in certain bacteria¹¹ and for the frequency of occurrence of sex-linked lethals in cultures of *Drosophila* which had been reared on an irradiated medium¹².

The data presented on *Tradescantia*, on *Vicia*, and on *Drosophila*, concerned with mitotic and meiotic chromosomes of plants and mutation in an insect supplementing the more extensive data on carrot cells in Tables 1-7, together comprise impressive evidence of the indirect biological effects of radiation that may be mediated by sugar—especially sucrose—in the ambient medium and presumably also in the cells. Before describing some further attempts to characterize chemically the biologically active radiolysis products from sugar some other information in the literature may be relevant. The reported incidence of chromosome aberrations in barley embryos¹³ and roots¹⁴ grown on irradiated media could be attributed to breakdown of the carbohydrate in the culture medium in the manner here described. Similarly the anti-mitotic action of extracts prepared from irradiated plants as claimed by Kuzin and Kryukova¹⁵ could be due to breakdown products of carbohydrate. Evidence on these points awaits further enquiry.

Further Attempts to characterize the Biologically Active Radiolysis Products from Sucrose. Relatively large amounts of carbon-12 sucrose were radiated with cobalt-60 at 2 Mrads delivered over a 2-h period. The properties of the radiolysis products (see Table 8 or Fig. 1) could now be used in the attempted isolation of the most active components. The radiated sucrose, after preliminary trials and tests by the carrot assay method, was fractionated as shown in Figs. 5 and 6.

The information contained in Fig. 6 shows the procedures by which the biologically active radiolysis products of sucrose may be isolated, assayed and eventually purified with the view of their final identification; this work is still proceeding. Fig. 7 summarizes the state of the cells, with and without the growth induction due to coconut milk, and emphasizes the effects (direct and indirect) of cobalt-60 radiation on them. Prior to the large synthesis of nucleic acid (and formation of ribosomes), which pre-

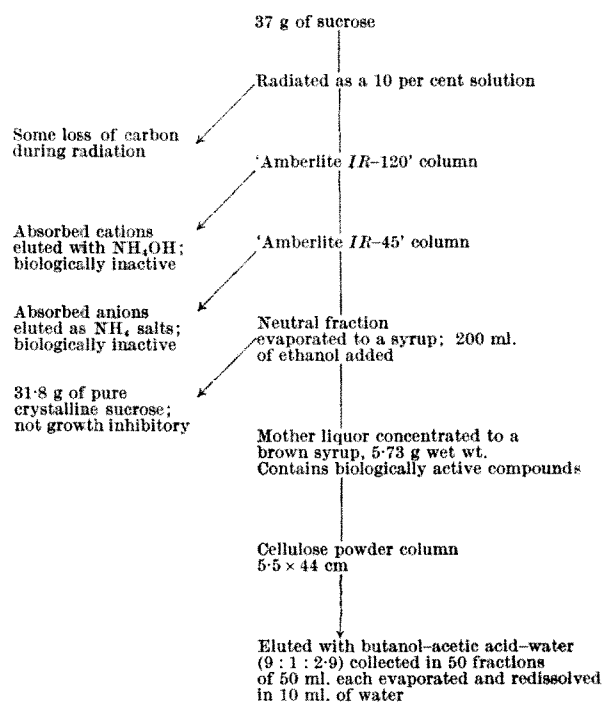
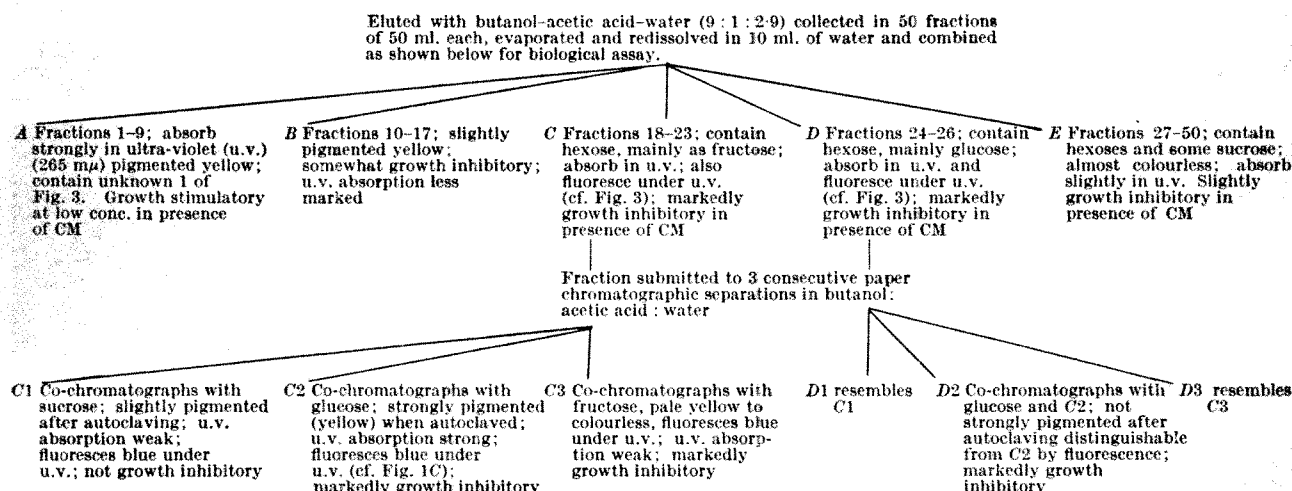


Fig. 5. Separation of radiolysis products from sucrose



In summary, cobalt-60 radiolysis produces in small amount many new substances; individually they account for only a small part of the weight of the sugar. Several of these compounds are strongly unsaturated, fluoresce, and become more pigmented on autoclaving (Fig. 1). Among these substances are those which are responsible for the growth-inhibitory properties at higher concentrations and the growth-stimulatory properties at lower concentrations; these effects are exerted at the site at which the growth induction due to coconut milk normally acts. They also have other biological effects to which reference has been made.

Fig. 6. Properties of radiolysis products from sucrose: sub-fractions of the neutral fraction eluted from the cellulose powder column

Table 10. EFFECT OF IRRADIATED SUCROSE ON MUTATIONS IN *Drosophila melanogaster*

	Frequency of sex-linked lethals	% lethals
Irradiated sucrose	5/1000	0.5
Control sucrose	1/500	0.2

Interpretation: This system was set up for the purpose in the laboratory of Prof. Bruce Wallace. *SC* wild-type flies were grown on a normal culture medium with and without 2 per cent irradiated sucrose (2 Mrad). The male progeny from these populations were mated to Muller-5 females. Mutations caused by the irradiated sucrose on the X-chromosome of the *SC* males will be carried in the female progeny of this mating. By brother-sister matings in this programme, the occurrence of sex-linked lethals may be revealed by the abnormal proportions of red-eyed male progeny. Thus the test of the incidence of mutation is the frequency of red-eyed males in the population, for a sex-linked lethal, due to the sucrose, would render the red-eyed males inviable. From these results the incidences of mutation could be calculated, and these are shown as percentages.

cedes the cell division caused by the coconut milk (that is, 0-4 days), the cells are very sensitive to cobalt-60 radiation (Tables 2 and 4); subsequent to this time and after cells are in the dividing state they are much more resistant (Tables 3 and 5). There is a suggestive parallel here with certain work on bacteria¹⁷.

Concluding Remarks. The work here described has a potential impact on currently important biological problems as follows:

(1) A strong antimetabolite from irradiated sugar, which can overcome the growth induction due to coconut milk, or even stimulate it further at low concentration, contributes to an understanding of the site of action of the growth stimulus and to the interpretation of a balanced set of chemical controls which either release or suppress the ability of the cells to grow.

(2) If the deleterious effects of radiation on cells may be mediated indirectly through its effects on sugar, present either in the cells or in the medium, this is important for the understanding of the mechanism of radiation-induced effects. These ideas lead to obvious biologically important questions that may now be investigated. In particular, the stability of the radiolysis products raises questions concerning their longevity in

cells, or organisms, for this has an important bearing on the relative importance of the direct and the indirect effects of radiation.

(3) The work has other and obvious implications for the radiation sterilization of food, especially in those cases in which the material so radiated is relatively rich in sugar. If radiation effects may be transmitted to cells via stable radiolysis products derived from sugar, one should clearly know whether or not these have biologically important consequences, both short and long term, before there is widespread use of radiation-sterilized foods that contain sugar.

(4) Much more needs to be known, than is available in the literature, or in this paper, of the chemical consequences of submitting sugar to radiation, particularly γ -radiation from cobalt-60. Sufficient has, however, been said to show that the biologically active substances produced from sugars by radiation can now be recognized and isolated and, in the carrot-coconut milk tissue culture system, there is a precise means available for the assay of their effects on growth by both cell division and cell enlargement. Further work of this kind is now in progress.

In short, the experiments which started as an investigation of radiation-induced mutations in isolated cells (still a worthy objective to which we shall return) have raised biologically important questions to which final answers should be found. The purpose of this article is to make these findings widely known, for, although the evidence is largely drawn from higher plants, it nevertheless has obvious potential bearing on other living systems, for it stresses that the effects of the radiation are neither confined to their direct effects on the cells nor to the immediate period of exposure to the radiation.

During part of this work Dr. Holsten worked under pre-doctoral fellowships of the National Science Foundation and the National Institutes of Health as directed by one of us (F. C. S.) and most of the results presented here were embodied in his thesis for a Ph.D. degree.

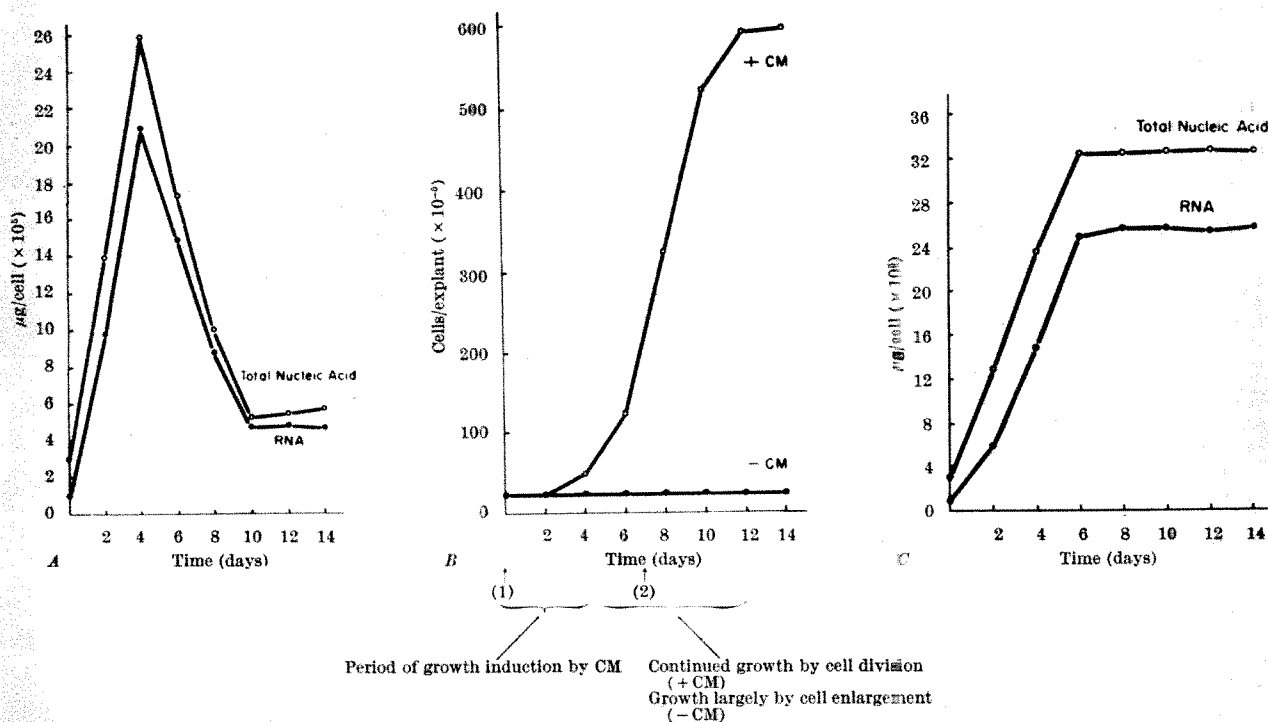


Fig. 7. Characteristics of the tissue culture system, with respect to growth and nucleic acid content, which responds to cobalt-60. A depicts the changes with time in the nucleic acid content of carrot cells in explants which undergo the growth induced by coconut milk (+ CM); B describes the changes in the number of cells in carrot explants which grow by cell division in the presence of coconut milk (+ CM) or merely enlarge in its absence (- CM); C depicts the changes in the nucleic acid content of carrot cells in explants which lack the growth stimulus of coconut milk (- CM). In B the arrow at (1) indicates the time of application of cobalt-60 radiation which thereby inactivated the cell division response otherwise due to CM, though it still permitted growth by cell enlargement. The arrow at (2) indicates the time of application of cobalt-60 radiation to growing cells, which were much more resistant to the radiation than those which had not undergone prior growth induction. A and C respectively show the nucleic acid content at these times.

Dr. M. Sugii, of the Institute for Chemical Research, Kyoto University, Takatsuki, Osaka, Japan, was able to work on this problem at Cornell University under arrangements made possible by a grant (GM09609) to one of us (F. C. S.) from the National Institutes of Health, U.S. Department of Health Education and Welfare; Dr. Sugii's contribution related to the more chemical fractionation of the radiated sucrose.

All other laboratory work was made possible by the research grant cited. This included the help with the aseptic procedures provided by Mrs. M. O. Mapes and by certain research assistants to one of us (F. C. S.).

For the radiation treatments we thank the Geneva Experiment Station of Cornell University and also the Nuclear Reactor Laboratory at Ithaca, New York, for access to their cobalt-60 sources.

¹ Holsten, Richard D., Ph.D. thesis, Cornell Univ., Ithaca, N.Y. (1965).

² Steward, F. C., Mapes, Marion O., and Smith, Joan, *Amer. J. Bot.*, **45**, 693 (1958).

³ Blakely, L. M., and Steward, F. C., *Amer. J. Bot.*, **51**, 780 (1964).

⁴ Steward, F. C., Blakely, L. M., Kent, Ann E., and Mapes, Marion O., *Brookhaven Symp. Biol.*, **16**, 73 (1963). Steward, F. C., Mapes, Marion O., Kent, Ann E., and Holsten, R. D., *Science*, **143**, 20 (1964).

⁵ Steward, F. C., Shantz, E. M., Pollard, J. K., Mapes, Marion O., and Mitra, J., in *Synthesis of Molecular and Cellular Structure*, edit. by Rudnick, Dorothea, 193 (1961).

⁶ Laurent, Tarvard C., *J. Amer. Chem. Soc.*, **78**, 1875 (1956).

⁷ von Euler, Hans, and Martius, Carl, *Ann.*, **505**, 73 (1933). Weygand, Friedrich, *Arkiv. Kemi.*, **3**, 11 (1950).

⁸ Phillips, G. O., Moody, G. J., and Mattok, G. L., *J. Chem. Soc.*, **52**, 3522 (1958).

⁹ Phillips, Glyn O., *Adv. Carbohydrate Chem.*, **16**, 13 (1961).

¹⁰ Melander, Y., and Wingstrand, K. G., *Stain Tech.*, **29**, 217 (1953).

¹¹ Stone, Wilson, Wyss, Arville, and Hass, Felix, *Proc. U.S. Nat. Acad. Sci.*, **33**, 59 (1947).

¹² Swaminathan, M. S., Nirula, S., Natarajan, A. T., and Sharma, R. P., *Science*, **141**, 637 (1963).

¹³ Natarajan, A. T., and Swaminathan, M. S., *Ind. J. Genetics and Plant Breeding*, **16**, 220 (1958).

¹⁴ Swaminathan, M. S., Chopra, V. L., and Bhaskaran, S., *Rad. Res.*, **16**, 182 (1962).

¹⁵ Kuzin, A. M., and Kryukova, L. M., *Doklady Acad. Nauk U.S.S.R.*, (AIBS Trans.), **137**, 205 (1961).

¹⁶ White, P. R., *The Cultivation of Animal and Plant Cells* (Ronald Press, New York, 1963).

¹⁷ Pollard, Ernest C., Ebert, Marlin J., Miller, Carolyn, Kolacz, Kathryn, and Barone, Thomas E., *Science*, **147**, 1045 (1965).

NATURE OF THE HEPATOMEGALIC EFFECT PRODUCED BY ETHYL-CHLOROPHENOXY-ISOBUTYRATE IN THE RAT

By Dr. R. HESS, W. STÄUBLI and W. RIESS

Research Laboratories, Pharmaceutical Department, Ciba, Ltd., and Laboratory of Electron Microscopy, University of Basle, Switzerland

ETHYL- α -(*p*-chlorophenoxy)isobutyrate (CPIB), a compound with hypolipidaemic properties in man¹ as well as in animal species², has been found^{3,4} to cause enlargement of the liver in male rats when added to the diet at a level of 0.25 or 0.3 per cent. This hepatomegalic effect occurred without accumulation of lipids in the liver tissue and it was supposed to be a non-specific change caused by metabolic conversion of CPIB (ref. 3). In livers of rats treated with CPIB for no longer than one week, an increase in the size of the hepatocytes was noted by Paget⁵ and this change appeared to be related to an accumulation of lysosome-like organelles within the cytoplasm.

In order to elucidate further the structural basis of the hepatomegalic effect, male albino rats on a standardized diet were given daily oral doses of 500 mg/kg CPIB for 14 days. This dosage caused a mean 41 per cent decrease in serum cholesterol concentration and also inhibited the animals' weight gain. As shown in Fig. 1, the relative liver weight rose rapidly during the first week of treatment and fell again to pre-treatment levels after CPIB was withdrawn. At the time-intervals indicated in Fig. 1, liver tissue was fixed in buffered 2 per cent osmium tetroxide using either the technique of Palade⁶ or that of Millonig⁷. Following embedding of the tissue in 'Araldite' or 'Epon 812' ultra-thin sections were prepared on a Porter-Blum (MT 1) microtome, stained with either lead hydroxide⁸ or with lead citrate⁹, and examined in a Siemens Elmiskop I operated at 80 kV.

Treatment with CPIB for 14 days gave rise to a marked increase in liver-cell organelles showing the morphological characteristics of microbodies^{10,11} (Figs. 2 and 3). In normal rat liver many of the microbodies contain an array of tubular elements that form a 'nucleoid' or core^{11,12}. In liver cells from treated rats, the microbody-like structures were mostly without cores, indicating that CPIB had caused the formation of an incomplete and aberrant type of organelle. Although the diameter of the 'microbodies' following CPIB treatment was found to vary within wider limits than in normal liver (mean diameter 0.5–0.6 μ), the single membrane and the appearance of the finely granular matrix were not visually altered.

The increased formation of 'microbodies' appeared to be a very rapid process since these organelles were much more numerous 24 h after the first dose of CPIB. The accumulating 'microbodies', some of them in clusters, were distributed throughout the cytoplasm. There was no obvious association with other cytoplasmic constituents except that, in part, the 'microbody' membranes were connected to the smooth-surfaced endoplasmic reticulum by means of short, hook-like or finger-like extensions¹³. CPIB treatment caused no increase in 'multivesicular bodies', that is, particulate structures which have been considered to develop into microbodies¹⁴. By contrast, elements of the smooth-surfaced endoplasmic reticulum proliferated in response to the first dose of CPIB.

Despite the fact that the liver weight returned to normal values following the withdrawal of CPIB (Fig. 1), there was still an increased number of microbodies present 14 days after the cessation of treatment. The microbodies appeared to be broken down in part by the mechanism proposed by Novikoff and Shin¹⁵ which leads to the formation of 'autophagic vacuoles'. In contrast to the slow removal of microbodies, the CPIB-induced pro-

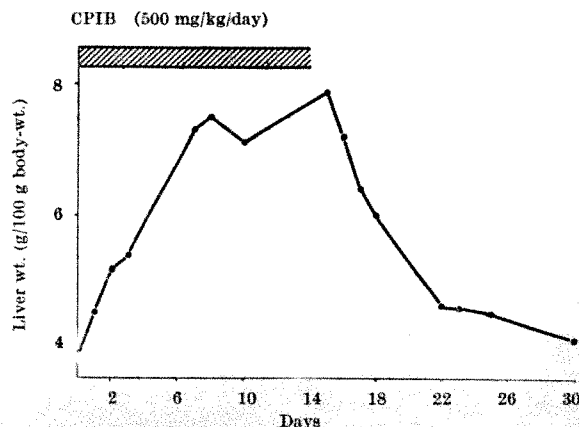
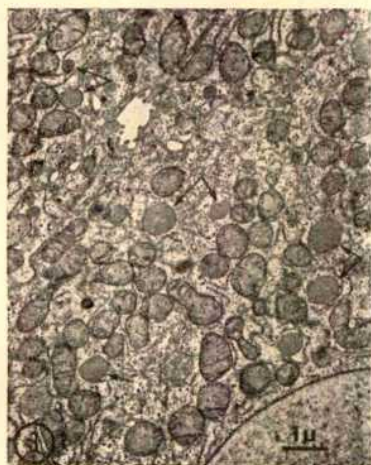


Fig. 1. Liver hypertrophy produced by oral administration of CPIB. Each point represents the mean of values for 2 male rats



Figs. 2 and 3. Electron micrographs of comparable cytoplasmic areas of normal liver cell (Fig. 2) and of liver following treatment with 500 mg/kg/day of CPIB for 10 days (Fig. 3). Microbody-like structures (arrows) are increased in number.

liferation of smooth-surfaced endoplasmic reticulum regressed rapidly after treatment was discontinued.

Biochemical changes occurring during treatment were examined in liver tissue from male rats following daily oral treatment with 300 mg/kg CPIB for 7 days. A mean 29.3 per cent increase in relative liver weight ($P < 0.01$) was obtained and this effect was unaccompanied by significant alterations in liver content of total lipid, phospholipid, and total cholesterol relative to the wet weight of tissue. A decrease in relative cholesterol content (mean 33 per cent) was attained in a separate experiment after 4 weeks of treatment with the same dose. In principle, these findings agree with earlier reports^{3,4}.

Since the protein content per g of liver was the same in treated animals as in the controls (Table 1), it could be assumed that the increase in the number of microbodies had in part contributed to the actual rise in total liver protein. These organelles have been shown to contain the bulk of hepatic catalase, urate oxidase and D-amino-acid oxidase activity¹⁵. In order to follow up, by

means of enzymatic parameters, changes in the population of cellular organelles we observed in the electron microscope, catalase¹⁶, urate oxidase¹⁷, and cytochrome oxidase¹⁸ were assayed in 1:10 (w/v) liver homogenates prepared at 0° in 0.25 M sucrose containing tris(hydroxymethyl)aminomethane 0.05 M pH 7.4 and 6 per cent 'Ficoll' (mol. wt. 400,000; Pharmacia, Uppsala). Mitochondrial glycerol-1-phosphate dehydrogenase (GPD) activity was determined in homogenates from the same livers¹⁹. The results (Table 1) demonstrate an increase in specific activity of the mitochondrial enzymes cytochrome oxidase and GPD, which was accompanied by a decrease in urate oxidase activity and a small but significant increase in catalase specific activity. Owing to the increase in liver weight, the decrease in urate oxidase activity possibly reflects dilution. This appeared to be related to the accumulation of microbodies deficient in crystalloid cores which were seen in the electron micrographs of livers following treatment with CPIB. The cores have been tentatively identified as bearers of urate oxidase^{12,15}.

An attempt was made to separate microbody-like particles from mitochondria (obtained at 480,000g min) by density equilibration centrifugation in 'Ficoll' density gradients (mixed from two stock concentrations, each containing 'Ficoll' in a mixture of isotonic sucrose:tris buffer pH 7.4 = 2:1). Particles from normal liver were fractionated at densities ranging from 1.1728 g/ml. to 1.1890 g/ml. This resulted in the resolution of catalase and urate oxidase activity in two peaks. By contrast, a gradient of lower density (1.1643–1.1748) had to be used to separate liver particles following CPIB treatment. A considerable overlap of all the cytoplasmic particles was observed in these preparations and no clear separation was achieved. This different behaviour of particles from normal and CPIB-treated livers might depend on some structural alteration caused by the treatment. That this may be the case was also suggested by a tendency for catalase to be released into the post-mitochondrial supernatant of preparations from treated liver. Catalase activity has been associated with the structureless 'sap' of microbody-like particles^{12,15}. After centrifugation of the particulate fractions in the tris-sucrose-'Ficoll' medium at 1.28×10^6 g min the supernatant fraction contained 36 per cent of the total catalase activity of normal liver, but 69 per cent of recoverable activity in the case of liver from CPIB-treated animals.

It is not readily apparent whether, in the rat, the hepatic enlargement associated with accumulation of microbody-like organelles has any relationship to the hypolipidaemic effect of CPIB. The physiological role of these particles remains virtually unknown¹⁵.

The inhibitory effect of CPIB on cholesterol synthesis *in vivo*⁴ might rather be explained by the changes we have observed to occur in the endoplasmic reticulum. Stimulation of microsomal systems is a property of a number of cholesterol synthesis inhibitors²¹. According to Thorp²², CPIB may act by raising the relative concentration of thyroxine in the liver. The increase in mitochondrial activity, particularly of GPD, observed in the present experiments, seems to fit in with this assumption. It has been shown^{19,23} that, at the tissue-level, both endogenous and exogenous thyroid hormone is capable of selectively stimulating mitochondrial GPD activity. Both

Table 1. EFFECT OF CPIB ON LIVER ENZYME ACTIVITIES OF RATS

Animals	Protein mg/g wet wt.	Catalase	Urate oxidase	GPD	Cytochrome oxidase
Controls	119.0 ± 8.6 (10)	8.20 ± 1.55 (6)	95.12 ± 49.7 (8)	0.313 ± 0.13 (5)	0.86 ± 0.058 (3)
CPIB	127.8 ± 14.4 (20)	10.46 ± 2.78 (15) $P < 0.05$	40.39 ± 32.2 (19) $P < 0.02$	1.95 ± 0.42 (7) $P < 0.001$	1.50 ± 0.540 (6) $P < 0.05$

Enzymatic activity per mg of protein of total homogenate is expressed as follows: catalase, μ moles of H_2O_2 decomp./sec.; urate oxidase, μ moles of uric acid ox./h; GPD, μ moles of formazan prod./h; cytochrome oxidase, μ moles of reduced cytochrome c ox./min. Protein was measured according to Lowry *et al.* (ref. 20). Mean values ± S.D. and no. of animals (in brackets) are given; significance of difference (P) is ascertained by t -test. CPIB was administered daily by gavage at a dose of 300 mg/kg for 7 days.

the latter effect and the hepatomegaly (including the accumulation of microbodies) produced by CPIB may be attributed to altered co-factor availability since, in preliminary experiments, the hepatic effects of this drug could be inhibited by simultaneous treatment with the ATP-trapping agent ethionine²⁴ (14×250 mg/kg/day) but were not affected by puromycin (3×3 mg/kg/day) or actinomycin D (1 mg/kg/day). (The latter substance was kindly supplied by Merck, Sharp and Dohme, International.)

¹ Oliver, M. F., *J. Atheroscler. Res.*, **3**, 427 (1963).

² Thorp, J. M., and Waring, W. S., *Nature*, **194**, 948 (1962).

³ Best, M. M., and Duncan, C. H., *J. Lab. Clin. Med.*, **64**, 634 (1964).

⁴ Avey, D. R., Swyryd, E. A., and Gould, R. G., *J. Lipid Res.*, **6**, 369 (1965).

⁵ Paget, G. E., *J. Atheroscler. Res.*, **3**, 729 (1963).

⁶ Palade, G. E., *J. Exp. Med.*, **95**, 285 (1952).

⁷ Millonig, G., in *From Molecule to Cell: Symp. Electron Microscopy*, edit. by Buffa, P., 347 (Modena, 1963).

⁸ Millonig, G., *J. Biophys. and Biochem. Cytol.*, **11**, 736 (1961).

⁹ Reynolds, E. S., *J. Cell Biol.*, **17**, 208 (1963).

¹⁰ Gansler, H., and Rouiller, C., *Schweiz. Z. Allgem. Pathol. Bakteriolog.*, **19**, 217 (1956).

¹¹ Stäubli, W., *J. Cell Biol.*, **16**, 197 (1963).

¹² Hruban, Z., and Swift, H., *Science*, **146**, 1316 (1964).

¹³ Novikoff, A. B., and Shin, W. Y., *J. Microscopie*, **3**, 187 (1964).

¹⁴ Bruni, C., and Porter, K. R., *Amer. J. Pathol.*, **48**, 691 (1965).

¹⁵ Baudhuin, P., Beaufay, H., and De Duve, C., *J. Cell Biol.*, **26**, 219 (1965).

¹⁶ Creasey, W. A., *Biochem. J.*, **77**, 5 (1960).

¹⁷ De Duve, C., Pressman, B. C., Gianetto, R., Wattiaux, R., and Appelmans F., *Biochem. J.*, **60**, 604 (1955).

¹⁸ Cooperstein, S. J., and Lazarow, A., *J. Biol. Chem.*, **189**, 665 (1951).

¹⁹ Lee, Y. P., and Lardy, H. A., *J. Biol. Chem.*, **240**, 1427 (1965).

²⁰ Lowry, H. O., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. Biol. Chem.*, **193**, 265 (1951).

²¹ Kato, R., Vassanelli, P., and Chiesara, E., *Biochem. Pharmacol.*, **12**, 349 (1963).

²² Thorp, J. M., *J. Atheroscler. Res.*, **3**, 351 (1963).

²³ Rueggamer, W. R., Westerfeld, W. W., and Richert, D. A., *Endocrinology*, **75**, 908 (1964).

²⁴ Villa-Trevino, S., Shull, K. H., and Farber, E., *J. Biol. Chem.*, **238**, 1757 (1963).

A LOCALIZED HAEMOLYSIS IN GEL METHOD FOR THE DETECTION OF CELLS PRODUCING 7S ANTIBODY

Detection of Cells producing 7S Antibodies by the Plaque Technique

THE kinetics of the appearance of antibody-forming cells after the injection of sheep red blood cell (sheep-RBC) antigen have been studied by the plaque technique^{1,2}. The number of plaque-forming cells before immunization is about 60 per 10^8 lymphoid cells; but after immunization the number rises to a maximum between the 4th and 5th day of about 30,000 in 10^8 lymphoid cells. After the 5th day there is a rapid decline in the number of plaques so that on the 13th day there are only 2,000 per 10^8 lymphoid cells. The kinetics of serum haemolytic antibody appearance are consistent with its production by plaque-forming cells. In contrast to this, serum haemagglutinating antibody does not reach a maximum until the 10th day post-immunization and afterwards persists considerably longer than haemolytic antibody. The differences between haemagglutinins and haemolysins could be related either to antibodies directed against different antigenic specificities on the erythrocyte or to the heterogeneity of antibodies directed against identical specificities, or both.

It has been found that the antigenic determinants evoking haemolysins can be dissociated from those evoking haemagglutinins³. When sheep-RBC were treated with 0.5 per cent or 1 per cent formalin for a few days they lost the capacity to induce haemolysin formation but retained unchanged their capacity to induce haemagglutinins. It follows that the haemolysins and haemagglutinins were directed against different antigenic groups.

In further experiments we have examined the plaque-forming ability of 19S and 7S producing cells. 19S antibodies are known to be much more effective in inducing haemolysis than the 7S antibody⁴. However, Riha found that he could increase the haemolytic activity of 7S antibodies by the addition of an antiserum specific for the 7S antibodies⁵.

In the experiments described here we used this principle in combination with the plaque technique to detect the production of 7S antibodies by individual cells. We have used the plaque technique previously described^{6,7}. Cells isolated from the spleen or lymph nodes were washed three times and suspended to the required concentration in Parker 199 solution with 0.5 per cent human serum albumin (HSA) and brought to 42° C. One part of the lymphoid cell suspension was added to two parts of agarose solution containing washed sheep-RBC (3 parts 1 per cent agarose with 1 part 6 per cent sheep-RBC; in Parker 199/HSA) which was also at 42° C. The mixture of cells,

sheep-RBC and agarose was pipetted dropwise from a height of about 60 cm into a Petri dish or on to a slide. This resulted in the drop forming a flattened spot with a diameter of 18 mm. The spots were allowed to harden and were then incubated at 37° C in a humid atmosphere with 5 per cent carbon dioxide for 6–20 h. After the incubation the individual 'spots' were overlaid with the anti- γ G serum. This antiserum was prepared by immunization of a different species with the γ G from the species the plaque-forming cells of which were to be examined. The antiserum was allowed to remain on the agarose spots for 1 h at 4° C and was then washed off with veronal buffer⁸. Finally, guinea-pig complement (20 units/ml.) previously absorbed with sheep-RBC was added. The anti- γ G serum was equally effective when incorporated into the agarose solution at the start of the procedure, and for routine work this procedure is recommended.

The number of plaques formed varied greatly with the concentration of anti- γ G serum (Table 1). There was an optimal concentration for maximal development of plaques; higher concentration resulted in inhibition. Plaque formation by 19S-producing cells was also inhibited at high concentrations, presumably because of the presence of antibodies directed to the L chain, which are common to 7S and 19S molecules.

In order to study the morphology of individual cells forming 7S and 19S antibodies, the following method was used. The medium with cells and sheep-RBC was incubated without anti- γ G and developed with complement. The plaques thus formed (mainly 19S) were then marked and the complement washed off; the anti- γ G was then added and followed by a second incubation with complement.

The kinetics of the appearance of antibody cells detected with and without added anti- γ G are shown in Fig. 1. On the 5th day after immunization with sheep-RBC the number of cells detected after the addition of anti- γ G

Table 1. INFLUENCE OF ANTI- γ G SERUM ON THE NUMBER OF ANTIBODY-FORMING CELLS

	Complement only	Anti- γ G serum + complement 1:5	1:50	1:100
Rabbits (secondary * reaction)	23,000	7	121,000	— (a)
	6,000	660	75,000	— (a)
	5,500	—	40,000	39,000 (b)
Piglets (secondary † reaction)	2,640	—	21,600	— (a)
Mice 10 days after primary stimulus‡	4,160	—	15,330	— (b)

(a) Antiserum added into gel medium.

(b) Antiserum overlays drops after incubation.

* Pig antiserum to rabbit γ G.

† Rabbit antiserum to pig γ G.

‡ Rabbit antiserum to mouse γ G.

was not greater than the number seen on the addition of complement alone. On the 10th day only 27 per cent of the total plaque-forming cells are detected by complement alone. By the 14th day 92 per cent of the plaques are detected only if the anti- γ G is added. The serum haemolysin titres of these mice are also shown in Fig. 1. The peak of haemolytic antibody detected by complement alone is reached within 5 days and falls after the 10th day. The peak of haemolytic antibody detected by complement plus anti- γ G is not reached until the 10th day.

It was demonstrated that the quality of haemolytic antibody in these mice also changes (Fig. 2).

The partition of serum by either 'Sephadex-G 200' or density gradient ultracentrifugation revealed that 5 days after immunization only high molecular weight antibodies were present. They were sensitive to treatment with

2-mercaptoethanol. By the same technique it was shown that serum from mice 14 days after immunization contained predominantly 7S antibody, which was resistant to treatment with 2-mercaptoethanol. However, it must be borne in mind that changes in serum antibody may appear later than changes observed at the cellular level.

We conclude that the addition of anti- γ G increases the detection by the gel-technique of 7S antibody-producing cells. This enables us to follow quantitative changes in cells producing 19S or 7S antibody.

J. ŠTERZL
I. ŘÍHA

Department of Immunology,
Institute of Microbiology,
Czechoslovak Academy of Science,
Prague.

- ¹ Jerne, N. K., Nordin, A. A., and Henry, C., in *Cell-Bound Antibodies*, 109 (Wistar Institute Press, Philadelphia, 1963).
- ² Šterzl, J., and Mandel, L., *Folia Microbiol.*, **9**, 173 (1964).
- ³ Šterzl, J., and Říha, I. (unpublished observations).
- ⁴ Humphrey, J. H., in *Complement*, 175 (Churchill, London, 1965).
- ⁵ Říha, I., *Folia Microbiol.*, **9**, 304 (1964).
- ⁶ Bernovská, J., Kostka, J., and Šterzl, J., *Folia Microbiol.*, **8**, 376 (1963).
- ⁷ Šterzl, J., Veselý, J., Jilek, M., and Mandel, L., in *Molecular and Cellular Basis of Antibody Formation*, 463 (Czech. Acad. Sci., Prague, 1965).
- ⁸ Kabat, E. A., and Mayer, M. M., *Experimental Immunology*, second ed. (C. C. Thomas, Springfield, Ill., 1961).

Use of an Antiglobulin Serum to detect Cells producing Antibody with Low Haemolytic Efficiency

THE microdroplet and immunofluorescent techniques have been used extensively for the study of antibody production by single cells¹. Recently, a third method has been established which uses a semi-solid supporting medium in which antibody-producing cells have been incorporated². The basis of this localized haemolysis in gel (LHG) technique is to set up a mixed suspension of sheep-RBC and dissociated lymphoid cells from an animal previously immunized against sheep-RBC. Antibody (haemolysin) diffuses from some of the lymphoid cells and binds to the sheep-RBC in the medium; complement is added and small areas of haemolysis (plaques) develop around these lymphoid cells.

It appears, although it has not formally been proved, that the LHG technique is limited to the detection of γ M antibody³. This conclusion is based on the observation of a parallel rise and fall of both serum haemolytic activity, mainly due to macroglobulin antibody, and plaque-forming ability. It is also consistent with the knowledge that 19S antibody is far more efficient than 7S in effecting complement-dependent lysis of sheep-RBC⁴.

This article reports the details of a method devised to adapt the LHG method for the detection of cells forming antibody with low haemolytic efficiency. Mouse antibody to sheep-RBC, produced by lymphoid cells suspended in agarose, is allowed to react with the sheep-RBC in the medium; a rabbit anti-mouse immunoglobulin serum is then allowed to react with the complex; finally, complement is added and in zones around certain lymphoid cells the sheep-RBCs lyse, forming plaques visible to the naked eye. This is illustrated diagrammatically in Fig. 1.

An antiserum against mouse immunoglobulin was raised in Himalayan rabbits, which were immunized with a preparation of a normal mouse γ G in Freund's adjuvant, followed by intravenous boosters of the mouse γ G in saline. The γ G was prepared from ascites fluid of CBA mice carrying a C3H plasma cell tumour (No. 5563) as an ascites. It was separated from γ G myeloma and other serum proteins on a DEAE-cellulose column⁵. Control sera (NRS) were obtained from Himalayan rabbits. Fresh guinea-pig serum was used as a source of complement. All sera used were absorbed with sheep-RBC and in addition the rabbit sera were heated at 56° C for 40

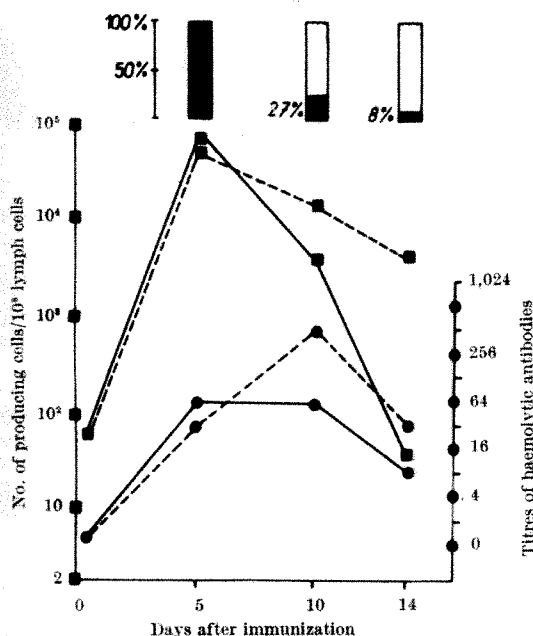


Fig. 1. The dynamics of 19 and 7S antibody formation by individual cells in mice. Top, black, percentage of cells detected by complement only; white, percentage of cells detected by anti- γ G serum and complement. Graphs, No. of cells detected by: —●—●—, complement only; —●—●—, anti- γ G serum and complement. Titres of serum haemolytic antibodies detected by: —●—●—, complement; —●—●—, anti- γ G serum and complement.

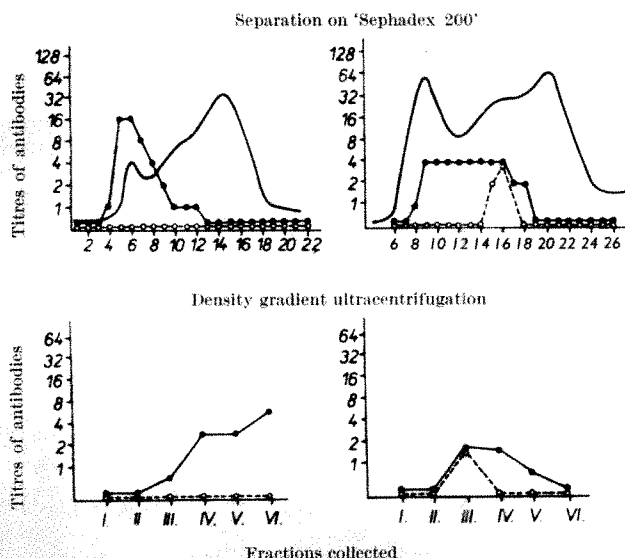


Fig. 2. Haemolytic antibodies. Left, mice 5 days after immunization with sheep-RBC. Right, mice 14 days after immunization with sheep-RBC. —, Protein; —●—●—, antibodies; —○—○—, antibodies after treatment with 2-mercaptoethanol.

min to destroy complement. The donors (CBA mice) of the lymphoid cells, in this case spleen cells, were immunized by a single intraperitoneal injection of 0.5 ml. of a 20 per cent suspension of sheep-RBC (2×10^9 cells) in Gey's solution. The technique was as follows:

(1) Disposable polystyrene Petri dishes, 9 cm in diameter, were used in these experiments.

(2) 10 ml. of 1.2 per cent (w/v) agarose (L'Industrie Biologique Française) in Dulbecco's phosphate buffered saline were added to each Petri dish and allowed to set on a level surface; these plates could be stored for up to 24 h at 2° C. All agarose solutions were made by melting the powder in 1/5 the final volume and then adding the rest of the buffer, suitably warmed. This was done because the smaller volume is more easily managed, thus helping to prevent loss of material due to boiling over. The prepared plates were warmed to 37° C immediately before addition of the medium containing the cells.

(3) Lymphoid (spleen) cells were suspended in Gey's solution with a very loose-fitting glass homogenizer. Cell suspensions were poured through a 150 μ hole stainless-steel wire mesh and the volume adjusted to give 10×10^6 viable cells per ml. Cell counts were made by phase contrast microscopy and estimates of viability were made by counting the number of cells which excluded eosin. More than 100×10^6 cells per spleen were always obtained and of these more than 75 per cent were scored as viable.

(4) Sheep-RBC in Alsever's solution (Burroughs Wellcome and Co.) were washed three times with Gey's solution (5-min centrifugation at relative centrifugal force of 800*g*) and then made up to a 20 per cent suspension in Gey's solution.

(5) For each plate, 2 ml. of 0.6 per cent agarose in Gey's solution were pipetted into a 1.3 \times 10 cm test-tube held in a rack in a water bath at 47° C.

(6) A tube was removed from the bath, the outside dried and 0.1 ml. of the 20 per cent sheep-RBC suspension rapidly pipetted into it. The contents were mixed and 0.1 ml. (1×10^6 cells) of the lymphoid cell suspension added. The contents were mixed again and poured into the prepared Petri dish. This top layer was spread by a swirling motion and allowed to set on a level surface.

(7) Plates were incubated for 1 h at 37° C in a moist atmosphere of 5 per cent carbon dioxide and 95 per cent air.

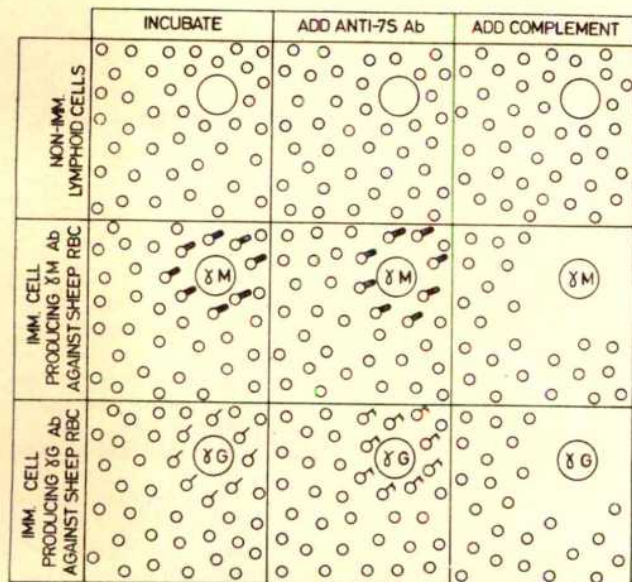


Fig. 1. A diagram to illustrate the theoretical basis of the technique. The large circles represent mouse lymphoid (spleen) cells producing no antibody, γ M type antibody or γ G type antibody. The small circles represent sheep-RBC which was the antigen and indicator cell used in these experiments. The broad and narrow bars represent γ M and γ G antibody molecules respectively, both directed against sheep-RBC. The bent bars (bottom centre) represent rabbit antibody molecules specific for mouse γ G globulins.

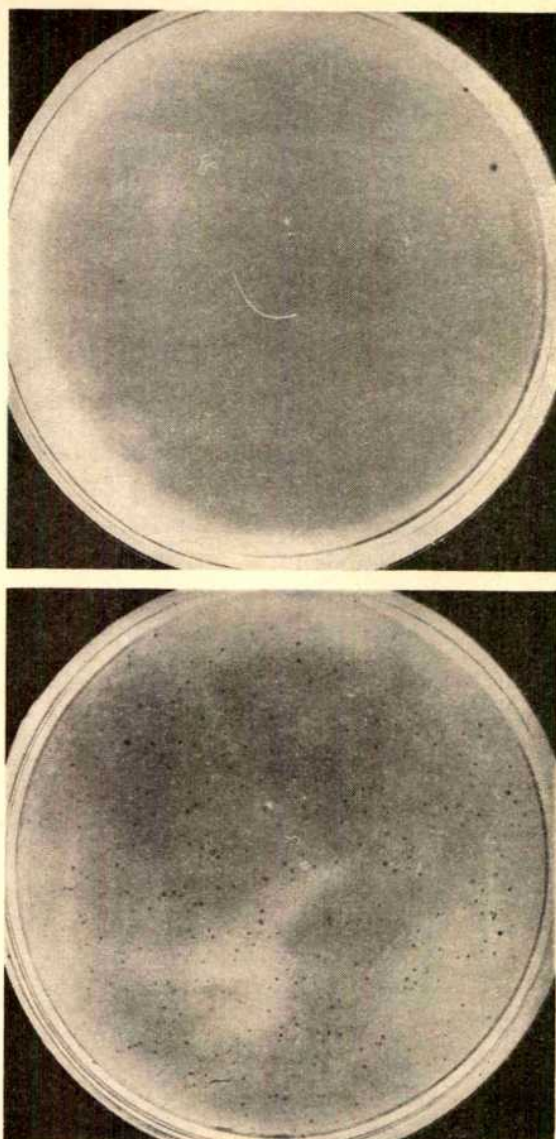


Fig. 2. This figure shows the difference between a control plate (16 plaques) and a plate treated with 1/10 rabbit anti-mouse immunoglobulin serum (548 plaques). The mouse spleen cells used in both these plates were obtained 14 days after immunization with sheep-RBC.

(8) 2 ml. of a stated dilution of the rabbit anti-mouse immunoglobulin serum were carefully pipetted on to the surface of the plate and gently spread. 2 ml. of normal rabbit serum at the same dilution were used in control experiments. Further control experiments were carried out without the addition of either antiserum or normal serum (see top line of Table 1). The plates were incubated for 1 h at 37° C.

(9) The added rabbit serum was then poured off. 1 ml. of a 10 per cent solution of fresh guinea-pig serum in Gey's solution was added to each plate as a source of complement. The plates were incubated for 30 min at 37° C.

(10) The complement was poured off, the plates inverted and the plaques counted with dark-field illumination with the aid of a slight magnification. This could be done immediately or after the plates had been stored for up to 24 h at 2° C.

Table 1 shows the effect on the development of the plaques of adding either rabbit anti-mouse immunoglobulin or normal rabbit serum. In each case the number of plaques seen are expressed as a factor of the number of plaques which developed in untreated control plates (see top line of Table 1). At 2 and 4 days after immuniza-

Table 1

The table summarizes the results of experiments designed to show the effect of normal rabbit serum (NRS) and rabbit anti-mouse γ G on the number of plaques developing *in vitro* at different times post-immunization of the spleen cell donors

Serum	Dilution	Days post-immunization of spleen cell donors					
		2	4	7	11	37	
None NRS	Control	17 (2)*	638 (3)	199 (3)	62 (2)	9 (2)	
	1/10	0.26†	0.45	1.03	0.78	0.75	
	1/30	0.58	0.76	0.95	1.37	1.00	
	1/90	0.60	0.88	1.04	1.10	1.25	
	1/270	0.70	0.89	0.89	1.47	1.00	
	1/810	0.80	0.98	0.94	1.45	1.50	
	1/2,430	0.84	0.95	0.83	0.82	1.25	
Rabbit anti-mouse immunoglobulin serum	1/10	0.08	0.26	2.32	11.45	10.40	
	1/30	0.32	0.56	3.67	11.72	10.80	
	1/90	0.51	0.66	2.80	13.10	11.00	
	1/270	0.70	0.89	2.31	8.79	8.00	
	1/810	0.66	0.83	1.38	2.51	4.00	
	1/2,430	0.83	0.99	1.06	1.39	2.50	

* No. of plaques per 10^6 spleen cells on untreated (control) plates. Figures in parentheses are the No. of mice used. Duplicate plates were made from the cells of each mouse.

† Each result is based on two plates from each of two donor mice (4 plates/result) and the figure given is a factor = $\frac{\text{No. of plaques with added serum}}{\text{No. of plaques on untreated plates}}$.

A factor of 0.08 shows a very marked inhibition of plaques; a factor of 13.02 shows a great increase of plaques, compared with controls.

tion of the lymphoid cell donors the addition of either NRS or antiserum depressed the number of plaques formed. At 7 days, NRS neither suppressed nor increased plaque development, but the plates treated with the antiserum at a 1/30 dilution showed a greater than three-fold increase in number of plaques. Eleven days after immunization there was only a slight increase in the number of plaques seen with NRS treatment but a very large increase with the addition of the antiserum; the optimum antiserum dilution was about 1/90. At 37 days post-immunization there was still a distinct difference between NRS and the antiserum, the latter giving at least ten times more plaques than the NRS with both sera at a dilution of 1/30. The effect of adding antiserum is shown in Fig. 2.

We believe that the plaques which appeared when the plates were treated with antiserum were due to antibody with low haemolytic activity, such as γ G antibody. We base this argument on the following observations: (a) the maximum number of these plaques occurred when γ M(19S) antibody was past its peak level; (b) antiserum

had opposite effects on the development of plaques formed soon after immunization and those formed later, which suggests that different types of antibody and/or cells were being affected; (c) the antiserum was primarily anti- γ G and was probably acting by virtue of its specific anti- γ G activity. The antiserum used reacted strongly with the F piece (piece III) of a papain digest of a γ G myeloma (plasma cell tumour No. 5563), but had only a minimal reaction with the S piece (pieces I and II) which contains the antigenic determinants common to all the immunoglobulins⁶. It is unlikely that the antiserum developed plaques solely because of its low reactivity with common determinants. If the serum action did depend on antibody activity against common determinants it would be difficult to understand how the same serum could decrease the number of visible plaques at 2 and 4 days post-immunization and yet increase the number of visible plaques at later times. It therefore seems unlikely that the antiserum acts by damaging γ M-containing cells that would otherwise not release their antibody. However, until more specific antisera are used it will be impossible to know the relative contributions of the cells producing different classes of immunoglobulins.

We thank Drs. R. B. Taylor and D. R. Bainbridge for suggestions, and Miss Doreen Cross for assistance. One of us (H. H. W.) was supported by an Arthritis Foundation (U.S.A.) post-doctoral fellowship.

D. W. DRESSER
H. H. WORTIS

National Institute for Medical Research,
Mill Hill,
London, N.W.7.

¹ Coons, A. H., *Harvey Lectures*, 113 (1957-58). Attardi, G., Cohen, M., Horibata, K., and Lennox, E. S., *Bact. Rev.*, **23**, 213 (1959). Nossal, G. J. V., *Adv. Immunol.*, **2**, 163 (1962).

² Ingraham, J. S., and Bussard, A., *J. Exp. Med.*, **119**, 667 (1964). Jerne, N. K., Nordin, A. A., and Henry, C., in *Cell-Bound Antibodies*, 109 (Wistar Institute Press, Philadelphia, 1963).

³ Sterzl, J., and Mandel, L., *Folia Microbiol.*, **9**, 173 (1964). Rowley, D. A., and Fitch, F. W., *J. Exp. Med.*, **121**, 671 (1964). Bussard, A. E., and Binet, J. L., *Nature*, **205**, 675 (1965).

⁴ Humphrey, J. H., in *Complement*, 175 (Churchill, London, 1965).

⁵ Askonas, B. A., *Biochem. J.*, **79**, 33 (1961).

⁶ Fahey, J., *Adv. Immunol.*, **2**, 42 (1962).

A NEW MODEL FOR VIRUS RIBONUCLEIC ACID REPLICATION

By DR. F. BROWN and DR. S. J. MARTIN

Animal Virus Research Institute, Pirbright, Woking, Surrey

SUBSTANTIAL evidence now exists to support the initial observations of Montagnier and Sanders¹ that the multiplication of virus RNA proceeds via a replicating form. This form is readily differentiated from the RNA which is present in the complete virus particles by its considerably slower rate of sedimentation in sucrose, lower buoyant density in caesium sulphate and resistance to degradation by ribonuclease. It has been postulated that this replicating form permits the preferential synthesis of new virus RNA (corresponding to the plus strand) on a primer (minus strand) which is initially coded for by the ingoing virus RNA. This replicating form is envisaged as containing a double-stranded region as well as a number of single-stranded tails, corresponding to partially formed plus strands (Fig. 1a)²⁻⁴. Such a molecule would possess a lower buoyant density than single-stranded RNA and it has been assumed that ribonuclease will remove the single-stranded tails, leaving only a double-stranded molecule (Fig. 1b).

Certain features of this model, however, do not account for all the experimental evidence available. In the first place, it has been shown that the ribonuclease-resistant RNAs present in cells infected with EMC⁵, foot-and-mouth disease⁶ and poliomyelitis⁶ viruses possess low but signi-

ficant infectivity. Unless the minus strand is infective, which is extremely unlikely, the infectivity must be contained in the plus strand. In the model which is at

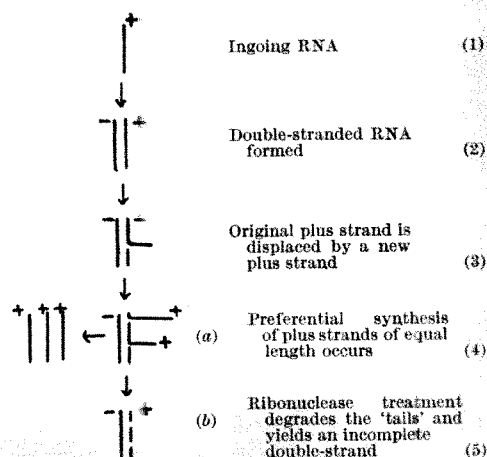


Fig. 1. Diagrammatic representation of the replicating mechanism of virus RNA which is at present accepted

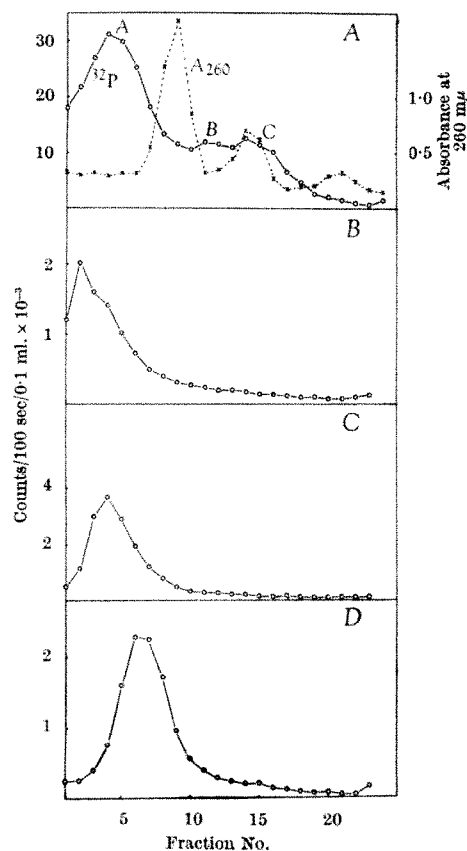


Fig. 2. Sedimentation in sucrose gradients (5–25 per cent sucrose in 0.1 M acetate, pH 5.0) of 'Sephadex G-200' filtered RNA from actinomycin D treated BHK cells, 4 h after infection with FMD virus. (A), Total RNA; (B), fraction 2 from first sedimentation recycled in a second gradient; (C), recycled fraction 4; (D), recycled fraction 6. The RNA was centrifuged for 16 h at 20,000 r.p.m. in an SW 25 rotor in a Spinco ultracentrifuge at 4°. Unlabelled BHK cell RNA was added to each sample before centrifuging to serve as an internal marker.

present favoured, the plus strand of the ribonuclease-resistant RNA would not be an intact virus RNA strand but a collection of RNA molecules, each shorter in length than the virus RNA. All the evidence so far accumulated in a number of laboratories has shown that the intact virus RNA molecule is required for infectivity^{7,8}. Secondly, the ribonuclease-resistant RNA in the model shown in Fig. 1b is a double-stranded molecule which has gaps between adjoining segments of the residual plus strands. It is questionable whether such a molecule would be stable to relatively high concentrations of ribonuclease, for example, 50 $\mu\text{g}/\text{ml}$.⁹

A model for the replicating form which accounts for these experimental findings should contain a complete double strand as the ribonuclease-resistant part of the structure. Additional experimental evidence which we have derived from an examination of the RNA molecules synthesized in baby hamster kidney cells infected with foot-and-mouth disease virus supports this supposition and has led us to propose a new hypothesis for virus RNA replication. Our hypothesis is based on a cyclic mechanism similar to that which is thought to be involved in the replication of DNA phages.

In a previous report from this laboratory, Brown and Cartwright⁶ demonstrated that three peaks of ³²P-labelled RNA were formed in the presence of actinomycin D during foot-and-mouth disease virus replication in baby hamster kidney cells. These fractions were separated by sucrose-gradient sedimentation and have approximate sedimentation coefficients of 37S, 20S and 16S (peaks A, B and C, Fig. 2A).

This work has now been extended to a more detailed investigation of the individual fractions obtained from

Table 1. BASE COMPOSITION OF FOOT-AND-MOUTH DISEASE VIRUS RNA

Individual fractions from gradients were precipitated with 1 mg yeast RNA and hydrolysed in 0.3 N potassium hydroxide for 18 h at 37°. The 2'-3'-nucleotides were separated by paper electrophoresis at pH 3.5 in sodium citrate buffer. The distribution of phosphorus-32 in the ultra-violet absorbing regions was determined after elution.

Fraction No.	Base composition (counts/100 counts)			
	A	U	G	C
1	26.6	21.8	23.3	28.3
2	26.7	21.7	23.4	28.2
3	25.9	22.0	24.2	27.9
4	26.9	21.3	23.0	28.8
5	26.7	21.7	23.7	27.9
6	26.2	21.9	23.9	28.0
7	26.2	22.2	24.3	27.3
8	26.9	21.7	23.2	28.2
9	26.2	21.9	23.7	28.2
10	25.8	21.4	23.6	29.2
11	26.0	21.9	23.3	28.8
12	25.5	21.7	23.6	29.2
13	24.8	22.1	24.5	28.6
14	24.5	23.1	24.3	28.1
15	25.4	22.8	23.7	28.1
Purified virus RNA	26.0	21.8	24.1	27.8
RNAse-resistant RNA	23.8	24.2	25.6	26.4
Calculated duplex	24.0	24.0	26.0	26.0

sucrose gradients. For the determination of the base composition, aliquots of each fraction were precipitated with 2 volumes of ethanol at -20° following addition of purified yeast RNA. The precipitates were then hydrolysed in 0.3 N potassium hydroxide and the distribution of radioactivity in the 2'-3'-nucleotides was determined. Aliquots of individual fractions were precipitated with ethanol in the presence of unlabelled baby hamster kidney cell RNA, which was added to serve as an internal marker, and then recentrifuged in a second sucrose gradient. The results in Table 1 show that the RNA sedimenting in fractions 1–15 of virus-induced RNA has the same base composition as RNA extracted from purified virus^{10,11}. Recycling of the individual fractions showed, however, that peak A was not homogeneous but consisted of a spectrum of fractions which differed in their sedimentation coefficients (Fig. 2B, C, D). In contrast, different fractions of the RNA from purified radioactive

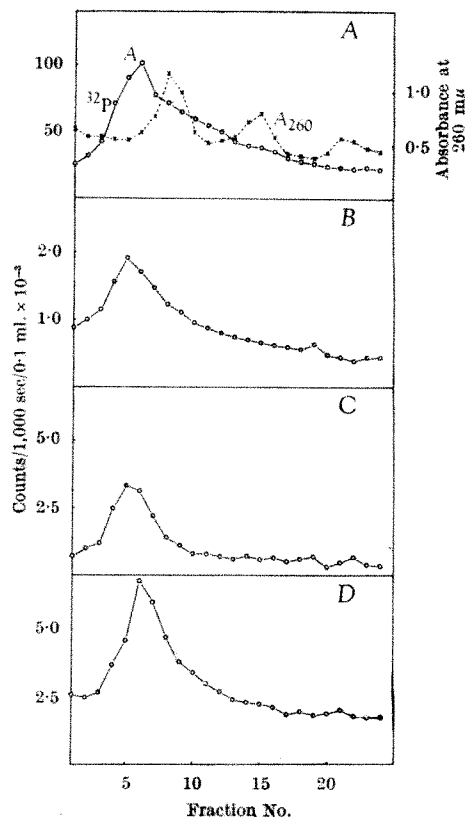


Fig. 3. Sedimentation in sucrose gradients of RNA from purified ³²P-labelled FMD virus. (A), Total RNA; (B), fraction 3 from first sedimentation recycled in second gradient; (C), recycled fraction 5; (D), recycled fraction 6. Unlabelled BHK cell RNA was added to each sample before centrifuging to serve as an internal marker.

virus obtained in a similar way by sedimentation in a sucrose gradient had the same sedimentation coefficient (Fig. 3). The spectrum of sedimentation values present in virus-induced RNA has therefore been interpreted as being due to molecules of different chain-lengths. It is considered unlikely that these differences in sedimentation values, which are not found in RNA from purified virus isolated by the same procedure, are due to the presence of different stable configurations. The results in Figs. 2 and 3 also reveal that peak A from virus-infected cells sediments more quickly than the RNA isolated from purified virus. This suggests that the RNA isolated from virus-infected cells contains some molecules which are larger than those which are incorporated into the virus particles. These molecules, as shown in Table 1, have the same base composition as virus RNA. Molecules of a length greater than that of virus RNA could not be formed on the basis of the displacement mechanism shown in Fig. 1. The maximum length that could be obtained by this mechanism would be the same as that of the primer RNA.

We have also examined the structure of the ribonuclease-resistant RNA obtained by two successive centrifugations in sucrose gradients of virus-induced RNA which had been treated with 0.01 μ g RNase/ml. This has a base composition which is in agreement with its being a duplex structure consisting of a plus and a minus strand (Table 1). Heating this molecule at various temperatures up to 110° C for 5 min, followed by rapid cooling, has failed to produce any molecules sedimenting at the same position as virus RNA (peak A, Fig. 3). A typical result is shown in Fig. 4, which indicates that both heated and unheated RNA sediment in approximately the same position. Nevertheless, after heating at 110° C the ribonuclease-resistant RNA is degraded by the enzyme. Single-stranded RNA isolated from purified virus also sedimented to the same position as the ribonuclease-resistant RNA after

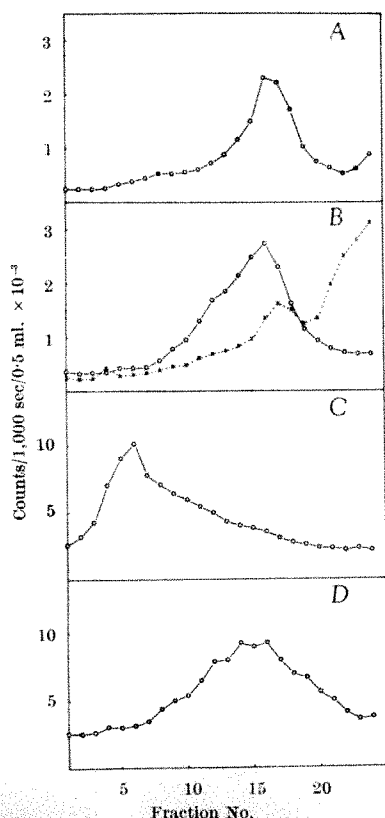


Fig. 4. Effect of heating at 110° in 10⁻³ M EDTA on the sedimentation in sucrose gradients of RNase-resistant RNA and virus RNA. (A), Unheated RNase-resistant RNA; (B), heated RNase-resistant RNA; (C), without RNase; (D), heated virus RNA.

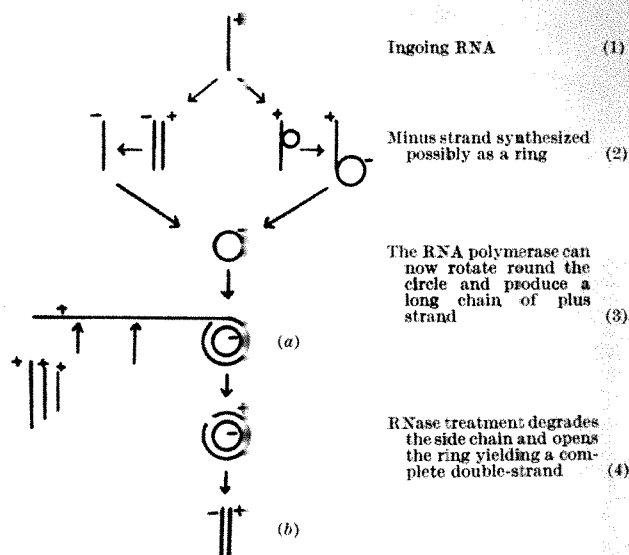


Fig. 5. Diagrammatic representation of the proposed model for virus RNA replication

heating under these conditions (Fig. 4C). Apparently, heating single-stranded virus RNA in this manner produces structural or configurational changes in the molecule which are sufficient to greatly reduce its sedimentation coefficient. The fact that heating the ribonuclease-resistant RNA at 110° C did not produce any molecules with sedimentation coefficients smaller than that of heated virus RNA has been taken as evidence for the absence of a double-stranded structure in which the plus strand is composed of segments (cf. Fig. 1b).

All the evidence at present available leads us to propose a replicating form which will release an intact double strand on ribonuclease treatment and also permit the synthesis of molecules with different chain-lengths. Neither of these requirements can be met with the displacement mechanism at present held. It is postulated that the ingoing virus RNA (plus strand) codes for a complementary minus strand (Fig. 5). This minus strand takes up the configuration of a circle, or is synthesized as a cyclic structure. The RNA polymerase which produces the new plus strands can rotate around this cyclic primer producing a long chain of plus strand, which is repetitive in sequence. This long chain is then broken down by an enzyme into segments of approximately the correct length for viral RNA. Molecules of shorter length may function as specific messenger RNAs for the synthesis of virus coat protein. This may be the function of the molecules present in peak B of the virus-induced RNA. Only those molecules of the correct length are finally incorporated into virus.

This cyclic replicating form is consistent with the evidence available at present. On ribonuclease treatment the side chain will be removed and the ring probably opened at the growing point to yield a complete double strand. The hypothesis also explains the presence of virus-like RNA in virus-infected cells that has longer chain-lengths than the RNA extracted from purified virus.

¹ Montagnier, L., and Sanders, F. K., *Nature*, **199**, 664 (1963).

² Weissmann, C., Borst, P., Burdon, R. H., Billetter, M. A., and Ochoa, S., *Proc. U.S. Nat. Acad. Sci.*, **51**, 682 (1964).

³ Fenwick, M. L., Erikson, R. L., and Franklin, R. M., *Science*, **146**, 527 (1964).

⁴ Hausen, P., *Virology*, **25**, 23 (1965).

⁵ Brown, F., and Cartwright, B., *Nature*, **204**, 855 (1964).

⁶ Pons, M., *Virology*, **24**, 467 (1964).

⁷ Gierer, A., *Nature*, **179**, 2297 (1957).

⁸ Ginoia, W., *Nature*, **181**, 958 (1958).

⁹ Weissmann, C., Billetter, M. A., Schneider, M. C., Knight, C. A., and Ochoa, S., *Proc. U.S. Nat. Acad. Sci.*, **53**, 653 (1965).

¹⁰ Brown, F., and Cartwright, B., *Nature*, **199**, 1168 (1963).

¹¹ Bachrach, H. L., Trautman, R., and Breese, S. S., *Amer. J. Vet. Res.*, **25**, 333 (1964).

CYTOPLASMIC ORGANELLES AND CELL GROWTH IN ROOT CAPS

By DR. B. E. JUNIPER and DR. F. A. L. CLOWES

Botany School, University of Oxford

CELLS are the products of expansion and division of other cells, and so mechanisms must exist for the division or the production *de novo* of cytoplasmic organelles to compensate for increase in the size and the number of cells. There are many references to possible mechanisms for the duplication or origin of cytoplasmic organelles, but no direct visual study has been carried out on their numbers in relation to cell growth and development of a homogeneous plant tissue.

We have chosen the cells of the root cap for the investigation recorded here because they lack large vacuoles, and this makes it easier to produce good estimates of the numbers of cytoplasmic organelles. The cells at the periphery of the cap contain small vacuoles, but they do not possess the single large vacuoles of other kinds of differentiated plant cells. In *Zea mays*, and the Gramineae generally, the cap is clearly demarcated from the rest of the root by a thick cell wall which we shall call the cap junction. In *Zea* the meristematic cells of the root cap, those nearest to the cap junction, cut off cells distally, approximately once every 12 h, and are the most rapidly dividing cells of the root. Meristematic activity ceases within 100 μ of the cap junction, and the cells expand from this point onwards, only ceasing to do so at the periphery where they are sloughed off. Along the axis of the cap in the primary roots of *Zea* there are approximately sixteen cells in this developmental sequence from initiation to detachment; the length of the root cap is about 450 μ and the overall increase in cell size is fifteen-fold. We shall consider only a column of non-vacuolate cells, extending from the distal side of the cap junction to a region one or two cells proximal to the very tip of the root. Median longitudinal sections through this zone have provided the material for the counts of organelles. The organelles that have been counted and measured are the mitochondria, the Golgi bodies, the plastids and the endoplasmic reticulum (ER). The first three have been counted and measured and the area of the ER has been calculated from the length of the profiles in the sections.

The techniques for embedding, sectioning and photographing large sections of plant tissue have already been described⁷. Complete mosaics of the cap tissue corresponding to a region about 400 μ long by 100 μ wide have been assembled. Such an area of a median section includes practically all of the cells of the cap that are non-vacuolate. The cap junction has been used as a base line and quadrats corresponding to 1,500 μ^2 of tissue have been marked off successively until the tip of the cap is reached. The numbers of organelles and length of ER have then been counted within each quadrat. We have assumed that the sections were a constant 50 μ thick and that the depth of focus of the microscope was greater than this. Allowances have been made for the variation in the size of the organelles by calculating the numbers of sections occupied by organelles of average dimensions at every level along the cap. The results have been expressed first on a per unit volume of cytoplasm basis after making a correction for the volumes of nuclei and secondly on a per cell basis using average cell dimensions at a range of levels in caps of similar size to estimate cell volumes and cytoplasm volumes. The amount of ER is given as an area, on the assumption that each profile seen in the electron micrographs is a section through a sheet of ER as wide as the thickness of the section.

Figs. 1 and 2 show the results from one single root cap mosaic. The results of two other complete mosaics have been compared and are, in all important respects, identical. However, root caps vary in length so that averages conceal trends.

The most striking feature of the quantities per cell is the difference between the numbers of the plastids on the one hand and the mitochondria, Golgi bodies and ER on the other. The number of plastids per cell varies very little, about three-fold, whereas the numbers or quantity of the other three components are more nearly constant per unit volume and therefore vary about 15-fold on a per cell basis. This means that the production of most, if not all, of the plastids probably takes place in the dividing cells, although growth of each individual plastid continues in the enlarging cells. On the other hand, the division or production of the other components takes place not only in the dividing cells but also in all the cells of the root cap.

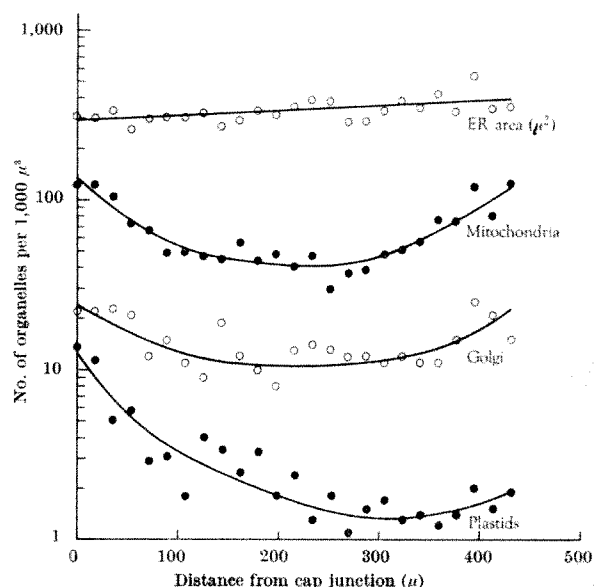


Fig. 1. Average numbers of organelles and areas of endoplasmic reticulum per unit volume of cytoplasm (log scale) at various levels along the length of the root cap of *Zea mays*. Levels near the cap junction include the meristematic cap initials and those beyond 400 μ are the peripheral cells at the tip of the root.

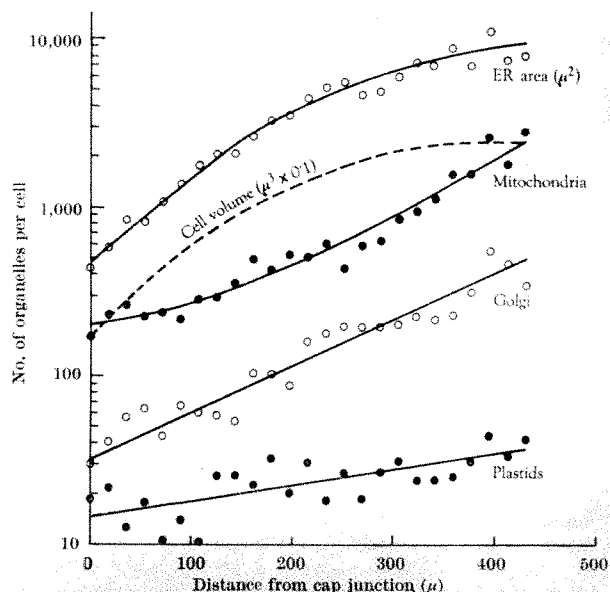


Fig. 2. Average numbers of organelles and areas of endoplasmic reticulum per cell (log scale) at various levels along the length of the root cap of *Zea mays*.

Although the numbers or amount of the components other than the plastids increase as cell expansion proceeds, they do not all appear to keep pace with the increase in cell volume. Only the ER maintains its level per unit volume, whereas both the mitochondria and the Golgi bodies fall in numbers per unit volume during the early phase of cell growth to approximately half the quantity found in the meristematic cells. In both, however, the total per unit volume in the peripheral cells is practically identical to the total in the meristematic cells. Our interpretation of this fall is that the rates of reproduction of the mitochondria and the Golgi bodies do not quite keep pace with cell expansion at first but recover their original concentration per unit volume as cell enlargement slows and ceases.

The ER maintains its level per unit volume, or even may increase it slightly throughout the changes which each cell undergoes in rates of division and growth. This contrasts with the subjective impression gained from the electron micrographs of sparsity in the meristematic cells and abundance in the peripheral cells. In the meristematic cells the profiles of the ER are short and irregularly dispersed; in the cells from 100–300 μ from the cap junction the profiles of the ER are longer and they are most often distributed parallel to the cell wall and to the nuclear membrane; in the peripheral cells the profiles remain long but form irregular rings and loops with no obvious orientation. This feature of the peripheral cells, a property of ordinary roots, is similar to the distribution of the ER described by Bouček⁴ as a product of centrifugation.

The mechanisms of origin and division of cytoplasmic components are still subjects for dispute. There is evidence, for example, for the *de novo* origin of the mitochondria^{2,6,11} and of the plastids³. There is, however, rather more evidence for the view that both mitochondria and plastids always arise by fission except perhaps in special cells. This evidence comes from electron microscopy^{9,10,15,16}, from light microscopy¹⁷ and from the radioactive labelling of mitochondrial structural precursors in *Neurospora*^{13,14}. In the latter the distribution of the labelling is consistent with fission rather than *de novo* origin. We have no evidence in this tissue for any method other than fission for the origin of mitochondria and plastids. Many electron micrographs show images consistent with such a process for the plastids in the meristematic cells and for the mitochondria in both the meristematic and enlarging cells.

The exact mechanism of the sub-division of the Golgi bodies is not known and it may well be that a number of systems operate. The number of cisternae of each Golgi body in this tissue varies from two to seven and this suggests that the division of the Golgi could be brought about by the separation of the cisternae. An alternative mechanism, proposed by Buvat⁸ for Golgi division in the leaves of *Elodea canadensis*, and illustrated by convincing

micrographs, is the division of a Golgi body at right angles to the planes of the cisternae. But we have not observed any images which would be consistent with such a mechanism in this tissue. If a cisternal separation (as opposed to a cisternal sub-division) mechanism were to operate, the multiplication of new cisternae could follow by some process similar to the formation of multiple membrane stacks from the nuclear membrane noted by Barer *et al.*¹.

The origin of the ER is even more difficult to understand. Lindgren¹² has suggested that in yeast the ER is assembled within the nucleus and fed through the nuclear membrane via an 'extruder duct'. Barer *et al.*¹, in animal tissue, have suggested a mechanism in which blebs from the nuclear membrane are converted either into flattened cisternae or annuli and thence migrate into the cytoplasm. We have no evidence in this tissue for either of these processes, but there is nearly always some ER lying close to the nuclear membrane in the expanding cells.

We have demonstrated that plastids differ in their pattern of division from the three other cytoplasmic components considered. The mitochondria, Golgi bodies and ER appear to be closely correlated with cell size. However, it is not only numbers of organelles that are significant when we come to study metabolism. Size and differentiation of the organelles may also be important. It is well known that the development of the cristae of mitochondria varies during differentiation of the cell and generally cristae become more prominent in metabolically active cells¹⁸. The size and shape of the Golgi bodies also vary considerably^{7,16,19}, possibly in relation to carbohydrate metabolism. Moreover the plastids, although remaining few in number per cell, increase their volume sixty-fold within 300 μ from the cap junction. Therefore the estimates of numbers of plastids, mitochondria and Golgi and area of ER presented here should be considered in conjunction with changes of size and differentiation of each kind of organelle.

¹ Barer, R., Joseph, S., and Heek, G. A., *Proc. Roy. Soc., B*, **152**, 353 (1960).

² Bell, P. R., and Mühlethaler, K., *J. Ultrastructure Res.*, **7**, 452 (1962).

³ Bell, P. R., and Mühlethaler, K., *Cell Biol.*, **20**, 235 (1964).

⁴ Bouček, G. B., *Amer. J. Bot.*, **50**, 1046 (1963).

⁵ Buvat, R., *C.R. Acad. Sci., Paris*, **264** (2), 2157 (1958).

⁶ Buvat, R., *C.R. Acad. Sci., Paris*, **248** (1), 1014 (1959).

⁷ Clowes, F. A. L., and Juniper, B. E., *J. Exp. Bot.*, **15**, 622 (1965).

⁸ Falk, H., *Protoplasma*, **55**, 237 (1962).

⁹ Fawcett, D. W., *J. Nat. Cancer Inst.*, **15**, Suppl., 1475 (1955).

¹⁰ Granick, S., in *Cytodifferentiation and Macromolecular Synthesis*, edit. by Lock, M., 144 (Academic Press, New York and London, 1963).

¹¹ Hoffman, H., and Grigg, W., *Exp. Cell Res.*, **15**, 118 (1958).

¹² Lindgren, C. C., *Nature*, **195**, 1225 (1962).

¹³ Luck, D. J. L., *J. Cell Biol.*, **16**, 483 (1963).

¹⁴ Luck, D. J. L., *Proc. U.S. Nat. Acad. Sci.*, **49**, 233 (1963).

¹⁵ Manton, I., *J. Mar. Biol. Assoc., U.K.*, **38**, 319 (1959).

¹⁶ Manton, I., *J. Exp. Bot.*, **12**, 421 (1961).

¹⁷ Michaelis, P., *Protoplasma*, **55**, 177 (1962).

¹⁸ Mollenhauer, H. H., Whaley, W. G., and Leech, J. H., *J. Ultrastructure Res.*, **5**, 193 (1961).

¹⁹ Simon, E. W., and Chapman, J. A., *J. Exp. Bot.*, **12**, 414 (1961).

²⁰ Whaley, W. G., Mollenhauer, H. H., and Kephart, J. E., *Fifth Intern. Cong. Electron Microscopy*, **2**, W-12 (1962).

THE BURSA PHARYNGEA IN THE GIANT PANDA (*Ailuropoda melanoleuca*)

By PROF. A. J. E. CAVE

Department of Anatomy, St. Bartholomew's Hospital Medical College, London, E.C.1

THE bursa pharyngea, first described in man by Mayer¹, occurs in foetal and young mammals as an insignificant, single, dorso-medial recess in the mucosa of the epipharynx below the basioccipital: in the adult it is often less readily detectable, or even wanting. Killian² summarized the early knowledge of this structure and examined it in a representative range of foetal and post-natal material, both human and mammalian: he showed it to be the centre of a specialized mucosal arrangement associated with the peculiar aggregation of mucous glands and lymphoid tissue known as the tonsilla pharyngea.

The morphology of this structure has, not unnaturally been most intensively studied in the human subject, and detailed accounts of its gross and microscopic anatomy therein have been provided by Symington³, Joannesco⁴ and others.

While there is general agreement that the mammalian bursa pharyngea is without developmental relationship either to the cranio-pharyngeal canal or to the cranial extremity of the notochord, no specific function has been attributed to it. It is invariably situated underneath the occipital component of the basis cranii and immediately

above the muscular roof (m. palatopharyngeus) of the true (oro-)pharynx; it is thus a minute posterior mucosal diverticulum of that respiratory chamber which lies behind the nasal fossae and above the pharynx proper, which is most aptly termed the epipharynx. It would appear that, in some mammals at least, the commonly insignificant bursa pharyngea may develop into an epipharyngeal diverticulum of gross, dissectable size, and the existence of such diverticula in bears has long been known, their presence having been reported (by Mayer¹, Rapp², Alix³ and Boulart⁷) in *Ursus arctos*, *U. americanus*, *U. horribilis*, *Melursus ursinus* and *Helarctos malayanus*. Such ursid diverticula are, however, dual structures of unequal size. Thus Alix³, in *Melursus ursinus*, found the dextral diverticulum to be 5 cm long by 3 cm wide, the sinistral diverticulum only 2 cm long by 0.5 cm broad. Such paired epipharyngeal diverticula appear to be confined to the Ursidae, for Killian² failed to observe any comparable structures in a variety of carnivores (*Canis familiaris*, *Nasua rufa*, *Viverra civetta*, *Herpestes griseus*, *Paradoxurus trivirgatus*, *Mephitis mephitis*, *Felis domestica*, *Lutra vulgaris*), and recently Davis⁴ has noted their absence in *Procyon lotor* and *Ailurus fulgens*. Davis did, however, observe the presence of a pair of unequal bursae pharyngeae in a sub-adult (16 months old) male specimen ('Sun Lin') of the giant panda, *Ailuropoda melanoleuca*, and regarded this finding as additional evidence indicative of the ursid rather than the procyonid nature of this form.

In this immature animal the dextral, larger sac had a slit-like orifice some 7 mm long, a narrow neck and a capacious body: it measured 130 mm in length by 30 mm in maximal breadth and its fundus was partially subdivided by a short septum; the smaller sinistral sac was only 15 mm long. Both sacs were thin-walled, with a longitudinally plicated lining. The larger sac extended caudally dorsal to the pharynx and the anterior oesophagus and ventral to the basioccipital and the bodies of the anterior five cervical vertebrae.

In 1939, during the post-mortem examination of the first *Ailuropoda* specimen to die in the London Zoo (the adult female animal 'Grandma'), I observed an obtrusive patch of thin, reddish-brown, mucous membrane clothing the under surface of the basioccipital region and extending caudally over the anterior cervical vertebral bodies between the bilateral eminences of the powerful longus colli muscles. Though the cut edge of this membranous patch was unmistakable, its connexions were not then ascertainable; it was therefore recorded as something unusual and possibly of a pathological nature. The subsequent examination of additional *Ailuropoda* specimens, however, made it clear that this curious mucosal patch was nothing other than the adherent dorsal parietes of an unidentified epipharyngeal diverticulum (enlarged bursa pharyngea) mutilated during necropsy.

In 1944, the adult (7 years old) female giant panda 'Ming' died in the London Zoo and in 1950 so did the 6-year-old male animal, 'Lien Ho'. By courtesy of the Council of the Zoological Society of London the throat parts of these two animals became available for anatomical examination. In each specimen a well-developed enlarged pharyngeal bursa was present in the shape of a single, median, thin-walled, piriform sac, presenting ostium, neck, body and fundus. In each specimen this sac lay between the basi-occipital and anterior cervical vertebrae dorsally and the pharynx and anterior oesophagus ventrally: to the former structures the dorsal sac wall was firmly adherent; to the latter the ventral sac wall was only loosely attached by blood-vessels and connective tissue.

The rounded ostium, some 6 mm in diameter, opened from the epipharynx immediately above the arcus palatopharyngeus: the sac neck was approximately 30 mm long and was longitudinally plicated interiorly; the sac body measured approximately 55 mm long by 28 mm in maximal width; the sac fundus overlay the

beginning of the oesophagus. The sacs of the two animals agreed in all anatomical particulars and their morphological nature could not be in doubt; they represented a particular and remarkable development of the otherwise insignificant bursa pharyngea (Figs. 1 and 2).

It is curious that in Davis's young male *Ailuropoda* specimen (as in bears), two enlarged pharyngeal bursae of unequal size should have been present, whereas both in the adult male and in the adult female specimen

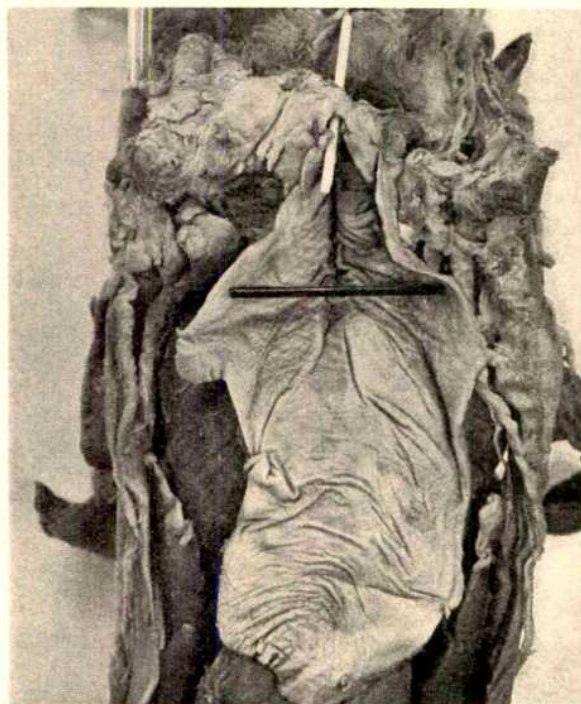


Fig. 1. *Ailuropoda melanoleuca* (giant panda) adult female. Dorsal aspect of pharynx with opened bursa pharyngea in situ. White rod in bursa ostium, dark rod at junction of neck and body of bursa



Fig. 2. *Ailuropoda melanoleuca* (giant panda) adult male. Dorsal aspect of pharynx with bursa pharyngea in situ: short transverse rod in opened bursa neck, longer transverse rod between bursa and pharynx, vertical rod traversing sac body

examined by me, only a single bursa should occur. The range of variation of this bursa cannot, however, be known until a wider range of *Ailuropoda* material has been investigated. There is, nevertheless, no doubt as to the morphological identity of these epipharyngeal diverticula, for, whether manifested as single or as dual structures, their topographical relationships are identical.

The occurrence of a comparably developed pharyngeal bursa is not recorded for non-carnivores. This may well be because of the rarity of suitably fixed material for specific investigation and because of the inevitable damage inflicted on the pharyngeal region during routine post-mortem examination.

I myself have evidence, however, of the occurrence of a large, single epipharyngeal diverticulum (enlarged bursa pharyngea) in mammals outside the order Carnivora. For in the excised pharynges of an adult male and an adult female white rhinoceros (*Ceratotherium simum*), of an adult Indian rhinoceros (*Rhinoceros unicornis*), of an adult okapi (*Okapia johnstoni*) and of a young giraffe (*Giraffa camelopardalis*), the dorsal aspect of the pharynx has, loosely attached to it, some portion of the ventral moiety of a mutilated but unmistakable pharyngeal bursa, the absent portion of which has remained adherent to the skull base and anterior cervical spine (Figs. 3 and 4). These (imperfect) diverticula are thin-walled, bluntly piriform sacs, communicating anteriorly with the epipharynx above the arcus palatopharyngeus; their narrow necks are succeeded by expanded bodies and their fundi overlie the anterior portion of the oesophagus. Histological examination of

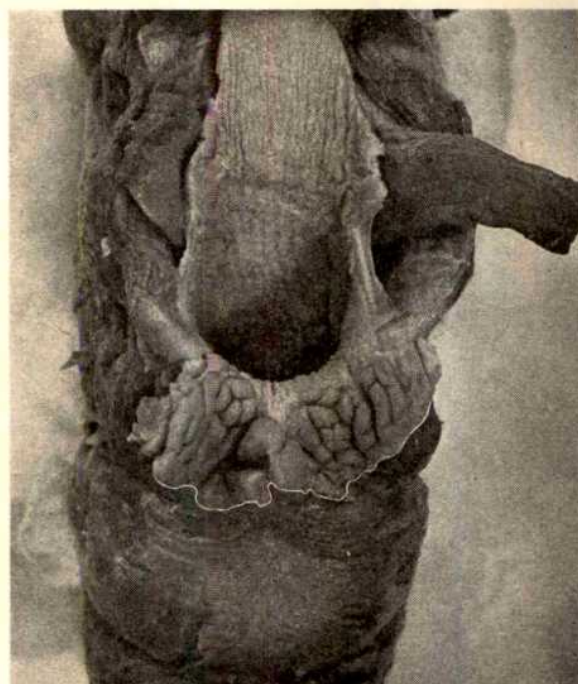


Fig. 4. *Giraffa camelopardalis* (giraffe) juvenile female. Dorsal aspect of soft palate and pharynx, with remnant of ventral wall of bursa pharyngea in situ.

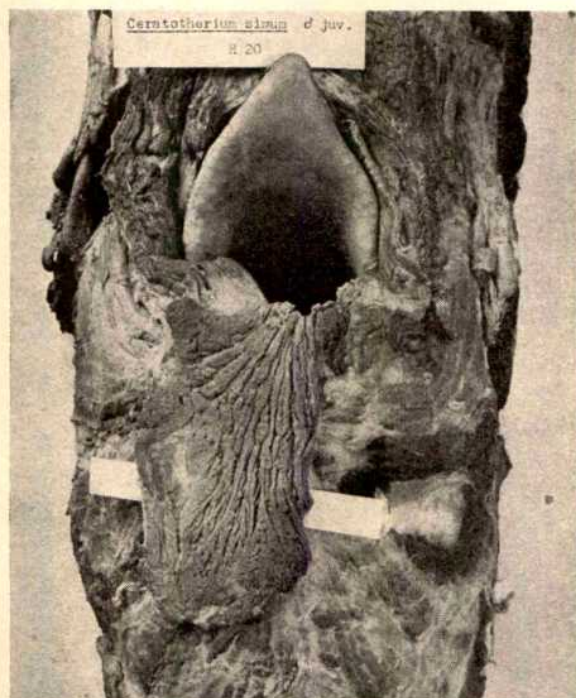


Fig. 3. *Ceratotherium simum* (white rhinoceros) juvenile male. Dorsal aspect of pharynx showing in situ (and separated by white card) remnant of ventral wall of bursa pharyngea.

their longitudinally plicated mucosa reveals a typical (that is, pseudo-stratified columnar ciliated) epithelium, an abundance of mucous (or muco-serous) glands, often opening into distinct crypts, an extraordinarily rich, specialized vasculature and an intense infiltration of mucosa and submucosa by lymphoid tissue manifesting germinal centres. A detailed account of these non-carnivore pharyngeal bursae will be published elsewhere.

The presence or absence of specialized pharyngeal bursae in mammals generally merits further investigation, as does the precise physiological role of the bursae themselves.

The general function of the mammalian epipharynx is partly mechanical (the reception of conditioned air from the nasal fossae) and partly defensive, since therein the inspired air is brought into particularly intimate contact with an abundance of highly active, protective lymphoid tissue. The elaboration of the customary bursa pharyngea into a single or double posterior diverticulum of some magnitude doubtless enhances the defensive role of the epipharynx, but it remains to be explained why such diverticula should be developed in some mammalian forms and yet be wanting in others.

¹ Mayer, A. F. J. C., *Neue Untersuchungen aus dem Gebiete der Anatomie und Physiologie* (Bonn, 1842).

² Killian, G., *Morph. Jahrb.*, **14**, 618 (1888).

³ Symington, J., *Quain's Elements of Anatomy*, eleventh ed., **2**, 50 (Longmans, London, 1914).

⁴ Jonnesco, T., in Poirier and Charpy, *Traité d'Anatomie Humaine*, second ed., **4**, 162 (Masson, Paris, 1901).

⁵ Rapp, W. v., *Muellers Archiv. Anat. Physiol.*, **189** (1839).

⁶ Alix, E., *Bull. Soc. Philomath.*, Paris, **1**, 47 (1878).

⁷ Boulart, J., *Anat. et Physiol.*, Paris, **6**, 535 (1885).

⁸ Davis, D. D., *Fieldiana: Zool. Mem.*, **3** (Nat. Hist. Mus., Chicago, 1964).

ELECTRON MICROSCOPE OBSERVATIONS ON THE FREE PROTEIN AND THE PROTEIN-POLYSACCHARIDE COMPLEX OF BOVINE SYNOVIAL FLUID

By DR. JAMES W. SMITH and DR. JOHN FRAME

Department of Anatomy, St. Salvador's College, University of St. Andrews

THE two major constituents of synovial fluid are hyaluronic acid and soluble protein: in the ox, the average concentration of hyaluronic acid is about 0.05 g per 100 ml.¹, while that of soluble protein is about 0.9 g

per 100 ml.². The protein fraction is entirely derived from the blood serum^{3,4}. The hyaluronic acid exists in large measure as the sodium salt, and this sodium hyaluronate is bound to a part of the soluble protein as a

hyaluronate-protein complex⁶. Although the existence of this complex is generally accepted, little is known of its nature: assessments of the amount of protein which the complex contains have varied from 30 per cent⁶ to 2 per cent⁷, and little is known of the manner in which the two parts of the complex are combined.

When synovial fluid is acidified to pH 3 with acetic acid, a mucin clot forms and can be readily separated from a clear supernatant fluid⁸. The supernatant contains a proportion of the protein of the synovial fluid⁸, and very little, if any, of the hyaluronate-protein complex¹. The mucin clot thus certainly contains the greater part of the complex but its content of free protein is controversial. Free protein can be separated from the clot by various means^{7,9}, but opinion varies as to whether this is to be regarded as a separation of distinct compounds or a degradation of the complex.

It was considered that a new approach to this problem might be made by electron microscopic examination of synovial fluid and its mucin and supernatant fractions after suitable precipitation and staining. The use of lead salts for the fixation of acid mucopolysaccharides was introduced for conventional histology by Holmgren and Wilander¹⁰, and various modifications of their method are now in use. Lead salts have also been used in the past few years as stains which enhance the electron-scattering properties of biological material¹¹⁻¹³.

It is well known that the sodium ion with which hyaluronic acid is normally associated in the tissues may be exchanged for other cations, the extent of the exchange depending on their concentration and valency^{5,14,15}. This type of exchange is utilized in Hale's stain¹⁶ and in the precipitation of hyaluronic acid by hexavalent cobalt salts⁵. When 1 per cent lead nitrate is added to an equal volume of 0.1 per cent potassium hyaluronate or hyaluronic acid (B.D.H.), a fine off-white precipitate forms immediately and can be spun down into a discrete pellet. On the addition of potassium nitrate the precipitate disappears. It is considered that this precipitate is insoluble lead hyaluronate.

At acidities of pH 3-7 small amounts of lead ions cause almost complete precipitation of comparatively large amounts of protein from solution¹⁷. Single lead ions are bound to individual carboxyl groups of the protein¹⁸ and the reaction, which is reversible, is complete within an hour. It is considered, therefore, that lead nitrate causes precipitation of both hyaluronic acid and soluble proteins.

In the present investigation, therefore, synovial fluid was separated by acidification into mucin clot and supernatant. The clot was washed in acetic acid and then in distilled water, and afterwards redissolved in water at pH 10. The reaction of this solution, and that of the supernatant, was adjusted to pH 7.5. Synovial fluid, the mucin clot solution and the supernatant fractions were then fixed and stained by the addition of equal parts of 1 per cent lead nitrate and 10 per cent neutral formalin at pH 5. With synovial fluid a discrete coagulum was formed, while with the two fractions diffuse precipitates were produced and were afterwards spun down into pellets. The coagulum and pellets were then dehydrated and embedded in 'Araldite', and sections were examined, without further staining, in a Siemens Elmiskop I microscope.

Fig. 1 shows the appearance in the light microscope of a thick 'Araldite' section of synovial fluid, stained with toluidine blue. The field is occupied by small particles of varying densities. Most appear to be approximately spherical and many are linked together to form short linear groups.

In the electron microscope, thin sections of the same material exhibit the appearance shown in Fig. 2. It is now apparent that the particles are irregular in shape and, as would be expected, the linear groupings, though still evident, are less frequent. All the larger particles

contain two distinct materials, a central group of dark (or electron dense) spherical bodies which vary in diameter from 300 to 1200 Å, being surrounded by a larger volume of lighter (or less dense) material. The particles which do not exhibit central dark bodies are always small, and this is consistent with the view that the dark bodies are a feature of all particles and that their apparent absence from some is due to tangential sectioning. On the other hand, dark bodies have not been observed without the enveloping lighter material.

In Fig. 3 the supernatant fraction is seen to consist predominantly of dark spherical bodies similar in both size and density to the central dark bodies in synovial fluid particles. In addition, there are a few areas which consist of lighter material, which has an appearance reminiscent of the peripheral material in synovial fluid particles. As has been noted, the supernatant contains a proportion of the original protein content of the synovial fluid and very little, or possibly none, of the hyaluronate-protein complex¹. On this basis it seems reasonable to postulate that the dark bodies in Fig. 3—and by virtue of their similar appearance the central dark bodies in synovial fluid particles (Fig. 2)—are aggregates of free protein molecules. In a very thin 'Araldite' section of the supernatant material (Fig. 4) it is indeed evident that the dark bodies in Fig. 3 are aggregates of smaller rod-like units and the dimensions of these are of the same order as the calculated dimensions of the molecules of albumin and γ -globulin¹⁹⁻²¹.

The appearance of the washed mucin fraction is shown in Fig. 5. It is evident that it is morphologically very similar to the peripheral light material of the synovial fluid particles (Fig. 2), exhibiting the same density and the same irregular outline. Moreover, the absence of dark bodies comparable with those in the synovial fluid particles (Fig. 2) and the supernatant fraction pellet (Figs. 3 and 4) appears to be absolute. It seems reasonable to conclude, therefore, that the washed mucin clot contains no free protein, and consequently that both the washed mucin clot and the peripheral light material in synovial fluid particles consist entirely of hyaluronate-protein complex.

As a result of the present investigation, it is considered that in sections of the coagulum formed by treatment of synovial fluid with lead nitrate, free protein and hyaluronate-protein complex are both visible in distinct

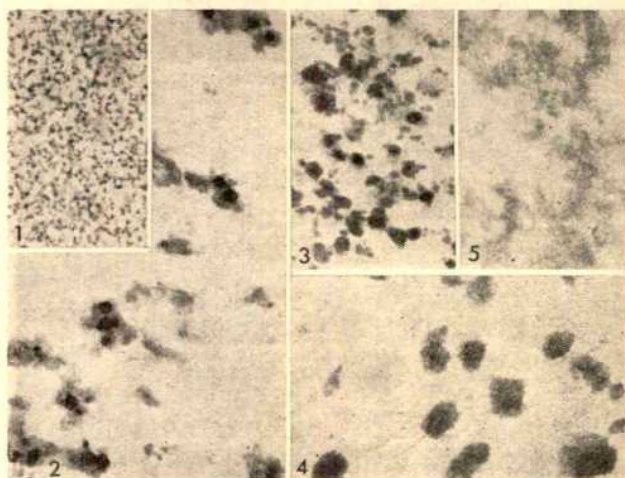


Fig. 1. Light photomicrograph. Synovial fluid. Toluidine blue. ($\times 700$)

Fig. 2. Synovial fluid. ($\times 20,000$)

Fig. 3. Supernatant fraction after acidification of synovial fluid. Centrifuged pellet. ($\times 20,000$)

Fig. 4. The material in Fig. 3. ($\times 70,000$)

Fig. 5. Mucin fraction after acidification of synovial fluid. Centrifuged pellet. ($\times 20,000$)

forms. The coagulum consists of particles, variable in diameter, joined together as a continuous network by thinner strands. Each particle contains a variable number of dark central spherical masses which are considered to be aggregates of free soluble protein molecules. These are surrounded by a lighter sponge-like mass of hyaluronate-protein complex which is continuous from particle to particle through the thin connecting strands. It is presumed that the different degrees of electron-scattering exhibited by the soluble protein, on one hand, and the hyaluronate-protein complex on the other, are to be related to differences in the amounts of lead bound to the two substances.

In the natural state, of course, both the complex and the free protein are much more dispersed, and the condensation of both moieties into an isolated network is an artefact produced by precipitation. Indeed, such an alteration from a dispersed state to a condensed network seems to be typical of the action of any coagulant fixative^{22,23}. The change involves both the binding of adjacent molecules to one another and the formation, in certain situations within the tissue, of planes of cleavage on either side of which condensation occurs in opposite directions. In synovial fluid particles the dark, free protein bodies almost invariably lie centrally and never occur without a covering of hyaluronate-protein complex. One possible explanation is that in untreated synovial fluid the free protein molecules and the complex are not evenly mixed but are preferentially situated in separate zones, and that, on fixation, condensation of both fractions takes place with the planes of cleavage occurring always through zones occupied by complex.

It was noted earlier that the composition of the hyaluronate-protein complex of synovial fluid is still highly controversial. Ogston and Stanier⁶ showed that although the mucin clot formed on acidification of synovial fluid contained entrained free protein, after washing, first in 1 per cent acetic acid and then in distilled water, the weight of the clot reached a constant value. It then contained about 70 per cent hyaluronic acid and 30 per cent protein. It was further demonstrated that the residue, obtained after filtration of synovial fluid through sintered

glass filters, which allowed the passage of free protein, again contained between 30 per cent and 25 per cent of protein^{6,24}. These authors considered that any reduction in this amount of protein was to be regarded as a degradation of the complex rather than a purification by removal of free protein. More recently, it has been suggested^{7,9} that the amount of protein actually combined to hyaluronate is considerably less, being of the order of 2 per cent, and this view has been rather widely accepted²⁵⁻²⁷. The results of the present investigation are in keeping with the older view of Ogston and Stanier. The morphology of the washed mucin clot suggests that it contains no appreciable amount of free protein and is to be regarded as identical with the hyaluronate-protein complex.

¹ Hamerman, D., and Schuster, H., *J. Clin. Invest.*, **37**, 57 (1958).

² Ropes, M. W., Rossmiesl, E. C., and Bauer, W., *J. Clin. Invest.*, **19**, 795 (1940).

³ Curtin, C. C., *Biochem. J.*, **31**, 688 (1955).

⁴ Hesselvik, L., *Acta Med. Scand.*, **105**, 153 (1940).

⁵ Schubert, M., *Connective Tissue: Intercellular Macromolecules* (Churchill, London, 1964).

⁶ Ogston, A. G., and Stanier, J. E., *Biochem. J.*, **46**, 364 (1950).

⁷ Sandson, J., and Hamerman, D., *J. Clin. Invest.*, **41**, 1817 (1962).

⁸ Meyer, K., *Physiol. Rev.*, **27**, 335 (1947).

⁹ Blix, G., *Acta Physiol. Scand.*, **1**, 29 (1940).

¹⁰ Holmgren, H., and Wilander, O., *Z. Mikr.-anat. Forsch.*, **42**, 242 (1937).

¹¹ Dalton, A. J., and Ziegel, R. F., *J. Biophys. Biochem. Cytol.*, **7**, 409 (1960).

¹² Reynolds, E. S., *J. Cell Biol.*, **17**, 208 (1963).

¹³ Watson, M. L., *J. Biophys. Biochem. Cytol.*, **4**, 727 (1958).

¹⁴ Farber, S. J., and Schubert, M., *J. Clin. Invest.*, **36**, 1715 (1957).

¹⁵ Vouras, M., and Schubert, M., *J. Amer. Chem. Soc.*, **79**, 792 (1957).

¹⁶ Hale, C. W., *Nature*, **157**, 802 (1946).

¹⁷ Gurd, F. R. N., and Wilcox, P. E., in *Adv. Protein Chem.*, **11**, 387, edit. by Anson, M. L., Bailey, K., and Edsall, J. T. (Academic Press, New York, 1965).

¹⁸ Gurd, F. R. N., and Murray, G. R., *J. Amer. Chem. Soc.*, **76**, 187 (1954).

¹⁹ Edsall, J. T., and Foster, J. F., *J. Amer. Chem. Soc.*, **70**, 1860 (1948).

²⁰ Low, B. W., *J. Amer. Chem. Soc.*, **74**, 4830 (1952).

²¹ Oncley, J. L., Scatchard, G., and Brown, A., *J. Phys. Colloid Chem.*, **51**, 184 (1947).

²² Hardy, W. B., *J. Physiol.*, **24**, 158 (1899).

²³ Schwarz, F., *Beit. Biol. Pflanz (Cohn)*, **5**, 1 (1887).

²⁴ Ogston, A. G., and Stanier, J. E., *Biochem. J.*, **52**, 149 (1952).

²⁵ Fitton-Jackson, S., in *The Cell*, edit. by Brachet, J., and Mirsky, A. E., **6**, 394 (Academic Press, New York, 1964).

²⁶ Hamerman, D., and Schubert, M., *Amer. J. Med.*, **33**, 555 (1962).

²⁷ Muir, H., in *Intern. Rev. Connective Tissue Res.*, edit. by Hall, D. A., **2**, 101 (Academic Press, New York, 1964).

POSSIBLE RELATIONSHIP BETWEEN TRACE METALS AND BLOAT IN RUMINANTS

By P. J. HARRIS and PROF. F. SEBBA

Department of Chemistry, University of the Witwatersrand, Johannesburg, South Africa

FROTHY bloat occurs in ruminants when they are fed green lucerne or similar lush green fodder. It is due to the formation in the rumen of a froth of remarkable toughness and stability. This froth builds up inside the rumen, owing to the entrapped gases produced during bacteriological fermentation of the feed, until it rises above the level of the reflex mechanism and prevents eructation. Death usually follows from internal pressure on the vital organs lying close to the rumen. Plant proteins, saponins and bacterial polysaccharide and peptide slimes have severally been suggested, singly or in combinations, as the cause of this stable foam. Nothing is known concerning the critical conditions under which they may become foam-formers in the rumen, nor the part, if any, which each plays in the formation of the foam. Further understanding of this problem requires an investigation of the physical and chemical properties of the surface-active agents in green lucerne with particular reference to their ability to produce tough foams. Investigations of this kind have been started at this university,

and the following preliminary results suggest a new approach to the problem.

Traces of metal hydroxides and, in some cases, metal cations have been shown to affect the stability of foams. Lucerne extracts were made up in a sodium bicarbonate buffer to simulate the pH conditions inside the rumen, and by bubbling air through such solutions, and polarographic examination of the foam fractions, traces of nickel and zinc were found to have been concentrated in the foam.

The foam fractionation apparatus (Fig. 1) consisted of a large foaming vessel 3.5 in. in diameter and 12 in. in height with a sintered glass disk of porosity 4 sealed into the base. A glass ring 1.5 in. in length and of the same diameter as the foaming vessel was placed on top of it. Air was bubbled through the solution at a fixed rate, and at the appropriate time a thin copper metal sheet was slid between the vessel and the ring. The foam fraction was thus retained inside the ring and could be removed for analysis. An identical clean ring was immediately placed on top of the vessel to collect the next fraction of

foam. Samples could be obtained every 30 sec and were prepared for polarography using the method of Reed and Cummings¹. The sample was ashed below 500° C, treated with concentrated hydrochloric acid and evaporated to dryness. The residue was taken up in dilute hydrochloric acid and adjusted to pH 4.6 using a glass electrode. The solution was then filtered to remove any aluminium and iron present and evaporated to dryness. The residue was afterwards dissolved in 10 ml. of a solution of 0.1 N ammonium acetate, 0.025 N with respect to potassium thiocyanate and of pH 4.6. This solution was examined polarographically at a mercury electrode dropping at a rate of 1 drop every 1.8 sec.

Samples of lucerne from two different sources were used:

(a) Fresh green lucerne was obtained from a Johannesburg City Council farm. One portion was extracted immediately and the juices diluted 10 times in a 0.5 per cent sodium bicarbonate buffer of pH 4.5 before foaming. The second portion was allowed to dry and age for 2 weeks before extraction and dilution of the juices in the same manner.

(b) A sample of fresh lucerne, grown in a hot-house at the University of the Witwatersrand, was extracted and diluted 10 times in the sodium bicarbonate buffer. One-half of the solution was immediately foamed, while the other half was kept at 4° C for 6 days before being foamed.

In both cases the foaming rate was maintained constant at a fixed rate.

In order to examine the stabilities of the foams, a foam drainage apparatus was used, based on that of Ruysen and Louwers² (Fig. 2). The liquid in the vessel was foamed by bubbling air through a small sintered glass disk of porosity 3 at a constant rate. As the foam was formed, the decreasing level of liquid was periodically re-adjusted to its initial height by the addition of liquid from the burette. During the drainage of the foam the liquid level was kept constant by draining off the liquid at definite time-intervals. This permitted the determination of the total volume of liquid in the foam as a function of time.

Sample (a). The decrease in stability of the foam formed from the aged lucerne in comparison with that formed from the fresh lucerne was very noticeable. The foam

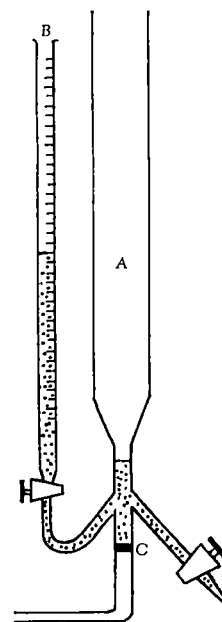


Fig. 2. Foam drainage apparatus. A, Foam drainage vessel; B, burette; C, sintered glass disk

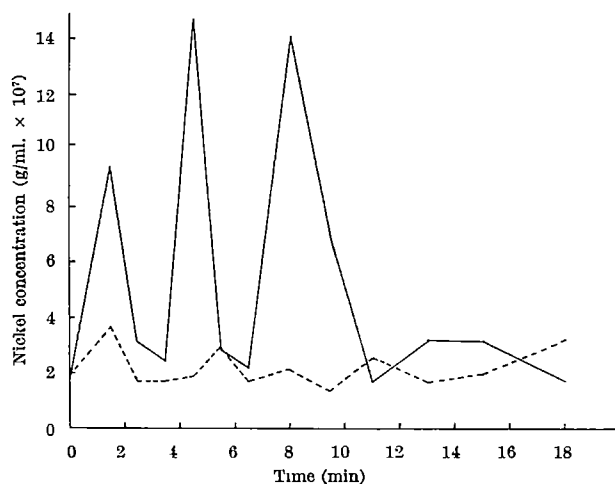


Fig. 3. Sample 1. Concentration of nickel in foam versus time. —, Fresh lucerne solution, ----, aged lucerne solution

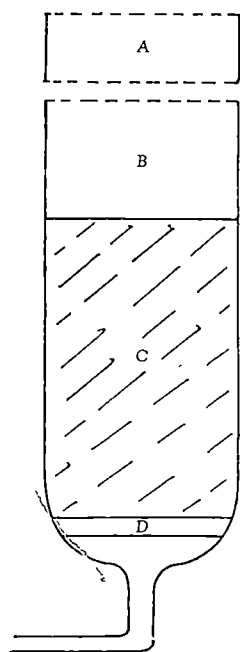


Fig. 1. Foam fractionation vessel. A, Glass ring; B, foaming vessel; C, solution; D, sintered glass disk

from the aged solution was too unstable to give reasonable measurements using the foam drainage apparatus. The foam fractions obtained from these two solutions were examined polarographically for nickel and zinc. During the first 10 min of foaming the concentration of nickel in the foam fractions from the fresh lucerne was five times greater than the nickel concentrations in the foam from aged lucerne (Fig. 3). Similarly, although not so marked, the zinc concentrations were higher in the foam than in the very much more stable fresh lucerne solution (Fig. 4).

Sample (b). The difference in stability between the foams of the fresh lucerne solution and the solution which was allowed to stand for 6 days was checked using the foam drainage apparatus. During the actual foam fractionation, samples of the solution were removed at definite time-intervals and these were used for measurements of drainage rate. In Fig. 7 it can be seen that there was a definite decrease in the stability of the foam formed from the 6-day-old solution. On examination of the nickel concentration in the foam fractions it was found that the

nickel content had decreased slightly in the older solution (Fig. 5) while the zinc concentration had remained more or less the same (Fig. 6).

The overall patterns of the nickel and zinc concentrations in the foams are different for the two samples. This is possibly due to the different soils in which the plants were grown and the different fertilizers used. In each case a decrease in stability of the foam has been accompanied by a decrease in the nickel concentration in the foam. In the solution of fresh lucerne the nickel had been concentrated in the foam whereas in a solution which formed an unstable foam the nickel concentration in the foam was very much the same as that in the solution. The concentration of nickel in the solution was approximately 3×10^{-7} g/ml. and in a stable foam this increased to nearly 15×10^{-7} g/ml. The highest concentrations of nickel were found in the first four foam fractions only, and from Fig. 7 it can be seen that the stability of the foam decreased with time.

Examination of the nitrogen content of the foam fractions of fresh lucerne by the Kjeldahl method showed an increase in nitrogen in the first foam fractions. This indicates that the surface-active agent is initially a protein. It is presumed that it is the negatively charged sites on the protein which combine with the positive nickel ions. This would well be a naturally occurring example of ion flotation in which the protein acts as collector and the metal ion is collected³. In the fresh lucerne the nickel appears to be available for attachment to the protein, while in the aged lucerne, although it is still present, it is no longer in an available form. This has been verified in preliminary experiments where small traces of nickel sulphate added

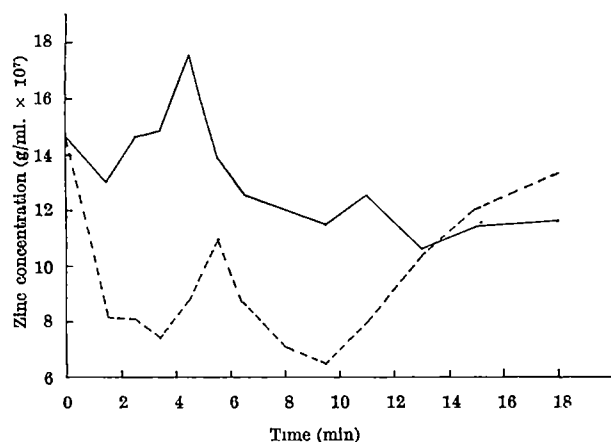


Fig. 4. Sample 1. Concentration of zinc in foam versus time. —, Fresh lucerne solution; ---, aged lucerne solution

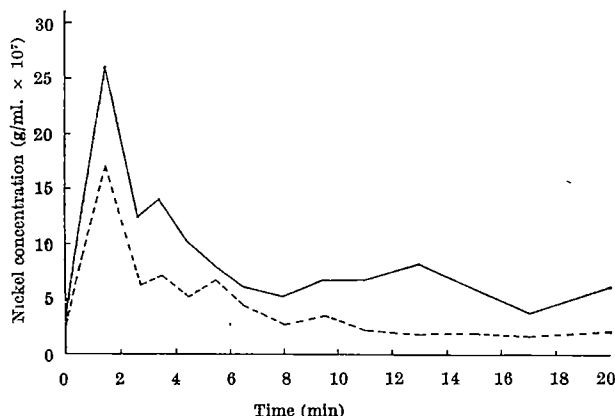


Fig. 5. Sample 2. Concentration of nickel in foam versus time. —, Fresh lucerne solution; ---, 6-day-old lucerne solution

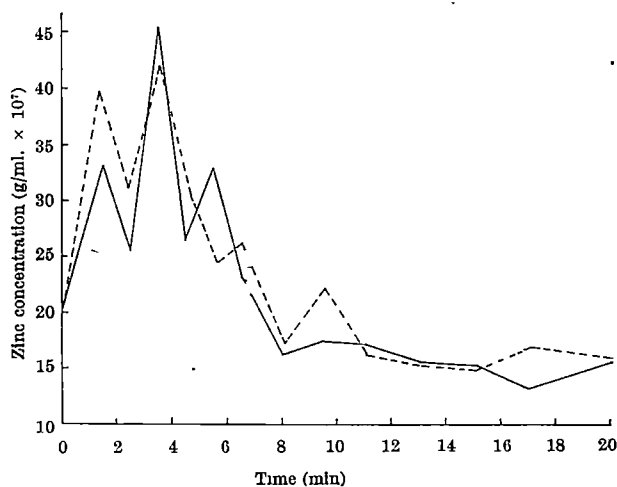


Fig. 6. Sample 2. Concentration of zinc in foam versus time. —, Fresh lucerne solution; ---, 6-day-old lucerne solution

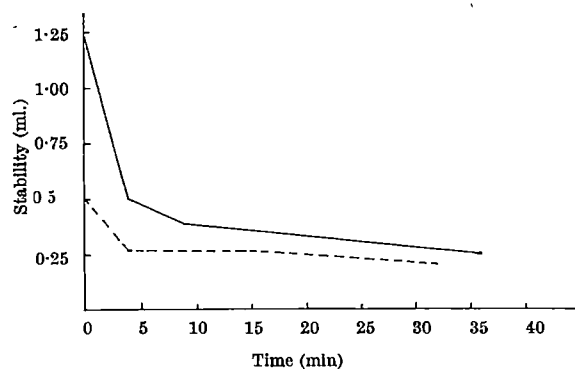


Fig. 7. Stability (vol. of liquid after draining for 10 min) of foam versus time after foaming commenced. —, Fresh lucerne solution; ---, 6-day-old lucerne solution

to aged, and therefore unstable, lucerne solutions immediately caused a substantial increase in the stability of the foam from this solution. Initial tests show that the alkali metals have no measurable effect when added to aged lucerne solutions, nor has manganese, but zinc increases the stability slightly. However, none of these is nearly so effective as nickel in increasing the stability of the foam.

It may be surprising that the element nickel, which is not a usual trace metal in plants, should be found in lucerne. It should be pointed out, however, that the quantity present in the lucerne extract is barely detectable, and it is only when concentrated in the foam that its presence can be unequivocally confirmed.

It must be mentioned that foam drainage is not an ideal method for measuring foam stabilities, as drainage is not the only factor involved. A more refined and accurate technique than those available at present is required before the effects of various metal ions on the stability of the lucerne foam can be accurately measured. At present an apparatus is being developed here which, it is hoped, will give a more accurate measure of the factors determining foam stability.

We thank the Livestock and Meat Industry Control Board for financing this work, and Dr. F. M. C. Gilchrist, of the Veterinary Research Institute, Onderstepoort, for advice.

¹ Reed, J. F., and Cummings, R. W., *Indust. Eng. Chem. (Anal. Ed.)*, **12**, 489 (1940)

² Ruyssen, R., and Louwers, A., *Mémoires. Koninkl. Vlaam. Acad. Wetenschap, Belg.*, **Kl. Wetenschap**, **21**, No. 5 (1959); *Chem. Abs.*, **54**, 9375.

³ Sebba, F., *Ion Flotation* (Elsevier, Amsterdam, 1962).

2,6-DIAMINO-3-HYDROXYPIMELIC ACID IN MICROBIAL CELL WALL MUCOPEPTIDE

By H. R. PERKINS

Twyford Laboratories, Twyford Abbey Road, London, N.W.10

THE diamino-acids hitherto identified in the cell-wall mucopeptide of various bacteria have been diaminopimelic acid, lysine and, more recently, ornithine and 2,4-diaminobutyric acid¹. Many years ago a species of *Micromonospora* (*LSTM*) was found to contain a component that moved slowly in paper chromatographic systems and gave with ninhydrin the same characteristic colour as diaminopimelic acid after chromatograms had been developed with methanol/pyridine/hydrochloric acid². Recently, examination of the cell-wall composition of many strains of *Actinomyces* showed that some members of the *Actinoplanes* and a strain of *Micromonospora* contained the same "slow-moving component"³. In particular, Yamaguchi³ reported that the cell walls of *Ampullariella regularis* contained glutamic acid, alanine, glycine and the "slow-moving component" with only traces of diaminopimelic acid.

A culture of *A. regularis* was obtained from the collection of Dr. J. N. Couch. The cells were grown and the cell walls were prepared as described by Yamaguchi³. After hydrolysis (6 N hydrochloric acid, 16 h, 105° C) samples were run on chromatograms in methanol/water/pyridine/conc. hydrochloric acid, 32 : 7 : 4 : 1 (solvent A). The dried chromatogram was treated with ninhydrin and heated. It showed the expected "slow-moving component", ($R_{\text{meso-diaminopimelic acid}} = 0.67$), giving with ninhydrin, like diaminopimelic acid, a greenish colour turning to permanent yellow. To recover larger quantities of this unknown component, 100 mg of dried cell wall was hydrolysed and the hydrolysate was run as a band on a sheet of Whatman No. 3 paper (previously thoroughly washed with 2 M acetic acid and then with water), in solvent B (methanol/water/pyridine/98 per cent formic acid, 80 : 19 : 10 : 1). This solvent was used instead of A to avoid contamination of the product with non-volatile pyridine hydrochloride. The "slow-moving component" gave $R_{\text{meso-diaminopimelic acid}} = 0.66$. The band was located by applying streaks of 0.025 per cent (w/v) ninhydrin in *n*-butanol at intervals across the paper. The unknown substance was eluted with water, the solution dried *in vacuo* and made up to a final volume of 0.2 ml.

A sample of the unknown substance subjected to paper electrophoresis at pH 7 in collidine acetate buffer⁴ gave a single neutral spot detectable with ninhydrin. The fact that the unknown ran slower than diaminopimelic acid in solvent A but gave a similar colour after ninhydrin treatment suggested that it might be a derivative with some neutral but polar substituent such as hydroxyl. If this hydroxyl were adjacent to one of the amino-groups, then the compound should be destroyed by periodate solution to yield glutamic semi-aldehyde and glyoxylic acid. On a similar basis 'tabtoxine', the amino-acid from the phytopathogenic toxin of *Pseudomonas tabaci*, was tentatively identified as 2,6-diamino-3-hydroxypimelic acid⁵. 1 μ l. of the solution of "slow-moving component" was treated with 2 μ l. of 0.2 M sodium periodate and 10 μ l. water at room temperature for 30 min. Then 2 μ l. of 20 per cent (v/v) ethylene glycol was added to destroy excess periodate. The mixture was spotted on Whatman No. 4 paper and run for 7 h in solvent B. Whereas an equal quantity of untreated material gave a clear ninhydrin-positive spot, the periodate had completely

destroyed the unknown substance. Confirmation of the destruction of the unknown substance by periodate was obtained by running two samples in solvent B, and spraying one with ninhydrin and the other with 0.5 per cent (w/v) sodium periodate followed after 5 min by benzidine in acetic acid/ethanol⁶. The white spot on a blue ground given by the second spray exactly corresponded with the ninhydrin-positive spot.

The overall length of the chain was confirmed by reduction as described by Stewart⁷. 5 μ l. of "slow-moving component" was sealed in a tube with a little red phosphorus and 0.2 ml. of freshly redistilled hydriodic acid, spec. grav. 1.7. The tube was heated at 120° for 8 h and then the excess acid was removed *in vacuo* and water added three times and similarly removed. The residue was dissolved in water and shaken with a small portion of cation exchange resin 'Zeo-Karb 225' (Na⁺). The resin was washed and then extracted with N ammonia solution. Ammonia was removed *in vacuo* and a sample of the extract run in solvents A or B gave clear spots of LL-2,6-diaminopimelic acid and meso-2,6-diaminopimelic acid (presumably mixed with the DD-form⁸). Similar racemization during reduction of synthetic diaminohydroxypimelic acid by hydriodic acid was observed by Stewart⁷. Thus the unknown was clearly a derivative of diaminopimelic acid, presumably hydroxylated.

The unknown substance was also degraded by permanganate. 4 μ l. of "slow-moving component" was heated at 60° C with 10 μ l. portions of 1 per cent (w/v) potassium permanganate in 20 per cent (v/v) sulphuric acid (five portions in all). The mixture was cooled and neutralized with saturated barium hydroxide solution. The concentrated supernatant gave, in addition to unchanged starting material, a spot corresponding to glutamic acid both on paper electrophoresis at pH 7 and on chromatography in solvent B. On the assumption that permanganate oxidized a secondary alcohol group to a carboxyl group, this result supported the suggestion from the periodate degradation that the compound had a hydroxyl group on C-3, an amino group at C-6 and a carboxyl group at C-7.

Racemic mixtures of the four possible pairs of optical antipodes of 2,6-diamino-3-hydroxypimelic acid have been synthesized and characterized^{7,9}, and crystalline samples of these substances were kindly supplied by Dr. John M. Stewart. Comparison of these substances with the unknown in solvent A gave the result shown in Table 1. It was evident that the unknown could only correspond to the slow component of either synthetic isomer B or isomer C. The fact that three of the synthetic substances gave more than one spot presumably meant that this solvent was capable of separating their optical enantiomorphs, just as it can separate DD- and LL-diaminopimelic acid⁸. The

Table 1. CHROMATOGRAM RUN ON WHATMAN NO. 1 PAPER IN SOLVENT A FOR 20.5 H

Spots revealed by ninhydrin	
Substance	$R_{\text{meso-diaminopimelic acid}}$
"Slow-moving component" from <i>A. regularis</i>	0.67
2,6-diamino-3-hydroxypimelic acid	
Isomer A	0.90
Isomer B	0.67, 0.81*
Isomer C	0.68, 0.91*
Isomer D	0.85, 1.01*
LL-diaminopimelic acid	1.16

* This compound gave two spots.

unknown was evidently not a racemic mixture but one of the optically active forms of isomer *B* or isomer *C*.

A further means of comparison of the unknown substance with diaminohydroxypimelic acid was offered by the reaction with ninhydrin at acid pH values, which gives characteristic absorption curves with diamino-acids¹⁰. Samples of the unknown, diaminohydroxypimelic acid isomer *B* and diaminopimelic acid, each in 0.5 ml. water, were heated with 0.5 ml. acetic acid and 0.5 ml. of ninhydrin reagent (2.5 per cent (w/v) ninhydrin in a mixture of acetic acid/0.6 M phosphoric acid, 3 : 2) at 100° C for 5 min. The mixtures were cooled immediately, diluted to 5 ml. with acetic acid and read in a colorimeter against a reagent blank. The absorption curves given in Fig. 1 indicate that the unknown substance from *A. regularis* is the same as 2,6-diamino-3-hydroxypimelic acid (maxima at 360 mμ and 420 mμ). The difference from diaminopimelic acid (maximum 345 mμ) is very marked.

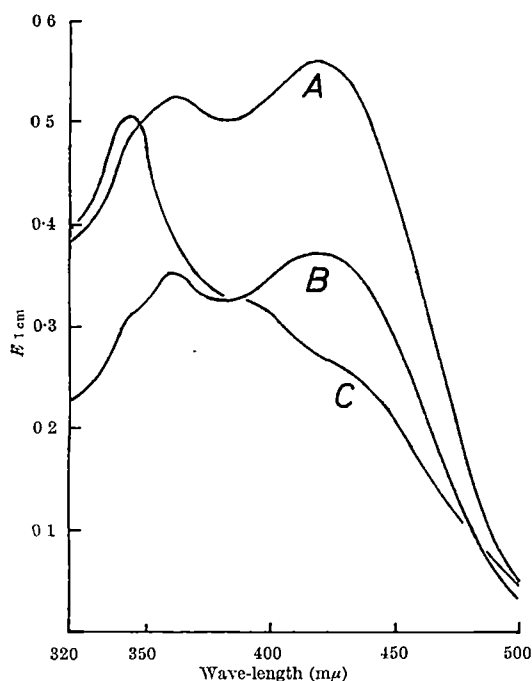


Fig. 1 Absorption curves given in the reaction with ninhydrin at pH 0.9 (ref. 10) by: A, "slow-moving component" from *A. regularis*; B, 2,6-diamino-3-hydroxypimelic acid, isomer B; C, 2,6-diaminopimelic acid

Stewart⁷ found that conversion to the *bis*-dinitrophenyl derivatives provided the only sure way of separating the four isomers of 2,6-diamino-3-hydroxypimelic acid. Samples of isomers A, B, C, and D and also of the "slow-moving component" were converted to their *bis*-DNP derivatives⁷. They were then run overnight in solvent C⁷ (*n*-butanol/water/ammonia solution S.G.O.880, 20 : 19 : 1) giving the following values for R_{DNP} -isomer D: Unknown and DNP-isomer B, 0.51; DNP-isomer C, 0.84; DNP-isomer A, 0.94 (DNP-isomer D moved 21.2 cm). This result showed that the substance from *A. regularis* was isomer B and could not be isomer C.

Stewart⁷ observed that 'tabtoxinine' corresponded to one isomer of 2,6-diamino-3-hydroxypimelic acid when compared as the *bis*-DNP derivative, and to a different isomer when the free amino-acids were run on an ion-exchange column¹¹, indicating that it was not either of these substances. The "slow-moving component" was run in the EEL automatic amino-acid analyser on a 150-cm column of 'Amberlite CG 120' at 50° and in buffer at pH 3.25. It was eluted just before cysteine and in exactly the same position as isomer B of 2,6-diamino-3-hydroxypimelic acid. Mixtures of the two substances could not be separated, and furthermore the colours

developed with ninhydrin both gave the same ratio of extinction at 440 mμ compared with 570 mμ. This identity on column chromatography confirmed the previous results and allowed quantitative estimation of the composition of the isolated cell walls of *A. regularis*. The results are given in Table 2. They show that 2,6-diamino-3-hydroxypimelic acid is the only diamino-acid present in appreciable quantity in the cell walls of this *Actinomyces*.

Table 2

Component	Mole/10 ⁴ g	Molar ratio (muramic acid = 1)
Glucosamine	2.43	0.70
Muramic acid	3.49	1.00
Glutamic acid	3.79	1.08
Glycine	4.85	1.39
Alanine	2.43	0.70
2,6-Diamino-3-hydroxypimelic acid (isomer B)	2.78	0.80
Ammonia	2.53	0.67
2,6-Diaminopimelic acid	0.35	0.10
Lysine	0.1	0.03

Cell walls of *A. regularis* were prepared by breaking the organisms with glass beads, differential centrifuging, washing, extraction with 0.5 per cent (w/v) potassium hydroxide in ethanol (48 h 37°) followed by digestion with trypsin and pepsin⁸. The washed product was freeze-dried, and a sample hydrolysed in 4 N hydrochloric acid for 4 h at 105°. It was analysed on the EEL automatic amino-acid analyser.

No other amino-acid was present in a molar ratio greater than 0.17.

The identification of the "slow-moving component" in the cell walls of *A. regularis* as isomer B of 2,6-diamino-3-hydroxypimelic acid means that this substance can now be added to the list of diamino acids known to occur in the mucopeptides of microbial cell walls. From recently published surveys³ it is evident that the "slow-moving component" is present in several strains of *Actinomyces* and may clearly be of value in taxonomy. In this respect the value of the specific solvent and colour reaction of Rhuland *et al.*⁸ cannot be over-emphasized. Although the substance from *A. regularis* has been shown to be isomer B, it may be that the cell walls of other strains contain different isomers of diaminohydroxypimelic acid. Almost all these run more slowly than *meso*-diaminopimelic acid on chromatography (Table 1) and might well not have been distinguished by the methods so far applied⁸. Isomer B has the *threo* configuration for the vicinal hydroxyl and amino groups and the *meso* configuration for the two amino groups⁷. The fact that the racemic mixture gives two spots on chromatography (Table 1), only one of which corresponds to the substance from *A. regularis*, suggests that the natural compound will be optically active. It is not possible to say anything at present about its absolute configuration.

Stewart⁷ showed that the substance from *Pseudomonas tabaci* designated 'tabtoxinine'⁵ was not, in fact, identical with any of the four isomers of 2,6-diamino-3-hydroxypimelic acid. This is therefore the first time that this substance has been identified in natural products.

I thank Dr. J. N. Couch and Dr. H. Gooder for supplying the culture of *A. regularis*, Miss M. F. Leyland for assistance, Mr. V. Okoro for preparations of cell walls and Mr. M. W. C. Hatton for expertise with the automatic amino-acid analyser. I also thank Dr. John M. Stewart for samples of the four isomers of 2,6-diamino-3-hydroxypimelic acid.

¹ Perkins, H. R., and Cummins, C. S., *Nature*, 201, 1105 (1964). Work, E., *Nature*, 201, 1107 (1964). Duc-Nguyen, H., and Weed, L. L., *J. Biol. Chem.*, 239, 3372 (1964). Veerkamp, J. H., Lambert, R., and Saito, Y., *Abst. Second Meeting Fed. European Biochem. Soc.*, 121 (1965). Cummins, C. S., *Nature*, 206, 1272 (1965).

² Work, E., and Dewey, D. L., *Proc. Sixth Congr. Intern. Micro.*, Rome 1, 50 (1955). Hoare, D. S., and Work, E., *Biochem. J.*, 65, 441 (1957).

³ Yamaguchi, T., *J. Bacteriol.*, 88, 444 (1965). Becker, B., Lechevalier, M. D., and Lechevalier, H. A., *App. Microbiol.*, 13, 236 (1965).

⁴ Newton, G. G. F., and Abraham, E. P., *Biochem. J.*, 58, 103 (1954).

⁵ Woolley, D. W., Schaffner, G., and Braun, A. C., *J. Biol. Chem.*, 198, 807 (1952); 215, 485 (1955).

⁶ Viscontini, M., Hoch, D., and Karrer, P., *Helv. Chim. Acta*, 38, 642 (1955).

⁷ Stewart, J. M., *J. Amer. Chem. Soc.*, 83, 435 (1961).

⁸ Rhuland, L. E., Work, E., Denman, R. F., and Hoare, D. S., *J. Amer. Chem. Soc.*, 77, 4844 (1955).

⁹ Stewart, J. M., and Woolley, D. W., *J. Amer. Chem. Soc.*, 78, 5336 (1956).

¹⁰ Work, E., *Biochem. J.*, 67, 4.6 (1957).

¹¹ Moore, S., and Stein, W. H., *J. Biol. Chem.*, 192, 663 (1951).

HETEROGENEITY OF WATER-SOLUBLE STRUCTURAL COMPONENTS OF HUMAN RED CELL MEMBRANE

By PROF. M. D. POULIK and DR. P. K. LAUF

Child Research Center of Michigan, and Department of Pediatrics, Wayne State University School of Medicine, Detroit, Michigan

THE methods available for extraction of water-soluble mucoids adopt enzymatic¹ and chemical procedures. Phenol², phenol-water³⁻⁵, methanol-chloroform⁶ or ethanol extraction⁷ have also been used. To prepare protein components stroma has been extracted with ether (Soxhlet)⁸. This latter method is efficient and a number of protein components were separated in starch gel containing urea⁹. Unfortunately, such material is not water-soluble unless urea is present in the solvent. The application of *n*-butanol for extraction of enzymes from muscle tissues by Morton¹⁰ opened a new approach to the preparation of structural components of cells and biomembranes. Maddy¹¹ used this technique for the isolation of carbohydrate-rich proteins of ox red cell membranes. Harris¹² used a similar method for the isolation of antigens of liver cell fragments associated with rejection of transferred allogeneic lymph-node cells. This method was therefore chosen for the extraction of the water-soluble components of human red cell membranes in this laboratory.

Out-dated blood of the major blood types which was collected in ACD-solution served as the starting material for the preparation of stroma by the method, slightly modified, of Dodge, Mitchell and Hanahan¹³. Stroma routinely so prepared contained less than 3 per cent haemoglobin. The leucocyte count in a typical preparation before haemolysis was less than 10 per cent of the original count. All preparations were screened also for bacterial contamination and spot tests were made with phase-contrast microscopy and electron microscopy to assess the integrity of the ghosts. Several methods for preparing the water-soluble protein of the ghosts were investigated. Removal of the lipid with *n*-butanol at -2° C was the procedure of choice because of its mildness. The effect of reductive cleavage in the absence or presence of urea^{14,15} on the recovery and solubility of the water-soluble material after butanol extraction was explored. The results are reported in Table 1. A typical recovery after butanol extraction of the treated and untreated stroma ranged from 70 to 80 per cent. The highest yield of the water-soluble material was obtained after reduction and alkylation in the absence of urea (16-18 per cent). Ether extracted stroma (Soxhlet) yielded a similar amount of water-soluble material after subsequent butanol extraction. A high degree of solubilization of the intact stroma was obtained in *tris*-mercaptoethanol mixture (0.55 M *tris*/HCl buffer, pH 8.2, containing 0.2 M mercaptoethanol) as used for the reductive cleavage. Irrespective of the method of preparation the water-phase material retained a high degree of solubility in aqueous solvents after lyophilization; low-speed centrifugation, however, was required to obtain a clear solution.

Purification of the water-phase material was attempted by gel-filtration on 'Sephadex G-200' using distilled water or saline as eluents. Two major peaks were separated (Fig. 1) with the water-phase of stroma which was reduced and alkylated in the absence or presence of urea prior to butanol extraction and of butanol-extracted stroma.

Table 1. THE YIELD OF WATER-SOLUBLE MATERIAL AFTER BUTANOL EXTRACTION
(Expressed in per cent of starting material)

	Stroma (untreated)	Stroma (reduced and alkylated)	Stroma (reduced and alkylated in 8 M urea)
Water-phase	11.0	16.3	10.8
Butanol-phase	30.6	35.0	33.8
Interface material	28.5	25.0	32.4

Ultra-violet absorption spectra of the peaks were compared with the starting material and revealed marked differences as shown in Fig. 2. Similar ultra-violet absorption spectra were found also with the water-phases of stroma which had been reduced and alkylated in the absence or presence of urea. The maximum of the first peak was that of a protein (276 mμ); the maximum of the second peak suggested the presence of lipid. This possibility was supported by the determination of the nitrogen content, where peak A contained 7.8 per cent and peak B 0.3 per cent nitrogen, respectively. Haemoglobin content was also determined in the three phases. On the dry-weight basis the water-phase usually contained between 1 and 3 per cent, the butanol-phase less than 2 per cent, and the interface approximately 3 per cent, haemoglobin which was detected as a haemochromogen-pyridine complex¹⁶. Small amounts of this complex were also found in the water-phase after gel-filtration. The biological activity of the water-phase preparations was now tested with the aid of the haemagglutination inhibition method using the Takatsi-micro-haemagglutination plates. The results of stroma and its phases of blood group A are presented in Table 2. It is

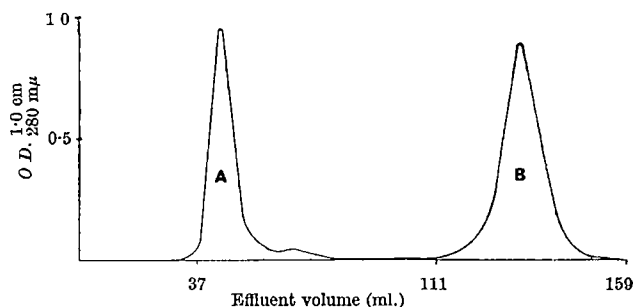


Fig. 1. Separation of water-phase material on 'Sephadex G-200'

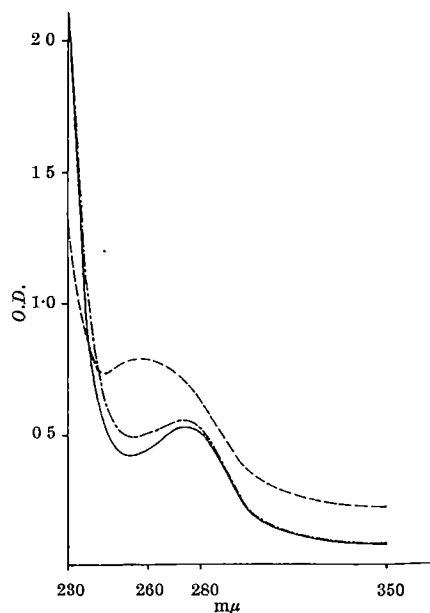


Fig. 2. Ultra-violet absorption spectra of water-phase (interrupted line) before and after gel-filtration. Peak A, solid line, peak B, dashed line

Table 2. SEROLOGICAL ACTIVITY OF DIFFERENTLY TREATED STROMA BEFORE AND AFTER EXTRACTION WITH *n*-BUTANOL

Material	Inhibition of four haemagglutination doses			
	Anti-A	Anti-Rh ₀ (D)	Anti-M	Anti-N
Stroma (untreated)	+	+	+	+
Water-phase	+	+	+	+
Interface	+	+	+	+
Butanol-phase	+	+	+	+
Peak A	+	n.t.	n.t.	n.t.
Peak B	+	n.t.	n.t.	n.t.
Stroma (reduced and alkylated)	+	+	+	+
Water-phase	+	+	+	+
Interface	+	+	+	+
Butanol-phase	+	+	+	+
Peak A	+	n.t.	n.t.	n.t.
Peak B	+	n.t.	n.t.	n.t.
Stroma (reduced and alkylated in 8 M urea)	+	+	+	+
Water-phase	+	+	+	+
Interface	+	+	+	+
Butanol-phase	+	+	+	+
Peak A	+	n.t.	n.t.	n.t.
Peak B	+	n.t.	n.t.	n.t.
Stroma (ether extracted)	+	+	+	+

n.t., Not tested.

evident that the Rh₀(D) antigen is destroyed by reductive cleavage as well as by butanol extraction alone. Both complete and incomplete antibodies were used for testing. Moreover, Rh₀(D), M and N antigens were not detected in the interface layer material or in the butanol-phase. The A-antigen was found in the interface, in the water-phase and also in both peaks obtained by gel filtration as well as in the butanol-phase. Irrespective of the pre-treatment of the stroma, the M and N activity was found in the water-phase and in the peak A of this material after gel filtration.

The presence of M and N antigens in the water-phase and their absence in the interface indicated a separation of water-soluble surface antigens. The water-phase was therefore analysed for its carbohydrate content. Three stroma preparations were compared and the results are tabulated in Table 3. It is evident that butanol extraction is able to release a water-soluble material which contains more than 90 per cent of the sialic acid of the stroma used. The interface contained a small amount of sialic acid as well as hexose and hexosamine as seen in Table 3, and is thus believed to contain a considerable amount of glycoprotein. It is to be noted that the content of sialic acid in the water-phase varied from preparation to preparation and it was considerably lower when determined in the water-phase of stroma which were reduced and alkylated prior to butanol extraction. Work is being carried out to explain the cause of these discrepancies.

The results suggested that water-phase material may be complex in nature, and were therefore investigated further with the aid of electrophoresis in starch gel containing urea and/or mercaptoethanol. Material from group A stroma obtained in the three phases as well as on the first peak was therefore subjected to starch-gel electrophoresis in buffers containing 8 M urea and 0.07 M mercaptoethanol. Two acidic buffers (sodium formate and aluminium lactate) were used. The patterns are shown in Fig. 3. A number of well-defined zones could be separated when untreated lyophilized stroma was dissolved in the reducing solution and applied to the gel (Fig. 3A). No differences were found among the major blood groups, regardless of whether the sodium formate or the aluminium lactate buffers were used for the electrophoretic experiment (cf. ref. 18).

Table 3. CARBOHYDRATE- AND NITROGEN-ANALYSIS OF DIFFERENT STROMA PREPARATIONS, A, B, C, BEFORE AND AFTER EXTRACTION WITH *n*-BUTANOL (Expressed as weight per cent)

	% Sialic acid (21)	% Hexose (22)	% Hexosamine (23)	% Total nitrogen
Stroma	1.2	8.7	1.3	6.5
A Water-phase	12.8	4.4	4.1	5.1
Interface material	0.3	5.6	1.3	10.5
Water-phase, peak A	17.6	n.t.	n.t.	7.8
B Water-phase	19.8	8.3	4.4	7.4
Interface	0.2	2.0	1.5	8.8
C* Water-phase	8.1	3.6	3.9	7.4
Interface	0.2	2.1	2.1	9.0

* Reduced and alkylated in absence of urea.
n.t., Not tested.

Comparison of patterns obtained with lyophilized stroma and with interface prepared by butanol extraction did not reveal any significant differences (Fig. 3A and B). Patterns of Soxhlet-extracted and untreated stroma were similar in the distribution of the separated components (Fig. 3A and F). The pattern obtained with water-phase material was less complex and most of the (Fig. 3C) zones present could be identified with those given by stroma. The constant feature of such a pattern was the presence of a component migrating to the anode. This zone is poorly represented in Fig. 3C due to the uneven thickness of the gel, but is clearly evident in the subsequent patterns (Fig. 3D and E). The pattern obtained with material in peak A differed only slightly from that of the water-phase; the mobility of the anodically migrating component was increased (Fig. 3D). Reduction and alkylation of the water-phase material afforded a pattern quite similar to that of unreduced water-phase and peak A material (cf. Fig. 3E). The anodically migrating component was also found. It should be noted that the patterns of these three materials (Fig. 3C, D and E) were further characterized by a 'white' zone which extended from the application point to the slowest of the cathodically migrating zones.

This phenomenon is most pronounced in the reduced and alkylated water-phase. A similar zone was also observed repeatedly with the water-phase material of other blood groups. The studies indicate that a reasonable amount of water-soluble serologically active material can be extracted with *n*-butanol. The yield could be increased by pre-treatment of the stroma by reductive cleavage, and yields up to 20 per cent were obtained depending on the content of haemoglobin in the original material. Inhibition of four haemagglutination doses of anti-A-serum was accomplished with 4-40 µg nitrogen of the water-phase of blood group A. Approximately four times this amount was necessary to inhibit the same dose in the case of blood group A interface material. The latter result may be dependent on the relative insolubility of the interface material rather than a reflexion of low content of A-substance. The loss of the Rh₀(D) activity after reductive cleavage, reported by Green¹⁷, is corroborated in this work. Furthermore the Rh₀(D) activity is also destroyed by butanol- or Soxhlet-extraction. This is an indication that a lipid as well as a protein component is probably implicated in its structural integrity. The serological activity of the M and N substances present in the water-phase was not destroyed by the chemical treatment used. It is to be noted that no M and N activity was found in the interface. This finding is supported by the low sialic acid content of the material. Since about 75 per cent of the recovered nitrogen was present in the interface, the material was re-extracted

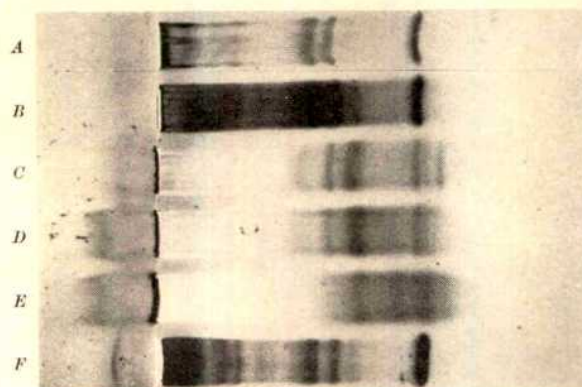


Fig. 3. Starch-gel electrophoretic patterns of A, stroma; B, interface; C, water-phase; D, peak A (cf. Fig. 1); E, water-phase (reduced and alkylated in absence of urea); F, ether extracted stroma (Soxhlet). Gel prepared in aluminium lactate buffer (0.015 M; pH 3.2) containing 8 M urea and 0.07 M mercaptoethanol.

with butanol in order to increase the yield of the water-phase. More than 50 per cent of the water-soluble material was obtained after this first extraction and the yield does not increase after the fourth extraction. The electrophoretic pattern, however, is improved after repeated extraction.

The application of urea starch-gel electrophoresis to the investigation of the red cell membranes^{9,18} offers new insight to the complexity of the constituents of such membranes. In spite of the fact that none of the zones except haemoglobin can be linked with definite functions as yet, the means are now available for monitoring further subfractionation of the water-soluble material. The number of separable zones may vary, depending on the age of cells, mode of preparation and electrophoretic conditions. Comparative investigations will now be feasible with mucoids and other membrane constituents obtained by the method described or by other methods. On the basis of electrophoretic results some purification of the water-phase was achieved by gel-filtration (cf. Table I). Peak A contained A, M and N substances. These materials are included in small amounts in the gel. Hence their molecular weight cannot be accurately estimated. However, their molecular weights must be higher than those obtained by Kathan and Winzler¹⁹. Despite the fact that this finding controverts the results obtained by these authors, the type of cells used and methods of their preparation may play a part in the final chemical state of such substances. Our results are, however, in agreement with those obtained by Lisowska²⁰ with respect to the ultra-violet absorption spectra and the molecular weight. The heterogeneity of each of the phases as demonstrated by starch-gel electrophoresis indicates a need for isolation and characterization of each of the components. Only

then can a more rational concept about the assembly of these sub-units in the red cell membrane and their specific biological role be obtained.

This work was supported in part by grants from the National Institutes of Health, AI 05785 from the National Institute of Allergy and Infectious Diseases, and HE 07495 from the National Heart Institute, and in part by the Children's Leukemia Foundation of Michigan.

- ¹ Springer, G. F., and Rapaport, M., *Proc. Soc. Exp. Biol. and Med.*, **96**, 103 (1957).
- ² Morgan, W. T. J., and King, H. K., *Biochem. J.*, **37**, 640 (1943).
- ³ Hohorst, H. J., *Z. Hygiene*, **139**, 561 (1954).
- ⁴ Klenk, E., and Uhlenbruck, G., *Hoppe Seyler's Z. Physiol. Chem.*, **319**, 151 (1960).
- ⁵ Nagai, Y., and Springer, G. F., *Fed. Proc.*, **21**, 67 (1962).
- ⁶ Howe, C., Rose, H. M., and Schneider, L., *Proc. Soc. Exp. Biol. and Med.*, **96**, 88 (1957).
- ⁷ Stadler, K., and Springer, G. F., *Proc. Eighth Congr. Europ. Soc. Haematol.*, Vienna 1961, 489 (Karger, S., Basel, New York, 1962).
- ⁸ Moskowitz, M., and Calvin, M., *Exp. Cell Res.*, **3**, 33 (1952).
- ⁹ Azen, E. A., Orr, S., and Smithies, O., *J. Lab. Clin. Med.*, **65**, 440 (1965).
- ¹⁰ Morton, R. K., *Nature*, **166**, 1092 (1950).
- ¹¹ Maddy, A. H., *Biophys. Biochim. Acta*, **88**, 448 (1964).
- ¹² Harris, T. N., *Proc. Soc. Exp. Biol. and Med.*, **118**, 1083 (1965).
- ¹³ Dodge, J. T., Mitchell, C., and Hanahan, D. J., *Arch. Biophys. Biochem.*, **100**, 119 (1963).
- ¹⁴ Fleischman, J. B., Pain, R. H., and Porter, R. R., *Arch. Biophys. Biochem.*, Suppl. I, 174 (1962).
- ¹⁵ Edelman, G. H., and Poulik, M. D., *J. Exp. Med.*, **113**, 861 (1961).
- ¹⁶ Rimington, C., *Brit. Med. J.*, **3**, 177 (1942).
- ¹⁷ Green, F. A., *Vox Sang.*, **10**, 32 (1965).
- ¹⁸ Poulik, M. D., Lauf, P. K., and Shuster, J., *Intern. Symp. Macromolecular Chemistry (Prague)*, Abst. A 256, p. 142; Sept. 1965.
- ¹⁹ Kathan, R. H., and Winzler, R. J., *J. Biol. Chem.*, **238**, 21 (1963).
- ²⁰ Lisowska, E., *Arch. Immun. Ther. Exp.*, **10**, 1011 (1963).
- ²¹ Aminoff, D., *Biochem. J.*, **81**, 384 (1961).
- ²² Schultze, H. E., Schmidtberger, R., and Haupt, H., *Biochem. Z.*, **392**, 490 (1958).
- ²³ Randle, C. J. M., and Morgan, W. T. J., *Biochem. J.*, **61**, 586 (1955).

PECTIC POLYSACCHARIDES IN THE GROWTH OF PLANT CELLS: MOLECULAR STRUCTURAL FACTORS AND THEIR ROLE IN THE GERMINATION OF WHITE MUSTARD

By SHEILA E. B. GOULD, DR. D. A. REES, N. G. RICHARDSON and I. W. STEELE

Chemistry Department, University of Edinburgh

THE part that the plant cell wall plays in growth has been discussed very thoroughly by plant biologists in recent years¹. Much of the speculation and experiment has stemmed from the hope that wall extensibility, which must play a major part in cell growth and which may have a controlling influence on it, might be adjusted through one set of molecular interactions. 'Cross-linkages' between pectic components of the wall have often been suggested to have this kind of key role; a discussion of the molecular interactions which could exist between pectic polysaccharides in the cell wall is therefore given here from a chemical point of view together with a preliminary account of a chemical approach to the investigation of these interactions in one system.

The possible nature of the association between pectic molecules in the cell wall is suggested by the gels that these substances form *in vitro*. Such gels contain a network of macromolecules that is cross-linked at various points; the number of cross-linkages need only be small, provided they are strong enough to withstand disruptive forces such as osmosis and coulombic repulsion. The presence of repulsive forces is necessary if the gel is to exist in a swollen state. When the galacturonic acid units are methyl-esterified, gels can be formed in the presence of a high concentration of a low-molecular-weight solute such as sucrose, glycerol or ammonium sulphate². Minor changes in molecular structure can prevent gelation^{3,4}, suggesting that the cross-linkages are secondary valence forces between parallel polysaccharide chains that contain

large numbers of consecutive methyl galacturonate units in close contact. The strength of the association in this type of gel is probably, therefore, affected by the distribution of the methyl galacturonate units available for bonding (that is, not carrying any other substituent). Gel formation can occur whether or not esterification is complete⁵, but a proportion of any non-esterified units must occur largely in the undissociated state, or in association with divalent cations (see following). The gel must be stabilized by the presence of solutes which presumably act, in large part at least, by diminishing the osmotic pressure which would otherwise be disruptive.

A different type of gel formation occurs *in vitro* in the presence of divalent cations such as calcium when the polysaccharide is de-esterified to a sufficient degree. Although charge neutralization might be one factor in the mechanism, it seems likely that other effects such as hydrogen bonding between chains and/or co-ordination of the cation by hydroxyl groups are also involved. Otherwise it would be difficult to understand why partial acetylation should affect gel formation^{6,7}, why cation binding should be influenced by the stereochemistry of the uronic acid units⁸, and why acidic polysaccharides such as polysaccharide sulphates should not form gels with divalent cations. The strength of the association in this type of gel is dependent on the intermolecular, and possible intramolecular, distribution of ionized uronic acid units⁹.

Neutral sugars occur in two types of covalent combination in galacturonans. Units of rhamnose, and possibly of other sugars, occur (normally in small amounts) as interruptions in the galacturonan chain; without a more detailed understanding of the stereochemistry of the secondary bonding involved in cross-linking, their effect on gel properties cannot be predicted. The second type is in side-chains which are sometimes very large; it appears that they can often be isolated by degradation of the galacturonan chain with alkali¹⁰. These side-chains would be expected to prevent the alignment of galacturonan chains, and polysaccharides with numerous such side-chains might lose the capacity to form a stable gel structure in the cell wall¹¹, or to do so by different mechanisms. A number of plant slimes appear to be examples of this type. These are laid down in cell walls, often associated with cellulose microfibrils¹². In water they swell to such an extent that the cell is burst; but this effect is not observed if the water contains solute to diminish the osmotic pressure sufficiently. The swelling might be due to an inability to form a compact gel structure, owing to the lack of strong inter-chain binding between the molecules. The slimes seem often to contain a high proportion of neutral sugar units in the main 'galacturonan' chain, numerous short side-chains also being present (for example, cross seed¹³ and linseed¹⁴ mucilages). A similar effect of increased branching in preventing alignment and aggregation of polysaccharide chains has been noted with certain arabinoxylans¹⁵. In the 'normal' pectic polysaccharides that have so far been thoroughly studied^{10,16,17}, relatively few side-chains occur (although this proportion seems rarely to be negligible), and the continuity of the galacturonan chain is less interrupted by other sugar units.

This brief discussion of the chemistry of pectic substances indicates the way in which these molecules are likely to be associated in the middle lamella and perhaps also in the cell wall proper, though here especially their association might be modified by the presence of other types of molecule. The fact that naturally occurring pectic substances are often highly esterified¹⁸ suggests that association via methyl-esterified galacturonic acid units might sometimes be important. The polysaccharide molecules might be synthesized and laid down such that there is a higher proportion of intermolecular relative to intramolecular cross-linkages than can be achieved in synthetic gels. The structure might not then need to be stabilized to exist, as it must *in vitro*, by the control of solute concentration, pH and/or cations present. Even should osmotic stabilization be required, it might be provided by other wall components such as different polysaccharides¹⁹. Calcium, and perhaps other cations, probably participate in the natural cross-linking of pectic polysaccharides. This is suggested by the maceration and improved extraction of pectic substances that is brought about by chelating agents and agents that precipitate calcium ions^{20,21} and by the co-occurrence of calcium and pectic substances in the same region^{20,22}. Such 'cation bridges' might add extra stability to a 'methyl ester linked' gel, or function independently. The presence of covalent ester linkages between wall polysaccharides is another possible type of cross-linkage to be borne in mind. There are therefore many types of molecular change which would alter the association between pectic substances *in vivo*, including changes in the molecular distribution of acetyl and methyl ester groups and neutral sugar units. Although the metabolic control of extensibility could conceivably be achieved through only one of these, it is equally likely that the situation is more complicated. The discussion presented here has been simplified by considering only the association between pectic polysaccharides, and no attention has been given to the role of other wall components which might indeed be more important, especially in some species where the concentration of pectic polysaccharides is very low²³.

Against this background, we have undertaken a chemical study of the pectic polysaccharides of white mustard cotyledons, to look for molecular changes which might correlate with biological changes in the cell walls during germination^{24,25}. The pectic extract was readily separated into a neutral araban and a mixture consisting chiefly of acidic polysaccharides²⁴. Several types of structure are consistent with the results of structural studies on the araban, but it is only relevant here to give one of them (Fig. 1). The other possibilities contain the same structural units in the same proportions, but differ in the way in which the units are arranged. After germination, the araban was present in smaller amount and methylation analysis coupled with quantitative gas chromatography showed that the proportion of branched units in the 'main chain' (Fig. 1) has diminished from 56 to 40 per cent.

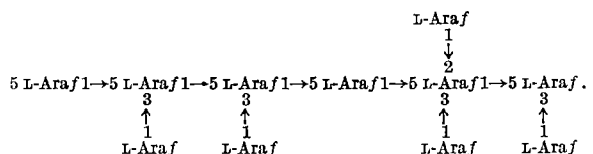
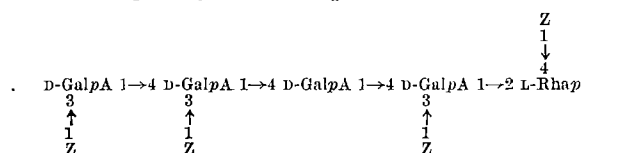


Fig. 1 Possible structure for mustard araban; note that this is only one of the types of structure consistent with present evidence

The acidic polysaccharide fraction is very complex and contains several components which appear to be related in that they give similar mixtures of sugars on hydrolysis. The main structural features indicated by experiments on the mixture are shown in the form of a composite typical molecule (Fig. 2). The average degree of branching is quite remarkable, and might be related to the observation that the cotyledon cell walls appear to swell markedly at the onset of germination (cf. the discussion of plant slimes here). One of the acidic pectic components showed a specific tendency to aggregate in the presence of low concentrations of calcium ions, with the formation of an insoluble gel. This behaviour was most conveniently examined using the ultracentrifuge. The relative concentration of this aggregating component increased very much during the germination period.



where Z is D-Xylp . . . or highly branched side-chains containing only arabinose units, perhaps resembling the neutral araban shown in Fig. 1

Fig. 2. Structural units present in the mixture of acidic polysaccharides from mustard embryos

The general conclusions to be drawn from these experiments are as follows. During the early stages of germination, the pectic polysaccharides in the cotyledons remain little changed in terms of total amount present, sugar composition on hydrolysis—and even in electrophoretic mobility which implies similarity with respect to such properties as charge density and perhaps degree of esterification. Dramatic changes are only revealed by more detailed examination of the chemistry of the wall components. The araban, a highly soluble polysaccharide which by analogy with other systems would be expected to have little capacity for intermolecular bonding, decreases in amount during germination to about one-third of the original level. At the same time, the degree of branching is diminished and therefore the capacity for cross-linking of the fraction of araban that remains is probably increased. The acidic polysaccharides (very much the major pectic components) change in such a way that they would appear by their aggregation behaviour to have an increased capacity for intermolecular bonding by 'cation bridging' after germination. These various factors would be expected to result in a firmer, less-extensible overall association. A decrease in the deformability of the cell wall would be expected on biological grounds,

because the period in which the alteration in visible form of the call was most rapid was complete at the point at which cotyledons were taken for analysis (after 4 days, germination). In view of this correlation, it seems possible that pectic polysaccharides are involved in the metabolic control of cell enlargement; their role could either be confined to the middle lamella¹, or be more general throughout the whole wall. The detailed chemistry of the process is not yet clear, but the general factors that might apply are outlined in the first part of this article and the evidence suggests that the chemistry is more complicated than has often been imagined in previous discussions. This function of the pectic polysaccharides would not exclude the possibility that enlargement is dependent on other biochemical changes as well.

We thank Mr. J. W. B. Samuel for a preliminary methylation examination of the mixture of acidic polysaccharides from resting cotyledons; Sir Edmund Hirst for advice; Prof. Robert Brown for sectioning experiments and for advice; Dr. G. O. Aspinall for many reference samples and for advice concerning the chemistry of pectic substances; and Prof. K. Wilson, Dr. R. O. Gould and Mr. A. Haug for further advice.

This work was supported by a grant under the Pioneering Research Program of the Institute of Paper Chemistry, Appleton, Wisconsin. We thank Dr. C. T. Greenwood for the use of the ultracentrifuge and boundary electrophoresis apparatus.

- ¹ Wilson, K., *Intern. Rev. Cytol.*, **17**, 1 (1964)
- ² Kertesz, Z. I., *The Pectic Substances* (Interscience Publishers, New York, 1951).
- ³ Neukom, H., Ph.D. thesis, Zurich (1949) quoted by Deuel, H., and Stutz, E., *Adv. Enzymol.*, **20**, 341 (1958)
- ⁴ Phippen, E. L., McCready, R. M., and Owens, H. S., *J. Amer. Chem. Soc.*, **72**, 813 (1950).
- ⁵ Deuel, H., Huber, G., and Leuenberg, R., *Helv. Chim. Acta*, **33**, 1226 (1950)
- ⁶ Solms, J., and Deuel, H., *Helv. Chim. Acta*, **34**, 2242 (1951).
- ⁷ Schweiger, R., *J. Org. Chem.*, **29**, 2973 (1964)
- ⁸ Haug, A., and Smidsrød, O., *Acta Chem. Scand.*, **19**, 341 (1965), and earlier papers
- ⁹ Speiser, R., Copley, M., and Nutting, G. C., *J. Phys. and Colloid Chem.*, **51**, 117 (1947)
- ¹⁰ Barrett, A. J., and Northcote, D. H., *Biochem. J.*, **94**, 617 (1965).
- ¹¹ Solms, J., *Adv. Chem. Ser.*, **25**, 37 (1960)
- ¹² Muhlethaler, K., *Exp. Cell Res.*, **1**, 341 (1950)
- ¹³ Tyler, J. M., *J. Chem. Soc.* (in the press).
- ¹⁴ Hunt, K., and Jones, J. K. N., *Canad. J. Chem.*, **40**, 1266 (1962).
- ¹⁵ Perlman, A. S., *Cereal Chem.*, **28**, 382 (1951).
- ¹⁶ Aspinall, G. O., and Cañas-Rodríguez, A., *J. Chem. Soc.*, 4020 (1958)
- ¹⁷ Aspinall, G. O., and Fanshawe, R. S., *J. Chem. Soc.*, 4215 (1961).
- ¹⁸ Gee, M., Reeve, R. M., and McCready, R. M., *J. Agric. Food Chem.*, **7**, 34 (1959).
- ¹⁹ Compare Boyer, R. F., *J. Chem. Phys.*, **13**, 363 (1945).
- ²⁰ Whistler, R. L., and Smart, C. L., *Polysaccharide Chemistry*, 163 (Academic Press, New York, 1953).
- ²¹ Letham, D. S., *Exp. Cell Res.*, **27**, 352 (1962)
- ²² Albersheim, P., Muhlethaler, K., and Frey-Wyssling, A., *J. Biophys. Biochem. Cytol.*, **8**, 501 (1960).
- ²³ Ray, P. M., and Rottenberg, D. A., *Biochem. J.*, **90**, 646 (1964)
- ²⁴ Hirst, E. L., Rees, D. A., and Richardson, N. G., *Biochem. J.*, **95**, 453 (1965).
- ²⁵ Gould, S. E. B., and Rees, D. A., *J. Sci. Food Agric.* (in the press).

QUANTITATIVE GAS CHROMATOGRAPHY OF AMINO-ACIDS AS TRIMETHYLSILYL DERIVATIVES

By PROF. EDGAR D. SMITH and HOWARD SHEPPARD, jun.*
Graduate Institute of Technology, University of Arkansas, Little Rock, Arkansas

BECAUSE of its speed, accuracy and sensitivity, gas chromatography offers substantial advantages over other methods at present in use for the determination of amino-acids. A critical review of the literature on this problem so far is given by Lamkin and Gehrke¹. In this review, however, the pioneering work of Ruhlmann on the synthesis and separation of the trimethylsilyl derivatives²⁻⁵ is given little attention because of the reported instability of these derivatives. Although Ruhlmann himself is credited with having stated that these derivatives were too unstable for satisfactory quantitative analysis⁶, we feel that this statement is entirely too broad and is possibly in error. It is the purpose of this article to present results showing that quantitative analyses of amino-acids are possible with at least three representative protein amino-acids.

(A) *Synthesis of amino-acid derivatives.* Three methods of synthesizing the trimethylsilyl (TMS) derivative of D,L-leucine were briefly explored as follows:

(1) The leucine was mixed with a 50-mole per cent excess of hexamethyldisilazane (HMDS) and refluxed until a clear solution was obtained and the evolution of ammonia practically ceased. The excess HMDS was removed by distillation at atmospheric pressure, the pot residue cooled, and the TMS-leucine distilled at 10-mm mercury pressure.

(2) The leucine was mixed with 100-mole per cent excess HMDS and refluxed as in method 1. The resulting solution was cooled to room temperature, diluted with about an equal volume of dry petroleum ether, and 50 mole per cent of triethylamine added to serve as a hydrochloric-acid scavenger. Finally, 50-mole per cent of trimethylchlorosilane (TMCS) was added dropwise with vigorous agitation, and the mixture stirred continuously for 2 h. This reaction mixture was then filtered under a nitrogen atmosphere to remove triethylamine hydrochloride and

the clear filtrate distilled as in method 1. This method is a slightly modified version of the procedure proposed by Birkofer and Ritter⁷.

(3) The leucine was mixed with a 30-mole per cent excess of trimethylsilyldiethylamine (TMSDEA) and refluxed on a Nester-Faust semi-micro spinning band still until the overhead temperature dropped to 55.5°C, the boiling-point of pure diethylamine. The diethylamine was then removed at a 10 : 1 reflux ratio. The overhead temperature remained practically constant until the theoretical quantity of diethylamine had been removed and then rose rapidly to 126.5°C, the boiling-point of pure TMSDEA. The excess TMSDEA was removed at this temperature, after which the pot was cooled and the crude TMS-leucine distilled at 10-mm mercury pressure as before. This procedure constitutes a minor variation of the procedure described by Ruhlmann⁶. The TMSDEA used in this work was synthesized in about 50 per cent yield by the method described by Hurwitz, Park and Benneville⁸.

All fractionations of the TMS-leucine derivative were carried out on the Nester-Faust still mentioned in method 3. The purified product yield in method 1 was only about 33 per cent of theoretical and was contaminated with a white solid. Our tests indicated that this solid was pure leucine, confirming the observations of Birkofer and Ritter. These workers believed that this product formed in the distillate because of the incomplete conversion of the amine group to the TMS derivative; two moles of the partially silylated amino-acid reacting to yield a fully silylated product plus free amino-acid.

The two-step synthesis procedure (method 2) was designed to eliminate the difficulty mentioned here and did lead to slightly better yields and appreciably lower contamination of the distillates. However, method 3 was clearly superior to method 2 in our hands and gave nearly quantitative yields of pure TMS-leucine. The former

* Present address E. I. du Pont de Nemours Co., Old Hickory, Tennessee

Table 1. PHYSICAL PROPERTIES AND YIELDS OF TRIMETHYLSILYL DERIVATIVES

No.	TMS-Derivative of	Yield % of theory	BP ° C/press (mm)	Relative retention time *
1	D,L-Alanine	61	76/15	1.00
2	Glycine	82	88/15	1.06
3	D,L-Valine	78	104/10	1.72
4	D,L-Leucine	87	108/10	2.07
5	L-Proline	76	87/5.5	2.63
6	D,L-Serine	50	114/4	3.07
7	D,L-Aspartic acid	95	113/4	4.70
8	D,L-Methionine	89	134/5	4.72
9	D,L-Phenylalanine	91	145/5	5.72
10	L-Lysine	81	147/4	6.67

* On 1 meter column of 10 per cent DC 200 on Gas-Chrom Z, programming from 92° to 200° C at 4 degrees/min, flow rate 100 cc/min helium.

method was therefore used in synthesizing the amino-acid derivatives listed in Table 1. It should be noted in this connexion that the yields of derivative shown in this table are the actual yield of purified product obtained. No attempt was made to account for losses due to still hold-up, intermediate cuts, etc. Ruhlmann has indicated yields of 82–97 per cent for all the amino-acids tested, and we feel that his figures are probably more nearly correct than ours.

(B) *Gas chromatographic analysis conditions and procedure.* As noted in Table 1, columns of 'DC 200' fluid (12,500 centistoke viscosity) on 'Gas-Chrom Z' gave good separations of most of the TMS amino-acid derivatives synthesized in this work. A number of other partition liquids were briefly tested and symmetrical peaks were obtained on such standard materials as 'DC 710', 'Apiezon L', 'SE 52' and 'QF-1'. However, none of these partition liquids was any better than 'DC 200' fluid. Attempts to use other standard partition liquids containing active hydrogen atoms, such as polyglycols or polyesters, failed to give recognizable peaks even after repeated injections or after attempts to react these partition liquids with excess TMSDEA before preparing the columns. In view of the distinct possibility that the TMS derivatives might prove to be too unstable or too reactive for gas chromatography anyway, it was decided to carry out a preliminary quantitative evaluation on the 'DC 200' column. It was further decided to carry out these analyses under isothermal conditions to eliminate errors which might occur due to non-reproducible programming of temperature. D,L-Leucine, D,L-serine and D,L-aspartic acid were the amino-acids chosen for this work as being representative of a neutral, hydroxy-, and acidic amino-acid respectively. An Aerograph model A-350-B dual column temperature-programmed gas chromatograph equipped with a dual 4-filament detector cell was used with a Sargent model SR recorder. Helium was used as the carrier gas and 2 metres of standard 0.25-in. diameter copper tubing was used to contain the packing of 10 per cent 'DC 200' fluid (12,500 centistoke viscosity) on 'Gas-Chrom Z'. The carrier gas flow-rate was set at 100 ml./min, and the column temperature at 160° C, in order to obtain a clean separation of the three amino-acids in an over-all analysis time of 8 min. Initially the injector and detector cell blocks were both set at 230° C, but a few preliminary semi-quantitative tests soon showed that better peak shapes and slightly more reproducible responses were obtained with on-column injection. The injector block temperature was therefore reduced to 160° C in subsequent quantitative work. In view of the known sensitivity of the TMS-amino-acid derivatives to traces of moisture, peak responses were also checked semi-quantitatively before and after the installation of a Matronic XF-100 gas chromatography scrubber in the carrier gas line between the helium tank and the gas chromatographic column. This unit is designed to remove traces of moisture, oil and dirt often found in commercial tanks of helium. Comparison of the data with and without the scrubber showed that the sample responses increased by nearly 40 per cent with the scrubber in place. This definitely showed the desirability of scrubbing the helium for this work even though it was not possible to detect any gain in weight in conventional magnesium perchlorate drying

tubes after the passage of 30–40 l. of this gas through them.

Standard samples of the TMS derivatives of leucine, serine, and aspartic acid were prepared by injecting each of the pure derivatives into a dry, tared, and nitrogen-flushed vial through a self-sealing silicone-rubber septum. The vial was re-weighed after each injection, and finally a calculated volume of TMSDEA was injected to bring the final solution to the desired concentration and the vial again weighed. In this way the weight per cent of the final solution could be calculated accurately.

Reproducibility of sample size injection was checked by injecting replicate 2, 4, and 8 µl. samples into a tared vial. The vials were re-weighed after each injection so that both the reproducibility of injection and the average sample weight per injection could be calculated. The assumption was made in subsequent work that these average weights were also injected into the gas chromatograph. Thus, the size of each amino-acid component could be calculated from a knowledge of the weight-per-cent composition of the sample mixture. Calibration curves were then prepared by injecting triplicate samples of 2–8 µl. of several standard mixtures. Under the gas chromatographic conditions specified, the elution times of the three amino-acids chosen for analysis were 2.2, 3.3, and 6.8 min for leucine, serine, and aspartic acid respectively.

Finally, several mixtures of leucine, serine and aspartic acid were weighed out and converted to their trimethylsilyl derivatives by the following procedure: a mixture of 80–300 µmoles of each of the three amino-acids was suspended in 4–5 ml of TMSDEA in a tared distillation flask. The suspension was refluxed on a Nester-Faust micro spinning-band still for 5–10 min, and then the resulting clear solution was slowly distilled so as to collect 2–3 ml. of distillate over a period of 50 min. Initially the overhead distillate temperature was 55°–60° C, but it gradually climbed to about 120° C at the end of the distillation time. The pot contents were removed from the still and the flask re-weighed so that the amino-acid content could be calculated. An aliquot of this solution was injected into the gas chromatograph and analysed under the conditions previously described.

The statistical correlations observed between the sample size of pure TMS-derivative injected and the average peak areas or peak heights are summarized in Table 2. Attempts were made to improve the precision of the peak area measurements by doubling the recorder chart speed, but no significant improvement was observed. It was concluded that the major cause of the relatively poor precision (95 per cent confidence limits were about ± 10 per cent of the actual amount present) was due to the non-reproducibility of sample injection. This conclusion was confirmed by the following facts: (1) in every case where an unusually high or low response was obtained for leucine, correspondingly high or low responses were also obtained for serine and aspartic acid; (2) when analyses were calculated on a relative, rather than on an absolute, basis, the precision of the analyses was markedly improved (95 per cent confidence limits were ± 0.6 per cent of the actual amount present).

Table 3 summarizes the results obtained on analysing four synthetic mixtures of pure leucine, serine and aspartic acid. Recorder peak height responses were used in this work since there seemed to be no advantage of the more complicated peak area determinations. Minor variations of the procedure described in the experimental section were used in these runs, and it is felt that the reproducibility shown in this table could be improved by strict adherence

Table 2. STATISTICAL CORRELATION OF SAMPLE SIZE AND RECORDER RESPONSE FOR PURE TMS DERIVATIVES*

Amino-acid	Peak heights		Peak areas	
	Corr. coeff.	S.D.	Corr. coeff.	S.D.
Leucine	0.995	0.019	0.995	0.019
Serine	0.995	0.019	0.996	0.018
Aspartic acid	0.995	0.022	0.996	0.019

* Range of sample size, was 0.10–0.70 µmoles for each amino-acid.

Table 3. ANALYSIS OF SYNTHETIC AMINO-ACID MIXTURES

Sample No.	Component	Absolute amount in μ moles			Relative amount (%)		
		Present	Found	% Dev.	Present	Found	% Dev.
1	Leucine	101	81.4	-19.4	33.6	34.4	+2.4
	Serine	100	78.6	-21.4	33.2	30.2	-9.0
	Aspartic acid	100	76.5	-23.5	33.2	32.4	-2.4
2	Leucine	100	67.2	-32.8	32.8	31.9	-2.7
	Serine	80	54.1	-32.4	26.2	26.5	+1.1
	Aspartic acid	125	84.4	-32.5	41.0	41.7	+1.7
3	Leucine	91.1	66.7	-26.8	28.8	29.1	+1.0
	Serine	93.9	68.0	-29.7	29.7	29.4	-1.0
	Aspartic acid	131	92.2	-29.6	41.5	41.5	0.0
4	Leucine	138	114	-17.4	23.2	24.3	+4.7
	Serine	162	131	-19.1	27.2	27.9	+2.6
	Aspartic acid	296	225	-24.0	49.6	47.7	-3.8

to a single procedure. Nevertheless, the data in Table 3 are useful to demonstrate two points: (1) the average yield of amino-acid derivative via the procedure used was roughly 75 per cent for each of the three amino-acids; (2) the relative percentage composition of the mixtures could be calculated with an average precision of ± 3 per cent (95 per cent confidence limit). The latter fact appears

to substantiate the conclusion that all three amino-acids were being converted equally to trimethylsilyl derivative.

In summary, we feel that this work strongly indicates that the trimethylsilyl derivatives may yet prove to be the simplest and best derivatives for the gas chromatographic analysis of amino-acids. Work is continuing to extend this method to additional protein amino-acids and to refine the analytical procedures outlined in this article.

This work was supported by U.S. Public Health Service research grant GM 09776 from the National Institutes of Health.

¹ Lamkin, W. M., and Gehrke, C. W., *Anal. Chem.*, **37**, 383 (1965).

² Ruhlmann, K., and Giesecke, W., *Angew. Chem.*, **73**, 113 (1961).

³ Ruhlmann, K., *J. Prakt. Chem.*, **9**, 86 (1959).

⁴ Ruhlmann, K., *J. Prakt. Chem.*, **315** (1959).

⁵ Ruhlmann, K., *Chem. Ber.*, **94**, 1875 (1961).

⁶ Ettre, L. S., *J. Gas Chromatog.*, **1**, 15 (1963).

⁷ Birkofer, L., and Ritter, A., *Chem. Ber.*, **93**, 424 (1960).

⁸ Hurwitz, M. J., Park, E., and Benneville, P. L. (to Rohm and Haas Co.), U S Patent 2,876,254 (March 3, 1959).

CORRELATED ATOMIC COLLISIONS IN IRRADIATED CRYSTAL LATTICES

By DR. LEWIS T. CHADDERTON and IAN McC. TORRENS

Physics and Chemistry of Solids, Cavendish Laboratory, University of Cambridge

WHEN crystalline matter is bombarded with energetic radiation—protons, neutrons, α -particles, electrons, fission particles, etc.—the atoms of the lattice are disturbed and some are displaced, either singly or collectively, to form defects. The detailed examination of these processes is the physics of radiation damage, in which interest has been stimulated by the onset of the nuclear age, and with the realization of the importance of crystal defects in many solid-state devices^{1,2}. Only comparatively recently, however, has close attention been paid to the fact that the atoms of an irradiated crystal are arranged in an ordered way on a three-dimensional lattice, though as long ago as 1957 Silsbee predicted that this would be of profound importance³.

The way in which the regularity of the crystal can modify the problem is perhaps best seen in a qualitative way by considering a primary radiation damage event; that is, a collision between an incoming particle and a lattice atom. If the collision is a close one, then sufficient energy is communicated to the struck atom to displace it and, indeed, a cascade or shower of subsequent displacements may ensue. If the energy of the 'primary knock-on', however, is not so great, then the surrounding lattice is able to impose rigid conditions on the possible modes of momentum transfer. Thus, when the struck atom cannot penetrate the surrounding lattice it can only transfer energy and momentum to one of its immediate neighbours, and so the possibility of collision correlation becomes apparent. More particularly, where close-packed rows of atoms exist in the crystal, one may expect an energy pulse, or a sequence of collisions, to be propagated along them. This is the phenomenon of focusing, first suggested by Silsbee³ and discovered in practice more recently through a series of elegant experiments performed by M. W. Thompson *et al.* at Harwell⁴⁻⁶. The bundle of energy which passes down a row of atoms is called a 'focuson', and it is said to have propagated by means of a 'focused collision sequence'. If the close-packed row of atoms is bordered by nearby atoms, then these also may exert some influence on the behaviour of the focuson, and an 'assisted focusing' process, which is very similar to the action which a simple thin converging lens exerts on a beam of light, may occur. When the nearby atoms lie close to the 'optic' axis, then the atomic lens is strong, focusing is enhanced, and the effective focal length is small. Moreover, if the focused collision sequence

is sufficiently energetic, then it can be accompanied by a series of sequential atomic replacements. This is called a dynamic crowdion. Afterwards, of course, owing to the dissipation of energy to the atoms comprising the atomic lenses and because of thermal vibrations of all the atoms, a dynamic crowdion is degraded into a focuson, an interstitial atom is deposited, and the focuson fades away⁷.

One other very important process derives from the existence of close-packed chains of atoms in the crystal, or rather from the open layers, or channels between them. If a primary knock-on enters and moves down such a channel, its interaction with the lattice will be small. It will travel long distances in an almost force-free space and will be constrained to stay there by an effectively infinite potential well created by the atoms in the close-packed chains. By analogy with the focuson, where the particular nature of the process was also emphasized through a high degree of energy localization, a primary of this kind has been called a 'channelon'⁷. The channelon, like the dynamic crowdion, but unlike the focuson, always transfers energy and mass.

The one single factor which perhaps best serves to distinguish between the phenomena of focusing and channelling is the energy at which they occur. Thus, while focusing is essentially a low-energy process (of the order of a few hundred electron volts in the close-packed metals), channelling is generally more favoured at higher energies (of the order of several keV); without any essential upper energy limit.

Computer methods. Both focusing and channelling are amenable to some kind of analytical treatment⁷, but a comprehensive and accurate analysis is ruled out by the difficulties encountered in allowing for many simultaneous atomic interactions—the very core of the correlation problem. Moreover, our knowledge about the interaction potential itself is not very satisfactory, so that in many circumstances a numerical approach can be of greater value at this time. For this reason, Vineyard *et al.* at the Brookhaven National Laboratory have initiated computer calculations of radiation damage in face-centred cubic metals⁸⁻¹¹, and the work has since been extended by Erginsoy and others to the body-centred cubic structure¹². The computer is asked to consider a crystallite containing a reasonably large number of atoms which interact with realistic forces. Atoms on the surface of the crystallite are supplied with extra forces simulating the reaction of

atoms outside, as though the crystallite were embedded in an infinite crystalline matrix, and a radiation damage event starts with all atoms on their lattice sites and all but one at rest. That one atom is initially endowed with arbitrary kinetic energy and direction of motion, as though it has just been struck by an incoming particle. The computer then integrates the classical equations of motion for the set of atoms, showing how the knock-on transfers energy to neighbouring atoms, how the dynamic stages evolve, and how the kinetic energy finally dies away and the atoms of the set come to rest in a damaged configuration.

Our own interest is the particular problem of fission fragment damage in crystals and in determining the part which focusing and channelling are likely to play in the different crystal types. For this reason a computer programme has been established which is similar in general form to that of Vineyard *et al.* but which differs in detail. In particular, a section is taken across the crystallite by the computer and the projected orbits of atoms originally at rest on lattice sites in that plane are automatically plotted. The programme is so constructed that it is a relatively simple matter to change the section taken, the nature of the material—element or compound, and the nature of the binding. The energy of the primary event is very easily controlled. The computation begins with the struck atom directed along a major crystallographic direction and the orbits of all the disturbed atoms in the plane selected, including the struck atom, are plotted out. After a sufficient interval of time the computation is arrested and the direction of the initial knock-on in the primary plane is slightly changed, so that it now makes a small angle with the original major crystallographic direction. The programme is then reset and restarted and a further set of atomic orbits are plotted out on the same diagram. This procedure is continued, the same increment of angle being added to the previous direction at each resetting, until such time as a full 360° has been covered in the plane or, if the plane contains one or more axes of symmetry, until the diagram contains all the necessary information. A new section through the crystallite—a new crystallographic plane—is then selected, and the entire computation followed through again for the same initial atom, and another diagram obtained. Each of these composite diagrams, which we call 'energy correlation diagrams', contains information about the importance of focusing and channelling following primary radiation damage events in the crystal at that particular energy. The idea is best illustrated by choosing a few examples.

We begin by considering an alkali halide lattice—potassium chloride. This is a face-centred cubic structure with singly charged potassium and chlorine ions (both electronic configurations identical with argon) arranged alternately at the lattice points of a simple cubic lattice. The ions are close-packed with an inter-ionic spacing of 3.14 \AA ; so we would expect focusons to be favoured and channelons to be rare. Fig. 1 shows the 80-eV correlation diagram for a struck potassium ion in the $\{100\}$ plane. All the events depicted were run under identical conditions, and continued for the same length of time ($1.2 \times 10^{-13} \text{ sec}$) so as to allow any focusons created to leave the immediate vicinity of the knock-on. The diagram illustrates very effectively the relative importance of the low-index directions in the focusing of energy away from a primary event. In particular, the predominance of the $[110]$ assisted focusing is extremely marked and much more intense than the $[100]$ focusing. The main $[110]$ lines carry some energy from almost all the events, and a large fraction of the energy from some initial projections close to 45° , whereas in the $[100]$ case, only events where the ion is set moving at under 20° focus energy over more than about three lattice spacings. Study of the diagram reveals that subsidiary $[110]$ focusing in adjacent lines is beginning and, if the events

were permitted to continue into the relaxation period, this would undoubtedly become more obvious. Disturbance of the $[110]$ lens by the traversal of a focuson is clearly illustrated. The size of the disturbed region, moreover, is governed by the energy of the primary knock-on, and in the potassium chloride structure the symmetrical spread of this region is very marked. Activity is concentrated along or near to the principal focusing axes.

Now consider a lattice which is characterized by a measure of covalent binding—lead iodide. This possesses a hexagonal crystal structure of the cadmium hydroxide group ($a_0 = b_0 = 4.54 \text{ \AA}$, $c_0 = 6.86 \text{ \AA}$) and consists of 'sandwiches' of planes parallel to the basal (0001) plane, each sandwich being composed of a plane of lead atoms between two planes of iodine atoms. The atoms are packed somewhat loosely if we compare the lattice with potassium chloride, so that channelling of atoms, rather than focusing, might be expected to be a possibility. Fig. 2 shows the 80-eV correlation diagram for a struck lead atom in the $\{1210\}$ plane. Strongly assisted focusing occurs in one of the events for atoms moving in the $[1011]$ direction, since a lead atom travelling in this direction passes through an assisting lens of two iodine atoms, which aids the focusing. However, the distance it must travel before colliding with its successor in the chain is 10.43 \AA , and consequently it does not require to be more than a few degrees off-axis in order to defocus rapidly, due to over-correction by the iodine/iodine lens. Some subsidiary focusing takes place in the $[0001]$ direction, but this is generally short-lived, due to the asymmetry of the adjacent lines of atoms. The relatively long range of the secondary knock-ons which the diagram reveals, and the lack of regularity in the spreading of the disturbance, are both consequences of the high spacings in the open lead iodide lattice. This contrasts markedly with Fig. 1, though undoubtedly some trajectories would cross in this diagram if the events were run into the relaxation period, where $[110]$ replacements and diffusive ionic motions would occur.

So far we have only referred to examples where the correlated atomic motions lead to the generation of focusons. Now we consider the case of channelling, for which we shall in general require higher energy correlation diagrams, though, of course, we dare not go to such high energies that energy losses to electrons would become important, since the programme does not acknowledge

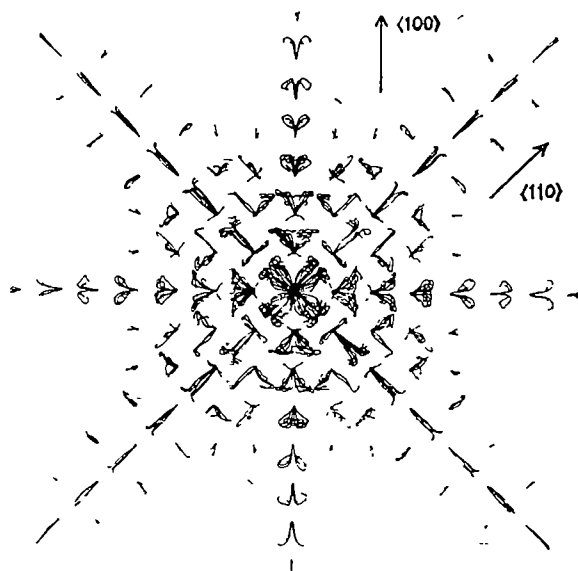


Fig. 1 80-eV correlation diagram for a struck potassium ion in the $\{100\}$ plane of the potassium chloride structure. The diagram consists of many superimposed atomic trajectories. Both simple and assisted focusing occur strongly in the $[100]$ and $[110]$ directions respectively.

the existence of a fine atomic structure. Here we introduce the concept of a primary energy correlation diagram, in which only the orbits executed by the primary knock-on are plotted out. This is simply a device to remove the background of the many secondary orbits which in this case would only complicate the issue. Fig. 3 is the 80-eV primary correlation diagram for the events shown in Fig. 2. The orbits of the knocked-on lead atom show minor variations with angle but do not vary significantly in length. Fig. 4, on the other hand, which is the 1,000-eV primary correlation diagram for a struck lead atom exhibits primary orbits with a very marked angular variation in length and shape. These indicate that within two angular bands of about 30° channelling occurs in the $\langle 1011 \rangle$ directions. There is a transverse oscillation superimposed on atomic motion along this channel, and this tends either to decrease in amplitude ('active' channelling), or to increase rapidly until the atom is knocked out of the channel. This oscillation of an imperfect channelon about the axis, if extrapolated to three dimensions, becomes a spiralling movement, though it is somewhat irregular since the atomic 'lenses' are rather astigmatic.

Conclusion. The primary energy correlation diagram, which is essentially a polar plot of channelon probability, is of value to those interested in this particular phenomenon. The displacement energy correlation diagram, however, which we have not so far discussed, will be of more general interest. In this, for a knock-on directed at a particular angle, the atomic orbits are plotted out for the lowest energy leading to production of a permanent Frenkel pair. Information about this displacement energy, and about the final positions of the vacancy and interstitial, is stored before the computer is instructed to add another increment of angle. The displacement energy correlation diagram may then consist of a plot of those atomic orbits which lead to displacement, including the positions in the lattice of the vacancies and interstitials produced, and, if necessary, of a true polar plot of the displacement probability.

While the methods which have been described here have been developed with a specific purpose in mind, we believe that the correlation diagram can be of use to workers in the general field of radiation damage. Since

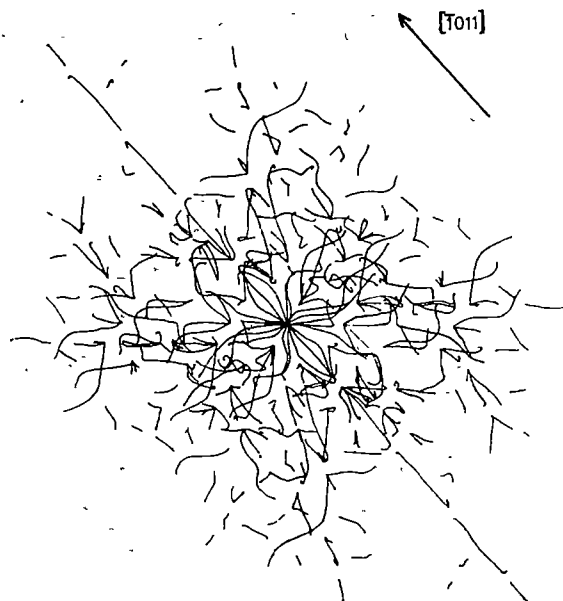


Fig. 2. 80-eV correlation diagram for a struck lead atom in the (1210) plane of the hexagonal lead iodide lattice. Strongly assisted focusing occurs in the 40° event for atoms moving in the [1011] direction, but this requires projection within a small angle of this direction, otherwise defocusing takes place immediately. The relatively long range of the secondary knock-ons is a consequence of the very open lattice of lead iodide.

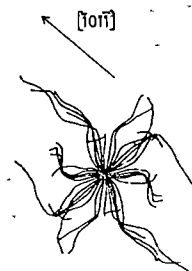


Fig. 3. 80-eV primary correlation diagram for the events shown in Fig. 2. Only the orbits executed by the primary knock-on are plotted out, and though these show minor variations with angle they do not vary significantly in length.

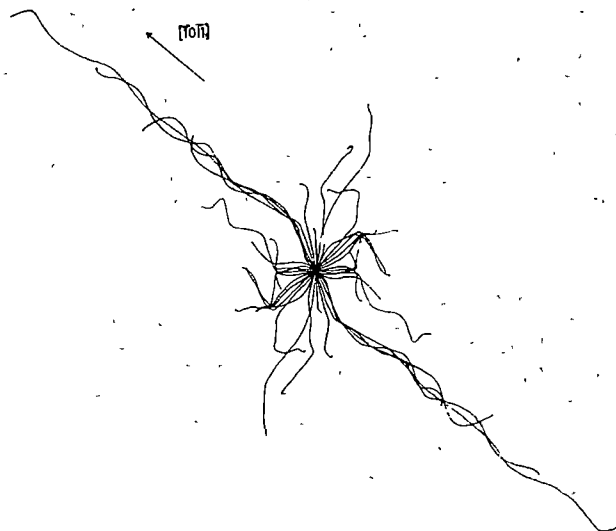


Fig. 4. 1,000-eV primary correlation diagram for a struck lead atom in the (1210) plane of the lead iodide lattice. Orbits with a very marked angular variation in length and shape indicate that within two angular bands of about 30° channelling occurs in the $\langle 1011 \rangle$ directions.

the computer programme can be easily adapted to different structures, a first step might be to catalogue the non-displacement correlation diagrams for the principal planes in the elemental crystal lattices—cubic, tetragonal, hexagonal, monoclinic, etc. This would make available a neat, but qualitative, summary of the relative probabilities of focuson propagation along the major directions in crystals of the elements. A second step would be to see how these diagrams vary with the nature of the interatomic binding—metallic, ionic, covalent, etc.—in going from an element to a compound, and with atomic mass. Thirdly, and somewhat more sophisticatedly, it might be informative to introduce defects—dislocations, interstitials, impurities, stacking faults, etc.—of the type to which the particular crystal is susceptible.

We thank Dr. F. P. Bowden for his advice, the Ministry of Education, Northern Ireland, for a research scholarship (I. McC. T.), and the Science Research Council for the award of a research grant.

- ¹ Dienes, G. J., and Vineyard, G. H., *Radiation Effects in Solids* (Interscience, 1957).
- ² Billington, D. S., and Crawford, J. H., jun., *Radiation Damage in Solids* (Princeton Univ. Press, 1961).
- ³ Silsbee, R. H., *J. App. Phys.*, **28**, 1246 (1957).
- ⁴ Thompson, M. W., and Nelson, R. S., *Proc. Roy. Soc., A*, **259**, 458 (1961).
- ⁵ Thompson, M. W., and Nelson, R. S., *Phil. Mag.*, **7**, 2015 (1962).
- ⁶ Thompson, M. W., Nelson, R. S., and Montgomery, H., *Phil. Mag.*, **7**, 1385 (1962).
- ⁷ Chadderton, L. T., *Radiation Damage in Crystals* (Methuen, 1965).
- ⁸ Gibson, J. B., Goland, A. N., Milgram, M., and Vineyard, G. H., *Phys. Rev.*, **120**, 1229 (1961).
- ⁹ Vineyard, G. H., *J. Phys. Soc. (Japan)*, **18**, Supp. III, 144 (1963).
- ¹⁰ Vineyard, G. H., *Energia Nucleare*, **8**, 9 (1961).
- ¹¹ Vineyard, G. H., *Disc. Faraday Soc.*, **31**, 7 (1961).
- ¹² Erginsoy, C., Vineyard, G. H., and Englert, A., *Phys. Rev.*, **133**, A, 595 (1964).

LETTERS TO THE EDITOR

PHYSICS

A Wave Mechanical Approach to the Plasma Interaction Problems

WHEN an object moves rapidly in an ionized gas, its surface, if electrically conducting, will become charged due to the differential in mobilities of the electrons and ions in the gas. Assuming that the medium is in thermodynamic equilibrium and consists of singly charged particles only, the surface will acquire a negative potential. The equilibrium surface potential will be reached when the incident fluxes of electrons and ions become equalized by the biased surface potential. A charged body influences, by electrostatic interaction, the trajectories and concentrations of the neighbouring charged particles which, in turn, modify the potential field by their presence as space charges. This electrodynamic phenomenon, which occurs in the immediate vicinity of a body moving rapidly in a rarefied ionized medium, is the plasma interaction of interest. A variety of scientific experiments have led to the discussion of this problem. As an example we may cite the measurement of the upper ionosphere by the use of a satellite probing scheme. The ionospheric quantities of interest, for example, electron density and temperature, must be inferred from the planned measurements made at the satellite body which is, of course, shielded by a cloud of charged particles and their associated potential field. The theory of measurement, therefore, must be based on an understanding of the specific plasma interaction developed.

The conventional approach to the problem consists of treating the Poisson equation which governs the potential field jointly with the Boltzmann equations which determine the concentrations of the charged particles in question. These equations are coupled by the space-charge terms of the Poisson equation and the external-force term of the Boltzmann equation. It would be out of place to review the extensive publications on the subject. Interested readers are referred to the recent review by Al'pert *et al.*¹. Generally speaking, the method of iterative approximations is used starting with a quasineutral particle solution for the particle concentration. Some pertinent references which are exceptions to the iterative approach are discussed later.

I shall present here an alternative approach to the plasma interaction problem which leads to a more rigorous treatment of the charge-field coupling effect, thus shedding more light on the physical process of interaction. In this approach, the motion of the particles is represented by the Schrödinger wave equation instead of the Boltzmann equation as used in the conventional approach. In the present communication, we are interested in the stationary state of the interaction, thus eliminating the considerations of hydrodynamic stability and wave excitations. The absence of magnetic field is also assumed although the formulation can be generalized to include the effect of magnetic field. This omission is justifiable if we are interested in the particle distributions in the zone close to a body moving in a weak magnetic field.

Consider a rarefied plasma flowing toward a fixed (conducting) body at hypersonic speed such that the free stream velocity V_0 is much larger than the thermal velocity of the ions and much smaller than that of the electrons. It is assumed that the characteristic dimension of the body is small compared with the minimum mean free path of the charged particles. Thus the particle flow in

this zone close to the body can be treated as collisionless. To make our problem definite we postulate that the surface of the body absorbs and neutralizes the encountering electrons and ions respectively. This is a reasonable assumption for particles impinging with energy of the order of a few electron volts. It is further assumed that the body is electrically charged to a high negative potential, hence the electron density (n_e) can be approximated by the Maxwell-Boltzmann distribution:

$$n_e(\mathbf{r}) = n_0 \exp[e\varphi(\mathbf{r})/k T_e]$$

where e , k , T_e , φ and n_0 denote the electron charge, Boltzmann constant, electron temperature, electrical potential and electron density at infinity where $\varphi = 0$, respectively.

The Schrödinger wave equation for an ionic particle (m) in steady motion is:

$$h^2 \nabla^2 \psi(\mathbf{r}) + 8\pi^2 m [E - e\varphi(\mathbf{r})] \psi(\mathbf{r}) = 0 \quad (1)$$

where h denotes Planck's constant; φ , potential; E , total energy of the particle in question. The wave function $\psi(\cdot)$ has meaning² such that $|\psi|^2 d\mathbf{r}$ is the probability of finding a particle in volume element $d\mathbf{r}$ at \mathbf{r} . Thus if there are n non-interacting identical particles per unit volume, $n|\psi|^2$ represents the probable number density at \mathbf{r} . It can be shown that for the ionic particles of interest in the plasma interaction problem here concerned, the de Broglie wave-length is negligibly small compared with the Debye shielding distances¹ in which φ varies significantly. We may therefore simplify the solution of equation (1) by using a short-wave-length approximation. In so doing all the 'diffraction' effect (a term borrowed from optics), which is important for the shadow (wake) zone of the flow field, vanishes in this limit, as might be expected. In other words, in the present level of approximation, we limit our discussion to the flow field in the frontal zone facing the stream. It is noted from geometric optics that if the coefficient of the ψ -term were a constant, say α , $\exp[i\alpha(\vec{\beta} \cdot \mathbf{r})]$ would be a solution to equation (1), where $\vec{\beta}$ is a unit vector in the direction of propagation. This result, together with the Wentzel-Kramers-Brillouin-Jeffreys approximation to equation (1) in the one-dimensional case, suggests the following form for ψ (if several rays pass through a point, we may write instead of (2):

$$\psi = \Sigma A \exp[iW(\mathbf{r})],$$

the sum includes one term for each ray through the point):

$$\psi = A \exp[iW(\mathbf{r})] \quad (2)$$

Equation (1), after the substitution of (2), becomes:

$$h^2[(\nabla W)^2 - \vec{\nabla}^2 W] = 8\pi^2 m(E - e\varphi)$$

which reduces by short-wave-length approximation to

$$h^2(\nabla W)^2 = 8\pi^2 m(E - e\varphi) \quad (3)$$

The first-order partial differential equation (3) for W is equivalent to a system of ordinary differential equations for the rays (or trajectories) of the particles. It is a form of Hamilton-Jacobi equation in classical mechanics, as might be expected²; W is proportional to the action function. The possible trajectories of ions are orthogonal to the surfaces of constant W and hence to the surfaces of constant phase of the wave function ψ . The formal solution for ψ can be written:

$$W(\mathbf{r}) = \int 2\pi \sqrt{2mh^{-1} \{E - e\varphi(\mathbf{r}(s))\}} ds \quad (4)$$

where s is a parameter in terms of which the trajectories of ions are described.

The advantage of the present method results from the fact that the Schrödinger equation for ψ is linear for a given φ . It is admitted that the Hamilton-Jacobi equation can be exactly and generally integrated only in a few simple cases. On the other hand, particular solutions with correct initial conditions can often be fairly easily obtained by numerical or graphical methods. In the following we shall illustrate the use of the present method in a plasma interaction problem for which an exact solution for the potential field can be obtained.

Consider a rarefied hypersonic plasma stream with properties as already described impinging on a large conducting plate at a small angle θ to its normal, the x -axis. It is assumed that the plate, which absorbs and neutralizes the electrons and ions respectively, is large, hence the edge effect is negligible and the potential field can be treated one-dimensionally, $\varphi = \varphi(x)$. We have now the Schrödinger equation for the motion of the ions:

$$\frac{\partial^2 \psi}{\partial x^2} + \frac{\partial^2 \psi}{\partial y^2} + \frac{8\pi^2 m}{h^2} [E - e\varphi(x)] \psi = 0 \quad (5)$$

and the Poisson equation governing the variation of φ :

$$\frac{d^2 \varphi}{dx^2} = -4\pi en_0 [\psi^2 - \exp(e\varphi/kT_e)] \quad (6)$$

with the boundary conditions:

$$\varphi = 0, d\varphi/dx = 0, \text{ at } x = -\infty$$

Let $\psi(x, y) = \psi_1(x)\psi_2(y)$ be substituted into equation (5), we obtain the separated equations:

$$\frac{d^2 \psi_1}{dx^2} + \frac{8\pi^2 m}{h^2} [E - e\varphi(x)] \psi_1 = C_1^2 \quad (7a)$$

and

$$\frac{d^2 \psi_2}{dy^2} = C_1^2 \quad (7b)$$

where C_1^2 is the separation constant. Using the short-wave-length approximation (or WKBJ-method) and the upstream condition:

$$\text{at } x = -\infty, \psi \sim \exp[2\pi i(2mE/h^2)^{1/2}(x \cos \theta + y \sin \theta)]$$

and also $|\psi|^2 = 1, \varphi = 0$ we obtain from (7):

$$|\psi|^2 = (1 + e\varphi/E \cos^2 \theta)^{-1} \quad (8)$$

It is interesting to note that the result (8) agrees with that obtained by Hays³, who used the Boltzmann equation method. It is also of interest to compare the present result with that of Ginzburg⁴, who studied a special case ($\theta = 0$) using hydrodynamic equations derived from continuum flows. They check when θ is put equal to zero in (8) and the pressure-term contribution in Ginzburg's result is neglected to comply with the hypersonic assumption.

Equation (6) re-arranged⁵, after $|\psi|^2$ is replaced according to equation (8), and multiplied by $d\varphi/dx$, is integrated to:

$$x - x_0 = \int (8\pi n_0)^{-1} [2E(1 - e\varphi/kT_e)^{1/2} - 2E - kT_e + kT_e \exp(e\varphi/kT_e)]^{-1/2} d\varphi \quad (9)$$

where φ_0 denotes the potential at $x = x_0$; the total ionic energy $E = mV_0^2/2$. In equation (9) we have the structure of the electrical potential shield in front of an inclined plate with negative surface potential φ_0 facing a hypersonic plasma.

The generalization of the present approach to incident ions having a thermal velocity which is small but not negligible compared with V_0 should not prove difficult in view of Bohm and Vigier's interpretation of quantum theory in terms of a fluid with irregular fluctuations⁶.

In rarefied-gas dynamics, an issue of much controversy⁷ exists as to the limit of validity of the hydrodynamic equations. With charged particles the appearance of

collective phenomena which arise because of the long-range electrostatic forces between charges tends to widen the range of validity of the continuum hydrodynamic equations. Still, the discussion has been at the best qualitative. The present formulation helps to shed some light on the transition from micro- to macro-mechanics of plasma flows. Furthermore, the present method appears natural for the treatment of some unsolved problems, such as: (1) unsteady plasma flows; (2) the distribution of ions with bound orbits. In fact it was during an investigation of these problems that I was attracted to the idea of using the Schrödinger equation.

This work has been supported by research grant NsG660 from the National Aeronautics and Space Administration. I thank Ming-Young Yu for his help in the calculations.

V. C. LIU

Department of Aerospace Engineering,
University of Michigan,
Ann Arbor.

¹ Al'pert, V. A. L., Gurevich, A. V., and Pitaevskii, L. P., *Soviet Phys Uspekhi*, **6**, 13 (1963).

² Schiff, L. I., *Quantum Mechanics* (McGraw-Hill, 1955).

³ Hays, P. B., Ph.D. thesis, Univ. of Mich. 1964 (available from Univ Microfilm Co., Ann Arbor).

⁴ Ginzburg, M. A., *Trudy Izmiran, Akademii Nauk S.S.S.R.* No. 17 (1960) (Engl. Trans. *A.R.S.J.*, **32**, 1794, 1962).

⁵ Kamke, E., *Differential Gleichungen Lösungsmethoden und Lösungen*, Bd. I, Chelsea, 1948.

⁶ Bohm, D., and Vigier, J. P., *Phys. Rev.*, **96**, 208 (1954).

⁷ Liu, V. C., Pang, S. C., and Jew, H., *Phys. Fluids*, **8**, 788 (1965).

Direct Ultramicroscopic Observation of Thermal Scattering in Sodium Chloride

THERMALLY scattered light emitted from nominally very pure single crystals of sodium chloride is directly seen in ultramicroscopic examination of impurity centres^{1,2} using illumination at right angles to the direction of observation (Fig. 1).

This is proved by photographic intensity measurement of the light scattered by a crystal region which is free from individual scatterers. For the reference photograph the intensity of the illuminating light is optically attenuated to 10^{-14} of its original value with a specially designed system using neutral density filters, multiple reflexions and optical sampling.

The relative intensity of the scattered light is determined with an experimental error of ± 15 per cent for the wavelengths of 400 nm and 546 nm of a high-pressure mercury discharge lamp. The results comply with the theoretical values³.

L. Taurel⁴ was able to show this by careful selection of the illuminated portion of the crystal measuring the total intensity of the light emitted by it. With this method one cannot distinguish between the thermal scattering itself and the contributions of individual scattering centres which are due to impurities in the crystal. The measurement presented here overcomes this difficulty.

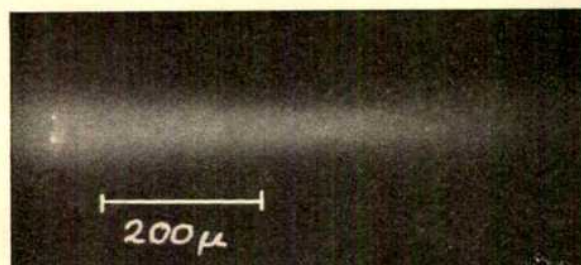


Fig. 1. Illuminated portion of sodium chloride crystal as observed with an ultramicroscopic arrangement, exposure time 18 h. Only some few scattering centres show up in the right-hand side of the scattering volume.

This work was carried out under the sponsorship of the United Kingdom Atomic Energy Authority. I thank Dr. E. Schneider for his advice.

GERHARD HEILMANN

Department of Physics,
University of Newcastle upon Tyne.

* Present address: Universität des Saarlandes, Institut für Experimentalphysik, 66 Saarbrücken, 15.

Heilmann, G., *Z. angew. Physik*, **17**, 482 (1964).

Heilmann, G., *Applied Optics*, **4**, 1201 (1965).

Leontovitch, M., and Mandelstam, jun., S., *Physik. Z. Sowjetunion*, **1**, 817 (1932).

Taurel, L., and Humphreys-Owen, S. P. F., *Proc. Phys. Soc. (London)*, **75**, 473 (1960).

GEOLOGY

Corona Structures in the Basic Igneous Masses of East Aberdeenshire

THE presence of corona structures in the east Aberdeenshire intrusions is well known. Read¹ described and figured such reaction relations from olivine-gabbros of the Huntly mass. Stewart² noted in the Belhelvie mass common reaction rims surrounding the olivines of the troctolites, the inner being colourless orthopyroxene, the outer amphibole. Sadashivaiah³ described from ultrabasic rocks of the Bourtie area of the Inch mass "Very beautiful double coronas . . . are developed around the olivines at the contacts with feldspar; the narrow inner rim is usually of orthopyroxene and the broader outer rim of fibrous amphibole . . .". Read, Sadashivaiah and Haq⁴ recorded from olivine-gabbros of the Inch mass "intergrowths of various kinds and reaction-associations are beautifully developed. . . . The discontinuous reaction series involved in the reaction association consists of the following members: olivine, hypersthene, augite, hornblende and biotite." In my investigation of the southern end of the Huntly mass I have observed in troctolites double coronas of enstatite and hornblende, and in olivine-gabbros and olivine-norites double coronas of hypersthene and hornblende.

These facts are of considerable importance in the light of recent investigations into the effects of pressure on the melting of enstatite by Boyd, England and Davis⁵. From the results presented, they suggest that "... rocks which show textural evidence of an olivine-liquid reaction relation have developed this relation at relatively shallow depth, probably less than about 9 km".

Stewart² stated that the most reasonable explanation of the form and banded nature of the Belhelvie mass is "that of gravitative differentiation in place, and subsequent tilting to an almost vertical position". Blundell and Read⁶, from palaeomagnetic evidence, state "There is no significant difference between the magnetism of any of the basic masses, and it is therefore suggested that they have remained relatively undisturbed since their formation". Stewart and Johnson⁷ "... feel that the evidence in favour of differentiation in place and subsequent folding, provided by the primary igneous structures, is strong". Read *et al.*⁴ advanced a provisional conclusion for the Inch mass that crystal-fractionation of gabbro magma occurred in place.

From the results obtained by Boyd, England and Davis⁵, it would appear that the basic intrusives of east Aberdeenshire are high-level types, and do not represent a large 'Bushveld type' layered intrusive broken up at depth. The presence of perfect corona and other reaction structures in each of these masses suggests a common factor in their crystallization history; perhaps as one fractionation sequence, for example, Read *et al.*⁴ "... the exposed basic masses of north-east Scottish Province might make one huge sheet"; perhaps as individual bodies of magma crystallizing under similar conditions of depth and

metamorphism. Stewart and Johnson⁷ suggest that the 'older' rocks near the western margin of the Huntly mass south of Knock Hill are in reality 'younger'. If this is correct, and indeed if the Port Soy ultrabasics are also 'younger' and hence correlate with the southern end of the Huntly mass, crystallization of these rocks must have occurred before some, at least, of the regional metamorphism. In contrast, the thermal aureole surrounding part of the Inch mass shows it to be younger than the MacDuff Slates.

In the symposium: "Depth and Tectonics as Factors in Regional Metamorphism", it was suggested by Chinner⁸ that: "On the load pressure assumption, kyanite-sillimanite sequences appear to require not only low geothermal gradients . . . but also depths of burial of the order of 30 kilometres". Any depth burial approaching this figure is at odds with the evidence of the presence of corona and reaction structures in the Aberdeenshire basic masses, which suggest late-tectonic, high-level crystallization.

Variation in regional metamorphism in the country rocks surrounding each of these masses, coupled with investigation of the variation of contact aureoles and the nature of the reaction structures present in each mass, will be necessary before the general history of these masses is elucidated.

D. S. WEEDON

Department of Geology,
University of Glasgow.

¹ Read, H. H., *Mem. Geol. Surv. U.K.* (1923).

² Stewart, F. H., *Quart. J. Geol. Soc. Lond.*, **102**, 465 (1947).

³ Sadashivaiah, M. S., *Geol. Mag.*, **91**, 137 (1954).

⁴ Read, H. H., Sadashivaiah, M. S., and Haq, B. T., *Proc. Geol. Assoc. Lond.*, **72**, 391 (1961).

⁵ Boyd, F. R., England, J. L., and Davis, B. T. C., *Geophys. Res.*, **69**, 2101 (1964).

⁶ Blundell, D. J., and Read, H. H., *Proc. Geol. Assoc. Lond.*, **69**, 191 (1958).

⁷ Stewart, F. H., and Johnson, M. R. W., *Trans. Edin. Geol. Soc.*, **18**, 104 (1960).

⁸ Chinner, G. A., *Proc. Geol. Soc. Lond.*, **15**, 1594 (1962).

Oligocene in California ?

RECENTLY, Eames, Banner, Blow and Clarke¹ stated that strata of Oligocene age are missing in most of America. To support their conclusions, they noted that the Relizian Stage of California was Aquitanian in age and correlated it with the Chickasawhay Formation of the Gulf Coast region. They also noted that the Tumey Formation of California, formerly considered as Oligocene, contains Eocene *Discocyclus clarki*. By ignoring the strata between the Tumey Formation and rocks of the Relizian Stage in the context of a "Brief Review of Significant Faunas from Important Parts of the Central American Region", they created the impression that California also lacks strata of Oligocene age. Indeed, for America they "conclude that in the whole region we have considered (and even as far north as the state of Washington), there are no published records of stratigraphical sections of fossiliferous marine beds which can be dated as Oligocene". Klempell long ago suggested that his 'Miocene' stages below the Relizian were equivalent to the Oligocene of Europe². Later, Klempell and Weaver³ discussed 'Oligocene' molluscs and benthonic foraminifera from part of California, but stated that their local sequence did not necessarily coincide in age with that of the typical European Oligocene.

Planktonic foraminifera, indicative of the Oligocene *Globigerina oligocenic* Zone (= *G. selli* Zone) of Eames *et al.*, have been found in rocks assigned previously to the Zemorrian Stage of the California 'Miocene'. These strata occur immediately below the type section of the overlying Saucian Stage and above the Vaqueros Formation in Los Sauces Creek, Ventura County, California. The benthonic fauna has been documented

previously, but the planktonic foraminifera have been largely ignored⁴.

The planktonic foraminiferal fauna of this section is listed in Table 1 together with the stratigraphic distribution of the species according to Eames *et al.* Although certain of the species of Eames *et al.* are regarded as synonyms of others, I have utilized their names without reviewing the taxonomic complications for lack of space.

Table 1. STRATIGRAPHIC DISTRIBUTION OF PLANKTONIC FORAMINIFERA, ACCORDING TO EAMES *et al.*¹, OCCURRING IN THE *Globigerina oligocaenica* ZONE

Species also occurring in the Zemorrian Stage at Los Sauces Creek are indicated. Zones, characterized by species of *Globigerina*, and their age assignments are from Eames *et al.*¹

Zemorrian Los Sauces Creek	Species	U. Eocene <i>G. turritilina</i> zone	Oligocene <i>G. oligocaenica</i> zone	L. Miocene <i>G. ampliapertura</i> zone
	<i>Pseudohastigerina micra</i>	X	X	
X	<i>Globigerinita unicava primitiva</i>	X	X	
	<i>Globigerinita pera</i>	X	X	
X	<i>Globorotalia (Turborotalia) increbescens</i>	X	X	
X	<i>Globigerina yeguaensis pseudovenezuelana</i>	X	X	
	<i>Globigerina yeguaensis yeguaensis</i>	X	X	
X	<i>Globigerina senilis</i>	X	X	
X	<i>Globigerina ouachitaensis gnaucki</i>	X	X	
X	<i>Globigerina turritilina turritilina</i>	X	X	
	<i>Globigerinita dissimilis</i>	X	X	X
X	<i>Globorotaloides suteri</i>	X	X	X
X	<i>Globorotalia (Turborotalia) permicra</i>	X	X	X
X	<i>Globorotalia (Turborotalia) opima nana</i>	X	X	X
X	<i>Globigerina ampliapertura ampliapertura</i>	X	X	X
X	<i>Globigerina ampliapertura euapertura</i>	X	X	X
X	<i>Globigerina ouachitaensis ouachitaensis</i>	X	X	X
X	<i>Globigerina officinalis</i>	X	X	X
	<i>Globigerina tripartita tripartita</i>	X	X	X
X	<i>Globigerina praebulloides leroys</i>	X	X	X
X	<i>Globigerina praebulloides occlusa</i>	X	X	X
X	<i>Globigerina praebulloides praebulloides</i>	X	X	X
X	<i>Globigerina angustiumbilicata</i>	X	X	X
X	<i>Globigerinita martini scandretti</i>	X	X	
X	<i>Globorotalia (Turborotalia) postcretacea</i>		X	
	<i>Globigerina tripartita lapuriensis</i>		X	
X	<i>Globigerina oligocaenica</i>		X	
X	<i>Cassigerinella chipolensis</i>		X	X
X	<i>Globigerina ouachitaensis ciperoensis</i>		X	X

The *Globigerina oligocaenica* Zone, according to Eames *et al.*, is characterized by the restricted occurrence of *Globigerinita martini scandretti*, *Globorotalia (Turborotalia) postcretacea*, *Globigerina tripartita lapuriensis*, and *G. oligocaenica*; by the last appearances of *Pseudohastigerina micra*, *Globigerinita unicava primitiva*, *G. pera*, *Globorotalia (Turborotalia) increbescens*, *Globigerina yeguaensis yeguaensis*, *G. yeguaensis pseudovenezuelana*, *G. senilis*, *G. ouachitaensis gnaucki* and *G. turritilina turritilina*; and the first appearances of *Cassigerinella chipolensis* and *Globigerina ouachitaensis ciperoensis*. As can be seen in Table 1, the species listed by Eames *et al.* from this zone which were not found at Los Sauces Creek have stratigraphic ranges broader than the *G. oligocaenica* Zone. Of the missing species, only the absence of *Pseudohastigerina micra* is critical as the zone is most clearly defined by the overlapping ranges of this species and *Cassigerinella chipolensis*¹. However, in view of the abundant occurrence in the Los Sauces Creek section of all the species elsewhere restricted to the *G. oligocaenica* Zone, and the overlapping ranges of *Cassigerinella chipolensis* with *Globigerinita unicava primitiva*, *Globorotalia (Turborotalia) increbescens*, *Globigerina ouachitaensis gnaucki*, *G. senilis*, *G. yeguaensis pseudovenezuelana* and *G. turritilina turritilina*, these strata cannot be assigned to any other than Eames *et al.*'s *G. oligocaenica* Zone.

This fauna clearly establishes the presence of Oligocene marine sediments in California, contrary to previous implications and statements. Together with the now established presence of this zone in the Gulf Coast region⁶, these results suggest that widespread uplift, resulting in non-deposition of sediments throughout these parts of

America as suggested by Eames *et al.*¹, did not occur during the Oligocene.

JERE H. LIPPS

Department of Geology,
University of California,
Los Angeles.

¹ Eames, F. E., Banner, F. T., Blow, W. H., and Clarke, W. J., *Fundamental of Mid-Tertiary Stratigraphical Correlation* (Camb. Univ. Press, 1962).

² Klempell, R. M., *Miocene Stratigraphy of California* (American Assoc. Petrol. Geol., Tulsa, Okla., 1938).

³ Klempell, R. M., and Weaver, D. W., *Univ. California Publ. Geol. Sci.*, 4 (1963).

⁴ Cushman, J. A., and Laiming, B., *J. Paleontol.*, 5, 2 (1931).

⁵ Saito, T., and Bé, A. W. H., *Science*, 145, 3633 (1965).

GEOCHEMISTRY

Enrichment of Iron during Accretion in the Solar Nebula

THE anomaly between the abundances of iron in the solar system derived from solar spectra and of that from meteorites is well known¹. Relative to silicon, the abundance of iron in the chondritic meteorites appears to be several times larger than that observed in the Sun. Normalization to any other common element introduces a variation by a factor of two at most. The data are given in Tables 1 and 2. The solar and chondritic abundances of cobalt and nickel show a much smaller and perhaps not significant variation. Pottasch² determined the abundances of several elements in the solar corona, finding an increased abundance of iron relative to silicon, compared with the abundances derived from the photosphere by Aller *et al.*^{3,4}. These workers who have provided the most recent estimates of solar abundances, conclude, after careful examination of the problem, that "there seems no escape from the conclusion that the solar Fe/silicon or Fe/Fe group ratio is smaller than in the meteorites"⁴. Results by Fowler *et al.*^{5,11} on the relative abundances of the iron-group elements predicted from theories of nucleosynthesis, which correctly reproduce isotope ratios, are in substantial agreement with the solar-derived iron abundance. The chondritic iron/silicon and iron/cobalt/nickel ratios are securely based on extensive chemical analyses and are unlikely to be seriously in error.

It is assumed in the following discussion that the solar abundance of iron is close to that of the original solar nebula and that the meteorites have been enriched in iron. It is not proposed to argue the alternative, which seems less likely from consideration of the overall element abundances and the relative amounts of material involved. It is difficult to devise methods of fractionating iron from nickel and cobalt in geochemical processes. For example, reduction processes⁶ will preferentially concentrate nickel in the metallic phase and thus processes involving addition or removal of such a phase will not account for the observed discrepancy in iron/nickel ratios. These chemical difficulties indicate that probably other processes have been effective in causing the enrichment of iron in chondritic meteorites.

Latimer⁷ and Urey⁸ showed that elements in the original solar nebula would be oxidized at the prevailing low

Table 1. ATOMIC IRON/SILICON RATIOS IN THE SUN AND CHONDRITIC METEORITES

	Fe/Si
Chondrites (high iron group) (ref. 1)	0.80
Solar photosphere (ref. 3)	0.12
Solar corona (ref. 2)	0.44

Table 2. ATOMIC CHONDRITE/SOLAR ABUNDANCE RATIOS FOR SEVERAL ELEMENTS

	Chondrite (high iron group) (ref. 1)	Solar (ref. 3)	Chondrite/solar
Si	10 ⁶	10 ⁶	1.0
Mg	9.6 × 10 ⁵	7.9 × 10 ⁵	1.22
Al	6.0 × 10 ⁴	5.0 × 10 ⁴	1.20
Ca	4.9 × 10 ⁴	4.46 × 10 ⁴	1.10
Ti	3,120	1,514	2.06
Fe	8.0 × 10 ³	1.18 × 10 ³	6.78
Co	1,990	1,380	1.44
Ni	4.8 × 10 ⁴	2.6 × 10 ⁴	1.85

temperatures, and also showed that the common metals could occur as oxides. According to the data of Ringwood⁶, iron would exist as magnetite, and partly in iron silicates, at low temperatures of about 400° K. Magnetite is ferromagnetic and retains this property to low temperatures despite a transition to an orthorhombic form at 133° K.

Recent workers on the early history of the solar system, notably Hoyle *et al.*⁹⁻¹¹, have appealed to a magnetic torque coupling between the early Sun and the disk of dust and gas, to solve the long-standing problem of transfer of angular momentum from the Sun to the planets. In Hoyle's model the transfer of angular momentum causes the material of the disk to move out to the region of the major planets, leaving the region of the terrestrial planets and meteorites free of dust and gas. Condensed bodies larger than a metre or so in diameter are left behind. The material forming the meteorites and terrestrial planets must have condensed prior to the removal of the dust and gas. If accretion takes place during the outward movement of material in a magnetic field as proposed by Hoyle⁹, then ferromagnetic material may preferentially accumulate. If the field is non-uniform, such aggregations may trigger the condensation process for non-magnetic material. It is hoped to investigate several possible models in detail.

Magnetite can thus be selectively enriched. Nickel and cobalt oxides are not ferromagnetic and will condense in proportion to their original abundances, in the solar nebula, with the other non-volatile elements. Probably minor amounts of nickel and cobalt will be incorporated in the magnetite, depending on conditions in the nebula. This could account for the apparently higher chondritic abundances for these elements, if the differences are significant. Magnetite from the Orgueil carbonaceous chondrite is nickel-rich, but the entry of nickel could have occurred during recrystallization. Thus iron can become selectively enriched by this process and afterwards accreted into the parent bodies of the meteorites and, presumably, the terrestrial planets. This process, which is unlikely to be uniform, provides an additional possibility to explain the varying density of the terrestrial planets.

I thank Prof. W. A. Fowler for his advice.

S. R. TAYLOR

Department of Geophysics and Geochemistry,
Australian National University,
Canberra.

Compared with palladium, the generally much lower solubility of hydrogen in platinum cathodes is reflected by much more rapid changes of E following electrolysis. This, taken together with inexact knowledge of the bulk solubility of hydrogen in platinum as a function of electrode potential near 25° C, and of the diffusion constant governing hydrogen in platinum, makes it more difficult to assess directly the magnitude of overpotential components reflecting the hydrogen content of either the surface or of the bulk of platinum cathodes.

In Fig. 1, values of η , as functions of current density i (m.amp cm⁻²), at platinized platinum cathodes (in the form of 2-cm lengths of wire) are compared with both η and the component η_a recorded at geometrically similar palladized palladium cathodes under similar experimental conditions, in 1.0 N hydrochloric acid, over a low range of current density where η_a can be the major component of η . The catholyte was saturated and stirred by a stream of bubbles of hydrogen. Both η and E were measured with respect to a platinum/hydrogen electrode immersed beside the specimen. Palladium specimens were palladized from a solution of 1 per cent palladium chloride in 0.1 N hydrochloric acid; platinum was platinized from chloroplatinic acid containing 1 per cent platinum chloride and 0.2 per cent lead acetate at current densities from 15 to 150 m.amp cm⁻² for 1 min.

Fig. 1 illustrates that for wires of equivalent diameters there is good agreement between plots of $\log i$ against both η and η_a for palladized palladium and η for platinized platinum. Also illustrated in Fig. 1 are experimental values of η_a compared with plots of $\eta_a = 0.0295 \log (i + i_0)/i_0$, in which i_0 equals $2PFk_0/N$, where P is the pressure under which hydrogen is dissolved, F and N are Faraday's constant and Avogadro's number respectively, and the rate constant k_0 has been calculated from measurements of the open circuit potential E of the palladized palladium electrodes in the hydrogen-saturated catholyte prior to cathodization^{2,3}. The plots in Fig. 1 indicate that, again for wires of the same diameter, experimental values of η for platinized platinum also would show good agreement with the appropriate plot of $\log (i + i_0)/i_0$. This corollary seems sound evidence that, over this relatively low range of current densities, the total overvoltage η at platinized platinum can be comprised primarily—as a limiting case—of the component η_a , which is in turn governed by the transport of hydrogen molecules away from the cathode through the Brunner-

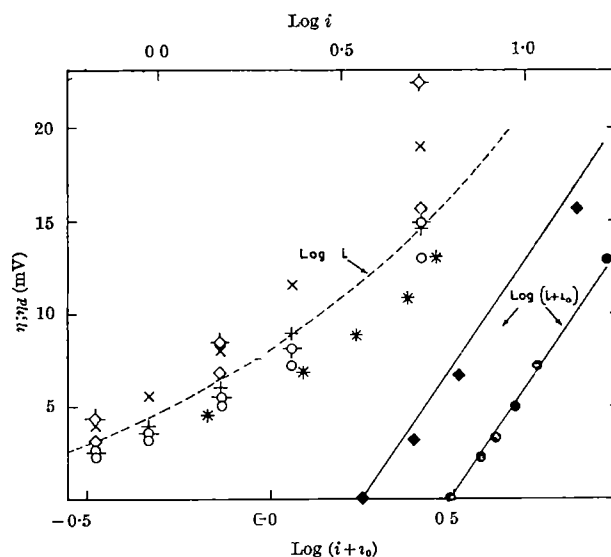


Fig. 1. Plots of η against $\log i$ for platinized platinum cathodes. (*, 0.10 mm; +, 0.25 mm; ×, 0.457 mm diam. wires). Also, η (barred symbols) and η_a plotted against $\log i$; and η_a (filled symbols) against $\log (i + i_0)$ for palladized palladium cathodes (○, 0.122 mm, and ◇, 0.274 mm diam.).

CHEMISTRY

Diffusion or Hydride Component of Hydrogen Overpotential at Platinized Platinum and Palladized Palladium Cathodes

At suitably activated palladium cathodes, the diffusion or hydride component of hydrogen overpotential η_2 (or η_d) can be separated conveniently from the total apparent overpotential, η , by extrapolation of plots against time of changes in the open circuit potential, E , measured after cessation of electrolysis¹⁻³.

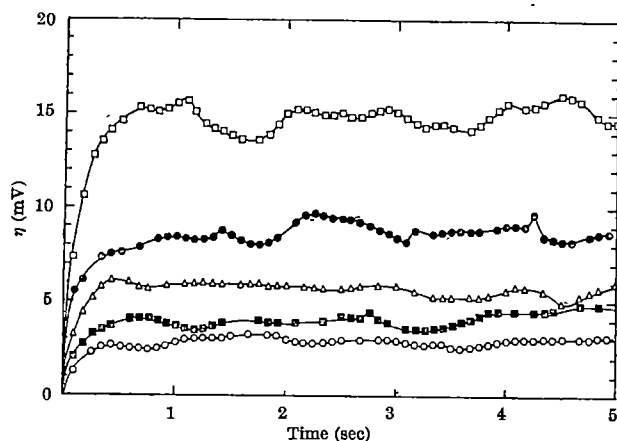


Fig. 2. Examples of fluctuations of η with time found with platinized platinum cathodes as recorded during electrolysis with a specimen of 0.25 mm diam. Current density (mA cm⁻²): □, 5.20; ●, 2.27; △, 1.47; ■, 0.93; ○, 0.67.

Nernst boundary layer^{2,3}; this conclusion lends support to a similar interpretation by Kandler, Knorr and Schwitzer⁴ of their results obtained at 'bright' platinum cathodes in 2.0 N sulphuric acid.

The lower value of i_0 obtained with the larger diameter palladium wire, and the consistently higher values of η at a given current density for the platinum wires of larger diameter, are both typical of the findings in repeat experiments. These results seem consistent with the increase in the effective thickness, δ , of the Brunner-Nernst layer with increased wire diameter, reported by Fallon and Castellan⁵.

Electrode-potential measurements were recorded using a 'register printer' (Solartron model ED 1176) printing 12 readings per sec, fed from a digital voltmeter (Solartron model LM 1010); and over these short time-intervals substantial fluctuations of the total overvoltage, η , at platinized platinum cathodes were revealed—as shown, for example, in Fig. 2 (results plotted in Fig. 1 represent mean readings). The rapidity of these fluctuations was very much reduced by interrupting the stirring of the solution, but slow oscillations of η with time still remained. In analogous experiments with palladized palladium these fluctuations of η were not observed (nor have they been observed in preliminary measurements with 'bright' platinum cathodes): it seems possible that they reflect local changes of the hydrogen content of the surface, which, paralleling the low solubility of hydrogen in platinum, may be expected to be relatively sensitive to variations in the rate of desorption of hydrogen molecules away from the surface by a transport mechanism—which, in turn, is governed by the stirring conditions prevalent at all points on the cathode surface.

M. N. HULL
F. A. LEWIS

Department of Chemistry,
The Queen's University of Belfast.

¹ Clamroth, R., and Knorr, C. A., *Z. Elektrochem.*, **57**, 399 (1953).

² Barton, J. C., and Lewis, F. A., *Z. Physik. Chem. Neue Folge*, **33**, 99 (1962).

³ Green, J. A. S., and Lewis, F. A., *Trans. Faraday Soc.*, **60**, 2234 (1964).

⁴ Kandler, L., Knorr, C. A., and Schwitzer, C., *Z. Physik. Chem. Lpz.*, **A**, **180**, 281 (1937).

⁵ Fallon, R. J., and Castellan, G. W., *J. Phys. Chem.*, **64**, 4 (1960).

Electron Spin Resonance Spectrum of Cu⁺⁺ in Diamine Oxidase

THE electron spin resonance spectrum of hog kidney diamine oxidase (DO) has been measured. DO is a cupric enzyme and is similar in properties to histaminase and monoamine oxidase (MO). The electron spin resonance spectrum of the cupric ion in MO was reported by Yamada *et al.*¹. They have shown that copper is the only ion of those tested (Cu⁺⁺, Fe⁺⁺, Fe⁺⁺⁺, Co⁺⁺, Ni⁺⁺, Zn⁺⁺) which

was able to reactivate their MO. The work reported here i additional evidence that copper is associated in some manner with the activity of amine oxidases. This work indicates that Cu⁺⁺ forms either a chelate binding one of the DO prosthetic groups (pyridoxal phosphate or flavin adenine dinucleotide) to the apoenzyme, or else a chelate which holds the DO in a configuration in which it is an active enzyme. Changing or breaking this chelate inactivates the enzyme.

Powdered and liquid (100 mg DO/10 ml. 0.1 M phosphate buffer at pH 7.10) samples of DO were examined using a Varian V-4500 X-band spectrometer with 100 kc/sec magnetic field modulation. Fig. 1 shows spectra for powdered and liquid samples of active DO at room temperature. The lines shown are characteristic of Cu⁺⁺ in tetragonal symmetry. Table 1 gives the experimental values for the g -factors and the hyperfine constants.

The powdered sample showed two other resonances, a very broad (~600 gauss) slightly asymmetric line at $g = 2.05 (\pm 0.05)$, and a symmetric line at $g = 2.02 (\pm 0.02)$ which was 28 gauss wide. These lines did not appear in the liquid sample. My DO was rather impure and, when dissolved in the buffer, a large amount of residue collected in the bottom of the sample. This residue is probably protein impurity and was filtered out of the liquid sample. The fact that these lines did not appear in the filtered liquid sample indicates that they are probably the result of some magnetic ion or ions (probably Fe⁺⁺⁺) associated with the protein impurities. The liquid spectrum resembles very nearly the electron spin resonance spectrum for Cu⁺⁺-histidine complexes reported by Malmström and Vänngård².

When the powdered and liquid samples of DO were heated for 1 min in a temperature bath at 70° C, the cupric spectrum disappeared. At lower temperatures the spectrum faded more slowly as shown in Fig. 2. This is the temperature range in which DO loses its activity. This disappearance could result from two factors: (a) the Cu⁺⁺ could be reduced to Cu⁺; (b) the crystal structure surround-

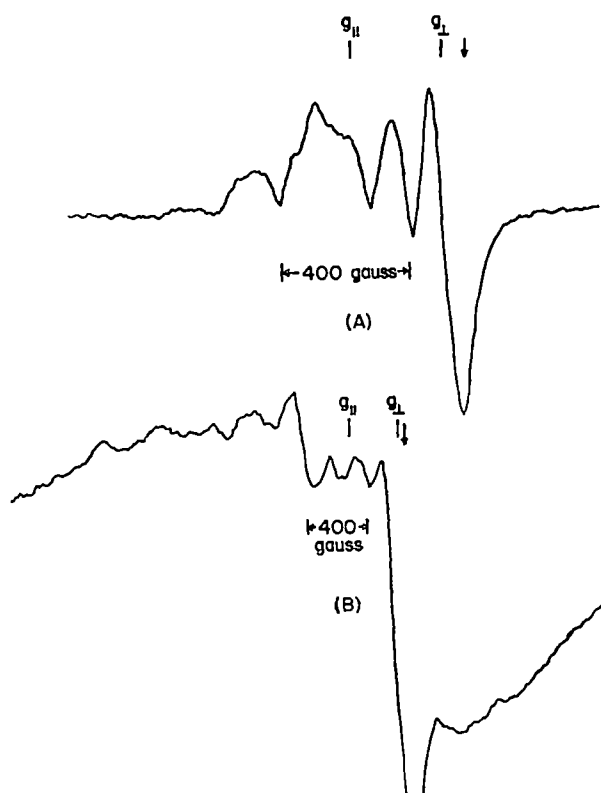


Fig. 1. Electron spin resonance spectrum of room temperature samples of diamine oxidase: (A) liquid sample; (B) powder sample. The arrow indicates the resonance position of free electrons.

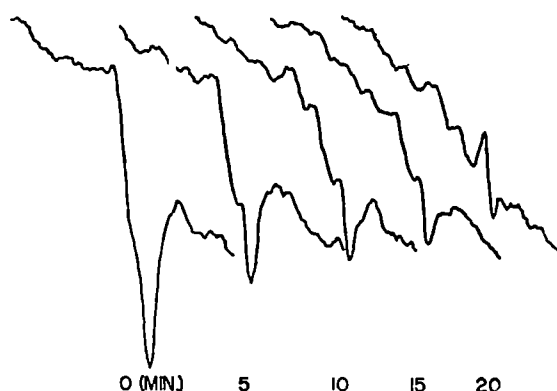


Fig. 2. Electron spin resonance spectrum of powder samples of diamine oxidase heated for various times in a temperature bath at 55°C

ing the Cu^{++} could change in such a fashion as to cause the spectrum to disappear. The latter choice is preferred for reasons given here. Cu^{++} normally co-ordinates strongly with four ligands in a square-planar structure. In such a structure the Cu^{++} valence electrons could form covalent bonds in such a way as to have no unpaired electrons (diamagnetic). If, however, the bonds are more ionic, the Cu^{++} can also co-ordinate less strongly, with two ions perpendicular to the plane, thus forming a distorted octahedral structure with tetragonal symmetry. The Cu^{++} in this case is paramagnetic. Lifschitz *et al.*³ have shown that differences in stability of the two forms are in some cases very slight, since a given complex of chelate may be converted between the diamagnetic form (covalent dsp^2 electron states) and the paramagnetic form by only a slight change in conditions⁴.

Table 1. THE g -FACTORS AND HYPERFINE CONSTANTS FOR Cu^{++} IN DIAMINE OXIDASE AND OTHER Cu^{++} -PROTEIN COMPLEXES
All data except for DO are from Malmström and Vånnård (ref. 2)

Protein	g_{\perp}	g_{\parallel}	$A_{\perp}(\text{cm}^{-1})$	$A_{\parallel}(\text{cm}^{-1})$
Diamine oxidase	2.05	2.25	≤ 0.004	0.021
Histidine	2.083	2.230		0.018
Laccase	2.048	2.197		0.009
Ceruloplasmin	2.056	2.209		0.008

The experimental values of g_{\parallel} , g_{\perp} , and A_{\parallel} given in Table 1 can be used to calculate several parameters which indicate the degree of covalent bonding of Cu^{++} to its neighbours. An investigation by Maki and McGarvey⁵ has shown that a parameter a^2 (related to g_{\parallel}) is reduced in value from a maximum of one as the covalency in the σ - and π -bonds in the plane of the ligands becomes stronger. Another parameter $4/7 a^2 + x$ is reduced by the covalency of in-plane σ -bonding. The results for DO ($a^2 = 0.60$ and $4/7 a^2 + x = 0.83$) indicate that there is some covalent bonding of Cu^{++} to its ligands, especially π -bonding in the plane. Nitrogen is known to form stronger covalent bonds than oxygen, and the value of a^2 for DO conforms to those reported by Malmström and Vånnård² when Cu^{++} is in a plane of four N. This result is certainly not conclusive.

Malmström and Vånnård have found that the hyperfine constant A_{\parallel} in laccase and ceruloplasmin is much smaller than in other protein-copper complexes. In these two enzymes the copper changes valence when the enzyme performs its catalytic action. If the unpaired electron of Cu^{++} in these enzymes were highly delocalized, this would facilitate the valence change and could also reduce the value of A_{\parallel} . However, in DO, A_{\parallel} is very large, indicating that the unpaired electron is not free for valence changes.

In summary, it appears that Cu^{++} is necessary as a chelating agent in active DO. The Cu^{++} in the active enzyme is co-ordinated strongly with four ligands (probably N) in a plane, and loosely with two ligands perpendicular to the plane. When the enzyme is de-activated by heating, the Cu^{++} forms diamagnetic covalent bonds in the plane, and is no longer co-ordinated perpendicular to the plane. The unpaired Cu^{++} electron of the active enzyme is not delocalized

as it is in other enzymes that change valence during their catalytic action (laccase and ceruloplasmin).

ALAN VAN HEUVELEN

Department of Physics,
New Mexico State University,
University Park,
New Mexico.

¹ Yamada, H., Yasunobu, K., Yamano, T., and Mason, H. S., *Nature*, **198**, 1092 (1963).

² Malmström, B. B., and Vånnård, T., *J. Mol. Biol.*, **2**, 118 (1960).

³ Lifschitz, B., and Dijkema, Z., *Anorg. Chem.*, **242**, 97 (1939).

⁴ See a discussion of this in Martell, A. E., and Calvin, M., *Chemistry of the Metal Chelate Compounds* (Prentice-Hall, Inc., New York, 1952).

⁵ Maki, A. H., and McGarvey, D. R., *J. Chem. Phys.*, **29**, 31 (1958).

Use of Dimethyl Sulphoxide in Liquid Scintillation Counting

DIMETHYL sulphoxide is a polar solvent which is miscible with many organic solvents including toluene. It is an excellent solvent for polysaccharides, proteins and macromolecules generally. Though we expected that it would be a quencher we thought that its solvent properties might make it useful for holding water-soluble substances in solution in liquid scintillators.

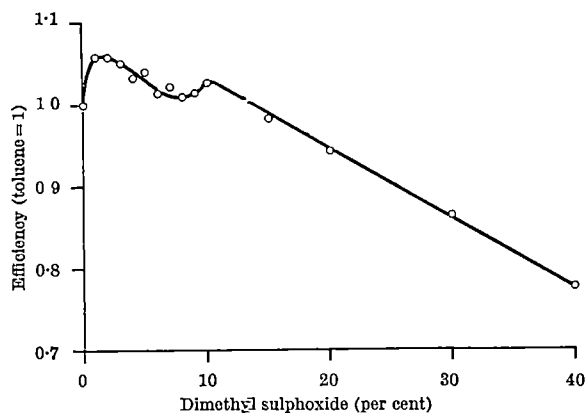


Fig. 1. Effect of dimethyl sulphoxide on counting efficiency of toluene phosphor

Dimethyl sulphoxide, far from being a serious quencher, is itself a scintillation solvent, albeit an inefficient one. To obtain the results shown in Fig. 1, phosphors were made by dissolving 40 mg polyphenol oxide and 5 mg 1,4-bis-2-(5-phenyloxazolyl)-benzene in 10 ml. of a varying mixture of toluene and dimethyl sulphoxide. The activity added was in the form of carbon-14-labelled hexadecane and was counted in the Packard 'Tricarb' model 3303. Though there is no obvious explanation of the shape of the early part of the curve it is clear that up to 13 per cent of dimethyl sulphoxide enhanced rather than quenched the scintillations. The results are sufficiently encouraging for us to continue investigations on the use of dimethyl sulphoxide as a solvent for counting tissues.

EVAN WRIGHT
K. R. MILLAR

Wallaceville Animal Research Centre,
Private Bag, Welling-on, New Zealand.

Rate of Micelle Formation of Sodium Alkyl Sulphates in Water

THE description of the properties of aqueous detergent solutions in terms of a chemical equilibrium between monomers and micelles has proved very fruitful. Thermodynamic as well as transport behaviour has been analysed from this point of view. The detergents most extensively investigated in the past are the sodium alkyl sulphates, and for these a satisfactory explanation may be given of critical micelle concentrations (c.m.c.),

micellar weights, and the influence of temperature, electrolyte concentration and the like, on these quantities.

Less attention has been paid to the kinetics of micelle formation, that is to the question of how long it takes to reach chemical equilibrium in a solution at a given temperature, pressure and total detergent concentration. This question becomes important when the conditions vary with place and/or time, such as during diffusion in a concentration gradient. Another example is the adsorption of a detergent, primarily of monomer molecules, on a freshly-formed oil-water interface as, for example, in suddenly-formed emulsions: micelles have to disintegrate to maintain the equilibrium monomer concentration. Apart from this, micelle formation in itself is an interesting multi-step consecutive chemical reaction.

Some recent papers have been devoted to this subject. From flowing-junction experiments Jaycock and Ottewill¹ concluded that the time of micelle disintegration of sodium dodecyl sulphate and of alkylpyridinium salts was certainly shorter than 10 millisecc. Nagakawa and Inoue² performed nuclear magnetic resonance measurements on a number of ω -phenylalkyltrimethylammonium bromides; the average time a monomer forms part of a micelle was found to be shorter than 10^{-4} sec.

We have made a direct measurement of the relaxation time of micelle formation for sodium dodecyl sulphate (SDS) and sodium tetradecyl sulphate (STDS) in water.

Chemical relaxation was studied with the pressure-jump method as developed by Strehlow and Becker³. This method can be used if a chemical reaction is accompanied by a change in electrical conductivity. This condition is satisfied in the case of SDS and STDS, the equivalent conductivity of the micelles being about one third of that of the monomers; the same property was selected by Jaycock and Ottewill¹ to observe micelle disintegration. An additional (though not necessary) circumstance that favours application of the pressure-jump method is the appreciable influence of pressure on the micellar equilibrium: the partial specific volume of micellar SDS exceeds that of monomeric SDS by about 5 per cent.

The method is based on the principle that the primary effect of a sudden pressure increase, in a solution originally in chemical equilibrium, is an equal relative increase in concentration of all species; the ratio of the concentrations therefore remains the same. Subsequently, in a way determined by the kinetics of the particular chemical reaction, the concentrations will change to their new equilibrium values which correspond to the new pressure and the new total solute concentration (which is higher, owing to the compression). These processes are followed conductometrically. For small deviations from equilibrium the chemical reaction may be described in terms of chemical relaxation times τ_c , of which there are as many as there are independent reaction steps.

Experimentally an instantaneous pressure-jump cannot be brought about. In general an exponential pressure decrease is applied; the relaxation time, τ_p , of this decrease has a lower limit of about 5×10^{-5} sec. In the original apparatus, which was a copy of Strehlow's instrument except for a few details to be described elsewhere, only τ_c -values down to this value could be measured.

Measurements on SDS solutions with this apparatus did not reveal any chemical relaxation; for SDS, therefore, τ_c is certainly smaller than 5×10^{-5} sec.

By introducing a simple but essential change in the way of following the conductivity change, we were able to lower the limit of measurable τ_c -values to about 10^{-6} sec. To explain this, the principle of the measuring device, a Wheatstone bridge, is shown in Fig. 1. Cell *m* contains the solution (in this case SDS in water) to be investigated, cell *b* a solution of a reference electrolyte (KCl) the conductivity of which changes exactly in phase with the pressure.

By means of a simple calibration one finds the relation between V , the measured voltage difference between the

points 1 and 2, and $\Delta R(t)$, the unbalance of the bridge. In the simple case when there is only one chemical relaxation time $\Delta R(t)$ obeys the following equation:

$$\begin{aligned} \Delta R(t) &\equiv R_{m,0} + R_m(t) - R_{b,0} - R_b(t) \\ &= -R_m(t=0) \left[K_1(1 - e^{-t/\tau_p}) - \right. \\ &\quad \left. \left(\Delta \frac{\Delta \kappa}{\kappa} \right) \left(1 - \frac{\tau_c}{\tau_c - \tau_p} e^{-t/\tau_c} + \frac{\tau_p}{\tau_c - \tau_p} e^{-t/\tau_p} \right) \right] \\ &\quad + R_b(t=0) K_2(1 - e^{-t/\tau_p}) \end{aligned} \quad (1)$$

provided the bridge is in balance before the pressure release. The constants K_1 and K_2 are related to equilibrium properties of the systems in cells *m* and *b*, respectively. The quantity $\Delta \frac{\Delta \kappa}{\kappa}$ represents the difference between the relative conductivity changes (for a pressure change equal to the total pressure-jump) of the particular SDS solution above the c.m.c. and that below the c.m.c.; the values of $\Delta \frac{\Delta \kappa}{\kappa}$ can be found from separate experiments.

One sees that in this original arrangement the more τ_c decreases, the more ΔR approaches a simple exponential function of t governed by τ_p .

We then decided to have the bridge in balance not only before releasing the pressure but also at the end of the relaxation processes. Equation (1) shows that the only way to do this is by changing $R_b(t=0)$, that is by varying the KCl-concentration in cell *b* until this requirement is met; K_2 depends on the type of electrolyte in cell *b* but not on its concentration. If conditions for balance before and after the release are satisfied, the expression for $\Delta R(t)$ becomes:

$$\Delta R(t) = R_m(t=0) \left(\Delta \frac{\Delta \kappa}{\kappa} \right) \frac{\tau_c}{\tau_c - \tau_p} [e^{-t/\tau_p} - e^{-t/\tau_c}] \quad (2)$$

and the bridge signal will, temporarily, differ from zero only if there is a chemical relaxation. τ_c is then easily found from the extreme value, $\Delta R_{\text{extr.}}$, of $\Delta R(t)$. From equation (2) one derives:

$$\frac{\Delta R_{\text{extr.}}}{R_m(t=0)} = \left(\Delta \frac{\Delta \kappa}{\kappa} \right) \frac{\tau_c}{\tau_p} \quad (3)$$

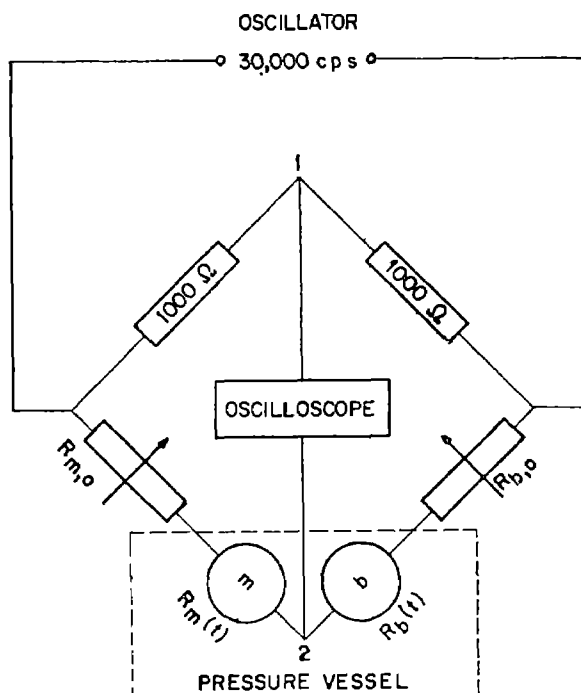


Fig. 1 Diagram of experimental arrangement

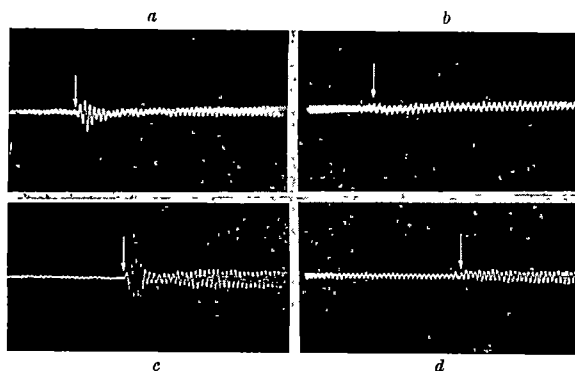


Fig. 2 Bridge signals for SDS and STDS. Time proceeds from left to right. Pressure release starts at the arrows. Frequency used 30,000 c.p.s. The c.m.c. of SDS is at 0.23 per cent w , that of STDS at 0.06 per cent w . a, SDS, $T = 15^\circ \text{C}$, conc. 0.3 per cent w , b, SDS, $T = 15^\circ \text{C}$, conc. 0.18 per cent w ; c, STDS, $T = 35^\circ \text{C}$, conc. 0.095 per cent w , d, STDS, $T = 35^\circ \text{C}$, conc. 0.048 per cent w .

SDS solutions, investigated in this arrangement, did show chemical relaxation, but only at concentrations exceeding the c.m.c. This indicates that micelle formation is responsible for the relaxation signals, for only above the c.m.c. does $\Delta \frac{\Delta x}{x}$ differ from zero.

Representative oscillograms are shown in Fig. 2. For SDS at $T = 25^\circ \text{C}$ and a concentration of 0.5 per cent w , and for a 50 atmospheres pressure drop, we found:

$$\Delta \frac{\Delta x}{x} = 0.0060; \frac{\Delta R'_{\text{extr.}}}{R_m(t=0)} = 0.0008 \pm 0.0003$$

With $\tau_p = 6.5 \times 10^{-5}$ sec this leads to:

$$\tau_c = (1.3 \pm 0.5) \times 10^{-5} \text{ sec}$$

The τ_c values scarcely depend on the SDS concentration and they decrease only slightly with increasing temperature; we made experiments at 15° , 25° , 35° and 45°C . The average value for all experiments is:

$$\tau_c = (1.2 \pm 0.5) \times 10^{-5} \text{ sec (SDS)}$$

Analogous results were obtained for STDS, which was only investigated at $T = 35^\circ \text{C}$. We found:

$$\tau_c = (2.0 \pm 0.5) \times 10^{-5} \text{ sec (STDS)}$$

A complete account of this work, as well as a discussion of the kinetics of micelle formation, is being prepared. Here we will mention one problem: the signals of the type shown in Fig. 2 were analysed as if they were caused by one relaxing mechanism only. Admittedly, a more detailed analysis, even in terms of as few as two relaxing mechanisms, would have been impossible. Still, our procedure is correct only if from a whole set of relaxation times, numbering $n-1$ in the case of micelles containing n monomers, a single one predominates completely. Calculations on the kinetics which will justify or invalidate our procedure are now being carried out.

P. F. MIJNLIEFF
R. DITMARSCH

Koninklijke/Shell-Laboratorium,
Badhuisweg 3, Amsterdam-N.

Jaycock, M. J., and Ottewill, R. H., IVth Intern. Cong. Surface Active Substances (Brussels, September, 1964), Section B, Paper 8.

Nagakawa, T., and Inoue, H., IVth Intern. Congr. Surface Active Substances (Brussels, September 1964), Paper 11.

Strehlow, H., and Becker, M., *Z. Elektrochemie*, **63**, 457 (1959)

Polarization and Hybridization

Two types of polarization that can occur by bond formation are: (i) the result of concentration of charge due to the overlap of atomic orbitals; and (ii) the actual polarization of the basis orbitals. Mulliken¹ has described this second type of polarization and its effect as a function

of the overlap integrals for certain diatomic molecules. In all cases it was observed that the addition of a given amount of a $2p$ Slater type orbital (STO) into a hybrid orbital produced the largest change in the overlap if the hybrid orbital was a $2s$ STO. This observation led to the well-known phrase "A little hybridization goes a long way". The lowering of the energy of the system due to the increase in the overlap integral must be larger than the increase in energy incurred by the inclusion of the higher energy $2p$ STO if the polarization is to be useful. We are inclined to reserve the word 'polarization' for this type of hybridization for the following reasons: (a) the process described displaces the centroid of the electronic charge distribution from the nucleus; (b) this concept can be readily extended to polyatomic molecules and is not dependent on the 'localized bond' approximation (this will prove particularly useful for molecules containing lone-pair electrons); (c) it distinguishes this type of hybridization from the hybrid orbitals obtained from molecular orbitals by a unitary transformation.

It will be useful to have a measure of polarization in the preceding discussion. As an example, let us set up the four molecular orbitals for H_2O (ignoring inner shell orbitals) using a minimal basis set of orbitals consisting of $2s$ and $2p$ STO's on oxygen and $1s$ orbitals (h) on each hydrogen. This is the most general set that is useful to the chemist:

$$\varphi_1 = \lambda_1[(2s) + \alpha(2p_x)] + \mu_1(h_1 + h_2)$$

$$\varphi_2 = \lambda_2[\beta(2s) + (2p_x)] + \mu_2(h_1 + h_2)$$

$$\varphi_3 = \lambda_3(2p_y) + \mu_3(h_1 - h_2) \quad \varphi_4 = (2p_z)$$

where the oxygen nucleus is the origin of the co-ordinate system and the x axis bisects the $\text{H}-\text{O}-\text{H}$ angle. The polarization of the valence shell orbitals on oxygen will be defined as the coefficient of the product $(2s)(2p_x)$ obtained by squaring each orbital φ_i and adding. This is basically a function of α and β . This type of polarization is not possible for atoms in molecules with highly symmetrical environments such as carbon in methane. In methane the hydrogen orbitals can be polarized in this way.

Mulliken's results are all included in this type of polarization as long as there are no lone-pairs in the valence shell. Thus we should distinguish between bond polarization, specifically considered by Mulliken, and shell polarization, as already described. Bond polarization is well defined for a few diatomic molecules because each bond involves just two nuclei, but in general it is not particularly useful since other hybridizations may be involved, the hybrids may not necessarily lie along the internuclear axis of a bond, and more than two nuclei may be included.

It has been customary to assign similar electron distributions² for the series of molecules CH_4 , NH_3 , H_2O and HF . As Ellison and Shull³ showed, hybridization does not necessarily determine the molecular geometry for H_2O . More recently, Bader and Jones⁴⁻⁶ have seriously questioned even a suggestion of similarity by observing that the lone-pair electrons in their calculations possess the maximum amount of s character. It seems qualitatively useful to compare the polarization of the valence shell for the various calculations on these molecules.

The calculations of Pople and Duncan⁷ on NH_3 , H_2O and HF are frequently quoted to support the concept of directed lone-pairs. If the electron distribution of NH_4^+ is similar to NH_3 , the valence shell of nitrogen must be polarized to substitute for the removal, and the associated polarizing effect, of a proton. Since it seems natural to define positive polarization along the symmetry axis towards the protons, this polarization is negative. In Table 1, the polarizations extracted from the calculations of Pople and Duncan⁷ are given. Bond polarizations are also given, but since these contain other hybridizations only the sign is pertinent.

Molecule	Table 1 Shell polarization	Bond polarization
NH ₃	-0.93	0.037
H ₂ O	-0.86	0.071
HF ($\beta = 95^\circ$)	-0.56	0.286
HF ($\beta = 116^\circ$)	-0.52	0.105

Since, as Mulliken¹ showed, positive bond polarization is conducive to larger overlap, this might compensate for the comparatively large negative polarization. Although the expectancy of negative shell polarization is reasonable for molecules with lone-pair electrons, the same expectancy for at least positive bond polarization is not realized in recent calculations. For example, bond polarization for HF is negative in recent work (for example, -0.27 (ref. 8), -0.46 (ref. 6)) as it is for the shell polarization (for example, -0.46 (ref. 8), -0.25 (ref. 6)). The principle of maximum overlap⁹ is obviously not a good criterion for these molecules.

There is some justification to write the equivalent orbitals for methane to a good approximation in the form:

$$\psi_i = \lambda[\cos \varepsilon_b (2s) + \sin \varepsilon_b (2p_i)] + \mu\psi_i$$

where $2p_i$ is an STO defined with its axis along a line connecting carbon and the i^{th} proton. The bond polarization is certainly positive in this case. This type of polarization is reflected by an expansion or contraction of the valence shell on bond formation. This possible difference in sign of the bond polarization between methane and hydrogen fluoride suggests that overlap is not of the same importance in these two molecules. In Table 2, a comparison of selected calculations on water is given. Strict comparison is not possible owing to the different requirements that the different electron densities reproduce.

Reference	Table 2 Shell polarization	S/S_0	ψ l.p.
7	-0.87	2.34	0.791 (2s) - 0.612 (2p _z)
3	-0.54	1.87	0.962 (2s) - 0.272 (2p _z)
5	-0.28	1.6	(2s)
11	0	1	0.492 (2s) + 0.871 (2p _z)

ψ l.p. is one of the lone-pair orbitals, the other being the $2p_z$ STO, and S_0 is the overlap integral S for the zero polarization case. All but the first calculations indicate negative bond polarization.

A number of points for discussion derive from Table 2. As shell polarization becomes less negative, so the overlap decreases. If we maintain that large polarizations are energetically unfavourable, it seems that the lone-pair electrons must be involved in the bonding. Bader¹⁰ has already reached this conclusion, but prefers to use the term 'binding' rather than 'bonding'. However, whether binding, for lone-pair electrons, or bonding, for bonding electrons, both are functions of the energy. There does not seem to be any convenient way to determine a value for the shielding so that a comparison with the overlap can be made. Bader has plotted the difference between a proposed molecular density distribution and one obtained from the original atomic densities centred on the same nuclear configuration, to show, in the case of water, that for small polarizations (-0.28) there is an excess charge build-up in the binding region for the molecule. Significantly, there is charge loss in this region for large overlap (-0.87). The binding region is within the H-O-H triangle, the region where the lone-pair electrons are concentrated for small polarizations (see Table 2). Obviously, the more positive the polarization, the more lone-pair electron density will be put into the binding region. Unless we know the relative importance of each term and the effect on the energy, further qualitative discussion is purely speculative. However, it appears that a small amount of negative polarization for the valence shell is reasonable. The actual amount will depend on the geometry of the molecule and the number of lone-pair electrons (NH₃ -0.34 (ref. 4); H₂O -0.28 (ref. 5); HF -0.25 (ref. 6)).

Some of the more pertinent conclusions are: (a) the long-range electrostatic fields involved in molecules with

lone-pair electrons may invalidate the localized bond approach dependent on the principle of maximum overlap; (b) the shielding of the lone-pair electrons in the binding regions of the molecule can more than compensate for loss in the overlap of atomic orbitals involved in bonding; (c) in terms of orbitals so important to chemists' theories of electronic structure the principle of 'A little polarization goes a long way' seems to be important for the valence shell of atoms containing lone-pair electrons. I thank Dr. K. E. Banyard for his advice.

T. A. CLAXTON

Department of Chemistry,
University of Leicester.

¹ Mulliken, R. S., *J. Chem. Phys.*, **19**, 900 (1951)

² Nyholm, R. S., and Gillespie, R. J., *Quart. Rev.*, **11**, 339 (1957).

³ Ellison, F. O., and Shull, H., *J. Chem. Phys.*, **23**, 2348 (1955).

⁴ Bader, R. F. W., and Jones, G. A., *J. Chem. Phys.*, **38**, 2791 (1963).

⁵ Bader, R. F. W., and Jones, G. A., *Canad. J. Chem.*, **41**, 586 (1963).

⁶ Bader, R. F. W., and Jones, G. A., *Canad. J. Chem.*, **41**, 2251 (1963).

⁷ Pople, J. A., and Duncan, A. B. F., *Trans. Faraday Soc.*, **49**, 217 (1953).

⁸ Peters, D., *J. Chem. Soc.*, 2003 (1963).

⁹ Maccoll, A., *Trans. Faraday Soc.*, **46**, 369 (1950).

¹⁰ Bader, R. F. W., *J. Amer. Chem. Soc.*, **86**, 5070 (1964).

¹¹ Claxton, T. A., *Leicester Chem. Rev.*, **6**, 31 (1964).

Breakdown of Simple Hydrocarbons subjected to a Silent Electric Discharge

THE gassing tendencies of insulating oils used in the electrical industry are assessed by measuring the rate of gas evolution or absorption when they are exposed under an atmosphere of hydrogen in an electrodeless discharge tube of the Siemens Evers-type. Previous investigations¹⁻⁴ have been confined to the investigation of a wide variety of commercial oils and the effect of additions of aromatic rich oil fractions and other additives chosen for their ability to impart a desirable hydrogen gas absorbing characteristic to the mixture. The different hydrocarbon types present in the oils, paraffins, naphthenes, olefines and aromatics cause the discharge reaction to be a complex competitive process in which the gas evolved by the paraffins and naphthenes is partially or completely absorbed by the olefinic and aromatic fractions. To avoid this complexity and enable a clearer understanding of the reaction processes to be reached, certain pure saturated and unsaturated hydrocarbons representative of the types found in oils have been selected for investigation. The experiments have shown that the discharge reaction is closely related to the corresponding radiolytic breakdown of simple hydrocarbons⁵.

A new design of gassing cell (Fig. 1) was used to measure the gassing rates. No radical change has been made in the basic design, since the concentric electrode system, a feature common to most other cells, has been retained. The design is such that hydrocarbons can be saturated with hydrogen after being transferred to the cell. Similarly, the more rigorous pre-treatment involving degassing at a pressure of a few microns prior to saturation with hydrogen gas, necessary for the more viscous commercial oils, can be carried out on the sample in the cell. In this way all risk of contamination with air or grease from taps and joints is avoided. The rate of gas evolution or absorption is followed by observing the rise or fall of the liquid under test in the central manometer tube.

After saturating the hydrocarbon with hydrogen by bubbling the gas through the liquid for 5 min at the test temperature, maintained by a metal heating block, a stress, calculated to be 43 kV/cm in the un-ionized gas phase, was applied at a voltage of 12 kV root mean square. Two distinct types of behaviour were observed. The saturated hydrocarbons, both straight-chain and ring structure, evolved gas at a constant rate, while the unsaturated hydrocarbons, including benzene, absorbed gas at a constant rate, after the first few minutes of

Table 1. GAS EVOLUTION AND ABSORPTION RATES FOR SIMPLE HYDROCARBONS

Saturated hydrocarbons	Rate of gas evolution ml./min	Unsaturated hydrocarbons	Rate of gas absorption ml./min
Cyclohexane	0.020 ± 0.001	1 decene	0.047 ± 0.001
Decane	0.013	1 octadecene	0.016
Hexadecane	0.006	1.19 eicosadiene	0.010
		Benzene	0.012

The rates were measured at 40° C using an applied voltage of 12 kV which gave a calculated stress of 43 kV/cm in the un-ionized gas phase.

reaction. Values of the rates obtained at 40° C are shown in Table 1. Vapour-phase chromatographic analysis of the gaseous products from *n*-decane showed that the major product was molecular hydrogen (96.4 per cent) while the remainder consisted of methane, ethane, propane and butanes. The main reaction must therefore be the production of molecular hydrogen by C—H scission while some C—C scission occurs yielding the small quantities of lower-molecular-weight hydrocarbons. This suggests that the reaction is similar to that observed in the radiolytic breakdown of liquid hydrocarbons⁶ where the molecular hydrogen yield has been accounted for by regarding the dominant reaction as the formation *in situ* of hydrogen atoms which then take part in competitive abstraction and addition reactions. Gas evolution rates for the discharge reaction of the saturated hydrocarbon

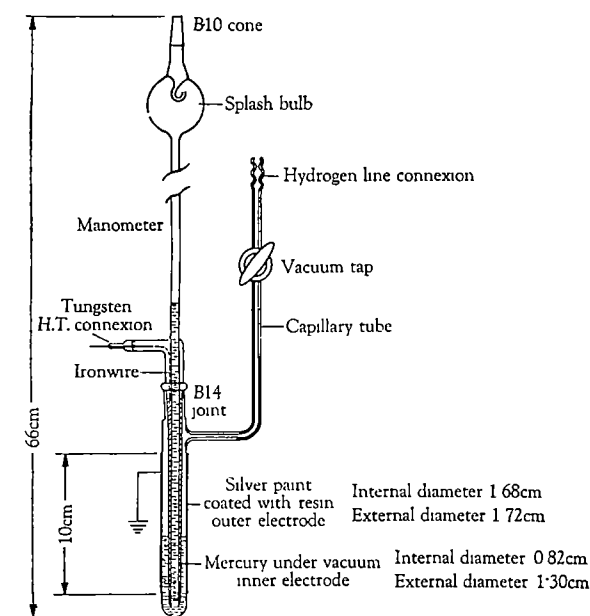


Fig. 1. Diagram of new British Insulated Callender's Cables, Ltd., gassing cell

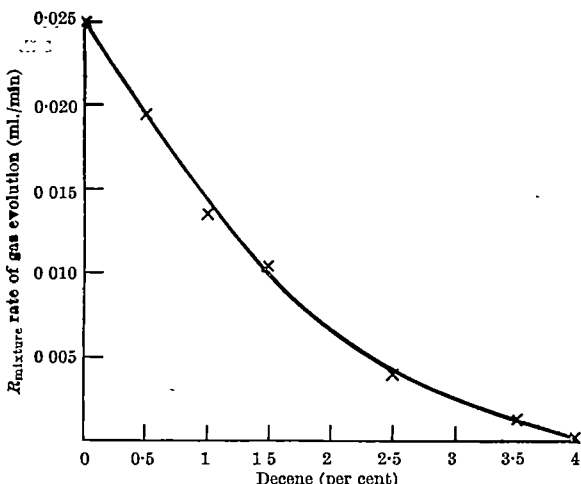
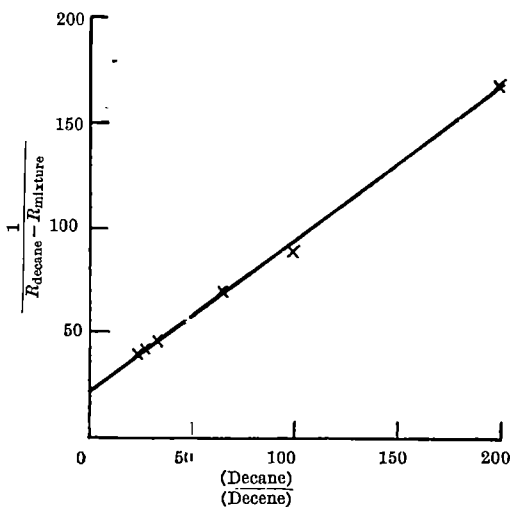
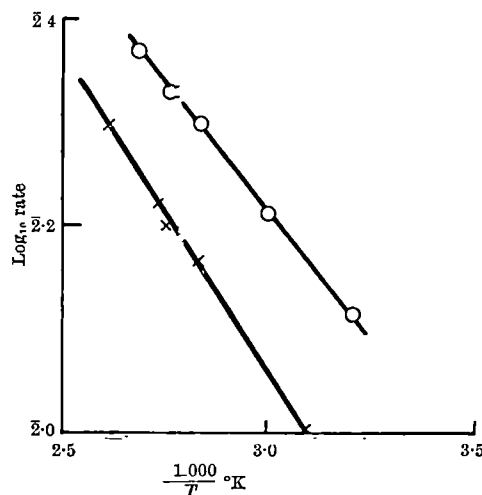


Fig. 2. Rate of gas evolution from decane-decene mixtures stressed under hydrogen at 60° C, 12 kV

Fig. 3. $1/R_{\text{decane}} - R_{\text{mixture}}$ versus (decane)/(decene)Fig. 4. Arrhenius plots for the reactions of *n*-decane (x) and 1 octadecene (O) stressed at an applied voltage of 12 kV

n-decane and mixtures containing low concentrations of the congruent unsaturated hydrocarbon 1-decene obey the same form of kinetic equation as that proposed for the radiolysis process⁷:

$$\frac{1}{R_{\text{decane}} - R_{\text{mixture}}} = A \frac{(\text{decane})}{(1\text{-decene})} + C$$

where *R* represents the rate of gas evolution measured in ml./min and *A* and *C* are constants (Figs. 2 and 3).

A slow stream of hydrogen gas, passed through an empty cell, the inner electrode of which had been previously dusted with molybdenum trioxide, reduced this oxide to the blue oxide when the electric stress was applied. This confirms that atomic hydrogen is formed in the gas phase under discharge conditions and affords further evidence for the suggested reaction mechanism.

Arrhenius plots of rate against absolute temperature (Fig. 4) for 1 octadecene and *n*-decane gave values of 2.5 kcal/mole and 2.9 kcal/mole for the respective activation energies, values about eight times those for the radiation-induced reactions in hydrocarbons.

These experiments suggest that the discharge reaction is explicable in a similar way to the radiolytic reaction, although the existence of a gaseous phase, a gas liquid interface and the lower energy of the electric discharge give rise to different reaction conditions, such that thermal collision processes are more prominent, as is evident from the higher activation energies. By examining other hydrocarbon mixtures and working under different gas atmospheres at different temperatures, it is

hoped to elucidate the reaction mechanism further and calculate absolute rate constants for comparison with those obtained radiolytically.

I thank Dr. A. L. Williams, director of research and engineering, British Insulated Callender's Cables, Ltd., for permission to publish this communication, and Dr. R. M. Black and Mr. E. H. Reynolds for their advice.

P. E. CROSS

British Insulated Callender's Cables, Ltd.,
Central Research and Engineering Division,
38 Wood Lane, London, W.12.

¹ Bassaches, H., and Mclean, P. A., *Indust. Eng. Chem.*, **47**, 1782 (1965).

² Church, H. F., and Garton, C. G., *Proc. Inst. Elec. Eng.*, **100**, IIA (528) (1953).

³ Clarke, S. A., and Reynolds, E. H., *Dielectrics*, **1**, 26 (1963).

⁴ Nossler, A., and Lawrence, R., *Nature*, **206**, 86 (1965).

⁵ Black, R. M., and Reynolds, E. H., *Proc. Inst. Elec. Eng.*, **112**, 1226 (1965).

⁶ Hardwick, T. J., *J. Phys. Chem.*, **64**, 1623 (1960).

⁷ Hardwick, T. J., *J. Phys. Chem.*, **66**, 291 (1962).

BIOCHEMISTRY

Molecular Weight of Urokinase

UROKINASE is a proteolytic enzyme in human urine¹. Its ability to activate plasminogen has stimulated interest in its potential clinical use as a thrombolytic agent.

Investigations were initiated in this laboratory to further characterize urokinase. Purified preparations of urokinase of specific activity approximately 7,000 Ploug units/mg dry weight (ref. 2) were obtained from Leo Laboratories (London) and other samples used were prepared from human urine. As a crystalline urokinase of about 100,000 C.T.A. units/mg protein (1 C.T.A. unit (ref. 3) = 0.7 Ploug units) has been described⁴, the material we have employed cannot be regarded as pure.

We have estimated the molecular weight of urokinase by a gel-filtration method⁵. Columns of 'Sephadex G-75' and 'G-100' were prepared and the elution volumes determined for a number of pure proteins by monitoring the ultra-violet absorption of the column effluent. The buffer routinely used was 0.05 M *tris*-HCl (pH 7.5) containing 0.1 M potassium chloride. Proteins used for calibration of the columns were cytochrome C, ribonuclease, myoglobin, soya-bean trypsin inhibitor, α -chymotrypsinogen, β -lactoglobulin, ovalbumin, serum albumin and γ -globulin. The relation between elution volume and \log_{10} mol. wt. was linear over the molecular weight-range investigated (12,000–70,000) for both columns (Fig. 1).

The elution volume of various urokinase samples was determined on both 'G-75' and 'G-100 Sephadex' columns. As it was known that the enzyme preparation was impure, detection of protein in the column effluent by ultra-violet absorption was not used. Instead, the urokinase

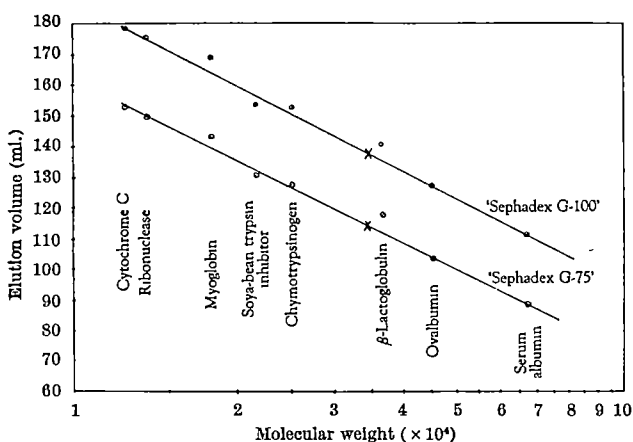


Fig. 1. Molecular weight calibrations of columns of 'Sephadex G-75' and 'G-100' at pH 7.5. 'X' indicates the position on the lines corresponding to the elution volume of urokinase

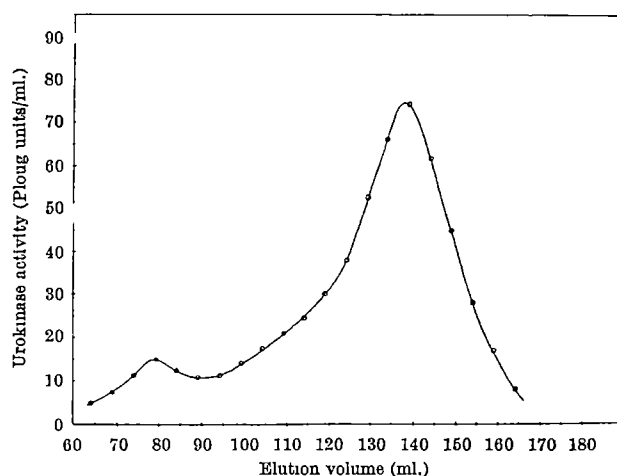


Fig. 2. The elution profile of a sample of urokinase on 'Sephadex G-100' at pH 7.5

present was assayed by its ability to activate plasminogen using the fibrin plate method². The elution volume obtained on 'G-75 Sephadex' was 114 ± 2 ml. and on 'G-100 Sephadex' was 138 ± 2 ml. (Fig. 2). In each case this corresponds to a molecular weight of $34,500 \pm 2,000$ for urokinase.

Evidence that some urokinase samples were contaminated with a small quantity of high-molecular-weight material capable of activating plasminogen was observed; but the possibility that this represents urokinase bound to a high molecular weight material could not be excluded. This material was completely excluded from both gels and therefore had a molecular weight of at least 100,000. No contamination of the urokinase by other proteolytic enzymes of less than 100,000 molecular weight was observed.

In order to minimize the possibility of the observed urokinase elution volume being modified by absorption effects, a similar experiment was performed using a 'G-75 Sephadex' column equilibrated with borate buffer at pH 10. An experiment was also performed on 'G-100 Sephadex' at pH 7.5 in the presence of 0.7 M potassium chloride instead of 0.1 M potassium chloride. In neither case was any change observed in the estimated molecular weight of urokinase.

These experiments indicate that the molecular weight of urokinase is approximately 35,000. This is much lower than the value of 53,000 recently reported for a crystalline sample of urokinase by Lesuk *et al.*⁴, based on ultracentrifugation measurements. The reason for the discrepancy between these two estimates is not clear.

R. A. BURGESS
K. W. BRAMMER
J. D. COOMBS

Biochemical Research Department,
Therapeutic Research Division,
Pfizer Ltd., Sandwich, Kent.

¹ Sobel, G. W., Mohler, S. R., Jones, N. W., Dowdy, A. B. C., and Guest, M. M., *Amer. J. Physiol.*, **171**, 768 (1952).

² Ploug, J., and Kjeldgaard, N. O., *Biochim. Biophys. Acta*, **24**, 278 (1957).

³ Sherry, S., Alkjaersig, N., and Fletcher, A. P., *J. Lab. Clin. Med.*, **64**, 145 (1964).

⁴ Lesuk, A., Terminiello, L., and Traver, J. H., *Science*, **147**, 880 (1965).

⁵ Andrews, P., *Biochem. J.*, **91**, 222 (1964).

Localized Strand Separations within Deoxyribonucleic Acid during Selective Transcription

MOLECULES of deoxyribonucleic acid isolated under gentle conditions are found to possess a double-stranded helical structure, in which the bases of each DNA strand are exactly paired within the interior of the helix with

complementary bases of the opposite strand¹. During gene transcription *in vivo* or under gentle conditions *in vitro*, only one of the two DNA strands serves as a template for new RNA synthesis². Furthermore, within a well-differentiated tissue, only a fraction of loci within the total genome are transcribed *in vivo*, each such selection of loci being characteristic of the particular tissue³. It has recently been demonstrated that stable localized strand separations are induced within the double-stranded DNA helix during selective gene transcription within higher organisms^{4,5}. Within such localized separation loops, one DNA strand appears to function as a template for RNA synthesis⁵. The complementary DNA strand of each separation loop appears to function as a binding site for specific de-repressor RNA molecules⁵ in an epigenetic control mechanism which selectively restricts gene transcription to only such strand-separated portions of the genome^{5,6}.

During cell interphase in higher organisms, the DNA molecules of the cell nucleus are found arrayed either within condensed masses of heterochromatin^{7,8} in which RNA synthesis is repressed⁷⁻⁹, or within extended microfibrils of euchromatin^{7,8} in which RNA synthesis is active⁷⁻⁹. When such repressed-heterochromatin and active-euchromatin complexes of a single tissue are each isolated in parallel under identical gentle conditions⁷, the isolated heterochromatin retains its original repressed and condensed state⁴, while the isolated euchromatin retains its original active and extended state⁴. The native associations between DNA and the other molecular species of chromatin, such as repressor histones, de-repressor RNA, and endogenous RNA polymerase, have been examined within isolated heterochromatin and isolated euchromatin in a variety of physical, chemical, and metabolic investigations^{4,6}.

DNA molecules isolated from either type of chromatin of one tissue are not significantly different in their base composition^{4,6} or in their thermal denaturation profiles⁴. Similarly, both types of chromatin contain the same quantities and patterns of histones in relation to their DNA contents^{4,6}. By contrast, active euchromatin contains an excess of such nuclear polyanions as RNA⁴, phosphoproteins¹⁰, and phospholipids¹¹, and localizes testosterone during stimulation of RNA synthesis by this steroid hormone¹².

When DNA molecules are examined while within the active euchromatin complex, they display a 60 per cent reduction of their usual thermal hyperchromicity⁴, indicating a significant degree of separation of the strands within their DNA double helices^{5,6}. By contrast, such DNA molecules of the sample display an entirely normal degree of thermal hyperchromicity when they are first isolated away from the histones and polyanions of the euchromatin complex before being examined⁴. Such contrasting behaviour suggests that the DNA strand separations observed within the intact euchromatin complex depend on an association with the other molecular species of euchromatin for the stabilization of such strand separations^{5,6}. The regaining of normal hyperchromicity following DNA isolation also suggests that these strand separations are only partial or looped within any given DNA double helical molecule, with some portion of each helix remaining in exact register during selective gene transcription^{5,6}. In parallel investigations under identical conditions, the DNA molecules while within the repressed heterochromatin complex do not reveal any reduction of their usual hyperchromicity⁴, but display an increased stability to strand separation while within the heterochromatin complex⁴.

Both active euchromatin and repressed heterochromatin contain equal amounts and types of polycationic histones in relation to their polyanionic DNA^{4,6}. Such histones function as non-specific repressors of the template function of DNA by interacting electrostatically with the negative phosphate groups on the exterior of the DNA double

helix^{13,14}. This histone-DNA interaction neutralizes the multiple negative charges along the length of the DNA molecule^{13,14}, overcoming the mutual repulsion otherwise present between these highly charged DNA molecules¹⁵, and favouring the formation of condensed masses of repressed heterochromatin^{6,16}. In such an interaction, the histones cross-link two or more DNA strands¹⁷, thus stabilizing the double-stranded DNA helices against strand separation and consequent transcription¹⁸.

The polycationic histone repressors can be partially displaced from the DNA double helices by several types of those polyanions found in excess within active chromatin⁴. In such action, these polyanions function as natural de-repressors, permitting the now-charged DNA double helix to assume the extended state⁶ and to undergo its normal degree of spontaneous strand separations¹⁹. Strand separation loops formed spontaneously appear to be stabilized and perhaps extended within specific loci of the genome by the co-operative binding of specific de-repressor RNA molecules to one of the two separated DNA strands at these loci^{5,6}. The remaining complementary DNA strand within the separation loop is then free to serve as a template for selective RNA synthesis^{5,6}.

These investigations indicate that the physical state of the DNA molecules during selective transcription in higher organisms is characterized by local stabilized separations of the strands of the DNA double helix within specific portions of the genome⁵. By contrast, repressed portions of the genome are characterized by an increased stability of the DNA helix⁴, with no evidence of strand separations⁴ or of active transcription^{7,9}, and appear as condensed masses of heterochromatin during cell interphase^{7,8}. Later in the cell cycle, during DNA replication, these same repressed heterochromatin portions of the genome display a late phase of DNA synthesis compared to the rest of the genome²⁰. Since *in vivo* DNA replication involves some degree of strand separation of the replicating DNA molecules²¹, the delay in DNA replication observed within these repressed heterochromatin regions may reflect the increased stability of the DNA helices within these regions found earlier in the cell cycle during selective transcription⁴.

Still later in the cell cycle, after the completion of DNA replication, these same repressed heterochromatin regions of the genome²² may now extend and form secondary constrictions²³ in localized areas of the metaphase chromosomes²². Such secondary constrictions map identically within each of the sister chromatids²³, indicating that each daughter cell receives an identical complement of chromosomes the DNA of which is physically altered in those same gene loci that were repressed in transcription and delayed in replication in the maternal cell⁶. Indeed, following cell division, it has been shown that at least some of the same gene loci that were repressed for transcription in the maternal cell are similarly repressed in each of the daughter cells for many subsequent cell generations²⁴, indicating a continuity of the specific transcription selection through the entire course of many cell divisions⁶. Such a continuity could be mediated by the passive symmetric transfer of maternal de-repressor RNA species to each of the daughter cells during cell division^{6,25}.

The strand separation loops within the DNA molecules of active euchromatin are the likely sites of action of those molecular species which increase or decrease the rate of RNA synthesis non-specifically⁶. Thus, such steroid hormones as oestradiol and testosterone bind preferentially to the single-stranded form of DNA²⁶, tending to stabilize the strand separation loops within DNA²⁶, and non-specifically increasing the rate of RNA synthesis within pre-selected loci of the genome²⁷. In this regard, testosterone is found localized preferentially within active euchromatin during the stimulation of nuclear RNA synthesis by hormone¹². Conversely, such inhibitors as histones¹⁴, actinomycin D²⁸, acridine orange²⁹, and chloroquine³⁰ all bind preferentially to the double-

stranded form of DNA, tending to stabilize the DNA helix against strand separations, and non-specifically decreasing the rate of synthesis of all RNA species⁶.

Recent investigations suggest that the strand separation loops within active euchromatin may be sensitive sites of action during radiosensitization³¹ and during the interaction of the host genome with oncogenic viruses³².

This work was carried out during the tenure of a research career development award (CA-17857) from the U.S. Public Health Service.

JOHN H. FRENSTER

Laboratory of Cell Biology,
Roeckefeller Institute, New York.

- ¹ Watson, J. D., and Crick, F. H. C., *Nature*, **171**, 737, 964 (1953).
- ² Gelduschek, E. P., Tocchini-Valentini, G. P., and Sarnot, M. T., *Proc. U.S. Nat. Acad. Sci.*, **52**, 480 (1964); Bassel, A., Hayashi, M., and Spiegelman, S., *Proc. U.S. Nat. Acad. Sci.*, **52**, 796 (1964); Green, M. H., *Proc. U.S. Nat. Acad. Sci.*, **52**, 1388 (1964).
- ³ McCarthy, B. J., and Hoyer, B. H., *Proc. U.S. Nat. Acad. Sci.*, **52**, 915 (1964).
- ⁴ Frenster, J. H., *Nature*, **206**, 680 (1965).
- ⁵ Frenster, J. H., *Nature*, **206**, 1269 (1965).
- ⁶ Frenster, J. H., in *The Chromosomes: Structural and Functional Aspects* edit by Dawe, C. J., and Yerganian, G. (Williams and Wilkins, Inc., Baltimore, 1965).
- ⁷ Frenster, J. H., Allfrey, V. G., and Mirsky, A. E., *Proc. U.S. Nat. Acad. Sci.*, **50**, 1026 (1963).
- ⁸ Frenster, J. H., *Nature*, **205**, 1341 (1965).
- ⁹ Littau, V. C., Allfrey, V. G., Frenster, J. H., and Mirsky, A. E., *Proc. U.S. Nat. Acad. Sci.*, **52**, 93 (1964).
- ¹⁰ Langan, T. A., and Lipmann, F., *J. Biol. Chem.* (In the press).
- ¹¹ Rose, H. G., and Frenster, J. H., *Biochim. Biophys. Acta* (In the press).
- ¹² Loeb, P. M., and Wilson, J. D., *Clin. Res.*, **13**, 45 (1965).
- ¹³ Kawade, Y., *Biochim. Biophys. Acta*, **26**, 61 (1957); Phillips, D. M. P., *Prog. Biophys. Chem.*, **12**, 211 (1962).
- ¹⁴ Akinrimisi, E. O., Bonner, J., and Ts'o, P. O. P., *J. Mol. Biol.*, **11**, 128 (1965).
- ¹⁵ Schildkraut, C. L., and Lifson, S., *Biopolymers*, **3**, 195 (1965).
- ¹⁶ Dyer, A. F., *Cytologia*, **29**, 155 (1964).
- ¹⁷ Wilkens, M. H. F., Zubay, G., and Wilson, H. R., *J. Mol. Biol.*, **1**, 179 (1959); Luzzati, V., and Nicolaleff, A., *J. Mol. Biol.*, **7**, 142 (1963); Lloyd, P. H., and Peacocke, A. R., *Biochim. Biophys. Acta*, **95**, 522 (1964).
- ¹⁸ Huang, R. C., and Bonner, J., *Proc. U.S. Nat. Acad. Sci.*, **48**, 1216 (1962); Allfrey, V. G., Faulkner, R., and Mirsky, A. E., *Proc. U.S. Nat. Acad. Sci.*, **51**, 786 (1964).
- ¹⁹ Printz, M. P., and von Hippel, P. H., *Proc. U.S. Nat. Acad. Sci.*, **53**, 363 (1965).
- ²⁰ Klevecz, R. R., and Hsu, T. C., *Proc. U.S. Nat. Acad. Sci.*, **52**, 811 (1964).
- ²¹ Rolfe, R., *Proc. U.S. Nat. Acad. Sci.*, **49**, 386 (1963); Rosenberg, B. H., and Cavaliere, L. F., *Proc. U.S. Nat. Acad. Sci.*, **51**, 826 (1964); *Nature*, **206**, 999 (1965).
- ²² Schmid, W., *Cytogenetics*, **2**, 175 (1963).
- ²³ Dyer, A. F., *Cytologia*, **29**, 171 (1964); Sasaki, M. S., and Makino, S., *Amer. J. Human Gen.*, **15**, 24 (1963).
- ²⁴ Davidson, R. G., Nitowsky, H. M., and Childs, B., *Proc. U.S. Nat. Acad. Sci.*, **50**, 481 (1963).
- ²⁵ Love, R., *Nature*, **180**, 1338 (1957); Feinendegen, L. E., and Bond, V. P., *Exp. Cell Res.*, **30**, 393 (1963); Jackson, L. G., Barr, M. A., and Wiess, A. J., *Proc. Amer. Assoc. Cancer Res.*, **5**, 31 (1964).
- ²⁶ Ts'o, P. O. P., and Lu, P., *Proc. U.S. Nat. Acad. Sci.*, **51**, 17 (1964).
- ²⁷ Schmirke, R. T., Sweeney, E. W., and Berlin, C. M., *J. Biol. Chem.*, **240**, 322 (1965).
- ²⁸ Reich, E., *Science*, **143**, 684 (1964); Gellert, M., Smith, C. E., Neville, D., and Felsenfeld, G., *J. Mol. Biol.*, **11**, 445 (1965).
- ²⁹ Lerman, L. S., *J. Cell Comp. Physiol.*, **64**, suppl. 1, 1 (1964).
- ³⁰ Cohen, S. N., and Yielding, L. K., *J. Biol. Chem.*, **240**, 3123 (1965); *Proc. U.S. Nat. Acad. Sci.*, **54**, 521 (1965).
- ³¹ Kaplan, H. S., and Howsden, F. L., *Proc. U.S. Nat. Acad. Sci.*, **51**, 181 (1964).
- ³² Axelrod, D., Habel, K., and Bolton, E. T., *Science*, **146**, 1466 (1964).

Properties of Hyaluronic Acid from a Human Myxosarcoma

It is known that the degree of polymerization of purified hyaluronic acid depends on the sources of preparation and the method of isolation. Caputo and I previously reported¹ the occurrence in Rous sarcoma of highly polymerized hyaluronic acid complexed with protein. Investigations of the effect of proteolytic enzymes on this complex have revealed the prominent part played by the protein in the molecular conformation of the complex².

This communication reports some properties of a very special hyaluronic acid which I have purified from a human myxosarcoma.

The polysaccharide was isolated by precipitation with cold, acid acetone, according to the technique previously

Table 1. MOLECULAR CHARACTERISTICS OF A HYALURONIC ACID-PROTEIN COMPLEX ISOLATED FROM HUMAN MYXOSARCOMA

$S_{20,w}^*$	16.8×10^{-13}
$D_{20,w}^\dagger$	5.7×10^{-7}
\bar{V}	0.710
Mw	2.5×10^5

$S_{20,w}$, sedimentation coefficient; $D_{20,w}$, diffusion constant; \bar{V} , partial specific volume; Mw , molecular weight.

* Extrapolated to zero protein concentration.

† Protein concentration 5 mg/ml.

reported¹. At the end of the preparation several further attempts to reduce the nitrogen content of the compound failed, and therefore it seemed certain that the polysaccharide was stably complexed with a protein. The resulting material behaved as a monodispersed system in the free boundary electrophoresis and was homogeneous in the ultracentrifuge. When compared with other types of hyaluronic acid, the sedimentation boundary of the myxosarcoma polysaccharide was always found to be perceptibly less sharp and to have a great tendency to diffuse. Some molecular characteristics of myxosarcoma hyaluronic acid-protein complex are given in Table 1.

The molecular weight of 2.5×10^5 is very low compared with values of about 1 million described previously for the same polysaccharide isolated from different sources.

After treatment with hyaluronidase, a decrease in the viscosity of the hyaluronic acid complex solution and the disappearance in electrophoresis and the ultracentrifuge of the homogeneity of the boundary were observed.

Preliminary chemical analyses have shown that the protein content of hyaluronic acid complex from myxosarcoma is about 45 per cent. Until now this value is the highest found for all types of hyaluronic acid-protein complex.

The fact that proteolytic enzymes have a very weak effect on the hyaluronic acid complex from myxosarcoma provides strong evidence that the polysaccharide occurs in this tumour in a completely different molecular state, not only from that isolated from normal sources, but also from Rous sarcoma. Stronger evidence for this hypothesis has been provided by additional chemical analyses of the amino-acid composition of the protein moiety of the complex. In this connexion it is interesting to note that hydroxyproline was found in the hydrolysate, among seventeen different amino-acids. The fact that myxosarcoma hyaluronic acid containing hydroxyproline has a low molecular weight indicates that there is a similarity between this polysaccharide and that already isolated from vitreous humour and that it is completely different from that previously isolated from the Rous sarcoma.

MARIA LUISA MARCANTE

Instituto 'Regina Elena' per lo
Studio e la Cura dei Tumori,
Rome.

¹ Caputo, A., and Marcante, M. L., *Cancer Res.*, **19**, 1010 (1959).

² Caputo, A., and Marcante, M. L., *Clin. Chim. Acta*, **5**, 477 (1960).

Oleic Acid labelled with Iodine-131, and Triolein De-iodination *in vitro*

THE de-iodination of ¹³¹I-labelled Rose Bengal¹ and thyroxine² has been studied before, and it can be assumed that a thermostable factor is present in the organs in different concentrations³ and that some proteins⁴ have a stimulative effect on the de-iodination.

This experimental work was carried out to study the de-iodination *in vitro* of ¹³¹I-labelled oleic acid and triolein, widely used as diagnostic radiopharmaceuticals. Some variable results obtained in the investigation of fatty-acid metabolism with these radioisotopes could be related to the de-iodination *in vivo*⁵.

The tested rat organs were homogenized with saline to give a final organ concentration of approximately 200 mg/ml. (wet organ weight). Three series of experi-

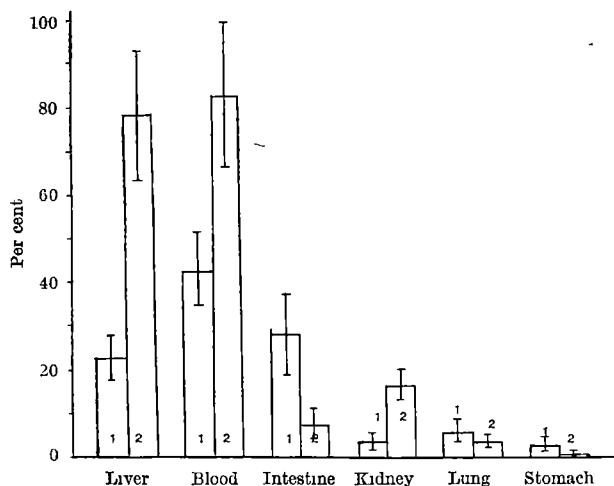


Fig. 1 Iodine-131 found as iodide after 15 h incubation 1, ¹³¹I-labelled triolein; 2, ¹³¹I-labelled oleic acid

ments using ¹³¹I-labelled triolein (specific activity 29 μ c./mg) and three other series using ¹³¹I-labelled oleic acid (specific activity 38 μ c./mg, were performed. 10 μ l of the labelled compound (approximately 50 μ c.) were added to each series of test-tubes containing 0.5 ml. of homogenate and 0.2 ml. of isotonic phosphate buffer, pH 7.2. In each series a test tube containing the same activity in 0.2 ml. of isotonic phosphate buffer pH 7.2 and an equal volume of saline instead of the homogenate were used as a blank. All the tubes were incubated under the same conditions: 15 h at 37° C with occasional stirring and avoiding the evaporation.

After the incubation was completed, 2 ml. of ethyl alcohol was added to each tube and then stirred and centrifuged. The residue was washed once with 1 ml. of ethyl alcohol and finally extracted twice with 1 ml. of ethyl ether. The alcohol and ether extracts were mixed and a sample of it was analysed by paper electrophoresis. Under these experimental conditions the formed iodide as well as the labelled oleic acid or triolein are completely extracted from the tissue residue.

The electrophoresis was performed using Whatman 3 MM paper, sodium bicarbonate, 0.2 per cent, as a buffer and with a voltage gradient of 12 V/cm. After 1 h the iodide migrated approximately 6 cm while the labelled triolein or oleic acid remained at the starting line.

The scanning of the electrophoresis was carried out using an automatic graphic recorder. In this way it was possible to evaluate, by integration, the percentage of activity corresponding to each peak (iodide and labelled oleic acid or triolein; no other intermediate compound was detected). Fig. 1 gives the values corresponding to the formed radioiodide (as percentage of the total activity present) after 15 h of incubation at 37° C. No iodide formation was found in the blank test-tubes.

The ¹³¹I-labelled oleic acid was rapidly de-iodinated by liver and blood homogenates; the ¹³¹I-labelled triolein was more stable. Intestine was twice as active for ¹³¹I-labelled triolein as for ¹³¹I-labelled oleic acid. The stomach had practically no action on ¹³¹I-labelled triolein and ¹³¹I-labelled oleic acid *in vitro*.

As a result of these experiments it can be stated that the blood and liver are rich in the dehalogenase responsible for the de-iodination of both labelled compounds.

LEOPOLDO JOSE ANGHILERI*

Comision Nacional de Energia Atomica,
Buenos Aires.

* Present address. Institut du Radium, Laboratoire Curie, Paris, France

¹ Raban, P., Gregora, V., and Jirsa M., *Nature*, **204**, 885 (1964).

² De Escobar, G. M., Del Rey, F. E., and Rodriguez, P. L., *J. Biol. Chem.*, **237**, 2041 (1962).

³ Magas, S., and Rozynkova, *Acta Biochim. Polon.*, **10** (4), 337 (1963).

PHYSIOLOGY

Heat Production in a Cardiac Contraction

THE energetics of cardiac contraction, a field of much physiological interest, has remained incompletely developed because of the difficulty of performing heat measurements on a heart muscle preparation. We have recently succeeded in solving some of the technical difficulties and have developed a procedure for myothermic measurements on isolated papillary muscles of the rabbit heart.

The papillary muscles used are about 1.0 mm in diameter and 4–8 mm in length. To obtain an adequate thermopile output, a sufficient number of junctions has to be situated in this range of lengths. The present pile has 32 active and 4 'protective' junctions of silver constantan over a length of 5 mm; it has an output of 815 μ V/°C. The junctions are situated at the bottom of a hemicylindrical trough, about 1 mm diameter, that holds the muscle.

The problems associated with myothermic measurements have been discussed in detail by Hill^{1–3}. A major difficulty encountered with cardiac heat measurements is the large heat capacity of the thermopile relative to that of the small muscle. This heat capacity was determined, on the one hand, by liberating a condenser discharge or an RF current pulse into a saline-gelatin rod placed in the thermopile groove; on the other hand, using the cardiac pile, measurements were made on a 20-mg strip carefully dissected from a frog sartorius muscle on which heat measurements had previously been made with a standard thermopile. Both methods agreed; the present thermopile has a heat capacity equivalent to 3.0 ± 0.5 mg of muscle. The muscles themselves generally weigh 3–10 mg. The average heat loss from the muscle-thermopile system is 13 per cent per second; this is corrected for electronically. The correction method and the technique for thermopile construction will be described by one of us (N. V. R.) in a later paper. The muscles were bathed in a modified Tyrode solution containing 4.0 mM calcium/l. and aerated with 98 per cent oxygen and 2 per cent carbon dioxide. The experiments were run at room temperature which was between 18 and 22° C. The muscles were not left out of solution longer than 15 min and were never stimulated at frequencies above 60 beats/min.

In the work recorded here we have concerned ourselves solely with the magnitude of heat production, and some of the results obtained are given in Table 1. Our main objectives were to determine: (1) the rate of resting heat production; (2) the relationship between heat production and tension development; (3) if there was a heat equivalent to the activation heat of skeletal muscle. Resting heat was measured by first zeroing the amplifier input with the thermopile immersed in solution. The papillary muscle was then brought into contact with the pile and the change in input voltage was measured when the bath was drained of Tyrode solution. The heat output was measured over a period of several minutes. The experiments were all run with a resting tension of 1.0 g on the muscle and hence the measurement makes no distinction between the basal resting heat and the heat due to the existence of tension in the resting muscle⁴. The mean value of seven individual measurements was 25.7 mcal/g muscle/min.

Table 1

Exp. No.	Blotted weight (mg)	P ₀ — M	P ₀ — H	Resting heat (mcal/g/min)	Activation heat (mcal/g/twitch)	Heat* (mcal/g/twitch)	Tension (g)
1	5.0	433	4.05	—	—	1.59	3.3
2	6.5	270	4.0	26.0	—	1.53	2.5
3	8.0	525	5.8	36.6	0.96	2.04	5.3
4	3.2	750	5.1	25	0.80	2.14	3.0
5	6.1	496	5.5	35	0.47	2.46	6.0
6	4.5	392	6.1	24.4	0.61	1.38	1.9
7	5.9	395	5.3	15.4	0.31	1.52	2.9
8	4.5	555	5.7	38.6	0.61	2.3	4.9
Mean	5.5	477	5.2	28.7	0.63	1.87	3.7

* Tension and heat measured in a single contraction after a period of stimulation at 30 beats/min.

Records of heat produced during a cardiac contraction are shown in Fig. 1. The rate of heat production seems to be much slower than in skeletal muscle, but at present we cannot be certain that this is not an artefact. The muscle was first stabilized at a chosen frequency. Stimulation was then stopped; the heat quickly returned to its resting level. A single stimulus given at this point gave a tension output similar to that in the steady state and the heat produced was measured a few seconds after the contraction was completed. The heat production so measured was 10–20 per cent less than that determined from the total heat produced in a long series of contractions divided by the twitch number. There can be at least two reasons for this: (1) the resting heat production may increase during prolonged activity, a possibility suggested by Whalen⁶ in his oxygen consumption studies, or (2) there may be a small and slow phase of delayed heat that we miss by reading too early after a single contraction. To investigate this point we need a thermopile with more output and one that possesses an accurate exponential heat loss over several minutes. The relationship between isometric tension and the heat produced can either be curvilinear as shown in Fig. 2 or linear, and seems to resemble skeletal muscle in this property⁶.

Activation heat was determined either by allowing the muscle to shorten until little or no active tension was developed on stimulation⁷, or by eliminating tension development with Tyrode solution made hypertonic with sucrose⁸. The mean of six such measurements was 0.6 mecal/g.

The results so far obtained allow interesting preliminary comparisons to be made to frog sartorius muscle. The lesser

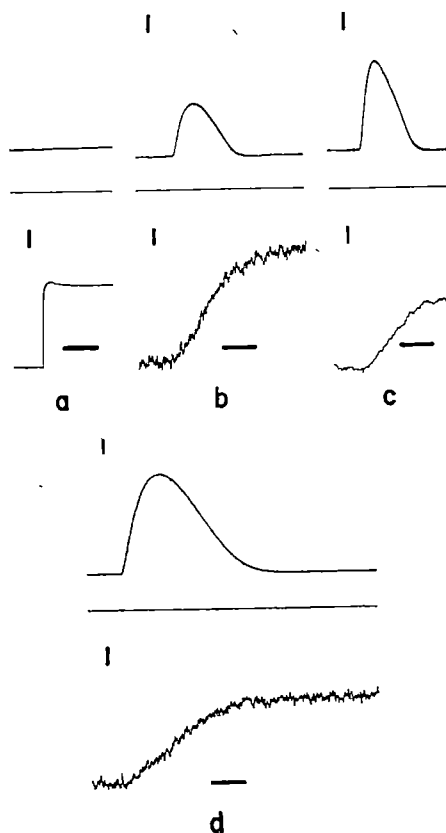


Fig. 1. Records of tension developed (top trace) and heat production (lower trace). The heat records are corrected for heat loss. Traces from three muscles a, b, c and d. The filter cut-off frequency was 25 c/sec in (b) and (d) and 4 cycles in (c).

In (a) a 10 msec RF pulse; bottom abscissa 10 sec, ordinate 1.0 μ V; (b) a contraction; top abscissa 2.0 sec, ordinate 1.0 g; bottom abscissa 2.0 sec, ordinate 0.1 μ V; (c) a contraction; top abscissa 2.0 sec, ordinate 0.4 g, bottom abscissa 2.0 sec, ordinate 0.2 μ V; (d) a contraction, top abscissa 0.5 sec, ordinate 1.0 g, bottom abscissa 0.5 sec, ordinate 0.2 μ V.

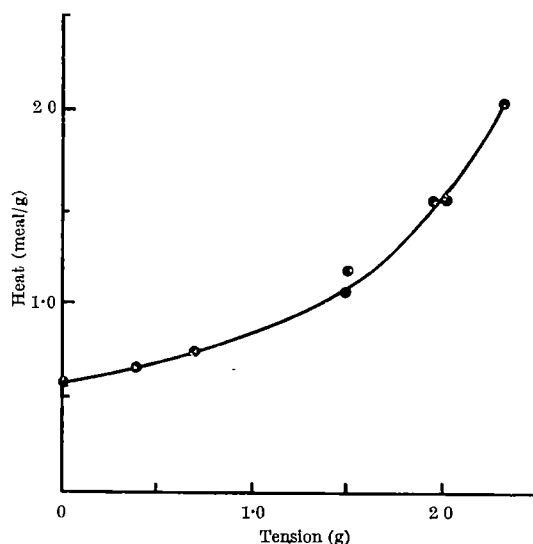


Fig. 2. A plot of heat production against twitch tension. Tension varied by altering stimulus frequency, muscle length and Tyrode tonicity. Muscle length 8 mm, blotted weight 4.5 mg.

activation heat, 0.6 as compared with 1.2 mecal/g (ref. 9), possibly correlates with the lesser capability for tension development. The slower rate of heat production in a twitch, if confirmed, might have a two-fold cause: the slower onset of the active state¹⁰, and the fact that the aerobic recovery heat is presumably not a separate entity but occurs in close temporal association with each twitch to which it belongs. The latter circumstances would also explain why the ratio P/H is 5.2 rather than close to 10 as found by Hill¹¹.

We thank Dr. W. F. H. M. Mommaerts for his advice.

This work was supported by research grants HE 03067 of the U.S. National Heart Institute, National Institutes of Health, Bethesda, and AFOSR 146-63 of the Air Force Office of Scientific Research, and the Muscular Dystrophy Associations of America, Inc.

N. V. RICCHIUTI
C. L. GIBBS*

Department of Medicine (The Los Angeles County Heart Association Cardiovascular Research Laboratory), University of California, Los Angeles.

* Research Fellow of the National Heart Foundation of Australia

- ¹ Hill, A. V., *Proc. Roy. Soc. B*, **124**, 114 (1937)
- ² Hill, A. V., *Proc. Roy. Soc. B*, **126**, 136 (1938)
- ³ Hill, A. V., *Proc. Roy. Soc. B*, **136**, 228 (1949)
- ⁴ Lee, K. S., *J. Physiol.*, **151**, 186 (1960).
- ⁵ Whalen, W. J., *J. Physiol.*, **157**, 1 (1961).
- ⁶ Ricchiuti, N. V., Gibbs, C. L., and Mommaerts, W. F. H. M., *Fed. Proc.*, **24**, 101 (1965).
- ⁷ Hill, A. V., *Proc. Roy. Soc. B*, **138**, 195 (1949)
- ⁸ Hill, A. V., *J. Physiol.*, **137**, 57 (1957)
- ⁹ Gibbs, C. L., and Ricchiuti, N. V., *Science*, **147**, 162 (1965).
- ¹⁰ Brady, A. J., *Pharmacology of Cardiac Function*, Prague (1963) 15 (Pergamon Press, Oxford, 1964)
- ¹¹ Hill, A. V., *Proc. Roy. Soc. B*, **149**, 58 (1958)

Somesthetic and Visual Responses of Superior Colliculus Neurones

APART from visual responses¹, somesthetic responses have been repeatedly observed in the superior colliculus². We have previously reported that under chloralose anaesthesia the somesthetic responses to forelimb stimulation recorded with gross concentric electrodes could be divided into a short-latency group (9 ms) and a long-latency group (70 ms), and that long-latency responses disappeared after ablation of the visual cortex³. This indicated a convergence of two somesthetic pathways towards the colliculus, as well as a convergence of somesthetic and visual afferent pathways. A microelectrode analysis has

been undertaken in order to determine the extent of these convergences at the cellular level.

Cats were anaesthetized with chloralose (80 mg/kg) administered intravenously, placed in a stereotaxic head holder and later paralysed with 'Flaxedil' and artificially respired. A hole drilled in the skull over the visual cortex, plugged with a 4-per cent solution of agar to reduce the pulsations of the brain, allowed the penetration of capillary microelectrodes filled with 3-M potassium chloride. Punctures were made only in the central part of the superior colliculus, mostly along the line A2, L3, from $H + 4.5$ to $H + 2.5$ to the zero plane⁴, thereby avoiding the insecurity in studying responses near the margins of the nucleus. Stimulation of the contralateral eye was a 500-ms flash obtained from a neon tube. The pupil was dilated with 1 per cent atropine chloride. Electrical stimuli were delivered to each of the four limbs through a pair of hypodermic needles thrust into the skin. At the end of the experiment the brain was perfused with saline, followed by 10 per cent formalin, and the approximate locations of the electrodes tips were checked in frozen sections.

Extracellular records were analysed in 82 units, all of which maintained a constant spike height and responded to at least one of the stimulations used.

Somesthetic responses were observed in 79 of the 82 cells examined. These responses showed short or long latencies. In both cases they were characterized by slow waves that after their onset gave rise to a burst of spikes (usually two or three but sometimes more). With forelimb stimulation the onset of the slow wave for short and long latency responses began after 9 ms and 68 ms respectively. It may be appreciated that the values for latencies of the slow waves are similar to those of responses recorded with gross concentric electrodes. The mean average latency of the first spike for the short-latency responses was 18 ± 5 ms (forelimb stimulation) and 23 ± 5 ms (hindlimb stimulation), and for long-latency responses was 72 ± 12 ms (forelimb stimulation) and 81 ± 12 ms (hindlimb stimulation). The histogram in Fig. 1 shows the distribution of latencies of the first spike of response appearing with short and long latency in a series of collicular neurones. Among the 79 units included in the histogram of Fig. 1, 18 responded only with a short latency (Fig. 1a); 47 responded only with a long latency (Fig. 1c) and 14 responded with a double response, in which the latencies of the two components fell within the range of the two previous groups (Fig. 1b). Somesthetic units belonging to the various groups already described here may be indistinctly found at variable depths of the superior colliculus. However, more than half of the short-latency responses (including those followed by a long-latency one) were encountered in the lowermost third of the superior colliculus (Fig. 2). This finding may suggest some degree of topographical distribution of these different types of cells along the vertical axis. Sections of the spinal dorsal funiculi at the level of T8 did not modify the responses to stimulation of any leg. On the other hand, ablation of the visual cortex and surrounding cortical areas above the colliculus suppressed the long-latency ones.

These results confirm that the superior colliculus receives two independent groups of somesthetic impulses: one of these groups seems to depend on a relay in the visual cortex, and thus on cortico-collicular connexions; the other one is probably conveyed by the spinotectal pathway. In addition, it is apparent that a large number of collicular neurones receive a convergent input from these two pathways.

Visual responses were observed in 72 of the 82 units investigated. As already observed by Altman and Malis¹ some units responded to light-on, others to light-off, and still others to light-on and to light-off. Among the 10 units which apparently responded only to somatic stimuli, activating and inhibiting effects of light could be demon-

strated when tested on a response to somesthetic stimulation. These effects were independent of the type of somesthetic response one (short-latency, long-latency or double).

Considering the whole sample of neurons studied, it can be seen that only 3 units responding to visual stimuli were not activated by somesthetic stimuli, and 4 were in the reverse situation. The other 75 units received visual and somesthetic inputs. Once again the distribution of the neurons along the vertical axis did not seem to be correlated with their responsiveness to somatic or visual stimuli (Fig. 2).

Thus at the cellular level there is a high degree of convergence not only between the two somesthetic pathways, but also between the somesthetic and visual pathways. Since the colliculus has a dual visual input^{1,5} (some impulses reaching it directly from the retina, others relating in the visual cortex), a part of the observed

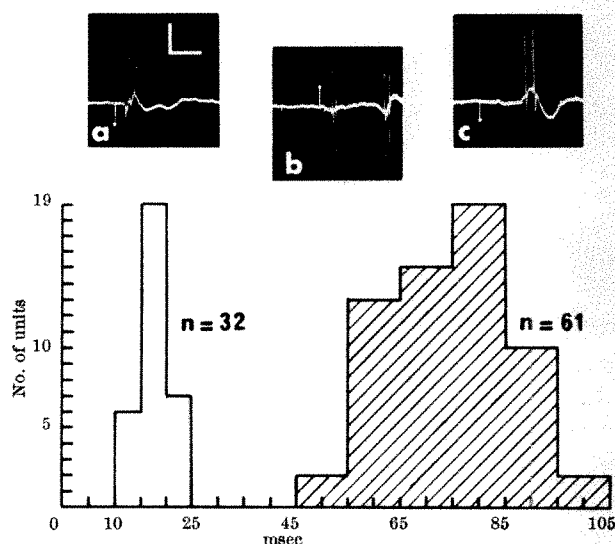


Fig. 1. Histograms of latencies of superior colliculus units to somesthetic stimulation. The abscissa corresponds to the latencies of the responses in msec and the ordinate to the number of units discharging at given latencies. Two populations of cells according to their latencies are clearly shown by the histograms. The units responding with both short and long latency are included in both histograms. Latency for each unit is average for stimulation of all four extremities. *n*, Number of units studied in each group. The upper figures give examples of the firing of each type of unit: (a) cell firing only with short latency; (b) with both short and long latency; (c) with long latency alone. Calibration: 50 msec and 1 mV.

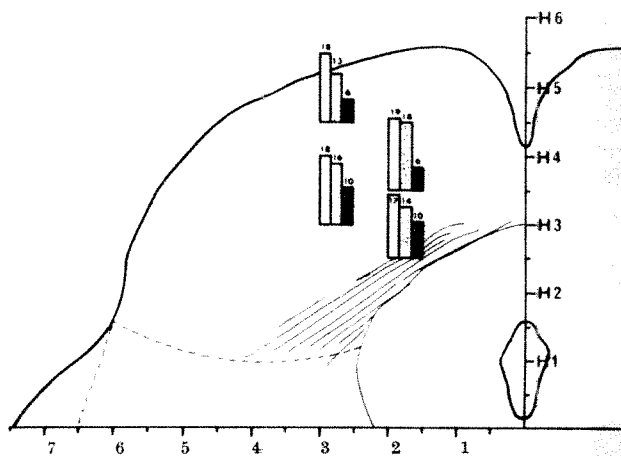


Fig. 2. Distribution of units in the superior colliculus. Histograms represent the number of units recorded at different levels of the superior colliculus expressed according with the type of stimulation (visual or somesthetic) and the latency of somesthetic responses. White bands, units responding to visual stimuli; dotted bands, units responding with long-latency somesthetic responses; black bands, units responding with short-latency somesthetic responses. Units responding with both short and long latency to somesthetic stimuli were included in both dotted and black bands. The number of each type of units is expressed at the top of each band. The levels correspond to stereotaxic co-ordinates.

convergences can occur in the visual cortex. But another part must obviously occur in the superior colliculus itself, as, for example, the convergence between spino-tectal and retino-collicular impulses.

Anatomical studies⁶ have emphasized that retina and visual cortex project to more superficial collicular layers than spino-tectal fibres. Thus it is interesting to find so few 'modality-specific' neurones: that is, neurones responding only to visual or only to somesthetic stimulation. Further investigations, and especially, better localization of the electrodes tips, may tell if the 'specific' neurones were missed because of their small size or their anatomical localization. Perhaps another possibility might be that intracollicular connexions were responsible for the fact that nearly all collicular neurones studied received visual and somesthetic inputs.

I thank Dr. P. Ascher for his advice. This work was supported by a grant from the Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina, and by grant AF-AFOSR-656-65 from the U.S. Air Force Office of Scientific Research.

DORA JASSIK-GERSCHENFELD*

Instituto de Anatomía General y Embriología,
Facultad de Ciencias Médicas,
Buenos Aires.

* Investigation careere, Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina.

¹ Altman, J., and Malis, L. I., *Exp. Neurol.*, **5**, 233 (1962).

² Horn, G., and Hill, R. M., *Nature*, **202**, 296 (1964). Bell, C., Sierra, G., Buendía, N., and Segundo, J. P., *J. Neurophysiol.*, **27**, 961 (1964).

³ Jassik-Gerschenfeld, D., and Ascher, P., *Experientia*, **19**, 655 (1963).

⁴ Jasper, H. H., and Ajmone-Marsan, C., *A Stereotaxic Atlas of the Diencephalon of the Cat* (Nat. Res. Coun. Canada, Ottawa, 1954). Snider, R. S., and Niemer, W. T., *A Stereotaxic Atlas of the Cat Brain* (Univ. Chicago Press, 1961).

⁵ Beever, C. E., and Horsley, V., *Brain*, **25**, 436 (1902). Harman, P. J., and Berry, C. M., *J. Comp. Neurol.*, **105**, 393 (1956). Beresford, W. A., in *Neurophysiology and Psychophysics of the Visual System*, edit. by Jung, R., and Kornhuber, K. (Springer, 1961). Altman, J., *J. Comp. Neurol.*, **119**, 77 (1962). Jassik-Gerschenfeld, D., Ascher, P., and Guevara, J. A., *Arch. Ital. Biol.* (in the press).

⁶ Sprague, J. M., *Anat. Rec.*, **145**, 288 (1963). Lund, R. D., *Nature*, **204**, 1283 (1964).

PHARMACOLOGY

Thromboses in Large Veins and Pulmonary Embolisms induced by Catecholamines or Serotonin

It has recently been noted that a singular thrombohaemorrhagic phenomenon can be elicited in rats by the intravenous injection of various metals or sulphated polysaccharides (which act as 'sensitizers'), followed by subcutaneous treatment with catecholamines or serotonin (which act as 'challengers'). The basic structural lesions always consist of multiple haemorrhages and micro-thromboses, thus resembling those of the Sanarelli-Shwartzman phenomenon (induced by two properly spaced injections of bacterial endotoxins). In its simplest form, this thrombohaemorrhagic phenomenon is obtained by the intravenous administration of a sensitizer (for example, indium chloride) and the concurrent subcutaneous injection of a challenger (for example, adrenaline). In this case the lesions occur topically at the site of challenge; however, after similar sensitization, the systemic administration of suitable challengers can produce thrombohaemorrhagic lesions more or less selectively at various sites such as the salivary glands, heart, lung, kidneys, intestinal tract or even transplantable neoplasms¹⁻⁴.

The question arose whether this technique could be adapted to the production by naturally occurring compounds (such as catecholamines or serotonin) of experimental thromboses in large veins with subsequent pulmonary embolisms.

Eighty female Sprague Dawley rats with a mean initial body-weight of 100 g (90-100 g) were subdivided into eight equal groups and treated as indicated in Table 1.

Table 1. PRODUCTION OF VENOUS THROMBI BY SYSTEMIC SENSITIZATION WITH ScCl_3 AND TOPICAL CHALLENGE BY CATECHOLAMINES OR SEROTONIN

Treatment	Incidence (%) of thrombosis in femoral vein
NaCl	0
Adrenaline	0
Noradrenaline	0
Serotonin	0
ScCl_3 + NaCl	0
ScCl_3 + adrenaline	100
ScCl_3 + noradrenaline	100
ScCl_3 + serotonin	100

20 μg of adrenaline (adrenaline bitartrate, Brickman and Co.), 20 μg of noradrenaline ('Levophed' bitartrate, Winthrop Laboratories) and 200 μg of serotonin (serotonin creatinine sulphate, Nutritional Biochemicals, Ltd.) were used as challengers. All these amines were injected in 0.1 ml. of physiological saline into the musculature of the left thigh just underneath the femoral vein before its entrance into the inguinal canal. For control purposes, physiological saline was administered in the same manner. Scandium chloride (ScCl_3 , K and K Laboratories)—a particularly potent sensitizer for the production of het thrombohaemorrhagic phenomenon¹—was injected at a dose of 4 mg in 1 ml. water under light ether anaesthesia into the jugular vein 1 min before the administration of the challengers. All the animals were killed with chloroform 24 h later. The presence of thrombi was verified by inspection with a dissecting loupe and subsequent histological investigation of the femoral veins using the periodic acid-Schiff and multipurpose polychrome stains⁵.

As shown in Table 1, by itself the intramuscular injection of NaCl, adrenaline, noradrenaline or serotonin never produced thrombosis in the adjacent vein, while after sensitization with ScCl_3 all three biological amines (unlike the solvent itself) consistently gave positive results. The thrombi thus obtained were easily detectable by the naked eye (Fig. 1). They consisted of a white 'head' and a red 'tail'. On histological examination, the 'head' proved to be composed of aggregated platelet strands (Zahn's lines) surrounded by degenerating polymorphonuclear leucocytes; the 'tail' consisted of erythrocytes and fibrin threads. The 'heads' of the thrombi always pointed towards the heart and their composition was the same, irrespective of the challenger used.

The experiment was repeated afterwards under essentially similar conditions; but now the challengers were injected into muscles adjacent to the vena cava just distally from the origin of the renal veins. Here, large, almost occlusive thrombi of the cava were regularly



Fig. 1. Large thrombus occluding the left common iliac vein. The sharp line (arrow) dividing the white 'head' from the red 'tail' is clearly visible through the vessel wall.

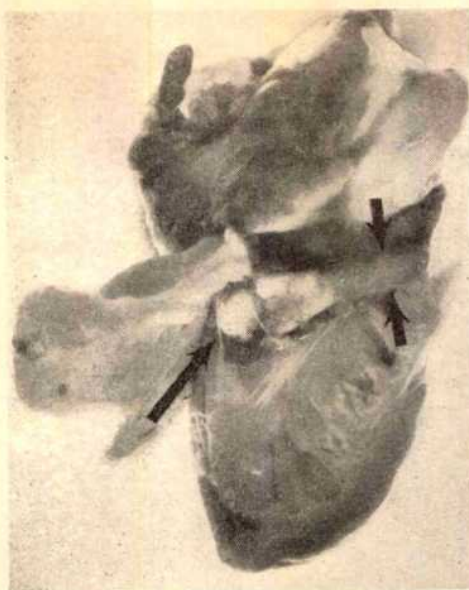


Fig. 2. Large embolus exposed by reflecting the lateral wall of the right ventricle. The white 'head' is lodged in the tricuspid valve (long arrow) while the red 'tail' protrudes into the pulmonary cone.

obtained in the scandium-sensitized animals (but not in the controls) given adrenaline, noradrenaline or serotonin.

In a third experiment, thrombi were produced in the iliac veins of ten rats given the same amount of ScCl_3 intravenously and then adrenaline into the pelvic muscles adjacent to the left iliac vein. Twenty-four hours later, the injection site was exposed under ether anaesthesia through a suprapubic incision and the thrombi mobilized by gentle massage with the thumb applied to the thigh pushing towards the inguinal ligament. In each instance, the detachment of the thrombus and its propulsion into the vena cava could be followed by naked-eye inspection. Somewhat unexpectedly the resulting large emboli did not kill any of the rats, although autopsy of five animals 15 min after mobilization of their thrombi showed these firmly lodged in the almost totally occluded pulmonary artery (Fig. 2). In the remaining five animals, which were killed 24 h after the operation, the lysis of the pulmonary emboli was virtually complete.

In all these as well as in many similar experiments, it was incidentally noted that (irrespective of any additional treatment) after death the blood of rats sensitized with ScCl_3 clots in the vessels much more rapidly than that of normal controls. This is of interest in connexion with the currently popular view that the haemorrhages in various thrombohaemorrhagic phenomena—including the Sanarelli-Shwartzman reaction—are due to a "consumption coagulopathy"⁶. According to this concept the clotting factors are largely used up during the thrombotic phase, whereupon the blood gradually becomes incoagulable. This explanation does not appear to be easily applicable to the metal-induced type of thrombohaemorrhagic phenomena with which we are concerned here.

This work was supported by the John A. Hartford Foundation and the Canadian Heart Foundation.

HANS SELYE
BEATRIZ TUCHWEBER
PAVEL ROHAN

Institut de Médecine et de Chirurgie expérimentales,
Université de Montréal,
Montréal, Canada.

Labelled Depolarizing Drugs in Normal and Denervated Muscle

THE antagonism between *d*-tubocurarine and depolarizing relaxant drugs has been investigated by means of a decamethonium derivative labelled with radioactive iodine, the uptake of which in muscle is reduced in the presence of the antagonist^{1,2}. The present investigations have shown that *d*-tubocurarine also reduces the uptake of decamethonium and of carbachol which have been labelled with tritium, and, in addition, the muscles have been sectioned so that a localized concentration of drug in the region of the nerve endings could be demonstrated.

Fig. 1 shows the uptake of 6 μM decamethonium dichloride (*N*-methyl-³H) in isolated rat diaphragm. After 2 h at 38° C the muscle was removed, secured to a strip of aluminium and frozen on a block of solid carbon dioxide. The nerve could be seen in the frozen muscle. The tissue was trimmed with a razor blade to provide a rectangle of muscle, which was then sectioned transversely between tendon and rib at intervals of 1 mm. The sections were weighed, dissolved and counted by methods used previously³. The uptake in each section was expressed as ml./g. being (activity per g muscle)/(activity per ml. solution). The uptake shows a pronounced peak in the section which contained the nerve and a similar distribution was found in 20 muscles which were sectioned. The presence of *d*-tubocurarine in a concentration of 7 μM reduced the uptake as shown by the shaded portion of Fig. 1, though the peak was not entirely abolished even with high concentrations (up to 70 μM) of the antagonist.

The uptake of decamethonium by the phrenic nerve was found to be low and not affected by 7- μM *d*-tubocurarine, and the peak in Fig. 1 may be attributed to the presence of the endplates which are known to be in a narrow zone close to the nerve⁴. Decamethonium injected into mice has been shown to become localized in the endplates of the diaphragm muscle^{5,6}.

The unshaded part of Fig. 2 shows the uptake of labelled decamethonium in a diaphragm muscle of the guinea-pig, which is pharmacologically more sensitive to decamethonium than the rat⁷. In the guinea-pig the terminal branches of the phrenic nerve are not distributed in a regular manner. A flattened peak was found in all guinea-pig diaphragms which were sectioned, and this peak appeared to be abolished in the presence of a high concentration of *d*-tubocurarine (Fig. 2).

The light shading of Fig. 2 shows the uptake of decamethonium in a muscle which had been denervated 10 days previously by section of the left phrenic nerve in the thorax. The uptake is markedly increased, and a similar raised level of radioactivity was found in denervated muscle as compared with the control in each of 12 pairs of muscles which were tested. In most cases no peak could be demonstrated in denervated muscle. The increased uptake of the drug may be correlated with the

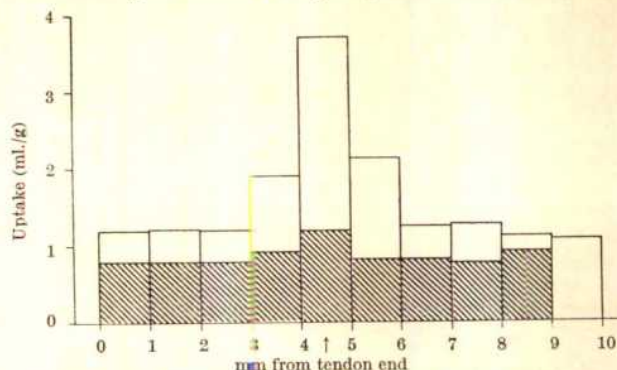


Fig. 1. Radioactivity of rat diaphragm cut transversely after 2 h in 6- μM labelled decamethonium. The uptake is given as ml./g. The section which contained the nerve is indicated by the arrow. The shaded area shows the uptake of another strip of muscle from the same diaphragm in the presence of 7- μM *d*-tubocurarine.

¹ Gabbiani, G., Jacquin, M. L., and Selye, H., *J. Pharmacol. Exp. Ther.* (in the press).

² Selye, H., and Tuchweber, B., *Proc. Soc. Exp. Biol.* (N.Y.), **118**, 680 (1965).

³ Selye, H., *Science*, **149**, 201 (1965).

⁴ Selye, H., *Perspect. Biol. Med.* (in the press).

⁵ Selye, H., *The Mast Cells* (Butterworth, London and Washington, 1965).

⁶ McKay, D. G., *Disseminated Intravascular Coagulation* (Harper and Row, Publ., Inc., New York, N.Y., 1965).

PATHOLOGY

Conversion of the Inconvertible L-1 Lymphosarcoma to Ascitic Form

DURING the course of experiments with the L-1 lymphosarcoma it was often desirable to have large numbers of single cells available. Suspensions of cells dissociated from minced tumour by standard techniques (cytosieve¹, trypsinization², etc.) contained variable numbers of morphologically dead cells and required large inocula ($\geq 10^4$ cells) to induce growth on subcutaneous re-inoculation. It seemed that the required numbers of free cells might be obtained if the tumour was converted to ascitic form. Following the method described by Klein and Klein³, cell suspensions prepared from the tumour by various techniques were intraperitoneally injected into strain A/J hosts. At graded intervals afterwards, the mice were killed, the open peritoneal cavity washed, and the collected washing re-inoculated into new mice. This was repeated for several fluid-transfer generations. In a course of more than twenty-five such attempts, a small growth of ascites was obtained in only a single instance. This one ascites grew slowly for three fluid-transfer generations and then failed to multiply on the fourth re-inoculation. In all other attempts, this procedure failed to produce ascitic growth. In many animals, however, nodular growths were obtained at the site of injection of the initial suspension, or on the peritoneal surface.

During the course of previous investigations in this laboratory, it was found possible to obtain relatively small numbers of highly viable tumour cells by trypsinization of minces of the liver and spleen, using the method described by Hewitt⁴. Comparable cell suspensions were also obtainable from the blood by modifications of the technique of Romsdahl *et al.*⁵. While neither of these techniques provided sufficient cells for certain of our requirements, it was reasoned that cells collected in this manner might be more amenable to the establishment of an ascites. Accordingly, a number of mice was injected with suspensions of cells collected in the foregoing two ways. In both groups, the peritoneal washings from almost all of the mice gave rise to the development of ascites when re-inoculated into new hosts.

Lines selected from these ascites have been serially transplanted at intervals ranging from 1 to 2 weeks for over one year and have continued to show good growth. Other lines, prepared by the same techniques, have also been successfully maintained for shorter periods. The ascites is composed predominantly of reproductively active cells, requiring somewhat less than ten cells on the average, to induce tumours in 50 per cent of previously untreated strain A/J mice (TD-50) when injected under the capsule of the kidney. The TD-50 for intraperitoneal injection is about an order of magnitude higher, while subcutaneous injection requires a yet larger inoculum. Although this aspect has not been examined in detail, it appears that the solid tumours resulting from the injection of ascites are morphologically similar to the original tumour and do not display any marked deviations in radiosensitivity, although they grow somewhat more rapidly. Interestingly, the lines initiated from circulating cells have consistently shown more rapid growth, both in solid and ascitic forms, than those started from cells obtained from the liver and spleen.

Of the various explanations for the foregoing results, a selection process seems most likely. Thus, cells which successfully enter the blood and which continue to circulate and, to a lesser extent, those which have travelled through the lymphatic or haematogenous circulation to lodge in the visceral organs, represent a selected population of cells which are better able to survive (and proliferate) in the free state. On the other hand, it may be that less mechanical damage occurs during the freeing of the cells

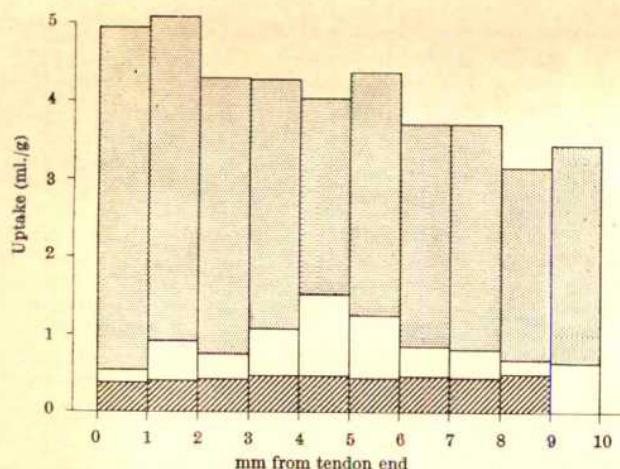


Fig. 2. The unshaded portion shows the uptake of labelled decamethonium in guinea-pig diaphragm treated as in Fig. 1. The dark areas show the uptake in another muscle in the presence of 70 μ M *d*-tubocurarine. The lightly shaded area shows the uptake in a muscle which had been denervated 10 days previously.

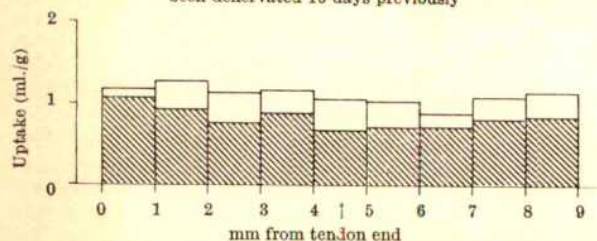


Fig. 3. Uptake of labelled carbachol in rat diaphragm (2 h in 1 μ M). The arrow indicates the section which contained the nerve. The shaded portion shows the uptake in another muscle which was treated with 7- μ M *d*-tubocurarine.

increased pharmacological sensitivity of denervated muscle^{6,7}. In similar experiments it was found that *d*-tubocurarine was also effective in reducing the uptake of labelled decamethonium in denervated guinea-pig diaphragm.

A localized peak was not found in the case of carbachol (carbaminoyle choline chloride, *N*-methyl-³H). Fig. 3 shows the uptake of labelled carbachol in rat diaphragm at a concentration of 1 μ M. The radioactivity of the muscle shows little variation from tendon to rib, and a similar distribution was found with concentrations of 5 and 50 μ M. Diffuse application of depolarizing drugs to the muscle is known to produce depolarization along the whole fibre¹⁰. Fig. 3 shows that *d*-tubocurarine (7 μ M) also diminished the uptake of labelled carbachol as compared with the control, and a similar effect was found in each of six pairs of muscles which were tested.

This work was supported by U.S. Public Health Service grant NB 00738, and also by a Wellcome research travel grant.

D. B. TAYLOR
R. CREESE
O. A. NEDERGAARD
R. CASE

Department of Pharmacology and Brain Research
Institute,
University of California Center for Health Sciences,
Los Angeles.

¹ Creese, R., Taylor, D. B., and Tilton, B., *J. Pharmacol.*, **138**, 8 (1963).

² Taylor, D. B., Creese, R., and Scholes, N. W., *J. Pharmacol.*, **144**, 293 (1964).

³ Creese, R., and Taylor, D. B., *Nature*, **206**, 310 (1965).

⁴ Hebb, C. O., Krnjević, K., and Silver, A., *J. Physiol.*, **171**, 504 (1964).

⁵ Waser, P., and Lüthi, U., *Arch. Intern. Pharmacodyn.*, **112**, 272 (1957).

⁶ Waser, P., *J. Pharm. Pharmacol.*, **12**, 577 (1960).

⁷ Bergh, N. P., *Scand. J. Clin. Lab. Invest.*, **5**, suppl. 7 (1953).

⁸ Axelsson, J., and Thesleff, S., *J. Physiol.*, **147**, 178 (1959).

⁹ Miledi, R., in *Enzymes and Drug Action* (Ciba Found. Symp.) edit. by Mongar, J. L., and de Reuck, A. V. S., 35 (Little, Brown and Co., Boston, 1962).

¹⁰ Thesleff, S., *Acta Physiol. Scand.*, **34**, 386 (1955).

lodged in the organs or collected from the circulating blood, so that a greater percentage is capable of reproduction. Also, the smaller number of dead cells and decreased amount of cellular debris in these cell suspensions may decrease the possibility of deleterious interaction with partially damaged cells.

MELVIN R. SIKOV*

Department of Radiology,
Wayne State University,
Detroit, Michigan.

* Present address: Battelle-Northwest, Pacific Northwest Laboratory P.O. Box 999, Richland, Washington, 99352.

¹ Snell, G. D., *J. Nat. Cancer Inst.*, **13**, 1511 (1953).

² Madden, R. E., and Burk, D., *J. Nat. Cancer Inst.*, **27**, 841 (1961).

³ Klein, G., and Klein, E., *Ann. N.Y. Acad. Sci.*, **63**, 640 (1956).

⁴ Hewitt, H. B., *Brit. J. Cancer*, **12**, 378 (1958).

⁵ Ronsdahl, M. M., Chu, E. W., Hume, R., and Smith, R. R., *J. Nat. Cancer Inst.*, **26**, 19 (1961).

Primary Tissue Phase of *Plasmodium berghei* in Different Experimental Hosts

In a previous communication¹, we reported the finding of pre-erythrocytic tissue schizonts of *P. berghei* in the liver of an experimentally infected young hamster. The forms observed were few in number, involving examination of many hundreds of stained liver sections. The sporozoites used in the former experiments were derived from experimentally infected *Anopheles quadrimaculatus*. In this laboratory vector only small numbers of viable sporozoites reached the salivary glands. The introduction of *Anopheles stephensi* as an experimental vector of *P. berghei*² has greatly facilitated and advanced our work, for in this mosquito species invasion of the salivary glands by very large numbers of sporozoites takes place with regularity after exposure to gametocyte carriers and maintenance at 21° C.

Using techniques of homogenation of infected *A. stephensi* or the trituration of infected salivary glands and midguts, we have been able, by intravenous and intraperitoneal inoculation, to inject large numbers of viable sporozoites, and we have easily found pre-erythrocytic growth forms of *P. berghei* in parenchyma cells of the liver of experimental rodent hosts.

Tissue schizonts have been found in liver sections stained in Giemsa colophonium³ in experimentally infected laboratory-bred tree rats (*Thomomys surdaster*), in hamsters (*Mesocricetus auratus*) in young albino rats^{4,5}, and in white mice.

The inoculation of 500,000–750,000 sporozoites in each of these mammalian hosts of *P. berghei* has produced four to seven tissue schizonts per histological section (1 cm × 0.5 cm in diameter and 4μ in depth). The pre-erythrocytic schizonts show similar morphological features in the different hosts. Growth forms of 18 h, 22 h, 36 h, 48 h, and mature schizonts 51–52 h old were found in the different experimentally infected animals. A search for the early stages of 3–18 h is now in progress.

The regular experimental production of pre-erythrocytic tissue stages of rodent malaria under well-defined laboratory conditions may facilitate research in the chemotherapy and chemoprophylaxis of mammalian plasmodial infections as well as in other fields of malaria research.

A detailed description of the exo-erythrocytic development of *P. berghei* and the techniques involved will be published shortly.

This work was conducted under the sponsorship of the Commission on Malaria,

Armed Forces Epidemiological Board, and supported in part by the Office of the Surgeon General, U.S. Department of the Army, and by research grant AI-02423 from the National Institute of Allergy and Infectious Diseases, U.S. National Institutes of Health.

MEIR YOELI
JEROME VANDERBERG
RASMA S. UPMANIS
HARRY MOST

Department of Preventive Medicine,
New York University School of Medicine,
New York.

¹ Yoeli, M., and Most, H., *Nature*, **205**, 715 (1965).

² Yoeli, M., Nawrot, R., Vanderberg, J., and Most, H., *Amer. J. Trop. Med. and Hyg.* (in the press).

³ Shortt, H. E., and Cooper, W., *Trans. Roy. Soc. Trop. Med. and Hyg.*, **41**, 427 (1948).

⁴ Yoeli, M., and Most, H., *Amer. J. Trop. Med. and Hyg.* (in the press).

⁵ Yoeli, M., *Trans. Roy. Soc. Trop. Med. and Hyg.*, **59**, 255 (1965).

Virus Aetiology for Down's Syndrome (Mongolism)

For many years now, we have been working on the epidemiology of Down's syndrome (mongolism) in the State of Victoria, Australia, and have charted its occurrences during 1942–64. Peaks of incidence, of 2-year duration have been recorded at 5–7 year intervals from 1942 until 1957 and, as a result of this, a further peak of occurrence for this congenital anomaly was forecast for 1962–63. This was the first time ever that such a forecast had been able to be made and, in fact, this eventuated^{1,2}. On the basis of our original findings^{1,2}, we had postulated a hypothesis of an infective virus, of long incubation, affecting mostly, but not exclusively, the ovum of the ageing mother, either directly or through some immunity pattern. Our reasons for this were not only the perception by one of us (A. S.) of a possible clinical relationship between the exposure of the mother to infective hepatitis prior to conception, but also the epidemiological findings that cases of mongol births clustered significantly in time and place, that urban peaks of annual incidence were in every case greater than rural peaks (higher contact rates), and rural peaks followed on

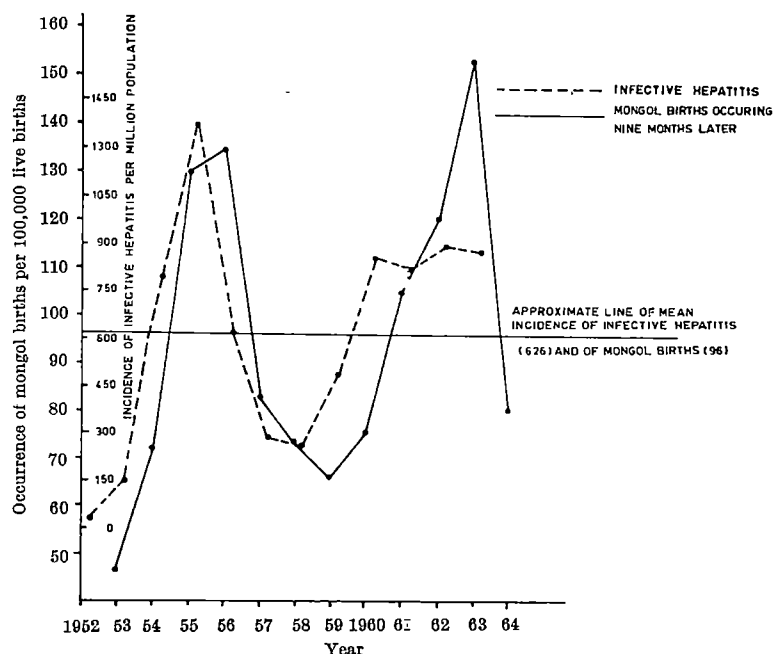


Fig 1 Annual incidence of infective hepatitis in Melbourne, and of mongol births 9 months later for the period 1952 to 1964

one year after urban peaks (suggesting a slow spread of infection out of the high-contact urban areas into the rural areas). Our latest investigations have consisted of an attempt to match the annual occurrence of mongolism in the Melbourne area with the annual occurrence of notifiable infectious diseases during 1952-64, 1952 being the year that infective hepatitis first became notifiable in the State of Victoria. Of all infectious diseases, the incidence of infective hepatitis, charted nine months prior to that of mongolism, has alone shown concordance (Fig. 1); another link in the chain of evidence we had forged relating mongolism to the virus of infective hepatitis, or some process associated with the infection, affecting the ovum prior to, or about the time of, conception. The correlation coefficient between the incidences of mongols, per 100,000 live births, and those of infective hepatitis, per million of population, was 0.81, significant at the level of $P < 0.01$.

We would, in fact, postulate that such a process of virus/human interaction could well be the basis of other genetic anomalies. We have already noted some degree of concordance between peaks of hydrocephaly and mongolism³, even though, in the former, no visible chromosomal abnormality is apparent. A prospective clinical investigation is being put into operation to test the association between infective hepatitis and the occurrence of mongolism; and immunity patterns for this virus, as soon as feasible, will need to be tested on a large group of mothers 'at risk'. Meanwhile, also, examination of the effects of viruses on human cells in culture, and of ovarian tissue in particular, might well open up an undreamed-of prospect of obtaining insight into the production, and thence prevention, of many gene disturbances.

ALAN STOLLER
R. D. COLLMANN

Mental Health Research Institute,
Royal Park, N.2,
Victoria, Australia.

¹ Collmann, R. D., and Stoller, A., *Amer. J. Publ. Hlth.*, 52, 818 (1962).

² Stoller, A., and Collmann, R. D., *Med. J. Austral.*, 1, 1 (1965).

³ Collmann, R. D., and Stoller, A., *J. Ment. Defic. Res.*, 76, 22 (1962).

RADIOBIOLOGY

Modification of Radiation 'Cerebral Death' by Hypoxia

PROTECTION from radiation damage by induction of brief periods of hypoxia at the time of irradiation has been demonstrated for a number of intact mammalian tissues¹⁻⁴. There is at present no information available concerning this 'oxygen effect' in the central nervous system. This knowledge is essential if radiotherapy under hypoxic conditions is planned for cerebral tumours. Cerebral damage in the experimental animal following single doses of 2,000-3,000 rads does not normally appear for 6 months or more, so in our attempt to demonstrate this phenomenon we used much higher doses of radiation that give survival times of the order of hours and minutes.

Young adult male Swiss albino mice from a closed colony were used. The animals were anaesthetized with 'Nembutal' (60 mg/kg intraperitoneally) 15 min before irradiation, and irradiated singly in a 'Perspex' box fitted with a gas supply. The box was inserted into a pressed wood block so that the mouse was irradiated head on by a beam of 8-MeV electrons (delivered from the Medical Research Council Linear Accelerator at Hammersmith Hospital) at a dose-rate of 1,600-1,800 krads/min with doses ranging from 50 to 400 krads. Dosimetry was carried out with 'Perspex' disks, and the maximum absorbed dose was recorded at a position level with the brain, 1 cm in from the animal's snout.

Control irradiated mice were supplied with air at a flow rate of 3.5 l./min. Mice irradiated under hypoxic condi-

tions were given pure nitrogen to breathe for 50 sec, with the irradiation given in the last half of this period so that the end of the irradiation coincided with an immediate change back to air. Five seconds or so after irradiation the mice were removed from the chamber and if necessary given positive-pressure artificial respiration with oxygen for 2-3 min or until they breathed spontaneously. One group of animals was given oxygen at 1 atmosphere (A) to breathe for at least a minute before and during irradiation and another set were injected with cysteamine (200 mg/kg, intraperitoneally) 15 min before irradiation in air. All survivals were timed from the end of irradiation.

The figure shows the fitted regression lines of log survival time on dose for air-breathing, air-breathing after cysteamine injection, oxygen-breathing and hypoxic animals. With air-breathing animals, a straight-line response was obtained over a dose range of 50-230 krads. Each point represents results from 6 to 9 animals. The regression lines of the air-breathing and air-breathing-plus-cysteamine animals were identical ($P < 0.001$). The 'oxygen' line, although parallel to the 'air' line, was not identical with it, but showed a shift to the right. The slope of the 'nitrogen' line was not parallel to the 'air' line ($P < 0.001$) and extrapolated below 50 krads crosses the 'air' line at 34 krads. Thus at approximately 50 krads the empirical radiosensitivity of air- and nitrogen-breathing mice would appear to be the same, while the protective effect of nitrogen increases with increasing dose. If one uses the ratio of the regression coefficients from the lines fitted for doses of radiation greater than 50 krads, then the dose modifying factor for nitrogen obtained here is 1.79 (95 per cent confidence limits, 1.64-1.94).

If radiosensitivity is judged by period of survival it is clear that nitrogen breathing has produced a definite protective effect for very high doses of radiation, but the detailed interpretation of the curves is extremely difficult. The apparent lack of effect of nitrogen breathing on radio-sensitivity below 50 krads might be due to the dose approaching the 'plateau' section (10-30 krads) of the curve that Rajewsky⁵ described for survival time-dose on a log log plot. (The survival times for air-breathing animals obtained here with 8-MeV electrons are in good general agreement with Rajewsky's data using 50-kV X-rays at

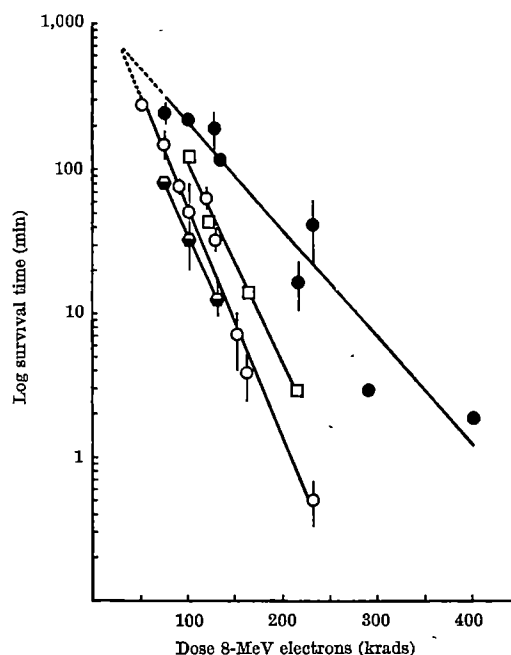


Fig. 1. Survival times of mice (min) plotted against dose of 8-MeV electrons (krad). ○, Animals breathing air throughout; ●, animals given cysteamine, 200 mg/kg, 15 min before irradiation; □, animals given pure oxygen for at least 1 min before and during irradiation; ●, animals irradiated during last 6 to 12 sec of 50-sec period of breathing nitrogen

10^6 r./min.) An alternative suggestion is that the whole effect is due to the consumption of oxygen by the radiation as demonstrated by Dewey and Boag⁶. They showed that approximately 60,000 rads were required to consume all the oxygen dissolved in an agar gel which was in equilibrium with air at one atmosphere pressure. A mammal breathing air contains much oxygen in its oxyhaemoglobin and oxy-myoglobin and is continually renewing its supplies by breathing. Thus, even with the very high dose rates used here, it is probable that the consumption of oxygen by radiation is of little significance in air-breathing animals. The situation is, however, different for the animals at the end of a period of breathing nitrogen, for not only is the animal's oxygen content reduced but the supply of oxygen is cut off. The consumption of oxygen by the radiation might now be all-important. It would have to be argued that the oxygen tension is not reduced to a level that significantly affects radiosensitivity until 40 to 60 krad have been delivered. Thereafter, increasing doses increasingly render the tissues hypoxic. If this idea is correct it implies that nitrogen breathing, which is known to produce radio-resistance of other tissues, does not under these conditions directly produce radio-resistance in the central nervous system, and that to demonstrate an effect some other additional method of consuming oxygen must operate.

The lack of effect of cysteamine is not surprising in view of the demonstration that the uptake of 35 S-labelled cysteamine in the mouse brain 15 min after injection reached only 1/5 of the amount taken up by the kidneys, liver and gut⁷.

The slight protective effect of oxygen cannot be explained. Protective cerebral vasoconstriction as a defence against oxygen poisoning was suggested in 1921⁸ and there is experimental evidence that breathing O_2 at 1 atm. results in cerebral vasoconstriction⁹ and decreased cerebral blood flow¹⁰. But polarographic studies show increased brain oxygen tension when O_2 at 1 atm. is given^{11,12}. Rectal temperature measurements of anaesthetized mice placed in a stream of O_2 show, in our hands, no greater fall than do those of mice in a stream of N_2 , so a differential cooling effect is not involved.

In conclusion, we can say that nitrogen breathing produces a marked change in radiosensitivity to massive doses of radiation. The mechanism by which this change is brought about is not yet understood, and probably awaits greater knowledge of the fluctuations in available tissue oxygen in the brain.

We thank Mrs. Ann Silvester, Cyclotron Unit, Hammersmith Hospital, for carrying out the irradiations, and for the dosimetry of these experiments. The work was supported by a grant from the British Empire Cancer Campaign for Research.

E. A. WRIGHT
JENNIFER SHEWELL

Department of Pathology,
St. Mary's Hospital Medical School,
London, W.2.

Acute Radio-sensitivity as a Function of Age in Mice

To understand the 'ageing' effects of the irreparable component of radiation injury, it is essential to accumulate much base-line data on natural chronological ageing. Earlier work¹ was designed to determine radiation sensitivity at different ages, using the fractionated dose method, which allows for biological repair between acute exposures. With this type of exposure, which is primarily a measure of repair from bone marrow injury, radio-resistance remains relatively stable through the first half of adult life and then deteriorates quite rapidly to the end of normal life expectancy². The work described here was designed to investigate variations in sensitivity to single acute X-ray exposures in various age-groups of mice.

Some 2,900 RF-strain virgin female mice of 14 age-groups were used (Table 1). Animals in each age-group were randomly divided into a minimum of 5 and a maximum of 12 acute X-ray exposure groups to bracket the LD_{50} dose. X-ray exposures were made with a 250-kVp X-ray machine with a beryllium window tube under conditions of 30 mamp, 'Thoraeus II' filter, 2.6 mm Cu HVL, 60 cm target-to-specimen distance, and 50 rads/min dose rate.

Table 1. LD_{50} VALUE FOR RF-STRAIN VIRGIN FEMALE MICE AT VARIOUS AGES THROUGHOUT THEIR NORMAL LIFE SPAN

Age (weeks)	No. of mice	LD_{50} (rads)	Standard error
3.6	26	593	14.6
6.0	136	647	18.9
8.0	210	691	14.8
12.8	190	733	13.6
21.4	200	697	15.8
30.0	233	634	20.3
42.8	151	487	18.7
51.4	120	547	36.9
60.0	57	537	16.0
68.6	125	453	5.16
77.1	179	424	22.8
85.7	149	442	17.6
94.3	52	441	12.5
103.6	32	343	18.6

To avoid seasonal effects, LD_{50} evaluations on all 14 age-groups were made within a 60-day period. Standard exposure methods described earlier² for determining LD_{50} values were used. Mice were observed for 30 days after exposure, and lethality data were analysed by standard methods of probit analysis³ to obtain the LD_{50} for each age-group.

Results are presented in Table 1 and Fig. 1. Radio-resistance increased with age from age 3.6 weeks, reaching maximum resistance at 13–17 weeks. At about 18–20 weeks of age, resistance declined throughout the remainder of the life span, reaching a minimum at 104 weeks. There was no apparent period of relative stability in radio-resistance, as was shown earlier¹ with the fractionated technique. Fig. 1 shows increased variability in radio-resistance with increased age after approximately 1 year

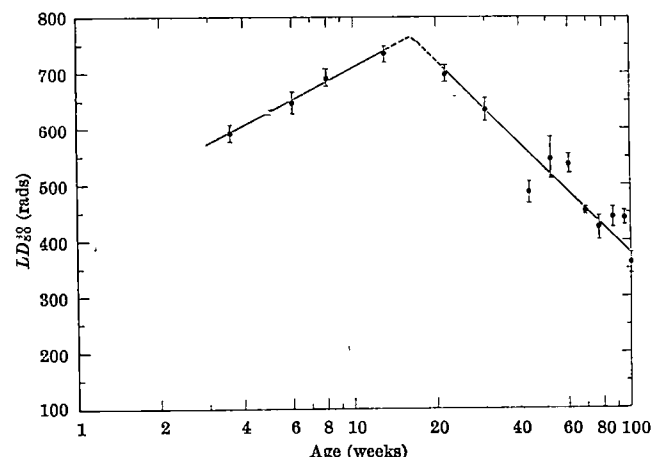


Fig. 1. Log plot of variation in radio-sensitivity with age in RF-strain virgin female mice

¹ Dowdy, A. H., Bennett, L. R., and Chastain, S. M., *Radiology*, 55, 879 (1950).

² Wright, E. A., and Batchelor, A. L., *Brit. J. Radiology*, 32, 168 (1959).

³ Wright, E. A., and Bewley, D. K., *Rad. Res.*, 13, 649 (1960).

⁴ Lindop, P. J., and Rotblat, J., *Nature*, 185, 593 (1960).

⁵ Rajewsky, B., *Radiobiology Symposium* 1954, p. 81, edit. by Bacq, Z. M., and Alexander, P. (Butterworths, London, 1955).

⁶ Dewey, D. L., and Boag, J. W., *Nature*, 183, 1450 (1959).

⁷ Verley, W. G., Bacq, Z. M., Rayet, P., and Urbain, M. F., *Biochim. Biophys. Acta*, 13, 233 (1954).

⁸ Dautrebande, L., and Haldane, J. S., *J. Physiol.*, 55, 296 (1921).

⁹ Kety, S. S., and Schmidt, C. F., *J. Clin. Invest.*, 27, 484 (1948).

¹⁰ Jacobson, I., Harper, A. M., and MacDowall, D. G., *Surg. Gynec. Obstet.*, 119, 737 (1964).

¹¹ Bean, J. W., *Amer. J. Physiol.*, 201, 1192 (1961).

¹² Davies, P. W., and Bronk, D. W., *Med. Prac.*, 16, 689 (1957).

is reached. This is a characteristic commonly associated with advancing age. An exponential function was a better fit to the data than a linear one. This also is characteristic of many biological parameters.

The difference in response to radiation exposure with age by the acute LD_{50} and the fractionated mean accumulated dose (MAD) methods is not surprising. The sub-lethal fractions given in the fractionated method primarily affect the bone marrow, causing the destruction of one or more stages in the maturation sequence of the erythrocytic series. However, body functions are maintained by the mature radio-resistant erythrocytes in the circulating blood until the bone marrow recovers sufficiently to provide new cell replenishment. Death from a series of widely spaced sub-lethal doses of ionizing radiation is due primarily to repeated damage to the bone marrow to the point at which the red blood cell level is reduced below that necessary to sustain life. Single acute exposures (used in the LD_{50} method), which result in lethality, cause injury to lymphatic tissue and gut, as well as to the bone marrow, and at the time of death in the majority of cases the total erythrocyte count is within the normal range or at least well above a lethal level. In short, death from exposure to ionizing radiation administered by the two different methods is the result of damage to different physiological functions. The comparative results of this and the earlier study¹ suggest a relatively stable repair rate from sub-lethal exposure throughout the first half of the normal life span but waning with age in the last half. However, there appears to be a constantly changing sensitivity in terms of lethality (irreparability) in the critical organs with increasing age. These results are in quite good agreement with those of Crossfill *et al.*⁴ and of Storer⁵. Pertinent literature in this field has been discussed elsewhere^{1,2,4-7}.

This work was performed under the auspices of the U.S. Atomic Energy Commission.

Biomedical Research Group,
Los Alamos Scientific Laboratory,
Los Alamos, New Mexico.

J. F. SPALDING
O. S. JOHNSON
R. F. ARCHULETA

¹ Spalding, J. F., and Trujillo, T. T., *Rad. Res.*, **16**, 125 (1962).

² Spalding, J. F., Trujillo, T. T., and LeSturgeon, W. L., *Rad. Res.*, **15**, 378 (1961).

³ Finney, D. J., *Probit Analysis*, second ed. (Cambridge University Press, London, 1952).

⁴ Crossfill, M. L., Lindop, P. J., and Rotblat, J., *Nature*, **183**, 1729 (1959).

⁵ Storer, J. B., *Rad. Res.*, **17**, 878 (1962).

⁶ Spalding, J. F., Trujillo, T. T., and LeSturgeon, W. L., *Rad. Res.*, **15**, 754 (1961).

⁷ Spalding, J. F., Trujillo, T. T., and McWilliams, P., *Health Phys.*, **10**, 709 (1964).

Distribution and Effective Half-life of Cobalt-58 in Habrobracon

COBALT isotopes appear in the wastes of reactor installations and are widely used in the control processes of other industries. They have also featured in biology and medicine for nutritional trace-element studies, particularly those involving vitamin B₁₂ (refs. 1 and 2). As an external source of γ -rays for therapeutic and experimental purposes, cobalt-60 has offered many advantages³. However, even though several cobalt isotopes occur in plankton, clams and fish near Bikini⁴, surprisingly few studies of the biological consequences of radiocobalt ingestion have appeared^{5,6}. Even the behaviour of inorganic cobalt has been neglected, despite interesting features of metabolism and transport⁷. Our observations made on a braconid wasp reveal differences between the insect and mammalian pattern of retention and suggest serious consideration of the role of insect vectors in dispersal of fourth period nuclides from contaminated areas.

Young habrobracon (*Bracon hebetor*) females from Whiting Stock No. 33 were fed a single meal from a mixture containing 100 μ c. of carrier-free $^{58}\text{CoSO}_4$ per ml. of

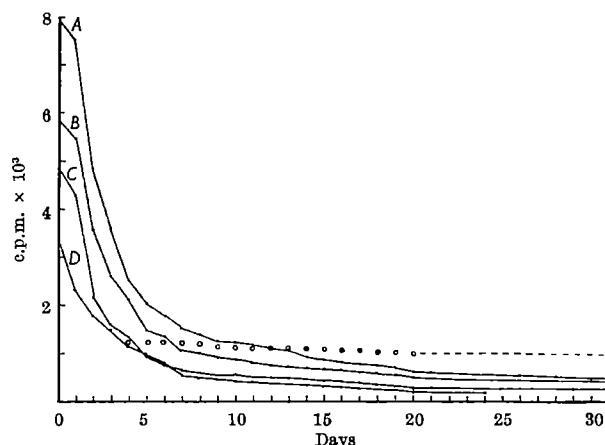


Fig. 1. The average daily radioactivity in c.p.m. of four groups of habrobracon females fed $^{58}\text{CoSO}_4$. Values are corrected for background but not for physical decay. Open circles which represent counts made on three dead wasps demonstrate the slight effect due to physical decay (72 day half-life). No. of wasps used in sub-samples: A, 6; B, 10; C, 9; D, 6.

saturated sugar water. Thus, the average wasp weighing 1.32 mg ingested about 0.5 μ c. of radiocobalt. The radioactivity of each of a sample of 31 wasps was determined daily until death, using a conventional scintillation well counter connected to an integrating decade scaler. The eggs deposited by these females were collected daily and their hatchability determined after 48 h. Another sample of 70 wasps provided groups of 5 individuals which were killed each day for the first nine days plus groups which were killed on days 13, 14, 16, 20 and 25. By counting, the radioactivity was assessed for the whole individual. Then the anterior and posterior parts transected at the petiole were counted separately. Finally, the gut, the fat body with urates and the genital system obtained by dissection were counted. Routinely the average of three counts per specimen was calculated.

Fig. 1 shows the pattern of decline in radioactivity for four sub-samples of wasps followed until death. The average life-span for wasps fed cobalt-58 was 25.6 ± 3.1 days, which compares well with 26.4 ± 2.0 days for controls. To avoid pooling individuals of extremely high and low radioactivity, the 31 females were divided into sub-samples according to their initial counts. Nevertheless in all sub-samples the effective half-life was attained by the third day following a meal containing cobalt-58. During this period most of the radioisotope was abdominal, falling from 98 per cent of the first 2 h through 94 per cent at the end of the first day to 90 per cent on the third day. Even after 20 days, 89 per cent of the cobalt-58 burden was abdominal.

The level of radioactivity of the gut and its contents paralleled that of the abdomen. On the first day, 46 per cent of the abdominal radioactivity was localized in the gut. On the twentieth day the gut still held 43 per cent of the abdominal radioactivity. Much of the remainder was associated with the fat and urate complex. The radioactivity of this tissue climbed from 35 per cent on day 1 to 42 per cent by day 3, and continued to increase in a long sloping plateau to a high of 47 per cent achieved on day 16. After the twentieth day, values fell to a 35 per cent average, understandable on the basis that only live survivors were considered.

Neither ovaries nor eggs became appreciably radioactive, although up to 20 per cent of the abdominal radioactivity during the first three days was associated with the poison apparatus. In contrast, by the third day, ovaries were barely above background, never having contained more than 3 per cent of the abdominal radioactivity. Egg radioactivity follows a simple pattern apparently correlated with the radioactivity of the mother:

Day	1	2	3	4	5	6	7 and later
Counts/mln.	5.52	2.03	2.39	0.73	0.43	0.35	Not above background

Unimpressive egg incorporation differs strikingly from the quantity of phosphorus-32 eliminated via oviposition⁸ and also from the notable third-day peak characteristic of zinc-65 experiments⁹.

Hatchability was low only on the first day (76 per cent). On the second day 98 per cent of the eggs hatched. This and subsequent values were not significantly different from control values.

In mammals, cobalt has been demonstrated to be one of the 'liver-seeking' elements^{10,11}, and on the basis of a rough analogy, localization in the insect fat body might be expected. In addition, the insect tissue has a propensity for storing excess amounts of metabolic products, not only as nutritional reserve but also as storage excretion. Thus histological conditioning towards a persistent radioisotope burden exists. Such an aspect of insect physiology helps to explain an effective half-life several times that in vertebrates where up to 67 per cent of a comparable radiocobalt dose is excreted by the end of the first day¹⁰; but there is also a prolonged association of cobalt with the wasp gut not entirely explained by the Malpighian-tube association. Most of the isotope can be precipitated along with the protein from homogenates of ground wasps. Such preparations on resuspension yield little dialysable cobalt-58, which suggests a firm association of cobalt-58 with tissue components. Furthermore, autoradiographs of ingested nickel-63 demonstrate that transition elements are retained by the cytoplasm of the cells of the mid-gut wall¹². Recently, Wiser and Nelson⁸ have reported a considerably longer time for the elimination of cobalt-60 from crayfish. Here integument adsorption plays an important part in addition to absorption by the gut and hepatopancreas.

Investigations of the bioaccumulation of isotopes of the transition elements appear to be essential for an understanding of potential environmental hazards in an 'atomic age', even though reproductive capacity is not specifically threatened.

This work was supported by U.S. Atomic Energy Commission contract AT-(40-1)-2836.

DANIEL S. GROSCH

Department of Genetics,
North Carolina State of the University of North Carolina,
Raleigh, North Carolina.

¹ Comar, C. L., *Radioisotopes in Biology and Agriculture*, 239 (McGraw-Hill Book Co., Inc., New York, 1955).

² Wilson, T. H., *The Physiologist*, 6, 11 (1963).

³ Glasstone, S., *Sourcebook of Atomic Energy* (D. Van Nostrand Co., Inc., New York, 1950).

⁴ U.S. Congress, *Hearings before the Special Subcommittee on Radiation. The Nature of Radioactive Fallout and its Effects on Man* (Gov. Printing Office: Washington, D.C., 1957).

⁵ Catsch, A., *Ann. Rev. Pharmacol.*, 3, 243 (1963).

⁶ Wiser, C. W., and Nelson, D. J., *Amer. Midland Nat.*, 72, 181 (1964).

⁷ Maynard, L. S., *Ann. N.Y. Acad. Sci.*, 72, 227 (1958).

⁸ Groesch, D. S., and Sullivan, R. L., *Biol. Bull.*, 105, 296 (1953).

⁹ Groesch, D. S., *Nature*, 195, 356 (1962).

¹⁰ Lee, C.-C., and Wolterink, L. F., *Amer. J. Physiol.*, 183, 167 (1955).

¹¹ Abrunina, G. A., in *The Toxicology of Radioactive Substances*, trans. by Travers, R. E., edit. by Lloyd, E., 2, 14 (Pergamon Press, Oxford, 1963).

¹² Groesch, D. S., Lin, J. C.-H., and Smith, R. H., *Radiation Res.*, 25, 194 (1965).

BIOLOGY

Experimental Infections of Cattle with *Fasciola hepatica*: a Comparison of Low and High Infection Rates

It has previously been demonstrated, in single infections of cattle with 200–1,300 metacercariae, that an increase in the infection level does not alter the percentage of the infection which becomes patent and that a few of the flukes are inhibited in the damaged parenchyma, particularly in the ventral lobe where a preferential migration of the parasite occurs¹. In subsequent investigations, parasite-free calves were given single infections of

Table 1

Infection level	Percentage take	Mature to immature ratio	Infection length
2,500 m	3	6 to 1	24 and 30 weeks
5,000 m	3.5	1 to 13	30 weeks
15,000 m	0.2	3 to 4	56 weeks
1,300 m	29	20 to 1	23 weeks

2,500, 5,000 and 15,000 metacercariae and killed 24–56 weeks post-infection.

The percentage takes, relative proportions of immature to mature fluke from calves which received 2,500, 5,000 and 15,000 metacercariae, and were killed 24, 30 and 56 weeks post-infection, are shown in Table 1 and compared with previous infections of calves with 1,300 metacercariae, killed at the 23rd week. When the level of infection is increased to 2,500 or more, the numbers of fluke reaching the bile duct is drastically reduced and at the higher levels of infection (5,000 and 15,000), many immature flukes are trapped in the liver parenchyma. That a large percentage of the infections proceeded to the parenchymal migrating stage is confirmed by the greater extent and severity of the cirrhosis present in the 2,500–15,000 infection livers compared with that present in the 1,300 group. The severity and extent of the fibrosis in the bile ducts in the high-level infections are considerably less than that present in infections of 1,300 metacercariae, and this observation, in conjunction with the absence of anaemia in the high-level infections compared with a severe anaemia which developed in the 1,300 infection group in association with the bile duct state infection, confirms that very few of the parasites in the high-level infections reached the bile ducts.

As was previously observed in lower level infections, the distribution of the cirrhosis produced by the migrating fluke was mainly confined to the ventral lobe of the liver. This preferential migration in the ventral lobe of the liver must, in high-level infections, enhance the local reaction and produce inhibition in the later migrating parasites. Many of these inhibited parasites were observed to be trapped within granulomatous tissue and many were dead and in varying stages of disintegration. Within these fibrous lesions considerable bile-ductule proliferation was observed and in some instances fluke eggs were present, indicating that a few parasites achieve patency within the granulomatous mass.

The inhibition phenomenon observed in these high-level infections may explain the rare occurrence of acute fascioliasis in cattle as compared with its high incidence in sheep. With massive infection levels in cattle there is quite considerable destruction of liver tissue, and it may perhaps be important to assess minimum liver tissue requirements in cattle if pathogenesis is to be fully understood.

J. G. ROSS

Veterinary Research Division,
The Farm,
Stormont, Belfast.

¹ Ross, J. G., *Proc. First Intern. Congr. Parasit.*, Rome (1964).

Haemoglobin in *Ceriodaphnia quadrangula* (O. F. Müller)

In the reports on the presence of haemoglobin in some of the representatives of the Daphniidae^{1–4}, I have found no data concerning haemoglobin in *Ceriodaphnia quadrangula* (O. F. Müller), quite frequently found in ponds and lakes.

In the marginal areas of ponds, large concentrations of this crustacean are quite often observed just below the surface of the water, giving it a red hue. I observed such a phenomenon in the middle of July in the ponds located on the park surrounding Białystok Medical Academy. Hydrochemical data of these ponds are given in Table 1. It is significant that the surface layer of the water con-

Table 1

Temperature °C	21.5
Colour mg/l. Pt	320.0
pH	7.1
O ₂ mg/l (surface)	0.6
Oxidizability mg/l. O ₂	107.0
Cl mg/l.	355.0
Fe mg/l.	—
N (NO ₃) mg/l.	0.3
N (NH ₃) mg/l	0.8
PO ₄ mg/l.	0.1
Ca mg/l.	210.0
Alkalinity mg/l. CaCO ₃	600.5
Total hardness mg/l. CaCO ₃	385.6

tained 0.6 mg O₂/l. whereas at a depth of 0.10 m no oxygen was found.

The population of this crustacean consisted chiefly of mature specimens of the *typica* form. The majority of specimens were 0.713–1.120 mm long. Haemoglobin extraction was performed by the cyanide micromethod^{5,6} after which the absorption curve was determined by means of a Beckman 'DU' spectrophotometer. It was seen from this curve that maximum absorption occurs at a wave-length of 418 mμ (Fig. 1).

For comparison, the maximum absorption of haemoglobin of *Daphnia magna* Straus was determined. It should be mentioned that Hildemann and Keighley⁷, using the same solvent, obtained maximum absorption of *Daphnia magna* haemoglobin at a wave-length of 415 mμ. It has frequently been noted that, although two species of the same genus do not essentially differ morphologically, their haemoglobin has a different maximum absorption. I noted this phenomenon in larvae of the genus *Tendipes*⁸.

In addition to the above investigations, an attempt was made to determine the amount of haemoglobin present in one specimen of *Ceriodaphnia quadrangula*. For the standard curve, crystalline haemoglobin produced by 'Riedl' was used. The four estimates obtained were 1.0, 1.1, 1.1 and 1.2 γ/individual (mean 1.1 γ). The amount of haemoglobin in one specimen varies between 1.0 and 1.2 γ.

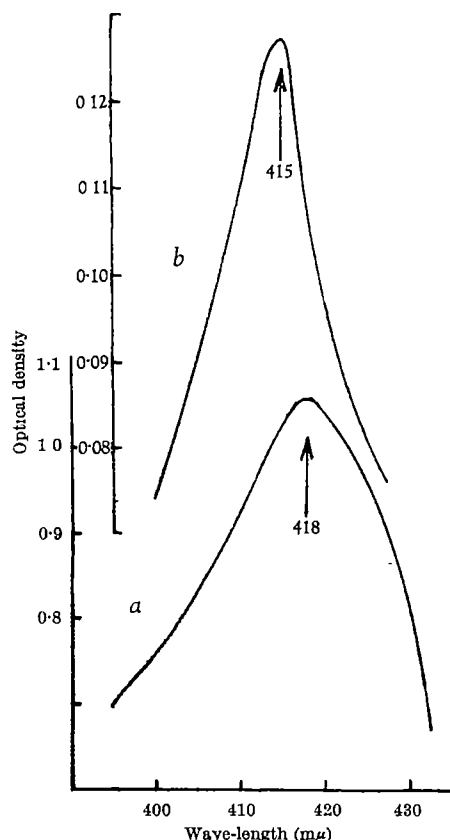


Fig. 1. Absorption curve of the haemoglobin of (a) *Ceriodaphnia quadrangula* and (b) *Daphnia magna*

These findings should be considered in relation to the environment, particularly with the amount of oxygen dissolved in it, since cases are known where the amount of haemoglobin increases where there is a small amount of oxygen in the environment^{9,10}.

Red specimens of *Leydigia acanthocercoides* (Fischer) were found in summer, 1963, in the layer just above the bed of Lake Sniardwy. The region round the heart, and the embryos in the spawn, were of a particularly intensive red colour.

These observations suggest that the presence of haemoglobin in invertebrates is usually associated with the occupation of ecological niches containing very little oxygen.

B. CZECZUGA

Department of Biology,
Bialystok Medical Academy,
Poland.

¹ Fox, H. M., *Proc. Roy. Soc.*, B, 135, 195 (1948)

² Green, J., *Proc. Roy. Soc.*, B, 145, 214 (1956).

³ Hoshi, T., *Sci. Rep. Tohoku Univ.*, 4th Ser., 23, 35 (1957).

⁴ Hoshi, T., and Nagumo, S., *J. Fac. Sci. Niigata Univ.*, Ser. D, 1, 85 (1964).

⁵ Czeczuga, B., *Rocz. Akad. Med. w Białymstoku*, 4, 143 (1958).

⁶ Czeczuga, B., *Rocz. Akad. Med. w Białymstoku*, Suppl. 8, 1 (1962).

⁷ Hildemann, W. H., and Keighley, G., *Amer. Nat.*, 89, 169 (1955).

⁸ Czeczuga, B., *Nature*, 186, 484 (1960)

⁹ Fox, H. M., and Taylor, E. R., *Proc. Roy. Soc.*, B, 143, 214 (1955)

¹⁰ Gilchrist, B., *Proc. Roy. Soc.*, B, 143, 136 (1954)

Kinesis

STUDIES on the behaviour of two stored-products beetles, *Carpophilus dimidiatus* F. and *C. hemipterus* L. (Col., Nitidulidae), in humidity gradients prompted a consideration of orientation mechanisms. It was evident that some clarification of the generally accepted classification^{1,2} and its amended forms³⁻⁵ was required, particularly with respect to the definition and interpretation of the various components of kinetic responses.

Kineses, that is variations in random locomotory activity due to changing intensity of stimulus, are classified into ortho- and klino-kineses². The former are defined as variations in either activity, speed or both. The activity of an animal is generally recognized as its frequency of movement. There appears, however, to be some confusion regarding the speed component, which indicates the animal's rate of movement, that is, the distance it covers in unit time. In many studies the time spent motionless by the animal is often included in the time factor of the relationship, distance covered in unit time. Consequently the animal's speed will also involve its frequency of movement (activity) and in this context it may be considered as representing a parameter approaching the 'overall' orthokinetic response. Use of this interpretation of speed will thus invalidate comparison with other species, as it will not be known to what degree the animal's frequency of movement is contributing to the speed component of orthokinesis. The exclusion of resting periods (that is, time spent motionless) from the time factor in the relationship, distance covered per unit time, will therefore indicate the animal's true speed, completely dissociated and independent of activity. An interpretation of this nature provides an objective basis for any comparative studies.

Another point worthy of mention concerns klinokinesis, the concept of which is based on the work of Ulyott⁶, where alteration in rate of change of direction (r.c.d.), later termed angular velocity¹, results in aggregation. It was assumed that changes in angular velocity reflected changes in rate of turning, a parameter which has been the subject of many studies. Alteration in angular velocity, however, may also be brought about by alteration in size of turn alone or by both rate of turning and size of turn. A mathematical analysis of Ulyott's data by Patlak⁷ indicated that it was the size of turn and not

the rate of turning which altered, and hence invalidated the previous assumption that alteration in r.c.d. (angular velocity) reflected a change in rate of turning. It is clear that both rate of turning and size of turn must be taken into account when analysing klinokinesis. Further details are to be published fully elsewhere.

In investigations to determine the existence and nature of kinesis orientation mechanisms, apparatus is invariably employed with little or no regard to the type and nature of the sub-stratum on which the behaviour of the experimental animal is to be observed. Such an item in experimental design may be considered of little importance in affecting behaviour, particularly as Chapman⁸ demonstrated that the surface texture of a heated hardboard plate was unimportant in group formation in nymphs of *Locusta*. It is possible that the animal's behaviour will be distinctly different when observed on various types of substratum, such as perforated zinc, brass wire gauze, steel screening, voile, bolting silk, nylon net and filter paper, all of which have been used in behavioural investigations. Moreover, the position of the apparatus relative to its immediate environment is relevant. Graham and Waterhouse⁹ found that minor inclinations in a 'Perspex' trough resulted in a downward slipping of *Tribolium castaneum* and a significantly uneven distribution. Consequently, studies preliminary to an investigation on kinesis orientation mechanisms in *Carpophilus hemipterus* L. (Col., Nitidulidae) were undertaken in which two of the four parameters of the kinesis response (see above), namely linear velocity (orthokinesis) and rate of turning (klinokinesis), were studied, with respect to two types of substratum.

Untreated adults of *C. hemipterus*, bred at 25° C and 65–70 per cent relative humidity and 7–21 days old, were examined individually under dim diffuse red illumination in uniform humidities of 100 per cent and 0 per cent relative humidities maintained in a round 'Perspex' chamber similar to that used by Gunn and Kennedy¹⁰. The time and route taken by each individual on the cambric (closely woven cotton fabric) false floor and the 'Perspex' roof of the chamber were recorded with a grease pencil on the 'Perspex' roof. Routes taken near and at the sides of these two regions were not taken into consideration, as were those made on the side walls of the chamber, because of the probable limiting influence of the apparatus on the animal's behaviour⁷. A number of tracks were obtained for each individual and these were then transferred to paper where the distance covered and the number of turns made per track were computed with respect to time. At least five individuals of each sex were studied for each humidity and substratum condition. An analysis of variance of linear velocity (cm per sec) and rate of turning (turns per sec) for the different conditions of humidity and substratum was carried out and a summary of the results is given in Table 1, where *H*, *S* and *P* represent humidity, sex and substratum factors respectively.

It can be seen from Table 1 that the type of substratum significantly affects linear velocity, and further analysis showed that a higher linear velocity occurred on cambric (0.51 cm/sec) than on 'Perspex' (0.42 cm/sec).

Table 1. SUMMARY OF RESULTS FOR ANALYSIS OF VARIANCE ON LINEAR VELOCITY (LV) AND RATE OF TURNING (RT) OF *C. hemipterus*

Nature of effect	Source	d.f.		Variance		'F' ratio	
		LV	RT	LV	RT	LV	RT
Main factors	<i>H</i>	1	1	0.0483	7.0×10^{-4}	3.3	1.7×10^{-4}
	<i>S</i>	1	1	0.0793	0.0285	5.4*	0.7
	<i>P</i>	1	1	0.5076	0.4379	34.8†	10.5*
Interaction between pairs	<i>H</i> × <i>S</i>	1	1	0.0281	0.0668	1.9	1.6
	<i>H</i> × <i>P</i>	1	1	0.0083	0.0995	0.6	2.4
	<i>P</i> × <i>S</i>	1	1	0.0218	0.0067	1.5	0.2
Interaction of all factors	<i>H</i> × <i>S</i> × <i>P</i>	1	1	0.0371	0.386	2.5	0.9
Replication	Residual	237	230	0.0146	0.0417		
	Total	244	237				

* Significant at 1 per cent level.

† Significant at 0.1 per cent level.

It is interesting to note that there is a sex reaction difference which was found to be due to the females being faster (0.47 cm/sec) than males (0.44 cm/sec). The effect of humidity on linear velocity was not marked, being significant only at the 10 per cent level, with a tendency for a higher rate of movement at 0 per cent relative humidity (0.47 cm/sec) than at 100 per cent relative humidity (0.44 cm/sec). The rate of turning was only affected by a difference in substratum with further analysis indicating a higher rate of turning on cambric (0.45 turns per sec) than on 'Perspex' (0.36 turns per sec). A factor which may be of importance in interpretation is the dimension in which the behaviour was observed, that is, vertical/horizontal, as it is known that, for example, the activity behaviour of *Porcellio scaber* differs between the horizontal and vertical planes¹¹. The behaviour of *C. hemipterus* individuals was studied in the same horizontal plane when on the two types of substratum, although their primary orientation² was different, as they were moving upside down on the 'Perspex' roof of the chamber whereas on the cambric false floor they were orientated normally. It is believed, however, that the differences in behaviour reported here are due principally to the difference in the type of substratum. Although concerned with only two kinetic orientation parameters, these results lend further support to the view that more consideration should be given to the type of substratum on which the animal's behaviour is to be investigated.

Part of this work was carried out while I was in receipt of a Treasury bursary.

T. G. AMOS*

Department of Natural History,
Queen's College,
University of St. Andrews,
Dundee, Angus.

* Present address: Tropical Stored Products Centre, Ministry of Overseas Development, London Road, Slough, Bucks.

¹ Gunn, D. L., Kennedy, J. S., and Picon, D. P., *Nature*, **140**, 1064 (1937).

² Fraenkel, G., and Gunn, D. L., *Orientation of Animals* (Oxford, 1940).

³ Ewer, D. W., and Bursell, E., *Behaviour*, **8**, 40 (1950).

⁴ Gunn, D. L., *Nature*, **155**, 17* (1945).

⁵ Kennedy, J. S., *Nature*, **155**, 78 (1945).

⁶ Ulliyott, P., *J. Exp. Biol.*, **13**, 265 (1936).

⁷ Patlak, C. S., *Bull. Math. Biophys.*, **15**, 431 (1953).

⁸ Chapman, R. F., *J. Exp. Biol.*, **32**, 126 (1956).

⁹ Graham, W. M., and Waterhouse, F. L., *Anim. Behav.*, **12** (2 and 3), 368 (1964).

¹⁰ Gunn, D. L., and Kennedy, J. S., *J. Exp. Biol.*, **13**, 450 (1936).

¹¹ Den Boer, P. J., *Arch. Neerl. Zool.*, **14** (3), 283 (1961).

Generation of Nutational Movements

In 1953, Arnal¹ described a characteristic of nutation in *Triticum coleoptiles* which had been hitherto unreported. His observation was that the movement of the coleoptile consisted of the propagation of a wave of bending, of more or less constant amplitude, from the tip of the growing zone towards the base of the organ. He noted that the increasing displacement of the tip from the mean position is caused by the increasing distance of the bend from the tip rather than by an increasing intensity of curvature of a fixed region of the organ. The organ was sometimes observed to possess two or more curvatures of opposite sense at different points along the length of the growing zone.

The purpose of this communication is to report a similar characteristic of the nodding nutations of seedlings of two varieties of *Phaseolus multiflorus* (runner bean). The observations reported here were made by means of time-lapse photographic techniques. The experimental conditions are outlined in Table 1. Bean seedlings at the 1st–2nd internode stage photographed under these conditions exhibited waves of bending as shown in Fig. 1. This shows silhouettes of bean seedlings at 15-min intervals, drawn from the time-lapse photographs. The numbered

brackets trace the course of each successive bend as it passes down from the tip. The positions of the bending regions were determined by careful comparison with the preceding frame. The rate of propagation of the bend was in all cases in the region of 2 cm/h. A similar pattern of movements has also been observed by me in horizontally growing, etiolated potato stems shown in a commercially available film *Plant and Light*².

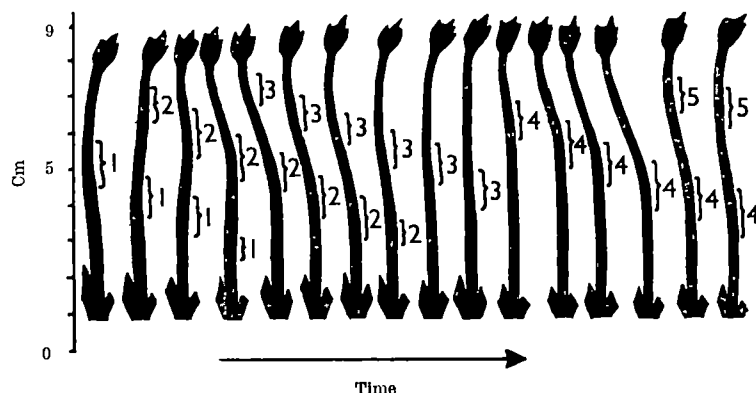


Fig. 1. Silhouettes of 'Prizewinner' beans at 15-min intervals. The numbered brackets trace the course of each bend.

From the rate of travel of the waves of bending, it is likely that the nutational oscillations are transmitted through some mechanism similar to the auxin transport system. The theory put forward by Arnal¹, which suggested that the nutational oscillation was a modulation of auxin production at the tip, has been disproved by Joerrens³, who observed nutation to occur in decapitated *Triticum* coleoptiles supplied with auxin-lanolin paste. It is possible that such patterns of movement could be generated by a modulation of the volume of polar transport, with the oscillations of opposite sides of the organ 180° out of phase. These observations are also compatible with Gradmann's⁴ theory which describes nutation as geotropic 'hunting' of the growing organ for the vertical position. This interpretation is doubtful in the case of the two varieties of *Phaseolus* examined by me which frequently do not 'overshoot' the vertical position, but 'nod' to one side only. Moreover, in the case of the orthogeotropic oscillations described by Bennet-Clark and Ball⁵ in *Aegopodium podagraria* rhizomes, the oscillations induced by a change from the normal orientation rapidly damp out whereas the oscillations of nutating plants continue throughout the growing period.

Table 1		
Variety	Series 1 'Prizewinner'	Series 2 'Desirée Stringless'
Lighting	Fluorescent Continuous, 10 lm/ft. ²	12/12 h photoperiod, 20 lm/ft. ²
Additions	None	2 × 200 J., 400 μsec duration. Electronic flash at 5-min intervals
Film	Ilford HP 35 mm roll	Kodak Tri-X reversal 16 mm
Temperature	19° ± 2° C	20° ± 1° C

The experimental work of Series 1 was carried out in the Department of Botany of the University of Birmingham and was supported by a Department of Scientific and Industrial Research studentship. I thank Dr. D. B. Idle for his advice.

DAVID G. HEATHCOTE

Department of Botany,
University College of South Wales and Monmouthshire,
Cardiff.

¹ Arnal, C., *Ann. Univ. sarav, Naturw. Sci.*, 2, 92 (1953)

² Film directed by Calibec, J., available from Educational and Television Films Ltd, London.

³ Joerrens, G., *Z. Bot.*, 49, 403 (1959)

⁴ Gradmann, H., *Jb. Wiss. Bot.*, 65, 224 (1926).

⁵ Bennet-Clark, T. A., and Ball, N. G., *J. Exp. Bot.*, 2, 169 (1951)

Thermal Contraction in Twigs

CHANGES in linear dimensions or volume of plant parts often are assumed to represent changes in growth. However, plants routinely undergo reversible changes in size resulting from hydration and thermal effects. Sometimes such reversible changes are not very large and do not complicate growth studies. At other times, however, they may actually exceed changes resulting from accretion through meristematic activity. For this reason more attention should be given to distinguish between total dimensional changes of plants and those caused by tissue growth alone. Hydration effects on plants are considered elsewhere^{1,2}. The present discussion deals primarily with thermal influences on dimensional changes in woody plants.

As early as 1906 Weigand³ noted that woody plants shrank considerably after exposure to cold. Daubenmire and Deters⁴ demonstrated that unseasonal drops in temperature not only decreased cambial activity but also caused considerable stem contraction. When temperatures dropped abruptly in the winter, contraction of tree stems occurred rapidly and often amounted to much more than the amount of cambial growth the previous summer. Such shrinkage was primarily a function of species and stem size⁵. Byram and Doolittle⁶ noted in North Carolina that, shortly after a cold spell beginning on January 19, the outer layer of living tissue of *Pinus echinata* stems contracted about 10 times as much as the mean daily shrinkage that occurred during the period of rapid spring growth. During the winter Small and Monk⁷ found highly significant correlations of average radial change of *Fraxinus* stems and air temperature. The highest temperatures usually occurred at noon, and major radial increases occurred at the same time. These experiments have been extended to compare the influence of low temperatures on twig diameters of both gymnosperms and angiosperms.

In the first experiment 10 twigs, each 6 in. long and 0.6–0.9 in. in diameter, were collected from each of 6 species including *Pinus sylvestris* L., *P. strobus* L., *P. ponderosa* Laws., *Acer platanoides* L., *Betula papyrifera* Marsh., and *Quercus robur* L. To minimize loss of water the cut ends of the twigs were coated with shellac and all twigs placed in sealed polythene bags. Small plastic disks had been glued to opposite sides of each twig to identify the points of measurement. A micrometer gauge with fine anvils and very light pressure override was used to determine twig diameters to 0.001 in. Small sections of bark were removed from each twig for determinations of thickness. After 2 days at 20° C the diameters of each twig were determined and the specimens were then transferred to a cold room at –20° C. Diameters were remeasured daily for three successive days and the twigs were then returned to a room for thawing at 20° C and final measurement.

After transfer from 20° C to –20° C stem contraction in all species, except *Pinus sylvestris*, was most marked after 24 h (Table 1). Thereafter further contraction was small. Retransfer to 20° C caused expansion, and in most cases diameters after thawing exceeded the original diameters. This anomaly probably resulted from some experimental error associated with loss of the plastic disks during thawing. Large differences in thermal contraction occurred among species. Shrinkage varied from less than 1 per cent of the diameter in *Pinus strobus* twigs to near 5 per cent in *Acer platanoides*. Twigs of angiosperms as a group contracted much more than those of gymnosperms. This was consistent with data of Winget and Kozlowski⁸, who noted a much lower winter contraction of stems of *Tsuga canadensis* than those of several species of angiosperms. In the work recorded here, thickness of bark did not

Table 1. EFFECT OF TEMPERATURE CHANGE ON TWIG DIAMETERS. MEASUREMENTS WERE TAKEN AFTER 24 H AT EACH INDICATED TEMPERATURE

Species	Mean diameter (0.001 in.)	Mean bark thickness		Diameter change (as % of original diameter at 20° C)				
		(0.001 in.)	(% of diameter)	-20° C	-20° C	-20° C	3° C	20° C
<i>Pinus sylvestris</i>	780	47	12.0	-0.41	-0.87	-0.93	0.55	0.90
<i>Pinus strobus</i>	486	56	25.7	-0.23	-0.14	-0.52	-0.14	0.01
<i>Pinus ponderosa</i>	680	97	28.5	-1.26	-1.25	-1.35	0.52	0.44
<i>Quercus robur</i>	794	93	23.4	-2.50	-2.85	-2.88	-0.15	0.68
<i>Acer platanoides</i>	708	48	13.5	-3.90	-4.58	-4.49	-1.38	-1.30
<i>Betula papyrifera</i>	745	34	9.1	-2.31	-2.60	-2.55	0.72	1.29

correlate well with thermal contraction. Bark was about as thick in *Pinus ponderosa* as in *Quercus robur*, but twigs of the latter species shrank much more. *Pinus sylvestris* had the thinnest bark of the three pines examined; but its twigs contracted more than those of *Pinus strobus* and less than those of *Pinus ponderosa* (Table 1).

In another experiment 10 branches, each 8 in. long and 0.7-1.0 in. in diameter, were collected from each of three species including *Quercus robur*, *Acer platanoides*, and *Fagus sylvatica*. The bark was then removed from five

of the specimens of each species. All specimens were coated with shellac to minimize loss of water, and plastic disks were positioned at opposite sides of each specimen to identify points of diameter measurement. Initial diameters were recorded in a room at 20° C. The specimens were then placed in polythene bags and transferred to a cold room at -4° C for remeasurement. The temperature was then decreased to -20° C and diameters were measured twice at 24-h intervals before transfer of the specimens to a room at 20° C. Then the twigs were stored for 14 days at -20° C and afterwards thawed at 20° C. Diameters of specimens with and without bark were measured in these warm and cold environments. After transfer from a warm to a cold environment all specimens with bark shrank much more than those without bark (Fig. 1). Twigs with bark often contracted several times as much as those without bark, even though bark thickness was much less than wood thickness. This emphasized that most of the contraction occurred in the bark. Some contraction of wood alone occurred, but this was usually a small amount.

To determine how much the bark alone would contract and expand in warm and cold environments, small samples of bark, each 2 in. × 1 in. × 0.5 in., of *Quercus robur* were used. Points for measurement were marked on each sample prior to coating them with shellac to minimize loss of water by evaporation. To test the efficiency of the seal the specimens were kept at 20° C for 24 h and then transferred to a cold room at -20° C. Thickness measurements were made at 20° C immediately after sealing, 24 h later at 20° C, and after 24 h at -20° C. The specimens were then thawed at 20° C for remeasurement. They were measured again 48 h later at 20° C, and once more 6 days later at 20° C, when they appeared to have lost considerable moisture. They were then transferred to -20° C for 48 h and remeasured. Five specimens of each species were then placed in polythene bags and 5 were left exposed. Final measurements were made after an additional 48 h at 20° C.

Marked shrinkage of the pieces of bark occurred during the first 24 h at 20° C because of water loss (Fig. 2), but

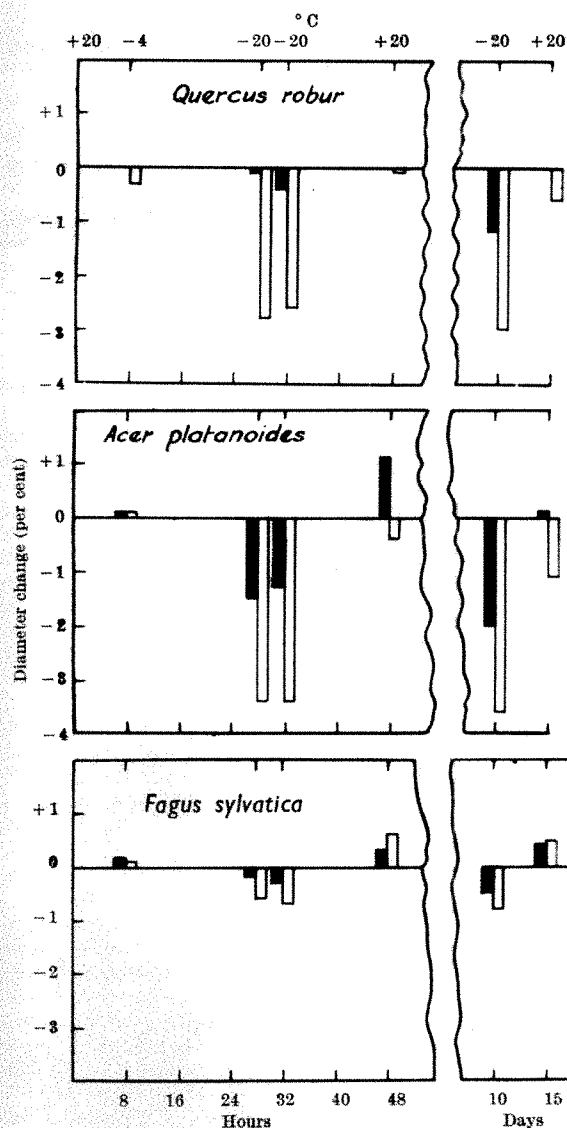


Fig. 1. Thermal changes in diameters of twigs of three species of angiosperms. Diameter changes are given as percentages of original diameters at 20° C. Black, without bark; white, with bark.

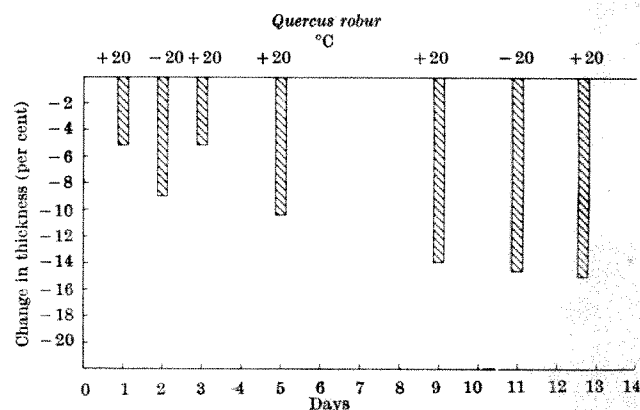


Fig. 2. Thermal and desiccation changes in isolated bark samples after exposure to warm and cold temperatures. Much of the contraction was caused by desiccation.

shrinkage was accelerated in the cold room at -20°C . On thawing the specimens expanded to the thickness at which freezing began, but shrinkage continued as water thereafter was lost at 20°C . The amount of contraction in the final freezing was negligible in comparison to that which occurred earlier, and recovery was slight. No recovery was observed in the five exposed specimens, and slight but not complete recovery occurred in the specimens in the polythene bags. This indicated that loss of water was occurring from the exposed specimens causing further contraction rather than expansion.

I. J. McCracken

Department of Forestry,
Commonwealth Forestry Institute,
University of Oxford.

T. T. KOZLOWSKI*

Department of Forestry,
University of Wisconsin,
Madison, Wisconsin.

* Fulbright research scholar in the Department of Forestry, University of Oxford.

¹ Kozłowski, T. T., *J. Forestry*, **61**, 655 (1963).

² Kozłowski, T. T., *Water Metabolism in Plants* (Harper Row, New York, 1964).

³ Wiegand, K. M., *Bot. Gaz.*, **41**, 373 (1906).

⁴ Daubenmire, R. F., and Deters, M. E., *Bot. Gaz.*, **109**, 1 (1947).

⁵ Winget, C. H., and Kozłowski, T. T., *J. Forestry*, **62**, 335 (1964).

⁶ Byram, G. M., and Doolittle, W. T., *Ecology*, **31**, 27 (1950).

⁷ Small, J. A., and Monk, C. D., *Forest Sci.*, **5**, 229 (1959).

Occurrence of Gnetalean Pollen Grains in the Katrol Formation (Upper Jurassic) of Kutch, India

WHILE carrying out palynological investigations of the samples from the Katrol formation (Upper Jurassic) of Kutch, pollen grains with close morphological affinity to *Ephedra* and *Welwitschia* pollen grains were observed along with plenty of Upper Jurassic spore pollen types and microplanktons (*Hystriosphæridium*, *Baltisphaeridium*, *Michrystidium*).

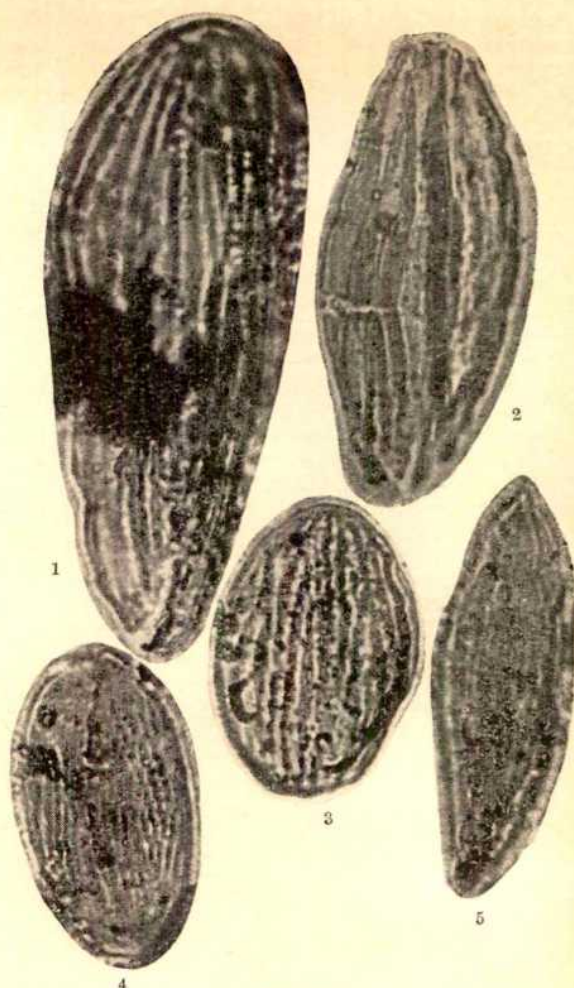
The samples consisted of black carbonaceous gypsiferous shales. These were digested in hydrofluoric acid for 24 h and the residue treated for 12 h with nitric acid and potassium hydroxide. The lighter fraction was separated by means of a heavy liquid of specific gravity 2.3 (a mixture of potassium, cadmium and zinc iodides). Permanent slides were prepared in glycerine jelly and sealed with vinyl acetate solution.

Ephedroid grains (Figs. 1-3) are perprolate, $48-106\mu \times 22-42\mu$ in size, ends rounded, exine thin with 14-24 ridges. The ridges were $2-3\mu$ thick, narrowing and converging towards the poles, $1-2\mu$ apart, alternating with furrows. Hyaline lines are not visible in any of the grains. Exine is characteristically Gnetalean. The morphological characters of the sporomorphs show close similarities to the pollen grains of *Ephedra*.

The sporomorphs with a morphological similarity to *Welwitschia* (Figs. 4 and 5) are perprolate, monocolpate colpa extending from one end to the other of the larger axis, exine scabrate, thick, marked by thin ridges comparatively close-spaced, size $67-50\mu \times 28-20\mu$.

The order Gnetales has a poor macrofossil record. In recent years, however, considerable evidence has been obtained on the occurrence of the group in the geological past throughout the world, based on pollen finds. The earliest record so far of pollen grains related to Gnetales comes from the Middle Permian of Oklahoma. Wilson¹ designated them as *Ephedripites* sp. and *Vittatina* sp., and considered them to be the ancestral forms of *Ephedra* and *Welwitschia*.

From Indian sediments, Bharadwaj² reported the occurrence of Gnetalean pollen in the Raniganj stage (Upper Permian), East Raniganj Coalfield; Ghosh *et al.*³ recorded *Ephedra*-type pollen from the Dharmasala beds (Lower Miocene to Oligocene) of Kangra District, Punjab;



Figs. 1-5

Wodehouse⁴ recorded pollen from the Pleistocene of Kashmir.

That the distribution of *Ephedra* during the Jurassic period extended as far as Kutch ($22^{\circ}45' \text{N}$. and 24°N . Lat. and $68^{\circ}15' \text{E}$. and 71°E . Long.) is interesting, for its present-day distribution is restricted to Kashmir and north-west India. It may be mentioned that *Ephedra*, a xeric plant, is to be found in dry regions, warm areas and also at high altitudes in the mountains.

Welwitschia, a monotypic genus, is restricted only to certain regions of south-east Africa, where extreme desert conditions prevail.

We thank Shri M. C. Poddar, deputy director of geology, for the collection of samples and advice. We also thank Shri V. V. Sastri, superintending geologist, Oil and Natural Gas Commission, for advice.

Y. K. MATHUR
K. MATHUR

Palynology Laboratory,
Oil and Natural Gas Commission,
Dehra Dun.

¹ Wilson, L. R., *Oklahoma Geol. Notes*, **18**, No. 2, 35 (1959).

² Bharadwaj, D. C., *The Palaeobotanist*, **9**, Nos. 1 and 2, 68 (1960).

³ Ghosh, A. K., Banerjee, D., and Srivastava, S. K., *Nature*, **198**, 408 (1963).

⁴ Wodehouse, R. P., *Pollen Grains (Mem. Conn. Acad.)*, **9**, 1935.

Increase during Winter in Capacity for Root Regeneration in Detached Shoots of Fruit-tree Rootstocks

THE ability of plants to form adventitious roots in detached portions of shoots has provided the horticulturist with a ready method of multiplying plants vegetatively by cuttings.

Fluctuations in the regenerative capacity of shoots throughout the year are well known, and plants which are considered difficult to propagate are often only rooted from cuttings during a limited period, usually when the shoot is in active growth^{1,2}. Hardwood cuttings, taken during the dormant season, are usually preferred wherever possible, however, mainly because elaborate control of the rooting environment is not necessary to ensure their survival.

The traditional time to plant hardwood cuttings in England is in the autumn³, when soil conditions are most suitable; but recent experiments with fruit tree rootstocks have shown that the capacity of the shoot to regenerate roots is at a low level at that time, but increases to a high level prior to regrowth the following spring (Fig. 1a, b, c). Hardwood cuttings of 'Crab C' and 'M.26' apple rootstocks and 'Myrobalan B' plum rootstock showed an increase in the number of rooted cuttings, and usually in the mean roots per cutting and mean root weight during winter. So far it has not been possible to observe this effect clearly when planting cuttings directly into the soil, as is the usual custom, because the environment changes markedly throughout the winter; but the development of a rooting environment for hardwood cuttings^{4,5}, in which they are subjected to a standard period of controlled temperature, has now overcome this difficulty.

Preliminary experiments involving low-temperature storage and bud removal suggest that this increase in

rooting is associated with the decreasing level of dormancy in the buds following winter chilling, a factor shown by other workers to be important^{6,7}.

B. H. HOWARD

East Malling Research Station,
East Malling, Maidstone,
Kent.

¹ Hartmann, H. T., and Brooks, R. M., *Proc. Amer. Soc. Hort. Sci.*, **71**, 127 (1958).

² Zimmerman, P. W., and Hitchcock, A. E., *J. Geront.*, **1**, 27 (1946).

³ Percival, J., *Agricultural Botany*, sixth ed., 260 (Duckworth and Co., 1921).

⁴ Hatcher, E. S. J., and Garner, R. J., *Rep. East Malling Res. Sta. for 1956*, 101 (1957).

⁵ Howard, B. H., and Garner, R. J., *Rep. East Malling Res. Sta. for 1964*, 88 (1965).

⁶ Lek, H. A. A. van der, *Meded. Landb. Hoogeschool Wageningen*, **38**, (2), 1 (1934).

⁷ Wareing, P. F., and Smith, N. G., *Rep. Forest Res., Lond., for 1962*, 120 (1963).

VETERINARY SCIENCE

Carcinogenic Activity of Bracken

THE bracken plant (*Pteridium aquilinum*) is known to contain radiomimetic activity. Ingestion by cattle of the whole plant, or of extracts, produces a syndrome in which there is pancytoid bone-marrow damage, pyrexia, and often gut-lining damage and ulceration¹; also typical are the widespread petechial haemorrhages².

Rosenberger³ reported the presence of haematuria in cattle fed bracken over long periods, and that changes of a polypous-tumorous nature occurred in the bladder mucosa. Georgiev⁴ obtained an extract from the urine of cattle fed hay from haematuria districts, which, when introduced into the urinary bladders of dogs, produced changes similar to a haemangioma. Applications of the same extract to the skin of white mice produced papilloma-type excrescences. Since many radiomimetic chemicals have a delayed carcinogenic effect it was felt worthwhile to test the possible carcinogenic activity of bracken directly.

Bracken frond was collected in June 1964 and oven-dried at 40° C for 48 h, after an initial period of 60 min in a tumbler dryer at 50° C, which removed most of the moisture. This material was then milled, mixed with milled Levers No. 4 rat diet in the proportion diet 66 per cent and bracken 34 per cent by weight, made into pellets and dried to avoid mould growth.

This diet was fed to 7-week-old, Glaxo strain, hooded Lister non-inbred rats, twenty of each sex (average male weight 125 g, female 110 g). It was found impossible to estimate the amount consumed due to the wastage incurred, but no other food was offered and the diet fed *ad lib.* for 64 days, from August 14 to November 11, 1964, inclusively and from January 8 to 18, 1965; the rats were then returned to the normal diet.

Since bracken also has a thiaminase enzyme quite distinct from the 'cattle factor'⁵, it was necessary to provide extra-dietary B₁. This was given by subcutaneous injection of B₁ (3 mg in 0.2 ml. physiological saline/rat) in the flank, while the animals were under light ether anaesthesia, on September 30, October 14 and October 30, 1964, respectively. Forty control animals of the same age and weight receiving the normal rat diet were given the same treatment; all these animals are alive and healthy.

To date (July 26, 1965) all 20 male rats and 14 of the females from the group fed the bracken diet have died or have been killed in poor condition, the first 3 males dying 29 weeks after the start of the experiment.

Post-mortem examination revealed the presence of numerous multiple tumours protruding into the lumen of the intestine; these occur throughout the small intestine, but predominantly in the ileal region. While most are of the order of 5–18 mm in diameter (Fig. 1), ten of the

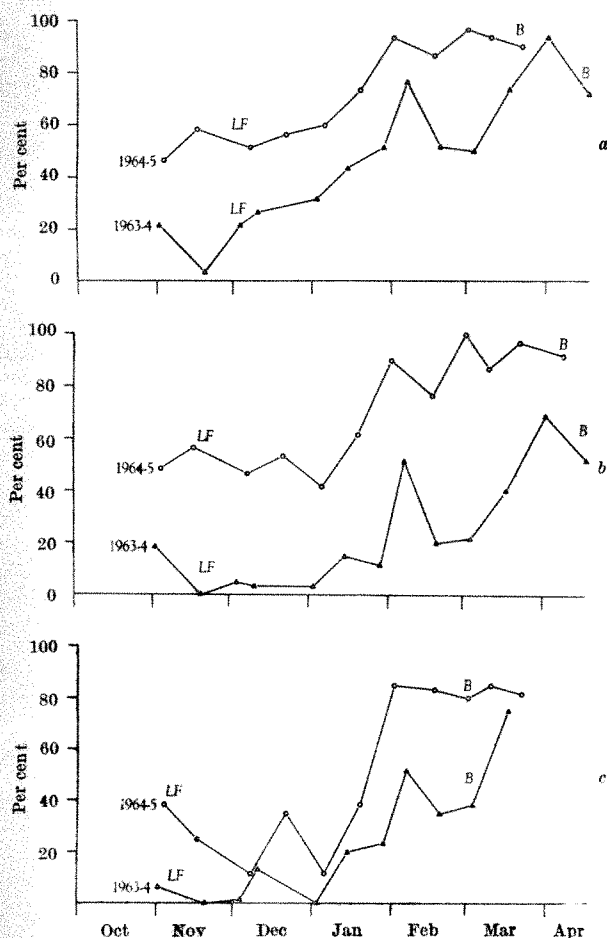


Fig. 1. Percentage rooting of 24-in. basal hardwood cuttings collected from randomized portions of hedges on the occasions shown and, after treatment with a root-promoting substance (γ -(3 indolyl)-butyric acid) rooted for 28 days in a peat-sand compost at 65° F minimum bottom heat in 1963-64 and 70° F minimum bottom heat in 1964-65. LF is the mean date between the beginning and end of leaf-fall, and B the inclusion of swelling buds on the cutting. Each value is the mean of 6 replicates of 10 cuttings, trends being significant, $P < 0.001$. a, 'Crab C'; b, 'M.26'; c, 'Myrobalan B'.

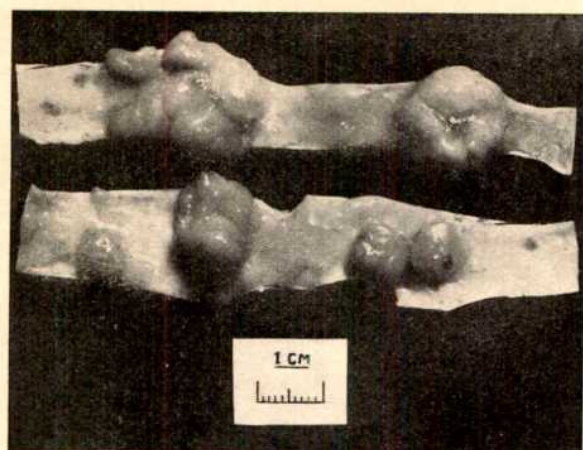


Fig. 1. Ileum opened longitudinally, showing adenocarcinoma

animals had developed a very large tumour 2-4 cm in diameter, as well as the other smaller widespread tumours. Frequently the larger tumours protruded through the muscle wall of the intestine, often with adhesions to the surrounding tissues and organs. Most of the animals scoured badly and some had intussusceptions caused by one of the tumours; in other cases a diverticulum or a ballooning of the intestine was found. The only animal not conforming to this general pattern was a female which developed a mammary tumour. One tumour has been found so far in four white rats fed the diet, but of 50 Swiss white mice fed, none has yet shown any effects.

Histological examination of these tumours has revealed proliferation of the epithelial layer of the intestine. Numerous mitoses can be seen in the columnar cells and, while the original structure of the villi is visible, the cells are more closely packed and several layers thick. Dr. R. Schoental of the Medical Research Council Toxicology Research Unit, Carshalton, kindly examined both animals and sections prepared from the tumours, and confirmed the presence of malignant adenocarcinoma of the mucosa.

Intestinal tumours in rodents are rare, although Schoental^{5,6} reports induction by polycyclic aromatic hydrocarbons and irradiation. Of interest is a recent private communication from Mr. W. H. Parker and Mr. C. T. McCrea (Veterinary Investigation Centre, Thirsk, Yorkshire) noting that in the course of a survey on the causes of death in sheep on the North Yorkshire Moors⁷, a number of older animals from bracken-infested areas were found to have died of the same type of tumour.

Work is now in progress to find the causative agent by trials with bracken extracts; the residue of fibrous material after extraction is also under test, to eliminate the possibility of an irritant action.

We thank Dr. Schoental and other members of the Toxicology Unit for their help and advice, Mr. I. S. Farr for the photography and Mrs. N. Mason for histological

preparations. One of us (J. M.) was supported by a grant from the Department of Scientific and Industrial Research.

I. ANTICE EVANS
J. MASON

Department of Biochemistry and Soil Science,
University College of North Wales,
Bangor, Caernarvonshire.

¹ Evans, W. C., Evans, I. A., Thomas, A. J., Watkins, J. E., and Chamberlain, A. G., *Brit. Vet. J.*, **114**, 180 (1958).

² Evans, W. C., Evans, E. T. R., and Hughes, L. E., *Brit. Vet. J.*, **110**, Parts I, II, III, 295, 365, 426 (1954).

³ Rosenberger, G., *Deutsche Tierärztliche Wochenschrift*, No. 8, 201 (1960).

⁴ Georgiev, R., Vrigasov, A., Antonov, S., and Dimitrov, A., *Wiener Tierärztliche Monatsschrift*, **196**, 589 (1963).

⁵ Schoental, R., and Bensted, J. P. M., *Brit. J. Cancer*, **17**, 242 (1963).

⁶ Schoental, R., *Nature*, **208**, 300 (1965).

⁷ Parker, W. H., and McCrea, C. T., *Vet. Rec.*, **77**, 861 (1965).

MICROBIOLOGY

Accumulation of Keto-acids in Tissues during Diphtheria Intoxication

AN earlier investigation¹ revealed that during citrate dissimilation by non-proliferating suspensions of *Klebsiella aerogenes*, diphtheria toxin caused increased accumulation of pyruvic and α -ketoglutaric acid. This observation suggested that diphtheria toxin acts by diminishing the utilization of keto-acids in tissues. It was, therefore, decided to investigate the effect of diphtheria toxin on the keto-acid content of blood and tissues in guinea-pigs.

A solution of Pope's purified diphtheria toxin was prepared by dissolving the toxin contained in a vial (kindly supplied by Dr. C. G. Pope) in 5 ml. distilled water. The solution contained 4,200 Lf/ml. Different doses of purified toxin were injected subcutaneously into guinea-pigs. After 18-24 h the animals were killed by stunning and bleeding from carotid arteries. Various tissues, including heart, kidney, adrenals, spleen and liver, were immediately removed. Tissue slices weighing approximately 0.5-1 g (or the entire organ when it weighed less than 0.5 g) were used in keto-acid estimations. 5 ml. blood was collected by cardiac puncture, immediately before killing the animals, for estimating keto-acids in blood. The tissue slices were homogenized in trichloroacetic acid, and α -ketoglutaric and pyruvic acids were determined by the method of Friedemann and Haugen². Similar techniques were used in normal guinea-pigs.

The results (Table 1) show that in diphtheria intoxication there is an increase in keto-acids in guinea-pig tissues. This is observed in liver, heart, spleen, kidney and adrenals, but it is fairly marked in spleen and adrenals. There is little or no increase in blood keto-acids.

It still remains to be seen as to how far this observation, that is the diminished utilization of keto-acids in diphtheria intoxication, can be interlinked with another observation reported earlier³ that diphtheria toxin inhibited acetylcholine synthesis in rat brain slices and this was probably due to a diminution in the content of the parent substrates, acetyl CoA and choline, which are necessary for acetyl

Table 1. KETO-ACIDS IN NORMAL AND DIPHTHERIA-INTOXICATED GUINEA-PIGS

Chemical estimations	Normal values average in 8 guinea-pigs	1	2	3	4	5	6	7	Average values	Difference*
(1) Blood pyruvic acid (μ g/ml.)	34 (24-42)	—	33	40	40	49	26	35	37.1	No difference
(2) Ketoglutaric acid in tissues (μ g/g moist tissue)										
Heart	15.7 (10.0-20.4)	52.8	32.6	35.0	22.0	18.8	17.5	21.8	28.6	Increase (82.1)
Liver	19.2 (15.7-24.6)	58.2	33.1	29.0	36.1	20.1	23.5	23.2	31.8	Increase (65.6)
Spleen	18.9 (10.0-27.6)	82.4	121.7	95.0	66.4	42.7	70.7	57.0	76.5	Increase (304.7)
Kidney	17.2 (10.8-31.8)	24.2	41.7	40.0	40.1	24.6	25.0	29.1	32.2	Increase (87.2)
Adrenal	24.04 (13.4-38.8)	89.8	56.3	42.0	57.8	52.2	30.0	37.4	52.2	Increase (112.9)
(3) α -Ketoglutaric acid in blood (μ g/ml.)	42.7 (30-50)	—	—	37.0	46.6	39.0	29.0	33.0	36.9	No difference
(4) Dose of diphtheria toxin (MLD)	—	10	40	70	18	18	70	18	—	—
(5) Weight of guinea-pig (g)	—	300	500	450	270	320	460	230	—	—

* Figures in parentheses indicate the per cent increase over the normal average values.

choline synthesis. It may be that diphtheria toxin causes a block in the conversion of pyruvate into acetyl CoA resulting in increased accumulation and non-utilization of pyruvate and diminished synthesis of acetyl CoA.

S. C. AGARWAL

Department of Microbiology,
Institute for Postgraduate Medical Research
and Education,
Chandigarh, Punjab, India.

¹ Agarwal, S. C., *J. Path. Bact.*, **87** (1), 186 (1964).

² Friedemann, T. E., and Haugen, Gladys E., *J. Biol. Chem.*, **147**, 415 (1948).

³ Agarwal, S. C., *J. Path. Bact.*, **79** (2), 313 (1960).

VIROLOGY

Inhibition of Influenza Virus Haemagglutination by Polymerized Orosomucoid

THE inhibition of haemagglutination by indicator influenza virus using mucoprotein inhibitors is a well-established observation¹. It has been proposed that for a molecule to inhibit viral haemagglutination it must not only contain sialic acid as an end-group susceptible to neuraminidase, but also be of 'minimum size'². Orosomucoid³, a serum glycoprotein containing 11 per cent of neuraminidase-susceptible sialic acid, however, does not inhibit viral haemagglutination⁴. It has been suggested that orosomucoid may fail as an inhibitor because its molecular weight of 41,000 is too low². We have found that incubation of this glycoprotein at 80° C caused it to polymerize in solution and to become an active inhibitor of certain strains of influenza virus⁵. Further observations on this phenomenon are reported here.

Orosomucoid was isolated from the urine of adult patients with the nephrotic syndrome, by means of chromatography on DEAE cellulose⁶. The preparation had been shown to be of high purity by electrophoretic, ultracentrifugal and immunochemical analysis⁶. Solutions of orosomucoid were prepared, 1 per cent in 0.9 per cent saline, and subjected to incubation for 1 h at the following temperatures: 37°, 56°, 66° and 80° C. A control solution was maintained at a room temperature of 20° C. Each of these solutions were then tested against indicator influenza virus as detailed here.

A further solution of orosomucoid in saline was subjected to analysis in the analytical ultracentrifuge before and after heating at 80° C for 1 h. In addition another solution of orosomucoid in 0.9 per cent saline was prepared of 1.6 per cent concentration and incubated at 37° C for 24 h. After specific time intervals, samples of the solution were withdrawn for testing against influenza virus *A/ENG/1/63*. These samples were then stored at 4° C for one month and then, with normal laboratory handling, re-tested against the same strain of virus. The control solution in this series was a solution of identical concentration in saline and stored at 4° C.

Indicator viruses were prepared as follows:

Influenza *A/PR8*, *A₁/ENG/55*, *A₂/ENG/1/63* and an *A₂* isolate of 1957 were diluted 1 in 5 in borate-citrate buffer (Clarke and Lubs's borate buffer⁷ pH 8.5, 1 vol., 0.2 per cent sodium citrate in 0.9 per cent saline, 9 vol.)⁸ and heated at 56° C for 30 min. Influenza *B* Lee strain was heated in undiluted allantoic fluid at 56° C for 30 min. Sufficient material was produced to carry out all titrations of orosomucoid at one time, except where samples of orosomucoid were re-tested one month later, when fresh indicator viruses were prepared under identical conditions from the original seed viruses. Eight haemagglutinating doses of indicator viruses were used with the exception of the Lee strain where four were used. Serial two-fold dilutions of orosomucoid were made from 1:100 to 1:51,200 for the heated preparations and from neat to 1:512 for the untreated controls. The cell suspension was 1 per cent fowl red blood cells. The unit volume was 0.25 ml., and the diluent was veronal buffered saline pH 7.2.

Table 1. INHIBITION OF INFLUENZA VIRUS BY HEATED OROSOMUCOID (Orosomucoid 1 per cent in 0.9 per cent saline)

Virus strain	Haemagglutination dose	Unheated	Haemagglutination inhibition titres*
LeeB	4	16	37°/1 h 2,560
<i>A₂/ENG/1/63</i>	8	128	56°/1 h >8,192
<i>A₁/ENG/55</i>	8	Neg.	67°/1 h >8,192
<i>A₁/57</i>	8	Neg.	80°/1 h >25,600
<i>A/PR8</i>	8	8	2 <50
			32 2,048 >8,192 12,800

* Expressed as reciprocal of initial orosomucoid solution diluted with veronal buffer.

Table 2. INHIBITION OF INFLUENZA VIRUS *A₂/ENG/1/63* BY OROSOMUCOID INCUBATED AT 37° C (Orosomucoid 1.6 per cent in 0.9 per cent saline)

Incubation period (h)	Haemagglutination inhibition titres
Control	160
1	320
3-25	1,280
5-25	>1,280
6-5	2,560
10-5	5,120
24	>5,120 <10,240

A haemagglutination dose of 8 units was used.

The results of ultracentrifugation are given in Fig. 1 and the inhibition investigations in Tables 1 and 2.

The interesting feature of the polymerization of orosomucoid on incubation at 80° C is the extent and degree of specificity of the reaction. Of the original monomer some 72 per cent had polymerized to a polymer of sedimentation constant 20.1S. Although γ -globulins are known to polymerize on incubating, the extent of polymerization is usually much smaller and of a much less specific nature⁹. These results were confirmed on two preparations of orosomucoid from individual patients. The results of inhibition (Table 1) confirmed that native unheated orosomucoid is not a significant inhibitor of haemagglutination by influenza virus. However, the heated material strongly inhibited haemagglutination by the strains *A/PR8* and *A₂/ENG/1/63* but not *A₂/57* or *A/55*. The possibility of orosomucoid becoming active against influenza virus at physiological temperatures is evident from Table 2, showing the results of incubation at 37° C.

Independent work by Morawiecki and Lisowska¹⁰ has also demonstrated inhibition of haemagglutination by influenza *B* virus (Lee strain), using orosomucoid polymerized by heating at 50° C for 24 h a 5 per cent solution in phosphate-acetate buffer pH 4.3 containing 1.3 per cent acetaldehyde. However, these workers stated that their heated 'control' solution, in acid buffer without acetaldehyde, gave polymers so unstable that the inhibiting effect against the Lee strain disappeared after 2-3 h. All the original incubated solutions described here retained their inhibition titre for several days, and it was specifically recorded that the solutions incubated at 37° C did not lose their activity against virus strain *A₂/ENG/1/63* when stored for 4 weeks at 4° C.

Morawiecki and Lisowska¹⁰ recorded an increase of 600-fold inhibition using their system of polymerization against their Lee strain of influenza virus. The present

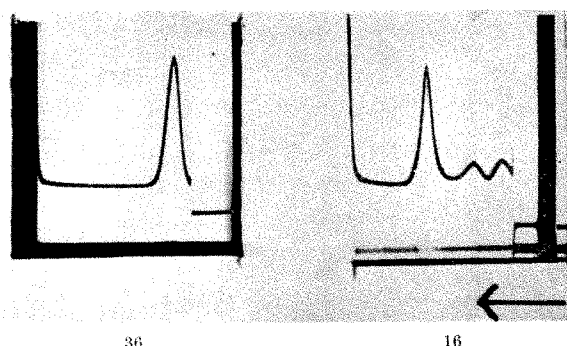


Fig. 1. The ultracentrifugation of orosomucoid (1.6 per cent in 0.9 per cent saline) before and after incubation at 80° C for 1 h. The figures below each photograph give the time of spinning in minutes

work would suggest that the net inhibition increase varies with the strain of virus. Of the strains listed here, the inhibition against the PR8 strain was increased by a factor of 1,600, against the $A_2/ENG/1/63$ by 200, and against the Lee by 160. However, no increase was recorded for the $A_2/57$ and $A/55$ strains.

Electron microscopic observations on the fraction heated at 80° C and negative stained with potassium borotungstate regularly recorded structures of diffusely fibrous appearance which were never present in the unheated material.

The observations recorded here must add new significance to the 'criteria of viral inhibition' as developed earlier by Gottschalk². However, it is still conceivable that polymerization is not the cause of this new biological property of orosomucoid, as other physical or chemical changes as yet undetected may ultimately prove responsible.

We thank Dr. D. R. Stanworth of the University of Birmingham for his assistance in the ultracentrifuge investigations.

P. H. WHITEHEAD
T. H. FLEWETT
J. R. FOSTER
H. G. SAMMONS

Pathology Laboratory and
Regional Virus Laboratory,
East Birmingham Hospital,
Birmingham, 9.

¹ Buzzell, A., and Hanig, M., *Adv. Virus Res.*, **5**, 289 (1958).

² Gottschalk, A., *The Chemistry and Biology of Sialic Acids and Related Substances*, 82 (Camb. Univ. Press, 1960).

³ Winzler, R. J., in *The Plasma Proteins*, edit. by Putnam, F. W., **1**, 309 (Academic Press, New York and London, 1960).

⁴ Tyrrell, D. A. J., *J. Immunol.*, **72**, 494 (1954).

⁵ Whitehead, P. H., *Biochem. J.*, **95**, 8p (1965).

⁶ Easton, J. A., Hardwicke, J., and Whitehead, P. H., *J. Clin. Path.*, **15**, 585 (1962).

⁷ Clarke, W. M., and Lubs, H. A., *J. Bact.*, **2**, 1 (1917).

⁸ Stone, J. D., *Austral. J. Exp. Biol. and Med. Sci.*, **27**, 229 (1949).

⁹ Edelman, M. D., Kunkel, H. G., and Franklin, E. C., *J. Exp. Med.*, **108**, 105 (1958).

¹⁰ Morawiecki, A., and Lisowska, E., *Biochem. Biophys. Res. Comm.*, **18**, 4 (1965).

CYTOLOGY

Demonstration of the Spiral Structure of Human Chromosomes

CHROMOSOMES have generally been regarded as consisting of coiled chromonemata. Although a number of cytological observations on the structure of chromosomes in plant germ cells have been conducted, as well as many studies on the fine structure of animal chromosomes at the molecular level, structural relations of chromonema and its molecular components are still poorly defined^{1,2}. Since the Denver Conference of Human Chromosomes Study Group, a number of morphological investigations have been carried out on the identification of each chromosome to establish the standard normal human karyotype, particularly with respect to the sites and relative frequencies of secondary constrictions³⁻⁵. There is general agreement regarding the normal karyotype; however, little or no morphological evidence has been presented to confirm the spiral structure of human somatic chromosomes. As an innovation to the technique for chromosome preparation, the following method has been developed which clearly demonstrates somatic coils through the partial dissociation of human chromosomes.

Peripheral blood leucocytes, harvested and maintained by standard methods, were used. Three-day cultures were treated with colchicine (final concentration of 0.06 µg/ml.) for 1 h at 37° C and the cells were suspended by shaking. The leucocytes were then concentrated by centrifugation in 15-ml. tubes and the supernate was discarded. The partial dissociation of the chromosomes was effected by treatment with 2-3 ml. of a 4:2:1 mixture of equimolar solutions of KCl, NaNO₃ and CH₃COONa. Originally,

this solution was developed by Matsuura *et al.*⁶ to obtain clear demonstrations of chromosome structure of the pollen mother cells of *Trillium*. Their procedure utilized 0.33 molar concentrations of these salts for 1.5-2.0 min. The optimal chromosomal dissociation of human leucocytes was obtained only after testing many combinations and ranges of the three parameters: concentration (range from 0.041 to 0.33 moles), time (20-150 min), and temperature (4°-50° C). The application of 0.055 mole of the three salts in the 4:2:1 ratio already described for 90 min at room temperature (21°-22° C) appeared to yield the best results, showing clear spiral figures without serious distortions of the chromosomal configuration (Fig. 1).

The salt concentration was found to be critical. Higher concentrations gave more severe uncoiling but did not give a clear figure. Lower concentrations gave slender, well-defined chromosomes but no visible spiralization. High temperature (37°-50° C) produced an indistinct chromosomal outline.

At the end of this treatment, 5 or 6 ml. of freshly prepared cold fixative solution (3 parts absolute methanol: 1 part glacial acetic acid) were added to this cell suspension, followed by a very gentle mixing with a pipette. About 30 min later, the fixative was exchanged with a fresh solution at least three times. One or two drops of cell suspension containing an adequate cell number were then dropped on a clean slide and allowed to dry. The preparations were scanned at this stage using phase contrast microscopy. After 1 or 2 h, slides were lightly stained with Giemsa, avoiding the loss of details by over-staining. Slides were then washed with running water for a few seconds and allowed to stand at room temperature for complete dehydration. After slides had been dipped in xylene for about 5 min, the coverslips were mounted with 'Permount'. The chromosomes were photographed using bright-field microscopy (Fig. 1) for an analysis of the number of gyres and the appearance of the secondary constrictions (Fig. 2).

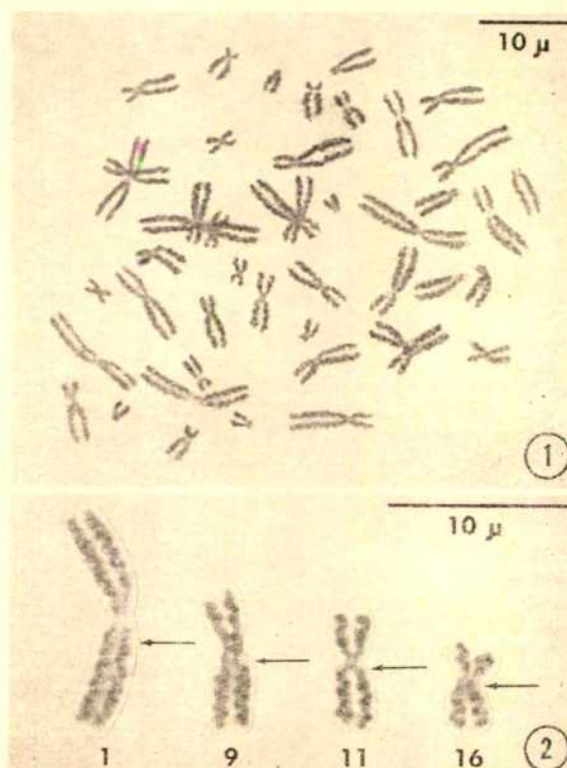


Fig. 1. Metaphase from a cultured blood cell of a male. The spiral structure (somatic coil) is clearly demonstrated.

Fig. 2. Chromosome Nos. 1, 9, 11 and 16 from the cell in Fig. 1 at higher magnification. Arrows indicate the loci at which secondary constrictions appear. Note the configuration of the chromonemata.

In addition to confirming the hypothetical helical configuration of human chromosomes, this study demonstrated that secondary constrictions are less tightly coiled regions of the chromonemata. Moreover, this technique offers the possibility of a more quantitative analysis of chromosomes at the chromonema level. If a reproducible gyre number is established, coil counts may become a more accurate method of determining arm ratios. The analysis of chromosome breaks or translocations, induced by treatment with chemicals or ionizing radiation, may be described more accurately with partial dissociation of the chromosomes. Quantitative studies regarding the number of helices and the measurement of the length and diameter of the coils are now in progress and will be published elsewhere.

This work was aided by grant GB-15 from the U.S. National Science Foundation, administered by Dr. Donald E. Rounds. I thank him for his advice.

This communication is dedicated to Prof. Sajiro Makino, Zoological Institute, Hokkaido University, Sapporo, Japan, in honour of his sixtieth birthday on June 21, 1966.

YASUSHI OHNOKI

Pasadena Foundation for Medical Research,
Pasadena, California.

Swanson, C. P., *Cytology and Cytogenetics* (Prentice-Hall, Inc., Englewood Cliffs, N. J., 1957).

² Bonner, J., and Ts'o, P. (eds.), *The Nucleohistones* (Holden-Day, Inc., San Francisco, 1964).

³ Sakela, E., and Moorhead, P. S., *Cytogenetics*, 1, 225 (1962).

⁴ Ferguson-Smith, M. A., Ferguson-Smith, M. E., Ellis, P. M., and Dickson, M., *Cytogenetics*, 1, 325 (1962).

⁵ Sasaki, M. S., and Makino, S., *Amer. J. Human Genet.*, 15, 24 (1963).

⁶ Matsuura, H., Tanifuji, S., Saho, T., Iwabuchi, M., and Takizawa, S., *J. Fac. Sci. Hokkaido Univ.*, Ser. V, 8, 201 (1962).

GENETICS

Chimerical Nature of the Entire-leaf Variant in the Potato Variety 'Majestic'

THE docken, or entire-leaf variation (Fig. 1), like several other variations for leaf shape in potatoes, has not been shown previously to be a chimera. Leaf shape in potatoes is largely determined by the constitution of L_2 (ref. 1), and the two standard methods of investigating potato chimeras, breeding behaviour and eye-excision, give information on L_2 in the variant and not on L_1 . It is thus not possible by these two methods to show that L_1 in a variant is unchanged. Information of L_1 may, however, be obtained by X-ray treatment².

Crossing 'Majestic' docken-leaf with 'Ulster Prince' resulted in a family with a segregation of 23 seedlings with entire leaves: 17 seedlings with compound, pinnate leaves

(the control cross of normal 'Majestic' with 'Ulster Prince' gave 53 seedlings with a compound, pinnate leaves and none with entire leaves).

Eye-excision experiments with docken-leaf tubers produced only plants with entire leaves, no normal 'Majestic' plants being obtained. Root buds, produced by the method of Howard³, also resulted only in plants with entire leaves. It thus seems that docken-leaf in 'Majestic' results from a mutation for entire leaves in the L_2 layer and that the L_2 layer also now has the same constitution as that of L_1 ; a similar replacement of L_2 by L_1 cells has been observed in triploid-hexaploid potato chimeras⁴.

Five apical (rose) ends of tubers of docken-leaf 'Majestic' were given an X-ray dosage of 3,500 r. Four of them produced plants and one of the four had a mixture of normal and docken-leaf shoots. The production of the shoots with normal leaves was presumably because in the docken-leaf variant L_1 is still normal, unchanged 'Majestic'. The frequency (about 12.5 per cent) of shoots with all layers with the constitution of L_1 in the variant is similar to that of full 'Red King' from normal 'Red King' and of white from 'Bante' sports after X-ray treatment⁵.

The finding that the docken-leaf sport is a chimera in which L_1 is still normal 'Majestic' suggests that certain other variants, such as wildings, feathery wildings and bolters, which do not appear to be chimeras from the results of eye-excision experiments, may be chimeras in which L_2 and L_3 have the mutated character but in which L_1 is still unchanged.

H. W. HOWARD

Plant Breeding Institute,
Trumpington, Cambridge.

¹ Howard, H. W., Wainwright, J., and Fuller, J. M., *Genetica*, 34, 118 (1963).

² Howard, H. W., *Rad. Bot.* 4, 361 (1964).

³ Howard, H. W., *Nature*, 203, 1303 (1964).

Estimation of the Frequency of Functioning Gametes in Monosomics

WE regret that the estimates of p and q of the functioning male gametes with n and $(n-1)$ chromosomes respectively given in our recent communication¹ are not consistent. The correct estimates should be as follows.

On applying the maximum likelihood method, the estimate of p may be obtained by taking the positive root of the quadratic equation:

$$p^2[N(p' - q')] + p[q'(a_1 + a_2) - p'(N + a_1)] + a_1p' = 0 \quad (1)$$

and the variance of the estimate will be:

$$V_p = \frac{p^2q^2(p'q + pq')^2}{(p'q + pq')^2(a_1q^2 + a_2p^2) + a_2p^2q^2(q' - p')^2} \quad (2)$$

where a_1 , a_2 and a_3 ($a_1 + a_2 + a_3 = N$) are the observed frequencies of disomic, monosomic and nullisomic respectively and p' and q' ($p' + q' = 1$) are the relative frequencies of the functioning female gametes with n and $(n-1)$ chromosomes respectively.

The square root of V_p will give the standard error (S.E.) of the estimate.

It can be shown that:

$$\text{when } p = q' = \frac{1}{2}$$

$$p = \frac{2a_1}{N} \text{ and } q = \frac{2a_3}{N}$$

and the variance of the estimate will be reduced to $\frac{2pq}{N}$.

By using (1) in our numerical example¹, we obtain:

$$-59.28p + 12.08p + 3.12 = 0$$

giving $p = 0.3529$ and $q = 1 - p = 0.6471$.

Similarly, from (2) we have

$$V_p = 0.00324967$$

$$\text{and } S.E. = 0.0570$$

It may be observed that the estimate of p is not significantly different from the experimental results² 0.39.

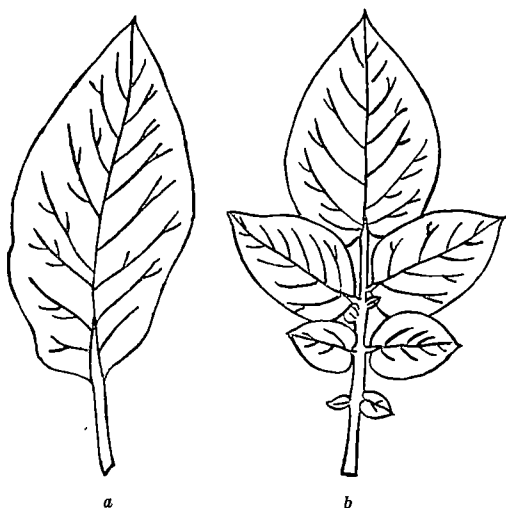


Fig. 1. (a) Docken-leaf and (b) normal, compound pinnate leaf of shoots after X-ray treatment

According to the correct estimates of p and q , the expected frequencies of disomic, monosomic and nullisomic presented in Table 2 in our communication¹ will be as follows:

Types	Disomic	Monosomic	Nullisomic	Total	χ^2
Chromosome No (42)	(41)	(40)			(d.f. 1)
Obs.	13	43	58	114	
Exp.	9.66	48.28	56.06	114.00	1.80

A. K. ROY CHOUDHURY

Bose Institute, Calcutta.

J. G. BHOWAL

Botany Division,
Indian Agricultural Research Institute,
New Delhi 12.

¹ Bhowal, J. G., and Roy Choudhury, A. K., *Nature*, 206, 1067 (1965).

² Bhowal, J. G., *Canad. J. Bot.*, 42, 1321 (1964).

PSYCHOLOGY

Memory Changes with Age

MANY studies have shown that increasing age results in a deficiency in ability to acquire new skills and information. However, the commonly held view that older people manifest an impairment in the retention of acquired material has received equivocal support from laboratory investigations^{1,2}. Even Inglis's recent demonstration³ of a loss with age in short-term memory is confined to an hypothesized storage system with a limit of approximately 4 sec. I wish to direct attention to the results of an experiment which suggests that the aged show special defects in the remembering of acquired material stored over longer periods of time. These defects seem to be due to a loss in ability to retrieve memories from storage rather than a deficiency in the storage system itself.

The material for the experiment consisted of two lists of 24 words. The words were presented on a screen at intervals of 4 sec. Memory was tested by the voluntary recall method on one list and the recognition method on the other list immediately after the last word on a list was exposed. For the recognition method, subjects were shown the 24 learning words, each in a group of four other words, and asked to underline those previously seen on the screen. One hundred and thirty-four subjects, aged between twenty and seventy-five, participated in the experiment, with approximately half the group performing the recognition test first, the others beginning with the recall test.

The scores showed no significant differences between results on the recognition test when this preceded or followed the recall test on the alternate list. Similarly, there was no difference between recall scores when these were obtained before or after a recognition test. The results on the two lists have, therefore, been combined.

Table 1 shows that there is no deterioration with age in the recognition scores, while there is a consistent drop in the recall scores. The differences between recognition and recall scores shown in the final column have a ρ correlation with age groups of +1.00. The Pearson product moment correlation between individual recognition minus recall scores and age is +0.66 ($P < 0.001$).

The age disparity between recognition and recall scores cannot be due to a difference at the acquisition stage since this should affect both types of memory tests. The disparity must, therefore, be the result of the different demands of the two testing procedures. The psychological distinction between voluntary recall and recognition seems to be that the former requires retrieval from storage while the latter does not. In the recognition test, a response is provided and the subject needs only to match a stimulus

with the stored trace. These findings, therefore, suggest that age-related impairment of long-term memory may be confined to situations which involve the retrieval of acquired material from storage.

The work described was supported by grant APA-89 from the Canada National Research Council; the testing was carried out by Miss Betty-Anne Robertson.

DAVID SCHONFIELD

Department of Psychology,
University of Alberta at Calgary,
Calgary, Alberta, Canada.

¹ Welford, A. T., *Aging and Human Skill* (Oxford Univ. Press, 1958).

² Jerome, E. A., in *Handbook of Aging and the Individual*, edit. by Birren, J. E. (Univ. Chicago Press, 1954).

³ Inglis, J., *Nature*, 204, 103 (1964).

MISCELLANEOUS

Ethology and the 'Baconian' Method

AMONG the many stimulating ideas elaborated by Sir Peter Medawar in his Presidential Address¹ to Section D of the British Association for the Advancement of Science is the claim that the foundation and subsequent development of what has come to be known as 'ethology' have "demonstrated the sterility of the old experimental approach" of test-stimulus and response, and illustrate "the danger of doing experiments in the Baconian style; that is to say, the danger of contriving 'experiences' intended merely to enlarge our general store of empirical knowledge rather than to sustain or confute a specific hypothesis or pre-supposition".

This rejection, by one with Sir Peter's authority, of the notion of a unique 'scientific method' applicable to all natural enquiry will fall gratefully on the ears of many. Nor can it be denied that the 'older experimental method' exemplified the weaker aspect of Bacon's incompletely worked out 'inductive method'. But does not Sir Peter imply a contrast that is in fact absent? For, in describing the 'new' and, in his view, effective ethological method, he says, "They [the pioneers of ethology] studied natural behaviour instead of contrived behaviour, and were thus able for the first time to discern natural behaviour structures or episodes—a style of analysis helped very greatly by the comparative approach." In the *Distributio Operis* prefixed to the *Instauratio magna* Bacon says, "all depends on keeping the eye steadily fixed upon the facts of nature and so receiving their images simply as they are. For God forbid that we should give out a dream of our own imagination for a pattern of the world."

That this ideal did not suffice to effect the so-called 'scientific revolution' is now abundantly clear; nor is it the method by which sciences greatly advanced by reason of their simplifying assumptions can be further enlarged. But, where an almost infinite number of possible variables leads to an almost limitless possibility of complexity, there is no alternative but to "sit down before facts like a little child" (Faraday)—but all the facts, in as great a variety of natural circumstances as possible. "Then, and only then", Sir Peter concludes, "was it possible to start to obtain significant information from the study of contrived behaviour . . . for it is not informative to study variations of behaviour unless we know beforehand the norm from which the variants depart." Being more interested in the "mastery of Nature for the relief of Man's estate", Bacon applied the method of comprehensive observation to the wrong data. But, so far from giving support to the now fashionable use of the term 'Baconian' as a wholesale term of disparagement, Sir Peter has shown how fertile, even indispensable, the 'Baconian' method may be in the appropriate context.

WILLIAM P. D. WIGHTMAN

Department of History and Philosophy of Science,
King's College,
University of Aberdeen.

¹ *Nature*, 207, 1327 (1965).

Table 1. MEAN RECOGNITION, RECALL, AND RECOGNITION MINUS RECALL SCORES BY AGE

Age range	No.	Recognition	Recall	Recognition minus recall
20-29	36	20.01	13.78	6.42
30-39	23	19.48	12.30	7.17
40-49	32	19.53	10.01	9.47
50-59	21	19.90	9.57	10.24
60+	22	20.09	7.60	12.59

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, November 29

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 10 a.m.—Colloquium on "Integrated Circuits".

INSTITUTION OF MECHANICAL ENGINEERS, RAILWAY ENGINEERING GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Specification and Inspection of Railway Materials".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Prof. Colin Cherry: "The Future of World Communication". (Last of three Cantor Lectures on "World Communication".)

ROYAL SOCIETY OF MEDICINE (at 1 Wimpole Street, London, W.1), at 3 p.m.—Prof. R. H. S. Thompson: "A Biochemical Approach to the Problem of Multiple Sclerosis" (Jephcott Lecture).

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, W.C.2), at 6.30 p.m.—Symposium on "Control and Its Applications".

PLASTICS INSTITUTE, LONDON SECTION REINFORCED PLASTICS SUB-GROUP (at the Wellcome Building, Euston Road, London, N.W.1), at 6.30 p.m.—Mr. B. A. Clark and Mr. D. F. Bailey: "Some Aspects of Design and Construction of Reinforced Plastics Building Panels".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Mr. A. R. Hanbury-Tenison: "From the Orinoco to the Plate".

Tuesday, November 30

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 9.30 a.m. and 2.30 p.m.—Colloquium on "Artificial Reverberation Techniques".

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Mr. H. S. Smith: "The Story of Nubia".*

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 2.30 p.m.—Anniversary Meeting.

UNIVERSITY OF LONDON (at King's College, Strand, London, W.C.2), at 5 p.m.—Prof. Ilmari Hustich (Finland): "Finland—a Developed and Underdeveloped Country".*

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, London, S.W.1), at 5.30 p.m.—Mr. S. E. H. Ford and Mr. S. C. Elliott: "Investigation and Design of Plover Cove Water Scheme".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 5.30 p.m.—Prof. G. Porter, F.R.S.: "Chaos and Chemical Equilibrium". (Afternoon lecture for Sixth Form Boys and Girls from Schools in London and the Home Counties. To be repeated on December 1, 7 and 8.)

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6.30 p.m.—Prof. R. Boyland: "The Causes of Cancer". (Tenth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation).*

INSTITUTION OF MECHANICAL ENGINEERS, AUTOMATIC CONTROL GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Relevance of Control Theory to Optimum Design in Engineering".

SOCIETY FOR ANALYTICAL CHEMISTRY, ATOMIC-ABSORPTION SPECTROSCOPY GROUP (at the Chemical Society, Burlington House, Piccadilly, London, W.1), at 6.30 p.m.—Annual General Meeting, followed by Mr. G. I. Goodfellow: "Some Observations on the Application of Atomic Fluorescence Spectrometry to Trace Analysis".

Tuesday, November 30—Wednesday, December 1

IRON AND STEEL INSTITUTE (at the Heare Memorial Hall, Church House, Great Smith Street, London, S.W.1, and at the Offices of the Institute, 4 Grosvenor Square, London, S.W.1), at 10 a.m. daily—Autumn General Meeting.

Wednesday, December 1

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (joint meeting with the I.E.R.E., at Savoy Place, London, W.C.2), at 2 p.m.—Discussion on "Telemetry in Medicine".

SOCIETY FOR ANALYTICAL CHEMISTRY, THIN-LAYER CHROMATOGRAPHY GROUP (at the Chelsea College of Science and Technology, Manresa Road, London, S.W.3), at 2.15 p.m.—Annual General Meeting, followed by Mr. J. W. Grindlay, Mr. P. M. Wraight and Mr. J. C. Wright: "A Study of the Oxidation of Nitroso-Aromatics to Nitro-Aromatics in Propellant Stabilisers Using T.L.C. and Mass-Spectrometry"; Mr. G. B. Campbell: "The Determination of Antioxidants and Other Additives in Plastics by T.L.C."; Mr. J. Scotney: "Study of the Auto-Oxidation of Lanosterol by T.L.C.". *

INSTITUTION OF MECHANICAL ENGINEERS, AUTOMATIC CONTROL GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 4.30 p.m.—Three papers on "High-Reliability Servos".

ROYAL STATISTICAL SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Mr. E. Seneta: "Stationary Distribution and Time-Reversion in Genetics".

UNIVERSITY OF LONDON (at King's College, Strand, London, W.C.2), at 6 p.m.—Prof. Ilmari Hustich (Finland): "The Polar Timber (Forest) Line".*

ROYAL SOCIETY OF MEDICINE, HISTORY OF MEDICINE SECTION (at 1 Wimpole Street, London, W.1), at 5.15 p.m.—Prof. A. Sorsby: "Gregor Mendel—Some Biographical Aspects"; Prof. E. B. Ford: "Mendelian Heredity and the Polymorphism Concept in Medicine" (Mendel Commemoration Meeting).

INSTITUTE OF PETROLEUM (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. A. S. Plane: "Calculation and Measurement of Quantities of Oil and Gas Evolved in Five-Stage Separation of Reservoir Crude".

INSTITUTION OF CIVIL ENGINEERS, HYDROLOGICAL GROUP (at Great George Street, London, S.W.1), at 5.30 p.m.—Informal Discussion on "Obtainable and Desirable Accuracies in Gauging Structure" introduced by Prof. J. R. D. Francis.

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2), at 5.50 p.m.—Mr. N. W. Notani, Mr. T. B. Burnett and Mr. J. H. Cheetham: "Parallel Operation of Transducer-Controlled Silicon-Rectifier Equipment with Waterwheel-Driven D.C. Generators Supplying an Aluminium Potline".

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, ELECTRO-ACOUSTICS GROUP (at 9 Bedford Square, London, W.C.1), at 6 p.m.—Dr. V. G. Welsby: "Non-Linear Effects in Acoustics, Radio and Optics".

PHARMACEUTICAL SOCIETY OF GREAT BRITAIN (at 17 Bloomsbury Square, London, W.C.1), at 6 p.m.—Mr. A. G. Fishburn: "The Future of the Pharmaceutical Sciences" (Harrison Memorial Lecture).

SOCIETY OF ENVIRONMENTAL ENGINEERS, PACKAGING GROUP (in the Mechanical Engineering Department, Imperial College, London, S.W.7), at 6 p.m.—Mr. D. G. Evans: "Atmospheric Corrosion".

Thursday, December 2

IRON AND STEEL INSTITUTE, ENGINEERS GROUP (at 4 Grosvenor Square, London, S.W.1), at 10 a.m.—Meeting on "Weighing in Iron and Steelworks".

ROYAL INSTITUTION OF Naval ARCHITECTS (in the Weir Lecture Hall, 10 Upper Belgrave Street, London, S.W.1), at 5 p.m.—Prof. E. V. Telfer: "Sir Charles Parsons and the Naval Architect" (Sir Charles Parsons Memorial Lecture).

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. J. A. Ratcliffe, F.R.S.: "Appleton's Contribution to Radio Science" (First Appleton Lecture).

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. G. Alan Rose: "Some Thoughts on Osteoporosis and Osteomalacia" (Eleventh of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation).*

INSTITUTE OF REFRIGERATION (at the National College for Heating, Ventilating, Refrigeration and Fan Engineering, Southwark Bridge Road, London, S.E.1), at 6 p.m.—Mr. W. A. Hines: "The Control of Air Conditioning Plant Noise in Buildings".

INSTITUTION OF MECHANICAL ENGINEERS, EDUCATION AND TRAINING GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Should Engineering Science be a G.C.E. Subject Taught in Schools?".

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (joint meeting with the Microbiology Group, at 14 Eelgrave Square, London, S.W.1), at 6 p.m.—Dr. W. Howard Hughes and Dr. P. N. Cardew: "The Technique and Application of Time-lapse Cinemicrography"; Mr. R. S. M. Revell: "The Use of the Electron Microscope in Microbiology".

SOCIETY FOR ANALYTICAL CHEMISTRY, THERMAL ANALYSIS GROUP (at the Chemical Society, Burlington House, Piccadilly, London, W.1), at 6.30 p.m.—Annual General Meeting, followed by Dr. D. Fitzgerald: "Coal Carbonization Kinetics—Simple Thoughts on a Complex Matter"; Dr. S. C. Bevan and Mr. S. Thorburn: "The Analysis of Volatile Materials by Mass Detection Gas Chromatography".

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (joint meeting with the Northampton College Chemical Society, at the Northampton College of Advanced Technology, St. John Street, London, E.C.1), at 7 p.m.—Prof. C. E. H. Bawn, F.R.S.: "Cationic Polymerisation".

SOCIETY OF COSMETIC CHEMISTS OF GREAT BRITAIN (at the Royal Society of Arts, John Adam Street, Adelphi, London, W.C.2), at 7.30 p.m.—Mr. R. Stokes: "The Selection of Scientific Personnel".

Thursday, December 2—Friday, December 3

ROYAL PHOTOGRAPHIC SOCIETY (in conjunction with the Polytechnic School of Photography, at the Polytechnic, Regent Street, London, W.1)—Symposium on "Techniques in Applied Photography".

Friday, December 3

NUTRITION SOCIETY (at the Nuffield Institute of Comparative Medicine, Outer Circle, Regents Park, London, N.W.1), at 10 a.m.—Original Communications.

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 4 p.m.—Prof. W. S. Fearl: "The Renin/Angiotensin System".*

ROYAL ASTRONOMICAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.15 p.m.—Sir Edward Bullard, F.R.S.: "Electromagnetic Induction Within the Earth" (Third Harold Jeffreys Lecture).

ASSOCIATION OF SCIENTIFIC WORKERS (in the Kent Room, Caxton Hall, Westminster, London, S.W.1), at 6.30 p.m.—Discussion Conference on "Technology and the Nation's Needs" opened by Lord Snow, C.B.E.

SOCIETY OF CHEMICAL INDUSTRY, FINE CHEMICALS GROUP (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. D. Jack: "Physicochemical Aspects of Drug Activity".

TELEVISION SOCIETY (in the Conference Suite, I.T.A., 70 Brompton Road, London, S.W.3), at 7 p.m.—Mr. B. Eastwood, Mr. A. Landman and Mr. P. L. Mothersole: "A Survey of Television Receiver Development".

Saturday, December 4

ASSOCIATION OF CLINICAL BIOCHEMISTS, SOUTHERN REGION (at Charing Cross Hospital Medical School, 62 Chandos Place, London, W.C.2), at 10 a.m.—Meeting on "Steroid Determination in the General Clinical Laboratory". Chairman: Prof. C. J. Gray.

INNER LONDON EDUCATIONAL AUTHORITY (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Dr. I. M. Lewis: "Somaland—Peoples and Poets".*

Monday, December 6

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 4 p.m.—Dr. J. C. Gilson: "Asbestos, Recent Work on the Biological Importance of Fibre Type".*

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Acoustic-Wave Amplification" opened by Dr. E. G. S. Paige and Mr. C. P. Sandbank.

INSTITUTION OF MECHANICAL ENGINEERS, THERMODYNAMICS AND FLUID MECHANICS GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Is Heat Transfer Theory of Any Use?"

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 6 p.m.—"The Underwater Search" (Colour film).

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (Joint meeting with the Heavy Organic Chemicals Group, at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. H. L. Riley: "Recent Developments in the Tonnage Production of Aromatic Chemicals".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

CHAIR OF BOTANY AND HEAD OF THE DEPARTMENT—The Registrar, University of Leicester, Leicester (December 4).

CHAIR OF THEORETICAL PHYSICS—The Registrar, University of Leicester, Leicester (December 4).

LECTURER AND AN ASSISTANT LECTURER (graduates in sociology, psychology or history) IN THE DEPARTMENT OF SOCIOLOGY—The Registrar, University of Leicester, Leicester (December 4).

SENIOR LECTURER IN CONTROL ENGINEERING—The Registrar, University of Leicester, Leicester (December 4).

LECTURER IN THE DEPARTMENT OF PURE MATHEMATICS—The Registrar, University College of North Wales, Bangor, North Wales (December 6).

LECTURER (with a medical qualification) IN PHYSIOLOGY; and a JUNIOR LECTURER (with a medical qualification or an honours degree in physiology) IN PHYSIOLOGY—The Secretary, Trinity College (University of Dublin), Dublin 2, Republic of Ireland (December 6).

PROGRAMMER (with a degree or equivalent qualification, at least two years programming experience, and preferably a knowledge of Algol, Fortran or Elliot 803 machine code) IN THE COMPUTING LABORATORY to help in the provision of a computing service, by advising on the use of programmes and writing new programmes—The Registrar, University College of North Wales, Bangor, North Wales (December 6).

SENIOR LECTURER (with high academic qualifications and appropriate teaching and/or research experience) IN PHYSICS—The Principal, Lancaster College of Technology, Priory Street, Coventry (December 6).

LECTURERS IN PURE MATHEMATICS—The Registrar, The University of Warwick, Coventry, Warwickshire (December 10).

CHAIR OF PHYSICS—The Secretary, The Queen's University, Belfast, Northern Ireland (December 15).

LECTURER IN SOCIOLOGY at the University of Singapore—The Inter-University Council, 33 Bedford Place, London, W.C.1 (December 15).

LECTURER OR ASSISTANT LECTURER (medically qualified or non-medically qualified candidate) IN MEDICAL GENETICS to be responsible for cytogenetics—The Registrar, The University, Manchester, 13, quoting Ref 196/65/Na (December 15).

CHAIR OF MICROBIOLOGY—Secretary to the University Court, University of Glasgow, Glasgow (December 18).

SENIOR LECTURER (preferably with considerable teaching and research experience) IN THE DEPARTMENT OF PHILOSOPHY, University of Ghana—The Assistant Registrar, Ghana High Commission, Higher Education Section, 16 Gordon Square, London, W.C.1; or The Registrar, University of Ghana, P.O. Box 25, Legon, Accra, Ghana (December 18).

LECTURERS (2) (with a good honours degree and preferably with a minimum of two years experience in industry and/or research) IN THE DEPARTMENT OF CIVIL ENGINEERING—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (December 20).

RESEARCH ASSOCIATE (with a degree, preferably Ph.D., in physics, chemistry or metallurgy) IN CRYSTAL GROWTH IN THE DEPARTMENT OF PHYSICAL METALLURGY, to join an established research group working in the general field of crystal growth in metals and non-metals—The Assistant Registrar (Science and Engineering), University of Birmingham, Birmingham, 15 (December 22).

BOTANIST OR AGRICULTURAL BOTANIST (with a good honours degree) IN THE BIOCHEMISTRY DEPARTMENT, to study the factors affecting the production and extraction of leaf protein from agricultural crops and other plants—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (January 1).

LECTURER (with a good honours degree and preferably teaching experience and a qualification and interest in logic) IN PHILOSOPHY at the University of Newcastle, New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (January 1).

CHAIR OF AGRONOMY within the Faculty of Agriculture, University of Western Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 15).

ENTOMOLOGISTS (2), NEMATOLOGISTS (2); a PLANT PATHOLOGIST (Mycologist); a SOIL CHEMIST; and a STATISTICIAN (Scientific Officer or Senior Scientific Officer grades)—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (January 15).

PROFESSOR OF GEOPHYSICS (Solid Earth) at Victoria University of Wellington, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, January 15).

LECTURER OR ASSISTANT LECTURER (man or woman graduate, preferably with qualifications and interest in social psychology) IN PSYCHOLOGY—The Secretary, Bedford College (University of London), Regent's Park, London, N.W.1 (January 30).

SENIOR LECTURER OR LECTURER (electrical engineer with experience of control problems in one of the process industries) IN CONTROL ENGINEERING IN THE DEPARTMENT OF SYSTEMS ENGINEERING—The Secretary, University of Lancaster, Bailrigg House, Lancaster (January 31).

RESEARCH TECHNICIAN OR SENIOR TECHNICIAN (woman, with a knowledge of histological and general laboratory techniques) for work with a medical research group—The Administrator, Department of Zoology, University of Oxford, Parks Road, Oxford.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Bulletin of the British Museum (Natural History). Entomology. Vol. 17, No. 2. Diptera from Nepal. Empididae. By Kenneth G. V. Smith. Pp. 61-112. 20s. Vol. 17, No. 3. An introduction to the Aleoidea of Western Africa (Homoptera). By L. A. Mound. Pp. 113-160. 18s. Vol. 17, No. 4:

Cicadelline Types in the British Museum (Natural History). Homoptera: Cicadellidae. By D. A. Young. Pp. 161-199. 13s. Historical Series. Vol. 3, No. 3: Supplementary Letters of Sir Joseph Banks, Second Series. Edited by Warren R. Dawson. Pp. 71-93. 9s. Zoology. Vol. 13, No. 2: Japanese and other Ophirurids from the Collections of the Munich Museum. By Ailsa M. Clark. Pp. 37-71 + 1 plate. 14s. Vol. 13, No. 3: The Freshwater Gastropod Molluscs of West Cameroon. By C. A. Wright. Pp. 73-98 + plates 1-3. 18s. (London: British Museum (Natural History), 1965.) [110]

The Overseas Development Institute, Ltd. British Private Investment in East Africa: Report of a Survey and a Conference. By D. J. Morgan. Pp. 63. (London: The Overseas Development Institute, Ltd., 1965) 7s. 6d. [110]

The Institute of Navigation. Annual Report 1964-65. Pp. 20. (London: The Institute of Navigation, 1965) [110]

The Institution of Mechanical Engineers. Engineering Among Schools: School Activities in Applied Science and Engineering—a Survey of 290 Schools. By G. T. Page. Pp. 328. (London: The Institution of Mechanical Engineers, 1965) 15s. [110]

H. M. Treasury. Report of a Committee Appointed to Review the Organization of the Scientific Civil Service. Pp. 28. (London: H. M. Stationery Office, 1965.) 2s. 3d. net. [110]

On the Nature of African History. By Prof. J. D. Fage. (An Inaugural Lecture delivered in the University of Birmingham on 10th March, 1965.) Pp. 20. (Birmingham: The University, 1965.) 2s. 6d. [110]

The Radiochemical Centre. Catalogue of Radioactive Products, 1965/1966. Pp. 203. Descriptive Brochure. Pp. 24. (Amersham: The Radiochemical Centre, 1965) [110]

UFAW News-Sheet, No. 1 (September, 1965). Pp. 12. (London: The Universities Federation for Animal Welfare, 1965.) [410]

British Broadcasting Corporation. B.B.C. Engineering Monograph No. 59: The Acoustic Design and Performance of a New Free-Field Sound Measurement Room. By D. E. L. Shorter, Dr. C. L. S. Gifford and H. D. Harwood. Pp. 23. (London: British Broadcasting Corporation, 1965) 5s. [410]

Other Countries

United States Department of the Interior: Geological Survey. Professional Paper 422-G: The Distribution of Branches in Rover Networks. By Ennio V. Giusti and William J. Schneider. Pp. iii + 10. 20 cents. Professional Paper 448-C: Cretaceous Aquifers in the Mississippi Embayment. By E. H. Boswell, G. K. Moore, and L. M. MacCary. Pp. iv + 37 + plates 1-8. Professional Paper 451: Geology and Artesian Water Supply, Grand Junction Area, Colorado. By S. W. Lohman. Pp. v + 149 + plates 1 and 2. Professional Paper 459-B: Ore Deposits of the Antler Peak Quadrangle, Humboldt and Lander Counties, Nevada. By Ralph J. Roberts and D. C. Arnold. Pp. v + 94 + plates 1-19. Professional Paper 465: Tectonic and Igneous Geology of the Northern Shoshone Range, Nevada. By James Gilly and Olcott Gates. With sections on Gravity in Crescent Valley by Donald Plouff, and Economic Geology by Keith B. Ketner. Pp. vi + 153 + plates 1-6. (Washington, D.C.: Government Printing Office, 1965.) [410]

Transactions of the Royal Society of New Zealand. Zoology. Vol. 6, No. 13 (May 19, 1965): Pre-Zoea Larva of *Petrolisthes novaezelandiae* Filhol, 1885 (Crustacea, Decapoda, Anomura). By Robert G. Wear. Pp. 127-132. Vol. 6, No. 14 (May 19, 1965): Three New Species of Gall-Mites (Acarina: Eriophyidae). By D. C. M. Manson. Pp. 133-139. Vol. 6, No. 15 (May 19, 1965): Seasonal Changes in Metabolism of Four South Temperate Marine Fishes. By R. W. Morris. Pp. 141-152. Vol. 6, No. 16 (May 19, 1965): A Description of the Larva of *Scopelopsis penicillatus* White (Curculionidae: Eucneminae). By E. M. Cawthra. Pp. 153-154. Vol. 6, No. 17 (June 16, 1965): A Redescription of Some A. O. Walker 'Types of 'Southern Cross' Lysianassidae (Crustacea Amphipoda) from the Ross Sea. By D. E. Hurley. Pp. 155-181. Vol. 6, No. 18 (June 16, 1965): A Redescription of *Orchomenella chilensis* (Heller) (Crustacea Amphipoda: Family Lysianassidae), from the Original Material Collected by the "Novara" in Chilean Waters. By D. E. Hurley. Pp. 183-188. Vol. 6, No. 19 (June 16, 1965): Some New Zealand Pedicellinidae (Entoprocta), and a Species New to Europe. By J. S. Ryland. Pp. 189-205. (Wellington: The Royal Society of New Zealand, 1965.) [410]

Transactions of the Royal Society of New Zealand. Zoology. Vol. 6, No. 20 (May 28, 1965): The Osteology of *Cheumatichthys fosteri* Haast (Pisces, Percomorpha). By E. D. Lane. Pp. 207-219. Vol. 6, No. 21 (June 16, 1965): Rocky Shore Ecology of a Southern New Zealand Fjord. By E. J. Batham. Pp. 215-227 + 2 plates. Vol. 6, No. 22 (June 16, 1965): Notes on Some New Zealand Plecoptera. By I. D. McLeall. Pp. 229-234. Vol. 6, No. 23 (June 16, 1965): The Aradidae of New Zealand (Hemiptera Heteroptera). II.—The Isoderminae. By J. G. Pendergast. Pp. 235-243. Vol. 6, No. 24 (May 28, 1965): The Distribution of Echinoderms Along the East Coast of New Zealand. By David L. Pawson. Pp. 245-252. Vol. 6, No. 25 (May 28, 1965): New Records of Echinoderms from the Snares Islands to the South of New Zealand. By David L. Pawson. Pp. 253-260. (Wellington: The Royal Society of New Zealand, 1965.) [410]

United States Department of the Interior: Geological Survey. Water-Supply Paper 1675: Magnitude and Frequency of Floods in the United States. Part 3-A: Ohio River Basin Except Cumberland and Tennessee River Basins. By Paul R. Speer and Charles R. Gamble. Pp. xiv + 630 + plate 1. (Washington, D.C.: Government Printing Office, 1965.) [410]

Instituto de Investigação Científica de Mocimboque, Lourenço Marques. Novos Taxa Entomologicas (Suplemento à *Revista Entomologica de Mocimboque*). No. 41: Contribuição para a Estudo dos Dinastíneos Africanos. I. Um Novo Genero da Tribo *Phileurini*. Por M. C. Ferreira. Pp. 8. (Lourenço Marques: Instituto de Investigação Científica de Mocimboque, 1965.) [410]

Editorial and Publishing Offices of "NATURE"

MACMILLAN (JOURNALS) LIMITED

4 LITTLE ESSEX STREET, LONDON, W.C.2

Telephone Number: Temple Bar 6633. Telegrams: Phusis London W.C.2

Annual subscription: Inland £14, Overseas £13 15s., payable in advance, postage paid to any part of the world.

Advertisements only should be addressed to

T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2

Telephone Number: Holborn 4743

Registered as a newspaper at the General Post Office

Copyright © Macmillan (Journals) Limited, November 27, 1965

NATURE

No. 5014

SATURDAY, DECEMBER 4, 1965

Vol. 208

Published by
MACMILLAN (JOURNALS) LTD.

CONTENTS

	<i>Page</i>
SCIENCE, INVENTIVENESS AND CREATIVITY	921
TECHNICS AND THE NATURE OF MAN By Lewis Mumford	923
THIRD DIMENSION OF MANAGEMENT. By John J. Haywood	928
STRUCTURES INSIDE A LUNAR CRATER PHOTOGRAPHED BY <i>Ranger VII</i> . By Captain Vern G. Smalley and Luciano B. Ronca	930
NEWS AND VIEWS	934
CENTENARY OF THE QUEKETT MICROSCOPICAL CLUB. By Prof. G. J. Cunningham, M.B.E.	937
TEACHING IN THE HISTORY AND PHILOSOPHY OF SCIENCE. By Dr. W. Mays	937
EDUCATION AND APPLICATION OF THE MATERIALS SCIENTIST. By Dr. G. F. Freeguard	939
EVIDENCE FOR STRUCTURES RESEMBLING CRYSTALLINE CARBONS AT THE ACTIVE CENTRE OF ENZYMES By Dr. E. D. Wachsmuth	942
THE WOLF CREEK IRON METEORITE. By Prof. S. R. Taylor	944
INITIATION OF CHAIN REACTION UNDER AN ULTRASONIC WAVE EFFECT. By Prof. I. E. Elpiner, A. V. Sokolskaya and M. A. Margulis	945
EVIDENCE FOR MICROFOSSILS IN THE ALAIS AND ORGUEIL CARBONACEOUS METEORITES. By Sam L. VanLandingham	947
INHIBITION OF TRANSPLANTABLE AND SPONTANEOUS MURINE TUMOURS BY THE M-P VIRUS. By Dr. Norman Molomut and Dr. Morton Padnos	948
IMMUNE SYSTEM IN A MONOTREME: STUDIES ON THE AUSTRALIAN ECHIDNA (<i>Tachy- glossus aculeatus</i>). By Dr. E. Diener and Dr. E. H. M. Ealey	950
PHOSPHORUS EXCRETION OF CATTLE FED ON HIGH-ENERGY DIETS. By W. D. C. Reed, Dr. R. C. Elliott and Dr. J. H. Topps	953
HYPERLIPOAEMIA FOLLOWING VIRAL INFECTION IN THE CHICKEN EMBRYO: A NEW SYNDROME. By Dr. S. E. Grossberg and Prof. W. M. O'Leary	954
CHROMOSOME MARKER STUDIES ON THE DEVELOPMENT OF THE HAEMOPOIETIC SYSTEM IN THE CHICK EMBRYO. By M. A. S. Moore and Dr. J. J. T. Owen	956
ORIGIN OF THE GEOELECTRIC EFFECT IN PLANTS. By Prof. Malcolm B. Wilkins and A. E. R. Woodcock	990

(Continued overleaf)

Elsevier

AMSTERDAM

P.O. Box 211
Amsterdam

LONDON

Rippleside Commercial
Estate
Barking, Essex

NEW YORK

52 Vanderbilt Ave.
New York, 17

GEO-TECHNOLOGY

Mineral Processing (Third Edition). E. J. Pryor

Formerly Reader in Mineral Dressing, University of London

Contents: General introduction; acceptance into the mill; primary crushing; secondary crushing; wet-grinding mills; forces in wet grinding; dry grinding; laboratory sizing control; industrial sizing and sorting; grinding circuit control; methods of separation; dense media separation; separation in vertical currents; separation in streaming currents; physics and chemistry in ore treatment; chemical extraction; principles of froth flotation; flotation practice; magnetic and electrical separation; testing and research; sampling and controls; unit processes and machines; selected ore treatments; appendices; index

140s.

The Mineral Resources of Africa. Nicolas de Kun

Department of Mining, Mineral Engineering and Metallurgy, Columbia University, New York

Contents: Part I. Industrial Development and Mineral Economics—Africa's share of world resources and production; the value of African production; the distribution of African output and resources; the history and development of mining; Northern Africa; Northeastern Africa; West Africa; Middle Africa; Southern Africa; the islands and the sea. Part II. Economic Geology—The mineralogical provinces of Africa; iron-bauxite group; gold group; base metal group; ultra-basic group; carbon-fuel group; sediments, miscellaneous; water and soil; appendices; index

240s.

The Mineral Resources of the Sea. John L. Mero

Newport News Shipbuilding and Dry Dock Co., Newport News, Va.

Contents: Preface; introduction; minerals from sea water, the continental shelves; strata underlying the soft sea-floor sediments; the deep-sea floor, ocean mining methods, some economic and legal aspects of ocean mining; appendices; references; index.

60s.

Sedimentology and Ore Genesis. Edited by G. C. Amstutz

Institute of Petrology and Mineralogy, University of Heidelberg, Germany

Contents: Introduction; redoxate deposits; oxidate deposits; sulphate and phosphate deposits; index.

55s.

The Techniques of Sedimentary Mineralogy. F. G. Tickell

Department of Mineral Engineering, Stanford University, Stanford, California

Contents: Introduction; size analysis; bulk properties; preparation of specimens; identification of minerals; description of minerals occurring in sedimentary rocks; appendices; references; index

90s

Identification of Mineral Grains. Meurig P. Jones and Marston G. Fleming

Department of Mining and Mineral Technology, Royal School of Mines, London

Contents: Introduction; summary of determinative scheme; list of equipment and reagents; procedure; recording test results; explanation of the determinative tables; references; alphabetical list of minerals; determinative table; index.

30s

Determination Tables for Ore Microscopy. C. Schouten

Technological University, Delft, The Netherlands

Contents: Preface; introduction; explanations and abbreviations; scheme of the tables; index

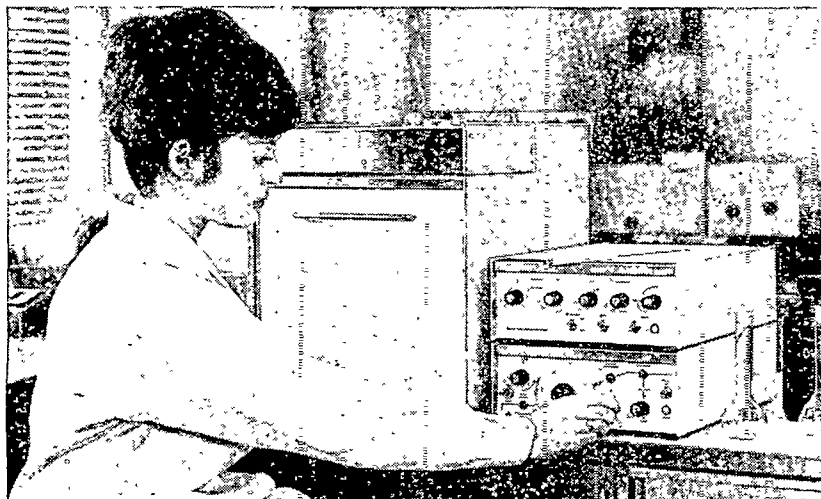
Cloth 55s, Paper 35s.

REVIEW SUPPLEMENT

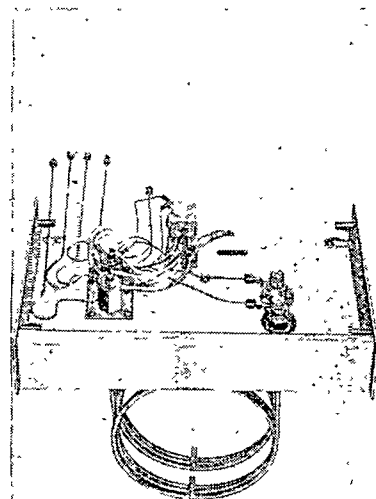
	<i>Page</i>
TOWARDS A PUBLIC UNDERSTANDING OF SCIENCE. By Dame Kathleen Lonsdale, F.R.S	957
AFRICAN STUDENTS IN BRITAIN. By Lord Twining, G.C.M.G., M.B.E.	957
VIEWS OF NINETEENTH-CENTURY SCIENCE. By Dr. W. P. D. Wightman	958
RACES, GOALS AND ALL THAT. By Prof. C. H. Waddington, C.B.E., F.R.S	959
FROM FORSTER TO FISHER. By Prof. W. H. G. Armytage	960
KEPLER'S DREAM. By Dr. Bernard Pagel	960
MOON AND PLANETS. By Dr. G. Fielder	961
A SURVEY OF THE EARTH. By Patrick Moore	961
LILIENTHAL'S ATOMIC YEARS. By Sir John Cockcroft, O.M., K.C.B., C.B.E., F.R.S	962
QUASARS. By Sir Bernard Lovell, O.B.E., F.R.S.	963
HURWITZ-COURANT. By Dr. J. C. Burkill, F.R.S.	963
AN ADVANCED THEORY OF VIBRATIONS. By K. R. Weiss	964
VIBRATION PROBLEMS IN ENGINEERING. By K. R. Weiss	964
EXPERIMENTAL PLASMA PHYSICS. By A. E. Dangor.	965
ASPECTS OF RHEOLOGY. By Dr. G. W. Scott Blair	965
MAGNETOHYDRODYNAMIC POWER GENERATION. By Prof. M. W. Thring	966
ADVANCES IN FLUID DYNAMICS. By Prof. K. Stewartson, F.R.S.	967
PALAEONTOLOGICAL TECHNIQUES. By Prof. D. T. Donovan	968
SOME ASPECTS OF BORON. By Dr. L. H. Long.	968
PROGRESS IN PETROLEUM CHEMISTRY AND REFINERY. By H. B. Milner	969
CHEMISTRY 1830 TO 1963. By Dr. R. G. Partington	969
TWO STEREOCHEMICAL KEYS. By Prof. W. Klyne	970
CLASSICAL OPTICS. By Dr. W. J. Bates	971
SUGAR METHODOLOGY AND DATA. By Prof. M. Stacey, F.R.S.	971
ACTIVITIES OF ENZYMES AND ENZYMOLOGISTS. By Dr. D. T. Elmore	972
BIOLOGICAL RHYTHM RESEARCH. By Dr. A. D. Lees	973
RADIATION PRESERVATION OF FOODS. By G. Birch.	973
PHOTOBIOLOGY UP TO DATE. By Dr. A. Knowles	974
ZOOLOGICAL MICROPALAEONTOLOGY. By Dr. R. H. Bate	974
CHEESE. By Dr. J. A. B. Smith, C.B.E.	975
WHEAT. By Dr. G. D. H. Bell	976
WEST AFRICAN FLOWERING PLANTS. By F. Nigel Hepper.	976
A SULPHURETUM OF BACTERIA. By Prof. T. W. Goodwin.	977
EVOLUTION OF STATE MEDICINE. By Sir Arthur MacNalty, K.C.B.	977
PHOTOPHYSIOLOGY. By Dr. Edith Harrison	978
THE THYROID. By Dr. E. Eric Pochin	979
THE EXPANDING EYE. By Dr. R. A. Weale	979
CHEMOTHERAPY RESEARCH. By Prof. E. P. Abraham, F.R.S.	980
SYNTHÈSE MANQUÉE By Prof. O. L. Zangwill	981
AGEING IN EASTERN EUROPE. By Dr. David A. Hall	981
THINKING BROKEN DOWN. By Prof. R. C. Oldfield	982
PHARMACY IN HISTORY. By Dr. Frank Hartley	982
SCIENCE IN HUNGARY. By Prof. D. Gabor, F.R.S.	983
EARLIEST CIVILIZATIONS OF THE NEAR EAST. By M. C. Burkitt.	984
ALGEBRA OF THE LATTICE. By Prof. T. A. A. Broadbent	984
SHORT REVIEWS	985

(Continued overleaf)

Pye precision in gas chromatography



Oven accommodates interchangeable head units incorporating different detector and column configurations and is designed for isothermal or programmed temperature control.



Oven head unit with dual flame detectors, columns and injection ports

NEW PYE SERIES 104 CHROMATOGRAPHS SPEED ROUTINE ANALYSES

Analysts are changing to the new low-cost Series 104 Chromatograph because its greater speed and convenience save money. They have acknowledged the functional design, the simplified operating features, the comprehensive specification and the overall economy of the system. They are now experiencing its complete reliability under continuous operating conditions.

Four versions of Series 104 are available which cover everyday analytical applications, including steroids, fatty acids, wide boiling range mixtures and high boilers.

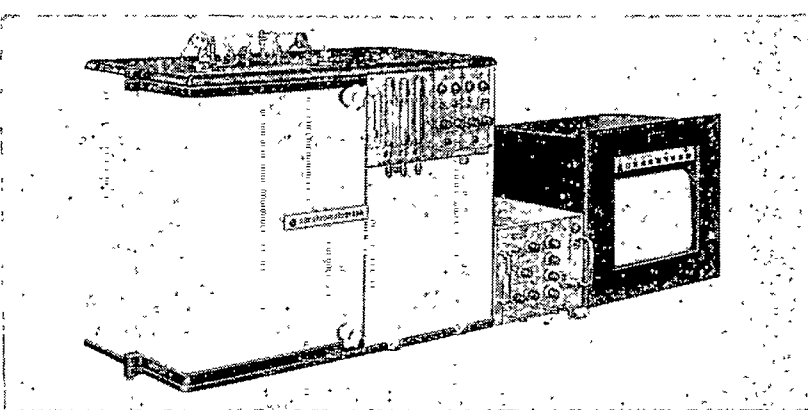
The Pye Series 104 Chromatograph gives *faster analysis* with warm-up rates of up to 48°C per minute and operating temperatures as high as 500°C, *easier analysis* due to excellent oven accessi-

bility and design of controls, and *cheaper analysis* with lowest possible outlay and maintenance costs.

Moreover, the Pye Series 104 is designed for continuous development and updating as new techniques emerge. Please send for the new 20 page illustrated brochure which provides all the technical facts and figures including oven performance data.

PYE PANCHROMATOGRAPHS AID RESEARCH

Leading research establishments in many parts of the world depend upon the versatility of the Pye Panchromatograph to satisfy their most stringent requirements including investigations into the technique and the teaching of gas chromatography. This analytical system can accommodate different detector combinations and column arrangements in two independent ovens, and it features a patented column effluent splitting device which permits further analysis of the column outputs. Numerous optional features including temperature programming, pyrolysis, preparative scale and radiochromatography are available. The Pye Panchromatograph is the first choice for an all-purpose instrument for research.



The Panchromatograph will accommodate six different types of detector, singly or in pairs.

W. G. Pye & Co. Ltd., P.O. Box 60, York Street, Cambridge
Telephone: Cambridge 53866 Telegrams: Pye Cambridge Telex: 81215

SCIENTIFIC  INSTRUMENTS

LETTERS TO THE EDITOR

	Page		Page
ASTRONOMY		PATHOLOGY	
Hydrogen Content of Virgo Cluster Galaxies.—Dr. B. J. Robinson and J. A. Koehler	993	A Screening Test for Substances inhibiting the Cancer Coagulative Factor.—Dr. E. M. Glaser, P. Spink and Prof. R. A. Q. O'Meara	1008
PHYSICS		Distribution of the Intestinal Factor of Normal Mice which inactivates Murine Virus Hepatitis.—Prof. M. Piazza, Dr. A. Amadio and Dr. G. Pane	1009
Townsend's First Ionization Coefficient for Compressed Nitrogen.—Dr. B. W. Ward	994	IMMUNOLOGY	
CHEMISTRY		Reaction of Adenine-specific Antibodies with Denatured Deoxyribonucleic Acid.—Dr. Sergio Estrada-Parra, Amanda Schmill and Rosa-Maria Martinez	1010
Phosphorus-31 Chemical Shifts in Secondary and Tertiary Phosphines.—Prof. Samuel O. Grim and William McFarlane	995	BIOLOGY	
Relative Ionization Efficiencies of Hydrocarbon Molecules as a Function of Bombarding Electron Energy.—J. A. Takacs and Prof. G. R. Freeman	996	Experimentation with Plants at Sub-atmospheric Oxygen-levels: Effects of Oxygen Pressure and Salts on Germination of Winter Rye.—Dr. S. M. Siegel, Olive Daly and Constance Giumarro	1012
Androgenic Steroid Complexes with <i>p</i> -Bromophenol.—Dr. Chaim Eger and Dr. Dorita A. Norton	997	Morphactins, a Novel Group of Plant-growth Regulators.—Gerhart Schneider, Dietrich Erdmann, Sigmund Lust, Gunther Mohr and Konrad Niethammer	1013
Crystalline Modifications of Ampicillin.—K. W. B. Austin, A. C. Marshall and H. Smith	999	Winter Scale Rings in <i>Lates niloticus</i> (Pisces: Centropomidae) from Lake Chad.—A. J. Hopson	1013
BIOCHEMISTRY		Technique for the Removal of the Brain in Bats.—Arthur M. Greenhall	1014
Photochemical Synthesis of Condensed Phosphates.—A. Beck and L. E. Orgel	1000	MICROBIOLOGY	
Effect of Hydrolysed Glucose <i>Cyclo</i> acetoacetate in the Prevention of Fatty Infiltration in the Liver.—Prof. N. Nath, V. D. Yawalkar and Prof. M. C. Nath	1001	Micro-organisms associated with Dead Insect Larvae in Nigeria.—Dr. N. Okafor	1015
Rapid Method for Selenium Assay of Plant Material.—R. Duff and Prof. M. Chessin	1001	A Rapid Screening Test for Transfer Factors in Drug-sensitive Enterobacteriaceae.—Dr. E. S. Anderson	1016
Disruption of Protozoa by Indole and Related Compounds.—Dr. R. W. Bailey and G. B. Russell	1001	Effect of Incubation Temperature on the Flagellation of Gram-negative Rods.—Dorothy A. Orr and Dr. Margaret M. Taylor	1017
PHYSIOLOGY		VIROLOGY	
Cerebello-rubral Connexions in the Cat.—Pierre Angaut and Dr. David Bowsher	1002	Mechanical Transmission of Hop Mosaic Virus.—Dr. J. T. Legg	1017
Delayed Mortality in Adult Chickens exposed to 1 Atmosphere Oxygen.—Prof. Harold S. Weiss, David L. Beckman and Ronald A. Wright	1003	CYTOLOGY	
Nerve Potential Recordings during electrically and mechanically evoked Monosynaptic Reflexes in Man.—Dr. M. M. Gassel and Dr. E. Diamantopoulos	1004	Cytoplasmic Labelling with Uridine-5- ³ H in Human Lymphocytes cultured with Phytohaemagglutinin.—Dr. G. C. B. Winter and Prof. J. M. Yoffey	1018
PHARMACOLOGY		GENETICS	
A New, Active Metabolite of 'Miracil D'.—David Rosi, George Peruzzotti, Dr. E. W. Dennis, Prof. D. A. Berberian, H. Freele and Dr. S. Archer	1005	Differential Deoxyribonucleic Acid Replication in the Giant Foot-pad Cells of <i>Sarcophaga bullata</i> .—Dr. Joan M. Whitten	1019
Pronethalol-induced Reversal of Adrenergic Vaso-depression.—Dr. M. Gonçalves Moreira and Dr. W. Osswald	1006	PSYCHOLOGY	
Inhibition of Anaphylactic Release of Vascular Permeability Factor or Histamine by Specific Protease Inhibitor in Tissue Culture.—Prof. H. Hayashi, Y. Kinuwaki and M. Yoshinaga	1007	Behavioural Deficit in the Rat induced by feeding Phenylalanine.—Dr. Russell C. Leaf, Dr. Peter L. Carlton and Dr. Sidney M. Hess	1021

MALATE DEHYDROGENASE**(Pig Heart)****MDH****CRYSTALLINE
SUPERNATANT****MDH****CHROMATOGRAPHICALLY
PURIFIED
MITOCHONDRIAL**

These products and many ENZYMES and RELATED BIOCHEMICALS of the highest possible integrity are produced entirely in our own laboratories.

Send for descriptive brochure

SERAVAC LABORATORIES (PTY.) LTD.

Moneyrow Green, Holyport,
Maidenhead, Berks., England.

GLASS

**All Glass Requirements for Scientific
Instrument Manufacturers and
Research Workers**

- SHEET & PLATE-GLASS—cut and ground to exact dimensions.
- LENSES, PRISMS & FLATS—to any standard of accuracy.
- MIRRORS—back silvered and front surface aluminised, etc.
- BLOOMING of optical components.

C. J. WHILEMS, LTD.

ILFORD OPTICAL WORKS

**FOREST ROAD, BARKINGSIDE,
ESSEX**

Telephone: HAInault 5454-5

**PRECISION INSTRUMENTS**

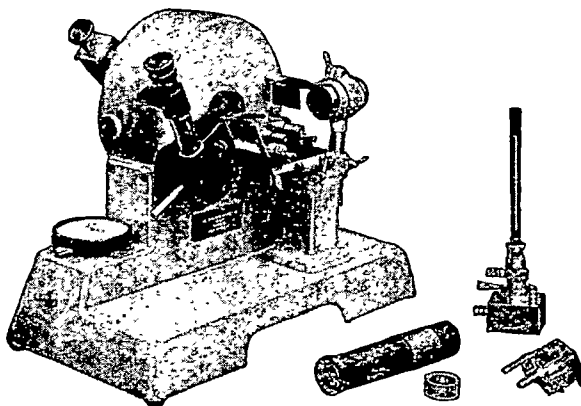
This Instrument, in its modern styling, still embodies the requirements laid down by Mr. Guild of the National Physical Laboratory and the advice of research workers in various laboratories.

- * CAN BE SUPPLIED WITH A DIFFERENTIAL CELL COMPLETE WITH COLLIMATOR AND SLIT FOR MEASUREMENT OF REFRACTIVE INDICES OVER THE RANGE 1.26 TO 1.95.

The interchangeable prisms, each covering a recommended range of refractive index, are of 60° refracting angle and are mounted in individual water jackets.

We are the sole distributors in the U.K. of Schott Monochromatic Interference Filters.

The Improved Type Pulfrich Refractometer



Please ask for Details 'N'.

Bellingham & Stanley Limited

71 HORNSEY RISE, LONDON, N.19. PHONE: ARCHWAY 2270

SCIENCE, INVENTIVENESS AND CREATIVITY

IN a reference to Sir Peter Medawar's Tizard Memorial Lecture when addressing Section L (Education) at the Cambridge meeting of the British Association for the Advancement of Science, Sir Edward Boyle expressed the hope that Sir Peter would later develop, at greater length, his comment that the more creditable part of our reverence for pure science might derive from the accident that in Britain the quintessential form of activity had always been poetic invention. Sir Edward appeared to agree with Sir Peter that imaginative thought played an important part in discovery and invention, and also that imagination could be trained and guided and deliberately stocked with material for imaginative use. However, neither he nor Sir Peter pursued further the place which the imagination holds in creative work. Sir Peter, indeed, questioning the view of Coleridge as expounded in *The Road to Xanadu*, insisted that poetic inspiration was no valid guide to imaginative activity in all its forms.

Earlier in his Lecture, Sir Peter had recognized that a scientist must be freely imaginative and yet a sceptic, creative yet critical. His reference to the imaginative and creative aspect of scientific activity, to the distinctions which Haslett, Coleridge and Shelley had drawn between science and poetry, was designed essentially to support his argument that applied or commissioned science was in no way inferior to what we characterize as 'pure' science. This creative or imaginative aspect was, however, pursued in other addresses at Cambridge to the same section and also to Section X (General) at the pragmatic level. Mr. Gerd Sommerhoff revealed it in his address, 'Creative Technology', when he described to the Education section some of the work and philosophy of the Technical Activities Centre which he directs at Sevenoaks School. The theme was maintained, at the philosophical level, by Mr. A. Koestler in an address to Section X on evolution and revolution in the history of science. It also received some passing comment in Dr. Magnus Pyke's presidential address to the latter Section on "Scientific Understanding and the Chief End of Man".

Dr. Pyke early in his address repudiated the charge of materialism that is often brought against science. He agreed that scientific understanding had relieved us of much of the need to pre-occupy ourselves with providing food and that it could equally remove the burden of earning a living—if 'burden' is an appropriate word to use in that context. The most powerful impact of applied science on the daily life of human society lies in the reduction it makes possible in the proportion of time necessarily spent on paid work, but, as Dr. Pyke continued, food, health, material prosperity, and a happy communal life, though desirable in themselves, are not the chief end of man. A civilized community should also direct its efforts towards developing the general powers of the mind, which makes possible the attainment of a higher purpose.

To such a goal, scientific thinking has much to contribute, and it is at this point that, after quoting a passage from Robert Oppenheimer about the delight of a scientist in the experience of discovery, which reflects the motif of Sir Cyril Hinshelwood's opening address, Dr. Pyke con-

sidered the way in which science shared the same attributes as art as one of man's creative activities. He did not suggest that this applied to all scientific work and he made a scathing comment about published scientific work which failed to advance scientific understanding. Unlike Sir Edward or Mr. Koestler, he did not refer to Prof. Kuhn, but in this passage of his address Dr. Pyke's thoughts obviously ran on similar lines to those expressed by Prof. Kuhn in his book, *The Structure of Scientific Revolutions**. Dr. Pyke passed on swiftly to urge that modern society had been created by the peculiar application of intellectual logic and imagination in search of truth. The real schism in our culture, he believed, was between those who based their behaviour on dogma and assertion and those who put their faith in reason and the conviction that the exercise of reason aided by imagination in search of truth is the basic human goal. The whole universe of science is built on the belief that this is a rational universe.

Dr. Pyke affirmed his own belief that the chief end of man is a moral one. While he contended that scientific understanding was not morally neutral but must radically affect our attitude to moral values, he agreed with Prof. D. M. MacKay, of the University of Keele, that it was the concern for truth and the like which begot science, not science which begot the concern for truth. He stated that such ethical values were essential to the practice of science, and the more people we taught to be scientists the more we should disseminate these values. At every point, scientific understanding, with its fundamental insistence on intellectual honesty, has a bearing on moral values. In its essentials, the search for truth, the chief end towards which man is finding himself driven to strive, is the pursuit of a moral goal. Dr. Pyke quoted Dr. A. Szent-Gyorgyi's observations that morals are the simple prescriptions which make living together possible and that the basic moral rule of the living society of scientists is mutual respect, intellectual honesty and goodwill. Because scientific understanding compels a reappraisal of the codes we learned mostly as children it is not destructive of moral behaviour.

Mr. Koestler began his lecture by referring to the framework he has developed at length in his book, *The Act of Creation*, at least sufficiently to insist that any discussion of creativity must start with a frame of reference for the ordinary routines of behaviour, and to argue that organic life, in all its manifestations, is governed by rules, whether innate or acquired, which are represented in coded form at various levels from the genetic code to the structures in the nervous system responsible for symbolic thought. He suggested that spontaneous insight usually means the emergence of a new synthesis of a whole, at a higher level than that of the parts which combined and fused into it. The combinational activity he regards as the key to creativeness and he appears to draw the line between solving routine problems and creative originality in much the same way as Prof. Kuhn does in *The Structure of Scientific Revolutions*.

* *The Structure of Scientific Revolutions*. By Prof. Thomas S. Kuhn. Pp. xiv + 172. (Chicago and London: University of Chicago Press, 1964. Originally published 1962.) 1.50 dollars; 10s. 6d. net.

As criteria of what we call 'creativity', Koestler used, as in *The Act of Creation*, four factors: originality; the lack of relation between the matrices which enter into the combination or, in other words, improbability; the intervention of extra-conscious processes; and the constructive-destructive element, which Koestler, like Kuhn, regards as characterizing any scientific revolution. The true scientist, he observes, like the true artist, is an uneasy mixture of the adventurer and the pedant, and he again reminds us that science is made by scientists and not scientists by science. His overall view of science as advancing by evolutionary and revolutionary cycles, a zig-zag progress, not a curve approaching its asymptote, thus closely resembles that of Kuhn.

Obviously this view of scientific advance and of creativity has implications both for the relations of science and technology and for their teaching, particularly when the whole content of syllabuses is under review. Kuhn insists that a new theory, for example, however special its range of applications, is seldom or never just an increment to what is already known. Its assimilation requires the reconstruction of prior theory and the reconstruction of prior fact, an intrinsically revolutionary process that is seldom completed by a single man and never overnight. The unexpected discovery is not simply factual in its import and the world of the scientist is qualitatively transformed as well as quantitatively enriched by fundamental novelties either of fact or of theory.

Prof. Kuhn is not directly concerned with this teaching aspect or with the relations between science and technology. He is concerned rather with the scientific community, which he regards as unique among professional communities in the extent to which individual creative work is addressed to and evaluated by other members of the profession. Moreover, this is facilitated by the comparative insulation of the scientific community from society in general. In this context, Prof. Kuhn's remark that, unlike the engineer and many doctors, the scientist need not choose problems because they urgently need solution or without regard for the tools available to solve them indicates the distinction he does in fact draw between the pure and the applied scientist—a distinction which he also draws between the former and the social scientist.

This effect of insulation from the larger society does lead Prof. Kuhn to touch on education, though apart from the remark that scientific training is not well designed to produce the man who will easily discover a fresh approach, he is concerned chiefly with the transmission of knowledge through text-books. These, he asserts, are systematically substituted for creative scientific literature, until the very last stages in the education of a scientist. That statement is certainly more open to challenge than a comment about the neglect of the historical aspects, where fortunately there are now indications that the past neglect is being repaired, but more important, perhaps, is Kuhn's comment on the implications of authority in science.

The very existence of science depends on vesting the power to choose between paradigms or the conceptual framework within which a discipline functions in the members of a special kind of community. The very tenuousness of humanity's hold on the scientific 'enterprise' indicates how special that community must be if science is to survive and grow. If authority alone, and particularly non-professional authority, were the arbiter of paradigm debates, the outcome might still be revolu-

tion; but it would not be scientific revolution. Nevertheless, while Prof. Kuhn recognizes also that the bulk of scientific knowledge is a product of Europe in the past four centuries and that no other place and time has supported the very special communities from which scientific productivity comes, he does not follow Dr. Pyke or draw the conclusion which Dr. Pyke has done about the moral basis of such a community or society.

Valuable as a clear understanding of the creative process may be, neither Kuhn nor Koestler establish the desirability of creativity as an important objective in our educational system. The important matter here is to ensure that society provides sufficient opportunities for fruitful creative work in science and technology, as in art, and that those opportunities are available to trained men and women at an early stage in their professional career (or, as Prof. R. V. Jones puts it, to bring them to the frontiers of their subject before they are so exhausted by their journey through knowledge already won that they cannot make the next advance). It is, of course, essential that their education and their professional training should be such as to encourage the capacity for clear and independent thought as well as to develop the critical faculty and sound judgment. This applies to university or higher education generally, however, and is now being realized in the technical colleges as well as in the colleges of technology and the universities, as not only the contribution of Mr. A. D. C. Peterson on liberal education and the British technologist but also Mr. F. Metcalfe's on the idea of a technical college plainly showed. Mr. Metcalfe was fully aware of the difficulties and handicaps of the technical colleges, but his paper was noteworthy because it gave a most encouraging picture of the way in which these colleges are now tackling the human problems of developing a new maturity in their students. Such a maturity should do much to ease the problem of communication between different disciplines and illuminate and encourage the understanding of the social values of their work. It is perhaps here, even more than in the universities and colleges of technology, that we must look for scientific understanding to make its fullest impact on the moral and the social values of the society in which we live.

The importance of a clearer and wider understanding of the creative process is also shown by a recent article in *Chemistry and Britain* (1, 449; October 1965). Under the title "Planning for Invention in Science-Based Industry", what is discussed is not so much the climate for invention, but rather the equally important problem of selecting the right projects; curiously enough, however, there is no reference to the difficult problem of deciding when to break off a project. It is true that the writer recognizes the importance of selecting staff with the right mental requirements and of ensuring effective communications, but the value of the article lies not in its contribution to the promotion of creative work but in its emphasis on the changes which the selection of problems now requires in the administration of research.

Now that most of the 'easy' problems have been solved, it is harder to obtain successful results in research, and this factor increases the importance of the research team, including specialists in various disciplines. The more penetrating background knowledge now available has, however, greatly increased the chances of solving the newer and more difficult problems. However, while research and invention remain as easy or difficult, and as challenging a routine because the two factors offset each other, the administrator of research must increasingly

strive to see that ideas are sought in the desired fields, and to choose those special fields correctly. It is obvious that this, in itself, could have profound implications for the balance between pure and applied science.

Leaving that aspect, however, the article in itself shows how timely is the decision which has led to the Summer 1965 issue of *Daedalus*, the journal of the American Academy of Arts and Sciences, being devoted to the subject of creativity and learning. Some of the essays, notably those of J. B. Wiesner on education for creativity in the sciences, Prof. L. S. Kubie on unsolved problems of scientific education, Dr. P. H. Abelson on relation of group activity to creativity in science, and Prof. E. P. Torrance on scientific views of creativity and factors affecting its growth, manifestly overlap with discussions at the Cambridge meetings of the British Association. This is also true, to a lesser degree, of Prof. D. Hawkins's essay on science education, "The Informed Vision", Prof. L. Eiseley's discussion of Darwin, Coleridge and the theory of unconscious creation, which is supported by an admirable bibliography, and Prof. J. D. Brown's essay on the development of creative teacher-scholars, on those who provide the vital core of a dynamic teaching university.

Not only does Prof. Brown maintain that a creative scholar provides a flow of new ideas, drawing on deep resources of accumulated knowledge; the art of teaching, well performed, is itself creative. He is well aware that he is concerned with only a small but precious minority, and that, while we can encourage the development of some attributes like the power of analysis and accumulation, the practice of self-discipline, or introspection, and resistance to external authority, with others like the inquiring mind, and most important of all, intuition, we can only hope to avoid dulling or repressing. He does not attempt to write a prescription for the development of a faculty of creative teacher-scholars but rather to point out some of the dangers which administration should seek to avoid; however, he recognizes that leadership and tradition have a contribution to make. Moreover, Prof. Brown reminds us that the true test of a good teacher is what he does to his students—developing their powers of clear thinking, analysis, orderly accumulation, evaluation and re-creation of ideas in their own terms. Higher education which fails to do just this is missing its objective and becomes a waste of precious resources.

There are other challenges to the thought and practice at present accepted in higher education in Britain, whether in science or technology, or in other fields, to be found in this symposium. Dr. Abelson, for example, considers the crucial element in creativity in science to-day is not dramatic illumination but judgment—a view which

would appear to put him with Koestler and Kuhn—though he does not ignore the importance of motivation and self-discipline. Torrance believes we have long known enough about the factors affecting creative growth to make a much better effort at creative education. His own investigations have convinced him that different kinds of children learn best when given opportunities to learn in ways best suited to their motivations and abilities, and he suggests that significant changes in methods of teaching could lead to many more people achieving a higher level of education as well as greater dignity and mental health in our society.

One of the most interesting contributions is that from Dr. Wiesner, who begins with some challenging remarks about professional obsolescence. Admittedly, he limits his assertion to the research or design engineer, but it may well be questioned whether ten years is, in fact, the longest time such a technologist can expect to be effective to-day without a continuing education or a major effort to refurbish his basic and specialized professional skills. If that is true, the validity of the original professional training may well be questioned, though no one would doubt that most new ideas come from a very small number of working scientists and technologists or that there is a scarcity of those with developed creative gifts.

Dr. Wiesner draws a distinction between creativity and productivity which is suggestive of Prof. Kuhn's thinking. A new idea or concept may be creative in a philosophical or artistic sense but, to be considered scientifically productive, it must also meet the criterion of being logically related, in quantitative terms, to the body of science. From this, he argues that creativity is a more general term than productivity and that when 'creativity' or 'creative' are used in association with science and technology, it is to be assumed that productivity is also involved.

The point is scarcely developed at sufficient length to command general assent before Dr. Wiesner proceeds to discuss the kinds of creative activity which society expects from scientists and technologists. Limiting that expectation, as he does, to scientific research, to applied scientific and technological research, and to engineering design, his thesis may perhaps be more readily accepted. Pointing out, however, that the student with a large fund of knowledge, polished techniques, and a total lack of creative ability is too common a product of our system, he emphasizes the need, at both school- and university-level, for education reform based on an adequate programme of research, if we are to develop the latent creative ability of our students—a conclusion which was also implicit in the Cambridge discussions.

TECHNICS AND THE NATURE OF MAN*

By LEWIS MUMFORD

President of the American Academy of Arts and Letters

THE last century, we all realize, has witnessed a radical transformation in the entire human environment, largely as a result of the impact of the mathematical and physical sciences on technology. This shift from an empirical, tradition-bound technics to an experimental

scientific mode has opened up such new realms as those of nuclear energy, supersonic transportation, cybernetic intelligence, and instantaneous planetary communication.

In terms of the currently accepted picture of the relation of man to technics our age is passing from the *primaeva* state of man, marked by his invention of tools and weapons for the purpose of achieving mastery over the forces of Nature, to a radically different condition, in which he will

* Substance of a paper read during the bicentennial celebrations commemorating the birth of James Smithson, held in Washington during September 16-18 (see *Nature* of October 23, p. 321).

not only have conquered Nature but detached himself completely from the organic habitat. With this new macro-technology, man will create a uniform, all-enveloping structure, designed for automatic operation. Instead of functioning actively as a tool-using animal, man will become a passive, machine-serving animal whose proper functions, if this process continues unchanged, will either be fed into a machine, or strictly limited and controlled for the benefit of depersonalized collective organizations. The ultimate tendency of this development was correctly anticipated by Samuel Butler more than a century ago: but it is only now that his playful fantasy shows many signs of becoming a far-from-playful reality.

My purpose in this article is to question both the assumptions and the predictions on which our commitment to the present form of technical and scientific progress, as an end itself, has been based. In particular, I find it necessary to cast doubts on the generally accepted theories of man's basic nature which have been implicit during the last century in our constant over-rating of the role of tools and machines in the human economy. I shall suggest that not only was Karl Marx in error in giving the instruments of production a central place and a directive function in human development, but even the seemingly benign interpretation by Teilhard de Chardin reads back into the whole story of man the narrow technological rationalism of our own age, and projects into the future a final state in which all the further possibilities of human development would come to an end, because nothing would be left of man's original nature that had not been absorbed into, if not suppressed by, the technical organizations of intelligence into a universal and omnipotent layer of mind.

Since the conclusions I have reached require, for their background, a large body of evidence I have been marshalling in a still unpublished book, I am aware that the following summary must, by its brevity, seem superficial and unconvincing. At best, I can only hope to show that there are serious reasons for reconsidering the whole picture of both human and technical development on which the present organization of Western society is based.

Now, we cannot understand the role that technics has played in human development without a deeper insight into the nature of man: yet that insight has itself been blurred, during the past century, because it has been conditioned by a social environment in which a mass of new mechanical inventions has suddenly proliferated, sweeping away many ancient processes and institutions, and altering our very conception of both human limitations and technical possibilities.

For a century man has been habitually defined as a tool-using animal. This definition would have seemed strange to Plato, who attributed man's rise from a primitive state as much to Marsyas and Orpheus as to Prometheus and Hephaestus, the blacksmith-god. Yet the description of man as essentially a tool-using and tool-making animal has become so firmly accepted that the mere finding of the fragments of skulls, in association with roughly shaped pebbles, as with Dr. L. S. B. Leakey's Australopithecines, is deemed sufficient to identify the creature as a proto-human, despite marked physical divergences from both earlier apes and later men.

Two substantial errors are embedded in this general interpretation. The first is the unintentional distortion of evidence, through the fact that the only durable remains of either early man or his hominid ancestors are an extremely scanty supply of bones and stones, presumably tools—though, apart from grubbing, pounding and ripping, one can only guess what purpose they served. The durability of stone artefacts has given this part of man's technical equipment a prominence it could never have claimed if the far richer store of organic materials, which early man shared with many primate ancestors, had been preserved. But it is an illusion to suppose that man's

technical development was confined to exploiting stone quarries, to flint-chipping, to the manipulation of tools alone: for the source of man's early technics was the whole natural environment—edible plants and animals, vines, leaves, shells, reeds, twigs, bark, fibre, skins—all of which, save the last, can be utilized without other tools than man's own unaided teeth and hands.

By fastening attention on the surviving stone artefacts, many anthropologists and ethnologists have gratuitously attributed to the shaping and using of tools the enlargement of the human brain and therewith the development of man's higher intelligence, though motor-sensory co-ordinations involved in this elementary manufacture do not call for or evoke any considerable mental acuteness. Since the sub-hominids of South Africa had a brain capacity about a third of *Homo sapiens*, no greater indeed than that of many apes, the capacity to make tools neither called for nor generated early man's rich cerebral equipment, as Dr. Ernst Mayr of Harvard has recently pointed out.

The second error in interpreting man's nature is a less pardonable one, since Francis Bacon should long ago have put scientists on guard against it; and that is, the present-day tendency to read back into prehistoric times modern man's overwhelming interest in tools and machines, to the exclusion of equally important items of technical equipment. Tools and weapons are specialized extrapolations of man's own organs for pushing, pounding, crunching, cutting, stabbing—all basic motor activities. No one can doubt that these dynamic processes, which man shares with many other species, formed an essential part of his earliest technical complex.

But just because man's need for tools is so obvious, we must guard against over-emphasizing the role of tools hundreds of thousands of years before they became functionally efficient. In treating tool-making as central to the palaeolithic economy, ethnologists have underplayed, or neglected, a mass of activities in which many other species were for long far more knowledgeable than man. Despite the contrary evidence put forward by R. V. Sayce, Daryll Forde and Andre Leroi-Gourhan, there is still a tendency to identify tools and machines with technology: to substitute a part for the whole. Even in describing only the material components of technics, this practice overlooks the equally vital role of containers: hearths, pits, houses, pots, sacks, clothes, traps, bins, byres, baskets, bags, ditches, reservoirs, canals and cities. These static components play an important part in every technology, not least in our own day, with its high tension transformers, its giant chemical retorts, its atom-reactors.

In any comprehensive definition of technics, it should be plain that many insects, birds and mammals had made far more radical innovations in the fabrication of containers, with their intricate nests and bowers, their geometric beehives, their urbanoid ant-hills and termitaries, than man's ancestors had achieved in the making of tools until the emergence of *Homo sapiens*. In short, if technical proficiency were alone sufficient to identify potential intelligence, man would, for long, have been rated a hopeless dunder alongside many other species. The consequences of this perception should be plain; namely, that there was nothing uniquely human in early technology until it was modified by linguistic symbols and aesthetic designs. At that point the human mind, not just the hand, made a profound difference.

At the beginning, then, I suggest that the human race had achieved no special position by reason of its tool-using or tool-making propensities alone. Or rather, man at the beginning possessed one primary all-purpose tool that was more important than any later assemblage: namely, his own mind-activated body, every part of it not just those motor activities that produced hand axes and wooden spears. To compensate for his extremely primitive working gear, early man had a much more

important asset that widened his whole technical horizon: he has a far richer biological equipment than any other animal, a body not specialized for any single activity, but, precisely because of its extraordinary plasticity, more effective in using a larger portion of both his external environment and his internal psycho-somatic resources.

Through man's over-developed and incessantly active brain, he had more mental energy to tap than he needed for survival at a purely animal level; and he was, accordingly, under the necessity of canalizing that energy, not just into food-getting and reproduction, but into modes of living that would convert this energy more directly and constructively into appropriate cultural—that is, symbolic—forms. Cultural work, by necessity, took precedence over manual work; this involved far more than the discipline of hand, muscle and eye in making and using tools. It likewise demanded a control of all man's biological functions, including his bodily organs, his emotions, his sexual activities, his dreams. Even the hand was no mere horny work-tool: it stroked a lover's body, held a baby close to the breast, made significant gestures, or expressed in ordered dance and shared ritual some otherwise inexpressible sentiment about life or death, a remembered past or an anxious future. Tool-technics is but a fragment of bio-technics: man's total equipment for life.

On this view, one may well hold it an open question whether the standardized patterns and the repetitive order which came to play such an effective part in the development of tools from an early period, as Braidwood has pointed out, derive solely from tool-making. Do they not rather derive even more, perhaps, from the forms of ritual, song and dance-forms which exist in a state of perfection among primitive peoples, often in a far more exquisitely finished state than their tools? There is, in fact, widespread evidence, first noted by Hocart, that ritual exactitude in ceremony preceded mechanical exactitude in work: that the first rigorous division of labour came through specialization in ceremonial offices.

These facts help to explain why simple peoples who easily get bored by purely mechanical tasks which might improve their physical well-being, will, nevertheless, repeat a meaningful ritual often to the point of physical exhaustion. The debt of technics to play and to play-toys, to myth and fantasy, to magic rite and religious rote, which, called attention to in "Technics and Civilization", has still to be sufficiently recognized, though J. Huizinga, in *Homo Ludens*, went so far as to treat play itself as the basic formative element in all culture.

Tool-making, in the narrow technical sense, may, indeed, go back to our hominid African ancestors. But the technical equipment of Chellean and Acheulian times remained extremely limited until a more richly endowed creature, with a nervous system nearer to that of *Homo sapiens* than to any primaeval hominid predecessors, had come into existence, and brought into operation not alone his hands and legs but his entire body and mind, projecting them, not just in tools and utensils but in more purely symbolic non-utilitarian forms.

In this revision of the accepted technological stereotypes, I would go even further: for I submit that at every stage, man's technological expansions and transformations were less for the purpose of increasing the food supply or controlling Nature, than for utilizing his immense actual resources, and expressing his latent potentialities, in order to fulfil more adequately his own unique super-organic needs. When not threatened by a hostile environment, the elaboration of symbolic culture was a more imperative need than control over the external environment—and, as one must infer, largely pre-dated it, and for long out-paced it.

On this reading, the invention of language—a culmination of man's more elementary forms of expressing and transmitting meaning—was incomparably more important to further human development than the chipping of a

mountain of hand axes. Beside the relatively simple co-ordinations required for tool-using, the delicate interplay of the many organs needed for the creation of articulate speech was a far more striking advance, and must have occupied a great part of early man's time, energy and mental concentration, since its collective product, language, was infinitely more complex and sophisticated at the dawn of civilization than the Egyptian or Mesopotamian kit of tools. For only when knowledge and practice could be stored in symbolic forms, and passed on by word of mouth from generation to generation, was it possible to keep each fresh cultural acquisition from dissolving with the passing moment or the dying generation. Then, and then only, did the domestication of plants and animals become possible. Need I remind you that this decisive technical transformation, sometimes termed a revolution, was achieved with no better tools than the digging stick, the axe, the mattock? The plough, like the cart-wheel, came later as a specialized adaptation to the large-scale field cultivation of grain.

To consider man as primarily a tool-using animal, then, is to overlook the main chapters of human prehistory. Even when one considers the technical milieu alone, this concentration on the dynamic components results in our having long treated as negligible that vast area of technics in which man's control of chemical changes through heat and fermentation, leaching and sterilizing—as in brewing, burning, melting, tanning, cooking—favourably modified the conditions of human existence. Opposed to this stereotype is the view that man is pre-eminently a mind-using, self-mastering animal; and the primary locus of all his activities is his own organism. Until he had made something of himself, he could make little of the world around him.

In this process of self-discovery and self-transformation, technics, in the narrow sense, served well as a subsidiary instrument, but not as the main operative agent in man's development; for technics was never until our own age dissociated from the larger cultural whole in which man—as man—has always functioned. Early man's original development was based on what Andre Varagnac happily called "the technology of the body": the utilization of man's highly plastic bodily capacities for the expression of his still unformed and uninformed mind, before that mind had yet achieved, through the development of symbols and images, its own more etherealized technical instruments. From the beginning, the creation of significant modes of symbolic expression, rather than more effective tools, was the basis of the further development of *Homo sapiens*. In approaching this conclusion I happily find myself reaching, by a quite independent route, Prof. Levi-Strauss's conception of cultural determinants.

Unfortunately, so firmly were the prevailing nineteenth-century conceptions committed to the notion of man as primarily *Homo faber*, the tool-maker, rather than *Homo sapiens*, the mind-maker, that, as is known, the first discovery of the art of the Altamira caves was dismissed as a hoax, because the leading palaeo-ethnologists would not admit that the Ice Age hunters, whose weapons and tools they had recently discovered, could have had either the leisure or the mental inclination to produce art—not crude forms, but images that showed powers of observation and abstraction of a high order. But when we compare the carvings and paintings of the Aurignacian or Magdalenian finds, with their surviving technical equipment, who shall say whether it is art or technics that shows the highest development? Even the finely finished Solutro laurel leaf points were plainly a gift of aesthetically sensitive artisans to functional efficiency. The Greek form for 'techniques' makes no distinction between industrial production and symbolic art; and for the greater part of human history these aspects were inseparable, one side respecting objective conditions and functions, the other responding to subjective needs.

Our age has not yet overcome the peculiar utilitarian bias that regards technical invention as primary, and aesthetic expression as secondary or superfluous; and this means that we have still to acknowledge that technics derives from the whole man, in his intercourse with every part of the environment, utilizing every aptitude in himself to make the most of his own biological and ecological potentials.

Even at the earliest stage, trapping and foraging called less for tools than for sharp observation of animal habits and habitats, backed by a wide experimental sampling of plants and shrewd interpretation of the effects of various foods, medicines and poisons on the human organism. And in those horticultural discoveries which, if Oakes Ames was right, must have preceded by many thousands of years the active domestication of plants, taste and formal beauty played a part no less than their food value; so that the earliest domesticates, other than the grains, were often valued for the colour and form of their flowers, for their perfume, their texture, their spiciness, rather than merely for nourishment. Edgar Anderson has suggested that the neolithic garden, like the gardens in many simple cultures to-day, was probably a mixture of food plants, dye plants and ornamentals—all treated as equally essential for life.

Similarly, some of early man's most daring technical experiments had nothing whatever to do with the mastery of the external environment: they were concerned with the anatomical modification or the superficial decoration of the human body, for sexual emphasis, self-expression, or group identification. The Abbé Breuil found evidence of such practices as early as the Mousterian culture, which served equally in the development of ornament and surgery. Plainly, tools and weapons, so far from dominating man's technical equipment, as the petrified artefacts too glibly suggested, constituted a small part of the biotechnical assemblage; and the struggle for existence, though sometimes severe, did not engross the energy and vitality of early man, or divert him from his more central need to bring order and meaning into every part of his life. In that larger effort, ritual, dance, song, painting, carving and, above all, discursive language—arts which utilize all the organs of the body—must for long have played a decisive role.

At its points of origin, then, technics was related to the whole nature of man, and that nature played a formative part in the development of every aspect of technology: thus technics at the beginning was broadly life-centred, not work-centred or power-centred. As in all ecological complexes, other human interests and purposes, other organic needs, restrained the overgrowth of any single component. As for the greatest technical feat before our own age, the domestication of plants and animals, this advance owed almost nothing to new tools, though it encouraged the development of clay containers. But it owed much, we now begin to realize, since Edouard Hahn, to an intense subjective concentration on sexuality in all its manifestations, abundantly visible in cult objects and symbolic art. Plant selection, hybridization, fertilization, manuring, seeding and castration were the products of an imaginative cultivation of sexuality, the first evidence of which one finds tens of thousands of years earlier in the emphatically sexual carvings of palaeolithic woman: the so-called Venuses.

But at the point where history, in the form of the written record, becomes visible, that life-centred economy, a true polytechnics, was challenged and in part displaced in a series of radical technical and social innovations. About five thousand years ago, a monotecnics, devoted to the increase of power and wealth by the systematic organization of workaday activities in a rigidly mechanical pattern, came into existence. At this moment, a new conception of the nature of man arose, and with it a new stress on the exploitation of physical energies, cosmic and human, apart from the processes of growth and reproduction,

came to the fore. In Egypt, Osiris symbolizes the older, life-oriented techniques: Atum-He, the Sun God, who characteristically created the world out of his own semen without female co-operation, stands for the machine-centred one. The expansion of power, through ruthless human coercion and mechanical organization, took precedence over the enhancement of life.

The chief mark of this change was the construction of the first complex, high-powered machines; and therewith the beginning of a new regimen, accepted by all later civilized societies—though reluctantly by more archaic cultures—in which work at a single specialized task, segregated from other biological and social activities, not only occupied the entire day, but increasingly engrossed the entire lifetime. That was the fundamental departure which, during the past few centuries, has led to the increasing mechanization and automation of all production. With the assemblage of the first collective machines, work, by its systematic dissociation from the rest of life, became a curse, a burden, a sacrifice, a form of punishment: and by reaction this new regimen soon awakened compensatory dreams of effortless affluence, emancipated not only from slavery but from work itself.

The machine I refer to was never discovered in any archaeological diggings, for a simple reason: it was composed almost entirely of human parts. These parts were brought together in a hierarchical organization under the rule of an absolute monarch, the commands of whom, supported by a coalition of the priesthood, the armed nobility, and the bureaucracy, secured a corpse-like obedience from all the components of the machine. Let us call this archetypal machine—the human model for all later specialized machines—the 'Megamachine'. This new kind of machine was far more complex than the contemporary potter's wheel or bow-drill, and it remained the most advanced type of machine until the invention of the mechanical clock in the fourteenth century.

Only through the deliberate invention of such a high-powered machine could the colossal works of engineering that marked the Pyramid Age in both Egypt and Mesopotamia have been brought into existence, often in a single generation. This new technics came to an early climax in the Great Pyramid at Giza. That structure, as J. H. Breasted pointed out, exhibited a watchmaker's standard of exact measurement. By operating as a mechanical unit, the 100,000 men who worked on that pyramid generated ten thousand horse-power: this human mechanism alone made it possible to raise that colossal structure with the use of only the simplest stone and copper tools—without the aid of such otherwise indispensable machines as the wheel, the wagon, the pulley, the derrick or the winch.

Two things must be noted about this new mechanism, because they identify it through its historic course down to the present. The first is that the organizers of this machine derived their power and authority from a cosmic source. The exactitude in measurement, the abstract mechanical order, the compulsive regularity of this Megamachine sprang directly from astronomical observations and abstract scientific calculations: this inflexible, predictable order, incorporated in the calendar, was then transferred to the regimentation of the human components. By a combination of divine command and ruthless military coercion, a large population was made to endure grinding poverty and forced labour at dull repetitive tasks, in order to ensure "life, prosperity and health" for the divine or semi-divine ruler and his entourage.

The second point is that the grave social defects of the human machine were partly offset by its superb achievements in flood control and grain production, which laid the ground for an enlargement in every area of human culture: in monumental art, in codified law, and in system-

atically pursued and permanently recorded thought. Such order, such collective security and abundance as was achieved in Mesopotamia and Egypt, later in India, China, in the Andean and Mayan cultures, was never surpassed until the Megamachine was re-established in a new form in our own time. But conceptually the machine was already detached from other human functions and purposes than the increase of mechanical power and order: with mordant symbolism, its ultimate products in Egypt were tombs and mummies, while later, in Assyria, the chief testimonial to its efficiency was typically a waste of destroyed cities and poisoned soils.

In a word, what modern economists lately termed the 'Machine Age' had its origin, not in the eighteenth century, but at the very outset of civilization. All its salient characteristics were present from the beginning in both the means and the ends of the collective Megamachine. So Keynes's acute prescription of pyramid building as an essential means of coping with the insensate productivity of a highly mechanized technology, applies both to the earliest manifestations and the present ones; for what is a space rocket but the precise dynamic equivalent, in terms of our present-day theology and cosmology, of the static Egyptian pyramid? Both are devices for securing, at an extravagant cost, a passage to heaven for the favoured few.

Unfortunately, though the labour machine lent itself to vast constructive enterprises, which no small-scale community could even contemplate, much less execute, the most conspicuous result has been achieved through military machines, in colossal acts of destruction and human extermination; acts which monotonously soil the pages of history, from the rape of Sumer to the blasting of Rotterdam and Hiroshima. Sooner or later, I suggest, we must have the courage to ask ourselves: Is this association of inordinate power and productivity with equally inordinate violence and destruction a purely accidental one?

Now the misuse of Megamachines would have proved intolerable had they not also brought genuine benefits to the whole community by raising the ceiling of collective human effort and aspiration. The least of these advantages was the gain in efficiency derived from concentration on rigorously repetitive motions in work, already, indeed, introduced in the grinding and polishing processes of neolithic tool-making. This inured civilized man to long spans of regular work, with higher productive efficiency per unit. But the social by-product of this new discipline was, perhaps, even more significant; for some of the psychological benefits, hitherto confined to religious ritual, were transferred to work. The sterile repetitive tasks imposed by the Megamachine, which in a pathological form we associate with a compulsion neurosis, nevertheless served, like all ritual and restrictive orders, to lessen anxiety and to defend the worker himself from the often demonic promptings of the unconscious, no longer held in check by the traditions and customs of the neolithic village.

In short, mechanization and regimentation, through labour armies, military armies, and ultimately through the derivative modes of industrial and bureaucratic organization, supplemented and increasingly replaced religious ritual as a means of coping with anxiety and promoting psychal stability in mass populations. Orderly, repetitive work provided a daily means of self-control: more pervasive, more effective, more universal than either ritual or law. This hitherto unnoticed psychological contribution was possibly more important than those gains in productive efficiency, which were too often offset by absolute losses in war and conquest. Unfortunately, the ruling classes, which claimed immunity from manual labour, were not subject to this discipline: hence, as the historic record testifies, their disordered fantasies too often found an outlet in reality through destruction and extermination.

Having indicated the beginnings of this process, I must regrettably pass over the actual institutional forces which have been at work during the past five thousand years, and leap, all too suddenly, into the present age, in which the ancient forms of biotechnics are being either suppressed or supplanted, and in which the continued enlargement of the Megamachine itself has become, with increasing compulsiveness, the condition of scientific and technical advance if not the main purpose of human existence. But if the clues I have been attempting to expose prove helpful, many aspects of the scientific and technical transformation of the last three centuries will call for reinterpretation and judicious reconsideration. For, at the very least, we are now bound to explain why the whole process of technical development has become increasingly coercive, totalitarian, and—subjectively speaking—compulsive and irrational.

Before accepting the ultimate translation of all organic processes, biological functions, and human aptitudes into an externally controllable mechanical system, increasingly automatic and self-expanding, it might be well to re-examine the ideological foundations of this whole system, with its concentration on centralized power and external control. We must, in fact, ask ourselves if the probable destination of this system is compatible with the further development of specifically human potentialities.

Consider the alternatives now before us. If man were actually, as present-day theory still supposes, a creature whose use of tools alone played the largest formative part in his development, on what valid grounds do we now propose to strip mankind of the wide variety of autonomous activities historically associated with agriculture and manufacture, leaving the residual mass of workers with the trivial task of watching buttons and dials, and responding to one-way communication and remote control? If man actually owes his intelligence mainly to his tool-using propensities, by what logic do we now take his tools away, so that he will become a functionless, workless being, conditioned to accept only what the Megamachine offers him: an automaton within a larger system of automation, condemned to compulsory consumption, as he was once condemned to compulsory production? What in fact will be left of human life if one function after another is either taken over by the machine, or else genetically suppressed, if not surgically removed?

But if the analysis of human development, in relation to technics sketched out in this article, proves sound, there is an even more fundamental criticism to be made; for we must then go on to questions that would now shift the locus of human activity from the organic environment and the human group to the Megamachine, and eventually reduce all forms of life and culture to those that can be translated into the current system of scientific abstractions, and transferred on a mass basis to machines and electronic apparatus. We are now in a position to question the dubious assumptions that have too long been treated as axioms, for the system of thought on which they are still based, antedated by three centuries anything like the present comprehension—scientific, humanistic and historic—of man's nature and special gifts. From the Pyramid Age to the so-called Nuclear Age, we note, the inventors and controllers of the Megamachine have been haunted by delusions of omniscience and omnipotence; and these delusions are not less irrational now that they have at their disposal all the formidable resources of exact science and a high-energy technology.

The Nuclear Age conception of 'absolute power' and infallible intelligence, exercised by a military-scientific elite, corresponds to the Bronze Age conception of divine kingship; and both belong to the same infantile magico-religious scheme as ritual human sacrifice. Living organisms can use only limited amounts of energy, as living personalities can utilize only limited quantities of knowledge and experience. 'Too much' or 'too little' is equally fatal to organic existence. Even too much abstract

knowledge, insulated from feeling, from moral evaluation, from historic experience, from responsible, purposeful action, can produce a serious unbalance in both the personality and the community. Organisms, societies and human persons are nothing less than delicate devices for regulating energy and putting it at the service of life. To the extent that our Megatechnics ignores these fundamental insights into the nature of organisms and human personalities, it is pro-scientific in its attitude toward the human personality, even when not actively irrational. When the implications of this weakness are taken in, a deliberate large-scale dismantling of the Megamachine in all its institutional forms must surely take place, with a re-distribution of power and authority to smaller units, under direct human control.

If technics is to be brought back again into the service of human culture, the path of advance will lead, not to the further expansion of the Megamachine, but to the development of all those parts of the organic environment and the human personality that have been suppressed in order to magnify the offices of the pure intelligence alone, and therewith to maximize its coercive collective exercise and quantitative productivity. The deliberate expression and fulfilment of human potentialities requires a quite different approach from that bent solely on the control of natural forces, and the modification of human nature in order to facilitate and expand the system of control. We know now that play and sport and ritual and dream-fantasy, no less than organized work, have exercised a formative influence on human culture and even on technics. But make-believe cannot for long be a sufficient substitute for productive work: only when play and work form part of a larger cultural whole, as in Tolstoy's pictures of the mowers in *Anna Karenina*, can the many-sided requirements for full human growth be satisfied.

Instead of liberation from work being the chief contribution of mechanization and automation, I would suggest that liberation for work, for educative, mind-forming work, self-rewarding even on the lowest physiological level, may become the most salutary contribution of a life-centred technology. This may prove an indispensable counter-balance to universal automation: partly by protecting the displaced worker from boredom and suicidal desperation, only temporarily relievable by anaesthetics and sedatives, partly by giving play to constructive impulses, autonomous functions, meaningful activities.

Relieved from abject dependence on the Megamachine, the whole world of biotechnics will at once become open to man; and those parts of his personality that have been crippled or paralysed by insufficient use should again come into play. Automation is indeed the proper end of a purely mechanical system; and in its place, subordinate to other human purposes, automation will serve the human community no less effectively than the reflexes, the hormones, and the autonomic nervous system—Nature's earliest experiment in automation—serve the human body. But autonomy is the proper end of organisms; and further technical development must aim at re-establishing autonomy at every stage of human growth by giving play to every part of the human personality, not merely to those functions which serve the machine.

I realize that in opening up these difficult questions I am not in a position to provide ready-made answers, nor do I suggest that such answers will be easy to fabricate. But it is time that our present wholesale commitment to the machine, which arises largely out of our one-sided interpretation of man's early technical development, should be replaced by a fuller picture of both human nature and the technical *milieu*, as both have evolved together.

THIRD DIMENSION OF MANAGEMENT

By JOHN J. HAYWOOD

Kingston College of Technology, Kingston on Thames

IT is known that management confrontation with problems is a daily event, yet doubt exists as to how, when, or if they are solved. Events generating problems and forces aiding their solution are marshalled both internally and externally with regard to the phenomenon of management. Its activity and involvement can be seen in a three-dimensional perspective. The individual unit, the firm, comprises the first dimension. In this sphere of management, establishment and control of problem-solving aids reflect growth and significance of those internal problems which management recognizes. Accordingly, the firm's individual and peculiar expertise is recruited as a personnel function.

The second dimension of management involvement concerns the group, a nucleus of individual firms. More and different problems are here involved, necessitating a wider and better source of expertise supply, reflecting more diverse problems, different methods of problem-solving, and differing aspects of the same problem found in the first dimension of management.

The third dimension of management involvement concerns industry, both nationally and internationally. The first two dimensions operate within internal constraints. They are introversive approaches to management of enterprise, while the third dimension is essentially extroversive. This outer-directed approach takes cognizance of environmental factors, determined by political, economic, social, and technical forces interacting to produce change, disturbing a given climate within which enterprise may function.

In this dimension, management needs to supplement individual firm and group expertise by use of external supplies of aid to combat those complex problems with which no one enterprise is fully equipped to deal. There is evidence that major problems arise externally, and are more difficult for management to control, a factor lending greater importance to investigation into sources and use made of external aids. Thus a decision was made to investigate and assess both quantitatively and qualitatively sources of external aids, institutional and non-institutional, formal and informal.

The Department of Scientific and Industrial Research financed a twelve-month pilot survey of firms and organizations in the Kingston area of Surrey, and completion was effected in May 1965 (Table 1). No fewer than 362 contact attempts were made, on a random sample basis. The local Ministry of Labour area was used for geographical definition. In all, 241 interviews were obtained, including 25 telephone interviews which were, however, excluded from the survey data. This left data on 189 organizations and 27 aid bodies interviewed in person. There were 68 firms who refused co-operation, 65 who could not be contacted, and 15 offered interviews but were unacceptable, primarily owing to distances involved. Non-contact firms were 18 per cent and refusals 18.75 per cent of the sample, leaving a 63.25 per cent coverage. A representative sample with a cross-section of industries and trades was grouped in conformity with the Standard Industrial Classification. Analysis of refusals of co-operation revealed that, of these, 21 confirmed some use of external aids, while 25 gave no

data, and the remaining 22 showed no common characteristics.

For purposes of analysis, firms were divided into manufacturing, service, and distributive trades, and differentiated over seven variables, *inter alia* with regard to size, type, technology, ownership and management characteristics. External aid bodies were grouped into research and trade associations, management and technical consultants, Government and public bodies, and trade suppliers. Interviewees consisted of managing directors or general managers.

After five revisions a suitable questionnaire *pro forma* report form was developed, while preliminary findings indicated the need for 'depth' interviews with four or five employees to obtain a more reliable guide as to aid usage. Many of the data gained in the investigation could well have been distorted by interviewees attempting to conceal ignorance or to maintain secrecy. Yet confidence was preserved throughout, and no evidence of unreliable data has emerged.

This investigation reveals a recognizable national and international amalgam of external aid-bodies, covering a wide variety of specialist services. This extant market of external aid, both produced and consumed, comprises the third dimension of management. Yet poor communication robs it and Britain of beneficence consequent on full impact. Ignorance by management personnel of where, when, and how to obtain relevant assistance is a functional malady partly due to their inadequate reading, the professional ethic, and indifference of Government agencies.

Nevertheless, our data reveal that advice and assistance were sought from 19 management consultants, 13 technical consultants, 35 professional bodies, 5 international bodies, 31 research associations, 53 Government bodies, and 23 miscellaneous bodies, which represents a minimal use of institutional aids of encouraging magnitude.

In conformity with expectations, it appears that large firms in all sectors used proportionally more external aids than medium- and small-sized firms. The manufacturing sector demonstrates that large firms consumed proportionally more external aids than medium-sized firms, and more absolutely than small firms, the most marked difference being use of management consultants.

A similar trend occurs within the services sector, but a more complex pattern of aid-consumption arises in the

distributive sector. In general, large firms used proportionally more aids, but the incidence of particular aid-consumption varies. There is a marked tendency for greater consumption of research association and technical consultant services by large firms compared with medium-sized firms, and a much greater consumption of research association and management consultant services over small firms.

Certainly large firms can better afford consultants' fees, and may need greater exposure to consultant expertise than smaller firms, which are less prone to complex organizational, production and marketing problems. Consumption of research association services seems more widespread in the manufacturing sector than in either services or distributive sectors. This points to a greater awareness of research benefits, and a more even deployment of internal technocracy in firms engaged in manufacturing activity. Yet it may also reflect a wider gap between the best and average performance firms in both the service and distributive sectors. Increased productivity in the latter sector would contribute much to national economic growth. But size, *per se*, is an indicator neither of effectiveness nor of competence within given areas of enterprise, despite the assumed greater technical expertise and resources available to management. In the science-based industries with dynamic growth-records since the Second World War, some small and medium-sized firms have made contributions to industrial growth out of all proportion to their size. This suggests that they make use of group resources, which are usually available to large organizations.

In the manufacturing sector, subsidiary firms used proportionately more aids than independently owned firms, especially of consultants and research associations. No trend was discernible in service trades, but subsidiaries were greater consumers of management consultant aids than independent firms in the distributive sector. This is encouraging, in that subsidiaries apparently seek the best, rather than the most accessible aids, that is, involvement in second dimension activities is supplemented by third dimension involvement.

In order to assess quantitative use of external aid, interviewees were requested to enumerate and evaluate usage. Informants invariably did not record usage, and though many felt able to evaluate benefits of aid, this was necessarily done in a superficial and subjective fashion. They all found difficulty in isolating the impact of external aid over long or short periods, and they also hesitated to evaluate their firm's efficiency and stability though the latter proved less difficult. Perhaps Prof. J. Johnson's article on the productivity of management consultants¹, regarding a quantitative analysis of the benefits of consultants to management, is indicative of the problems involved here.

Our investigation revealed an unawareness by top management of the extent to which its organization made use of external aid bodies, notably of trade and research associations. Similarly, reasons given for joining these bodies reflect a misunderstanding of the nature of services they can properly supply to management. Furthermore, incorrect use of consultants was not uncommon, for it was not recognized that consultants serve in a therapeutic role, are not magicians and cannot serve as a substitute for good management.

Government agencies are sometimes viewed with contempt and hostility, yet their utility is obvious in exporting activity, and in dissemination of technical knowledge in its customer/supplier relationships. Not least, the Ministry of Technology is responsible for numerous research stations, associations and national laboratories.

This pattern of using the right aids for the wrong reasons, and wrong aids for the right reasons, reflects ignorance and suspicion of external help in problem-solving. It produces a two-fold reaction. Internal resources and expertise may be under-utilized, while industry and

Table 1. ANALYSIS OF 189 INTERVIEWS BY EXTERNAL AID USED, 1963-65

	Ownership	R.A.	T.A.	M.C.	T.C.	Gov.	Supp.	Total firms
Manufacturing sector								
0-49 Employees	I	5	8	0	1	10	11	17
	S	3	2	1	0	1	3	4
50-999 Employees	I	12	26	5	6	17	18	29
	S	8	15	6	9	11	9	20
1,000+ Employees	I	6	7	3	3	6	5	7
	S	9	11	6	8	11	11	14
Total		43	69	21	27	56	57	91
Services sector								
0-49 Employees	I	2	7	2	8	12	11	23
	S	0	3	0	1	2	3	5
50-999 Employees	I	6	12	1	3	10	10	16
	S	3	4	1	3	3	5	8
1,000+ Employees	I	4	7	4	4	5	4	7
	S	0	1	1	0	0	1	1
Total		15	34	9	19	32	34	60
Distributive sector								
0-49 Employees	I	0	11	0	1	4	13	18
	S	0	0	0	0	0	2	2
50-999 Employees	I	2	5	4	1	4	4	8
	S	1	4	2	0	0	3	4
1,000+ Employees	I	6	6	4	3	4	5	6
	S	0	0	0	0	0	0	0
Total		9	26	10	5	12	27	38
Aggregate usage		67	129	40	51	100	118	189

Abbreviations: R.A., research association; T.A., trade association; M.C., management consultant; T.C., technical consultant; Gov., Government and public bodies; Supp., trade suppliers. Ownership: I, independent; S, subsidiaries.

This table only indicates minimum use of external aids, since firms may have used them more than once, in the past two years.

national opportunities fall short of what they could and should be.

External aids are the vehicle of technical and managerial advance via dissemination and propitious use of the latest techniques, systems and, above all, in the moulding of responsive attitudes.

While there is not necessarily a functional relationship between greater alertness and knowledge in management, and its willingness and ability to implement, some relationship does persist. Perhaps the momentum of international competition will stimulate the former and accelerate the latter.

If the impact of external aid consumption on management efficacy was difficult to measure, its incidence proved impracticable to quantify. The role of trade suppliers as an informal, non-institutional aid is probably a more active element in the incidence of aid, than any other. Such aid sources underline the quest for long-term relationships by management as does membership of trade and research associations. There appears to be a significant correlation between size and length of business relationships between suppliers and their customers, and the amount of technical aid received. Again, the earlier pattern of aid consumption is repeated, in that small and medium-sized firms, unable to order in bulk, lose benefits of research and development undertaken by suppliers, especially in diversifying the use of raw materials. In so far as large suppliers use external aids fully and incorporate such help in their products and services, they spread the incidence of third-dimensional involvement to trade buyers. Although analysis of such benefit is problematical, its importance cannot be overlooked.

In this pilot investigation, only eight firms alleged non-usage and non-involvement in external aids to management problem-solving. Analysis of their data and characteristics showed no clear pattern of trends, except that seven were very small, four were old-established independent firms, and only two had modern equipment. Subsequent behavioural characteristics of their top management revealed a comparative abnormality of attitudes for all eight firms, varying from excessive greed for power, to an obsessive desire to save money. The latter found expression in self-design of product packaging, self-advertising and extensive modification of standard machinery products imported from its parent firm. Apparently, management time and effort thus expended was regarded as a cost-free service to the firm.

Again, the impact on management from non-involvement in the third dimension was exceedingly complex and difficult to quantify.

But this survey has shown a different, probably more important, magnitude discernible in terms of the sociological and psychological aspects imbued in management, involved in third-dimensional activities. This is the unquantifiable element of management attitudes, the imagery formed from those impacts on the personality moulded by an environmental intercourse on an industrial, national and international plane. Attitudes are difficult to define and rarely expressed, but are the most vital component of receptiveness to change. This capacity interacts with innovational motivation, which in all functions and levels of management reveals the key to greater productivity. If continual confrontation with aid and involvement in the third dimension of management activity highlights individual shortcomings in knowledge and performance, compared with industry and world leaders, then perhaps the gap between the best and average performances can be narrowed. In this manner, elusive increases in productivity can fall within our grasp. Certainly if every firm increased its productivity by only a small amount, in aggregate national growth objectives would be realized. Enlightened management in leading British firms in all sectors can compete with their foreign rivals, a fact which holds good in the building industry as well as in the chemical and man-made fibres industries.

The third dimension of management in the form of supply and consumption of a variety of specialized services has potentially a powerful part to play in the improvement of industry and national performance. In this connexion, social and psychological aspects of impact and incidence on management imagery are vitally important for Britain's economic future; although not lending themselves to quantitative assessment, they are of such significance that more time and thought should be given to them.

This pilot investigation has many imperfections, but it is a beginning; more research resources are needed in this neglected field of management activity. For, while quantitative method has its place in management science, rigid attitudes of management towards its present role, and future responsibilities and needs, can seriously delay that improvement which is so urgently desired by industry and by Britain as a whole.

¹ Johnson, J., *J. Roy. Stat. Soc.*, 126, II (1963).

STRUCTURES INSIDE A LUNAR CRATER PHOTOGRAPHED BY RANGER VII

By CAPTAIN VERN G. SMALLEY* and LUCIANO B. RONCA

Lunar-Planetary Research Branch, Air Force Cambridge Research Laboratories,
L. G. Hanscom Field, Bedford, Massachusetts

FRAME number 199 of *Ranger VII*'s camera A reveals three structures in a 235-m diameter crater. These features have been the subject of considerable controversy, and their origin is the subject of this article. Fig. 1 is an enlargement of this crater, and the three structures and associated shadows are indicated by numbers and letters, respectively.

The features were analysed in three different ways, using three-dimensional clay models, tonal contour maps, and over-exposed under-developed prints. The first method involved the fabrication of scale models (1 : 1,500) illuminated at an angle of 23° to the horizon, corresponding to the angle of illumination of the lunar surface when the pictures were taken by the spacecraft. Using a Polaroid Land camera, the model was photographed at the *Ranger VII* angle of altitude and azimuth. Then, using a stereoviewer, we simultaneously viewed the model photograph

and the *Ranger VII* photograph, not trying to see a three-dimensional picture, but attempting to simultaneously compare the two photographs. In this manner one is able to pay very close attention to even small differences in the fine details in both photographs.

It was found that the shadows in this feature are so related that the placement of one shadow greatly influences the position of others. Changing the elevation of any one feature in the model results in a change of shadows which in turn results in a change of other elevations which in turn results in the change of other shadows, etc. By making innumerable modifications in our models, we made the photographs of the model match the *Ranger VII* photograph. To obtain an independent appraisal of this feature, we obtained the assistance of a professional artist, Mr. Emilius Ciampa, a sculptor trained in the investigation of light-and-shadow effects. Mr. Ciampa's model was identical in major outline to ours, strengthening

* At present at the Cosmic Radiation Branch.

the validity of the final interpretation, a stereo photograph of which is shown in Fig. 2.

The second phase of our investigation involved a tonal contour study. Using a first-generation 35-mm master negative of Frame A-199, we obtained a micro-analyser trace of the 235-m crater. The effective spot size was 11.2μ , and the traces were spaced 25μ apart. The output of the analyser was then digitized, and the results were plotted in a two-dimensional array and contoured (Fig. 3).

The third method of analysis undertaken was that which involved over-exposed and under-developed prints (Fig. 4). We found that by long exposures (5–6 min duration at

$f/5.6$) of high contrast paper (Kodak F/5), and by pulling the print from the developer prematurely, we were able to assess degrees of darkness of shadows which are normally completely black. The results thus obtained from these three investigations were analysed for clues which may suggest a probable origin.

A number of conclusions were obtained from this investigation:

(1) All three structures, Nos. 1, 2 and 3, are illuminated by primary sunlight. The possibility of at least one of the structures being illuminated by reflected light was discarded after an examination of scale-models which

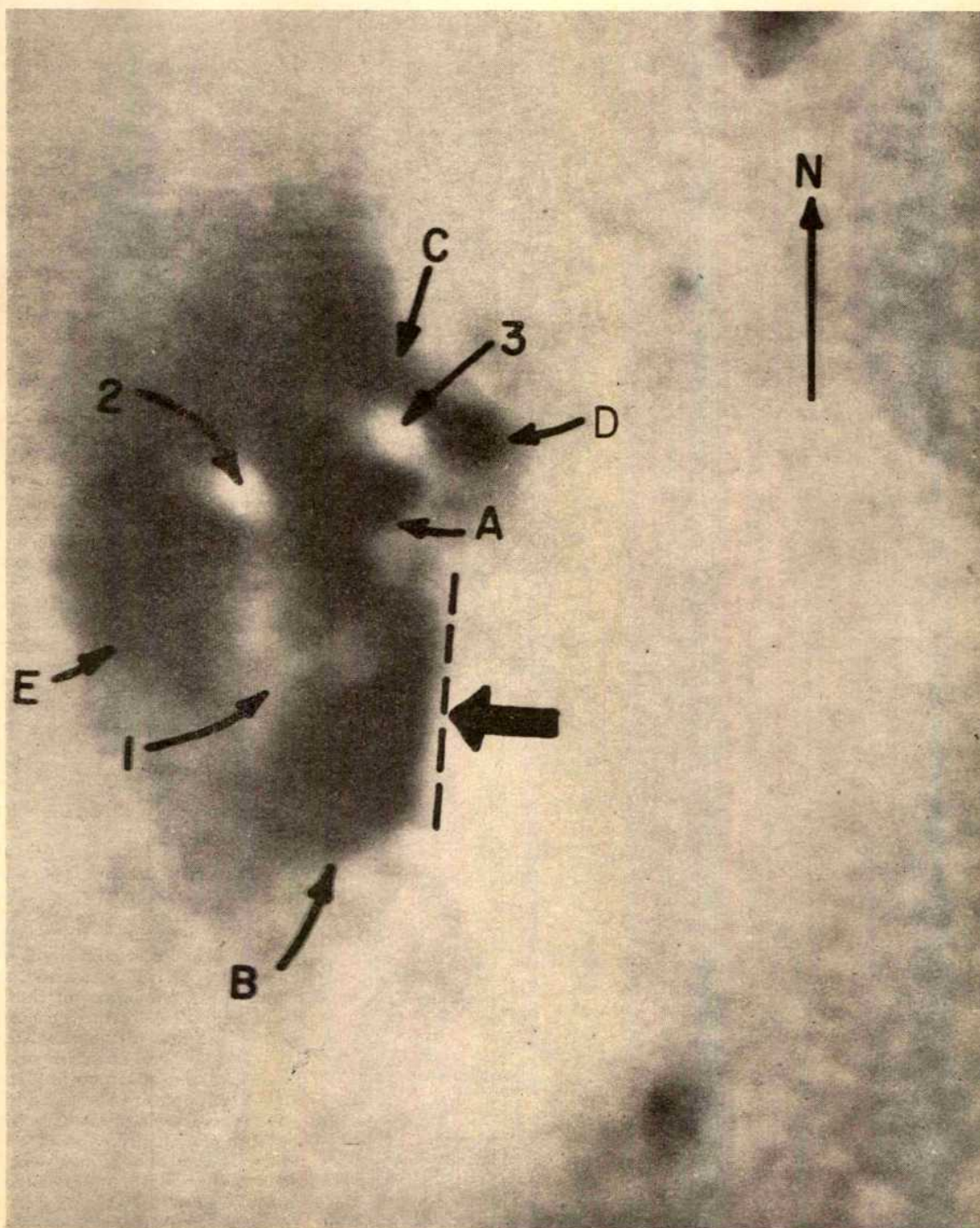


Fig. 1. Magnified detail of photograph 199 of *Ranger VII*'s camera A. Positive structures 1, 2 and 3 and shadows A, B, C, D, and E are indicated

were fabricated by the professional sculptor. In order to have reflected lighting, a steep cliff, shown as a dashed line in Fig. 1, would have to be the reflecting surface. From geometric considerations, it is difficult to explain reflected light to any one of the three structures and to maintain shadows *A* and *B* (Fig. 1) so extremely dark. Further, it is unlikely that the material of this structure could have a sufficient albedo to reflect the large amount of light necessary. Our conclusion is that all three structures are illuminated by direct lighting.

(2) The smooth portion of the 235-m crater floor is of lighter tone than the surroundings. It is impossible to reproduce the light tone of the crater floor without either tipping it strongly toward the light source or increasing the albedo. As the crater floor is unlikely to be steeply inclined, an increase in albedo is necessary.

(3) A lengthy examination of the results revealed that there were several steep slopes associated with the three structures. The northern side of structure 3 is very steep,

and an indication of this is seen in Fig. 1. There is no projection west of shadow *C*, but this shadow is seen to partially lie north of structure 3. This can only be explained by having a trench at that point. In other words, the north side of structure 3 is steep enough to have the terrain immediately north of it in shadow. This was easily verified in the three-dimensional analysis.

The southern slope of structure 3 is likewise steep. Shadow *A* is reasonably symmetrical about an east-west line which passes through the eastern-most point of that shadow. We argue that the slope south of structure 3 can only be steep, in view of the symmetry of shadow *A* and the close proximity of the dark portion of shadow *A*. A slight slope could not explain the sudden difference in quantity of reflected light, nor is it consistent with the geometry shown in the three-dimensional model study.

The west sides of structures 1 and 2 are thought to be steep, because gentle slopes could not account for the darkness of shadows *A* and *B*. The north and south sides of structure 2 are likewise thought to be steep. We experimented with various slopes and geometries, and found that only very steep slopes could reasonably account for the shape of shadow *A*.

We concluded, therefore, that very steep slopes, with more than 70° of inclination, exist on the north and south sides of structure 3, steep slopes with inclination of 45° – 60° exist on the east sides of structures 1, 2 and probably on the north and south sides of structure 2.

(4) The steep slopes in some cases do not stop at what would normally be the basin of the 235-m crater, but appear to be related to topographic lows, which are lower than the crater basin. These lows are also suggested by the over-exposed under-developed prints, which indicated uncommonly dark zones. The intensity of the darkness suggests that there exist topographic lows in these shadow zones, and that they are lower than the undisturbed floor of the crater. The darkness of a shadow is not linearly proportional to the depth of the low; but, other things being equal, the greater the depth within the shadow, the greater will be the intensity of darkness of the shadows. Considering this, and the topography of the terrain adjacent to the shadows, we conclude that shadows *A* and *B* traverse terrain which is lower than the adjacent terrain. This is supported also by the fact that, in order to place properly the eastern border of shadow *B* in the three-dimensional analysis, the terrain immediately to the east of this border had to slope slightly downward (see broad arrow in Fig. 1) towards structure 1.

(5) We were able to suggest a reasonable depth of the 235-m crater as a result of the three-dimensional model examined. We found the depth at the centre of the basin to be of the order of 40–50 m. Structures 1, 2 and 3 have heights above the basin of approximately 30, 20 and 20 m, respectively. The minimum volume of structure 1, that is, the portion which appears to rise above the floor of the crater, was determined by cutting that portion of the clay away with a sharp knife, rolling it into a ball, and measuring the diameter. The minimum volume was thus found to be 68,000 cubic metres.

(6) We also found that what was depicted as a circular summit crater on structure 1 in the Aeronautical Chart

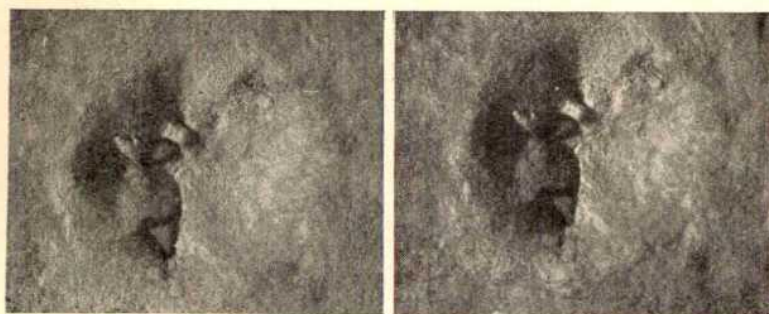


Fig. 2. Stereo pair of clay model

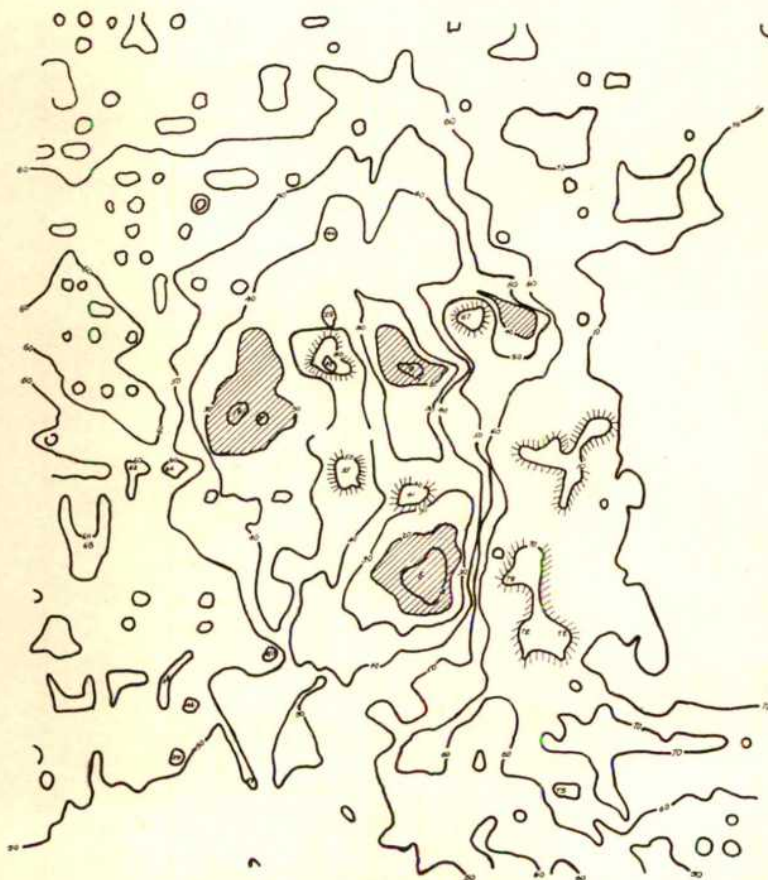


Fig. 3. Tonal contour map. The cross-hatched areas indicate shadows; areas with hachures indicate bright areas

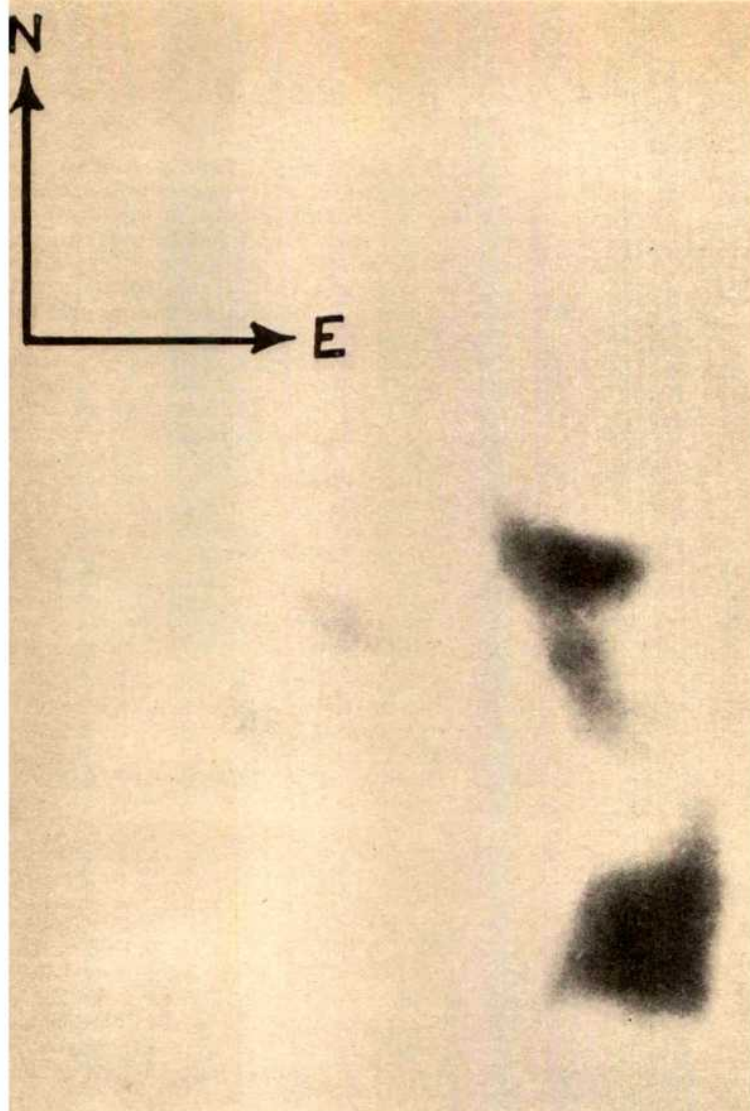


Fig. 4. Over-exposed under-developed print, showing the extremely dark areas, which are interpreted as being topographic lows

and Information Center's lunar chart, RLC 4, is in reality a U-shaped valley, extending downward to the north-east, and, rather than occurring at the summit, it occurs half-way down the gentle slope of that structure.

We therefore conclude that all three structures, 1, 2 and 3, are illuminated by primary sunlight; that the three structures are not the rims of, or are not due to, several minor craters in the 235-m crater; that associated with each of the three structures there is at least one steep slope; and that there are topographic lows within this crater that are lower than the crater floor. With this quantity of the topography known, we shall now proceed to interpret this information and suggest what we believe a reasonable origin.

Conceivably, the structures in the main crater could have one of the following origins:

- (1) The structures are pre-crater buried highs which are exposed during the formation of the crater.
- (2) The structures are contemporaneous or pene-contemporaneous with the crater. That is, they are the remnants of the projectile which caused the crater or they are chunks of local material dislodged by the impact.
- (3) The structures are post-crater. (a) The structures are the result of primary, secondary or tertiary impact in a pre-existing crater. (b) The structures were caused by

an endogenous process in a pre-existing crater.

It is unlikely that the structures are pre-crater, because the topographic lows, deeper than the floor of the main crater, and the steep slopes of the structures are unlikely to have survived an impact severe enough to have created a 235-m crater.

The second possibility, that the structures are contemporaneous or pene-contemporaneous with the main crater, also seems unlikely. Whether the structures tumbled down the slope of the crater immediately after its formation, or are the remnants of an impacting body, it would be surprising if they retained their large dimensions and steep slopes after undergoing the shock of either tumbling or deceleration. Lunar surface material is supposed to have low cohesive strength at this scale¹ and the angle of repose is not affected by the low lunar gravity².

Another argument against these structures being contemporaneous or pene-contemporaneous is based on the occurrence of other similar features. The mechanisms which we have discussed up to this point should be common to all other craters. We find it strange that the occurrence of structures in the basins of other similar craters is so rare. A reasonable conclusion is that the structures did not form, or were not placed, at the time of the formation of the crater, but at a later date. Thus, we are led to consider post-crater processes.

If we assume the structures to be post-crater, they could have been formed by either of two mechanisms: impact on a pre-existing crater or endogenous activity in a pre-existing crater.

The main objection to impact on a pre-existing crater is again the steepness of the walls of the structures. Whatever the origin of the 235-m crater (meteoritic impact, volcanism or collapse), it is likely that the material was brecciated. Impact on an already brecciated area should not have resulted in the stacking of material in three neat piles with nearly vertical walls.

Finally, endogenous activity is the last mechanism. There are several known volcanic processes on the Earth which would result in vertical walls and tall masses located near topographic lows, bearing in mind that on the Moon there may be other processes not known on Earth. It is not necessary to call for deep-rooted volcanism, as the structures could have been caused by a 'rootless activity centre', that is, a pocket where the gas content of a mare flow was unusually high. Ignimbrite flows on Earth are known to have such phenomena³.

It is also possible that the structures could be the result of deposition of minerals due to water percolating through the surface layer; the lows might be due to violent release of volatiles.

The structures inside a 235-m crater have therefore been carefully analysed. The conclusion that they are the result of an impact appeared unlikely due to the presence of steep-walled structures and topographic lows. The endogenous theory is favoured, and it is suggested that mechanisms such as rootless volcanic activity, real volcanic activity, or venting volatiles are responsible for this feature.

¹ Gault, D. E., and Quaide, W., *Forty-sixth Annual Meeting American Geophys. Union*, Paper No. 142 (1965).

² Halajian, J. D., *The Lunar Surface Layer*, 67 (Academic Press, 1964).

³ Cotton, C. A., *Volcanoes as Landscape Forms*, 416 (Wiley, 1952).

NEWS and VIEWS

The Royal Society of London:

Medal Awards

THE following awards of medals have been made by the President and the Council of the Royal Society: *The Copley Medal*, to Prof. A. L. Hodgkin, Foulerton research professor of the Royal Society (working in the Physiological Laboratory, Cambridge), for his discovery of the mechanism of excitation and impulse conduction in nerve, and his outstanding leadership in the development of neurophysiology; *The Davy Medal*, to Prof. H. W. Thompson, professor of chemistry in the Physical Chemistry Laboratory, Oxford, for his distinguished contributions to infra-red spectroscopy and its application to chemical problems; *The Hughes Medal*, to Prof. D. H. Wilkinson, professor of experimental physics in the University of Oxford, for his distinguished experimental and theoretical investigation in nuclear structure and high-energy physics.

Chemical Pathology at Guy's Hospital Medical School :
Prof. Sydney Cohen

DR. S. COHEN, reader in immunology in St. Mary's Hospital Medical School, has been appointed to the chair of chemical pathology at Guy's Hospital Medical School, in succession to Prof. R. H. S. Thompson, who has been appointed to the Courtauld chair of biochemistry in the Middlesex Hospital Medical School (*Nature*, 205, 1053; 1965). Dr. Cohen, a graduate of the Universities of Witwatersrand and London, spent a year at the Post-graduate Medical School at Hammersmith in 1947-48 and a year as Nuffield Dominion Fellow at the National Institute for Medical Research, 1954-55, so that he was familiar with London before he decided to come here permanently and to accept an appointment to the scientific staff of the National Institute in 1957. Dr. Cohen had been interested for some time in protein metabolism, and in collaboration with Dr. A. S. McFarlane he continued this work using experimental animals, but he also organized a Metabolic Unit for the Medical Research Council at the Archway Hospital so that this work could be extended to human subjects. He made important contributions during this period, particularly to knowledge of the metabolism of the immunoglobulin, and not surprisingly this aroused his interest in the rapidly developing field of immunology. In 1960 he became reader in the newly formed Department of Immunology at St. Mary's Hospital Medical School, where he played a large part in getting the teaching and research under way, and he soon established an international reputation in what quickly became a highly competitive field of research. Although still primarily a laboratory worker, he increased his clinical interests and he introduced several diagnostic techniques which were of great value to the hospital. This combination of clinical interest with high attainment in laboratory work is not very common in English medicine and there is no doubt that Guy's Hospital has been fortunate in finding in Dr. Cohen a person well equipped to make the most of the wider opportunities which this new position offers him.

Pharmaceutical Technology in the University of Strathclyde:
Prof. P. H. Elworthy

DR. P. H. ELWORTHY, senior lecturer in pharmaceutical chemistry in the Department of Pharmacy in the University of Strathclyde, has been appointed to the new chair of pharmaceutical technology at the University from January 1. Dr. Elworthy was educated at Mercers' School, Holborn, London, and at the School of Pharmacy, University of London, where he graduated with honours

in 1953, and where he was awarded a Ph.D. degree for research in physical chemistry in 1956. After a period on the staff of the School of Pharmacy, London, he became a lecturer in the Department of Pharmacy in the then Royal College of Science and Technology, Glasgow in 1959. He was appointed senior lecturer in pharmaceutical chemistry at the University in 1964. In 1966 he spent three months' leave of absence from the University in various pharmaceutical companies studying the application of physical chemistry to problems of pharmaceutical formulation. Six months' leave of absence granted during 1964, enabled him to work at the University of Southern California as a visiting lecturer on some properties of detergent solutions. Dr. Elworthy's research interests lie in investigations of the mode of action of detergents as emulsifying and suspending agents in pharmaceutical preparations, in producing models of the cell membrane, and in the relation of shape of certain drug molecules to their biological activity. His published work has resulted in the award of a D.Sc. degree this year.

Transport and Technology

THE debate in the House of Commons on the Queen's Speech on November 16 was concerned mainly with matters of transport and, to a lesser extent, of technology. The Minister of Transport, Mr. T. Fraser, in referring to the studies in the co-ordination of transport which were in hand, emphasized his belief that very great social benefit would arise from reversing the present trend of putting traffic now carried by road on to the railways apart from the full utilization of our investment in our railway system. He emphasized also that we could not continue to waste our resources on unproductive services but in dealing with closures he said he had not looked merely at the loss incurred. He hoped shortly to announce an arrangement under which British Railways and British Road Services will jointly operate a new parcels and sundries service throughout the country, each contributing the part for which it was best fitted. He was seeking further measures of freight co-ordination by which the railways would undertake the longer hauls for which they were best suited, and he was in touch with all main transport authorities in an endeavour to improve rail-road co-ordination for passengers. He also added, in reply to a question, that the practicability of the proposals for road pricing in the Sneed report was being intensively investigated. Apart from some questioning of the establishment of the Ministry of Technology and of the failure to set up the Industrial Research and Development Authority recommended by the Trend Committee, there was little mention of technology, as distinct from transport, in the debate.

Management Training in the Civil Service

IN a written answer in the House of Commons on November 12, the Financial Secretary to the Treasury Mr. N. MacDermot, stated that he had appointed a working party, with Mr. S. P. Osmond as chairman, to consider the training needs for middle and higher management in the Civil Service and to submit recommendations on the length, content and organization of such training taking account of the long-term future of the Centre for Administrative Studies and the desirability or otherwise of setting up a Civil Service Staff College. The members include Prof. Jean Blondel, Prof. D. C. Hague and Prof. E. A. G. Robinson, with R. Haynes, R. Hayward, C. D. E. Keeling, R. B. M. King, W. McCall, J. Parsons, C. H. Sisson, L. Williams, G. P. Hampshire and I. G. Gilber (secretary).

Regional Economic Development

IN a written answer in the House of Commons on November 18, the Joint Under-Secretary of State for Economic Affairs, Mr. W. Rodgers, stated that the National Institute for Economic and Social Research had been commissioned to carry out a three-year project under the direction, from the late summer or early autumn of 1966, of Prof. A. J. Brown, "to build up a theoretical and empirical framework for the analysis of regional economic development and the consideration of regional policy in the United Kingdom especially in relation to problems of national economic development". He had also recently discussed the priorities for regional research with representatives of regional planning councils, who were now considering which projects they would regard as most urgent. The Department was also prepared to consider proposals for projects on regional economic matters from those working in the field.

British Productivity Council

IN support of a proposed National Campaign for Quality and Reliability, plans for designating a Quality and Reliability Year (QRY), October 1966–September 1967, were announced on October 29 by the British Productivity Council and the National Council for Quality and Reliability. Details of the scheme are available from the British Productivity Council, Vintry House, Queen Street Place, London, E.C.4. The campaign is to be launched throughout Britain and its object is to emphasize the benefits of quality with built-in reliability, and the ways and means of achieving it. The turnover of British manufacturing industry in 1964 was £10,114 million. It has been shown that adoption of the methods which will be advocated during QRY can produce savings in the range of 1.5–5 per cent of gross turnover, while maintaining or raising product quality. Even on the lowest percentage, savings of £150 million a year should be well within the grasp of manufacturing industry. The technique which will be the *raison d'être* of the campaign, and which, as is rightly pointed out, must begin at the design stage of the product, involves: reduction of costs; reducing scrap and re-work; considerably reducing Britain's import bill for raw materials; ensuring that delivery dates are kept or improved; increasing earnings, not by harder work but by increased efficiency and "doing it right first time"; and giving more customer satisfaction by consistency of product. The Quality and Reliability Year is already assured of widespread support by representative industrial, national and professional bodies in Britain. Its patron is H.R.H. Prince Philip, Duke of Edinburgh, who, in the course of a message sent to the organizing council, commented that a good reputation for well-designed goods or components, fit for the purpose, which do not fail or break down, is the criterion for certain success. A bad reputation is a very costly luxury which Britain cannot afford.

Overseas Development Institute

THE Overseas Development Institute, set up in 1960, is an independent non-Government body aiming to ensure wise action in the field of overseas development. It is financed by grants from the Ford Foundation, British foundations, and by donations from British industrial and commercial concerns. Its declared functions are: to provide a centre for co-ordination of studies on development problems; to direct studies of its own; to be a forum for all concerned with development problems to facilitate contacts, discussions, and sharing of ideas; to spread information collected as widely as possible among those concerned with development problems; and to keep the urgency of such problems before the public and responsible authorities. An instance of one of the most recent activities of the Institute is provided by a publication entitled *British Private Investment in East Africa*,

by D. J. Morgan (Pp. 62. London: The Overseas Development Institute, Ltd., 1965. 7s. 6d.). The argument is that private investment overseas is a matter of controversy. It is sometimes blamed for balance of payment difficulties; the rate of such investment in developing countries is on the decline; Africa, in particular, has been affected by this changing pattern. For this reason, East Africa was chosen as a special area for study. This pamphlet is, in effect, a report of a survey and a conference. The survey was undertaken by the Institute in conjunction with the Federation of British Industries and the Dulverton Trust, and involved sending a questionnaire to 1,500 British firms concerned in East Africa; the replies are analysed and, at a conference held during June 1965, various obstacles to investment in that area were discussed and some vital economic questions posed. Among the latter are: Is East Africa a growth area? Are profit margins high enough to attract British investment? How important are tax and other concessions? What are the political risks? All these questions are examined in this pamphlet and to a large extent answered, but the conclusions reached still leave unsolved the basic problem of declining investment in East African enterprises, unless combined action by the African Governments concerned, the British Government, and equally important, British industry itself, materializes.

Science Papers of the Victorian Era

THE great majority of John Dalton's manuscripts were presented to the Manchester Literary and Philosophical Society in 1864 by his literary executor, William Charles Henry. More than three-quarters of the original collection was destroyed during the Second World War and many of the surviving items are in such a charred and brittle condition that their future preservation is problematical; careful separation and sorting of the charred sheets was a lengthy but necessary preliminary to the actual cataloguing of the collection. Subsequent to this partial destruction, the Society has received various gifts, including a collection originally presented to Dalton Hall, a hall of residence of the University of Manchester. These manuscripts in the possession of the Society have now been microfilmed, occupying four spools of a total length of 340 ft., of which printed matter occupies about 320 ft. The material consists of lecture notes, papers, notebooks, meteorological records, correspondence, domestic accounts and some rare printed items such as lecture syllabuses. The microfilm is available either in negative or in positive form; the former is judged to be more legible. The Society has also made available a microfilm of letters from J. P. Joule and John Mercer to Lyon Playfair dealing with scientific topics during 1841–49. The film consists of about 16 ft. of correspondence from Joule and 16 ft. of correspondence from Mercer.

Laboratory Photochemical Reactor

A PHOTOCHEMICAL reactor suitable for small-scale photochemical research in universities and industrial research laboratories is now available from Engelhard Hanovia Lamps (Slough, Bucks.). This welcome addition to their range of ultra-violet equipment was shown as a prototype at the Oxford International Congress of Photo-Biology in June 1964 and is now available from stock. The reactor consists of a three-necked glass flask fitted with standard ground glass sockets. One of these receives a clear quartz thimble which in turn holds a smaller quartz thimble containing the ultra-violet lamp. There is provision for water-cooling of the space between the inner and outer thimbles, and for nitrogen flushing of the lamp chamber to eliminate ozone. Use of high-purity synthetic quartz in the thimbles ensures good ultra-violet transmission characteristics. Two lamps are available for the reactor, a 100-W medium pressure lamp emitting a range of radiations from 185 to 366 mμ and a 2-W cold-cathode

low-pressure lamp emitting mainly at 185 m μ and 254 m μ . Both lamps have synthetic quartz envelopes. The reactor will undoubtedly be valuable for physical chemistry research as well as biological work, and the cone and socket arrangement makes it suitable for use also in the study of reactions in the gaseous phase. By circulating oxygen or air around the lamp it could, of course, be used as an ozone generator.

Alaskan Mammals

A USEFUL brochure entitled *The Distribution of Alaskan Mammals*, by R. H. Manville and S. P. Young, has been published by the U.S. Bureau of Sport Fisheries and Wildlife (United States Department of the Interior: Fish and Wildlife Service. Bureau of Sport Fisheries and Wildlife. Circular No. 211. Pp. iv+74. Washington, D.C.: Government Printing Office, 1965. 50 cents). A map showing the distribution within the State is given for each species, together with notes on the complete range of the species, its habitat, the races found in Alaska, and on related species. A black-and-white drawing illustrates each species competently; the scraper-board figures greatly exceed in effect those in pen and ink; that of the porcupine, *Erethizon dorsatum*, and especially that of the bearded seal, *Erignathus barbatus*, are splendid. The Cetacea are not treated in detail but are given an overall note and a list of recorded species. The introductory matter includes a short article on the distribution of Alaskan mammals, another on the mammalian fauna in general, and a list of 102 species in systematic order. The rapid opening up of the State to travel and tourism, and the establishment of the University and other centres of scientific activity, give great opportunities for an intensified study of Alaskan mammals, for which this publication will provide a valuable foundation.

Conservation of the Bee Orchis

RARE bee orchises (*Ophrys apifera*) were discovered on the Dykes of the Avebury Stone Circle, Wiltshire, during July 1965, by one of the custodians of the Office of Works, Mr. Hulbert, and the curator of the Museum, Mr. H. M. V. Young, who immediately alerted the headmistress of the parochial school, and her assistants and caretaker, all keen naturalists. The teachers found that the plants were flowering in the same spot in which they had appeared eight years ago, widely scattered over an area of 300 square yards. Seventeen were counted in bloom, and investigation showed that there had been at least twenty-two. Of the five known casualties, two had been picked by local adults and three were found discarded, probably by a coach party who may have carried some others away. Only nine Avebury people knew of the bee orchids and they agreed to conserve them if possible. Photographs were taken and further counts were made by the headmistress, who thinks one more was picked during the next week. Fourteen days from the first discovery, only one in the fruiting stage was seen, and this had well-formed seed pods. Bad weather and long grass prevented a prolonged search, but it is hoped that a few more remained to set seed. This small effort at conservation was prompted by a lecture on the Nature Conservancy Trust given by the Countess of Radnor to the Wiltshire Rural Studies Association. Further information can be obtained from Miss E. Dampier-Child, School House, Avebury, Wiltshire.

The International Commission on Radiological Protection

THE following have been elected officers and members of the International Commission on Radiological Protection for the period 1965-69: *Chairman*, Dr. E. E. Pochin (United Kingdom); *Vice-Chairman*, Dr. C. G. Stewart (Canada); *Council*, Prof. L. Bugnard (France),

Prof. O. Hug (Germany), Dr. H. Jammet (France), Prof. A. A. Letavet (U.S.S.R.), Prof. B. Lindell (Sweden), Dr. J. F. Loutit (United Kingdom), Dr. K. Z. Morgan (United States), Dr. H. B. Newcombe (Canada), Dr. C. C. Powell (United States), Dr. L. S. Taylor (United States) and Prof. B. Windeyer (United Kingdom); *Chairman Emeritus*, Prof. R. M. Sievert (Sweden); *Scientific Secretary*, Dr. F. D. Sowby (Canada). The Commission's secretariat is at Clifton Avenue, Sutton, Surrey, England.

The National Institute of Sciences of India

THE following have been elected members of the Council of the National Institute of Sciences of India for 1966: *President*, Dr. V. R. Khanolkar; *Vice-President*, Dr. Atma Ram and Dr. S. Bhagavantam; *Treasurer*, Prof. R. C. Majumdar; *Foreign Secretary*, Prof. B. F. Seshachar; *Secretaries*, Prof. F. C. Auluck and Dr. N. K. Panikkar; *Editor of Publications*, Prof. P. Maheshwari; *Members of Council*, Sri S. Basu, Prof. P. N. Bhadur, Prof. P. L. Bhatnagar, Prof. R. N. Chakravarti, Prof. N. F. Dhar, Dr. K. R. Dixit, Dr. T. R. Govindachari, Dr. A. C. Jhingran, Dr. M. S. Krishnan, Prof. T. S. Mahabale, Prof. K. C. Mukherji, Dr. K. R. Nair, Dr. B. P. Pal, Dr. R. E. Pal, Prof. T. R. Seshadri, Prof. S. M. Sircar, Prof. Venkateswarlu, Prof. P. N. Wahi and Dr. H. J. Bhabha (past president, *ex-officio*).

The Year Book of the National Institute of Sciences of India, 1964 (Pp. 133. New Delhi: National Institute of Sciences of India, 1965), includes, besides an account of the foundation of the Institute and the calendar for 1964, list of the Council senior office staff, of Fellows, of the members of committees, and of representatives on other organizations. The rules and regulations of the Institute are also included as well as lists of the Institute's medals, lectures and publications.

University and College News:

Cambridge

THE following have been elected to fellowships at Churchill College: *Title A*, Dr. I. T. Drummond (mathematics); *Title B*, Dr. B. B. Roberts.

Edinburg

THE following lecturers have been appointed: J. B. Penman (animal health); Dr. J. S. McKinley (biochemistry).

University College of Swansea

THE following lecturers have been appointed: Dr. R. C. V. Macario and D. Bell (electrical engineering).

Announcements

A SYMPOSIUM on "CNS-Drugs" will be held at the Regional Research Laboratory, Hyderabad, during January 24-30. Further information can be obtained from Dr. P. B. Sattur, Regional Research Laboratory, Hyderabad 9.

A SYMPOSIUM on "Regulation of the Antibody Response" will be held in Toronto during January 20-22. Further information can be obtained from Dr. B. C. Braden, Subdivision of Immunochemistry, Division of Biological Research, O.C.I., 500 Sherbourne Street, Toronto 5.

A ONE-DAY symposium on "Engineering the World's Airport Passenger Terminals" will be held at the Institution of Civil Engineers, London, on January 25. Further information can be obtained from the Secretary, Institution of Civil Engineers, Great George Street, London S.W.1.

A ONE-DAY symposium on "Food Safety", organized by the Luton College of Technology and the Institute of Biology, will be held in the College on January 26. Further information can be obtained from Dr. F. C. Webber, Luton College of Technology, Park Square, Luton, Bedfordshire.

CENTENARY OF THE QUEKETT MICROSCOPICAL CLUB

THE Quekett Microscopical Club celebrated its centenary with an exhibition at the Central Hall, Westminster, during October 8-9. This club, named in commemoration of Prof. John T. Quekett of the Royal College of Surgeons (1815-1861), has fostered the interests of microscopists by holding meetings and promoting field work throughout the past hundred years.

Foremost among the exhibits were a number of family relics loaned by Captain Charles Quekett, the great-grandson of John Quekett. Several examples of Quekett's work, including the manuscript of the *Catalogue of the Histological Collection* and a small selection of his microscopical preparations, were displayed by the Royal College of Surgeons. A fine display of microscopes made before 1865 was shown by Dr. H. Heywood. Mr. E. P. Herlihy's exhibit of portable microscopes, many of which dated from the past century, emphasized the great part these models have played in the history of the Club by facilitating the demonstration of microscopical preparations at the meetings. The historical display also included numerous examples of instruments and accessories designed or made by past members, and the work of Richard Beck, Andrew and Thomas Ross, Powell and Lealand, J. W. Stephenson, F. H. Wenham and Julius Rheinberg was well represented. Dr. Savile Bradbury contributed a special display devoted to E. M. Nelson—one of the Club's most famous members; and we were reminded that Ernst Abbe had been an honorary member of many years standing.

The rest of the space in the main hall was devoted to members' and guests' individual exhibits. The light reflexion in the eye of the spur dogfish was shown to be due to the presence of guanine crystals in the layer known as the silvery tapetum. Two exhibits of electron microscopy in the investigation of neurophysiology and neuropathology were shown by Drs. Bradbury, Harris and Salmon. Prof. Booth from Saskatoon demonstrated his Kerr-effect microscope for the visualization of magnetic domains. Dr. Gahan gave a demonstration of the cytochemistry of chromosomes. These were but a few of the

exhibits, which also included beautiful mounts of diatoms and mosses among the 'showpieces'.

The versatility and skill of members was shown by various pieces of apparatus for aiding microscopical techniques. These included various kinds of magazine slide-carriers, a home-constructed mounting cabinet incorporating an electrically driven ringing turntable, a simple but very effective mechanical finger for aiding the arranging and mounting of diatoms and similar minute objects, and a tube-length corrector mounted in the body-work of a microscope. Three members showed microscopes made by themselves. An original display showed the use of paper-sculptures to illustrate microscopic and natural-history specimens. A number of excellent drawings and paintings of entomological and botanical subjects were shown by Miss Dorothy Fitchew.

A programme of films included a 16-mm sound colour film on modern freeze-sectioning by the Pathology Department of the Royal College of Surgeons, and another, by a member, dealing with crystal growth. Sessions of micro-projection on a home-built machine by Mr. H. S. Henderson aroused admiration.

A trade exhibition was held in the smaller hall and many firms displayed their latest models of microscopes and photomicrographic apparatus. Many teaching aids for microscopy were on view, and a display of live *Daphnia* and *Volvox* projected by Flatters and Garnett evoked much interest. Dissection microscopes and equipment were shown in addition to knife-sharpening machines and an automatic staining apparatus.

It is estimated that about a thousand people attended the exhibition. These included a number of young people from schools and technical colleges, and it is anticipated that many will, in the future, want to join this flourishing club. The general opinion seemed to be that the centenary of the Club that commemorates that foremost microscopist of the past century, Prof. John T. Quekett, had been worthily celebrated.

G. J. CUNNINGHAM

TEACHING IN THE HISTORY AND PHILOSOPHY OF SCIENCE

By DR. W. MAYS

Department of Philosophy, University of Manchester

IN 1960 a questionnaire was sent out to British and Commonwealth universities to determine what teaching was being done in the history and philosophy of science¹. As it was felt that some of the information was now out of date, it was decided in April 1965 to make a further survey, restricting it this time to British and Irish universities. Since 1960 a number of new universities have been established; there have also been numerous debates on the so-called 'two cultures'. It was, therefore, of interest to see whether these factors had had any significant effect on the development of the subject. In all 34 replies were received. These covered the greater part of the universities to which circulars were sent, and they can be said to give a representative picture of the state of teaching in the subject at the moment.

What general impression does one gain when one looks over the replies? First, it seems clear that there

have been no dramatic developments. The past five years have been a period of consolidation rather than rapid advance. There has, it is true, been the establishment of two new departments in the history of science and technology. One at the Imperial College of Science and Technology, the other at the Manchester College of Science and Technology. A Department of the History and Philosophy of Science has also been set up at Queen's University, Belfast.

The consolidation has perhaps been greatest in those institutions where independent appointments have been made. Where the teaching has been largely on a voluntary basis, there has in some cases been an actual decrease in activity, either due to the interested member of the staff leaving the institution or getting disheartened. As was noted in the 1960 survey, one of the reasons why the subject has not grown commensurately with its educational

value is that it usually had to be a subsidiary enterprise of one or two interested people, who have not always been able to muster sufficient support for the subject.

Since the last survey there has been an increase in appointments, and a number of them have been in departments of philosophy. It would seem that philosophers are now taking a greater interest in the subject, at least in the philosophy of science, than are scientists and historians. This seems to reverse the trend noted previously¹, when scientists and historians appeared to be showing a greater interest. Some philosophers even complained that they found scientists not always willing to co-operate with them.

Although philosophers are now supporting the subject by the creation of appointments in their departments, there still is, however, something to be said in favour of separate departments of the history and philosophy of science. In some philosophy departments there could be a tendency to undervalue the history of science, thus overlooking that historical techniques can provide an intellectual discipline of a high order. In departments of history or history of science, there may be a tendency to neglect the philosophical aspects of the subject, and to regard them as speculative. Both aspects seem necessary, if one is to develop a balanced perspective of the nature of science.

In view of the debates on the 'two cultures' it is surprising that there has not been greater interest in the history and philosophy of science in universities. One would have thought that to offer courses in it was one way of getting arts students to learn something about the aims and methods of science, and of bridging the alleged gap between the 'two cultures'. As students can now take history and philosophy of science as a subject for the General Certificate of Education, one would have assumed that an increased provision of courses at the university-level might have stimulated more of them to take it at school.

It was noted in the 1960 survey that interest in the subject seemed dependent on the size of the university, date of foundation, and the priority given to cultural questions *vis-à-vis* bread-and-butter ones. To this one must add that the subject has a chance to grow in universities where departmental boundaries are flexible and inter-faculty co-operation exists. In universities with excessive departmentalism, inter-disciplinary subjects may be faced with difficulties. Teachers of established courses may feel that with a limited number of students to go round, the development of the history and philosophy of science can only be at the expense of their own subject.

At Oxford and Cambridge there has been a steady increase in activity. Oxford is developing the subject at a number of levels, and forging links with historians and philosophers. The history of science can, for example, be taken as a special subject by third-year historians. Classes and lectures of postgraduate standard go on throughout the year. These are taken by candidates for the B.Phil. and the diploma in the history and philosophy of science.

Cambridge has now replaced the certificate in the history and philosophy of science, which could be taken as a post-Part I, by a Part II of the natural sciences tripos (general honours) in which there is a choice of four papers from a panel of six, three of which emphasize the historical side and three the philosophical side. In addition the subject can be taken as an option in Part I of the natural sciences tripos. It is also planned to have an option in the projected M.Phil.

So far as the newer universities are concerned, Sussex seems to be the only one at which appointments have been made, and it teaches the history as well as the philosophy of science. York expects to have philosophy of science available as an optional paper from 1966 onwards, to be taken by undergraduates reading for the B.A. in a combined honours course. Lancaster hopes to

provide an optional undergraduate course in the philosophy of science and another in the philosophy of mathematics. The physics department there is providing a course in the basic ideas of physics for students majoring in the arts and sciences.

Warwick intends to put on a common course for all freshmen to deal with the nature and methods of the humanities and the natural and social sciences, and it is hoped to develop philosophy of science as a special option. East Anglia plans to give an optional course in the philosophy of science in the School of Mathematics and Physics in 1967; Kent hopes to have an option for philosophy and science students in 1965, and is also starting courses in science and religion and the methodology of science. As only a limited range of subjects can be taught in the newer universities, the older-established studies would seem to stand a better chance of being included in the syllabus. However, the philosophy departments of some of these universities intend to develop teaching in the philosophy of science and to appoint staff to teach it.

Among the other universities, the sub-department in the University of Leeds is very active. The subject is taught to undergraduates in the Faculties of Arts, Science and Technology, and with five staff members it can cover a range of topics. Both Belfast and Dublin gave courses in which the historical and philosophical aspects were equally stressed. Durham has appointments in both the history of science and the philosophy of science, and plans to integrate more closely the teaching of these subjects. In Wales, both Bangor and Aberystwyth have courses in the philosophy of science and the foundations of mathematics. Leicester gives a one-year course in the history of science, which can be taken by students in any faculty or year, and it also provides a course in the philosophy of science.

In London there are now three main university centres: the London School of Economics, University College and the Imperial College of Science and Technology. The latter, which is a new department, will give undergraduate courses and provide research facilities for the Ph.D. and M.Sc. degrees. In Scotland, Aberdeen shows some expansion in staff and courses; it also runs classes in conjunction with the Medical Faculty.

It is interesting to note that the courses offered by Cambridge and Dublin include topics in what might be called the 'history and philosophy of psychology', for example, the mind-body relationship and the unconscious. Furthermore, in view of the development of sociology in British universities in recent years, it would probably be worth while to have more courses in the history and philosophy of the social sciences. One gains the perhaps mistaken impression that the subject is sometimes rather narrowly interpreted in terms of the physical sciences. It might well be the case that the history and philosophy of the biological and social sciences would not only be more acceptable to mathematically unsophisticated arts students, but also more profitable educationally for them.

In conclusion, progress certainly seems to have been made during the past five years, though perhaps it has not been spectacular. Taken in conjunction with developments in the colleges of advanced technology (we know, for example, of one new department and a number of new appointments here) progress in the teaching of the subject is appreciably greater than would appear from our survey. It is probable that the increased activity in the colleges of advanced technology may feed back again into the universities and stimulate further interest.

Mention in detail cannot be made here of the other universities which provide, or intend to provide, teaching in the subject. These include Birmingham, Bristol, Exeter, Keele, Manchester, Newcastle, Nottingham, Liverpool, Reading, St. Andrews, Sheffield, Southampton and Swansea.

¹ Mays, W., *Nature*, 189, 971 (1961).

EDUCATION AND APPLICATION OF THE MATERIALS SCIENTIST

It has recently been said¹ that in an economy which is no better than its best materials, there is no lack of employment for the materials scientist. The Materials Science Club, composed as it is of members from a variety of disciplines, has endeavoured to direct attention to the factors that enable the country to benefit by the most advantageous utilization of the materials scientist. Consideration has already been given to collaboration between industrial and academic bodies², and it was a natural development to consider "The Education and Application of the Materials Scientist" as a main topic during the third Banbury Conference of the Club during October 1-3.

This was also the occasion of the presentation of the Club's A. A. Griffith Medal to the first recipient, Dr. A. H. Cottrell. The A. A. Griffith Medal will be awarded annually by the Materials Science Club to a British scientist who has made a distinguished contribution to the study of materials science or technology. In making the presentation, the Club president, Mr. L. Holliday, paid tribute to the generosity of Rolls-Royce, who had financed the fabrication of the medal die. Briefly sketching the career of A. A. Griffith, who is to be considered a pioneer in the field of materials science because of his work on crack propagation and the strength of materials, Mr. Holliday recalled that it was in 1917 that Griffith was presented with his first medal. Between 1914 and 1939 Griffith had a distinguished career at the Royal Aircraft Establishment, Farnborough, then moving to Rolls-Royce, Ltd., where during 1939 until 1960 he was engaged on advanced research, particularly in the field of vertical take-off aircraft. In 1941 he was elected to the Royal Society.

Turning to the distinguished career of Dr. Cottrell³, Mr. Holliday said that it was in 1954 that he was awarded his first medal, the Rosenhain Medal, and was elected a Fellow of the Royal Society in 1955. In research, Dr. Cottrell has been concerned with the plastic deformation of metal crystals, with creep and fracture phenomena, and more recently with whisker and composite materials. This year, Dr. Cottrell left the Department of Metallurgy at Cambridge, where he was Goldsmith professor, to take up the post of deputy chief scientific adviser to the Ministry of Defence. In presenting the Medal, the president referred to the fact that it was now forty-five years after the publication of the Griffith theory of crack propagation in brittle materials, and added by way of anticipation that he felt that by the year 2010 there would be somewhere an A. H. Cottrell Medal.

In thanking the president and the Club, Dr. Cottrell expressed his pleasure at being associated with A. A. Griffith in this way, since he had long been his admirer, particularly with regard to the theory of fracture and crack propagation. He considered the original treatment elegant and the power of the method profound. It was with regret that he was unable to deliver a lecture at the conference, but the pressures of his recent appointment made it necessary to defer this to a future meeting of the Club.

The opening lecture was given by Prof. D. D. Eley, on 'Adhesion'; he spoke immediately after the annual Club dinner. After briefly tracing the events which led to his involvement in this subject, which can conveniently be studied as a branch of surface chemistry, attention was

then directed to the classification of the process of joint formation: (1) wetting of the solid surface; (2) setting of the adhesive; (3) deformation to release any stress set up in the previous step. Consideration was given to the thermodynamic work of adhesion and to the measurement of heats of immersion by highly accurate calorimetric methods. In order to assess joint strength it is necessary to devise some form of standard joint, which has resulted in the Napkin ring test, and the various techniques involved were described. The penetration of the liquid adhesive into the porous solid and the nature of the adherend are of prime importance, and a variety of treatments to give an increase in surface area were considered. In determining the heats of wetting to this surface a variety of the problems involved were indicated and in particular the time to complete the research work was constantly emphasized. Prof. Eley indicated that this was the type of research that was perhaps only possible in a university, because the cost would be a severe drain on the financial resources of any industrial concern. Finally, the strengths of the molecular forces involved were considered. At the conclusion of the lecture a series of exhibits were passed to the audience which illustrated a variety of the uses of the modern adhesive, and in particular their use in the aircraft industry.

In the four papers which were next presented, a pattern for the future training of the scientist competent in the materials field gradually emerged. The first, by Mr. D. M. Cunningham (Woolwich Polytechnic), on 'Prospects in Materials Science', presented the results of a statistical survey, the objects of which were: (a) to test attitudes and opinions about this recently defined study; (b) closely connected with the first object—to obtain some idea of the kind of training thought most suitable for future materials scientists; (c) to make some kind of quantitative estimate of the number of people engaged in materials science, together with some growth projection into the future.

Mr. Cunningham explained the manner in which the survey had been conducted, together with the various tests which had been applied to establish randomness of samples and confidence in the results. It would appear that there is substantial agreement that materials science is a combination of 'pure' and 'applied' science and that physics and chemistry should provide the basis for undergraduate courses. The study of materials science as such should, in general, be reserved for the postgraduate level. It was of interest to note that approximately 5 per cent of pure scientists and the same proportion of mechanical, electrical and chemical engineers claim that their work directly encompasses materials science. Thus by 1968 there could be about 10,000 scientists and engineers effectively concerned in materials science. About 4,000 of these might well be chemists and/or physicists and about 5,000 engineers, the remainder being metallurgists, mathematicians, etc. There is a possibility that the demand for scientific workers in the field of materials science may well rise more rapidly than the demand for all scientists and technologists. This demand will have to be met, during the next three years, from the present resources since the output of materials scientists trained as such is likely to be small. Mr. Cunningham emphasized that the foregoing conclusions are rather pre-

curiously based on relatively small samples and that the estimates of the total numbers in particular should be regarded as approximate.

The part played by the schools in equipping candidates at advanced level with the necessary educational background is of fundamental importance in the structure of further-educational courses. Dr. J. E. Spice (Winchester), in his paper "Physical Science as an 'A'-Level Course", described the progress that has been made in this science teaching project which is sponsored by the Nuffield Foundation. It was Dr. Spice's view that physical science could be a more satisfying and exciting 'A'-level subject than physics or chemistry separately. It could be taken together with either mathematics or biology, so that it would be possible to satisfy university entrance requirements for courses in science, medicine and technology with two 'A'-level passes at a high enough level instead of three as at present. This would allow the better sixth-formers to take a third, unrelated subject at 'A'-level, for example, economics or a modern language. Perhaps as important, it would also enable the choice of a university course to be deferred until later in a student's school career than at present.

The Nuffield Science Teaching Project has now set up a Joint Committee for the Physical Sciences at 'A'-level, which is co-ordinating the work of three separate groups. These groups are producing Nuffield-style 'A'-level courses in physics, in chemistry, and in physical science, and it is hoped that trials will begin in selected schools in September 1966.

The physical science course would have a small number of key concepts (such as energy, and the structure of matter) as unifying themes. At the same time, it would introduce pupils to all the topics and techniques which entrants to honours courses in physics and chemistry should know. It is also hoped to introduce the pupil to experiments so designed that he is taught to find things out for himself and become less dependent on memorized facts.

Prof. R. W. Cahn next spoke about the teaching of materials science at the University of Sussex. He began by explaining the system of schools of study which was used for all teaching in the new University. Students who were going to specialize in materials science took preliminary courses in structure and properties of matter as a principal subject (an atomistic approach to physics and chemistry), mathematics and either chemistry or further mathematics. Thereafter, materials science would be taken as a major subject in either the School of Applied Sciences or the School of Molecular Sciences.

In the School of Applied Sciences, materials science courses were accompanied by a variety of courses in engineering topics such as electronics, mechanics of fluids, automatic control theory, etc. The planned materials science course included first a detailed course devoted to the electrical and magnetic properties of matter with detailed emphasis on semi-conductors and ferro-magnetic materials and to the mechanical properties and the physical factors determining them. Consideration is also given to the various techniques for preparing and refining materials and assessing their properties. The materials specialists also have an introduction to physical metallurgy with special emphasis on nucleation and transformation, and of course crystallographic and diffraction techniques. A great deal of emphasis was to be placed on laboratory experiments, and Prof. Cahn explained his philosophy about such experiments and emphasized their importance as a means of "costing out fear" of unfamiliar techniques while also developing dexterity and an awareness of the source of error.

It was also possible to major in materials science in the School of Molecular Sciences, and here the emphasis would be scientific rather than of an engineering nature. The programme included lectures on quantum mechanics, crystal chemistry, molecular spectroscopy and topics in

inorganic chemistry, all given by physicists or chemists. A materials scientist would be responsible for a detailed course in crystallography and defect theory with special attention to dislocations, and there would also be a course surveying the methods of mechanical and physical characteristics of materials. Students would, of course, attend a basic course on electrical and magnetic properties jointly with students of the School of Applied Sciences. In conclusion, Prof. Cahn said that it was too soon to say which of the two alternative ways of preparing a materials science specialist would prove to be more successful and more popular, and it might well be that one or other of these would prove to be so superior to the other that only one approach would eventually be offered.

The title "Management of Materials Science Research" enabled the next contributor, Dr. F. J. P. Clark (U.K. Atomic Energy Authority), to convince the Club that it should have a half-day's conference on management. He pointed out that in the majority of industrial concerns the expenditure on people attending management conferences is relatively much less than on technical conferences. It has been maintained by Holloman, among others, that good management is so important that it matters little whether the principles involved are applied to research and development or, for example, running a pickle factory. If management principles are as important as this example suggests, then more attention should be paid to them.

Examples were then given of some of the primary functions of a research manager. To begin with, a distinction was made between three levels of management: first, the most senior level at which general objectives are determined; secondly, the executive level at which a decision is made on how these should be achieved; and thirdly, the responsibility of the day-to-day organization of the work. Dr. Clark then particularly directed his remarks towards the second category in management. The primary job of a research manager is to define objectives, and the advantages of clearly defined objectives are numerous. Two were mentioned in particular: (1) diffuseness in research is prevented; (2) clear definition of objectives can provide opportunities for scientists to exercise responsible freedom in their research. In an example it was shown that scientists, given freedom to involve themselves in divergent research, in fact converged their research on objectives if those objectives had been sufficiently well defined. Another function of a manager is to provide the organizational and intellectual environment in which the objectives can be obtained. This means removing potential sources of frustration inherent in poor organization. On the intellectual side, this means an appreciation of the fact that the best results are obtained by a sharing of the thinking that leads to decisions. The speaker finally argued that there was definite experimental evidence supporting the contention that the efficiency of scientists as scientists was increased by their participation in administrative jobs, perhaps to the extent of a quarter of their total time.

The first of four papers on specific materials and problems in the materials field was given by Dr. H. Fessler (University of Nottingham) and was entitled "Some Problems in Strain Measurement of Non-Linear Materials". Dr. Fessler discussed the way in which the mechanical engineer, to utilize fully the capacity of a material, wishes to know the stress to failure within very fine limits. In considering the phenomena of confined plastic deformation and shape of mechanical components, reference was made to the unexpected failure of materials. The plastic-elastic distribution can only be obtained by direct measurement of the strain of the material in the component form; several techniques, such as point measurement, field methods, brittle coatings, Moiré fringe patterns and the birefringence of photo-elastic materials are all at present being used.

The photo-elastic method is of particular importance to the mechanical engineer, and Dr. Fessler made a plea for some effort to be directed towards the improvement of the materials at present available for this technique.

Factors which influence the choice of such a material may be listed as follows:

The model constructed in the photo-elastic material must reproduce the strain in the original.

The model must be loaded so as to imitate the structural component. Good optical sensitivity is required, it being desirable to increase the amount of birefringence for each given strain at least ten-fold.

The material should be chemically and mechanically stable, and preferably not very hygroscopic and should exhibit negligible volume change on setting. It should be as homogeneous and as isotropic as the material being copied.

The material should be castable in blocks by a curing reaction which should not be excessively exothermic since this could produce an undesirable initial birefringence, and the glass/rubber transition temperature should not be far removed from laboratory temperatures.

A Poisson's ratio of about one-third would be most convenient.

The part played by the epoxy resins as photo-elastic materials was then indicated, particularly in the light of the previous suggestions.

The examination of photo-processes is of obvious relevance to photoactive materials such as photographic emulsions, photosynthetic plants, photo-conductive electronic and laser devices. In his paper "Photoprocesses in Materials" Dr. D. J. Morantz (Woolwich Polytechnic) also emphasized the important information concerning the microstructure of materials that may be obtained by the study of these processes, three examples of photoprocesses being discussed:

(a) *The production of metastable excited states.* This phenomenon, which is well known, enables the inversion of the population of electronic states in photoactive material. This inversion is normally removed by spontaneous emission; alternatively, in a suitable optical cavity, inversion enables the onset of laser action.

(b) *Photochromism.* This refers to the production of a reversible colour change on exposure of a substance to light. The mechanism may involve a metastable state, a geometrical isomer or, more usually, a reversible chemical change. Such phenomena may be used for the photosensitized control of light transmission (for example, cryptocyanin in Q-switched lasers), and photochromism has been proposed for use in memory devices for computers. Although use of such devices is unlikely with present technology, Dr. Morantz proposed that, on a molecular level, interaction between photoactive chromophores in polymer chains may in the future provide feasible systems.

(c) *Photo-ionization.* The photo-ejection of electrons on irradiation may be followed by reaction in a liquid or gas medium. In a solid the electrons may be trapped and may subsequently be induced to recombine with the positive ions by thermal or radiational activation of the traps. The ionized material may be coloured and the act of recombination may result in luminescence. Dr. Morantz described work in his laboratory on the investigation of luminescence intensities, life-times and spectra, from which evidence is available that each of these parameters is dependent on the nature of the solid material which provides the trapping sites.

In concluding, correlations were discussed with the materials requirements of the previous speaker, when it was generally agreed that the use of photo-stimulated emission may lead to a more sensitive method.

A picture of present-day technology in the light of developments in solid-state physics was given by Dr. H. G. Howell (British Plaster Board Holdings, Ltd.) in

his paper, "Some Material Thoughts on Gypsum". In introducing the topic, Dr. Howell described the physical chemistry underlying the changes involved in going from rock gypsum to hemihydrate and then to dihydrate, with particular reference to crystal structure of the species, and the influence of hydrogen bonds on the crystal cleavage planes. In the case of hemihydrate it is found that properties are exhibited which are sensitive to the method of preparation, in spite of materials being identical with regard to their X-ray structures. The need for controlling the set of α -plaster and the part played by crystal size and habit in this operation are closely connected, and the elimination of voids becomes important with regard to realization of ultimate mechanical strength. It was interesting to learn that plaster of Paris is as strong as cement and could be its rival as a structural material except for its unfortunate solubility, and that many other hydrates form equally attractive structural materials.

In his paper, "Unorthodox Structural Materials", Mr. N. J. Parratt (E.R.D.E., Waltham Abbey) examined the strength of traditional materials, where the mean elastic bond strain at failure may be between 0.1 and 1.0 per cent and the tensile strength is therefore about that fraction of the Young's modulus. Since most structures are intended to be light in weight, as a first approximation Young's modulus divided by specific gravity may be used as a comparative criterion. The common structural materials have $E/s.g.$ close to 3.9×10^6 lb./in.², but this figure may be improved on by some high-specific modulus materials such as aluminium nitride, boron, carbon whiskers, silicon carbide and particularly silicon nitride, which has the figure 17×10^6 lb./in.². Mr. Parratt then explained how these materials may be exploited in the form of whisker crystals as reinforcing agents. To be of general use, an economic process for the mass production of whiskers would be desirable, and the developments towards this from laboratory experiments to semi-scale plant were described.

Delegates at the conference were able to visit a special exhibition of materials dealing with "Materials Science Technology at Aldermaston", which was organized under the direction of Dr. D. Deverell, of the Atomic Weapons Research Establishment. The range of work in materials carried out at Aldermaston is very wide and it is impossible to do more than mention some of these most interesting exhibits: glass-fibre reinforced concrete—to solve the problem of designing a non-magnetic clamping system to withstand repulsive forces between collector plates of a megajoule discharge bank; optical materials—special techniques have been developed to obtain high-grade optical properties in stainless-steel mirrors for high-speed cameras and in special light-weight aluminium mirrors; composite materials—reinforced metal matrices using whisker crystals, particularly with aligned fibres, the apparatus and product of each technique being displayed; joining—welding of less common metals, a variety of uranium and beryllium joints were shown, together with an example of a special technique, the electron beam welding of titanium-backed polythene.

In addition to these specialized subjects were examples of general materials research in the fields of, for example, adhesives, foam plastics, cellular rubbers, encapsulants, surface coatings and isostatic pressing. Since U.K. Atomic Energy Authority staff were in attendance at the stands, delegates were afforded the welcome opportunity to discuss in detail the various materials and techniques in the exhibition.

It would now seem opportune to direct attention to next year's Banbury Conference, when some consideration will be given to "The Selection of Engineering Materials", and to add that suggested contributions may now be submitted.

G. F. FREEGUARD

¹ *Discovery*, 26, No. 7, 41 (1965).

² *Nature*, 204, 831 (1964).

³ *Nature*, 208, 19 (1965).

EVIDENCE FOR STRUCTURES RESEMBLING CRYSTALLINE CARBONS AT THE ACTIVE CENTRE OF ENZYMES

By DR. E. D. WACHSMUTH

Institut für Biochemie, Universität Frankfurt am Main, Germany

THE bondings between the even-numbered members of a homologous series of aliphatic acids, amines and amino-acids are relatively stronger than those between the odd-numbered ones. This can be illustrated by the alternating melting points of such a series of compounds. Parallel to the melting points there is also an alternation in the adsorption of a series of hydrocarbons¹ and of a series of aliphatic acids^{2,3} on porous carbon. Thus non-crystalline structures are also apparently most stable when even-numbered chains of carbon atoms are adsorbed. Therefore an investigation of the effects of inhibitors and substrates containing hydrocarbon side-chains of different lengths should cast light on the steric configuration around the active centre of enzymes. This influence on an enzyme has been interpreted until now only as a hydrophobic interaction between enzyme and substrate. For example, of all the amino-acids existing in Nature, derivatives of leucine are very easily hydrolysed by the leucineamino-peptidase described by Smith and Spackman⁴ because of its most hydrophobic side-chain. In the case of alcohol-dehydrogenase, an increase in the size of substrate, or branching in the side-chain of the substrate, results in reduced activity⁵⁻⁹. These two types of enzymes were thus selected for detailed investigations to determine whether the molecular form in addition to the hydrophobic character of the substrate is important for interaction with the enzymes.

Inhibition experiments with pure aminopeptidase of pig kidney show that the longer is the side-chain of aliphatic acids, amines and amides, then the greater is the inhibition of hydrolysis of the leucine derivatives¹⁰. In contrast to the classical leucineaminopeptidase⁴ the aminopeptidase used in this work was prepared by a method¹¹ which avoided the use of denaturing agents. Since the inhibitions are competitive, the longer-chain inhibitors seem to be better able to replace the substrate on the hydrophobic binding site on the enzyme¹⁰. Such a competitive displacement is no longer possible when the substrate lacks a hydrophobic side-chain as is the case with glycine. Thus, when glycine derivatives were used as substrates¹⁰ the inhibition was un-competitive and showed an alternating pattern depending on the length of the side-chain of the inhibitor, as demonstrated in Fig. 1 for aliphatic acids. The even-numbered derivatives had relatively higher K_M and V'_{max} values than the odd ones and paralleled the

melting points of those inhibitors. As for liver alcohol dehydrogenase, aliphatic acids and amides are also known to have an alternating effect on the enzymatic reaction but these results were previously thought to be due to experimental errors¹² (Fig. 1). Fig. 2 shows that the K'_M values of a series of amino-acids used as inhibitors for the enzymatic hydrolysis of glycine *p*-nitranilide by amino peptidase also closely parallel the melting points.

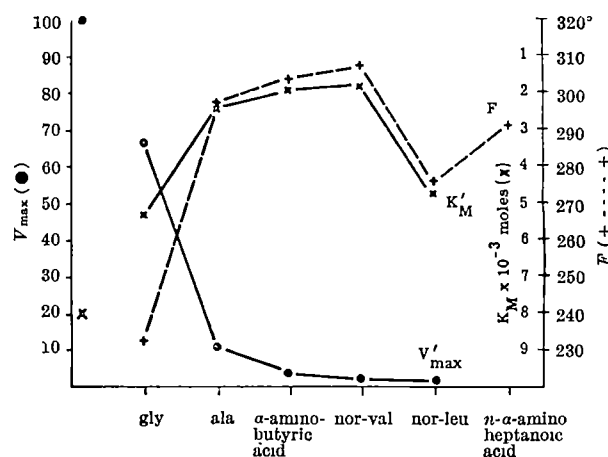


Fig. 2. Hydrolysis of glycine *p*-nitranilide by aminopeptidase measured in the presence of 0.01 M solutions of amino-acids. The Michaelis constants (molar) and maximal rates of hydrolysis (at 37°) in the presence of amino-acids are designated K'_M and V'_{max} and were measured from Lineweaver and Burk plots¹⁴. + - - +, melting points of amino-acids

The best criteria for judging the degree of interaction between an enzyme and its substrate are the V_{max} values. They represent directly the dissociation of the enzyme-substrate-complex, whereas the interpretation of K_M values is more complicated. Fig. 3 shows these constants for the hydrolysis of various amino-acid amides by aminopeptidase. The curve demonstrates that the even-numbered amino-acid amides are relatively more rapidly hydrolysed than the odd-numbered ones. The K_M and V_{max} values for alcoholdehydrogenase of liver (Fig. 4) have been measured and show similar variations according to the length of the hydrocarbon side-chain. The results in Fig. 5 illustrate the existence of similar relations for different alcohols used as substrates for yeast alcoholdehydrogenase. The oscillating variation of both the melting points and the V_{max} values with liver alcoholdehydrogenase ceases with alcohols longer than pentanol^{8,9}.

As for aminopeptidase of pig kidney, alcoholdehydrogenase of yeast and beef liver, a change in the length of the side-chain of the substrate results in relatively better substrates if the hydrocarbon side-chain is even-numbered than if it is odd-numbered. Very similar variations were found in the inhibitors. This parallels the melting points and the adsorption on porous carbon of all hydrocarbon derivatives tested. Since the melting points are decomposition points, they illustrate the adsorption of hydrocarbons on one another and analogously on porous carbon. Thus, it must be concluded, there should be a structure on the enzyme surface, which resembles that of a crystalline carbon.

By comparing aminopeptidase with the alcoholdehydrogenases it is apparent that both the adsorption of the substrates by the enzymes and the maximum rates of

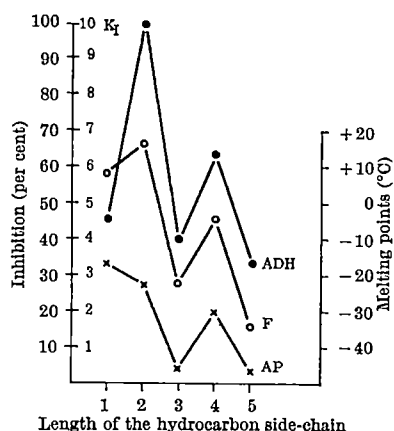


Fig. 1. Inhibition of enzymatic action by aliphatic acids. AP: V'_{max} of aminopeptidase in the presence of 0.5 M inhibitor. ADH: K_1 of liver alcoholdehydrogenase¹². F: melting points of aliphatic acids

hydrolysis vary in the same way with the length of the hydrocarbon side-chain (for aminopeptidase without carboxyl group, Figs. 3 and 4). As for alcoholdehydrogenases the hydroxyl group should be expected to be both recognized and specifically bound, whereas in the case of aminopeptidase we have proved the amino group and the carbonyl residue of the *N*-terminal amino-acid to be bound by the enzyme¹⁰. In this scheme the hydrocarbon side-chains serve as orientating groups which may be relatively favourably (C-even) or unfavourably (C-odd) bound by the enzyme.

The optimum type of side-chain is a straight chain of hydrocarbon. The introduction of an ether bridge instead of a $-\text{CH}_2-$ group into the substrate increases K_M and reduces V_{\max} (methyl- or ethyl-cellosolve, Table 1). Branched side-chains result in the same effect, as illustrated in Table 1 for alcoholdehydrogenase and in Fig. 3 for aminopeptidase (valine).

From the ratio of K_M to V_{\max} it may be possible to gain an idea of the size of the active centre itself. For example, both the aminopeptidase of pig kidney and the alcoholdehydrogenase of yeast exhibit their highest turnover numbers with substrates containing a 2-carbon side-chain. One explanation could be that these 2-carbon

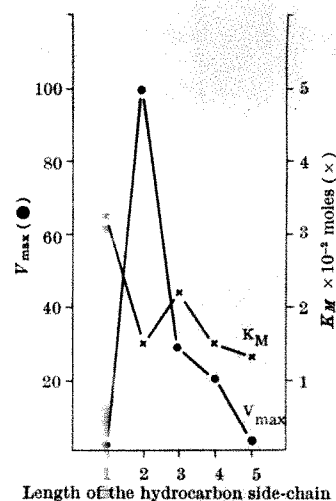


Fig. 5. The constants for the dehydrogenation of various straight-chain alcohols by yeast alcoholdehydrogenase measured from Lineweaver and Burk plots¹⁴. For experimental conditions see ref. 13

substrates offer the optimal orientation and thus the least steric hindrance for the conformational change of the enzyme-substrate-complex which occurs during the enzymatic reaction. This orientating effect is lacking in the case of the single carbon atom substrates, glycine and methanol. Bound substrates with more than two carbons in the backbone of the molecule present a greater steric hindrance to conformational change. In the case of liver alcoholdehydrogenase this hindrance is not apparent unless there are more than four carbon atoms in the substrate molecule^{6,9}.

Table 1. KINETIC CONSTANTS OF YEAST ALCOHOLDEHYDROGENASE WITH VARIOUS ALCOHOLS (For experimental conditions see ref. 13)

Alcohol	K_M (molar) $\times 10^{-2}$	V_{\max} (in % of ethanol) dehydrogenation
<i>n</i> -propanol	2.2	29
iso-propanol	20.8	3.6
<i>n</i> -butanol	1.5	21
sec.-butanol	18.2	3.6
tert.-butanol	—	0
<i>n</i> -butanol	1.5	21
methyl-cellosolve	5.2	0.5
<i>n</i> -amylalcohol	1.3	3
ethyl-cellosolve	10.4	0.5

These results, as a whole, show—analogueous to the melting points and the adsorption effects on porous carbon—that there is a high degree of order on the surface of these enzymes in the neighbourhood of the active centre which closely resembles the structure of crystalline carbon. If the orientation of the hydrocarbon side-chain starts from the tightly bound reactive part of the substrate molecule, the side-chain can either increase (even-numbered) or decrease (odd-numbered) the hydrophobic interactions between the enzyme binding site and a substrate or an inhibitor.

¹ McBain, J. W., Lucas, H. P., and Chapman, P. F., *J. Amer. Chem. Soc.*, **52**, 2668 (1930).

² Morrison, J. L., and Miller, D. M., *Nature*, **174**, 1188 (1954).

³ Morrison, J. L., and Miller, D. M., *Can. J. Chem.*, **33**, 330 (1955).

⁴ Smith, E. L., and Spackman, D. H., *J. Biol. Chem.*, **212**, 271 (1955).

⁵ Anderson, B. M., and Anderson, C. D., *Biochem. Biophys. Res. Comm.*, **16**, 258 (1964).

⁶ Aono, Y., *Osaka Daigaku Igaku Zasshi*, **10**, 619 (1958).

⁷ Gierer, A., *Biochim. Biophys. Acta*, **17**, 111 (1955).

⁸ Bulen, W. A., *Arch. Biochem. Biophys.*, **62**, 173 (1956).

⁹ Winer, A. D., *Acta Chem. Scand.*, **12**, 1695 (1958).

¹⁰ Wachsmuth, E. D., Fritze, I., and Pfeleiderer, G., *Biochemistry*, part II (in the press).

¹¹ Wachsmuth, E. D., Fritze, I., and Pfeleiderer, G., *Biochemistry*, part I (in the press).

¹² Winer, A. D., and Theorell, H., *Acta Chem. Scand.*, **14**, 1729 (1960).

¹³ Wallenfels, K., and Sund, E., *Biochem. Z.*, **329**, 27 (1957).

¹⁴ Lineweaver, H., and Burk, D., *J. Amer. Chem. Soc.*, **56**, 658 (1934).

¹⁵ Theorell, H., and Bonnichsen, R., *Acta Chem. Scand.*, **5**, 1105 (1951).

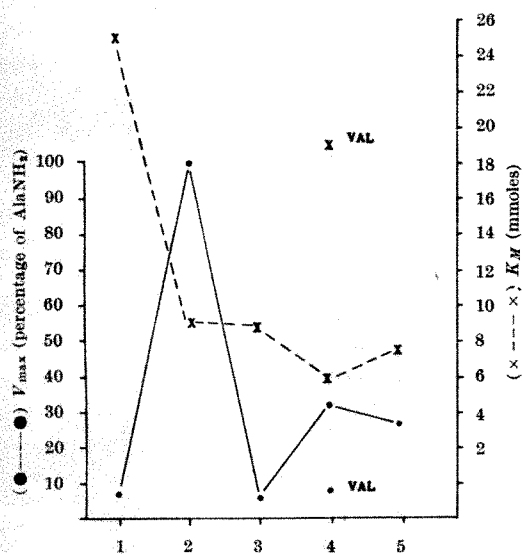


Fig. 3. The constants for the hydrolysis of various straight-chain amino-acid amides by pig kidney aminopeptidase measured from Lineweaver and Burk plots¹⁴. For experimental conditions see ref. 11. Left ordinate: V_{\max} values (percentage of AlaNH₂); Right ordinate: K_M values (mmoles) $\times \dots \times$

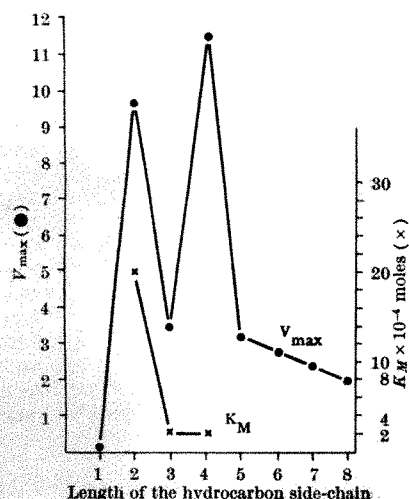


Fig. 4. The constants for the dehydrogenation of various straight-chain alcohols by liver alcoholdehydrogenase derived from refs. 8 and 15

THE WOLF CREEK IRON METEORITE

By PROF. S. R. TAYLOR

Department of Geophysics and Geochemistry, Australian National University, Canberra

THE discovery of the crater due to meteorite impact near Wolf Creek, Western Australia, was first reported by Reeves and Chalmers¹. Guppy and Matheson² gave a fuller description. The Wolf Creek crater (Fig. 1) is situated at long. 127° 48' E. and lat. 19° 11' S. (co-

ordinates of south-east sector of rim) 2,800 m. east of Wolf Creek in the East Kimberley District, Western Australia. The nearest town is Halls Creek, 66 miles north of the crater. The crater diameter (rim to rim) varies between 870 and 950 m (ref. 3). This size is



Fig. 1. Aerial view of Wolf Creek meteorite crater, looking north

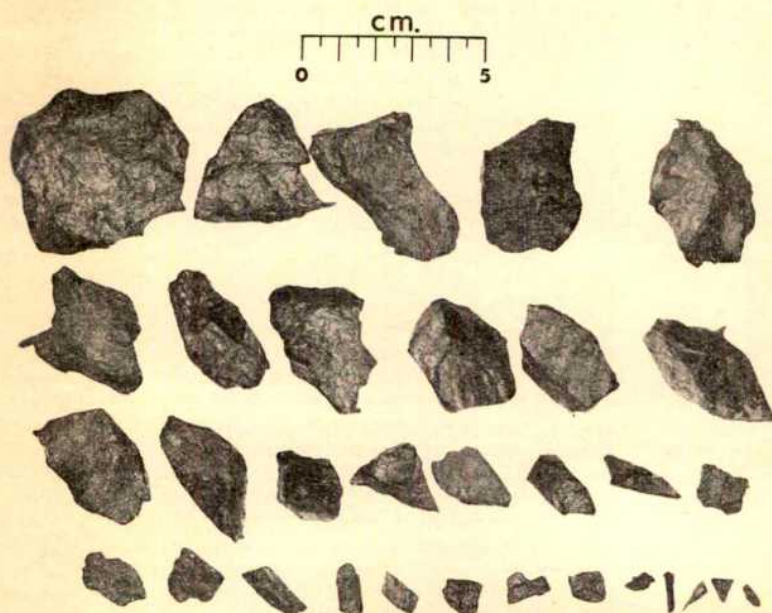


Fig. 2. Fragments of Wolf Creek iron meteorite showing size and form. Note the many angular fragments

exceeded only by the Arizona meteorite crater (Canyon Diablo meteorite) among craters with associated meteoritic material⁴.

Guppy and Matheson², Cassidy⁵, LaPaz⁶ and McCall⁷ have described the nickel-rich oxidized meteoritic material which was moderately abundant, but has now been extensively collected. No typical metallic meteorite specimens have been reported previously, although Guppy and Matheson² recovered trace amounts of metallic iron from the oxidized material, and LaPaz⁶ reported streaks and granules of metal in two large (120 and 125 kg) oxidized masses.

The purpose of this article is to report the discovery of fragments of a typical iron meteorite near the crater. In June 1965, Dr. P. Kolbe and Mr. E. H. Pedersen, of this Department, mapped Wolf Creek crater as part of a survey of Australian meteorite craters. In August 1965, Mrs. J. Moyle of Carranya Station, about 3 miles south-west of the crater, forwarded a small piece of iron meteorite found by Andy Timperley and a companion who are employed at the station. Following identification of the

fragment, the locality was visited by Mr. E. H. Pedersen and myself. On August 30 and subsequent days, 1,343 g were collected.

The fragments were found lying on the surface among small pebbles, overlying a calcareous clay soil without pebbles or meteorite fragments. The ground is partly covered by spinifex (*Triodia* sp.) but is otherwise barren. The fragments were found mainly in an elliptical area with axes of 30 and 20 m, 3,900 m south-west (235°) of the crater. The distance west from the creek on this bearing is 1,100 m. Other fragments were found about 100 m to the west and a large fragment (70 g) about 500 m west of the creek. Much of this area was traversed using a mine detector, but no large buried masses were located. A low ridge with a calcareous capping rises about 100 m north of the principal locality. Small sinkholes, not to be confused with impact features, have developed near the edge of the scarp facing the creek.

Fig. 2 shows the size-range collected, and the typical forms of the fragments. The largest piece (top left) weighs 72.6 g and the four smallest (bottom right) weigh 0.114, 0.114, 0.269 and 0.128 g respectively. The surface of the fragments is heavily oxidized. Their shape commonly is oriented parallel to the Widmanstätten structure developed parallel to octahedral planes. There is no definite evidence of modification by fusion of the surface, and the regular outlines, with occasional projecting spikes, are interpreted as due to secondary fragmentation of a larger mass, cleaving along the octahedral planes.

Fig. 3 shows polished and etched surfaces of four specimens, displaying well-developed Widmanstätten structure with alternating broad lamellae of kamacite (nickel-poor body-centred cubic or α -iron) and taenite (nickel-rich

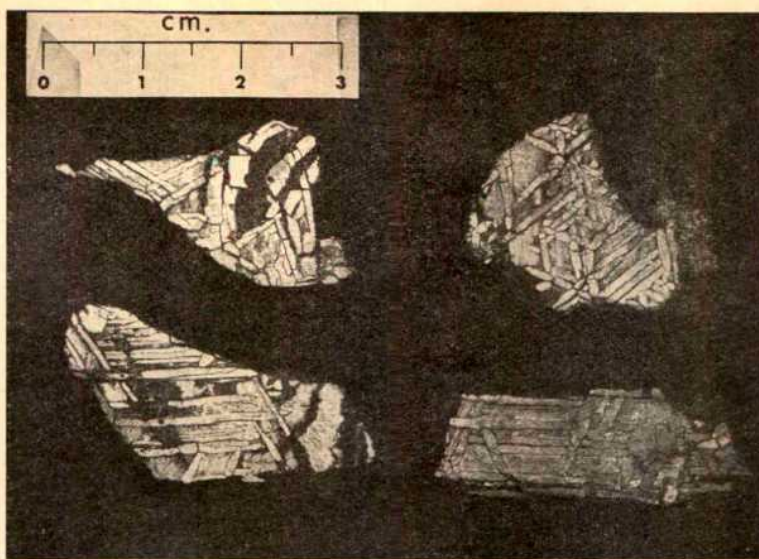


Fig. 3. Polished and etched sections showing Widmanstätten structure

face-centred cubic or γ -iron). The average width of the kamacite lamellae is about 0.9 mm. This falls within the range of kamacite band-widths (0.5–1.5 mm) of the medium octahedrite class of iron meteorites⁴. Chemical analysis gives a nickel content of 8.6 and 0.4 per cent of cobalt, rather close to the average of all iron meteorites reported by Mason⁴, and confirming the classification as a medium octahedrite.

¹ Reeves, F., and Chalmers, R. O., *Austral. J. Sci.*, **11**, 154 (1948).

² Guppy, D. J., and Matheson, R. S., *J. Geol.*, **58**, 30 (1950).

³ Kolbe, P. (personal communication).

⁴ Mason, B., *Meteorites* (John Wiley, 1962).

⁵ Cassidy, W. A., *Meteoritics*, **1**, 197 (1954).

⁶ LaPaz, L., *Meteoritics*, **1**, 200 (1954).

⁷ McCall, G. J. H., *Ann. N.Y. Acad. Sci.*, **123**, 970 (1965).

INITIATION OF CHAIN REACTION UNDER AN ULTRASONIC WAVE EFFECT

By PROF. I. E. ELPINER, A. V. SOKOLSKAYA and M. A. MARGULIS

Institute of Biological Physics, Academy of Sciences of the U.S.S.R., Moscow

CHEMICAL processes which proceed in an ultrasonic wave field are mainly conditioned by cavitation phenomenon. According to existing theories, gases, molecules of water and other compounds, penetrating into a cavitation bubble, undergo ionization or activation as a result of large electrical tensions¹ or large pressures². The oxidation and reduction reactions usually observed in sonicated aqueous media are related to the appearance of ions and short- or long-living radicals. To a great extent, the character of these reactions depends on the nature of the gas which saturates the sonicated aqueous solution. Moreover, it transpires that the character of ultrasonic reactions also depends on the nature of rare (noble) gases that are present in solution. Thus, oxidation processes proceed in an ultrasonic wave field not only in presence of oxygen but also in its absence if the sonicated solution has been previously saturated with argon or krypton (but not with helium)³⁻⁶. A suggestion was made that krypton or argon, excited in an ultrasonic field, 'catalyse' the process of splitting water molecules with the result that activated OH-radicals are formed which participate in oxidative reactions.

These observations revealed new possibilities of the control over ultrasonic chemical processes and suggested

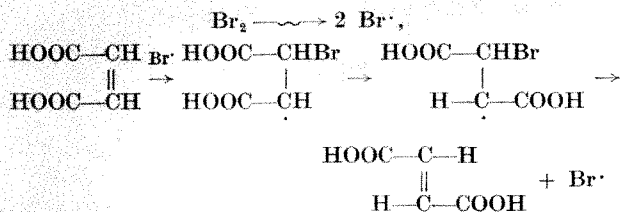
a way of investigating the elementary phenomena which form the basis of ultrasonic reactions. The problem of the intensifying of the reactions remains the urgent one.

We present here data which show that atoms and radicals, initiating reactions with large-quantum yield, can be formed by means of an ultrasonic wave effect. By 'initiating reactions' we mean the initiation of chain reactions, or, more precisely, the possibility of obtaining active particles which are necessary for chain formation. It has been suggested that for some admixtures (initiators), dissociation in the cavitation bubble with the concomitant formation of atoms or radicals provides the beginning of chain reactions under the influence of ultrasonic waves. This has now been confirmed by the following experiment.

We investigated the possibility of initiating a stereoisomerization effect in ethylene 'raw' compounds (ethylene-1,2-dicarboxylic acid and its dimethyl ether) when placed in an ultrasonic wave field.

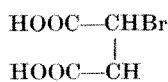
It should be noted that the isomerization reaction of maleic acid with the concomitant formation of fumaric acid proceeds under the influence of ultra-violet light or visible light in the presence of an initiator—a small amount of bromine. This process has the characteristic

features of a chain reaction which usually can be represented as the following scheme⁷:



and so on . . .

It can be seen that the primary reaction here is the appearance of $\text{Br} \cdot$ atoms. Bromine atoms react with *cis*-isomer molecule with the formation of free radicals:



which is transformed into more stable *trans*-isomer.

In darkness, isomerization of maleic acid into fumaric acid does not occur under the conditions mentioned even after 18 h (see Table 1). Later some experiments on sonication of the investigated mixtures were carried out in darkness.

In our work, ultrasonic oscillations having a frequency of 860 kc/s were used; 33 per cent aqueous solutions of maleic acid were sonicated in the presence of a small amount of bromine (0.0079 mole of Br_2 was added per mole of maleic acid). The sonicated solutions were saturated previously with oxygen, hydrogen, helium, argon or nitrogen.

Control of the course of the reaction is possible, owing to the fact that the fumaric acid formed, unlike maleic acid, is poorly soluble in water and precipitates as white needle-like crystals. Quantitative analyses of fumaric acid were carried out by the weighing technique.

As Table 1 shows, isomerization of maleic acid with the formation of fumaric acid proceeds at comparatively short exposures to ultrasonic waves. (The melting-point of the product obtained is 286° C, which corresponds to that of fumaric acid.) At the same time it appears that the reaction is inhibited if the reaction mixture has been previously saturated with oxygen. In the presence of the latter the reaction proceeds more slowly than in the presence of a rare gas.

Table 1. THE AMOUNT OF FUMARIC ACID, PRODUCED ACCORDING TO THE NATURE OF THE GAS, SATURATING THE AQUEOUS SOLUTION OF MALEIC ACID SONICATED IN DARKNESS

Five ml. of aqueous solution containing maleic acid (33 per cent) and 0.5 ml. of bromine water were sonicated under a frequency of 860 kc/s and intensity 12 W/cm²

Time of sonication (min)	Amount of fumaric acid produced on sonication in presence of:							
	Ar		He		O ₂		H ₂	
	g	%	g	%	g	%	g	%
5	0.1983	11.9	0.1472	8.8	0	—	0.1606	9.6
10	0.2034	12.2	0.1812	10.9	0	—	0.1383	8.3
15	0.2818	16.9	0.2161	13.0	0	—	0.2462	14.8
20	—	—	0.1294	7.8	0	—	—	—
35	—	—	—	—	0.043	2.6	—	—
20*	0	0	—	—	—	—	0	—
100*	0	0	—	—	—	—	0	—
18 h†	0	0	—	—	—	—	—	—

* Sonication was carried out in the absence of bromine.

† The solution studied was maintained in darkness and not sonicated.

Similar results were obtained when aqueous solutions of dimethyl ether of maleic acid were subjected to ultrasonic waves in the presence of catalytic amounts of bromine. Precipitation of small crystals of the dimethyl ether of fumaric acid occurred 2–3 min after the sonication commenced in darkness (after recrystallization m.p. was 151° C, which is characteristic of this compound).

The appearance of atomic bromine, which initiates the given chain reaction, apparently proceeds in the cavitation bubble. If we take into account the fact that the duration of life of the $\text{Br} \cdot$ is of the order of 10^{-7} – 10^{-8} sec, that is, smaller than the period of applied ultrasound or smaller than the duration of life of the cavitation bubble, the interaction between bromine atom and *cis*-isomer molecule should be considered to occur on the bubble-fluid interface. On the given interface the molecules of oxygen, penetrating into the cavitation bubble, halt the chain reaction which has begun, and form intermediate peroxide-inactive compounds with reaction products^{3,4}.

Later it was found that by ultrasonic chemical transformations some bromine-containing aliphatic compounds release atomic bromine which also initiates the chain reaction of isomerization of maleic acid into fumaric acid. The experiments were carried out on 1,2-dibromoethane ($\text{C}_2\text{H}_4\text{Br}_2$) and *n*-hexyl bromide ($\text{C}_6\text{H}_{11}\text{Br}$); 5 ml. of a 33 per cent aqueous solution of maleic acid plus 0.5 ml. of water, saturated at 60°–70° C with dibromoethane or *n*-hexyl bromide, were treated with ultrasonic waves.

The results of the investigation using dibromoethane are given in Table 2.

Table 2. THE AMOUNT OF FUMARIC ACID, PRODUCED ACCORDING TO THE NATURE OF THE GAS, SATURATING THE AQUEOUS SOLUTION OF 33 PER CENT MALEIC ACID SONICATED IN DARKNESS

Five ml. aqueous solution plus 0.5 ml. of water saturated with $\text{C}_2\text{H}_4\text{Br}_2$ were sonicated under a frequency of 860 kc/s and intensity 12 W/cm²

Gas, present during sonication	Time of sonication (min)	Amount of produced fumaric acid (g)
Hydrogen	75	0
Hydrogen	120	0
Argon	40	0.072
Argon	60	0.057
Oxygen	30	0.055
Air	60	0.050

In this case, it also seems that the bromine atoms are formed in a cavitation bubble where dibromo-ethane (or *n*-hexyl bromide) penetrates owing to the high elasticity of its vapour. However, the splitting of atomic bromine apparently proceeds with the participation of atomic oxygen and, in the case of argon (at the interaction with OH-radicals), products of water molecule splitting. The energy of the $\text{Br}-\text{C}$ bond is much greater than that of the $\text{Br}-\text{Br}$ bond. It accounts, probably, for the fact that the process of accumulation of a sufficient amount of fumaric acid proceeds more slowly than in the presence of bromine molecules. It is suggested that the oxygen molecules which are present in the cavitation bubble are mostly used for the breakage of the $\text{C}-\text{Br}$ bond; owing to this they do not influence further development of the chain reaction of the isomerization of maleic acid into fumaric acid.

It should be emphasized that we failed to initiate any isomerization reaction by means of bromo-benzene or sodium bromide instead of dibromo-ethane.

Thus, initiation of chain reactions in an ultrasonic wave field is probably due to the direct excitation of the initiators of these reactions, which penetrate into cavitation bubble. In some cases chemical transformations can bring about the formation of the intermediate reactions, the products of which can be initiators of chain chemical processes.

¹ Prudhomme, R. O., and Grabar, P., *J. Chim. Phys. Phys.-chim. Biol.*, **46**, 323 (1949).

² Neppiras, E. A., and Nolting, B. E., *Proc. Phys. Soc.*, **64**, B, 384 (1951).

³ Elpiner, I. E., *Ultrazvuk, Fiziko-khimicheskoe i biologicheskoe Deistvie, Fizmatgiz* (Moscow, 1963); *Ultrasound, Physical, Chemical, and Biological Effects* (Consultants Bureau, New York, 1964).

⁴ Elpiner, I. E., and Sokolskaya, A. V., *Dokl. Acad. Nauk U.S.S.R.*, **119**, 1180 (1958).

⁵ Sokolskaya, A. V., and Elpiner, I. E., *Akust. Zhur.*, **6**, 263 (1960).

⁶ Elpiner, I. E., and Sokolskaya, A. V., *Dokl. Acad. Nauk U.S.S.R.*, **153**, 200 (1963).

⁷ Reutov, O. A., *Teoreticheskie Osnovi Organicheskoy Khimii*, 284 (Moscow Universitet, 1964).

EVIDENCE FOR MICROFOSSILS IN THE ALAIS AND ORGUEIL
CARBONACEOUS METEORITES

By SAM L. VANLANDINGHAM

Department of Biology, University of Louisville, Kentucky

ACHEMICAL treatment of powdered samples of the Alais and Orgueil carbonaceous chondrites, using concentrated hydrofluoric acid or 6 N hydrochloric acid and/or a solution of saturated potassium chlorate with concentrated nitric acid (Schulze's solution), resulted in the isolation of a number of acid-resistant particles which morphologically resemble organized elements described by Claus and Nagy¹, Staplin², Timofejev³ and Palik⁴. Some of these particles appeared to be morphologically identical with the microstructures described by the same investigators and were found, by Nagy *et al.*⁵, to be partially mineralized. A few of the acid-resistant particles were identified as recent terrestrial contaminants; however, the majority of them appeared to be indigenous microfossils on morphological grounds. The fact that some of the microstructures in the Alais meteorite are identical with those in the Orgueil meteorite (obtained from a different museum) and that no microstructures were found in the Bruderheim meteorite (a non-carbonaceous chondrite used as a control) serves as a further indication of a possible indigenous origin of the microfossils.

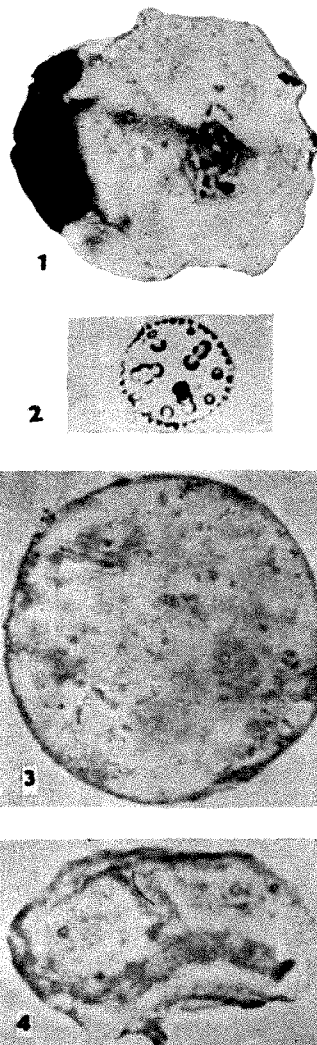
Approximately 670 mg of the Alais meteorite, 470 mg of the Orgueil meteorite and 680 mg of the Bruderheim meteorite were obtained from Dr. George Claus, New York University. These samples originally came from the American Museum of Natural History, New York, from the Smithsonian Institution, Washington, and from the University of Alberta, Canada, respectively. The Alais meteorite fell in 1806 in France, and was chemically studied by Berzelius⁶ in 1834. The Orgueil meteorite fell in 1864 in France and it has been the subject of extensive investigations in recent years. The Orgueil meteorite contains hydrocarbons⁷, fatty acids⁸, porphyrins⁹, and organic material which shows optical rotation¹⁰. Free-carbon radicals of possible biogenic origin were also found evenly distributed in some meteoritic organic phases¹¹. Mineralogical investigations^{12,13} have shown that its parent body had an aqueous, low temperature, slightly alkaline and somewhat reducing environment. The interior of the meteorite did not seem to be affected by the heat generated during the fall through the Earth's atmosphere. The Bruderheim meteorite fell in Canada in 1960 and it has been used widely in recent chemical and physical investigations. It is a non-carbonaceous chondrite containing minerals of high-temperature origin only; consequently it appears to be a suitable control for the present studies.

The Alais meteorite was crushed in a glass mortar. The powder was first treated with 75 ml. of concentrated hydrofluoric acid for 24 h at room temperature and then washed and siphoned six times with distilled water, allowing 2 h for each settling between siphonings. The distance of settling was 40 cm. The purpose of the hydrofluoric acid treatment was to remove silicate minerals. The material was then treated with approximately 75 ml. of Schulze's solution in order to bleach concentrated organic remains. After approximately 24 h in the Schulze's solution, the samples were washed and siphoned six times, allowing 2 h for each settling between siphonings. A water suspension of the cleaned organic and insoluble material was mixed with 'Clearcol' resin on glass cover-slips. The cover-slips were then placed on glass slides and allowed to dry. Fragments of the Orgueil meteorite were treated only with Schulze's solution.

In another experiment, approximately 373 mg of the Alais meteorite were crushed in a glass mortar and treated with 75 ml. of concentrated hydrochloric acid for 12 h. The samples were washed and siphoned six times, allowing 2 h for each settling of 40 ml. The acid-resistant remains were then mounted in 'Hyrax' resin. The same procedure was also followed for the Bruderheim and the Orgueil meteorites.

A microscopic examination of fifteen slides revealed some terrestrial contaminants. A fungus spore and some spores or pollen grains were noted in the Alais and Orgueil meteorites. The contamination level appeared to be low; only five slides out of the fifteen microscopic preparations showed microstructures that could be identified possibly with recent organisms. In the Bruderheim meteorite no contaminants were observed.

Figs. 1-4 show photomicrographs of some of the acid-resistant particles separated from the Alais and Orgueil carbonaceous meteorites. Thirty indigenous forms were



Figs. 1-4

found. Some of them appear to be identical with microstructures described by earlier investigators¹⁻⁴. Fig. 1 shows a laminar particle, slightly folded, having a small papilla, and with a width of 27μ . This form is from the Alais meteorite, and it is considered to be identical with forms described by Claus and Nagy from the Orgueil and Ivuna meteorites¹⁴. Fig. 2 shows a flat, disk-shaped body with perforations and spines. The body has a diameter of 10.5μ . This form was found in the Alais meteorite. Fig. 3 shows a spherical body, $28-31\mu$ in diameter, exhibiting a thin-wall structure. This form was found in the Orgueil meteorite. Fig. 4 shows an oblong body which is punctate to granulose and which has a conspicuous triradiate tubular wall thickening resembling a canal-like structure. The wall is thin. This body has dimensions of $17\mu \times 20\mu$ and was found in the Alais meteorite. A detailed micropalaeontological report on these and the remaining forms will be published elsewhere¹⁵.

The work recorded here confirms earlier findings regarding the presence of microstructures in carbonaceous meteorites. These have been identified by some investigators as only biological contaminants^{16,17}. The exact origin

of the organized elements is still not known; however, it does not seem to be likely that all microstructures can be accounted for as either recent biological contaminants or mineral artefacts.

- ¹ Claus, G., and Nagy, B., *Nature*, **192**, 594 (1961).
- ² Staplin, F. L., *Micropaleontol.*, **8**, 343 (1962).
- ³ Timofeev, B. W., *Grana Palynol.*, **4**, 92 (1963).
- ⁴ Palik, P., *Nature*, **194**, 1065 (1962).
- ⁵ Nagy, B., Fredrikson, K., Urey, H. C., Claus, G., Anderson, C., and Percy, J., *Nature*, **198**, 121 (1963).
- ⁶ Berzelius, J., *Kongl. Vetenskaps-Akad. Förhandl.*, 152 (1834).
- ⁷ Nagy, B., Meinschein, W. G., and Hennessey, D. J., *Ann. N.Y. Acad. Sci.*, **93**, 25 (1961).
- ⁸ Nagy, B., and Bitz, M. C., *Arch. Biochem. Biophys.*, **101**, 240 (1963).
- ⁹ Hodgson, G. W., and Baker, B. L., *Nature*, **202**, 125 (1964).
- ¹⁰ Nagy, B., Murphy, M. T. J., Modzeleski, V. E., Rouser, G., Claus, G., Hennessey, D. J., Colombo, U., and Gassarini, F., *Nature*, **202**, 228 (1964).
- ¹¹ Duchesne, J., Depireux, J., and Litt, C., *C.R. Acad. Sci., Paris*, **259**, 4776 (1964).
- ¹² Nagy, B., Meinschein, W. G., and Hennessey, D. J., *Ann. N.Y. Acad. Sci.*, **108**, 534 (1963).
- ¹³ DuFresne, E. R., and Anders, E., *Geochim. Cosmochim. Acta*, **26**, 1085 (1962).
- ¹⁴ Claus, G., and Nagy, B., *Phye. Soc. Amer. News Bull.*, **15**, 15 (1962).
- ¹⁵ VanLandingham, S. L., *Nova Hedwigia*, **10**, 161 (1965) (in the press).
- ¹⁶ Deflandre, G., *C.R. Acad. Sci., Paris*, **254**, 3405 (1962).
- ¹⁷ Fitch, F. W., and Anders, E., *Ann. N.Y. Acad. Sci.*, **108**, 495 (1963).

INHIBITION OF TRANSPLANTABLE AND SPONTANEOUS MURINE TUMOURS BY THE M-P VIRUS

By DR. NORMAN MOLOMUT and DR. MORTON PADNOS

Waldemar Medical Research Foundation, Inc., Woodbury, Long Island, New York

TWO years ago we reported that while studying subcellular fractions of Ehrlich ascites carcinoma (EAC), we noted that after 105,000g centrifugation the supernatant fluid was capable of transmitting EAC when injected into mice. Subsequent investigations revealed viable cells with vacuolated cytoplasm and nuclei compressed into evaginations of the cell wall present in the supernatant fluid¹.

Filtration of the final supernatant fluid on a 220-m μ 'Gradocol' membrane removed these cells and the filtrate did not transmit EAC when injected into mice. However, the filtrate, after injection, caused a mild disease grossly characterized by lassitude, ruffled fur, and swollen abdomen (ascites fluid) from which the mice recovered within 10-18 days varying with each inbred strain of mouse. The peripheral blood exhibited a marked lymphocytopenia, and our subsequent investigations determined the presence of a virus^{2,3}.

The major findings accompanying the viraemia in all strains of mice consist of a marked lowering of the peripheral blood lymphocytes, reduction in cells and size of the spleen, lymph nodes and thymus accompanied by the development of peritoneal and pleural fluids in Swiss mice. These reactions occur also in rats, and to a limited extent in young cats and dogs where the dose of virus may be too small to induce more marked symptoms. The peripheral blood lymphocytopenia is sustained on re-infection with virus given prior to development of neutralizing antibodies².

In contrast to the effect on lymphocytes and lymphoid tissues *in vivo*, the virus is not cytopathic for normal mouse cells in culture. We have quite recently observed that, after two culture passages in a primary isolate from a transplanted mouse passage lymphosarcoma, the M-P virus (designation of the new isolate) was cytopathic for the lymphosarcoma cells. The cytoplasm became vacuolated, cells swelled, nuclei appeared granular and many cells became multi-nucleated. The M-P virus appears to differentiate between normal and malignant cells of the same type, and we propose to pursue this aspect further.

The physical and chemical properties of the virus determined so far are the following: stable at -20°C in serum

for 16 months (the period of time so far tested); labile at 56°C in serum after 30 min, and in saline dilution it loses its infectivity rapidly, with retention of activity for less than one week at -20°C ; filtration on 180 m μ 'Gradocol' membranes retains the virus; the supernatant fluid after 5 h centrifugation at 105,000g in a Spinco model L is still infective for mice; the virus will not haemagglutinate bovine erythrocytes, which differentiates it from the Reo III and related viruses; it is not inactivated by RNase treatment; and is labile at pH 3.0 in glycine hydrochloride buffer.

The post-viraemic serum and organs of mice yield a haemagglutinin for sheep red blood cell (SRBC), which is not produced in cell culture. The haemagglutinin has been separated by differential filtration and absorption on SRBC from the M-P virus particle. Neutralizing antibody for the M-P virus does not react with the haemagglutinin and the haemagglutinin does not neutralize the M-P virus.

Serological tests utilizing haemagglutination inhibition, complement fixation, and infectivity neutralization methods were used to examine this agent. Serological cross-reactions were noted with antisera to the mouse leukaemia viruses of Moloney, Rauscher, and, to a lesser degree, the Friend virus. However, when these antisera were absorbed with normal mouse thymus cells, there were no cross-reactions.

No cross-reactions were noted with the following viruses: mouse hepatitis (MHV), Kilham (K), mouse pneumonitis (PVM), Reo III, and polyoma. Complement fixation tests with the antiserum to the M-P virus and LCM virus antigen indicate that there is a serological commonality between them. The control mouse sera from normal mice gave an anomalous reaction in that one of the sera was also positive for LCM virus antigen. The LCM virus is not infective by intracerebral inoculation in mice previously immunized to the M-P virus. The *in vivo* pathology induced by the LCM agent, however, in susceptible non-immune mice differs strikingly from that caused by the M-P virus. This would lead us to believe that the M-P virus and the LCM virus may both be part of a class of viruses which have some characteristics in common,

but differ markedly in size, pathological effects *in vivo* and anti-tumour action on both transplantable and spontaneous tumour in mice.

The marked lymphocytopenia induced by injection of the M-P virus directed our attention to the possible anti-leukaemic action of this virus and afterwards to its effect on other transplanted and spontaneous tumour in mice.

The transplanted tumours examined were the following: a lymphoid leukaemia induced by methylcholanthrene in BALB/C mice in 1960; Ehrlich ascites carcinoma obtained from Dr. Sugiura in 1960; a spontaneous C₃H mammary carcinoma in the second mouse passage; spontaneous lymphatic leukaemia in AKR mice.

Transplants of equal numbers of lymphoid cells in controls and mice injected with a single dose of M-P virus (500 × ID₅₀) administered 24 h after inoculation of the leukaemic cell result in a mean increase of 40 per cent in survival beyond the controls, and with the smaller transplanted dose of leukaemic cells, 55 per cent of the mice survived the leukaemia. No survivors had evidence of leukaemic involvement at 18 months.

Following the same experimental model with Ehrlich ascites carcinoma, 80 per cent of the M-P virus treated mice survive as compared to only 18 per cent controls².

Table 1 summarizes results from the second passage transplant of the spontaneous C₃H mammary carcinoma.

The experimental model varied with respect to the time of virus injection and tumour transplantation; one day prior to tumour transplant, and 12 days after tumour transplant when the graft was palpable, with appropriate tumour graft controls.

The virus-treated mice show a marked slowing of the progression of the subcutaneous transplant when the virus is administered one day before as well as 12 days after tumour graft. (Statistical probability less than 0.01 per cent, due to chance.) The tumour graft did not grow in four of the mice injected with M-P virus one day prior to tumour graft.

In an investigation (now in its tenth month) control mice have developed and died of lymphatic leukaemia, while two groups of mice treated with M-P virus (administered a single intraperitoneal injection of the MPV), one group treated at three months of age and another group treated at six months of age, reveal marked inhibition of leukaemia with the latter group showing complete suppression (Table 2).

The effect of the M-P virus in suppressing spontaneous C₃H mammary carcinoma is current, with the following data in hand; female mice from our C₃H breeding colony were weaned from their third litter, separated into a control and treated group using litter mates; one month after weaning, mice of one group were treated with a single intraperitoneal injection of the virus, and their respective litter mate controls were injected with the diluent only. The data at six months since the injection of the virus are presented in Table 3.

Table 1. M-P VIRUS INHIBITION OF TRANSPLANTED MAMMARY CARCINOMA IN C₃H MICE
(Second mouse passage from spontaneous tumour)

	No. mice	500 ID ₅₀ Virus dose injected	Average tumour size mm ² (after 1 month)	P(%)
Tumour control	100	None	308 ± 20.1	
Virus and tumour graft	95	1 day prior	202 ± 16.5	0.01
Virus and tumour graft	120	12 days after graft	175 ± 11.8	0.01

Table 2. INHIBITION BY M-P VIRUS OF SPONTANEOUS LEUKAEMIA IN AKR MICE

Group and treatment	No. of mice	Sex *	No. of mice dead with leukaemia/Total mice
(I) Control	28	F	17/28
No treatment	23	M	4/23
(II) M-P virus at 3 months	21	F	11/21
8 months	8	M	0/8
(III) M-P virus at 6 months	21	F	0/21
18 months	18	M	1/18

* No. of male mice reduced by early deaths due to injuries resulting from fighting within pens.

Table 3.

	No. of mice	Tumour incidence 3 months	6 months	No. of mice without tumours
Control	26	10	17	9
Virus treated *	26	3	10	16

* Litter mates were paired for each respective group.

The results from the experiments with transplanted and spontaneous tumours in mice, so far, encourage us to continue to examine the influence of this virus on other experimental tumour-host systems and to investigate the underlying mechanism for this antitumour action.

The most apparent and marked difference between this newly isolated virus and other reported mouse viruses lies in the pathological *sequelae* of the viraemia induced in mice. The pathological effects associated with this virus differ from those known to be associated with the viruses of mouse hepatitis, Reo III, the leukaemogenic murine viruses and the LCM virus.

The fifth to eighth day of the acute viraemia is characterized by a significant fall in total white blood cells, primarily the lymphocytes. Subsequent to this, the lymphocytes return to normal. The rise in total white blood cell count to levels greater than pre-infection is probably due to the stimulus of the infectious state itself. Virus is detectable in the circulating blood from 24 h to approximately 4 weeks after infection. After the 28th day no live virus is recoverable from either the blood or organs. The generalized lymphocytopenia accompanying infection with this virus is present in all strains of inoculated mice, but affects Swiss mice to a greater degree than any of the others.

A detailed report of the pathological *sequelae* of the M-P virus infection has been reported². There is an atrophy of the spleen to $\frac{1}{2}$ – $\frac{1}{3}$ the size of the normal organ resulting from two changes: first, a sharp reduction in the lymphocyte content of the pulp and of the follicles, and second, a curious gelatinoid degeneration of the perifollicular stroma with an increase of atypical megakaryocytes with multilobulated hyperchromatic nuclei. Essentially the same effects are seen in the lymph nodes and the thymus, and a degree of toxic hepatitis ranging from fatty degeneration to occasional central necrotic changes is also seen.

Both peritoneal and pleural fluids occur between the seventh and ninth day of the acute infectious stage (Swiss mice only). Live virus is recoverable from the exudate, circulating blood, and all internal organs of infected animals. In C₃H and C57BL/6 mice, in which the infection is usually less severe and which survive the acute viraemia, there is evidence of choriomeningitis in 5–7 per cent of the mice, but no actual cerebral changes are encountered.

The lymphocytopenia in the organs and blood is replaced by a lymphoid regeneration and hyperplasia with moderate congestion of the splenic pulp and disappearance of the acidophilic homogeneous matrix seen in the acute viraemic stage.

We have isolated and partially characterized a heretofore undescribed virus which differs from other viruses from murine sources. We have designated it the M-P virus. This virus does not induce any cytopathic changes in normal mouse cell cultures and, when inoculated into susceptible mice, induces a viraemia which is accompanied by a marked lymphocytopenia in the peripheral blood and lymphoid organs. Another intriguing property is the ability of this virus to suppress transplanted and spontaneous tumours in mice. Present experiments indicate that the M-P virus, though isolated from a murine source, is infective for rats, young cats and dogs, causing symptoms similar to those found in mice during the acute viraemia with no evidence of disease.

In considering the possibility that the M-P virus may be an agent worthy of trial for human anti-cancer therapy (especially leukaemias and lymphomata), we are aware of the fact that there are serious limitations to be borne in mind. These latter were commented on in an

editorial in the *Lancet* (February 13, 1965, p. 366): "... A serious limiting factor has been that immune response to the therapeutic agent renders it less effective for continued use. Also, the long-term infectivity and mutation possibilities of injecting live virus must be borne in mind".

We are, nevertheless, encouraged, because the M-P virus induces a mild, transient viraemia without serious disease of *sequelae* in experimental animals in doses which have inhibited experimental tumours.

Another point of interest from the laboratory point of view is that since the M-P virus affects lymphocytosis and reduces lymphoid organs (spleen, nodes, thymus) it lends itself to use as a laboratory tool for a re-evaluation and

examination of antibody formation, transplantation phenomena and tolerance, inflammation and wound healing in order to assay further the role of the lymphocyte in such phenomena.

We thank Miss Violet Satory, Mr. Gerard Shimonaski and Miss Judith Gruen for technical assistance during the course of these experiments.

This work was supported in part by a grant from the U.S. Public Health Service, CA 07114-02.

¹ Molomut, N., Padnos, M., Satory, V., and Gross, L., *Nature*, **203**, 740 (1964).

² Molomut, N., Padnos, M., and Smith, L. W., *J. Nat. Cancer Inst.*, **34**, 403 (1965).

³ Molomut, N., Padnos, M., Gross, L., and Satory, V., *Nature*, **204**, 1003 (1964).

IMMUNE SYSTEM IN A MONOTREME: STUDIES ON THE AUSTRALIAN ECHIDNA (*Tachyglossus aculeatus*)

By DR. E. DIENER*

Walter and Eliza Hall Institute of Medical Research, Melbourne

AND

DR. E. H. M. EALEY

Department of Zoology and Comparative Physiology, Monash University, Melbourne

THE phylogeny of the immune system has been investigated in some detail in lower vertebrates of different species by Good¹ and his co-workers and others. In the lower vertebrates, including bony fishes and Elasmobranchii, there is nothing corresponding to the peripheral lymph nodes of mammals. In the Amphibia we find for the first time in the phylogenetic series structures resembling lymph nodes, the so-called jugular bodies, the embryological development of which was described by German anatomists such as v. Braunmühl² in 1925. More recently, Kent *et al.*³ described the presence of pyroninophil cells in the jugular bodies of *Bufo marinus* after antigenic stimulation. Work on this species has been extended by Diener and Nossal⁴ with regard to the selective uptake and distribution of flagellar antigen and the antibody response.

There is general agreement that mammals probably derive from the mammal-like therapsid reptiles of the Triassic and Jurassic periods. However, anatomical investigations, such as those by Goodrich⁵ on the circulatory systems, demonstrate that mammals could not have evolved from any group closely related to modern reptiles. This would mean that the therapsid reptiles were derived separately from either labyrinthodont⁶ or microsaurs⁷ Amphibia. The evolutionary origin of the monotremes is obscure, but it has been suggested that they arose from the Docodonts along a line of evolution unrelated to the Pantotheria from which present-day marsupial and placental mammals are derived. It was of obvious interest to examine the immune system of the most readily available monotreme, the echidna or spiny ant-eater (*Tachyglossus aculeatus*), to determine whether there were any features intermediate between those of Amphibia and of eutherian mammals.

This article deals principally with the histo-anatomical aspects of the project. Preliminary results from an extensive experimental programme are mentioned only where relevant.

The work was based on a total of ten animals selected from a colony of 70 echidnas which is currently being used for physiological studies by the Department of Zoology and Comparative Physiology at Monash University, Melbourne. Seven echidnas were used for histological investigation which included examination of the distribution pattern of colloidal carbon and ¹²⁵I-labelled flagellar antigen from *Salmonella adelaide*, using the autoradiographic method described by Nossal, Ada and Austin⁸. In order not to deplete the colony, most tissue samples

were obtained by biopsy. Three other echidnas have so far been subjected to studies on serum antibody formation. Echidnas were kept at 20° C and maintained normal body-weight and growth rates on a diet of pulped egg, beef and milk mixed with earth and vitamins. For histological investigations colloidal carbon and radio-iodinated flagellar antigen were injected subcutaneously into the neck region for labelling peripheral lymph noduli which were sampled by repeated biopsies. Colloidal carbon was injected into the jugular vein and spleen biopsies were carried out. Tissues were fixed in 10 per cent formal-saline and sections stained with Unna-Pappenheim stain, toluidine blue, haematoxylin-eosin and periodic acid-Schiff stain. For the studies of humoral antibody-production sheep red cells were injected intraperitoneally; bovine serum albumin (BSA) and flagellar antigen were injected intramuscularly. Antibody titration was performed by the immobilization technique described by Nossal, Ada and Austin⁹ for anti-flagellar antibodies, by standard haemagglutination methods for sheep red cells and by BSA-precipitation methods using the technique of Farr¹⁰. However, neither haemagglutinin against sheep red cells nor BSA-precipitin was obtained. Even a secondary injection with alum-precipitated BSA could not demonstrate detectable antibody. The only antibody formation obtained in the echidna was after inoculation with 1 mg of flagellar antigen. However, the peak titres demonstrated by three echidnas treated with this antigen were remarkably low as compared with the normal antibody response of laboratory rats or mice. Treatment of the total antibody peak titre with 2-mercaptoethanol (ME) demonstrated the presence of 75 per cent ME-resistant antibody. Results of an extended experimental investigation of the antibody-forming capacity in the echidna will be presented elsewhere.

Lymphoid system of echidna. The major organs of the echidna, spleen, thymus and appendix, are found in the same anatomical situations as in the higher mammals. The spleen is of characteristic tri-radiate shape (Fig. 1). Great differences in spleen size in different individuals have been observed which may be the expression of different physiological states. The blood vessels supplying the spleen were large and numerous, suggesting a high rate of blood flow. Further evidence of this was found in the numerous thick-walled arterioles in the red pulp.

The most interesting feature was the presence of small and very numerous nodules of lymphoid tissue in most of the sites where lymph nodes would be expected in placental mammals.

* Supported by a Roche Fellowship.

They are present throughout the chest, neck and pelvic region characteristically in fatty tissue closely related to the main blood vessels. They can often be seen macroscopically to be related to a network of rather large lymphatic vessels. The mesenteric lymph nodules are very numerous and are solitary, although some are often larger than the nodules in peripheral tissues. They are interlinked in a web of lymphatic vessels. Tonsillar tissue was identified in the pharyngeal region closely associated with submandibular salivary glands (Fig. 2). Peyer's patches of the gut could not be found.

Histological findings

(1) *Lymph nodules*. The highly vascularized lymph nodules are the most interesting feature of the echidna lymphatic system, because they seem to be intermediate in histological organization between the jugular-bodies of the Amphibia and the eutherian lymph nodes. The jugular bodies of *Bufo marinus* merely contain an agglomeration of lymphatic cells throughout a reticulum; moreover, they have no cortical or medullary structure, neither follicles nor circular sinus. However, there is a

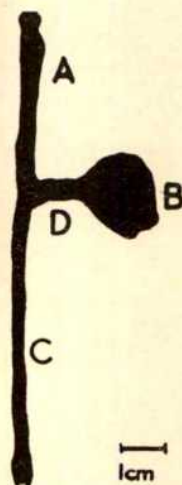


Fig. 1. Diagram of the echidna spleen showing the typical tri-radiate shape. Sections were taken out from parts indicated A, B, C and D

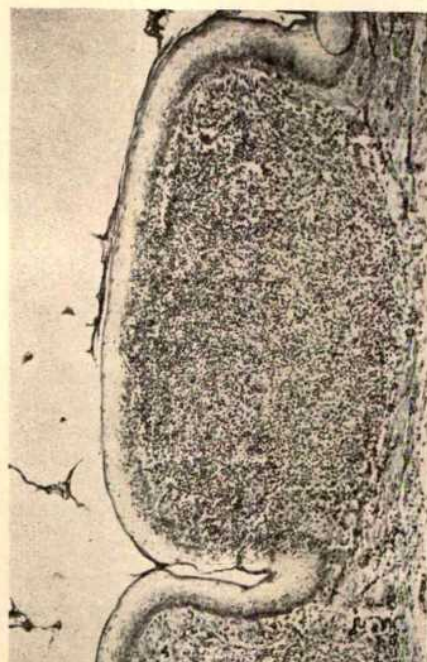


Fig. 2. Accumulation of lymphatic tissue in close relationship to the epithelium of the submandibular part of the pharyngeal region. The similarity with the tonsils of higher mammals is evident. (Periodic acid-Schiff stain: $\times 60$)

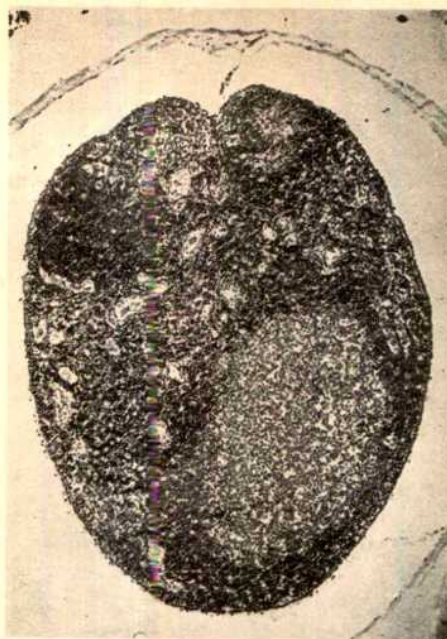


Fig. 3. Typical echidna lymph nodule showing a highly vascularized cortical zone of small lymphocytes. We believe the pale area of the nodule to be a germinal centre. (Haematoxylin and eosin: $\times c. 67$)



Fig. 4. Germinal centre of an echidna lymph nodule. Characteristic features of such centres are primitive cells often to be seen in mitotic activity. Note 'tingible body macrophages' indicated by arrows. (Haematoxylin and eosin: $\times c. 167$)

striking difference between the lymph nodes of the eutherians and the monotremes. (Histological preparations obtained from the other existing species of Monotreme, the platypus, *Ornithorhynchus paradoxus* (kindly provided by the Fisheries and Wildlife Department, Victoria), were similar to the ones obtained from the echidna.) Whereas in the eutherians a series of follicles is grouped within the diffuse cortex of each node, in the echidna each nodule appears to act as a single follicle. There is a peripheral cortical region of small lymphocytes enclosing a medullary zone which seems to represent a germinal centre containing primitive cells (Figs. 3, 4). After injection of colloidal carbon, the label accumulated within a well-developed circular sinus and the peripheral zone of the nodule, showing the fine web of phagocytic processes (Fig. 5), retained the carbon particles (as has been described in lymph nodes

of rats by Miller and Nossal¹¹). However, whereas in the rat lymph node the carbon retention occurs in close relationship to primary follicles, in the echidna the particles accumulate around the periphery of the entire nodule within 24 h and seem to spread undifferentially towards the centre. Later on, carbon can be seen throughout the medullary zone within periodic acid-Schiff-positive cells, sometimes seen particularly in association with the larger blood vessels which are evident in some of the nodules. Further investigation using the method of injecting ¹²⁵I-labelled flagellar antigen and subsequent autoradiography of the sections is in progress. Results so far have shown nothing comparable to the areas at the periphery of primary lymphoid follicles in the rat where antigen is selectively retained¹². Evidence of an immune response may, however, often be seen in the form of clusters of pyroninophil cells within zones of small lymphocytes.

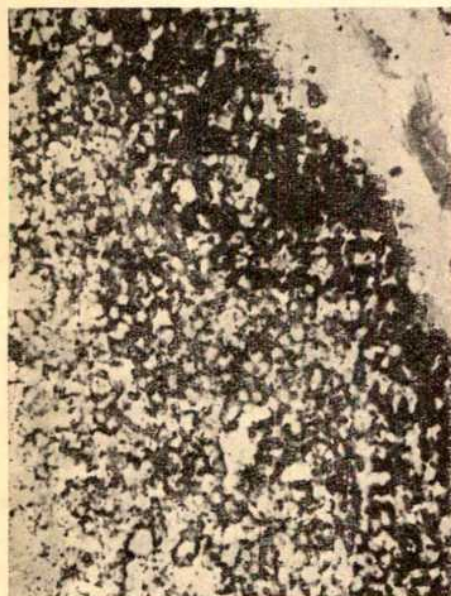


Fig. 5. Uptake of carbon at the periphery of the lymph nodule of the echidna, 24 h after injection. The web-like structure throughout the cortical zone may be produced by cytoplasmic processes, retaining carbon particles. (Toluidine blue: $\times 416$)

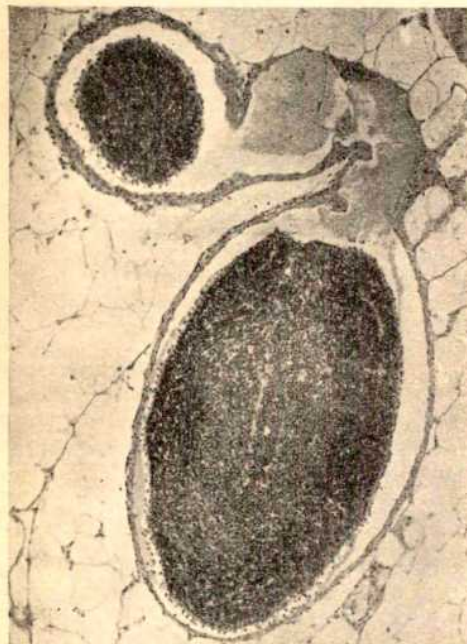


Fig. 6. Lymph nodules of echidna seen within the lumen of a lymphatic vessel. (Haematoxylin and eosin: $\times 60$)

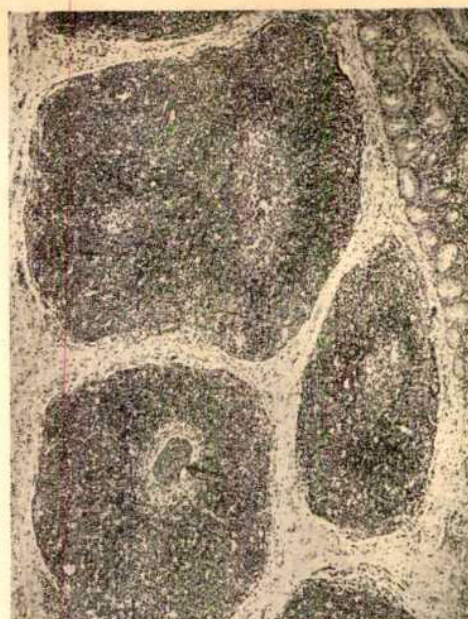


Fig. 7. Appendix of echidna showing lymphoid follicles each divided into cortical and medullary zone. Note the crypt in the centre of one of the follicles filled with polymorph nuclear cells (arrow). (Haematoxylin and eosin: $\times c. 41$)

In addition to this unique structure of the echidna lymph node it is of great interest that, in some regions at least, nodules are seen to be suspended within the lumen of a large lymphatic vessel (Fig. 6). This varies in its content; sometimes being empty, or with only a few lymphocytes, sometimes filled, perhaps as a result of trauma, with lymphocytes and red blood cells. It will be of great interest to follow the embryological and early post-hatching development of these structures. The appearance described might well arise by invagination of the lymphatic vascular wall into the lumen of the vessel. Subsequent vascularization of the invaginated wall could provide the appropriate meshwork, into which lymphatic cells could settle down to form a lymphatic nodule. Since the lymph nodule in the echidna seems to represent a single lymphoid follicle, one could think of a gradual evolutionary tendency of such follicles to fuse together to form the characteristic lymph node of eutherian mammals. That this process may even take place in the echidna is suggested by the occasional occurrence of such nodules which show more than one germinal centre. Autoradiographic studies of antigen distribution as well as embryological work will be necessary to work out the implications of the histological appearances.

(2) *Thymus*. Each half of the thymus is divided into numerous highly vascularized lobules. Each lobule has a cortical and medullary zone comparable with higher mammals. There are numerous Hassall's corpuscles in the medulla. As no significant difference in structure between the thymus of the echidna and that of Eutheria was recognized, further detail is not included in this article.

(3) *Spleen*. The echidna spleen was subjected to biopsies from five different positions indicated in Fig. 1. The relative proportion of white pulp to red pulp at these locations does not indicate significant structural differences within the organ. Erythrocytes can be seen not only within the red pulp but also dispersed in a characteristic way within the follicles of the white pulp, between the marginal zones and the germinal centres. These latter are similar to those found in the Eutheria, in that they contain primitive pyroninophil cells. Twenty-four hours after the intravenous injection of carbon, phagocytic cells containing carbon particles were scattered throughout the red pulp.

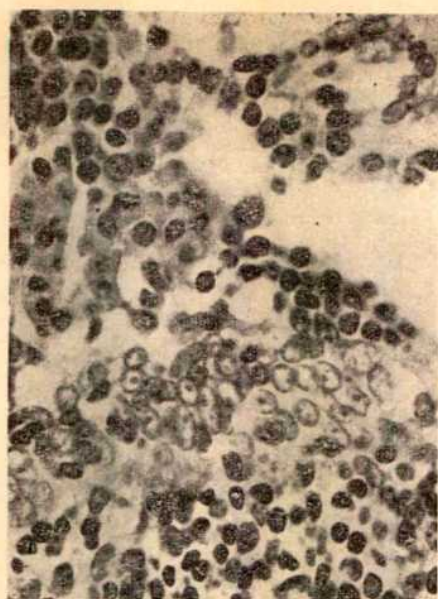


Fig. 8. High-power micrograph of the central medullary field of an appendix follicle. Note the cluster of epithelial cells among the lymphocytes and reticulum cells. (Haematoxylin and eosin: $\times c. 527$)

As in *Bufo marinus* and the *Eutheria* the white pulp was devoid of carbon.

(4) *Appendix*. In the appendix (Fig. 7), the lymphoid follicles generally resemble the nodules seen in subcutaneous tissue, but a very conspicuous feature in some is a central accumulation of epithelial cells (Fig. 8). The impression still to be confirmed by further investigations

is that each follicle may have arisen as an accumulation of lymphocytes around an epithelial crypt and that, as it developed, the structure of the crypt was gradually lost. In most, however, there are undoubted epithelial cells even though they are dispersed as single cells among a great excess of lymphocytes. The striking similarity between the appendix of the rabbit and the bursa of Fabricius in birds has been discussed by Archer, Sutherland and Good¹³. In comparison, the appendix of the echidna shows the same similarity in so far as there is a clear separation of the follicles into cortical and medullary zones. There is no reason, however, to suggest any homology between the echidna appendix and the bursa of Fabricius in birds. The anatomical situation is quite different, and palaeontological opinion is that birds were derived from the same archaic group, the Diadectomorpha, as modern reptiles and are far removed from the line of monotreme evolution.

We thank Sir Macfarlane Burnet for his advice, and Misses Carol Austin, Vera Hillel and Jenny Watson for their help.

¹ Good, R. A., and Papermaster, B. W., *Adv. Immunol.*, **4**, 1 (1964).

² v. Braunmühl, A., *Z. Mikr. Anat. Forsch.*, **4**, 635 (1925).

³ Kent, S. P., Evans, E. E., Attleberger, M. H., *Proc. Soc. Exp. Biol. and Med.*, **116**, 2, 456 (1964).

⁴ Diener, E., and Nossal, G. J. V. (in preparation).

⁵ Goodrich, E. S., *Proc. Roy. Soc.*, **89**, 261 (1916).

⁶ Romer, A. S., *Vert. Paleontology*, **3** (Chicago Univ. Press, 1945).

⁷ Vaughn, P. P., *Amer. Midl. Nat.*, **67** (1), 79 (1962).

⁸ Nossal, G. J. V., Ada, G. L., and Austin, C. M., *Austral. J. Exp. Biol. and Med. Sci.*, **42**, 311 (1964).

⁹ Nossal, G. J. V., Ada, G. L., and Austin, C. M., *Austral. J. Exp. Biol. and Med. Sci.*, **42**, 283 (1964).

¹⁰ Farr, R. S., *J. Infect. Dis.*, **163**, 239 (1958).

¹¹ Miller, J. J., III, and Nossal, G. J. V., *J. Exp. Med.*, **120**, 1075 (1964).

¹² Ada, G. L., Nossal, G. J. V., and Austin, C. M., *Austral. J. Exp. Biol. and Med. Sci.*, **42**, 331 (1964).

¹³ Archer, O. K., Sutherland, D. E. R., and Good, R. A., *Nature*, **200**, 337 (1963).

PHOSPHORUS EXCRETION OF CATTLE FED ON HIGH-ENERGY DIETS

By W. D. C. REED and DR. R. C. ELLIOTT

Henderson Research Station, Mazoe, Rhodesia

AND

DR. J. H. TOPPS*

Department of Agriculture, University College of Rhodesia and Nyasaland, Salisbury, Rhodesia

HIGH-ENERGY diets composed mainly of cereal grains are now widely used in beef production in Britain¹. Such diets have been shown to be the type of ration utilized most efficiently by ruminants for fattening². Rhodesia is a maize-exporting country where yields are high; for example, 6,000 lb. of maize grain per acre are frequently gathered. In the past, some of this cereal has been fed to cattle in conventional diets, which consisted of grass hay, maize silage and a balanced concentrate containing maize grain. Recently, interest has been given to feeding large quantities of maize in a complete high-energy diet to growing and fattening beef cattle. In 1964, several thousand head of cattle were fattened for slaughter in this way.

An investigation of the metabolism of cattle, fed on diets principally composed of maize, showed phosphorus excretion to be distinctly abnormal, while symptoms typical of acidosis were apparent. A summary of the findings is given in this article.

A balance trial was carried out with four 1-year-old Africander steers, average live-weight 550 lb. They were given four concentrate diets based on maize meal with a protein-mineral-vitamin A supplement. A four-unit Latin square design was used. Each diet contained a different amount of roughage, namely 0, 10, 20 and 30 per cent respectively of the quantity of concentrate. The roughage was grass hay of low nutritive value. The diets

and their preparation will be described in full in another communication. Each steer received each diet for a four-week period, in the last week of which the nitrogen and mineral balance of the animal was determined. The results, which show the phosphorus balance of the steers, are given in Table 1.

These results show two striking features. The steers excreted a large amount of phosphorus in their urine whereas with conventional diets cattle excrete very little. Tillman *et al.*^{3,4} found that 1-year-old Hereford steers excreted only 2-4 per cent of their phosphorus intake in their urine. Although the diets contained liberal amounts of phosphorus (0.38-0.47 per cent phosphorus) compared with suggested requirements of 0.21-0.28 per cent phosphorus (National Research Council⁵), the young rapidly growing steers retained relatively small amounts. In contrast, 1-year-old dairy heifers given 3.3 g phosphorus per 100 lb. live-weight have been found to retain 1.0 g phosphorus per 100 lb. live-weight⁶, while young steers examined by Tillman *et al.*³ showed a phosphorus retention

Table 1. MEAN VALUES (G/24 H) FOR INTAKE, FAECAL EXCRETION, URINARY EXCRETION AND RETENTION OF PHOSPHORUS FOR FOUR STEERS GIVEN FOUR DIFFERENT DIETS

Diets	Intake	Excretion		Retention
		Faecal	Urinary	
All concentrate	15.2	6.3	7.2	1.7
Concentrate + 10% roughage	19.2	9.6	7.1	2.5
Concentrate + 20% roughage	19.6	11.9	5.0	2.7
Concentrate + 30% roughage*	18.2	11.3	3.9	3.0

* Mean values for three steers.

* Present address: Rowett Research Institute, Bucksburn, Aberdeen.

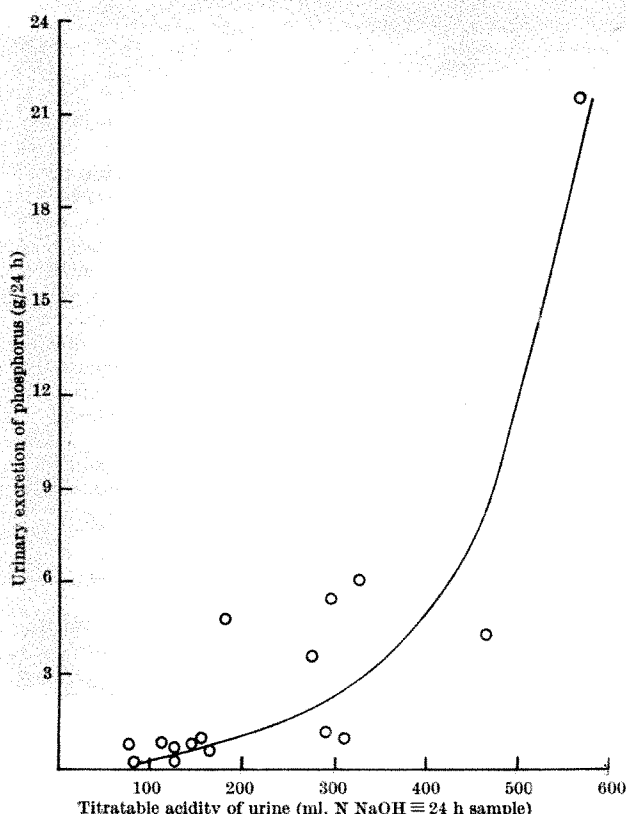


Fig. 1. Relationship between titratable acidity and phosphorus content of the urine of cattle given high-energy diets. $\log y = -0.74 + 0.0036x$

of 35 per cent of intake. High urinary excretion and low retention were most pronounced with the all-concentrated diet.

In a second trial, four mature Africander steers, average live-weight 1,450 lb. and fitted with rumen cannulae, were given four diets which were similar to those used in the balance experiment except that roughage was supplied by high-quality lucerne hay. Each steer received each diet for a 4-week period (Latin square design). During one day of the final week urine was collected and its titratable acidity, pH value and phosphorus content determined. Appreciable quantities of an amorphous white solid, which was found to be phosphate, followed the neutralization of many of the urine samples with sodium hydroxide in the determination of titratable acidity. A close relationship

($r = 0.804$, $P < 0.01$) was found between the titratable acidity and the phosphorus content of the excreted urine (Fig. 1). The linear regression obtained was $\log y = -0.74 + 0.0036x$ where y is the urinary excretion of phosphorus in g/24 h and x is the titratable acidity of the daily output of urine in ml. N NaOH.

Abnormally high amounts of acidic phosphate, $H_2PO_4^-$, are excreted in some forms of metabolic acidosis in humans. Through this loss, which is based on the reaction $HPO_4^{2-} + H^+ \rightarrow H_2PO_4^-$, the body excretes H^+ and conserves basic ions such as Na^+ and K^+ . The close relationship found between the acidity of the steers' urine and its phosphorus content indicates the presence of a similar effect in cattle fed on high-energy diets. It is relevant to note that Elam, Ham and Dyer⁷ found that the phosphorus content of urine was significantly increased when steers were given potassium acid phosphate.

The results of the two trials show that cattle given diets rich in maize excrete between 10 and 20 times the normal amounts of urinary phosphate, and that the titratable acidity of their urine is high. This is characteristic of metabolic acidosis. Previous workers^{1,8} have found that cattle fed cereal-rich diets suffer from a form of acidosis which is associated with depressed appetite and a stiff, inco-ordinated gait in the hind limbs. Attempts to alleviate this condition by the provision of basic sodium and potassium ions in the form of sodium and potassium acetates⁹ or sodium bicarbonate¹ have not been successful. The results of the work recorded here suggest that artificial increase in the phosphorus content of high-energy diets may counteract acidosis and maintain the food intake of the animal. Such practice may be needed also to supply rapidly growing young animals with sufficient phosphorus to meet metabolic requirements, since the retention of phosphorus supplied in high-energy diets appears to be relatively low.

This work was supported by the Rockefeller Foundation and we thank Dr. J. Oliver for advice.

¹ Preston, T. R., *Ann. Rep. Rowett Res. Inst.*, **19**, 54 (1963).

² Blaxter, K. L., *The Energy Metabolism of Ruminants* (Hutchinson, London, 1962).

³ Tillman, A. D., and Brethour, J. R., *J. Anim. Sci.*, **17**, 100 (1958).

⁴ Tillman, A. D., Brethour, J. R., and Hansard, S. L., *J. Anim. Sci.*, **18**, 249 (1959).

⁵ National Research Council, *Nutrient Requirements of Beef Cattle* (Washington, D.C., 1958).

⁶ Archibald, J. G., and Bennett, E., *J. Agric. Res.*, **51**, 83 (1935).

⁷ Elam, C. J., Ham, W. E., and Dyer, I. A., *J. Anim. Sci.*, **18**, 383 (1959).

⁸ Broberg, G., *Acute Overeating with Cereals in Ruminants* (Ab. Lovisa Nya Tryckeri, Lovisa, 1960).

⁹ Wise, M. B., Blumer, T. N., Matrone, G., and Barriek, E. R., *J. Anim. Sci.*, **20**, 561 (1961).

HYPERLIPAEMIA FOLLOWING VIRAL INFECTION IN THE CHICKEN EMBRYO: A NEW SYNDROME

By DR. S. E. GROSSBERG and PROF. W. M. O'LEARY

Department of Microbiology, Cornell University Medical College, New York, and Service de Physiologie Microbienne, Institut Pasteur, Paris

DURING investigations of viral interference with Japanese encephalitis (JE) virus in the chicken embryo, it was noted that plasmas from JE virus-infected embryos were turbid. This cloudiness rapidly progressed in relation to infection so that, by the third day after allantoic inoculation of virus, plasmas appeared creamy (Fig. 1). This opalescence occurred within two days after infection whether embryos were inoculated into the allantois at 12, 13, 14 or 15 days of embryonic life. A search of the literature has not revealed any report of such a phenomenon, even by workers studying growth of JE virus in the chicken embryo¹⁻³. In such investigations, however, either strains of virus were used which were rapidly lethal for the embryo¹, or embryos were utilized at an early stage of their development^{2,3} when they were unable to resist the virulent effects of JE virus⁴.

The JE virus strain M5/596 used in these investigations produced an inapparent infection in chicken embryos 12 days or older^{4,5}. This strain of virus was isolated from mosquitoes naturally infected in Japan⁶ and had been through 9-10 passages in suckling mouse brain. Stock virus was prepared as a 10 per cent suspension of suckling mouse brain in Hanks's salt solution containing 0.1 per cent bovine albumin, penicillin, 100 units/ml., and streptomycin, 100 µg/ml., and stored in sealed glass ampoules at -60° C. Virus was diluted in Hanks's solution with 0.1 per cent bovine albumin, penicillin, and streptomycin. Diluted virus (0.1 ml. containing 10^6 or 10^7 LD₅₀) was inoculated into the allantois of White Leghorn chicken embryos previously incubated at 38° C; infected embryos were subsequently incubated at 35° C. Embryos were bled into plastic syringes wetted with either heparin or 0.2-M

Table 1. GROWTH OF JAPANESE ENCEPHALITIS (JE) VIRUS IN THE CHICKEN EMBRYO AFTER INTRA-ALLANTOIC INOCULATION*

Embryonic material	Titre of JE virus after inoculation†		
	6 h	24 h	48 h
Allantoic fluid	2.5	4.1	5.1
Plasma	1.9	3.7	4.8
Brain‡	—	2.8	5.4
Liver‡	—	2.9	5.6

* $6.3 \log_{10} LD_{50}$ in inoculum.† $\log_{10} LD_{50}/\text{ml.}$ calculated from the mortality of mice following intra-cranial inoculation (0.03 ml.) of decimal dilutions (5 mice/dilution) of pools of material taken from 4 embryos at each time interval.‡ Supernatant of a 20 per cent suspension (centrifuged at 800 *g*) made in 0.1 per cent bovine albumin in Hanks's salt solution with penicillin and streptomycin.

versene from an allantoic vein through a window cut in the shell. Blood from four to nine embryos was usually pooled at a given bleeding, and the plasmas stored at -60°C .

JE virions inoculated into the allantois, an extra-embryonic organ, multiplied rapidly, then gained access to the embryonic circulation, and subsequently grew in intra-embryonic organs, for example, brain and liver (Table 1). Afterwards interferon was found in organs in the embryo and was thought to account for survival of the embryo until the time of hatching². Infected embryos delayed their hatching an additional 1–2 days beyond the usual 21–22 days, under the temperature and humidity conditions used. Infected chicks could peck a hole in the shell and appeared to breathe and cheep normally. Only a rare chick was able to peck its way completely out of the shell; for those which could not, removal of the shell allowed them to hatch. However, all chicks were unable to stand and were considerably smaller than uninfected chicks. Although no gross deformities or organic abnormalities were evident, the chicks usually died within another 48 h.

The remarkable opalescence of the plasma of JE virus-infected embryos was reminiscent of the fat-laden serum of diabetic patients in acidosis. To prove that turbidity was due to the lipid material, a series of experiments involving gravimetric analysis, thin-layer chromatography, and gas chromatography were instituted. Before these could be performed with safety, it was necessary to free the plasmas of infectious JE virus. Since JE virus contains lipids⁷, its infectivity should be readily inactivated by treatment with diethyl ether⁸. However, after treatment of infected plasma with as much as 5 volumes of ether at 4°C for 2 h, $1.5 \log_{10} LD_{50}$ of JE virions remained of the original $5.5 \log_{10} LD_{50}$. The plasmas, therefore, also had to be heated at 60°C for 60 min to free them of detectably infectious virus. Heating was afterwards incorporated into the first step of the extraction procedure.

Extraction and gravimetry were done by a modification of the method of Entenman⁹ using ethanol-ether extraction at 60°C followed by filtration, evaporation to dryness under nitrogen, and re-extraction of the residue. The results of gravimetric analysis of a representative experiment are shown in Fig. 2 where very marked increase in

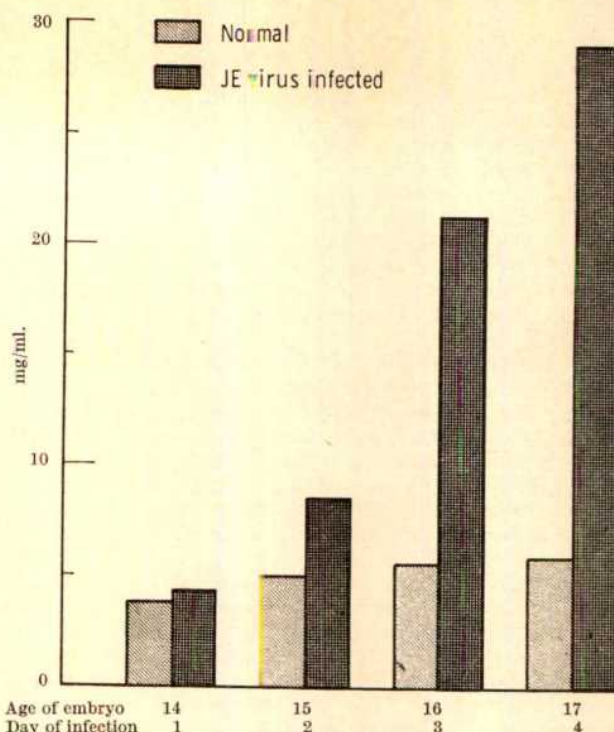


Fig. 2. Results of gravimetric analysis of total lipids in the plasmas of chicken embryos infected with Japanese encephalitis (JE) virus and in plasmas taken simultaneously from uninfected control embryos.

the amount of total plasma lipids was noted in JE virus-infected chicken embryo plasmas. A minimal increase in total plasma lipids of uninfected embryos was noted over a longer time period.

Thin-layer chromatography of total plasma lipids was performed on silica gel by standard methods¹⁰ utilizing petroleum ether/ethyl ether/acetic acid (90/10/1) or chloroform/methanol/water (65/25/4) as developing solvents. Spots were detected after development of the plates by two methods in sequence: (1) spraying with 2,6-dichloro-fluorescein for examination under ultra-violet light; (2) spraying with concentrated sulphuric acid and heating at 130°C for 30 min to char areas containing carbon. Plates were spotted with 300- μg samples dissolved in 100 μl ether. In normal plasmas there was a progressive increase of cholesterol esters with time and a decrease in triglycerides, free fatty acids, cholesterol, and phospholipids, in agreement with published data¹¹. In JE virus-infected embryos, however, there was a drop in cholesterol and cholesterol esters 3–4 days after infection, while there was an increase in triglycerides and phospholipids.

For gas chromatographic analyses, plasma lipids were hydrolysed, freed of non-saponifiable matter, and methylated with the use of boron trifluoride, as described in detail elsewhere¹². The ester mixtures were then analysed by gas chromatography using a Perkin-Elmer model 154-D fractometer fitted with a 2-metre 0.25-in. column with diethylene glycol succinate as a stationary phase. The analysis of the fatty acids recovered from a 16–18-day pool of normal embryonic plasmas showed the presence of tetradecanoic, hexadecanoic, hexadecenoic, octadecanoic, octadecenoic and octadec-dienoic acids in proportions that are in accord with published data¹¹. Analyses of fatty acid mixtures from the plasmas of JE virus-infected embryos of similar age revealed increases in the amounts of hexadecenoic and

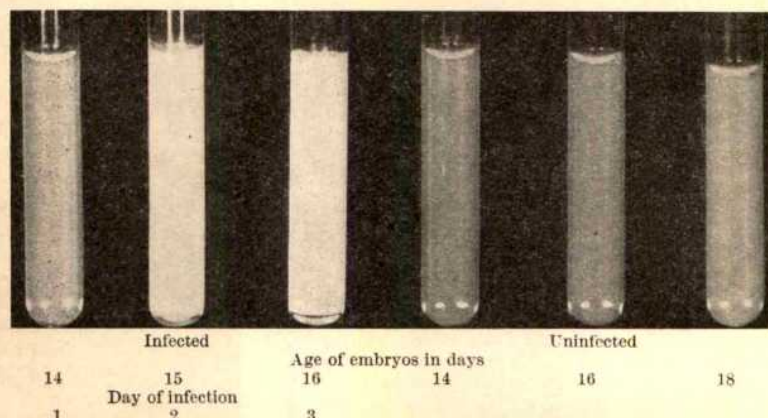


Fig. 1. Progressive appearance of lactescence in the plasmas of chicken embryos infected with Japanese encephalitis virus in contrast with those of uninfected control embryos.

octadec-dienoic acids. No unusual fatty acids were detected.

Porterfield and Rowe¹³ have demonstrated that certain serum fractions which contain lipid, including β -lipoproteins, inhibit the haemagglutination of JE virus, among other group B arboviruses. It was of interest to determine whether the increased lipids found could be associated with plasma protein components. Standard electrophoresis¹⁴ on normal and infected plasmas was done in agar gel using 0.05 M veronal buffer at pH 8.2; proteins were stained with azocarmine, and fats with sudan black. A striking increase in β -lipoproteins and the fat associated with them was noted by day 3 in JE virus-infected plasmas.

Whether this response of the chicken embryo to JE viral infection is a specific one is of interest. Preliminary investigations indicate that influenza virus and *Escherichia coli* inoculated into the allantois produce some lipaemia not comparable to that seen with JE virus, whereas *Staphylococcus* and Rous sarcoma virus do not.

The mechanism of the remarkable hyperlipaemia following Japanese encephalitis virus infection initiated in the allantoic sac is not clear.

It is possible that this neurotropic virus destroys fat-rich neural tissue with subsequent mobilization into the circulation. Histopathological study of the major tissues of the embryo did not show any inflammatory or destructive changes in neural tissue, but a hepatitis became evident by days 3 and 4. This was characterized by marked variation in fat distribution, diminished appearance of total fat, greatly increased haematopoiesis, and a few haemorrhagic lesions. No other organ was noticeably involved at this time. This hepatitis, heretofore unreported following JE virus infection in the chicken embryo, became evident long after the lipaemia was already pronounced, but the pathology seen undoubtedly reflects earlier sub-microscopic lesions.

The chicken embryo might be said to be on a relatively high fat diet, mobilizing lipids from the yolk for oxidative catabolism or lipid anabolism. Since many of the lipids of the embryo are produced as well as withdrawn from the circulation by the liver and partly by the yolk sac membrane^{11,15}, it is very possible that dynamic imbalances between the two processes may result in hyperlipaemia. Another possibility is that embryonic cells in liver and elsewhere are only sufficiently damaged by viral infection to initiate an increase in intracellular lipid stores, as has been demonstrated in cell culture¹⁶, with subsequent release of lipids into the circulation. The most attractive hypothesis, which does not exclude the above possibilities, is that a specific host enzyme or enzyme system, necessary for lipid metabolism, is suppressed by JE viral infection. The depression of host protein synthesis and enzymatic activity, which occurs early in viral infection, is

an apparently general cellular phenomenon¹⁷. Inhibition of lipoprotein lipase might account for this phenomenon; the turbidity of plasma of infected embryos bled after intravenous administration of heparin did not clear, whereas normal embryo plasma did show lipase activity. A viral-induced lipoprotein lipase deficiency would focus the defect in endothelial cells in which JE virus has been shown to multiply¹⁸.

It is tempting to speculate that such a lipaemia plays a role in host resistance. Certain lipid-containing, and therefore lipophilic viruses, bind to cholesterol, palmitic acid or hexadecylamine with varying affinities^{19,20}. Lipid inhibitors in serum might act by preferential binding of virions and thereby limit adsorption of virus to the lipoprotein receptors for virus which are normally present in cells.

Since the precise mechanism(s) involved remains obscure, the association of hyperlipaemia, hepatitis, and JE viral infection merits further study.

We thank Miss Janet Louis and Miss Claude Guyot-Jeannin for technical assistance, Dr. Robert Skarnes for aid with the electrophoresis of plasmas and Dr. J. C. Guillon for review of the histopathological sections. One of us (S. E. G.) thanks Dr. André Lwoff for his hospitality and for stimulating discussions.

This work was supported in part by grants from the New York Health Research Council (U-1432), the American Cancer Society (IN-73C), and the U.S. Public Health Service (AI-03327-D). One of us (S. E. G.) is Markle Scholar in Medical Sciences, on leave at the Pasteur Institute 1964-65 from Cornell University, and has received an award under the U.S. Public Health Service research career award programme (AI-K3-9915).

¹ Mitamura, T., and Kobayashi, E., *Tokai Ijishinshi*, **62**, 778 (1938).

² Koprowski, H., and Cox, H. R., *J. Immunol.*, **52**, 171 (1946).

³ Warren, J., and Hough, R. G., *Proc. Soc. Exp. Biol. and Med.*, **61**, 109 (1946).

⁴ Grossberg, S. E. (in preparation).

⁵ Grossberg, S. E., *Bact. Proc.*, 116 (1964).

⁶ Scherer, W. F., Moyer, J. T., and Izumi, T., *J. Immunol.*, **83**, 626 (1959).

⁷ Franklin, R. M., in *Prog. Med. Virol.*, **4**, 1 (Karger, New York, 1962).

⁸ Sunaga, H., Taylor, R. M., and Henderson, J. R., *Amer. J. Trop. Med.*, **9**, 419 (1960).

⁹ Entenman, C., in *Methods in Enzymology*, **3**, 299 (Academic Press, New York, 1957).

¹⁰ Randerath, E., *Thin Layer Chromatography* (Academic Press, New York, 1963).

¹¹ Schjeide, O. A., in *Progress in the Chemistry of Fats and other Lipids*, **6**, 251 (Pergamon Press, Oxford, 1963).

¹² O'Leary, W. M., *J. Bact.*, **84**, 967 (1962).

¹³ Porterfield, J. S., and Rowe, C. E., *Virology*, **11**, 765 (1960).

¹⁴ Grabar, P., and Burtin, P., *Immunoelectrophoretic Analysis* (Elsevier Publishing Co., Amsterdam, 1964).

¹⁵ Noble, R. C., and Moore, J. H., *Canad. J. Biochem.*, **42**, 1719 (1964).

¹⁶ Orsi, E. V., Love, R., and Koprowski, H., *Cancer Res.*, **17**, 306 (1957).

¹⁷ Joklik, W. K., in *Progress in Medical Virology* (in the press).

¹⁸ Grossberg, S. E., and Shope, jun., R. E. (in preparation).

¹⁹ Youngner, J. S., and Noll, H., *Virology*, **6**, 157 (1958).

²⁰ Noll, H., and Youngner, J. S., *Virology*, **8**, 319 (1959).

CHROMOSOME MARKER STUDIES ON THE DEVELOPMENT OF THE HAEMOPOIETIC SYSTEM IN THE CHICK EMBRYO

By M. A. S. MOORE and Dr. J. J. T. OWEN

Physical Anthropology Laboratory, Department of Human Anatomy, University of Oxford

A NUMBER of investigations have shown that placental interchange of blood in multiple mammalian pregnancies results in blood cell chimaerism in the adult animal¹⁻⁵. In addition, there is evidence that movement of erythrocyte precursors occurs during avian embryogenesis. Thus erythrocyte mosaicism has been demonstrated in chicks derived from double-yolked eggs⁶ and from eggs parabiosed during incubation⁷. These investigations suggest not only that there is movement of blood-forming cells between embryos sharing a common circulation, but also that such cells may become established and persist

into adult life. However, a direct study of the population of embryonic haemopoietic organs by blood-borne cells has not been made.

For a number of reasons the chick embryo provides an ideal subject for such a study. First, male (ZZ) and female (ZW) avian cells can be readily distinguished from each other in good chromosome preparations as the Z chromosome is the only large mediocentric element present. Secondly, avian embryos can be parabiosed at

(Continued on page 989)

Supplement to NATURE

No. 5014

DECEMBER 4, 1965

Vol. 208

TOWARDS A PUBLIC UNDERSTANDING OF SCIENCE

The Architecture of Molecules

By Prof. Linus Pauling and Roger Hayward. Pp. x+117 57 full-page drawings). (San Francisco and London: W. H. Freeman and Company, 1964.) 70s.; 10 dollars.

WE are now living in an atomic age. In order to understand the world, every person needs to have some knowledge of atoms and molecules."

This is the beginning paragraph of a fascinating work of art: *The Architecture of Molecules*, by Linus Pauling and Roger Hayward. The question as to how some understanding of science, however superficial, can be brought to the man and woman in the street has exercised many organizations as well as individuals. At a practical level, of course, it is unnecessary to know anything about electric currents in order to turn a switch and bring on the light. Babies love to do it before they are one year old. But for all too many people science is still magic even when they are twenty-one. They eat their food and hope that it will be digested. They give their car petrol, oil and water and expect that it will go. They put their vote into a ballot-box and leave someone else to make all political decisions for them. Furthermore, so long as there is a doctor to write out a prescription, a garage man to look at the motor when it stalls, a strike-leader to tell them when to stop work or a horoscope to tell them when to expect a change for the better, why worry?

Well, Linus Pauling worries. He thinks, quite rightly, that young people ought to want to know why the 'lead' of a pencil comes off on to the paper, what an atom of hydrogen or uranium 'looks like', how many different kinds of atoms there are and why a scientist refers to silver as Ag, why so many different models are used to represent molecules and what holds atoms together in molecules. He goes on to discuss the valencies, sizes and shapes of molecules, from H_2 to the antiviral aureomycin, $C_{22}H_{23}O_3N_2Cl$, and the even larger myoglobin and haemoglobin. He explains in terms of the strain of bent bonds and the eclipsed orientation of the hydrogen atoms why cyclopropane is a better fuel for rocket propulsion than cyclopentane or cyclohexane. He also shows how molecular shape may be responsible for disease and abnormality in human beings. Roger Hayward's drawings are superb. Every crystallographer will want to possess this book and so gloat over them. Undoubtedly many an arts sixth-former will pick the book off the school library shelf and will learn a great deal by browsing through it. So indeed will those science students who are bound to read anything that Linus Pauling writes. (Incidentally, it is a pity that in the definition of liganey, in section 29, the word is misprinted 'lignancy'.)

It will be interesting to see whether the book will appeal most to those who are already scientists or to the man-in-the-street for whom it seems to be intended. To an experienced reader this book tells a great deal about the interests of Linus Pauling himself. It is just a little difficult, otherwise, to explain the absence of benzene and the presence of $Pt_4(CH_3)_{16}$, or of the catenane ($C_{34}H_{66}O_2 + C_{34}H_{68}$). The linking of molecular structures and of crystals with the Platonic solids, and hence with the ancient symbols for earth, air, fire, water and the ether, is historically interesting, but one feels uneasily

that it could also be misleading. Nevertheless, it is difficult to be critical for long of a book with such unusual personality about it. It is too fascinating.

KATHLEEN LONSDALE

AFRICAN STUDENTS IN BRITAIN

New Commonwealth Students in Britain

With Special Reference to Students from East Africa. (A P.E.P. Publication.) Pp. 253. (London: George Allen and Unwin, Ltd., 1965.) 35s. net.

IN October 1964 there were 64,169 overseas students in the United Kingdom, of which 42,323 came from Commonwealth countries—the East African contingent numbering approximately 4,000. The figures for 1965 are not yet available but they are likely to show an increase. Moreover, there are probably as many again in other countries in Western Europe, the United States, India and in Communist countries. The magnitude of the problem does not seem to be appreciated by the general public, although it is a source of continuing anxiety to the authorities and organizations which have to deal with the students.

Political and Economic Planning has sponsored a study by a research team into the problems of East African students in Britain, financed by a generous grant from the Ford Foundation. The report which covers the thoroughgoing investigation is a valuable contribution to our understanding of the problem. It is not possible in a short review to cover the whole report and we must confine ourselves to a few salient points.

First we may enquire why there is an influx of East African students on this scale. The most obvious reason is that despite the considerable and costly expansion of post-secondary educational establishments in East Africa, it is quite insufficient to meet the demand. The newly independent countries are in a hurry to 'Africanize' and are doing everything possible to step up the output of secondary schools. In Kenya, for example, the Government plans to provide an additional 21,000 secondary school places by 1970, and Tanzania and Uganda are doing likewise. It seems most improbable that the University of East Africa and technical training establishments will expand sufficiently rapidly to meet the greatly increased demand for places. The inevitable answer is to seek places in educational establishments overseas. That this is recognized by the local governments is demonstrated by the fact that in Kenya the Ministry of Education's vote for post-secondary scholarships has been increased by £480,000 per annum. Another reason is that a number of students consider that an overseas qualification has a prestige value greater than one gained in their own country, although some suffer disillusionment when they return home and find that their degrees or diplomas are not of the standard required by their own Governments. This is not, however, the case with most qualifications gained in the United Kingdom, the standards of which are acceptable. Even so, in order to gain a professional qualification students sometimes have to learn subjects which are useless to them. For example, students training in England as accountants have to study the British income tax laws. But such anomalies do not deter those who are often more concerned with gaining an acceptable qualification than with what they have to learn.

The chapters of the report which are of particular interest and importance are those dealing with the life in Britain of new Commonwealth students. The report makes it clear that despite what students are told they find themselves in many important respects treated as foreigners and lead a life very different to that of British students. The material conditions—finance, climate, health, food and accommodation—are very different from those to which they are accustomed at home. This emphasizes the importance of adequate preparation before they leave their own countries. Of particular importance is the choice of a suitable course of study. In the past most students who come to the United Kingdom with Government scholarships have been free, on their return home, to choose such employments as they fancy. But now the tendency is for Government scholarships to be 'bonded', which means that successful students will be required to enter a career chosen for them by the Government of their country, at least for a number of years. There are many who disapprove of this interference with the individual freedom, but the Kenya Government thinks otherwise and this adds emphasis to the great care needed to ensure that a student studies a subject for which he has aptitude.

Government-sponsored students are usually provided with sufficient funds to live without being unduly troubled with financial worries and in any case they have their High Commissioners to turn to when in difficulties. On the other hand, private students are left to their own devices. In many cases they are financed by their parents or relations, who make great sacrifices on their behalf. The cost of study abroad is often underestimated and private students frequently find themselves genuinely short of money and have to earn in their spare time, which inevitably reduces their capacity for study. Except when looking for accommodation or work, coloured students do not complain unduly of racial discrimination, although it exists more than it should in places of entertainment, restaurants and public houses. The impressions students take home of attitudes of people in the United Kingdom are bound to affect their outlook and there is much scope for improvement in the relationship of the people of the United Kingdom with the overseas students in their midst.

Finally there is a formidable list of recommendations as to what the British Government, the Overseas Governments and the educational and other institutions in the United Kingdom should do to improve matters. The list is too long to comment on here, but all who have anything to do with overseas students should read this report and study the recommendations with the view of assisting in the alleviation of the lot of these men and women who are destined to play a vital part in the future of their own countries.

TWINING

VIEWS OF NINETEENTH-CENTURY SCIENCE

Science in the Nineteenth Century

Edited and with a Preface by René Taton. (A General History of the Sciences.) Pp. xix+623+69 plates. (London: Thames and Hudson, Ltd., 1965.) 126s.

OF no period more than the nineteenth century is the lack of a synoptic treatment of the history of science more marked; for that reason alone the appearance of the volume under review, purporting to be a translation of Volume 3 of *Histoire Générale des Sciences*, aroused in my mind keen expectations of interest and profit: it must be confessed that these expectations were realized only in a very limited degree.

In the "Préface générale" to the first volume of the original edition René Taton stated that the aim of the series

was to provide "un tableau objectif et suffisamment précis de l'évolution d'ensemble de l'histoire générale des sciences et des techniques considéré comme l'un des aspects essentiels de l'histoire des civilisations". In the volume under review the reader is prepared for the survey of "one of the most essential aspects of the history of civilizations" by means of three pages on "The Spirit of the Nineteenth Century". The result may well be imagined but when we are told, *inter alia*, that "until 1815 human geniuses were few and far between . . . in the nineteenth century, however, geniuses were both numerous and identifiable" it is difficult to avoid a feeling of chill as to what lies ahead of us. These fears are unfortunately soon realized. There are, for example, in the subsequent chapters far too many 'births', 're-births', 'true births' etc.—the 'real birth' of projective geometry was with Poncelet (p. 20), but only with Chasles (p. 21) was "the projective doctrine truly born"; "geometrical optics was born in the 17th century" (what then had Ptolemy and ibn al-Haitham been doing?); "the science of electricity began at the end of the 18th century" (p. 100), but (p. 178) "by the end of the 18th century all the basic principles of electrostatics and magnetostatics were known". Some of these parturitions seem to have involved irregular intra-uterine interference; for although spectrum analysis was 'born' only in 1859 (pp. 146-7) it was "given a tremendous boost" in 1814 (p. 146). In fairness to the authors of these and many similar doubtful statements the possibility could not be ruled out that some change of emphasis had occurred in translation; so having read the whole of the English version I turned to the French original with which I had had no previous acquaintance. By chance my first comparison was in respect of the statement (p. 250) that "it follows from the preceding discussion [Carnot cycle] that the work done in any reversible process must be zero". In the French original (p. 279) there is no reference to 'work done'; the discussion has merely to do with the emergence of the concept of entropy.

The discovery of such apparently gratuitous alteration of the French original (resulting in this case in a statement that is literally false) made it necessary to compare more than a score of similar cases. The results can only be described as disconcerting. A few examples must suffice: a footnote omitted (p. 50); the discussion of what the translator calls Poincaré's "commodism" ('conventionalism'?) follows (p. 107) lines quite different from those of the French (p. 118); "courant électrique" (p. 130) becomes "electromagnetic [-motive?] force" (p. 118) "théorie électromagnétique . . . de la lumière" (p. 162) becomes "discovery of the electromagnetic effect" (p. 145); a paragraph is omitted (p. 157); "à ce sujet" (p. 194) becomes "his explanation" (p. 171); the exposition of Raoult's work (p. 288) is largely rewritten (p. 258); "conception métaphysique" (p. 303) becomes "metaphysical fog" (p. 272). On p. 477 the translator, perhaps with the British market in mind, intercalates more than a page on the Huxley-Wilberforce incident to which there is no specific reference in the original. Finally, disconcerted by what seemed to me an oversimplified and inadequate supported version (allegedly by Taton, from whom one was surprised to find such a statement) of the organization of science in Great Britain, I was shocked to discover the complete omission of a scrupulously worded warning by Taton that, because of the paucity of detailed studies (Dr. Cardwell's excellent work finds no mention in his bibliography), the account presented must be accepted with great caution. Nor was this all: Taton's account had been so re-phrased as to convey the impression that the Cavendish Laboratory had been set up by the British Government instead of, in Taton's words, "grâce à des fonds privés"—incidentally of a member of the despised Establishment (*sic* referring to 1872!).

The translation of a work of this kind calls for very wide knowledge; and in providing a smooth and readable

ersion A. J. Pomerans has risen to the challenge. But hope enough has been said (and much more could be) to justify the complaint that the publication of this book 'as a translation', without any indication in the bibliographical note on the verso of the title-page that it is far from being so, constitutes a grave injustice to the original authors and does a signal disservice to British scholarship.

In the space at my disposal I can do no more than present a few personal impressions—with the caveat that they are based on the book which I have been called on to review and not on what the authors may or may not have written.

Apart from the brief but useful sketches of "Science and Society in Russia" (A. P. Yushkevitch and the late V. P. Zubov), "United States" (I. B. Cohen), "The Muslim World 1450–1900" (B. Ben Yahia), all the contributors are French; yet there is a marked freedom from national bias: M. Jacques Levy's evaluation of the Le-Verrier-Adams case is a model of human and technical insight. From the section (77 pages) on mathematics (Taton, Itard and Darrois), highly technical as at many points it inevitably becomes, a reader with no more than the very meagre mathematical understanding that I myself possess should be able to gain a valuable idea of the progressive 'arithmetization' and increased abstraction whereby mathematics acquired more and more relevance to natural science. M. Bauer's remarkable survey of the whole of "Electricity and Magnetism" from Galvani to Lorentz is nicely balanced between the 'continental' view and Faraday-Maxwell innovation (see p. 216). The "Rebirth" [original *Essor*] of Chemistry" by Daumas and Jacques is about as good as could be expected in 34 pages (out of 153 for "Physical Science", excluding mechanics and astronomy!), but Wilhelm Ostwald, for example, gets only a passing nod. The splitting of science into the traditional 'subjects' has the serious consequence that the long struggle to perfect the idea of 'atomicity' as such falls between 'chemistry' and 'molecular kinetics'; hence (together with the complete omission of Herapath and J. J. Waterston) the misleading statement on p. 265. The part played by "Geological Sciences" is attractively sketched by MM. Orcel and Furon. But the appalling problem of presenting a 'unified' but not over-simplified view of nineteenth-century biology has not yet been solved; I was struck by the fact that only where judicious citations of the original works were provided (as by M. Piveteau in "Comparative Vertebrate Anatomy" and "Vertebrate Palaeontology") did the history really come alive. The presentation of a mass of 'systematic' information (though well done by Mlle. Tétray) seems out of place in a work of this kind; similar remarks apply to almost the whole chapter on "Medical Sciences" (M. Astruc).

Among many old favourites there are some excellent lesser-known illustrations. The "Bibliography" should be very useful, as should the indexes of names and subjects; in the latter, however, the only possible reference to "Alternation of Generations in Plants" (pp. 467–8) is "Alteration", and this turns out to refer only to animals. One regrets the omission of 'life-spans' in the name index, and throughout the book there is a paucity of cross-references and, in many chapters, of dates.

W. P. D. WIGHTMAN

RACES, GOALS AND ALL THAT

Heredity and the Nature of Man

By Dr. Theodosius Dobzhansky. Pp. x+179. (London: George Allen and Unwin, Ltd., 1965.) 25s. net.

DOBZHANSKY's new book is based on a series of "Holiday Science Lectures" given under the auspices of the American Association for the Advancement of

Science, in the spring of 1963 and 1964. The purpose of these Lectures is "to broaden the scientific horizons of the audience, and to communicate to them some of the excitement and inspiration of the scientific endeavour". What one may expect, therefore, and what one gets in full measure from Dobzhansky, is popular science writing at its best. It would be improper to look for any radically new additions to the general theory of evolution, on which Dobzhansky is one of the world's foremost authorities. Nor do we find any quite novel suggestions as to how our understanding of evolutionary processes should be applied to human affairs.

The first half of *Heredity and the Nature of Man* is mainly devoted to giving a summary sketch of basic facts. Even in this Dobzhansky reveals his judicious middle position, by devoting much more space than is usual in elementary texts to the contributions which the environment, as well as the genes, make to the character of the final adult organism. He shows in simple and convincing terms that it is nonsense to attribute overwhelming importance either to heredity as some eugenicists have done, or to suppose that any human individual is infinitely malleable by suitable training, as some anthropologists have tended to argue. It is, however, in the second part of the book, when he applies the basic principles of genetics to such questions as human equality and race, that he best demonstrates his skill at steering between Scylla and Charybdis. The danger of a middle position is of wriggling out of a difficult situation by an exercise in 'double-think'. Occasionally one may get the impression that something of the kind is going to happen. For example, in discussing the relevance of the undoubted genetic differences between individuals to the concept of human equality, Dobzhansky writes, "The partisans of equality . . . simply failed to understand that to be equal is not the same thing as to be alike". However, he goes on to state explicitly what this implies, and demonstrates that it is by no means merely a semantic quibble: equality is an ethical and sociological, not a biological, ideal, and its practical effect is to give to each man an equal dignity and an equal opportunity to develop just because he is a unique (that is, 'unequal') individual. Dobzhansky is not afraid to point out that equality of opportunity does not mean mere uniformity. The opportunity must be judged in relation to the potentialities to which it is offered; the schooling provided for the average of the population may represent a most unfair deprivation for individuals who happen to be particularly talented in mathematics, music or various other special fields.

He is equally sensible about the other major areas in which genetics has impinged on public affairs. For example, he deals very justly with the evolutionary dangers of the extra mutations produced by ionizing radiation, which seemed so urgent when we first had to consider the effects of bomb fall-out. Discussing race, he castigates both "the racists who believe that culture is a matter of race . . . of the genes of the carriers of that culture", and the belief that, "as far as cultures and their changes are concerned, all people are genetically so completely identical that their genes may just as well be left out of consideration". The truth is somewhere in between the two and, although we do not know exactly where, it must be much nearer the second than the first. He makes the essential point that "human genetically secured educability enables most individuals of all races to be trained for most occupations". In his final chapter he is, I think, a trifle too conservative in his attitude to some of the more unconventional genetical procedures, such as the use of artificial insemination with selected gametes.

In all the great human questions that Dobzhansky discusses, genetics is only one aspect of a complex problem which also involves the human sciences, and politics and morals in addition. For example, the most important question about race at present seems to be the speed at

which the change of cultural level can be brought about by social means. Cultures certainly have a property analogous to inertia, and the problem now is not whether there is any genetical reason why you could not train an adequate number of Congolese or Indonesians to be efficient modern administrators and managers—Dobzhansky argues convincingly enough that there is no such reason—but how to do it as fast as possible and with the minimum damage to other important values such as 'equality'. Problems of this kind fall outside the scope of the book, but if the reader realizes that he must not expect to find the final answers to all problems, he can be assured that Dobzhansky provides, in a form suitable for the general reader, including scientists from other fields, a clear, concise and judicious summing-up of the main conclusions that genetics has come to about human nature and human affairs.

C. H. WADDINGTON

FROM FORSTER TO FISHER

Education and the Labour Movement 1870–1920

By Brian Simon. (Studies in the History of Education.) Pp. 387+29 plates. (London: Lawrence and Wishart, 1965.) 50s. net.

THE light industry of scholarship often obscures the fact that history is really concealed prophecy. Mandates to explain how things came about imply opinions as to where they are going. Those who accept them cannot avoid seeing their subjects in terms of the future. At the present our educational future is very much in the mind. Are we, the post-Robbinsians, structuring the 'open conspiracy' of which H. G. Wells was such a fervent advocate? Will our future Samurai of scientists, engineers and teachers demand an equally radical approach to those who sell the labour of their hands? What are we doing to counter the growth of Orwellian 'proles'? Will they transform themselves out of existence as we have known them?

Such questions need a long cool look at the role of the Labour movement in our recent history, and everyone will agree that in Mr. Brian Simon it has found a historian of outstanding merit. All his previous work has contributed to this role. His *A Student's View of the University* (1943) is now a minor classic; his *Intelligence Testing and the Comprehensive School* (1943) a pioneer work; his *Common Secondary School* (1955) offered a classic paradigm of the comprehensive school; and his *Psychology in the Soviet Union* (1957) brought together expositors from fields too little known in Britain.

Education and the Labour Movement 1870–1920 is virtually a continuation of an earlier, and by now standard, work: *Studies in the History of Education 1780–1870* (1960). It charts the development of Labour's aspirations up to the end of the First World War. As with its predecessor, it splices telling illustrations into a crisp and sparkling text, spikes narrative with pointed documentary evidence, and laces controversial issues by mature judgments and shrewd comments. If there are omissions (and Francis Galton is one) these have been in the interests of a flowing story. His story embraces the rise of Socialism, the pressures of the 'protean' working-class groups, the institutional and legislative embodiments of their aspirations, assessments of their abreaction to existing institutions, and their influence on the development of the welfare services.

The 'New Feudalism' of the settlement movement, the 'consolidation of caste' in the public and grammar schools, the pejorative adjective 'elementary', the militancy of the churches and the spice of sect all glow balefully in these pages as Mr. Simon threads his way, like an experienced galactic traveller, through the misty ways of educational debate and intrigue. He plots a fascinating course, since his characters were working for the future. His story is,

if anything, an apt illustration of the truth that you can only understand a movement by pointing to what it was before it becomes that which it will presently cease to be. For though writing about the period from Forster to Fisher, he is giving us the necessary canvas of the period from Fisher to Crosland, a canvas which I sincerely hope he will find time to paint to complete what will undoubtedly be a formidable trilogy of volumes.

For Socialism, as William Morris observed, could only be won by educating people into wanting it and then organizing them into claiming it. But educating people into wanting it was not as easy as organizing them into claiming it. Consider only Sidney Webb, surely the most mobile competitor in (and later the improver of) the nineteenth-century examination stakes. "Discreetly following behind the imperialist banner" (to quote Mr. Simon), Webb helped anaesthetize the school boards themselves a nursery of working-class politicians. Webb was more a portent than an educational apparatchik. Many another was to come up in twentieth-century Britain through the ladder he so eagerly rung. Those who profited by education did not always remember the steps by which they had ascended.

H. G. Wells described the Education Act of 1870—the year in which Mr. Simon begins his history—as one "to educate the lower class for employment on lower class lines". But he also wrote in the year after Mr. Simon's story ends: "Science will endure and rule, but Labour with a capital 'L', as the name of a class of human beings organized for distinctive class ends, will pass away". Labour's efforts tend to their own extinction. As a former editor of *Nature* (to whom Wells wrote those words) replied, "If labour will but listen and act it may become the most potent moving force of the future, but if it concentrates attention entirely upon scrambling with capital for the pennies in Tom Tiddler's ground of industry, the wise men around will give to it merely a tolerant smile".

Mr. Simon's story of how the Labour movement responded to this appeal will be eagerly awaited if it is written with the same verve and charm as this one.

W. H. G. ARMYTAGE

KEPLER'S DREAM

Kepler's Dream

By J. Lear. With the full text and notes of *Somnium, sive Astronomia Lunaris* Joannis Kepleri, translated by P. F. Kirkwood. Pp. 182. (Berkeley and Los Angeles: University of California Press; London: Cambridge University Press, 1965.) 40s.

FIRST written in 1609, but published only in 1634 four years after his death, Kepler's *Somnium, sive Astronomia Lunaris* introduces his ideas on the geography and physics of the Moon with a half allegorical, half science-fiction tale involving a "Daemon" who describes his travels there; the device of a didactic trip to the Moon had previously been used by the Greek writer Lucian in the second century A.D. Based on Copernican principles and therefore theologically 'hot', the Latin manuscript of 1609 was designed for limited circulation among scientists who were expected to divine the meaning hidden beneath the allegory. Unfortunately, the effect was precisely the opposite of that intended, since it was wholly ignored by scientists but fell into the hands of malicious persons in the small Württemberg town of Leonberg, who very probably seized on a fanciful reference to the hero's mother's ability to conjure the "Daemon" and twisted it in order to accuse Kepler's own mother of witchcraft. A copy of the manuscript was also believed by Kepler to have found its way to England, and its possible influence on contemporary English literature has been explored by Prof. Marjorie Nicolson, but the evidence

that this happened is not conclusive. During the decade between his mother's eventual acquittal in 1621 and his own death, Kepler took time off from the preparation of his major works of this period to embellish the original text of the *Dream* with several times its own length of footnotes giving those literary and scientific explanations of the narrative that had been proved necessary by bitter experience.

Most of the scientific part of the *Dream* is a sober exploration of the astronomical phenomena observable from the Moon as a result of its orbital motion with one face permanently turned towards the Earth; this part would be entirely acceptable to-day apart from the fact that the Sun's distance is underestimated by a factor of six. However, Kepler also believed that the Moon had air, water and inhabitants who sheltered from the extremes of heat and cold in caves; the arguments on these subjects in the notes, and the precautions suggested for the journey to the Moon, throw an interesting sidelight on his impressive physical knowledge.

John Lear has performed a useful service in commissioning this excellent translation and in providing a fascinating introduction in which the importance of this forgotten work is pointed out and the baleful effects of the misinterpretation of the 1609 manuscript are reconstructed for the first time on the basis of the notes in the 1634 edition. My only criticism refers to one passage where Lear seems to have confused nautical miles with statute miles and consequently credits Kepler with a less accurate estimate of the Moon's distance than he actually possessed.

BERNARD PAGEL

MOON AND PLANETS

Lunar and Planetary Surface Conditions

By N. A. Weil. (Advances in Space Science and Technology, Supplement 2.) Pp. ix+222. (New York and London: Academic Press, 1965.) 80s.

THE majority of books devoted to so wide a subject as planetary physics are composed of specialist sections that an editor attempts (rarely with high success) to weld together. Perhaps, in such cases, the initial conditions are not sufficiently firmly stipulated to ensure uniformity of style; or the authors who are selected are not able to present a full cross-section of the field defined in the title, and hence there are glaring omissions from the presentation.

Possibly these drawbacks may be overcome if one man alone prepares the whole account. Yet, with the rapidly multiplying amount of lunar and planetary literature, can one author have a sufficiently wide knowledge of the many and varied subjects involved to be in a position to present an authoritative review of the astronomy, physics, geology, and biological aspects of the planets? In *Lunar and Planetary Surface Conditions* Dr. Weil has bravely attempted this task.

An introduction serves to review the present hypotheses on the origin of the Solar System and describes the methods of acquiring planetary data. Approximately one-third of the book pertains to lunar studies—principally the surface aspects of the Moon—and the remainder deals with the conditions on the terrestrial and Jovian planets.

There has been drastic selection of the 293 references from the fund of available literature, but they have been well chosen and, pleasingly, there has been emphasis on recent work. Omissions from Dr. Weil's arguments are extensive and frequently yield to a biased account. For example: "Three theories exist regarding the origin of the maria" (p. 44). According to the author, these are Gold's, Urey's and Baldwin's. In fact, there are others—that of von Bülow, for example—that receive no mention although they may be even more important. Again, both the optical measurements of the way different parts of the

Moon scatter light and the polarization measurements on flat and inclined parts of the Moon point to a remarkably uniform structure of the Moon's surface layer, yet Dr. Weil writes, "... for exposed lunar surfaces, a totally different terrain is expected to be encountered on the maria as compared to the highlands" (p. 92). The inclusion of some arguments at the expense of others may have been imposed by the necessity for brevity, but is this justified in a review costing 80s.? Because of faults of this nature the book—which has a good index—will be of limited use to research workers. But the present work may be described as an excellent skeleton on which to build a future, somewhat enlarged, edition. If a second edition were to include a wider and deeper understanding of many of the vitally interesting points touched on by Dr. Weil, it could undoubtedly become of great use as a reference work.

G. FIELDER

A SURVEY OF THE EARTH

A Planet Called Earth

By Prof. George Gamow. Pp. x+257. (London: Macmillan and Co., Ltd., 1965.) 35s. net.

MORE than twenty years ago the book *Biography of the Earth*, by George Gamow, achieved wide popularity as an introduction to earth science written for the layman. There have been many scientific advances since then, and the author has wisely re-written the book completely instead of trying to patch up the original. The general plan remains the same; like its predecessor, *A Planet Called Earth* is informative and well written, so that it will no doubt be equally successful.

The first three chapters are mainly astronomical, and concern the formation of the Earth and other bodies of the Solar System, together with brief descriptions of the Moon and planets. This section is needed, because the Earth is, after all, only one of nine planets, and the comparisons between it and our neighbour worlds are well worth making. It is interesting to note that the author tends to favour the theory that the Earth and Moon were once one body, and that the Moon broke away in the fashion supposed by George Darwin in his book written during the nineteenth century. Most authorities have the gravest doubts about this process, but Gamow is careful to emphasize that there is no general agreement about the birth of the Moon.

The rest of the text deals with the Earth itself; its internal constitution, the shaping of the world map, weather and climate, and the atmosphere. The nature and origin of life, and the Earth's future, are also dealt with in a readable, concise manner.

The material has been selected with skill, and has been presented in a most attractive form, so that the reader with no previous scientific knowledge will be able to follow it throughout; mathematics are to all intents and purposes excluded. The line drawings are good, and the photographs are satisfactorily reproduced. It must be added, unfortunately, that there are a few slips, mainly in the astronomical section; for example, there are more than the stated 40,000,000,000 stars in our Galaxy (p. 22), and it is surely misleading to refer to a solar flare as "a tongue of flame" (p. 181). On page 43 the author falls into the trap, common among non-selenographers, of rejecting the volcanic theory of lunar craters simply because these main features of the Moon differ in form from Earth volcanoes such as Vesuvius.

These are, however, minor points, and in no way detract from the excellence of the book. Within its scope, it is probably the best of its kind, and it will further enhance Prof. Gamow's reputation as one of the most skilful of modern scientific writers. It deserves a wide circulation, and will certainly run to many editions before it, too, needs complete re-writing.

PATRICK MOORE

LILIENTHAL'S ATOMIC YEARS

The Journals of David E. Lilienthal

Vol. 1: The TVA Years, 1939-1945, including a Selection of Journal Entries from the 1917-1938 Period. Pp. xxxi + 734. Vol. 2: The Atomic Energy Years, 1945-1950. Pp. x + 666. (New York and London: Harper and Row, Publishers, 1964.) 150s.

THESE volumes contain the diary records of a great American, usually written by him in shorthand and later transcribed by a secretary. They were started during his freshman years as a youth of seventeen, and continued throughout the period when he was a director of the Tennessee Valley Authority (T.V.A.), and chairman of the United States Atomic Energy Commission. The entries were "jotted down at irregular intervals and in all manner of places at odd moments", in aeroplanes, even on holiday in Martha's Vineyard or Florida—holidays often interrupted by telephone calls from the T.V.A. or the Atomic Energy Commission.

Volume 2 opens with the appointment of Lilienthal, together with three others, to advise Dean Acheson, the Assistant Secretary of State, on action to be taken on the Truman-Attlee-King declaration of November 15, 1945, proposing the establishment of a U.N. Atomic Energy Commission to develop a proposal for international control of atomic energy.

After less than two months the 'Acheson-Lilienthal' Report proposed the establishment of an international atomic energy authority to have the exclusive control of all 'dangerous' aspects of atomic energy. The 'dangerous' areas included the operation of uranium mines, reactors, diffusion plants. The United States proposals were at once hindered by the appointment of Bernard Baruch, at the age of seventy-five—referred to in subsequent notes as "the old man"—as the member of the United States of the United Nations Atomic Energy Commission. Baruch immediately appointed his own panel of advisers, who had somewhat different views from the Lilienthal group. One of their early proposals was that the United Nations "should organise a world survey of raw materials and to send 50 Two-Man-Teams all over the world. In this way they would find out what was going on in Russia." The Baruch team appeared also to be obsessed with the idea of vetos and punishments for infringements. However, Acheson and Lilienthal were able to resist most of the proposals of the Baruch group.

Gromyko countered for the U.S.S.R. by proposing an international convention against atomic weapons, but without any inspection for clandestine activities. The East-West positions were incompatible and with our knowledge of the climate in the U.S.S.R. in the post-war years the United States plan was doomed to failure.

In September, 1945, after considerable congressional opposition, Lilienthal was appointed chairman of the Atomic Energy Commission established by the McMahon Act. An extremely difficult organization had been laid down in the Act—five Commissioners were jointly responsible for policy, but the chairman had no formal executive responsibility, this being devolved on a general manager. This form of organization led later to some difficulties, especially with Admiral Lewis Strauss, who was often in a minority of one. He sometimes refused to accept the majority decisions and intrigued outside the Commission to have their decisions overthrown.

The first major disagreement came over a trivial matter—the export of radioactive isotopes to Norway. This was agreed to by the Commission by a majority of 4 to 1, but Strauss attempted to take the decision to the State Department and to secure its reversal. This incident showed the dangers of a lack of scientific understanding in men in high places.

The journals give an account of co-operation with the United Kingdom. War-time collaboration had, of course,

been very close, and through it Britain had obtained a complete knowledge of the theory and construction of atomic bombs and nuclear reactors of outputs of up to a few megawatts. Britain was not allowed information about plutonium production plants; but this was of no great consequence. Churchill and Roosevelt at Hyde Park in 1944 agreed that the atomic bomb would continue to be constructed only in the United States, and that the United States would not use the bomb without the consent of the United Kingdom. The United States agreed to co-operate fully with the United Kingdom and Canada in post-war military and commercial atomic energy development unless and until terminated by Government agreement. The information about this agreement was not passed down by the President, and was not even known to Mr. Stimson, Secretary of State for War, six months later. The United States Senators were informed for the first time about this agreement by Lilienthal in December, 1946, and this came as a considerable surprise. Technical co-operation had, in fact, largely ceased after the passage of the McMahon Act. However, the agreement on the sharing of uranium supplies, operated by the Combined Development Authority, continued, and gave rise to some difficulties in the Senate, since in the years up to 1950 the United States was short of uranium supplies for its rapidly expanding programme of fissile material production, including the building of more Hanford reactors for the production of plutonium and the construction of more diffusion plants.

Eisenhower, who at this time was Chief of Staff, is quoted as saying that "denial of Atomic Energy information to the British was harsh and unfair". Lilienthal was in favour of increasing co-operation and was supported by the State Department, and in January 1948 a *modus vivendi* was arrived at in which co-operation was agreed in a number of areas. Some of these areas, such as health and safety, and nuclear physics, were unclassified areas. One of the important areas was co-operation on "Reactors in which the power was not wasted". This allowed discussions on power-producing reactors to proceed, but excluded discussion on production reactors such as Hanford. At this time ideas on power production were, in fact, rather nebulous, though E. O. Lawrence is quoted as saying that power could be produced by the Hanford reactors within a year—at that time the cooling water emerged at rather low temperatures.

The President, Mr. Truman, spoke to Lilienthal "of what wonderful things we could do with our stocks of nuclear material. I've been dreaming of T.V.A.'s in the Euphrates Valley to restore that country to fertility and beauty of ancient times, and also a T.V.A. in the Yangtze Valley and in the Danube. These things can be done and don't let anyone tell you different".

Lewis Strauss again opposed co-operation: "He feels Britain is far to the left and might give away the secrets to the communists".

Towards the end of 1949 the *modus vivendi* was extended in time and the United States was given a greater share of ore as a *quid pro quo*. Britain refused, however, to transfer bomb production to the United States and continued to ask for a complete interchange of all technical information.

In point of fact Britain was very little handicapped by lack of information from the United States on the nuclear reactors or on any civil application. Britain could, however, have been saved some expense and time by interchange of military information.

The journal records the conflict between the Atomic Energy Commission and the Department of Defence on the issue of civil versus military custody of atomic weapons. A crucial meeting was held between the President, the Heads of Service Departments, and the Commission. The Secretary of Air, Mr. Symington, remarked: "Our fellows at Sandia think they ought to have the bomb. They think they might get them when they need them and they might not work". The President replied: "You have to

understand that this is not a military weapon. It is used to wipe out women and children and unarmed people and not for military purposes. So we have got to treat them differently from rifles and cannons and ordinary things like that". The President decided that the Atomic Energy Commission should retain custody.

In October 1959, after the explosion of the first Russian atomic bomb, the issue of the hydrogen bomb known as the "Super" was debated within the Atomic Energy Commission's General Advisory Committee, chaired then by Robert Oppenheimer. The General Advisory Committee was very much divided. Conant was flatly against it: "We have built our Frankenstein"; Oppenheimer was against; Rabi was for; Lilienthal against. We know from later evidence that there were doubts about its technical feasibility and that later work transformed the situation.

A special committee of the National Security Council, Schleson, Johnson (Secretary of Defence) and Lilienthal, reported to the President, who ruled in favour of developing the "Super" (January, 1950).

Lilienthal was almost continuously attacked and vilified by some members of the Joint Congressional Atomic Energy Committee. Senator Hickenlooper charged the Commission in 1949 with gross mismanagement, and a Committee investigation proceeded under the glare of television lamps for several weeks. Complete trivialities were discussed, such as the loss of a few milligrammes of enriched UO_2 at the Argon Laboratory, later found in a dustbin. A great fuss was made about a *March of Time* film containing pictures already released in 1945. The Committee insisted on a full F.B.I. clearance for A.E.C. Fellows appointed for basic research in Universities. At one meeting of the Committee, "7 Senators and 8 Congressmen were busy reading a security file on a Miss B. employed at Los Alamos—about her drinking habits and where she slept—the great issues of co-operation with the U.K. had to wait".

President Truman told Lilienthal, "Don't let this tempest in a teapot get your goat, Dave. You let it get under your hide and you'll get like Forrester [committed suicide May 22, 1949]; no need worry about it".

The diaries reveal Lilienthal as a very humane man, much troubled by the implications of the nuclear bomb for the world; striving always to ensure that their implications were discussed at the highest policy-level. Truman is revealed as a very shrewd person; a person of vision, firm in decision and well able to handle military departments and their demands.

J. D. COCKROFT

QUASARS

Quasi-Stellar Sources and Gravitational Collapse

(Including the Proceedings of the First Texas Symposium on Relativistic Astrophysics.) Edited by Prof. Ivor Robinson, Prof. Alfred Schild and Prof. E. L. Schucking. Pp. xvii + 475. (Chicago and London: The University of Chicago Press, 1965.) 10 dollars; 72s.

QUASI-STELLAR *Sources and Gravitational Collapse*, appearing 18 months after the first Texas Symposium on Relativistic Astrophysics to which it refers, is already a historical document. It has been overtaken by the second 1964 Conference, by preparation for the third and by a series of discoveries of such moment and at such a pace that the normal media of publication have been more than usually inadequate.

In 1960 the combined work of the radio and optical astronomers led to the identification of the radio source known as 3C 295 (the number in the Cambridge Catalogue) with a cluster of galaxies in Bootes. This was the most remote object known—about 4,500 million light years distant with a red shift of 0.47. There seemed no immediate hope that a significant increase in the penetration of the telescopes might help to resolve the arguments

about the type of cosmology applicable to the real universe. Yet the search for more distant objects which was then in progress is really the starting point of this book.

Palmer and his collaborators at Jodrell Bank had found that two radio sources in the Cambridge Catalogue 3C 48 and 286 had angular diameters less than 2 sec of arc—much smaller than that of the Bootes source and hence by implication more distant. Accurate positional measurements of these sources at Cambridge and at the California Institute of Technology enabled Sandage to identify 3C 48 late in 1960 as a sixteenth magnitude blue star. For more than a year it seemed that 3C 48 and the other objects similarly identified were luminous blue stars in the Milky Way. Then early in 1963 came the famous announcement that the 'stars' were, in fact, objects with exceedingly large red shifts.

So much has happened since then that it is important to remember that at this Texas Symposium in December of that year only half a dozen of these quasi-stellar radio sources, or quasars, had been discovered and the red shifts of only four obtained. This included the red shift of 0.545 for 3C 147, making it the most distant object then known. Thus the observational and theoretical papers in this volume are based on these data, which are a small part of the information available to-day on the quasi-stellar sources. (At least 40 are now known with 18 red shifts.)

The theoretical papers are predominantly concerned with the properties of massive objects and with the possibility that the great energy of the radio galaxies and quasars (more than 10^{60} ergs in some cases) may be released through the processes of gravitational collapse. Of course, the energy difficulties existed for the radio galaxies long before the discovery of the quasars, but the latter added the additional problem of the energy needed to sustain the great luminosity of these objects. The papers offer no real solution, neither is any known to-day, although a repetition of this discussion would be likely to be far more critical of the gravitational collapse theory and speculate on possibilities of highly efficient nuclear burning or total annihilation as sources of the energy.

The observational papers include, of course, those concerned with the quasars and give the detailed arguments that the red shift is a cosmological effect and not a gravitational shift. There are, too, the valuable papers on the identification of the radio sources as a whole and their nature which have stood the test of time better than the papers on the quasi-sources.

Nearly all the papers in this volume are now reprints of existing or subsequent publications mainly from the *Astrophysical Journal* or *Nature*. The fortuitous timing of the conference does, perhaps, justify to some extent this collection as a unique mark of yet another revolution in astronomy and astrophysics which has already led to the discovery of objects with red shifts four times greater than those discussed here. The collection also enables one to express overall admiration for the outstanding observational and theoretical work of the authors, which has created a situation of unparalleled interest and potential in astronomy, astrophysics and cosmology.

BERNARD LOVELL

HURWITZ—COURANT

Funktionentheorie

Von A. Hurwitz und R. Courant. Mit einem Anhang von H. Rohrl. Vierte vermehrte und verbesserte Auflage. (Die Grundlehren der Mathematischen Wissenschaften in Einzeldarstellungen, Band 3.) Pp. xiv + 706. (Berlin: Springer-Verlag, 1964.) 49 D.M.

THE first edition (1922) of *Funktionentheorie* contained three parts. Parts 1 and 2 were based on lectures of Hurwitz. Part 1 (130 pages) expounded the

Weierstrass approach to function-theory through power series. Part 2 (110 pages) gave a fairly full account of elliptic functions, including modular functions and transformation theory. Part 3 (150 pages), due to Courant, supplemented the Hurwitz development by chapters in the geometric spirit originated by Riemann.

Even in the first edition Part 3 was largely independent of Parts 1 and 2, and in the second edition (1925) Courant expanded Part 3 into a self-contained presentation. In the third and fourth editions this tail has outgrown the dog, and I shall suggest later that two well-proportioned creatures could with advantage replace the one which has grown in an uncontrolled way.

Part 3 now fills nearly 300 pages and is an account, mainly of interest to the specialist, of the development of topological function-theory. There are other important branches of function-theory (for example, integral and meromorphic functions) for which one must look elsewhere.

The preparation of this new fourth edition is due to Prof. H. Rohrl, of Minnesota. He has revised and brought up to date the text of the third edition and has added an appendix of 150 pages (in two chapters). The additions expound the theory in the more abstract setting in which the work of the past two decades has placed it. Chapter 1 of the appendix deals with some problems of conformal representation, in particular new knowledge about prime-ends (introduced by Carathéodory in 1913) and quasi-conformal mappings. The 1-1 conformal representation of Riemann surfaces leads to a discussion of the Fuchsian groups involved. Chapter 2 of the appendix contains a clear account of selected recent investigations of compact and non-compact Riemann surfaces.

I suggest that the book, which has become unwieldy and costly, should, in future editions, become two. Parts 1 and 2 would form an excellent presentation of Weierstrass's theory for the undergraduate. Actually he would be unlikely to need so much of the detail of elliptic functions and a radical surgeon could excise some sections of Part 2. Only exceptional undergraduates would find time to read Part 3, and the others should not have to buy a large book for the sake of a small part of it. Any research worker interested in Part 3 will know what is in Parts 1 and 2 and would not refer to them except perhaps as a model of style.

J. C. BURKILL

AN ADVANCED THEORY OF VIBRATIONS

Vibrations

By Prof. D. K. Magnus. Pp. xi+299. (London: Blackie and Son, Ltd., 1965.) 65s.

VIBRATIONS certainly differs from other books about the same subject, inasmuch as it classifies the vibrations according to the mechanism from which they originate. This is reflected in an identical mathematical treatment and similar final solution. According to this classification the subject-matter is sub-divided into the following groups: (i) natural vibrations; (ii) self-excited vibrations; (iii) parametrically excited vibrations; (iv) forced vibrations; (v) coupled vibrations. This classification enables the author to deal with a variety of problems both in the mechanical and the electro-technological field in a relatively small and inexpensive book, and it seems astonishing that the subject-matter covered should form the syllabus of only one term (about four months) in a German university or technical university.

Throughout the book the emphasis is on as thorough and general a treatment as possible. For example, the section on "Damped Vibrations" covers viscous, Coulomb and square-law or turbulence friction and also non-linear

vibrations. Later in the book the response of linear systems to non-periodic inputs is dealt with, making use of Duhamel's integral. Statistically distributed excitations are also considered.

Wherever possible, exact mathematical methods are used. Extensive use is made of the phase-portrait (velocity-displacement diagram), thus, as in other ways providing a link with control systems analysis. However powerful and frequently indispensable approximation methods, such as the method of small oscillations, the harmonic balance (known as the describing function method in control systems analysis), the Ritz, the Rayleigh and the Van der Pol method of slowly varying amplitudes are not ignored.

Most of the book is devoted to single-degree-of-freedom problems and the most important methods are demonstrated on them. The treatment of systems with several degrees of freedom then offers no major difficulties.

Systems of an infinite number of degrees of freedom involving partial differential equations are only touched on in the few final pages (except the taut string which was considered at the beginning of the book), but at this stage the intelligent reader should already have grasped the most important aspects of vibrations, enabling him to embark on further problems with relative ease.

Vibrations includes carefully selected exercises for each chapter and their solutions, this making it a suitable text book for use in English-speaking universities. The translation is competent, although the translator adheres to places too closely to German wording and phrasing. It would have been helpful if, when quoting books or reference, English equivalents or existing English translations of German standard works were used, and in the case of books which had been translated from English into German, if the original titles (instead of back-translated one) were given. To sum up, it can be said that *Vibrations* is a valuable complement to existing treatises on vibrations and it can be highly recommended to all who want to penetrate more deeply into the field of vibrations.

K. R. WEISS

VIBRATION PROBLEMS IN ENGINEERING

Vibration

By Prof. R. E. D. Bishop. (Based on Six Lectures delivered at the Royal Institution, London, December 1962.) Pp. 120+20 plates. (London: Cambridge University Press, 1965.) 30s. net; 5.50 dollars.

VIBRATION is a lightheartedly written, completely non-mathematical book which arose from Christmas Lectures given at the Royal Institution to a mainly young audience. The primary purpose of the book is to explain what is involved in the profession of an engineer, and vibration was chosen as a particularly suitable field to do this. An introduction of this kind into the wide and complex field of vibrations could only be successful if supported by a number of experiments and demonstrations; these in fact accompanied the lectures, which must have been extremely interesting (and certainly sometimes amusing). The spirit of the lectures is reflected in the book, although experiments have to be described, aided by numerous plates and figures. Prof. Bishop covers many important aspects of vibrations such as: the effect of vibrations on the human body; the resistance of metals to vibration (fatigue, Wohler diagram); free vibrations; modes of vibrations (for example, demonstrated on the air-liner 'VC 10'); imposed vibrations including random vibrations; self-excited vibrations; shocks and waves.

The problems chosen as examples underline the importance of vibrations in engineering with their sometimes

pectacular effects as, for example, the resonance built up by soldiers marching in step across bridges, this having led to several disasters; whirling of shafts; the collapse of the Tacoma Bridge; fatigue failure in crankshafts and of engine compressor blades; rocking of railway trains and the wind-excited oscillations of electric transmission lines (causing short-circuiting), or of steel chimney stacks causing their collapse).

Returning to the basic purpose of the book—to explain what is involved in engineering—I should like to quote the following passage with which I heartily agree: “The idea . . . that progress is made by pure scientists having ideas, and engineers making them work, is ludicrously superficial. A successful flutter analyst has to make important decisions as a physicist, as a mathematician and as an engineer. . . . Rapid progress in any branch of engineering—not just flutter analysis—becomes possible when those who are engaged on research can cross the ill-defined frontier between pure and applied science without realizing what they have done so”.

The book will be particularly useful to the young reader looking for a comfortable way to make himself familiar with the broad aspects of vibration, or to get an idea of what is involved in engineering. He may soon, if his raining or profession lies in this field, feel the need to study a more comprehensive theoretical book (one of which has been written by the same author). Also the more advanced reader may find Prof. Bishop's elementary book pleasant reading and this may perhaps prevent him from “not seeing the wood for the trees”.

K. R. WEISS

EXPERIMENTAL PLASMA PHYSICS

Plasma Diagnostics with Microwaves

By Dr. M. A. Heald and C. B. Wharton. (Wiley Series in Plasma Physics.) Pp. xvii+452. (New York and London: John Wiley and Sons, Inc., 1965.) 102s.

THE interaction of electromagnetic waves with a plasma constitutes a very useful method of measuring three important parameters of a plasma—the electron density, the electron temperature and the magnetic field inside the plasma. The purpose of *Plasma Diagnostics with Microwaves* is to give a summary of the basic theory of this interaction, together with a description of the experimental techniques and apparatus involved. Most of the material presented has appeared in research reports written by the authors, who have been very active in the application of microwave diagnostics. However, the presentation here is in a manner and of sufficient detail to provide an excellent introduction to an experimentalist new in the field.

As implied by the title, the authors limit themselves to electromagnetic waves in the microwave region, though the theory and the basis of the experimental techniques presented are applicable to infra-red and optical wavelengths. Probing of a plasma at these wave-lengths has come into extensive use recently since the advent of masers and lasers. The plasmas under consideration are those characterized by high temperature, that is, those of high degree of ionization and low collision frequencies, and of dimensions greater than the electromagnetic wave-length. However, the diagnostics described are applicable to other plasmas.

The first six chapters are concerned with the probing of a plasma with radiation generated externally. In Chapter 1, an account of the theory of electromagnetic wave propagation through a cold plasma is given. In the next two chapters the treatment is extended to warm plasmas, and has a detailed discussion of collision processes in a plasma. Chapter 4 is concerned both with bounded plasmas and spatial non-uniformity. The choice of antenna systems for particular applications is also con-

sidered. In Chapter 5 plasmas confined in wave-guides are treated. Included is a discussion of wave-guide propagation in a plasma of both space-charge and electromagnetic waves. An extensive discussion of experimental techniques in general use is given in Chapter 6. The next two chapters are concerned with radiation in the microwave region emitted by the plasma. Both a theoretical description of the various processes by which this radiation is emitted and the details of the experiments concerned with this radiation are discussed. This is followed by a description of the experimental apparatus and the special circuits used in microwave diagnostic experiments. The final chapter is different in content and approach from the rest of the book. It consists of a list of some other plasma diagnostics with the ranges of their applicability. However, the discussion is limited to those diagnostic techniques which provide information similar to that obtained by microwaves. There are two appendixes—review of electromagnetic wave propagation and tensor and matrix algebra.

The book is very readable. The presentation is lucid and coherent. The reference list is very comprehensive and is designed to provide a means of following up those topics which of necessity could not be discussed fully. It is a very useful addition to the growing list of books available to the experimental plasma physicist.

A. E. DANGOR

ASPECTS OF RHEOLOGY

Proceedings of the Fourth International Congress on Rheology

Brown University, Providence, Rhode Island, August 26–30, 1963. Edited by E. H. Lee and Alfred L. Copley. Part 1: Pp. x+373. 120s. Part 2: Pp. xii+714. 226s. Part 3: Pp. xiii+637. 196s. Part 4: Symposium on Biorheology. Pp. xi+634. 189s. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, Inc., 1965.)

ABOUT ten years ago, a fellow-rheologist commented to me: “You know, there are two kinds of rheologist, the ‘P Q’ rheologists and the ‘i j’ variety”. Until recently, the former, mainly concerned with direct relations between experimentally applied pressures and induced rates of flow, formed a large majority. From the *Proceedings of the Fourth Congress on Rheology*, one concludes that the main interest now lies in tensor theory, except perhaps in the comparatively new and popular field of biorheology, which has scarcely reached this stage. One reason for this development is that in earlier times most rheologists were trained as chemists. The new generation is composed, as is proper, of young physicists with a prime interest in applied mathematics.

The *Proceedings of the Fourth Congress* are so extensive that it was necessary to publish them to four volumes, but the arrangement is not altogether a happy one. At first sight it sounds sensible: Part 1, invited papers and general lectures; Part 2, papers from members of the (American) Society of Rheology; Part 3, other contributed papers; Part 4, a biorheological symposium. But, in fact, biological subjects are to be found in Parts 1 and 3 and other subjects are fairly evenly scattered throughout the first three volumes. These three are edited by Prof. E. H. Lee, and the fourth, independently, by Prof. A. L. Copley.

It is difficult, in a short review, to do justice to nearly 200 papers ranging in subject-matter from aluminium as an unstable solid to the properties of the skin of the hippopotamus. Suffice it to say that, throughout, the standard is very high. Perhaps the most outstanding contribution is that of Prof. C. Truesdell, who was awarded the Bingham Medal for the year. His “Rational Mechanics

of Deformation and Flow" is clearly of fundamental importance but is very stiff reading.

In all volumes except the first, the papers are grouped according to subject-matter. For some reason, Prof. Truesdell's lecture appears in Part 2 and is followed by sections on continuum mechanics, testing techniques, stress and strain distribution analysis, birefringence, metals, technology, polymers, polymer fluids, glasses, dispersions. Part 3 has sections on continuum theory, testing techniques, stress and strain distribution analysis, metals, technology, polymers, polymer fluids, polymer solids, glasses, dispersions.

The biorheological symposium (Part 4) (which is dedicated to Prof. Robin Fåhræus) is sub-divided as follows: general lectures, laminar flow, cytoplasmic streaming, haemolysis and sap movements, pulsatile flow, blood flow in branching and tapered tubes, measurements and observations, haemorheology of red cell suspensions, medical biorheology, *in vivo* haemorheology, tissue materials, blood clotting and low shear haemorheology.

It is inevitable, but a pity, that the price of this massive work will limit its circulation almost exclusively to libraries: the *Proceedings* of previous congresses have been much more condensed; but such is the present-day trend.

This is certainly a work the usefulness of which will long outlive the period before the fifth congress in Japan in 1968. As a classic, it is also good that the production, printing and style are excellent throughout.

G. W. SCOTT BLAIR

MAGNETOHYDRODYNAMIC POWER GENERATION

Magnetohydrodynamic Electrical Power Generation (Proceedings of an International Symposium held in Paris, July 1964.) Vol. 1: Papers Communications, Sessions 1 and 2, Pp. 469; Vol. 2, Sessions 3, 4 and 5, Pp. 470-1048; Vol. 3, Sessions 6, 7 and 8, Pp. 1049-1721; Vol. 4: Technical Discussion, Pp. 1722-2042. Edited by J. A. Satkowski and H. B. Smets. (Paris: Organization for Economic Co-operation and Development, 1965.) 220s.

IN 1935 the Hungarian applied scientists, Karlowitz and Halasz, patented the idea of generating electricity from the combustion of fossil fuels by means of a cycle involving the compression of the combustion air followed by the highest possible pre-heat in a heat-exchanger, followed in turn by combustion to raise the temperature as high as possible. The high-temperature combustion products were then to be expanded adiabatically to a pressure approximately atmospheric and passed at supersonic velocity through a magnetic field. Provided the gases had sufficient electrical conductivity after the adiabatic expansion, the system would thus generate d.c. as originally proposed, by the Faraday effect owing to the presence of the strong magnetic field, and the energy for this current used in an external circuit would come from the destruction of the greater part of the directed kinetic energy of the fast-moving gases. Karlowitz worked on this with a large American organization until the work was closed down by the Second World War, but the basic difficulty was the insufficient electrical conductivity of the gases. I published essentially the same idea independently in my inaugural lecture in Sheffield in 1954, and gradually during the 11 years since then pilot plant experiments both on the small and large scale have been designed and presented in a number of countries, including the United States, France, Britain, Germany, Hungary, Poland and the U.S.S.R. Similarly, engineering conferences on the subject of electricity generation from fossil and fissile fuels have been held during the past 10 years, several in Britain (the first at Sheffield in 1959), in the United States and in the U.S.S.R.

The most ambitious international symposium on magnetohydrodynamic (MHD) electrical power generation was organized by the European Nuclear Energy Agency and the French Institut National des Sciences et Techniques Nucléaires in 1964. It was attended by 400 people from 25 countries, and 114 papers were presented divided into eight technical sessions. The *Proceedings* have now been published in the form of four thick paper-back volumes with more than 2,000 pages. The titles of the eight sessions were as follows: Session 1 "Physical Properties of the MHD Working Fluid (Combustion Gas)". Session 2, "Physical Properties of the MHD Working Fluid (Closed-cycle Fluid): (a) Elementary Processes and Plasma Diagnostics; (b) Non-equilibrium Aspects". Session 3, "Study of Magnetohydrodynamic Flows and Power Conversion Processes (Continuous Gas Flows): (a) Fluid Dynamic Aspects; (b) Conversion Processes". Session 4, "Liquid Metal and Unconventional MHD Conversion and AC Power Generation: (a) Striated Flows and Explosive; (b) AC Power Generation; (c) Liquid Metal MHD Converters". Session 5, "Production of Magnetic Fields". Session 6, "Problems of Materials". Session 7, "Open-cycle Converters: (a) Experimental Aspects; (b) Prospects". Session 8, "Closed-cycle Converters: (a) Experimental Aspects, (b) Prospects".

The classical method of electricity generation from heat involves passing the heat through a boiler tube to generate high-pressure steam, the steam then expanding and pushing against turbine blades and producing rotation which is mechanically coupled to a rotating generator in which copper windings are pushed through a moderately strong magnetic field. Compared with this, the open-cycle magnetohydrodynamic system has the obvious advantage that the temperature and use of the heat are not limited either by the need to pass the heat through a pressure-tight wall or by the need to have the hot working fluid pushing against metal blades. The fundamental disadvantage of the MHD system is the fact that the metallic copper conductor is replaced in it by the working fluid and that it is extremely difficult to obtain the necessary conductivity in the gas, which has to be of the order of 100 mhos/m. As a result of the work done in the past decade, reported in this symposium, it is now clear that for the open-cycle MHD, where the working fluid contains the triatomic molecules of combustion products, it is not possible to have non-equilibrium ionization, so that the necessary conductivity can only be obtained by having gases at a temperature of the order of 2,500° K and with a seeding of a low-ionization potential material (potassium is the only economic element and even that would have to be recovered with better than 99 per cent efficiency, although fine dusts with low-work functions are also being considered). This temperature and seeding must exist under the conditions of the gases when they pass through the magnetic field; whether one uses an impulse or reaction expansion cycle, the proportion of the enthalpy of the gases which can be converted ideally to electricity depends on the total temperature drop from upstream of the nozzle to the end of the magnetic field, so that to have a large temperature drop is desirable from the point of view of output. It is also necessary even with this conductivity to have a very high magnetic field since the power generation per unit volume of the magnetic field depends on $\sigma v^2 H^2$. Schemes in the 'fifties usually involved either iron-cored magnets with a maximum field strength of 10,000 gauss, or alternatively higher fields with water-cooled copper coils and no cores. A whole session was devoted to magnetic fields in this conference and work is now directed towards achieving 100,000 gauss over large volumes usually with super-conducting coils. For these very high fields iron is not much use and the papers deal both with the optimum design of the coil, methods of cooling to have a super-conductor near a very hot MHD channel and other design features. These very high gas temperatures also involve very severe materials

problems. The British work has mainly been concentrated on water-cooled walls and electrodes with a small amount of insulation, while the French work mainly uses hot walls. The cold-wall system gives large electrode potential drop of the order of 100 V. There is a very big-scale effect and it might well be possible on the scale of 1,000 MW to have water-cooled walls while smaller systems, for example of 200 MW, might require hot walls with considerable continuous erosion.

Although the conference was organized largely under the auspices of nuclear energy, and much of the work in all countries of the world is going on in nuclear agencies, yet there is considerably more pilot-plant work being carried out on open-cycle systems aimed at combustion-heated fossil fuel usage than on the closed-cycle systems which are being considered for nuclear heat. The closed-cycle systems have the advantage that one can choose the thermodynamic working fluid to have the optimum properties, for example, helium. In this case it is possible to work at appreciably lower temperatures and have on-equilibrium ionization to give the required gas conductivity, and although seeding is still needed it is now possible to use caesium. For the open cycle one can clearly classify all the work being done into three categories; the least exciting is that in which one expects a very small Mach number, for example 0.8, for the gases for the magnetic field, and with only about 10 per cent of the energy of the fuel converted into d.c. by the MHD system while the remainder is converted into electricity by means of a conventional boiler using the heat of the exit gases from the MHD duct. It is probable that this system can be made to work, although it may well be that the small increase in efficiency of electricity generation from the fossil fuels obtained in this way (with a substantial increase in capital cost) will be overtaken by improvements in economy of conventional steam generation from fossil fuels. The second category of open-cycle MHD development from combustion is that which aims at more than 50 per cent direct generation efficiency in the MHD generator either by using a very high efficiency re-heater capable of transferring heat from waste gases at about 1 atm. pressure and $2,500^{\circ}\text{K}$ to air at some 10 atm. and rising in temperature to nearly $2,300^{\circ}\text{K}$. These systems would have to be combined with either a gas turbine or a steam turbine cycle for the lower temperatures at least to produce the compression power for the air. Thirdly, we have the more sophisticated cycles, to which a whole session was devoted. These included, in particular, the separation of the thermodynamic working fluid into striations, an idea which was first put forward in Britain and is now being actively worked on in France, the use of detonation waves to give a high-temperature, electrically conducting shock and the very interesting liquid metal converters in which the hot-combustion gases are used to impart a high velocity to a highly conducting liquid metal. It is my opinion that within the next five years there will be a break-through in one or other of these more sophisticated cycles which will change the whole situation.

M. W. THRING

ADVANCES IN FLUID DYNAMICS

Research Frontiers in Fluid Dynamics

edited by Dr. Raymond J. Seeger and Dr. G. Temple. Interscience Monographs and Texts in Physics and Astronomy, Vol. 15. Pp. ix+738. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, 1965.) 230s.

RESEARCH Frontiers in Fluid Dynamics is a collection of some twenty articles on various aspects of modern fluid dynamics, each written by an acknowledged expert in his field. It is directed at the graduate student

and "intended to give him a panoramic view of some of the exciting research vistas" in the subject. The title is, in fact, slightly misleading. By no means all the research frontiers in fluid mechanics are covered and, of those omitted, some are very important and fashionable; for example, there is scarcely any mention of turbulence, instability of flow, supersonic flow, merged layers in hypersonic flow and the magneto-sphere. On the other hand, a number of very interesting fields of research are described, not all of which would be obvious to the young worker but all well worth his attention as possible areas in which useful work might be done.

It is not possible to discuss all the contributions and so I shall pick out a few which are of particular interest to me, asking the reader to bear in mind that it is a personal choice with which others might disagree. The longest chapter, by Prof. J. V. Wehausen, is on free surface flows and describes in reasonable detail the advances that have been made in this rapidly expanding field during the past few years. He covers exact mathematical theories, cavitating flow, ship waves, stratified and rotating fluids and approximate theories. Not least in importance is the list of about 600 papers in the bibliography giving the interested reader an excellent start in a subject which can be strongly recommended to graduate students. Dr. ter Haar has written an interesting article on the dynamics of liquid helium, a field in which fluid dynamicists led by C. C. Lin have begun to make notable contributions in recent years. It does not seem that the possibilities of the two-component model for helium II have yet been exhausted and it may well prove a basis for explaining its unique and fascinating dynamic properties.

Dr. Guderley gives a comprehensive account of hodograph methods in two-dimensional transonic flow, including the question of the existence of potential flows past profiles at high subsonic speeds. By way of contrast, in methods of describing research frontiers, Dr. Freeman describes one particular question in hypersonic flow—the nature of the asymptotic flow past bluff bodies—which involves a fascinating matching problem in mathematical analysis and which is still largely unsolved. One aspect of this question not discussed by the author is the possible appearance of secondary shocks.

For the more mathematically inclined student, there is a clear exposition of singular perturbation procedures, that is, Lighthill's strained co-ordinates and matched asymptotic expansions. The necessity for such sophisticated methods occurs too frequently in fluid mechanics for any theoretical worker not to have it in his armoury, and the account presented here is a good introduction. Further, the large computer is making an increasing impact in the subject and promises to be of decisive importance in the future. The article on numerical analysis by Dr. Polachek is therefore timely, for, whatever branch of fluid mechanics the student elects to work in, he will find the ideas in this chapter of assistance. There is, however, one omission, for Dorodnicyn's method of integral relations, which has had a number of successes in blunt-body aerodynamics and in boundary-layer theory, is not mentioned.

The remaining articles are: engineering aspects (W. R. Hawthorne); molecular theory of fluids (H. S. Green); viscoelastic fluids (R. S. Eivlin); anomalous viscosity (M. Reiner); second-order effects (M. Reiner); shock-wave phenomena (D. C. Pack); magneto-fluid dynamics (C. K. Chu and H. Grad); application of the Boltzmann equation of low-density flows (G. N. Patterson); high-temperature phenomena (J. D. Teare); high-density phenomena (J. S. Dahler); meteorology (J. G. Sutton); astrophysical phenomena (L. H. Aller) and fluid-dynamical problems associated with interplanetary space (E. N. Parker). Generally the contributors show a high standard of authority in their articles and infuse the reader with a feeling of the excitement, dynamism and the wide sweeping character of fluid mechanics. It should certainly be

put into the hands of postgraduate students to fire their enthusiasm for the field, but regrettably, it is doubtful whether there are any who can afford to pay the price asked.

K. STEWARTSON

PALAEONTOLOGICAL TECHNIQUES

Handbook of Paleontological Techniques

Edited by Bernhard Kummel and David Raup. Pp. xiii + 852. (San Francisco and London: W. H. Freeman and Co., 1965.) 130s.

PALAEONTOLOGICAL techniques, in the broadest sense, include searching for fossils, their collection, preparation, examination and eventual publication. Examination includes physical activities such as measurement, drawing and photography, and matters such as statistics and taxonomy. Publication involves writing and illustration. There is also storage and cataloguing. Palaeontologists generally work out methods suited to the fossils they are investigating by a combination of common sense and trial and error, although the larger museums and university departments have built up a repertory of techniques. There seems to have been an increasing number of new methods in recent years, taking advantage of new apparatus and materials, but most of the basic techniques and a number of specialized ones have been known a long time. Many papers describe individual techniques, but there has been no complete guide since the modest book by Camp and Hanna (*Methods in Paleontology*, University of California Press, 1937), which gave useful accounts of many techniques, with emphasis on vertebrates and micropalaeontology. *Handbook of Paleontological Techniques* is a new attempt to cover a field nearly as wide as that defined at the head of this paragraph.

The book consists of five parts: (1) procedures and techniques applicable to major fossil groups; (2) descriptions of specific techniques; (3) techniques in palynology (the study of pollen); (4) bibliography on palaeontological techniques; (5) list of bibliographies of stratigraphy and palaeontology. Each part consists of a number of short articles. Part 1 includes 21 essays covering most of the major fossil groups, although the ammonoids are a conspicuous omission. The essays range from chatty personal reminiscences to systematic notes on collecting and preparation. For the reader seeking practical information this is possibly the least useful part of the book, although several papers, notably that on ostracods, describe techniques in detail.

Part 2 is divided into sections on collecting, mechanical and chemical preparation, radiation techniques, casting and illustration. Most of the 13 articles in the section on collecting are either elementary (with remarks such as "Fossils are most commonly found in sediments or sedimentary rocks") or very specialized. The section on mechanical preparation has two omissions. Mechanical chisels have long been used at some laboratories, but it has been difficult to get satisfactory apparatus. An account of available machines, or constructional details, would have been useful, but only the Burgess vibro-tool is mentioned. Many makes of ultrasonic cleaning gear are now available and the palaeontologist needs guidance as to the merits of different frequencies, wide or narrow frequency ranges and different powers for cleaning fossils, but this is not given. The section on chemical methods is short (two papers) and deals only with acid treatment.

The section on radiation techniques is a good introduction for the worker contemplating the use of X-rays, ultra-violet or infra-red light, or electron microscopy in the examination of his fossils. The section on casting and moulding also gives a good account of the chief materials and methods. A fuller account of materials used for impregnating friable fossils would have been worth while.

Four of the seven articles on illustration are concerned with photography. There is also an account of ways of whitening fossils for photography, but some of the material in it is repeated in other articles throughout the book. This kind of duplication unfortunately affects a number of topics, for example micropalaeontology. The article on preparation of plates for publication is less useful than the notes for authors issued by the Palaeontological Association.

Part 3 seems to cover the specialized subject of palynology thoroughly. It occupies, however, no less than one third of the book, an unduly large proportion. There would have been a case for devoting other parts of the book to major branches of the subject. Micropalaeontology occupies about 130 pages scattered through the book. Would it not have been better to bring this material together? As it is, the information in the book is assembled partly under techniques and partly under fossil groups, which results in duplication.

Statistics might legitimately have been included. There is a useful article on sampling, but the only other paper on statistics is in the palynology section and has mathematical nomenclature beyond most palaeontologists. Similarly, zoological nomenclature concerns every palaeontologist, and an exposition of the main points of the latest rules of nomenclature would have been handy.

The difficulties of co-ordinating the work of 80 contributors are obvious. One wonders whether so many were really necessary, and whether a smaller number might not have resulted in a more compact, equally useful, book at a lower price.

D. T. DONOVAN

SOME ASPECTS OF BORON

Boron, Metallo-Boron Compounds and Boranes

Edited by Roy M. Adams. Pp. xxiii + 765. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, 1964.) 207s.

THE present upsurge of interest in the chemistry of boron—until 10 or 15 years ago a very neglected element—has created a genuine need for the recently amassed knowledge to be made readily available to the enquirer and for the demands of teachers and research workers in particular to be supplied. Fortunately the compilers of such volumes have not been idle, and several unrelated books about boron have appeared in recent years, not one of which, however, provides us with a comprehensive account of the chemistry of the element as a whole and in true perspective. For this we must wait, and, indeed, there are so many new and unexpected fundamental facts about the element still being discovered that it is doubtful whether such a volume (or volume) would be well timed to appear at the present moment. We must therefore be all the more grateful for the appearance of books that deal well with certain aspects of boron chemistry, of which *Boron, Metallo-Boron Compounds and Boranes* is an excellent example.

This book is in fact a collection of eight reviews by several experts, under the general editorship of the principal contributor, Roy M. Adams. In spite of a certain unevenness—the first two reviews are relatively slight—the impact of the book as a whole is considerable, the result of careful scholarship. The scope of the book is perhaps best conveyed by its chapter headings, which are: "History and Technology of the Borax Industry", by W. A. Gale (27 pp., 141 references); "Heterogeneous Equilibria in Aqueous Systems of Inorganic Borates" by W. A. Gale (24 pp., 31 references); "Inorganic Boron Oxygen Chemistry", by N. P. Nies and G. W. Campbell (179 pp., 980 references); "Elemental Boron", by A. J. Newkirk (67 pp., 374 references); "Refractory Binary Borides", by B. Post (71 pp., 160 references); "The Hydro-

oron Ions (Ionic Boron Hydrides)", by R. M. Adams and J. R. Siedle (134 pp., 624 references); "The Boranes or Boron Hydrides", by R. M. Adams (186 pp., 804 references); "Toxicology of Boron Compounds", by G. J. Nevinskas (45 pp., 182 references).

The book is all the more welcome because, of these topics, only two—elemental boron and the boron hydrides—can be said to have been anything like adequately covered by earlier reviews, and even these are now superseded by the present contributions, which are as comprehensive as one could wish. That so much ground was relatively untrodden is a far more important factor than any criticism one might wish to raise against the book on the grounds of heterogeneity. This latter characteristic may even be considered a virtue if it can be regarded as the outcome of sensitivity of the editor to former gaps in the fields surveyed. One result has certainly been the avoidance of overlap everywhere, except to a very minor extent between Chapters 2 and 3. Perhaps the most serious aspect of the heterogeneity is the consideration that, whereas the book is obviously mainly intended for the pure chemist, the latter may justly complain that the first and final chapters have little significance for him. This is partly mitigated by the fact that, taken together, they constitute less than 10 per cent of the book. Certainly these reviews needed to be written—especially the final one, since information concerning the physiological action of boron compounds is not otherwise conveniently accessible—but the wisdom of including them in the present book is questionable.

Deserving of special mention are the chapter on boron–oxygen chemistry and the chapters on hydroboron ions and boranes. That on boranes is sufficiently up to date to include reference to the recent isolations of B_6H_{12} , B_8H_{12} and $B_{10}H_{12}$, respectively. The weakest chapter is Chapter 1, which is too brief and generalized and markedly below the high standard of the others.

To conclude, the appearance of this book is welcome and timely. It will be frequently consulted by inorganic chemists and is a 'must' for all serious boron chemists and those who are concerned with the teaching of advanced boron chemistry. The style is good and very readable throughout. Moreover, the book is supplied with good formula and subject indexes. It is strongly recommended.

L. H. LONG

PROGRESS IN PETROLEUM CHEMISTRY AND REFINERY

Advances in Petroleum Chemistry and Refining, Vol. 10. Edited by John J. McKetta, jun. Pp. xvi + 571. New York and London: Interscience Publishers, a Division of John Wiley and Sons, 1965.) 210s.

In my review of Volume 9 in the series *Advances in Petroleum Chemistry and Refining*, I wrote: "This series of volumes does, within obvious limitations, at least provide a useful means of leisure-study in the specialized subjects so far treated; to this extent it undoubtedly fills a gap in contemporary technical literature of scientific progress of petroleum technology which future volumes may well gradually narrow" (*Nature*, 207, 0; 1965). That this gap will not now be bridged is made evident in the preface to Volume 10, where it is stated by the editor, John J. McKetta, jun., that this is the final volume of the series. The initial intent was an annual publication spread over the course of several years, during which "... the series of *Advances* will have reached upon all parts of the far-flung industry, and the totality of volumes will assume the character of a reference work". Unfortunately, the co-editor, Dr. Kenneth A. Kobe, died in November 1958, not long after the series was initiated; this was followed by the death in April 1964 of a prominent member of the advisory board, Dr. Cecil L.

Brown; in these circumstances one can understand that even attaining the goal of ten volumes must have seemed an almost hopeless task, as the present editor remarks. So far, the volumes in the series have covered 96 chapters, nearly 5,600 pages, and have required the collaboration of 162 authors. It may be wondered whether another reason was instrumental in the decision to terminate the series at this juncture, namely restriction of sales, at least internationally, due to the relatively high cost of each volume, to which Volume 10 is no exception, and to which I have previously directed attention.

However, from a technical point of view, the quality and particularly the range of subjects discussed in this most recent issue are well up to the standards set by previous volumes in the series. There is a modern exposition on "The Composition of Petroleum" by R. H. Hunt and M. J. O'Neal, jun. This is followed by a chapter on "Production and Distribution of Liquid Hydrogen", by C. R. Baker and L. C. Matsch. G. P. Hinds, jun., contributes an interesting paper on "Hydrogen Conservation in Petroleum Refining". "Hydrocarbon Gasification Processes" is the joint work of G. J. Van den Berg, W. R. Dammers, and L. W. ter Haar. Dewaxing of lubricating oils has long proceeded on conventional lines but the more recent reactions to the problem are admirably summarized by the chapter on "Modern Dewaxing Technology" by S. Marple, jun., and L. J. Landry. "In the near future petroleum may replace coal tar as the primary source of naphthalene . . ." is the theme of a dissertation on "Naphthalene from Petroleum", by H. D. Ballard, jun. On the more purely academic consideration of contemporary petrochemical processes, there is the chapter on "Nonconventional Polymerization of Vinyl Monomers", by N. G. Gaylord, D. E. Hoffenberg, and H. F. Mark. Rather tangential, but none the less a welcome addition to the *Advances in Petroleum Chemistry* series, is the inclusion in this latest volume of a lengthy chapter on "Nitrogen Fertilizers", by S. Strelzoff and L. H. Cook: this is an excellent summary of the subject which deserves a wider audience than it may perchance attract. R. S. Egly and E. S. Starkman give us a general outline of the nature, production and uses of nitroparaffin fuels, while this volume, and hence the present series, is brought to a conclusion by consideration under the group heading "Mechanical Equipment" of "Engine Fuel Additives", by M. R. Barusch and J. H. Macpherson.

The high-level theoretical and practical purpose of this series and its execution during the years are unquestioned. The present editor and his advisory board deserve praise for their persistence, and sympathy in that this ambitious project has had to be abandoned 'midstream', so to speak. It may later on be revived in another context; but I suggest that a far wider appeal would be satisfied by the inclusion of more authors of international fame (in four of the volumes, including this present one, taken at random, only five are other than American, out of a total of 65 contributors); and that the price bracket be favourably reassessed so that many other technologists, besides university and oil company libraries, may benefit from the wisdom this series purports to dispense. H. B. MILNER

CHEMISTRY 1830 TO 1963

A Hundred Years of Chemistry

By Prof. Alexander Findlay. Third edition revised by Trevor I. Williams. (University Paperbacks, No. 115.) Pp. 335. (London: Methuen and Co., Ltd., 1965.) 25s. net.

TO quote Dr. Williams's preface to the present edition of *A Hundred Years of Chemistry*: "It is hoped that this revised edition will serve the same purpose as its predecessors, to show that modern chemistry is firmly rooted in the past and to present the subject as the product of a process of continuous evolution".

The progress of chemistry in the period covered by this book (roughly 1830–1963) has been immense. Prof. Findlay himself was born only five years after Mendeleef and Lothar Meyer's discovery of the Periodic Law, and only nine years after Kekulé postulated the hexagonal structure of benzene. In revising the 1947 (second) edition, Dr. Williams had the task of extending the range of the book by nearly twenty years, and wisely chose to "indicate some of the main lines of advance and the more important results obtained", rather than to attempt to deal comprehensively with recent developments. His work has been successful, and the book now includes such recent developments as isotactic polymers and the synthetic herbicides based on dipirydyl. In view of the vast research and development expenditure from public funds on atomic energy and nuclear weapons, and their profound political significance, it is disappointing that in the chapter on "Radioactivity and Atomic Constitution" the atomic bomb is only mentioned specifically in a footnote reference, and the hydrogen bomb not at all. The chemistry involved in rocket propulsion and space travel, too, is a subject of which to-day's readers might justifiably expect a mention. There is some evidence that the authors found difficulty in deciding on the likely level of scientific knowledge and interest of their readers: for example, a reader interested in referring to Volume 8 of *Bihang till Kongl. Vetenskapsakad. Handlingar* would know already that photography "plays a part of great importance in the life of all peoples".

The book contains about 800 footnotes, most of which are references to the original publication of important nineteenth-century contributions to chemistry, and these will be valuable to readers wishing to extend their knowledge of chemical history. At the end of the book there are about seventy "Short biographical sketches of those, now dead, who have contributed most conspicuously to the work of building . . . the present day edifice of chemistry". The choice of some of the lesser known chemists will delight Scottish readers.

Some minor criticisms do not alter my opinion that the book, as a whole, fully justifies the publisher's claim to being "an invaluable and readable history", and it is to be hoped that the success of this edition will encourage Dr. Williams to make other revisions of this excellent book in the future.

R. G. PARTINGTON

TWO STEREOCHEMICAL KEYS

Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry

By Pierre Crabbé. (Holden-Day Series in Physical Techniques in Chemistry.) Pp. xv + 378. (San Francisco, London and Amsterdam: Holden-Day, 1965.) 12.95 dollars.

OPTICAL rotatory dispersion and circular dichroism are two techniques that depend on the interaction of polarized light with matter. Together they constitute a unique tool for investigating the chirality of molecules or (to use a more everyday expression) for distinguishing between a chemical right hand and a chemical left hand. In a more general sense, they may be considered as two keys for unlocking stereochemical problems.

Until the development of commercial instruments, these techniques were the preserve of those who were able and willing to build their own equipment, and the techniques could not therefore become routine tools for the structural chemist. The appearance of commercial instruments for optical rotatory dispersion in the United States in 1953 and for circular dichroism in France in 1960 has changed the situation dramatically; for all chemists working with optically active materials (organic, inorganic or polymeric) these techniques are now of great and growing importance. The instruments, although commercially

available, are still in an early stage of development and are not yet in very wide use. The potentialities of the two techniques are therefore still not fully developed.

The rapid developments during the past ten years have been due to fruitful collaboration between three different types of worker (although some might wish to be classified under two or three headings). These are (1) the designer and manufacturers of instruments; (2) the theoretical workers; and (3) the workers who collect experimental data. In a field of study which is still in a considerable measure empirical, the services of the last-mentioned group (to which I should certainly be allotted by my colleagues) are very necessary for the further development of the subject. The lack of rapid progress before commercial instruments became available was essentially due to the fact that theoretical workers had no large body of data with which to test their ideas.

Two standard books on these topics have previously appeared: Djerassi's book on *Optical Rotatory Dispersion* in 1960 and the book by Velluz, Legrand and Grosjean on *Optical Circular Dichroism* in 1965. Dr. Pierre Crabbé—who has been associated with two of the leading workers in the field, Prof. G. Ourisson in Strasbourg and late Prof. Carl Djerassi in Stanford and Mexico—has produced an excellent overall picture of both techniques from the point of view of a structural organic chemist. Although the techniques are applicable to all types of dissymmetric structures, they are chiefly of value to the natural product chemist or biological organic chemist, since nearly all natural products are dissymmetric.

After a brief but adequate theoretical and technical treatment (Chapters 1–3, 29 pp.), Dr. Crabbé outlines in Chapter 4 (22 pp.) the types of problem to which optical rotatory dispersion and circular dichroism can give useful answers; these are, first and foremost, absolute configuration, relative configuration, location of functional groups and preferred conformations.

Dr. Crabbé then considers the chromophores which have optically active absorption bands and hence give rise to Cotton effects; these chromophores may be divided into two classes: (a) symmetrical chromophores, which are perturbed by dissymmetric environment, and (b) inherently dissymmetric chromophores.

Chapters 6 and 7 (108 pp.) deal in great detail with the saturated carbonyl group, which has proved hitherto the most profitable single field for study. Chapter 11 (67 pp.) deals with the other perturbed chromophores, among which carboxyl and related groups, aromatic functions and the nitro group are becoming increasingly important and have indeed been investigated intensively since the book was published.

Chapters 8, 9 and 10 deal with the inherently dissymmetric or 'twisted' chromophores; these include biaryls (11 pp.), conjugated dienes (13 pp.), and the unsaturated ketones (53 pp.). The amount of the space allotted to the last-named group reflects their importance.

In Chapter 12 (17 pp.), polypeptides, proteins and nucleic acids are considered. Here the optical rotatory dispersion and circular dichroism techniques are especially valuable for the investigation of helical forms—and with the present rapid development of biochemistry any method for studying natural macromolecules can be sure of extensive use. There is a final appendix on the automatic processing of circular dichroism data.

Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry contains exhaustive references to work in the field, which are especially valuable at a time when optical rotatory dispersion and circular dichroism data are appearing in many structural papers; the references include many to work in the press. The book is well printed with numerous structural formulae and diagrams of optical rotatory dispersion and circular dichroism curves which are all models of clarity. Many well-prepared tables illustrate the value of the two techniques in structural problems and emphasize the striking differences

ften found between the curves for compounds differing only in one stereochemical feature.

The publishers and printers are to be heartily congratulated on the speed of production; the preface is dated December 1964, and the book was published in April 1965.

The subject is developing vigorously and the next few years will undoubtedly see rapid extensions of both techniques to shorter wave-lengths and to other functional groups, studies of conformational equilibria and solvation—and also (though this is perhaps a more speculative field) studies on magnetic optical rotatory dispersion and circular dichroism, that is, the production of what might be called 'artificial' optical rotatory dispersion and circular dichroism curves, by the application of powerful magnetic fields to otherwise symmetric molecules.

It cannot be claimed that optical rotatory dispersion and circular dichroism will ever play a part in as many fields of organic chemistry as some other widely used techniques (ultra-violet, infra-red, nuclear magnetic resonance and electron spin resonance); none the less, their place in the tool kit of the modern organic chemist is now certain. The book will provide a stimulating guide to anyone wishing to enter the field, or wishing to see how these techniques might help his research. Those working in the field will certainly have the book already.

W. KLYNE

CLASSICAL OPTICS

Mathematical Theory of Optics

By Dr. R. K. Luneburg. Supplementary Notes by M. Herzberger. Pp. xxx+448. (Berkeley and Los Angeles: University of California Press; London: Cambridge University Press, 1964.) 105s. net; 12.50 dollars.

MATHEMATICAL *Theory of Optics* originates in a collection of notes for a course of lectures given in 1944 by the late Dr. R. K. Luneburg at Brown University. It replaces mimeographed notes issued later by the University, and since the author did not often publish his work in the journals, the present book is one of the few means of access to his rigorous and original approach to the mathematical theory of optics.

Geometrical and diffraction optics have usually been treated as quite separate fields, and often in developing them little reference has been made to electromagnetic theory. In the first chapter of his book, Luneburg shows the way in which, by considering the propagation of electromagnetic discontinuities (wave-fronts), one is led, through the characteristic equations of Maxwell, to the identification of orthogonal trajectories of such discontinuities with light rays. He notes that the possibility of deducing the principles of geometrical optics in this way is not so much because they are inherently contained in Maxwell's equations but rather that in any event only a few of the general premises of a wave theory are necessary for this deduction. In this treatment the laws of reflexion and refraction, Huyghens's construction, Fermat's principle, and Fresnel's formulae appear in discussion of the transport of discontinuities through media of discontinuous optical properties, and not, as is more usual in the case of the latter, by the consideration of the transport of monochromatic periodic waves of small wave-length.

Two chapters are devoted to Hamilton's theory of geometrical optics, a derivation of the characteristic functions, and some instructive applications of this theory to special problems. Among the latter the theory of Cartesian ovals is discussed. There are a number of interesting examples of systems such as Maxwell's fish eye in which refractive index is a function of radius alone, and some formulae relating to the final correction of systems by aspheric surfaces are derived. Included, also,

is a detailed analysis of the 'Luneburg lens'—a lens of varying refractive index which images two spherical surfaces on to one another stigmatically. This type of system has, of course, been used for micro-wave antennae. Discussion of geometrical optics is completed by two further chapters on first and third order theory.

In a chapter on the diffraction theory of optical instruments, expressions are derived for the propagation from a periodic dipole, and for the resultant diffraction patterns near the focus of perfect and aberrant wave-fronts. Following consideration of the resolution of self-luminous and non-self-luminous points, there is a discussion of the effect of controlling the pupil function. This leads, for example, to the conclusion that while the contrast of the diffraction image of a periodic structure can be improved by apodizing, the resolving limit cannot be improved in this way. This latter work by Luneburg is among the first of its kind.

One appendix summarizes definitions and theorems of vector analysis, and in another, a vector form of the laws of reflexion and refraction developed earlier in the book is used to solve some practical problems of ray tracing through plane surfaces. From time to time throughout the book, reference is made to problems of electron optics, and at the end there is a set of supplementary notes prepared by Dr. A. Blank which deals with the associated theory of electron optics. In a second set of supplementary notes by Dr. M. Herzberger the properties of optical materials are discussed.

An advantage of this book, which perhaps stems from the fact that it is based on a series of lectures, is that there are frequent helpful digressions to prove detailed points in the analysis. This completeness offsets the scarcity of references, and in a similar way the fact that there is no index is offset by a long list of contents of what is to be found in each of the chapters, paragraphs and sub-paragraphs.

From all except perhaps the expert mathematician this book will demand painstaking study, but the rewards for this would be considerable. Despite the fact that, because of its earlier origins, modern topics such as coherence, wave-front reconstruction, and lasers are not mentioned, and that the optics of crystals and metals are not included, few will disagree with the comment by Prof. E. Wolf in the foreword, that it is a "highly original contribution to the optical literature", and one which is "by no means a compilation of generally available knowledge".

W. J. BATES

SUGAR METHODOLOGY AND DATA

Methods in Carbohydrate Chemistry

Edited by Roy L. Whistler. Vol. 4: Starch. Pp. xvi+335. 96s. 6d. Vol. 5: General Polysaccharides. Pp. xxii+463. 118s. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1964, 1965.)

Oligosaccharides

A Comprehensive Account of All Known Sugars of the Oligosaccharide Class of Compounds. By R. W. Bailey. (International Series of Monographs on Pure and Applied Biology. Division: Biochemistry, Vol. 4.) Pp. vii+179. (London and New York: Pergamon Press, 1965.) 60s. net.

THE first two volumes are a continuation of the now well-known and valuable series of *Methods in Carbohydrate Chemistry* originated by Profs. Whistler and Wolfson.

The policy of these editors is to select an important technique or analytical procedure and then invite an international authority to describe concisely and accurately the best proved method for a particular operation.

In Volume 4 on starch methods, more than sixty well-known carbohydrate workers united to produce seventy-five descriptive articles, and the result is a brilliant reference book for all those concerned with this important foodstuff and industrial chemical.

There are six divisions in the book which concern the preparation of starch and starch-fractions; chemical analyses; physical analyses; microscopy; starch degradation; starch derivatives and modifications, separate methods being provided for whole and modified starches, for starch bases, starch fractions and starch hydrolysates. The well-proved methods are precisely described in a manner very easy to repeat, and important and adequate references and cross-references are provided. The physical analytical descriptions are outstandingly good. No chemist or chemical engineer who deals in any way with starch can afford to be without this excellent reference book.

Volume 5, which, for the time being at least, ends the series, is concerned with the wider field of polysaccharide chemistry and the reader is introduced to new and rapidly expanding fields of macromolecules in the polysaccharide group. Here some seventy-five authors produced eighty-seven methods. Matters dealt with are: general isolation procedures, polysaccharide preparations, chemical analyses, physical analyses, determination of molecular weight, structural methods, derivatives, esterification and deacetylation, and etherification. It is difficult to pick out parts of the book for special mention, but the section on structural methods, Section 6, which includes descriptions of the methylation techniques, periodic and other oxidation techniques, degradation, etc., is of the greatest importance. In this section an up-to-date table of properties of carbohydrate methyl ethers is of special value.

Both volumes contain a wealth of information readily available for immediate reference. The presentation and printing are of the highest standards, and the editors, the authors and the publishers must be warmly congratulated on this excellent and valuable series. The chapters are extremely well referenced and cross-referenced, and the books contain suitable general and author indexes.

Oligosaccharides, by R. W. Bailey, will be welcomed by carbohydrate chemists. The oligosaccharides consist of a few simple saccharides units joined together by glycosidic union. It is now comparatively easy to obtain these compounds and to decide their molecular structure and they can readily be given unambiguous definitive names. It is less easy, however, without giving them quite trivial names, to speak or write about them.

Prof. W. Whelan, who writes a brief introductory note in this book, invented a series of short systematic names based on trivial names, which have been adopted by the *Biochemical Journal*, and the author has made use of these names in this work. The book is mainly an informative catalogue or source book (up to 1962) of the oligosaccharides, the number of which has grown rapidly in the past decade. The many origins, whether from naturally occurring compounds or by synthesis, of the compounds are first described (Chapter 2), and then in Chapter 3 their preparation, the methods of determination of their properties and structures are given. In a further eight chapters, each of which classifies a number of related oligosaccharides into a group, the compounds are individually described with key physical properties and explanatory notes provided. Where appropriate, the properties of derivatives are also given. The author has gathered together, evidently following a tremendous literature search, a mine of information in this important field. Excellent references are provided at the end of each chapter and there is also a classified index of oligosaccharides and a general index. This book makes quite fascinating reading and is a necessity for all reference libraries.

M. STACEY

ACTIVITIES OF ENZYMES AND ENZYMOLOGISTS

Structure and Activity of Enzymes

Federation of European Biochemical Societies, Symposium No. 1, held in London on 24th March, 1964. Edited by T. W. Goodwin, J. I. Harris and B. S. Hartley. Pp viii+190. (London and New York: Academic Press 1964.) 37s. 6d.

THE Federation of European Biochemical Societies came into being in January 1964. At its first annual meeting in London in March 1964 one of the main attractions was a symposium on the structure and activity of a few selected enzymes. *Structure and Activity of Enzymes* is a record of that symposium.

Bovine pancreatic ribonuclease provided the first subject for discussion. F. M. Richards described attempts to determine which of the 124 amino-acid residues are responsible for the manifestation of catalytic activity. Ribonuclease is particularly interesting, since it can be cleaved into two fragments, the so-called *S*-peptide and *S*-protein, which are separately inert but almost fully active when mixed. This observation, and the study of the behaviour of synthetic fragments of the *S*-peptide in association with the *S*-protein, have opened up fascinating possibilities of explaining how amino-acid residues interact in a polypeptide to determine the molecular conformation and, ultimately, the biological activity. Chemical modification has indicated that two histidine residues are essential for enzyme activity, and this view is supported by the extensive kinetic studies described by A. P. Mathias, A. Deavin and B. R. Rabin. While there is disagreement about the details and uncertainty about the role of some apparently essential residues, the main features of the reaction mechanism now seem reasonably certain.

Parallel efforts to determine the amino-acid sequence of chymotrypsinogen by F. Šorm and his colleagues in Prague and by B. S. Hartley in Cambridge are reported. The substantial measure of agreement between the two schools is a welcome demonstration of the validity of the methods for sequence determination when applied to fairly large proteins. The similarity of trypsin and chymotrypsin, pointed out by T. Hofmann, has been further underlined since this symposium was held (Hartley, Brown, Kauffman and Smillie, *Nature*, 207, 1157; 1965). Although X-ray crystallography played a remarkable part in the elucidation of the structures of haemoglobin and myoglobin, it is clear from two papers that it has not yet contributed much to our knowledge of the structure of chymotrypsin.

The third section of the symposium was devoted to investigations of the active sites of enzymes, and the variety of methods used illustrates the armoury of the molecular enzymologist. The technique of inhibiting an enzyme with a selective irreversible inhibitor and then determining the site of reaction by degradative methods is exemplified by investigations on esterases (R. A. Oosterbaan and J. A. Cohen) and dehydrogenases (J. I. Harris). A complementary procedure utilized with carbonic anhydrase (B. G. Malmström, P. O. Nyman, B. Strandberg and B. Tildander) involves the use of inhibitors containing heavy atoms followed by X-ray crystallographic investigations. The final outcome of this work will be awaited with considerable interest. In contrast, the site of binding of pyridoxal 5-phosphate to muscle phosphorylase and glutamic aspartic transaminase has been investigated (E. H. Fischer) by reducing the azomethine linkage, involving the aldehyde group of the co-enzyme and the ϵ -amino group of a lysyl residue in the protein, to a secondary amine which then survived degradative studies. Present evidence strongly suggests that azomethine linkages are a common method of binding of substrates or co-enzymes to enzymes.

The final section of the symposium was devoted to haemoglobin. While this protein is not an enzyme in the sense that it catalyses a chemical reaction, it nevertheless provides a useful model of several features of enzyme chemistry: equilibrium between protein and substrate and its dependence on pH, conformational changes induced by substrate, and molecular aggregation of the protein. All these topics received attention. An unexpected result of the X-ray crystallographic studies of oxyhaemoglobin and reduced haemoglobin was the discovery that the β -chains are further apart in the absence of bound oxygen.

This is a reasonably priced book which provides examples of the techniques used in the progress made by molecular enzymologists in recent years.

D. T. ELMORE

BIOLOGICAL RHYTHM RESEARCH

Biological Rhythm Research

By A. Sollberger. Pp. xx + 461. (Amsterdam, London and New York: Elsevier Publishing Company, 1965.) 140s.

IN this lengthy book, Prof. Sollberger sets out to discuss a very wide range of biological systems, many of which appear to have little in common except their overt rhythmical nature. 'Pure' physiological rhythms, such as heart beat, are featured, with medical examples particularly to the fore. Rhythms 'with external correlates', especially those with circadian (diurnal) periodicities, occupy a central position. But photoperiodism is also included, and even population cycles are given a place.

The study of biological rhythms has gained great impetus from the comparatively recent discovery that many circadian rhythms are remarkably persistent when studied in constant laboratory conditions. When free from the phase-setting stimulus of environmental cycles of light and darkness, the period of such endogenous rhythms usually differs slightly from the 24 h solar day. But it still exhibits a striking constancy, often remaining virtually unchanged for weeks or months on end. It is this attribute that has led to the common usage of the term 'biological clock'. Clearly, one of the main goals of rhythm research is to characterize these 'clock' systems as physico-chemical entities. So far, however, only slow progress has been made in this direction. The relationships between the environmental input and the output of the various 'indicator processes' have been explored in a wide range of animals and plants. But the clock itself remains a 'black box' which has defied further degradative analysis into separate components. Indeed, its very whereabouts has remained elusive. In these circumstances it is perhaps scarcely surprising that many physiologists have turned to the construction of model systems, the most popular of which has undoubtedly been an analogue, the electrical oscillator.

A substantial part of *Biological Rhythm Research* is devoted to laying the foundations for model building. There are chapters on the elements of general cybernetics, on servo-mechanisms, on oscillators of different types and on equations of motion. This leads into extended surveys of physical and mathematical models and of oscillator control and interaction. Although the general biologist may find this exposition salutary, he would certainly wish to be informed of the precise relevance of this information in the context of biological rhythms. A critical re-assessment of the validity of the proposed analogues in the light of the wealth of existing experimental data would certainly have been welcome. Can the models be tested? Do they permit prediction? Are they only qualitative? Do they materially aid the comprehension of what must be a biochemical system?

Unfortunately, these questions are neither posed nor answered. Indeed, the results of physiological research are given very cavalier treatment. Rather few biological examples are discussed in any detail; and, as a substitute, all too many paragraphs tend to finish in a volley of references (for example, p. 138 contains some ten lines of text and 175 authors' names).

The sections on photoperiodism are inadequate. The phenomenon is never defined nor is it clearly distinguished from photo-controlled circadian processes. Whether rhythmic endogenous mechanisms play any significant part in photoperiodic time measurement is, of course, a question which is attracting considerable present-day interest. But this problem has not been considered or even recognized. The selection of material is also most unrepresentative of the whole field of photoperiodism. Yet room is found for much entirely extraneous information on photosynthesis, chromatophore systems, the perception of polarized light, etc.

Chapters are sub-divided into a series of numbered paragraphs. Continuity of thought is not assisted by the fact that the subject-matter of adjacent paragraphs is often quite unrelated. The bibliography is immense (123 pages) but is far from being comprehensive. Its usefulness could have been improved by grouping papers under subjects.

As a text on the biological aspects of rhythms this book must be accounted a disappointment.

A. D. LEES

RADIATION PRESERVATION OF FOODS

Radiation Preservation of Foods

(Proceedings of an International Conference, Boston, Mass., September 27-30, 1964.) (Publication No. 1273.) Pp. 424. (Washington, D.C.: National Academy of Sciences—National Research Council, 1965.) 9 dollars.

FOOD scientists and technologists will be interested in a new book, *Radiation Preservation of Foods*. This book contains an excellent compilation of papers on the subject of the title. The conference was divided into four sessions dealing separately with technological aspects, source facilities, wholesomeness and food legislation, and microbiology. The appendix which follows consists of further papers dealing with the present scope and directions of food irradiation research.

Irradiation of fruits and vegetables appears to be more practicable than irradiation of flesh foods, because only the surface of the structure need be irradiated to eliminate infective yeasts and moulds. This can be done with relatively low-energy radiation which would cause only the surface of the fruit or vegetable to absorb a high dose, whereas the interior would absorb scarcely any radiation. 200-250 krad is the maximum dose most fruits can tolerate without excessive injury. This level of radiation decreases the ascorbic acid content slightly but the reduction is not nutritionally significant. On the other hand, at least 175 krad is necessary for beneficial effects in most fruits. This restricted dose range may make commercial fruit irradiators difficult to build. Off-odours and off-flavours may develop and afterwards disappear on storage of irradiated fruit. After disappearance of the off-odour the irradiated fruit is often rated organoleptically superior to the unirradiated material. Strawberries seem to be the most promising fruit for radiation preservation. Similar doses to these are suitable for extending the storage life of many species of fish, but radiation sterilization of fish does not appear to be possible. Irradiated beef develops undesirable organoleptic changes to a greater extent than lamb or veal. These, in turn, are more sensitive to flavour development than pork or chicken. The order of increasing sensitivity to undesirable flavour

development is approximately proportional to the increasing myoglobin content of the skeletal muscle tissue. These effects are to some extent caused by proteolytic enzyme changes on post-irradiation storage and can be minimized by pre-irradiation heat treatment. Undesirable pigment changes made radiation pasteurization of fresh meat unacceptable.

For radiation doses up to 1 Mrad most food packaging materials already in use are suitable, but above this dose some organoleptic changes tend to develop in the food-stuff. Only metal containers are as yet suitable for sterilizing dosages but research is under way to produce a flexible pack.

In Canada a clearance has been issued allowing commercial potatoes to receive 15 krad of cobalt-60 radiation, and in the United States irradiated canned bacon has been consumed. No adverse public reports have been received about these commodities, and extensive taste tests carried out by the United States army have shown that irradiated foods are acceptable as components of normal meals.

In the United Kingdom we are still awaiting action resulting from the recommendation of the working party on food irradiation.

G. BIRCH

PHOTOBIOLOGY UP TO DATE

Recent Progress in Photobiology

Edited by E. J. Bowen. (The Proceedings of an International Congress held at Oxford, July 1964.) Pp. vii + 400. (Oxford: Blackwell Scientific Publications, 1965.) 70s. net.

THE fourth International Photobiology Congress, held in Oxford in 1964, was the largest of the series and was attended by more than 500 scientists who represent the vanguard of the many fields of research falling within the term 'photobiology'. The *Proceedings* of the Congress reflect the determined effort made by the organizers to restrict the size of the sessions and the number of papers being read simultaneously by presenting about one-third of the submitted papers in review form, rather than allowing their authors to read them individually. These reviews were made by independent 'rapporteurs' who each selected about ten papers well before the Congress and from these prepared a 45-min summary. Thus the papers could be linked and their important features brought out while sparing the audience, and the readers of the *Proceedings*, the preambles of each of the authors.

The successful application of this technique was due almost entirely to the efforts of the rapporteurs, who are both good writers and leaders in photobiology. The seven rapporteur sessions covered the topics: basic photochemistry; photochemistry of nucleic acids; visual processes; structure of light-receptors; photo-environment; energy conversion in photosynthesis; and micro-irradiation of cells. The sessions comprised a 45-min plenary lecture by a distinguished speaker followed by the rapporteur's report. The authors of the reviewed papers were then allowed a short time to amplify any important points, and thus led into a general discussion. The *Proceedings* give these sessions in full and also the reports and a few papers from the symposia on space photobiology and on skin pigmentation.

This, however, is all that *Recent Progress in Photobiology* contains, and there is no mention of the papers—the majority of those presented—which did not fall into the confines of the rapporteur sessions and so were read by their authors in separate symposia. The editor points out in his preface that authors have been encouraged to publish their work in specialist journals, but not even a list of these papers or even a complete list of participants is given. It seems unfortunate that these *Proceedings* mention less than half the activities of the Congress. It will also be difficult for an interested reader to obtain

further information on the work abstracted by the rapporteurs, since in four of their reports no references are given, and in only one report are the addresses of the authors given. The blank pages separating the sections of the book might well have been used to give outline addresses of the participants in each session.

This book is well produced and is very reasonably priced. The editor and the publishers are to be congratulated on producing it so soon after the Congress. Bearing in mind the restrictions in scope due to limiting its size, this book forms a valuable and comprehensive survey of photobiology to-day. The introductory lectures are good reviews of the seven important topics listed, and, of these, the lectures of Dr. David Shugar on the photochemistry of nucleic acids and Prof. Melvin Calvin on energy conversion in photosynthesis deserve special mention. The rapporteur reports are easy to read and, while giving a rather limited picture of present-day research, are well worth reading. The book is recommended to all scientists from physicists to physicians who are interested in the action of non-ionizing radiation on biological material.

A KNOWLES

ZOOLOGICAL MICROPALAEONTOLOGY

Principles of Zoological Micropalaeontology

Vol. 2. (International Series of Monographs on Earth Sciences, Vol. 20.) By Dr. V. Pokorný. Translated by K. A. Allen. Pp. ix + 465. (Oxford, London and New York: Pergamon Press, 1965.) 80s.

MICROPALAEONTOLOGY has for a long time played an important part in geological science, especially as applied commercially. In recent months this aspect has been brought more to the notice of people in Britain with the present North Sea drilling project for oil and gas, and in the investigation of a possible location for a Channel Tunnel.

Besides a purely commercial aspect there is in addition a considerable academic interest in the sphere of micropalaeontology. From both points of view an essential tool is a good standard text-book which combines the various groups in a more general approach than is often the case in the specialist publications.

For some years now, the German edition of Pokorný's *Principles of Zoological Micropalaeontology* has been available as virtually the only book in its field. The English translation of these two volumes (of which the book under review is the second) has long since been overdue and should now prove, to an even greater extent, to be a valuable work of reference to students and professional workers alike.

Pokorný has provided in the two volumes a complete work on all aspects of zoological micropalaeontology and is to be congratulated on a first-class piece of work. Because *Principles of Zoological Micropalaeontology* is a straightforward translation of the 1958 German edition, certain aspects of the work are now slightly out of date. However, considering the pace with which scientific thought progresses these days, surprisingly little has been really affected.

The treatment, in the first three chapters, of the Porifera, Octocorallia and Scelcodonta indicate from the start the wealth of information to be found throughout this book. This is certainly true for the conodonts, which are dealt with in some detail. One point I should like to elaborate on here is the separation by Pokorný of the conodonts into two distinct groups, lamellar and fibrous. This distinction is not at present recognized, since a transition between lamellar conodonts and those showing a secondary fibrous state has been observed within a number of individuals.

The major part of this volume is devoted to a detailed study of the Ostracoda, a group which is becoming increasingly important. This section is introduced by a lively and informative consideration of ostracod morphology, ecology and evolution, together with a historical résumé of the classification. A common misunderstanding among palaeontologists is to suggest that the two ostracod valves are connected along the hinge margin by an elastic ligament. No such structure has yet been observed in living species. This idea of an elastic ligament is put forward in several places by Pokorny without further explanation, and all one can suggest is that he is regarding the outer chitinous layer of the carapace which passes over the hinge as having elastic properties in that region. Again there is no evidence of this.

The major part of the ostracod chapter is devoted to a detailed systematic description of a large number of genera. Here Pokorny has given the lead in raising the Ostracoda to the rank of sub-class and in re-introducing G. W. Muller's grouping (1894) of the Podocopa with the Platycopa and the Myodocopa with the Cladocopa, an arrangement now adopted by the majority of ostracod workers. A useful glossary in English, French, German and Russian is appended to this section. Moreover, fish otoliths and echinoid and crinoid skeletal remains, constituents of many microfossil samples, are considered in the final chapters of the book.

The author has produced an informative volume which is the result of much original thought. The reference section at the end will be especially appreciated by those wishing to take their studies further. One serious omission from this book, in my opinion, is a generic index. This is really essential when dealing with a large group like the Ostracoda.

On the production side, Pergamon Press have produced a book of high quality containing a wealth of illustrations, the reproduction of which is extremely good. Printing errors are rare and unimportant.

Pokorny's text-book is still the best in its field and is to be recommended to all who work with microfossils.

R. H. BATE

CHEESE

Cheese

By Dr. J. G. Davis. Vol. 1: Basic Technology. Pp. viii+463. 75s. net. Vol. 2: Annotated Bibliography with Subject Index. Pp. vii+275. 65s. net. (London: J. and A. Churchill, Ltd., 1965.)

FROM time immemorial cheese has been regarded as one of the most appetizing and nourishing of foods, and has been recognized as one of the best forms in which to preserve the greater part of the most nourishing constituents of milk for future use. To-day, as Dr. Davis points out in the preface to the first volume of *Cheese*, about 3 million tons of cheese are made in the world every year, and there are now probably more than a thousand different kinds. Dr. Davis has done a great service to dairy science and to the dairy industry in general in undertaking the compiling of this comprehensive work, which in all is to consist of four volumes. The first two volumes are already available, the third is to deal in detail with manufacturing methods and will be ready in 1966, and the fourth will give an account of scientific aspects of the subject and will appear in 1967.

Volume 1 consists of four parts and begins with a most interesting historical section which deals with what cheese really is, the origins of cheese-making and the development of the industry in Europe and Britain. This is followed, still in the introductory section, by a short but most informative outline of the scientific basis of cheese-making, and by a chapter on classification of cheese varieties. This classification in itself is most helpful in

these days when so many different types of cheese are readily available and when so many of them, with a great variety of names, are now made in countries other than those with which their particular types were first associated. Part 2 of Volume 1 deals with milk itself and with the importance of its physical, chemical and microbiological properties in the manufacture of cheese and how these properties affect the quality of the product. Descriptions are also given of the tests that are applied to the milk to detect abnormalities in it and of the treatment to which milk is subjected in a modern cheese factory. Since the making of cheese depends partly on the growth of lactic streptococci and its maturing partly on the development of lactobacilli, the author has dealt with the occurrence of milks that inhibit the growth of these organisms and also with the problem of antibiotics in milk.

Part 3 gives an account of the materials and the equipment used in the making of cheese. Five chapters here are devoted to 'starters', what they are, and factors affecting their preparation, behaviour and control. Another two chapters discuss the preparation, properties and use of rennet and the chemistry of the reaction which the enzyme rennin catalyses in milk. The additives that may be used in cheese-making, such as salt, annatto and other colouring matters, and materials such as wax and plastics that may be applied for protective purposes to the rind of cheese, are also very adequately discussed. At every stage in modern cheese-making scientific control is essential to ensure a first-rate product, and the methods used for this purpose are described in Part 3, which ends with a particularly informative chapter on traditional and modern equipment used in the manufacture of cheese. Finally, Part 4 gives an account of modern methods of packaging and also of the ripening and storage of cheese.

Throughout this first volume the author has maintained an excellent balance between the space he has given to descriptions of the older traditional methods, which are in themselves so interesting, and the amount of space he has devoted to modern developments such as the move towards complete mechanization of the Cheddar process, the invention of continuous cheese-making machines and the vacuum cheese presses which are now being evolved. This first volume is well illustrated with a large number of first-rate diagrams and photographs.

Volume 2 consists entirely of a list of 6,803 publications relating to cheese together with a most comprehensive subject index which alone occupies 47 pages. The references are arranged in alphabetical order according to the name of the first or sole author, and in addition to the usual information, such as date, journal and page number, a few words are added beside each reference such as "penicillin in milk and starters", "whey separation" or "gorgonzola manufacture", to indicate briefly but clearly what each paper is about. As the author of the volume rightly indicates in his preface, when this bibliography and subject index are used together it is possible to obtain in a few minutes a list of references to the literature available on any aspect of the subject. In the preparation of this volume, Dr. Davis was assisted by Miss Doris Knight, formerly librarian at the National Institute for Research in Dairying, Reading. They have attempted to cover the scientific and technical literature on cheese from the earliest times to 1961, and they have endeavoured to ensure that the period between 1940 and 1961 has been particularly well covered. Volume 4 will bring the bibliography up to date by dealing with the period from 1962 to 1966.

This bibliography and index should be of great value to research workers, dairy technologists, teachers and students and to all those whose job and interest it is to be well informed on cheese and related subjects. As the author rightly suggests, it is probably unique, and certainly it fills a gap so effectively that the pattern is one

that will surely be followed by others. The author and publishers are to be congratulated on the excellence with which these two volumes have been produced at what is in these days a relatively modest price.

J. A. B. SMITH

WHEAT

Wheat

(World Crops Books.) By R. F. Peterson. Pp. xxiv + 431 + 77 plates. (London: Leonard Hill Books; New York: Interscience Publishers, Inc., 1965.) 95s.

WH^{EAT} is the thirteenth in the *World Crops Books* series and follows the pattern of its predecessors in providing information on every aspect of significant knowledge from chromosomes to world trade. The objective is, to say the least, ambitious although the intention is stated to be only to give a "broad outline of the botany, cultivation and utilization of wheat" and to provide for the student a general introduction to wheat. For the specialist it is a reference book providing a guide to reading outside his speciality, and the text is supported by a subject index of fourteen pages for this purpose. It would have been impracticable to have given anything like a reasonably complete coverage in the bibliography of so wide a field of subjects as is dealt with in the book, and the relevant eight pages can only be regarded as a limited guide to what is probably the most extensive literature on any single crop.

Dr. Peterson, who is head of the Cereal Rusts Section of the Agricultural Research Station, Winnipeg, deals with his subject in nineteen chapters occupying 371 pages of text, and there are three appendixes of tables and a useful glossary. In planning a book which attempts such a complete picture of a crop it is obviously difficult to decide on an acceptable balance of subjects, and the final decision is important in giving the book its character. The author lays the greatest emphasis on the botany and general biology of wheat, including breeding and disease resistance, devoting 177 pages to these basic aspects of wheat. He then deals with the cultivation and handling of the crop in 59 pages, the grain and its utilization in 30 pages, and economic considerations with a similar allocation of space. The book is copiously illustrated by 77 photographic plates, some in colour; 46 text figures as diagrams and maps; and 36 tables. These various aids to the text are well chosen, the photographs being very good and the figures clear and explicit.

Of equal importance in gauging the value and use of such compendious volumes is the coverage of the individual subjects included and the technical and scientific level of treatment. The publishers have produced this book for the general reader or the student of agriculture, while providing a reference book for the specialist. The result is that much of the text dealing with the botany, for example, has to be at an elementary level and includes information that can be found in a number of standard books on agricultural botany. The genetics and cytology are similarly treated, starting with the cell and cell division. The author does manage to bring to a focus the most recent information and interpretation of such matters as the cytogenetics of wheat, however, and this achievement is valuable for the student and general reader. But the overall treatment does mean that it has been thought necessary to include a considerable number of elementary figures such as illustrations of plant cells and figures depicting mitosis and meiosis.

The situation is realized by the author, who remarks in the opening to the chapter on achievements in wheat breeding: "A full account . . . would require several volumes written by workers familiar with the details of the various breeding programmes". He deals with the subject in 37 pages, with 20 lines devoted to the United

Kingdom and just over 7 pages to the North American continent. It might also be commented that it is virtually impossible, in my opinion, to deal satisfactorily in 19 pages with the objectives, methods and organization of wheat breeding on a world basis. It would be easy, for example to quarrel with Dr. Peterson's allocation of space and treatment of such subjects as the induction of point mutations, hybrid wheat and plant breeding by "directed training" according to the precepts of T. D. Lysenko.

However, to produce a book of this kind is a considerable task, and Dr. Peterson has succeeded in writing lucidly and attractively on a wide range of topics to provide a useful addition to the series on world crops under the editorship of Prof. Nicholas Polunin. It would be difficult to find comparable information on wheat without consulting a number of books or even original scientific papers, and, no doubt, many enquirers on wheat topics will be most grateful for this contribution. G. D. H. BELL

WEST AFRICAN FLOWERING PLANTS

Introduction to the Flowering Plants of West Africa

By M. Steentoft Nielsen. Pp. ix + 246. (London: University of London Press, Ltd., 1965.) 42s.

AT a time when education in all the West African countries is surging ahead, suitable text-books must be in great demand. Perhaps of all scientific subjects, biology requires books specially written for each area of the world. West Africa is now served by several botanical works which should stimulate interest in the native plants of the region; this is a pre-requisite for an understanding of the need to conserve the natural flora and for its most suitable utilization.

Mrs. Steentoft Nielsen begins the work in a conventional way for a botanical text-book by describing the morphology of an easily obtainable plant: the cowpea. (It might be mentioned that as the cultivated plant has been selected the name *Vigna sinensis* rather than *V. unguiculata* would have been more appropriate.) A useful chapter deals with morphological modifications and adaptation using West African—although not always common—examples. Other chapters tackle classification and ecology, while a large one is devoted to an analysis of West African vegetation. It is based appropriately on the classification of vegetation types used in the *Vegetation Map of Africa*, published by Association pour l'Étude Taxonomique de la Flore d'Afrique Tropicale in 1958. A curious feature of the ecological section is the frequent digression in the text by the author to provide a morphological description of an important species not described elsewhere.

More than half the book is devoted to the description of 128 families in greater or lesser detail according to their "ecological importance". The same criterion is used for the selection of those genera and species discussed and described. There may be too much detail for the average student, but if his study is backed up by field work and by judicious selection by his teacher he will find the information easy to assimilate. The nomenclature and classification are based on the standard *Flora of West Tropical Africa*, using the revised edition for the dicotyledons and the first edition, with modifications, for the monocotyledons. Here and there certain statements and generalizations may be questioned, but this is almost inevitable in a work that contains so many small details, and occasionally names have been switched. The 68 figures by the author herself are a useful feature, providing the student and teacher with clear drawings of dissections and floral diagrams not easily obtainable elsewhere. However, some are rather stylized and one at least (Fig 48) has certain inaccuracies.

The publication of Mrs. Steentoft Nielsen's book is something of an event. The author (*née* Margaret Fox) herself taught at the University of Ibadan and her book should have a wide appeal to university and college students, also to school-teachers, interested amateurs, foresters and agriculturalists, who will find it a work of reference as well as an introduction to West African plants.

F. NIGEL HEPER

A SULPHURETUM OF BACTERIA

Photosynthetic Bacteria

By E. N. Kondrat'eva. Translated from the Russian by Jean Salkind. Edited by Dr. E. Rabinovitz. Pp. 243. (Jerusalem: Israel Program for Scientific Translations; London: Oldbourne Press, 1965.) 72s.

THE photosynthetic bacteria are divided into three main families, Athiorhodaceae, Thiorhodaceae and Chlorobacteriaceae; the first two are known as the purple non-sulphur and purple sulphur bacteria, respectively, and the last as the green sulphur bacteria. The Thiorhodaceae and Chlorobacteriaceae are strict anaerobes and utilize hydrogen sulphide and other sulphur compounds as a source of reducing power in photosynthesis, while the Athiorhodaceae are generally photoheterotrophes, although some are facultative aerobes and can grow in darkness.

The modern era of study of the photosynthetic bacteria began with the now classical investigations of van Niel in the 'thirties; since then these organisms have been the subject of numerous investigations relating not only to photosynthesis but also to their general metabolism. As experimental material for studies on photosynthesis, they have many advantages over the more intractable higher plants; but in one respect they, themselves, are intractable: to my knowledge no one has yet succeeded in producing satisfactorily active chromatophore fragments. Comparative biochemistry has also benefited greatly from studies with photosynthetic bacteria.

Although many excellent reviews exist on various aspects of the biochemistry of photosynthetic bacteria, Kondrat'eva's book is the first comprehensive monograph on the subject. His claim "to sum up the data concerning photosynthetic bacteria" is justified if one adds the phrase 'up to early 1962', when references cease. It can therefore be recommended as a satisfactory source book of the literature up to that time. The further aim to "provide a clearer concept of the prospects of future research in the field" is less successfully achieved because little or no critical assessment of the data is made and because, between the time the author completed his survey and its appearance in an English translation, a large number of important investigations have been reported. The topics treated include distribution, isolation, cultivation, morphology, chemical composition, physiology, photosynthesis and taxonomy. There is also a separate chapter on pigments, which have attracted the attention of chemists and biochemists for many years. There is unfortunately no subject index—presumably this was also missing in the Russian edition—and the bibliography is divided into two sections: (a) references to papers in Russian (including translations into Russian); and (b) 'others'.

Apart from a few peculiarities, such as the use of 'oxy' for 'hydroxy' and 'phenazine metasulphate' for 'phenazine methosulphate', the translation appears to be very satisfactory. A small number of obvious typographical errors were observed but errors of fact were few; the structures of farnesol (p. 88) and γ -carotene (p. 91) are, however, incorrect.

Photosynthetic Bacteria has been produced by a photo-offset technique, and while the typography is satisfactory,

one or two of the diagrams are not easy to decipher. The half-tone plates of electron micrographs are less satisfactory and one doubts if the authors who gave their 'kind permission' for reproduction of plates will feel that they have been kindly treated.

The price is high, 72s. for 243 pages, and compares rather unfavourably with the usual present-day cost of 25–30s. per 100 pages of a conventionally printed book, containing about the same number of words per page. One wonders whether the photo-offset technique is a sensible choice if the price of the final product is not considerably lower than that of a conventional book.

In summary, this is a book to be recommended for the library of any department concerned with research in photosynthetic bacteria.

T. W. GOODWIN

EVOLUTION OF STATE MEDICINE

Public Health in the Nineteenth Century

By Prof. C. Fraser Brockington. Pp. viii + 287 + 16 plates. (Edinburgh and London: E. and S. Livingstone, Ltd., 1965.) 42s. net.

PESTILENCE has always been a stimulus to public health administration and legislation. The edicts of Henry VIII, drawn up with the advice of Sir Thomas More, concerning plague and the sweating sickness, and those of Charles II and the Corporation of London on the Great Plague, are examples of this stimulus. The College of Physicians was frequently consulted as epidemics of disease were threatened or appeared. In the reign of George I, when bubonic plague ravaged Marseilles, the College appointed Dr. Mead to report, and he wrote his *Treatise on the Plague* (1720) in which he advocated a Central Board of Health with other precautions. The plague did not come to England and no action was taken on his recommendation.

In this general study of public health in the nineteenth century, Prof. Brockington first directs attention to early measures in State medicine which preceded the wider-known public health administration inspired by Southwood Smith, Arnott, Kay, Chadwick and John Simon.

Towards the end of 1804 the Privy Council consulted the College of Physicians as to how best the "Gibraltar Sickness" (an epidemic of yellow fever which had spread from Africa to Spain and killed 5,733 persons out of a population of 15,000) "might be prevented from extending its malignant effects, and what was proper and material to be known by the public at large on the subject of infectious complaints". The College promptly replied, arguing the case for strict quarantine together with services for early diagnosis, treatment and isolation, and a strict enforcement of the "Cordon Sanitaire". A central Board of Health was also recommended; this was set up in May 1805 with the Comptroller of His Majesty's Navy as president. It included the President and four Fellows of the College among its members. It established a centre of epidemic intelligence and made five important reports to the Privy Council. Sir Francis Milman, an active member, advocated its being made permanent, but, when the continental epidemic ceased, the Privy Council terminated the Board. "Frenzied action" was followed by torpor, until Asiatic cholera spread from India into Russia in 1830, and the College of Physicians was again consulted by the Privy Council.

A Central Board of Health was established in June 1831 with Sir Henry Hallford, then president of the Royal College of Physicians, as its president, six Fellows of the College and five Government officials. This Board was responsible for much good work. It investigated the disease, described it, advised on precautions against its spread and on its treatment, and recommended local boards of health, of which 1,200 were set up. In November

1831 the "physicians board" was dissolved through dispute with the Privy Council on the duration of quarantine. In its stead a Central Board of Health, unconnected with the College, was appointed. This Board was also short-lived and was dissolved at the end of 1832.

The local boards were zealous but lacked money to do their work, although cholera had reached Great Britain. By the middle of 1832 nearly 5,000 persons had died of cholera. The Cholera Act was passed enabling the Privy Council to make orders for the prevention of cholera; and provided that any expenses incurred in carrying out such orders should be "defrayed out of the relief of the poor of the parish, township or extra parochial place, maintaining its own poor". Still the financial difficulties of the local boards continued.

The Privy Council continued to act as a central health administration for a further 18 months and then abdicated. Public health was ignored as a permanent need. Prof. Brockington points out that these early struggles in the face of epidemics paved the way for the investigations and legislation promoted by Edwin Chadwick and John Simon which he describes in the later chapters of his book. Much new information is given about the first medical officers of health and other early workers in State medicine. There are brief biographies and portraits of some of the early public health pioneers. These essays when first published in the *Medical Officer* and other medical journals were highly appreciated, and Prof. Brockington has done well to assemble them in book form.

A. MACNALTY

PHOTOPHYSIOLOGY

Photophysiology

Edited by Arthur C. Giese. Vol. 1: General Principles; Action of Light on Plants. Pp. xiii+377. 100s. Vol. 2: Action of Light on Animals and Microorganisms; Photochemical Mechanisms; Bioluminescence. Pp. xiii+441. 107s. 6d. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1964.)

AS the volume of original publications continues to expand, so does the need for comprehensive and up-to-date coverage of the principles underlying any one field of investigation. The two volumes of *Photophysiology* succeed well in doing this. Together they comprise twenty-one chapters, each a review of a particular facet of the subject, which in his preface Prof. A. C. Giese defines as the study of the physiology of the action of non-ionizing radiations—ultra-violet, visible, and infra-red—on living things. Giese emphasizes that the two volumes should be considered as an integrated whole, and indeed the basic principles outlined in the opening chapters of Volume 1 are essential background for the accounts both of the action of light on plants, which make up the remainder of Volume 1, and on micro-organisms and animals in Volume 2.

The first four chapters will be of particular value to those readers whose own research field is not photophysiology. They provide the basic theory, describe apparatus used and discuss the difficulties involved in both experimentation and interpretation. In a brief historical introduction, Giese traces the development of photochemistry and indicates the various responses of plants and animals to radiation. Most photochemical reactions are wavelength dependent and it is therefore essential for the majority of experimental investigations to have a source of high-intensity monochromatic light. In the chapter by S. Claesson, light sources, filters and monochromators, measurement of light intensity, calculation of quantum yield and flash photolysis are described. An excellent account follows of the theory of electron spin resonance by M. S. Blois, jun., and E. C. Weaver. They describe

electron spin resonance, spectrophotometers and indicate the difficulties encountered in making measurements in biological systems. A critical evaluation is made of electron spin resonance measurements in photosynthesis. A. D. McLaren discusses the photochemical effects of light on macromolecules, including enzymes, nucleic acids and viruses. Data on absorption spectra and action spectra are essential for the elucidation of the mechanism of action of radiation effects. Mary Belle Allen gives a very clear account of the problems which may arise when measurements are made using biological systems, the instruments used and the interpretation of the data obtained.

Next there are three chapters dealing with the role of light in photosynthesis. After a brief résumé of the early work which led to the demonstration of photosynthetic phosphorylation in chloroplasts, Whatley and Losada postulate electron flow mechanisms and cite experimental evidence for the occurrence and participation of intermediates. They produce a persuasive account of the one quantum—one electron transfer scheme. R. K. Clayton follows with a short but informative discussion of other possible interpretations of the Emerson effect and enhancement phenomena, and rightly emphasizes the need for an open mind until more experimental evidence is available. The 'biophysical' problems of photosynthesis, behaviour of chlorophyll *in vivo* and *in vitro*, absorption of light quanta, nature of the excited state and events at the reaction centre, form the main bulk of the chapter. L. R. Blinks then gives a concise account of the nature and role of the accessory pigments.

The four remaining chapters of Volume 1 each deal with quite different topics. Phototropism in higher plants is reviewed by W. R. Briggs. It is of interest that such a large amount of experimental work has been carried out on such a small range of experimental material. Most of the work described has been performed with either oat or corn coleoptiles. A very clear account of possible relationships between auxin and curvature, action spectra, and kinetic studies is given. H. I. Virgin brings together much useful information on such diverse topics as chlorophyll formation, chloroplast movements and protoplasmic streaming. The photochemical aspects of photoperiodicity are discussed by S. B. Hendricks, and biological clocks, endogenous daily rhythms and the modifying effects of the environment are covered in the final article by J. W. Hastings.

The first chapter of Volume 2, written by A. Wolfson, deals with animal photoperiodism. He gives a detailed description of the effects of day-length on metabolic changes, migration and reproduction in birds and briefly records facts regarding other animals. The photoperiodic response is extremely complex and the problems involved in analysing it are discussed in relation to natural behaviour and to the experimental modification of the reproductive cycle by alteration of day-length. R. K. Clayton gives a second clear account; this one, on phototaxis in micro-organisms, deals mainly with green flagellates, blue-green algae and purple photosynthetic bacteria.

The following three chapters are concerned with vision. The photoreceptor process in lower animals is surveyed by D. Kennedy, vision as a photic process by W. A. H. Rushton, and the physical limits of visual discrimination by H. B. Barlow. These are followed by a chapter by A. C. Giese, who provides a comprehensive survey of ultra-violet radiation action on animals. Action spectra, the nature of possible absorbing substances and the effect of physiological status on the types of response recorded are discussed and there is an interesting account of the use of ultra-violet microbeams to investigate effects on different parts of the cell. G. Zetterberg discusses the mutagenic effects of ultra-violet and visible light, and C. S. Rupert that strange phenomenon, the reactivation of ultra-violet light damage by visible light. For the interpretation of many of the effects on living organisms,

lucidation of the effect of ultra-violet on nucleic acids is essential. K. C. Smith provides a critical account of our present knowledge of their photochemistry and clearly indicates those areas which stand in need of investigation. The final chapter, by A. M. Chase, is devoted to bioluminescence, and here the biochemistry of the reaction in a varied collection of organisms is described.

Prof. A. C. Giese is to be congratulated on bringing together such an excellent and well-balanced selection of articles. The standard of writing and clarity of exposition are consistently high. The treatment throughout is at an advanced level and the volumes should be of interest to research workers in neighbouring fields, university teachers and senior students. The high price will unfortunately prevent many who might like to possess the volumes from purchasing them, but they should be found on the shelves of most institutional libraries.

EDITH HARRISON

THE THYROID

The Thyroid

Edited by J. Brach Hazard and David E. Smith (International Academy of Pathology. Monographs in Pathology, No. 5.) Pp. xii + 288. (Baltimore, Md.: The Williams and Wilkins Co., 1964. Distributed in the United Kingdom by E. and S. Livingstone, Ltd., Edinburgh.) 108s.

FEW organs of the human body have been the subject of such detailed and effective investigation during the past 20 years as the thyroid gland. In particular, the use of radioactive isotopes of iodine has greatly facilitated a wide range of biochemical investigation, and has made possible very many measurements of the kinetics of iodine metabolism in the thyroid gland or in different body tissues in health and in disease. The applications of radioiodine are so varied and by now so familiar that it is particularly refreshing to see a work devoted mainly to other aspects of thyroid function and pathology, of which the examination does not depend on kinetic or metabolic tracer studies, but in which progress has been equally outstanding.

For example, the examination of auto-immune processes in thyroid disease has thrown light, not only on the forms of local or general 'thyroiditis' in which they are consistently involved, and on the development of thyroid deficiency without glandular enlargement, but also on auto-immune phenomena generally in human disease. Similarly, studies of the normal pituitary control of thyroid function have failed to explain conditions of thyroid over-activity as pathological exaggerations of the normal mechanism, and the demonstration of an abnormal and long-acting stimulator replacing and over-riding the normal hormonal control is one which may have wider implications than in thyroid disease alone.

The International Academy of Pathology's monograph is based on communications presented by a number of authors at a meeting on "The Pathologic Physiology and Anatomy of the Thyroid" held in Cincinnati in 1963. The review is thus a selective one, and deals with a number of important and developing subjects, with good co-ordination of treatment and little unnecessary overlap between the work of different contributors. An excellent group of four papers deals with aspects of thyroiditis: the histological patterns associated with different clinical forms of the condition, by Woolner; the experimental production of comparable lesions, by Witebsky; the techniques of antibody identification, by Beutner; and the types of immune phenomena occurring in thyroid disease by Senhauser, based on collaborative work with Roitt and Doniach. Beierwaltes gives a useful survey of genetic factors in thyroid abnormalities, and Brown Dobyns has discussed the pituitary and other stimulator processes involved in thyroid function and disease, including the

still somewhat nebulous 'exophthalmos producing factor'. Prominence of the eyes can be due to so many different causes in different circumstances that it remains difficult to be sure of the relevance to the human condition of biological assay methods. Revealing data are given by Klinck on the distribution of normal thyroid tissue outside the anatomical gland in man, and the same subject is referred to by Gorbman in his chapter on "Comparative Pathology", which describes also the response of the gland to radiation—a subject dealt with in detail by Vickery, who discusses autoradiographic methods and artefacts with authority.

Although the papers have been developed and extended since their oral presentation, the date of the original meeting is still reflected in the references cited, of which only about 10 per cent are more recent than 1961, with only 3 per cent since 1962. Reviews do not therefore refer to recent findings such as the curious associations of amyloid-producing medullary carcinomata with pheochromocytoma, neuroma and a familial incidence of similar conditions, or the newer work on the chemistry of the thyroid-stimulating hormone and the long-acting stimulator, and the inter-relations between the circulating levels of these substances in thyroid disease.

The format and clarity of publication and illustration are excellent, with the one unfortunate exception that the very clear maps of the geographical distribution of goitre prepared by Kelly and Snedden and published in the World Health Organization monograph on *Endemic Goitre*, with goitre belts clearly shown by red hatching on grey indications of mountain areas, have been reproduced in monochrome in an otherwise admirable chapter on "Geographic Pathology of Thyroid Disease" by Scrimshaw, so that it is no longer possible to distinguish the limits or even the presence of goitre areas hatched in black on the black of mountainous regions.

The work remains an important and authoritative review of many normal and pathological aspects of thyroid function, and of techniques required in their study, and will be of value and stimulus to many whose interests are in the control of organ function and the genesis of disease.

E. ERIC POCHIN

THE EXPANDING EYE

Development of the Eye

By G. V. Lopashov and O. G. Stroeve. Translated from the Russian. Pp. 177. (Jerusalem: Israel Program for Scientific Translations; London: Oldbourne Press, 1964.) 72s.

IF there is a flaw in the last movement of the "Fifth Symphony", it is that Beethoven gave it too many endings: this book has too many beginnings. But once it reaches page 44 it cracks ahead, and it would be a grave error if one were to imagine that this is Mann's or Barber's book on ocular embryology brought up to date. In fact, the authors disclaim to have written an ordinary textbook although their book is both this and a monograph.

As a text-book it forms an approximate introduction to the subject. The student who uses it must pray that he will not be asked a question on the layers of His and Chiewitz, nor on the development of the vitreous (other students may be pardoned for whispering prayers regarding the latter question). As a monograph it offers a newly refreshing, if mechanistic, outlook on the development of the eye. Organizers have gone out of fashion, although "inducing agents" are still with it: there is no index to help you remember where exactly they are defined. But the basic idea underlying Lopashov and Stroeve's view of the expanding ocular universe is that a shove here, a little pressure there will do the trick. What makes the tissues shove, what press? Ah—that would be telling. . . . The approach enables one to understand why the embry-

onic lens is both relatively large and spherical, for it helps to stretch the retina, thus speeding its differentiation into all those layers. It is a thousand pities that this subject of embryological mechanization is not pursued as regards the bearing it may well have on the aetiology of myopia where deformation of the eyeball may be involved. In this context it is sad to report that the authors find that "accurate experimental data concerning the origin of the scleral mesenchyme is lacking" (p. 110): One also wonders to what extent birth is no more than an accident, of which the development of the eye, perhaps retarded in myopia, may not officially take cognizance. At the same time, the authors lead one to revise some older facile explanations, such as that of the transparency of the cornea on the basis that it is due to arrested embryonic development: when pigmented epidermis replaces ectoderm that would normally form a cornea, or the eye-anlage is transplanted under the abdominal dermal epithelium, depigmentation and corneal formation follow. Again, while the probabilistic approach has replaced that of predestination in general embryology, it is refreshing to see references to eye formation of greater or smaller frequency even though they are not quantified. As the probability of most tissues to form parts of eyes is almost unity to begin with but, with the exception of the chosen few, they lose it rapidly as they have other business to attend to, the study of the eye, as the authors emphasize, may be of general embryological interest. One can almost watch someone compute the odds in favour of the formation of a monster—or a thalidomide baby. Lest you think this far-fetched, conditions favouring the development of cyclopia and anophthalmia are carefully set out. It remains to be seen, however, whether the importance tentatively attributed by the authors to the blood supply in connexion with the formation of the embryonic fissure may not be exaggerated.

Now for the criticisms. The production of the book is provocative. It is printed by a lithography-cum-type-writer process so that the right-hand edges are not squared off. I welcome this if it reduces cost, for so many technical books are produced for the greater glory of their publishers; inevitably out of date in two or three years' time, such books contain Vance Packard's "built-in waste". But in this case economy went too far. The reproductions of the photographs may be acceptable to the Russian eye, but are valueless by Western standards. Moreover, it seems utterly fantastic that there is scarcely a reference to electron-microscopic studies and no photograph obtained with the electron microscope. Other anatomical writers' habit of not considering a mention of the scale of magnification *de rigueur* is regrettably shared. And why Soviet references should be segregated from the rest in a list not free from error remains to be explained. The translation is adequate even though there is some talk about 'inner ocular pressure' and 'accommodation'. Those remarks are, however, addressed to the authors. Let the reader be told that the book is stimulating, thought-provoking and definitely worth owning—for three or four years.

R. A. WEALE

CHEMOTHERAPY RESEARCH

Antimicrobial Agents and Chemotherapy—1964

Edited by J. C. Sylvester. Pp. xiii + 789. Proceedings of the Fourth Interscience Conference on Antimicrobial Agents and Chemotherapy, New York, October 26–28, 1964. (Ann Arbor, Michigan: American Society for Microbiology; London: H. K. Lewis and Co., Ltd., 1965.) 15 dollars.

ANTIMICROBIAL Agents and Chemotherapy—1964 contains many of the papers presented at an Interscience Conference in New York in October 1964. Like previous volumes in the series, it presents work which spreadeagles several disciplines, and the raw material from

which it is manufactured (as is noted in a preface by Orville Wyss) is extremely uneven. Nevertheless, most of those who are concerned with chemotherapy, whether as clinicians, microbiologists, or chemists, will find in it something of interest.

The book opens with a well-balanced article by Maxwell Finland on "New Drugs and the FDA". He directs attention to the results of two opinion polls on the new governmental requirements relating to drug safety, which appeared to show that "it was just the ones who raised the greatest fuss" (the university clinical research workers, "who don't like the idea of being regulated and restricted"). Maxwell Finland also directs attention to the important role of these research workers in attempts to improve the evaluation of new drugs and makes a plea for programmes designed to increase the supply and quality of clinical investigators in this field.

The first group of papers is concerned with the production, structures and biological properties of a number of new antibiotics, most with antibacterial activity but some with antiviral and others with antitumour properties. The discovery of several members of the lincomycin and everninomicin families provides further examples of the common finding that a new antibiotic produced by a micro-organism turns out to be one of a group of compounds which have chemically related structures.

A further section of the book deals with the new penicillins and the cephalosporins, mostly with their pharmacological properties and clinical use in man. The value of these substances in medicine is now firmly established and useful assessments are made of their clinical status. However, H. J. Simon's wish for a 'panaceamycin', with all the desirable properties of this group of drugs but a much broader spectrum of activity, including *Pseudomonas* and the fungi, is unlikely to be easily fulfilled and it is arguable that problems of drug resistance may be exacerbated in the future by the extensive use now of substances with very broad spectra of activity.

Although the penicillins are non-toxic in the ordinary sense one of their disadvantages is an ability to cause hypersensitivity reactions. There is some reason to believe that the cephalosporins are not cross-allergenic with the penicillins in man, although they appear themselves to have caused some allergic reactions. However, an article by Paul H. Bunn directs attention to the complex nature of allergic reactions to the penicillins, to uncertainty about the hapten involved in the most serious of these reactions—anaphylactic shock—and to the absence of any simple and entirely reliable test for determining whether a person will react allergically to penicillin or not. Further basic studies of the immunological problems in this field are clearly desirable.

The value of some of the new penicillins and the cephalosporins depends on their resistance to hydrolysis by β -lactamase (penicillinase) produced by *Staphylococcus aureus*. But chemotherapy by members of these groups of compounds has now been extended to Gram-negative organisms, and the production by the latter of β -lactamases, which differ from the staphylococcal enzyme, has given the β -lactamase problem a wider clinical significance. M. R. Pollock has contributed a valuable review on the enzymes which destroy penicillins and cephalosporins, including a discussion of the probable role of conformational changes in relation to some of the varying 'penicillinase' and 'cephalosporinase' activities which have been encountered with β -lactamases from *B. licheniformis* and *Staph. aureus* on combination with antibody. He has also considered the different approaches which may be made, in theory, to the β -lactamase 'threat' to chemotherapy.

Antineoplastic antibiotics are discussed in a series of five papers. S. A. Schepartz gives a brief account of the activities of the U.S. Cancer Chemotherapy National Service Center, established in 1955, which has evaluated more than 120,000 fermentation broths and 20,000 plant

extracts in transplanted rodent tumour systems. Five fermentation products reached clinical trial and two of them, mithramycin and a quinone, streptonigrin, have shown reproducible activity against certain human tumours. Although the results of the programme so far are described as encouraging there appears to be no strong reason as yet to expect that the systematic screening of natural products will uncover substances which can do more than cause temporary remissions. Nevertheless, until other approaches to the cancer problem show greater sign of success a strong case can be made for extensive support of this one.

The remainder of the book contains a section concerned with the mode of action of antibiotics, including a paper on the effect of vancomycin on cell wall synthesis, and a variety of communications on subjects ranging from the increasing resistance to penicillin of the gonococcus to the quantitative estimations of antibiotics separated by thin-layer chromatography. The book is not one in which the reader will expect to find major contributions to the scientific basis of chemotherapy; but it brings together a great deal of useful information.

E. P. ABRAHAM

SYNTHÈSE MANQUÉE

Human Senses and Perception

By Prof. G. M. Wyburn, Prof. R. W. Pickford and Prof. R. J. Hirst. Edited by Prof. G. M. Wyburn. Pp. xii + 340. (Edinburgh and London: Oliver and Boyd, Ltd., 1964.) 45s.

THE authors of this unusual book occupy respectively the chairs of anatomy, psychology and logic in the University of Glasgow, and their avowed aim is "to make readily available within one cover the kind of information that a biologist, psychologist or philosopher might wish to have concerning those aspects of the subject outside his special interests". This is a praiseworthy intention, but one notoriously difficult to translate into practice. How well have the authors succeeded?

Prof. Wyburn provides an excellent outline of the anatomy and physiology of the senses along standard text-book lines. It is clearly written, agreeably up to date, and pitched roughly at the level of a first-year university course. While it would do excellently for budding biologists or medical students, one may be allowed to wonder whether it will prove so satisfactory for the students of philosophy or psychology for whom it is presumably intended. Already on p. 7 the author deals with decremental and non-decremental electrogenesis in the nervous system, which might be expected to scare the life out of any but the most intrepid philosopher. He does not seem to appreciate that students of philosophy or psychology, however intelligent they may be, seldom understand matters so elementary as basic physical units or the nature of the cell. While quick to acquire (and even quicker to question) scientific information, they have to be inducted into a whole new way of thinking. Indeed, what is really needed is a presentation of sensory physiology designed for highly intelligent scientific idiots. But this is no doubt crying for the Moon.

Prof. Pickford has an easier time, for what he has to say is, by and large, understandable to both biologists and philosophers. He deals almost wholly with experimental psychology, particularly in its application to vision. Yet a little more editorial bridging would have been a great help. Prof. Pickford's point of view is that of the traditional psychologist, concerned to delimit the conditions of perception with scant regard to its anatomical and physiological substratum. Yet psychophysiological bridges do nowadays exist, in neurophysiology, in clinical neurology and even in the applications of information theory to the analysis of sensory processes.

A little more recognition of their existence might well have caught the imagination of the young biologist or medical student, so often keenly interested in psychology but so often defeated by its seeming lack of coherence with the disciplines he understands.

Prof. Hirst's contribution is a straightforward essay in the philosophy of psychology. While incompetent to assess its technical merits, I believe that it has much to teach physiologists and experimental psychologists, prone as they are to flounder in quasi-philosophical bogs of their own making. Philosophy, it is true, cannot solve their problems, but some acquaintance with philosophical method may well sharpen argument and prevent much fruitless discussion. For the bright science student with an interest in perception, this account of the philosophical issues involved has much to commend it.

While *Human Senses and Perception* should have considerable value, and does to an appreciable extent justify the intention of its authors, it cannot be said to provide even the hope of a synthesis in the study of the human senses. Indeed, it is not even what is fashionably called 'inter-disciplinary'. There is no suggestion that any of the authors, in deploying his own expertise, has been influenced in the slightest degree by that of his colleagues. Perhaps this was intentional but it might seem the waste of an unusual opportunity. For all the many and varied merits of this book, those in search of a synthesis must still be advised to read their Helmholtz.

O. L. ZANGWILL

AGEING IN EASTERN EUROPE

International Conference on Gerontology

Edited by A. Balázs. Pp. 939. (Budapest: Akadémiai Kiadó, Publishing House of the Hungarian Academy of Sciences, 1965.) 19.60 dollars.

GERONTOLOGICAL research in Britain has progressed considerably since the third international congress was held in London in 1954. However, it is extremely unlikely that had Britain been host to another congress in 1962, it would have been able to provide nearly one hundred papers. That this was possible for gerontologists in Hungary in that year demonstrates the importance which ageing research has attained in that and other Eastern European countries. The geographical region from which the majority of the contributors are drawn is very circumscribed. It could more rightly be described as an Eastern European congress. 94 per cent of the papers originated from Hungary, Romania, Czechoslovakia, East Germany and the U.S.S.R. This does not, however, detract from the value of the publication. In 1964 the British Society for Research on Ageing organized a one-day meeting in London to which Eastern European gerontologists were invited, with the express view of permitting British workers in this field to learn more of work in progress in Eastern European countries. *International Conference on Gerontology* provides similar facilities on a much larger scale, and, without taking into account the intrinsic worth of the individual papers, can fulfil two important functions in Britain to-day. First, it can act as a check-list of the research projects being undertaken in these countries, and secondly, it must serve as a warning that Britain has still failed to appreciate to the full the importance of the ageing problem to the extent demonstrated by other countries with far smaller national budgets.

Two factors militate slightly against the general usefulness of the book. One, a general complaint of all congress memoirs, is the restricted length of each communication. This renders the book a specialist tool since no space is available for critical appraisal of the surrounding fields. The second is peculiar to the origin of the book. Nearly

8 per cent of the papers are in Russian, and for those whose linguistic attainments do not include this language neither summaries nor even title are included in any other.

The book is divided into three sections dealing broadly with the biological, medical and sociological aspects of ageing. It would, I think, be invidious to comment individually on selected papers, and the general impression given by the three separate sections may be of more help to would-be readers.

Although the biological section ranges from botany to palaeo-pathology, the main accent is on vascular changes, where the involvement of proteins, polysaccharides, lipids and hormonal secretions is considered.

The geriatric section is, as might be expected, much more varied in its approach, ranging from general questions of the care of the aged to specific topics and specific conditions which require particular control in aged subjects. For British geriatricians it is unfortunate that this section contains the highest proportion of papers in Russian.

One or two of the papers in the sociological section present studies which are peculiar to Hungary or to ageing in a socialist republic, but the majority touch on problems which are of universal interest. In this section in particular, I, as a biochemical gerontologist, found the shortness of the papers most frustrating.

In general this is a mammoth publication which should serve as very good general reading for all those concerned in similar work. It is only to be deplored that three years have elapsed between the congress and its publication.

DAVID A. HALL

THINKING BROKEN DOWN

The Pathology of Thinking

By B. V. Zeigarnik. (The International Behavioral Sciences Series.) Translated from the Russian by Basil Haigh. Pp. xvi+211. (New York: Consultants Bureau, 1965.) 12.50 dollars.

TO those who imagine contemporary Russian psychology to consist entirely of post-Pavlovian elaborations, experimental and theoretical, out of touch with the actual behaviour of individuals, this book may come as something of a surprise. Dr. Zeigarnik presents a great deal of empirical data from her investigations of the breakdown of normal intellectual processes in a variety of pathological conditions ranging from schizophrenia to arteriosclerosis, and sets them in a framework which seems to owe much to the inspiration of Vygotsky, who for a number of years exercised more effect in the West than in his native land.

In her introductory chapter, Dr. Zeigarnik accepts much from the classical German school of neuropsychiatry of the late nineteenth century, notably from Breuer, though she is perhaps rather unsympathetic and a trifle doctrinaire in stressing the dependence of its views on 'idealistic' and 'faculty' presuppositions. Her account of more recent American, German and British contributions is sometimes so compressed as to impose some distortion. But her basic point is clear. 'Classical' theories—whether they tend, on one hand, to doctrines of cerebral localization or of distinct, innate faculties, or, on the other, to cerebral mass-action or a *Gestalt* principle of function—are inadequate inasmuch as they neglect the essential characteristic of thought, which is to issue in action. It is in this latter conviction that Zeigarnik approaches the pathology of intellectual processes.

Her methods of investigation are quite largely ones similar to those used by others during the past forty years. Classification of objects, the perception of exceptions, understanding of proverbs, and the arrangement of pic-

tured events in logical time sequence are all test procedures of proved value and much used by clinical psychologists the world over. An interesting fresh element is, however, provided by the 'method of pictograms' (suggested by A. R. Luria) in which the patient is required to devise his own pictorial aids to memory for words, and the character of these aids provides data for an assessment of his abstractive capacities and tendencies.

So far as case-material is concerned, Zeigarnik has drawn her net wide. It comprises the major psychoses, traumata, vascular disease and perinatal injury and infective conditions. It is a pity that relatively little attention is paid, in any adequate statistical way, to assessment of the test results in terms of differences of pathology. But Dr. Zeigarnik's emphasis (in what is stated at the outset to be a students' text-book) is on the discernment of the main lines of intellectual breakdown regardless of its cause. These lines are three: (1) of abstraction and generalization; (2) of the logical course; (3) of the purposiveness, of thinking. That these three categories emerge so closely similar to the conclusions of other workers whose basic assumptions may have differed considerably is indeed a hopeful sign. Dr. Zeigarnik makes a contribution of considerable value, less on grounds of originality than through the admirable and fascinating way in which she illustrates her contentions by direct reference to her empirical material. Every now and then, however, her presentation seems to draw attention rather to the stereotyped character of normal behaviour adjudged 'correct' than to the true deviance of abnormal function.

It seems sad that academic Russian, at least in a field like this, apparently cannot go with any ease into simple, direct English. There is a cold didactic atmosphere about this translation which may perhaps be unavoidable, but which is notably at variance with many people's experience of conversational exchange with Russian colleagues. The book ought to be read, not only by those with direct concern with the breakdown of thought in pathological conditions but also by anyone interested in the analysis of the supposedly normal.

R. C. OLDFIELD

PHARMACY IN HISTORY

Pharmacy in History

By Prof. G. E. Trease. Pp. vi+265. (London: Baillière, Tindall and Cox, Ltd., 1964.) 50s.

MODERN materia medica is so largely concerned with defined and standardized substances that its evolution from the vegetable, animal and mineral materia medica of earlier times tends often to be overlooked. The isolation of chemical constituents from natural products and the evaluation of their biological effects, leading to the synthesis and evaluation of analogues, are relatively recent developments. It is fascinating, therefore, to recall our inheritance from earlier times and the periods of progress, stagnation and decline in medicine, science and the arts that have occurred. The history of medicine and pharmacy is not one continuous progress. It is important to be reminded of this lest we become too obsessed with the seeming miracles of modern therapy. There are many "diseases of medical progress" and many problems to be solved in the safe use of a vast and growing range of powerful therapeutic agents.

Man has learnt much by trial and error through the ages, and will doubtless continue to do so, however much scientific development encourages the planning of the trials. It is very interesting and instructive to read, in this concise and excellent monograph by Prof. Trease, of the development of pharmacy in history.

Pharmacy is concerned with medicines, and there have been many seemingly strange aberrations in its historical

development. There will, doubtless, be many more in the future.

For centuries, man has separated those plants which were good for food from those which were poisonous. That knowledge or "wortcunning" provided him with his first vegetable drugs. He learned that spices not only improved the taste of food but also had a preservative action. Extracts of both plants and animals provided arrow- and spear-poisons, and the effects of these and other poisons were noted but not understood. The preparation of drugs inevitably became associated with magic and religion and medical practice became the province of witch-doctors, priests and physicians.

Our older materia medica developed empirically from the folk medicines which were often beneficial but not dangerous. It included herbs used for flavouring and preserving food ('spices'), purgatives and remedies for expelling worms and killing lice. But it also included many preparations which were useless and unfortunately some of them are still used especially among poor people in many countries. There is undoubtedly, however, much yet to be learnt from investigation of the folk medicines of the world.

Trease briefly recounts our knowledge of ancient Egyptian materia medica, of the contributions of Greek civilization in the immediate pre-Christian era, through the investigations of Hippocrates and Theophrastus, and later, in the Roman Empire, through the work and writings of Pliny, Celsus, Dioscorides and Galen. He continues from Galen to the Middle Ages and then to the consideration of early technology and early alchemy. The development of weighing, of comminution, of pottery, ceramic and glass, of oils, perfumes and cosmetics, of sweetening agents and fermentation and of colouring matters all contributed to the development of pharmaceutical preparations. Alchemy, though particularly concerned with the transmutation of base metals into gold, was also concerned with attempts to discover the 'pill of immortality' and the 'elixir of life'. Thus it attracted many apothecaries. The words *apothēke* and *apotheca* originally meant a storehouse, but gradually came to mean a storehouse for the commodities sold by a pharmacist, and in time were applied to the whole of pharmacy. From an early date the pharmacy in Britain included areas for retail sales, for storage, and for the preparation of medicines. In medieval times drugs formed part of the miscellaneous collection of commodities known as 'spicery'. Later these were often roughly divided into the lighter, more expensive items known as 'apothecaries' wares' and the more bulky and less expensive 'grocery'. The grocers derived from the pepperers and spicers, the apothecaries from the spicers, and the chemists and druggists from the alchemists and the apothecaries. Trease re-tells the vicissitudes of the apothecaries and their long quarrels with the physicians, culminating in the Rose case of 1703-4, which had a profound effect on the subsequent practice of both medicine and pharmacy in Britain. The House of Lords found in favour of the apothecary, Rose, and against the College of Physicians in respect of the supply of medicines which had not been prescribed by any physician. Thereafter, apothecaries became increasingly concerned with the practice of medicine, and the term apothecary gradually changed its meaning from that of a pharmaceutical practitioner to that of a general medical practitioner. Although apothecaries continued to be interested in the preparation and supply of medicines, as well as in the advice of patients for a further two centuries, chemists and druggists developed separately from the apothecaries, forming their own associations. Eventually they founded the Pharmaceutical Society in 1841, and acquired headquarters for it at 17 Bloomsbury Square, London. They established there in 1842 the first School of Pharmacy in Britain.

Trease traces chronologically the development of English pharmacy against a concise background of relevant

general and social history and in so doing traces, too, the developments which have led to our modern materia medica and the widening scope for the pharmacist with the development of pharmaceutical education. The bibliographical references, the quotations from literature and the well-selected illustrations add much to the value of the book, which should appeal not only to students and practitioners of medicine and pharmacy, but also to a wider public.

FRANK HARTLEY

SCIENCE IN HUNGARY

Science in Hungary

Edited by Dr. T. Erdőy-Grúz and Dr. I. Trencsényi-Waldapfel. Pp. 316+38 photographs. (Budapest: Corvina Press, 1965.) £p.

THE Hungarian word for science is co-extensive with the German *Wissenschaft* and covers all fields of systematic study, from mathematics to musicology. The Hungarian Academy of Sciences, founded in 1825, was powerfully reorganized in 1949 under its president to this day, the eminent medical scientist Prof. István Ruzsnyák, and directs the scientific life of the whole country. It has eight sections—Language and Literary Sciences, Social and Historical Sciences, Mathematics and Physics, Agricultural Sciences, Medicine, Technical Sciences, Chemistry and Biology—but in fact its activities cover an even wider field, because it has attached to it an Institute for Musical Folklore, under Zoltán Kodály.

The present book, after an introduction by Prof. Erdőy-Grúz, contains nineteen reports by twenty-two specialists, extending over the whole vast area of sciences, with the notable exception of technology, which is represented in the excellent plates, but not in the text. This is regrettable, because Hungarian technology has many outstanding achievements to its credit, such as the flour rolling mill, the transformer, the rotary converter, the first tungsten lamp and many others. Perhaps this will be remedied in a later volume.

Hungary was long known as the greatest exporter of scientists among the small countries: of men such as v. Kármán, v. Hevesy, Bárány, Polányi, Szilárd, v. Neumann, Wigner, Teller, Szent-Györgyi and v. Békésy, with five Nobel Prizewinners among them. After 1948 the scientific leaders of the war-shaken and impoverished country made a determined effort to stop its scattering its treasures and to create the right environment for the rich native talent. The result is five universities of arts and sciences, six universities of technology and agriculture and four universities of medicine with altogether 650 departments. Even more important are perhaps the 125 independent research institutes, of which 40 belong to the Hungarian Academy of Sciences. The time is past when outstanding mathematicians and physicists, far too many for the few university chairs, had only the choice of becoming science teachers in secondary schools or of emigration.

Of the nineteen reports in *Science in Hungary*, seven can be considered as inside the field of natural science: "Physics", by Prof. Gyulai; "Chemistry", by Benedek and Kardos; "Earth Sciences", by Fülöp; "Biology and Medicine", by Törő; "Veterinary Science", by Mócsy; "Agronomy", by Koltay and Tamássy; "Mathematics", by Hajós. They all contain historical introductions on the pathetic struggles of the early pioneers, of great interest to all historians of science, rounded off by the much rosier picture of the new institutes *in statu nascendi* or already well established, and the achievements of the new men. A glance at the photographs of the new Medical Research Centre or of the Central Research Institute of Physics will give impressive proof to anybody that this small, and, by Western standards, still not rich

country has done everything for science that can be done in the way of organization. If one adds to this what one knows of Hungarian talent, there can be little doubt that these efforts must be followed by a rich harvest.

This handsome volume, in impeccable English, is not for sale through the trade, but scientists interested in Hungary may be able to obtain it through the Scientific Officer of the Hungarian Embassy, London.

D. GABOR

EARLIEST CIVILIZATIONS OF THE NEAR EAST

Earliest Civilizations of the Near East

By James Mellaart. (The Library of Early Civilizations.) Pp. 143. (London: Thames and Hudson, 1965.) 30s.

THE making of metal tools is a complicated process, more especially when the copper is derived from its sulphide ore. It would seem reasonable, then, to assume that the discoveries which led to the preparation and use of metals for tool-making purposes eventuated in only one or two places, and thence gradually spread around the world. The practice of agriculture and the domestication of animals, on the other hand, can result from climate changes and could have evolved independently in many different regions. Agriculture, especially, gave rise to the village and later to the articulated town where different groups of people performed different functions for the good of the community. Early Neolithic cultures as a rule are, therefore, more diverse in development than are the early metal cultures, and it is to the Near East that we must, perhaps, look for these earliest appearances. They succeed the so-called Mesolithic cultures which grew up following the changes of climate which took place at the end of Palaeolithic times. These in turn gave rise to a Protoneolithic period when tentative attempts at a simple agriculture and the domestication of animals can be observed. Then come the fully developed Neolithic cultures with, somewhat later, the manufacture of pottery. As has been indicated not a few slightly different developments of this true Neolithic civilization can be observed in the Near East. In *Earliest Civilizations of the Middle East* the author has described many of these for us, and the frequent illustrations, often in colour, help the reader to visualize how these early folk lived and the sort of objects they made.

In Anatolia a few naturalistic paintings and engravings have been observed which have been classed as late Palaeolithic, and, following these, there is a spread of Mesolithic cultures with typical industries including pygmy tools. One of the more evolved of these is the Natufian culture of Palestine. An illustration gives a good idea of the characteristic objects made. The protoneolithic is next considered and the succeeding chapter deals with Syria and Palestine in the seventh millennium. Here it may be mentioned that in the Near East the chronology is considerably 'longer' than has, until recently, been considered to be the case in the West. Almost certainly we in this part of the world will have to lengthen out our own Neolithic era. Succeeding chapters deal with the ceramic Neolithic period in Syria, Lebanon and Palestine, as well as in Mesopotamia and Northern Iran. Once again the illustrations are especially useful in enabling the reader to visualize the differences between the various culture groups. There follows a chapter on Anatolia where the author is very much on his own ground. Here he points out that it is a mistake to think of Anatolia as a barbarian fringe to the "fertile crescent". On the contrary, the region was a great cradle of fine Neolithic cultures. The most splendid site is Çatal Hüyük, covering some 32 acres. There are 12 successive building-levels

dating from 6500 to 5650 B.C. There are wall paintings, a rich industry including necklaces and many pottery statuettes of goddesses, etc. There is also a shrine elaborately furnished with bulls' heads. It would seem, too, that woven material was made. Çatal Hüyük was the high spot of the Near East Neolithic and after it the succeeding early metal age (Tell Halaf) seems almost to be a comedown. Nevertheless, the future, of course, lay with the spread of metal industries and the final chapter describes some of these in the area.

Admittedly the title of this little book confines us to the Near East, but it is perhaps sad to have to consider these regions, however important, without any chronological tie-up with the Neolithic cultures of Europe and elsewhere. None the less, this volume makes an admirable introduction to the subject and the author is to be congratulated.

M. C. BURKITT

ALGEBRA OF THE LATTICE

Introduction to Lattice Theory

By Prof. D. E. Rutherford. (University Mathematica Monographs.) Pp. x+117. (Edinburgh and London Oliver and Boyd, Ltd., 1965.) 35s.

A SET of elements x, y, \dots is partially ordered if there is a binary relation $x \geq y$ which is reflexive, anti-symmetric and transitive. This is a wide classification. In *Introduction to Lattice Theory*, Prof. Rutherford gives as an example, the set of all human beings, when $x \geq y$ means that either x and y are the same individual or y is a descendant of x . The idea goes back, at least implicitly, to Boole, whose algebra remained for a long time the only system in which the elements need have no numerical significance. C. S. Pierce remarked that, if an order relation exists, union and intersection can be defined as the least thing which contains x and y , and the greatest thing contained in both x and y , that is, as a least upper bound and a greatest lower bound, respectively. Schroeder cleared up some of Peirce's detail, and Dedekind directed attention to the importance of ordered sets, and lattices. A lattice is a partially ordered set such that any two elements possess both a least upper bound and a greatest lower bound; for example, the set of all positive integers when $x \geq y$ means that y is a factor of x and the bounds are then the lowest common multiple and the highest common factor.

Boolean algebra now appears as the first and still one of the most fascinating of investigated lattices. Lattices may be classified into main groups according to the various further restrictions laid on their relations, and Prof. Rutherford gives a very clear and comprehensive picture of these types, and their applications to logic, topology, geometry, and switching circuit theory. His book is meant for the undergraduate just beginning to make a serious study of abstract algebra, and is better suited for this purpose than its only competitor in English, the brilliant but somewhat highbrow volume by Garrett Birkhoff in the American Mathematical Society *Colloquium Series*. Rutherford knows that the omission of steps which would be obvious to the trained and sophisticated mathematician may cause endless trouble to the beginner; he therefore gives his proofs in full and adds a good deal of informal expository comment, providing also some sensible examples for the reader. His text has been remarkably well organized to allow him to do all this in little more than 100 pages, but, of course, it still demands, as by their nature do all texts on abstract algebra, a remarkably high degree of sustained concentration on the part of the reader. Granted this, the rewards to be obtained from a study of this live and growing field are considerable, as a pleasure in itself and as a stimulus to constructive work. T. A. A. BROADBENT

SHORT REVIEWS

God Beyond Time

by J. H. Reyner. Pp. 110. (London: Regency Press, 1965.) 21s.; 4 dollars.

GOD *Beyond Time* is a readable and interesting account of the various tenets which—largely by convention and unquestioning custom—we cherish with respect to time. It is an age-long problem, with 'Time's arrow' always facing towards the future, giving little encouragement to clear thinking as to the possible effects of time reversal and the now generally accepted principle that in certain circumstances entropy may remain constant, or even decrease.

Some of the most original parts of the work deal with states of mind commonly classed as abnormal: Mr. Reyner is sure that there is a (real) noumenal world having properties which a purely materialistic science cannot hope to reveal. It is certainly true that a subject under the influence of the alkaloid mescaline experiences visions akin to those of the biblical witnesses of the Apocalypse. But it is equally clear that these strange events are due to interference with the normal functioning of glucose in the brain: it is a matter for serious reflexion what mankind would have been like if this reaction had been different from what in fact it is.

Mr. Reyner deals bravely with the ether of space; rendered needless by the mathematics of relativity, but not thereby necessarily eliminated from the thought-processes of natural philosophers. Most probably many men of science feel that in ether physics they stand at the very frontier between the observable and the unknowable, where negative experiment may hide a world into which only metaphysics can enter, if at all. These deep waters are probed with a seemingly reverence, and a realization of the limits of the scientific method, as commonly understood.

F. I. G. RAWLINS

Science, Faith and Society

by Prof. Michael Polanyi. Pp. 96. (Chicago and London: University of Chicago Press, 1964.) 3.75 dollars; 28s.

Nature and God

by Prof. L. Charles Birch. Pp. 128. (London: SCM Press, Ltd., 1965.) 6s. 6d.

IN the second edition of his Riddell Memorial Lectures on the meaning and nature of scientific enquiry, originally published in 1946 under the title *Science, Faith and Society*, Prof. Polanyi contributes a new introduction, 'Background and Prospect'. In this he cites several writers such as W. I. Beveridge, J. Bronowski and S. S. S. Boulmin, who have published, in the interval, views on the nature of science and pursuit of discovery which overlap his own. He reiterates that we still have no clear conception of how discovery comes about and that the creative life of any community organized essentially on the line of scientific life rests on a belief in the continuing possibility of revealing truths still hidden, or, as he now refers to express it, in a belief in "the reality of emergent meaning and truth". Apart from this, Prof. Polanyi's argument for a symbiosis between thought and society leads him to insist that a general respect for truth is all that is needed for society to be free. Moreover, while he challenges the Marxist position as firmly as ever, he seems more hopeful of an ultimate *rapprochement* from the Communist side leading to a modern theory of freedom. The introduction and the original lectures are highly relevant to present-day discussions on the organization of science and on planning in general in Britain. It is

from this pragmatic point of view, perhaps, rather than for its contribution to the philosophy of science, that this second edition is welcome.

The book has in fact much less in common with Prof. Birch's little book than the titles would suggest. While Prof. Birch believes that the supernaturalist tradition in theology and traditional science are being driven farther and farther apart, he also believes that radical changes within science and within theology and philosophy are altering the whole traditional position. His book is an attempt to explain these changes and the new and constructive way of looking at the natural world, in the light both of science and of Christian insights, which these changes open up. He begins by reviewing the successive views of the universe from the sixteenth century to 'Darwin's century', which has a chapter to itself. A chapter on chance and purpose continues this historical exposition and this is followed by one in which he considers the modern concept of creation in the light of the concept which is based on The Bible. The essence of the book is in the final chapter on the meaning of creation in which Prof. Birch sets forth (without dogmatism) six affirmations about the nature and meaning of creation which he develops from the changes previously described. His argument is supported chapter by chapter, by lists of references which constitute an admirable bibliography. Whether or not Prof. Birch convinces his readers, he stimulates discussion and thought, and his little book should shatter complacency and dogmatism on either side.

Early Seventeenth Century Scientists

Edited by R. Harré. (Commonwealth and International Library, Science and Society, Vol. 1.) Pp. xi+188. (Oxford, London and New York: Pergamon Press, 1965.) 25s.

THE seventeenth century was a golden age in the history of science. The telescope had opened up vast new spaces in the cosmos and the microscope was revealing the infinite variety and wonder of new miniature worlds. Various academies of science were in being and, in particular, the Royal Society of London had been founded on the Restoration of the Monarchy. Galileo had died and Newton been born in the same year, 1642. On every side there was the joy in the use of eyes, ears and limbs in the study of Nature and the improvement of industry and navigation. Mathematics was becoming, to an increasing extent, both the handmaid and the language of science. The end of the Thirty Years War in Europe and the Commonwealth in England was marked with a great sense of freedom in observation, thought and invention, as well as in more trivial matters. By the study of seven scientists the authors present the state of science, both in method and in content, between 1590 and 1645.

The development of ideas of scientific method is followed through Bacon and Descartes, the development of the rudiments of biochemistry in Van Helmont, of physics in Gilbert, Galileo and Kepler, and of physiology in Harvey. Each of these men might be regarded almost as the founder of the branch of science in which he specialized, and laid the foundations on which the modern shapes of the respective branches of science were built. The "Harvard Case Books" have shown the way to a useful treatment of topics in the history of science. More important than details of the lives of scientists are the climate of thought in which they worked, their methods of working, their antecedents and advances in scientific

method which accrued from their ideas. This method allows each scientist to be studied in depth, and each biography is sufficiently long for the purpose. The contributing authors are R. Harré on Gilbert and Kepler, J. J. Macintosh on Bacon, M. Deutcher on Descartes, D. Knight on Galileo, D. Goodman on Harvey and J. Mepham on Van Helmont.

The paper-back of only 188 pages must be considered expensive when compared with other books of similar size.
W. L. SUMNER

Science in History

By J. D. Bernal. Third edition. Pp. xxvii+1039. (London: C. A. Watts and Co., Ltd., 1965.) 84s.

FOR this third edition, Prof. Bernal has almost completely re-written Part 6 of the earlier editions of *Science in History*, with the exception of the chapter on the social sciences in history. Part 7, which contains his conclusions and is entitled *Science and History*, is substantially unchanged, apart from some factual corrections bringing it more up to date and the addition of a section "Science in a Rapidly Changing World", as well as some new paragraphs dealing with, for example, the organization of scientists, information services, and secrecy: a few paragraphs have been omitted, and others, like that on science in the developing countries, revised. Apart, therefore, from the bulk of Part 6, constituting some 328 pages out of a total of 978 and containing in successive chapters brilliant reviews of the physical sciences and of the biological sciences in the twentieth century, the book is substantially unchanged, and reflects the weaknesses that have attracted criticism in the earlier editions. Prof. Bernal is too enthusiastic an exponent of Marxism for his book to be fairly described as history in any true sense: his enthusiasm leads him to make the most naive and questionable assertions as if they were generally accepted propositions. If his failure to discriminate between opinion and fact makes part of the book still misleading for the immature and less informed, the brilliance of those two chapters on the physical and biological sciences alone makes the book worth reading. The bibliography includes many references to publications since the first edition appeared in 1954. R. BRIGHTMAN

Mathematical Discovery

On Understanding, Learning, and Teaching Problem Solving. Vol. 2. By G. Polya. Pp. xi+191. (New York, London and Sydney: John Wiley and Sons, Inc., 1965.) 42s.

NO competent teacher of mathematics can afford to be ignorant of the sequence of books by Polya on the art and science of solving mathematical problems, in which the author shows how the seemingly subconscious processes of discovery and proof, as described by Poincaré and analysed by Hadamard, can be assisted and developed by conscious organization. His examples are taken from the elementary ranges of school mathematics, but they serve to illustrate how to discard blind alleys and find through routes, how to search for equivalent and possibly simpler results, how to strip away inessentials by looking at generalizations, how to acquire by experience the skill in selecting the right tool for the job. How would you set about finding the volume of the frustum of a pyramid? In the theorem that if three circles having the same radius pass through a point, the circle through the other three points of intersection has the same radius, how can we organize our knowledge in such a way as to lead us to the hinge on which the result turns? The whole book is packed with case-histories in the anatomy and psychology of mathematical discovery, chosen from simple algebra, geometry and trigonometry by one who has proved himself a master of recondite research. It should be in every school library, to be read by every teacher of

mathematics and by every pupil who aspires to a genuine knowledge and understanding of mathematics; and it will make all readers envious of those who have had the privilege of attending Polya's seminars in problem solving, given for high-school teachers at Stanford University.
T. A. A. BROADBENT

The Physics of Ice

By Prof. E. R. Pounder. (The Commonwealth and International Library: Geophysics Division.) Pp. vii+151. (London and New York: Pergamon Press, 1965) 17s. 6d. net.

ICE is a substance the properties and peculiarities of which are of interest in many fields of science and technology. Its more unusual properties can be attributed to a lack of symmetry and resulting dipole moment of the water molecule itself, which is preserved in the ice lattice. Prof. Pounder has found that the physics of ice in its broadest context is too extensive a subject to be discussed within the confines of a paperback, and, in *The Physics of Ice*, has limited himself to topics related to his own speciality, sea ice. Indeed, the "Physics of Sea Ice" would have been a much more realistic title, a fundamentals are examined only so far as is necessary for an understanding of this subject. The author is professor of physics in McGill University, which has had a research tradition in ice physics since the classic work of Barnes on ice structure forty years ago.

The first four chapters give an account of the formation, break up and climatology of sea and lake ice which will make interesting reading for sixth form students upwards. The structure of the ice depends on the meteorological conditions during its formation, and analysis of these sections in polarized light enables some deductions to be made about its history. The remainder of the book is concerned with crystallographic properties of ice, its rheology, and the influence of brine inclusions on thermal and electrical properties, is discussed at a somewhat higher level—to be easily followed by the second year undergraduate. It is a pity that the author did not lay greater emphasis in this section on the role of lattice defects in ice, in view of their fundamental influence on mechanical and electrical properties. The literature of this subject is necessarily rather scattered, and the book concludes with a list of useful references. Many of these are, however, in reports which are unfortunately difficult to obtain.

This is a book to be read by the physics student as a source of interesting problems for discussion, and to serve as a useful reference for those more directly concerned with polar research.
J. HALLETT

The Special Theory of Relativity

By Prof. David Bohm. (Lecture Notes and Supplement in Physics.) Pp. xiv+236. (New York and Amsterdam: W. A. Benjamin, Inc., 1965.) 7.70 dollars.

THE *Special Theory of Relativity* is neither a popular treatment of special relativity nor a conventional text-book but lies somewhere between the two. The author does not try to avoid mathematics in the way that a popular writer does, but on the other hand he only uses mathematics when it is essential, and relies on physical ideas whenever possible. This makes it a most satisfactory book for background reading for anyone learning special relativity. Moreover, the author brings to his subject the benefit of long experience, both in using the theory in teaching it, so that nearly every detail in the subject has a new and unexpected light thrown on it. His approach is by way of electrodynamics and the Lorentz electrodynamics. He uses the difficulty of determining when events are simultaneous in this theory to motivate the Lorentz transformation, and then he considers at length the way in which our common sense concepts of space and time have to be modified. Various applications of relativity

mostly grouped around the equivalence of mass and energy, are then considered. The setting up of the theory is taken up again in the later part of the book in terms of the perception of distant events by means of light signals (the so-called radar method or *k*-calculus). This enables him to discuss the clock paradox in a very clear manner. There is an appendix on physics and perception in which the author tries to extend to large areas of everyday life the general ideas (as opposed to specific mathematical concepts) that arise in special relativity. In some ways this part of the book is less satisfactory because less complete than the rest. In other ways it is the most interesting part because we see here a noted scientist trying to use the knowledge which he has acquired in his professional capacity to make sense of the whole world around him. The experience is a very exhilarating one.

C. W. KILMISTER

International Directory of Isotopes

Third edition. Pp. 487. (Vienna: International Atomic Energy Agency; London: H.M.S.O., 1964.) 54s.; 9.00 dollars.

THIS new edition of the International Atomic Energy Agency's *International Directory of Isotopes* is arranged in five main sections:

(1) An introductory section explaining its purpose and layout, and giving the essential information about 83 isotope suppliers (address and short description of materials and services offered). Reactor centres which will carry out irradiations are tabulated separately against their maximum neutron fluxes.

(2) An alphabetical list of 186 radioisotopes, showing which suppliers offer them and in what form. This section has more than 3,000 entries. A summary of the radioactive properties of each isotope is given (boxed so that it stands out on the page) and the various compounds, standard solutions, radiation sources, and so on are listed. Brief specifications (specific activity, dimensions) are given, but prices are not.

(3) A list of 250 separated stable isotopes, showing the degree of enrichment and quantity available from stock. There are only three primary suppliers—Harwell, Oak Ridge, and the Soviet export organization Soyuzchimexport—but several firms offer stable isotopes in the form of Mössbauer absorbers or labelled compounds.

(4) Compounds labelled with the radioisotopes carbon-14, tritium, iodine-125, iodine-131, phosphorus-32 and sulphur-35. Compounds of other radioisotopes are included in Section 2. The supplier and specific activity (in mc./m.mole) are given. There are nearly 1,800 compounds labelled with carbon-14 and 650 with tritium.

(5) Compounds labelled with the stable isotopes carbon-13, deuterium, nitrogen-15 and oxygen-18. The 100 compounds labelled with nitrogen-15 and the 40 labelled with oxygen-18 are of special interest as neither element has a long-lived radioisotope.

With this arrangement, the *Directory* admirably fulfils its purpose, to guide the research worker to the supply organization most likely to have the isotopic material he wants. It contains less detailed information than its predecessors, but it has many more entries. It will be useful for two or three years—after which it is to be hoped that the Agency will revise it again.

C. B. G. TAYLOR

Elements of Chemistry

By Antoine-Laurent Lavoisier. Translated by Robert Kerr. Introduction by D. McKie. Pp. xxxi+1+511+13 plates. (New York: Dover Publications, Inc.; London: Constable and Co., Ltd., 1965.) 3 dollars; 24s.

DOVER Publications, Inc., have published a facsimile reprint of *The Elements of Chemistry*. This is the translation by Robert Kerr, published in 1790, of Lavoisier's

famous *Traité élémentaire de Chimie*. It is unfortunate that the excellent introduction by Prof. D. McKie is limited to only 26 pages. Prof. McKie, an eminent historian of science and authority on Lavoisier, gives a fascinating account of the state of knowledge in chemistry when Lavoisier embarked on his researches, and provides many illuminating comments on Lavoisier's work which fully justify the statement that Lavoisier was the founder of modern chemistry, with the *Traité* as its first textbook.

The *Traité* itself contains the first explicit statement of the law of conservation of matter in chemical changes, the first modern list of the chemical elements, an account of the calorimetric experiments which were the foundation of thermochemistry, and an account and illustration of the apparatus used by Lavoisier in his famous experiments on the composition of the atmosphere in which he heated mercury in a confined volume of air. The illustrations in the *Traité* (there are 13 plates excellently reproduced) were from drawings by Lavoisier's wife (whom Lavoisier married in 1771 when she was aged nearly fourteen, and who later married Count Rumford).

Although a paper-back edition, the book is well printed on good quality paper and is sewn not glued.

Anyone interested in the history of science will welcome this opportunity of obtaining Lavoisier's classic. To anyone not interested, I challenge them to read Prof. McKie's introduction and remain uninterested.

ROGER PARTINGTON

Nucleotides and Coenzymes

By Dr. D. W. Hutchinson. (Methuen's Monographs on Biological Subjects.) Pp. viii+136. (London: Methuen and Co., Ltd.; New York: John Wiley and Sons, Inc., 1964.) 18s. net.

THIS addition to the Methuen monographs is very timely. It deals with matters which are central to the whole of biochemistry. Dr. Hutchinson has succeeded in encompassing the large field of nucleotide chemistry succinctly but in sufficient detail to convey the real chemistry underlying the complex molecules that come under his survey. How different this is from the situation to be found in so many larger text-books of chemistry and biochemistry. There, the structures alone of the nucleic acids, the nucleotide coenzymes and the many other classes of natural phosphate esters are considered to be sufficient for the student's understanding of their function. In this respect one suspects that this monograph will have a much wider appeal than to the undergraduate and research student body to whom it is primarily directed.

Beginning with the nucleosides and mononucleotides, the subject is developed through the nucleotide coenzymes, including those derived from thiamine and pyridoxal, to the oligo- and poly-nucleotides. At each stage the chemical and biochemical synthesis is considered, as is the function of the compound under consideration. The section dealing with the polynucleotides is short but, in the circumstances, adequate, as the book is designed as a companion to the well-established monograph on the nucleic acids by J. N. Davidson. The bibliography is well chosen to lead the student more deeply into the subject.

D. M. BROWN

Dictionary of Nutrition and Food Technology

By Dr. Arnold E. Bender. Second edition. Pp. viii+221. (London: Butterworth and Co. (Publishers), Ltd., 1965.) 47s. 6d.

THE second edition of this useful reference book provides a greatly increased collection of information. The sources of information are extended and Dr. Bender's wide knowledge of the science of nutrition and the application of nutrition to food technology allows him to give due attention to many aspects of the subject.

For the nutritionist who works mainly in the laboratory it is useful to be able to check the composition of products under their trade names, while the nutritionist concerned with the practical work of food processing or cooking may find the cryptic initials that come into scientific texts need interpretation. This is provided not only for such as ATP (adenosine triphosphate), DNA (deoxyribonucleic acid) and EDTA (ethylenediamine tetraacetic acid) but also for H.T.S.T. (high-temperature short-time pasteurization) and A.F.D. (accelerated freeze drying). The entries from 'Abalone' (a shellfish) to 'Zymotachograph' (an instrument that measures the gas produced in a fermenting dough and the amount escaping from the dough) make enlightening reading for all who have any interest in nutrition. Those who come into the field of food from the technical angle of the physicist or the chemical engineer will find help in defining unfamiliar terms.

For the merely curious there are entries like 'cibophobia' (dislike of food) or 'Dipsogen' (thirst-provoking agent). Products such as the Japanese ham and fish sausage are described. Fruits, vegetables, cereals, herbs of many countries are listed together with products commonly derived from them. Diseases due to nutritional failure are briefly noticed including coeliac disease, kwashiorkor, coronary thrombosis and cretinism. A final table gives the calorie contributions of average portions of common foods to aid the planning of a slimming diet.

In fact, this book is clearly so useful as a reference volume for many different people that it is unfortunate that the increase in size and content has involved an increase in price. This may prevent its use as a student handbook, but it should have a place on the shelf of any library that deals with nutrition. A. M. COPPING

Les Anticorps de Transplantation

Par André Govaerts. Pp. 148. (Brussels: Editions Arscia S.A.; Paris: Librairie Maloine S.A., 1964.) 250 Fr.B.

ANDRÉ GOVAERTS was the first person to report the destruction of target cells *in vitro* by sensitized lymphocytes. In this volume he describes his researches at greater length, in the form of a thesis. The introduction defines general notions of the biology of homografts and introduces us to an extensive bibliography. Though a clearly written summary, it is not always accurate—as when a proprietary brand of polystyrene dishes is described as being polyethylene. More serious, in defining immunological paralysis the author states that this is due to the persistence of depots of antigen which neutralize the antibody as fast as it is formed. But there is an accumulation of evidence, starting with a paper by Sercarz and Coons in 1959, that this is not so, and that a central inhibition, akin to that in immune tolerance, must be in operation.

The results themselves will be of much interest to both transplantation surgeons and immunologists. The *in vitro* homograft reaction by sensitized lymphocytes has now been obtained in several other laboratories. What needs further confirmation is Govaerts's observation that immune serum (inactive by itself) would greatly potentiate the action of the cells. Furthermore, cells immune to unrelated tissue (also inactive by themselves) became active when combined with antiserum specific for the target tissue. However, these lymphocytes may have had a low level of immunity to cross-reacting antigens, so it is a pity that the author did not make the control with non-immune lymphocytes. It is also of interest that an antibody was detected (by tanned cell haemagglutination) in homogenates of sensitized thoracic duct lymphocytes, where none could be found in sera from the same animals. Notwithstanding the present interest in transformation of lymphocytes to blast cells during immune reactions, the author does not mention the morphology of the active cells in his experiments. There

is an unbiased discussion, somewhat marred (as is the whole book) by too many indiscriminate references.

R. B. TAYLOR

The Freshwater Life of the British Isles

By John Clegg. Third edition. (Wayside and Woodland Series.) Pp. 352+67 plates. (London and New York: Frederick Warne and Co., Ltd., 1965.) 35s. net.

MR. JOHN CLEGG'S work has been a most useful introduction to limnology since its first appearance in the *Wayside and Woodland Series* in 1952. As a well-balanced, elementary text it has been greatly appreciated both by teachers and students. It is unnecessary here to review the text, which remains substantially the same as in the second edition of 1959.

Nevertheless, there are changes; Mr. Clegg has undertaken some extensive revisions, such as improvements in the sections on algae, bacteria and fungi, but the most noticeable differences are in the overall size of the book and in the introduction of some new illustrations. The new edition has been given a larger format (page size 8½ in. × 5½ in.) and a correspondingly bigger type face compared with the earlier pocket size, although the pagination is virtually unchanged. This allows larger plates, but many will find this new edition rather bulky to carry on field trips.

A number of colour photographs, chiefly of ecological scenes, have been withdrawn and have been replaced by eight excellent colour plates, by Mr. E. C. Mansell, that have already appeared in the author's *Observer's Book of Pond Life*. These new inclusions are plates illustrating planktonic organisms, sponges and *Hydra*, rotifers, cases of caddis larvae, beetles, water mites, frogs and toads. Among others, there are also some new black-and-white photographs of polyzoa and of the eggs of trout and amphibia. Most of the other monochrome pictures have been improved through being printed in a larger size, but a few have lost their crispness.

The value of this new edition lies in its excellent new colour plates, but those who already have the 1959 issue plus the author's *Pond Life* will find little excuse for buying this new volume. W. J. REES

Metaphysik der Naturwissenschaft

By W. Schapp. Pp. x+141. (Den Haag: Martinus Nijhoff, 1965.) 13.90 guilders.

IN *Metaphysik der Naturwissenschaft* a metaphysician seeks to communicate his thoughts over a very wide range of subjects to those whose primary interest is physics. This is by no means a commentary on natural philosophy as such, but rather a meditation on the world, as the author sees it, in terms of history and its impact on the way in which we regard phenomena. There are three main divisions: (1) criticism of cases; (2) colours and the world; (3) world, object, concept. From these the main trend can be discerned. Much of it is a kind of distillation of Einstein and Infeld's *Evolution of Physics*, even including the familiar cloud of particles constituting the 'real' table, which reminds one of Eddington. All this is by no means to be dismissed as mere playing with words; it resembles the outlook of Goethe in contemporary dress. It is significant how deeply the place of colour is envisaged in the life of mankind, without troubling about any quantitative character. (Newton was not blind to beauty, but he set about measuring—that is the essential difference.)

There is an element of the numinous here and there, which is rather charming: for example, in the introduction of thunderstorms in close proximity to a discussion of simultaneity. Nobody but a Teutonic scholar would do quite that. These are not pages to trouble the down-to-earth man of science, but they are well worth studying by anybody with the requisite time and patience.

(Continued from page 956)

various stages of development and afterwards the haemopoietic organs of pairs of opposite sex can be analysed for cell chimaerism using the sex chromosomes as markers. Recently a technique has been developed in this laboratory for the preparation of abundant, clear metaphases from various haemopoietic organs of the chick embryo^{8,9}, thus making feasible experiments of this design.

White Leghorn fertile eggs of 6–11 days' incubation were parabiosed using a modification of the technique of Hasek¹⁰. The use of a tissue bridge was found unnecessary for the production of a parabiotic union and vascular anastomosis was readily obtained by direct apposition of the chorioallantoic membranes.

After a further 7–13 days' incubation, each egg was injected, by way of the allantois, with 0.1 ml. demecolcine solution (containing 0.05–0.1 mg demecolcine according to the stage of incubation). Three hours later, parabiosed pairs were removed and sexed. Where partners were of opposite sex, cells of the thymus, spleen, bone marrow and bursa of Fabricius were prepared for chromosome analysis using a modified hypotonic citrate–air drying technique⁸. Staining was carried out in lactic-acetic-orcin and the preparations were viewed by phase-contrast microscopy.

In an additional experiment, double-yolked eggs were incubated for up to 13 days. Unfortunately, the very high mortality found in embryos derived from such eggs meant that only two pairs of embryos of opposite sex, of 11 and 12 days' incubation, were obtained for investigation. Chromosomal preparations were made from cells of the various haemopoietic organs as before.

A very high mortality was observed immediately after parabiotic union and this no doubt was due to trauma associated with the procedure. The pairs surviving to late incubation appeared perfectly normal when sampled. Although it is probable that there were histocompatibility differences between partners, no macroscopic or microscopic evidence of an immune reaction between them was found. As well as the initial operative mortality some embryos were killed by the demecolcine treatment. Unfortunately it appears that the dose of demecolcine which enables abundant metaphases to be obtained is very close to the toxic dose for the embryo. Thus only a very small proportion of the original parabiosed eggs were eventually analysed.

In Table 1 it can be seen that in embryos parabiosed at 6–8 days' incubation and sampled at 17–20 days' incubation, 14–42 per cent of the dividing cells in marrow, 19–46 per cent in the spleen and 26–50 per cent in the bursa of Fabricius are derived from the opposite partner. The percentage in the case of the thymus is much lower (0–12 per cent).

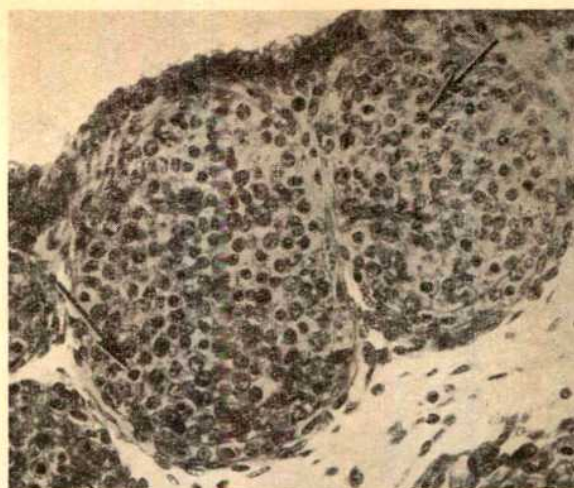


Fig. 1. Bursa of Fabricius of demecolcine-treated 19-day embryo showing a large number of metaphases (arrowed) in the lymphoid follicles. ($\times c. 900$)

In two embryos sampled at 15 days' incubation a considerable degree of chimaerism was found in the marrow and spleen of both and in the bursa of one. In a pair parabiosed as late as 11 days' incubation, chimaerism was found in the spleen and bursa; thymus and marrow were not sampled. In the twin embryos investigated, a high degree of chimaerism was found within the spleen (27–39 per cent), a low degree of chimaerism in the thymus and marrow (0–8 per cent), and no chimaerism at all in the bursa.

In order to gain some idea of the cell type being sampled for chromosome analysis, sections of spleen and marrow were examined after demecolcine treatment and it was noted that abundant mitotic figures were distributed throughout the whole tissue in both granulocytic and non-granulocytic areas. Phase contrast microscopic examination of cell suspensions prepared from these organs showed that both cell types were present. In sections of bursa (Fig. 1) and thymus (Fig. 2) it is quite clear that the overwhelming majority of mitoses are confined to the lymphocyte series. Bursal and thymic suspensions were predominantly lymphoid though a few granulocytic elements were present.

The spleen and marrow have similar haemopoietic functions during avian embryogenesis¹¹ although the spleen develops about 4 days earlier than the bone marrow. Both are sites of production of granulocytes, erythrocytes and thrombocytes, but, while the spleen is predominantly granulopoietic after an initial erythroid phase, the marrow continues to produce both types of cell throughout

Table 1

Pair No.	Age at parabiosis (days)	Age at sampling (days)	Sex	Bone marrow			Spleen			Bursa of Fabricius			Thymus		
				No. of cells scored	No. of cells of opposite sex	Percentage of cells of opposite sex	No. of cells scored	No. of cells of opposite sex	Percentage of cells of opposite sex	No. of cells scored	No. of cells of opposite sex	Percentage of cells of opposite sex	No. of cells scored	No. of cells of opposite sex	Percentage of cells of opposite sex
1	6	19	♂	100	24	24	100	20	20	261	97	37	35	0	0
2	6	19	♀	100	18	18	50	13	26	30	8	27	—	—	—
3	7	20	♂	54	10	18	57	26	46	300	78	26	100	3	3
4	8	19	♀	50	7	14	27	5	19	68	34	50	—	—	—
5	8	17	♂	51	15	29	54	12	22	31	13	42	52	6	12
6	8	15	♀	50	12	24	50	14	28	50	19	38	50	0	0
7	8	15	♂	100	42	42	100	46	46	100	29	29	100	0	0
8	11	19	♀	33	11	33	50	19	38	41	13	32	—	—	—
				50	24	48	50	18	36	10	5	50	50	0	0
				100	14	14	50	14	28	50	0	0	50	0	0
							60	16	27	30	14	47			
Embryos from double-yolked eggs 1	12		♂	50	2	4	100	27	27	50	0	0	100	1	1
Embryos from double-yolked eggs 2	11		♀	50	2	4	100	39	39	50	0	0	100	4	4
							—	—	—	50	0	0	50	1	2
							—	—	—	50	0	0	50	1	2

Notes: (1) In those cases where only one embryo is represented from the initial pair the partner had died following demecolcine injection. (2) No metaphases were obtained for study from the thymic tissue of three embryos and the spleens of two others.

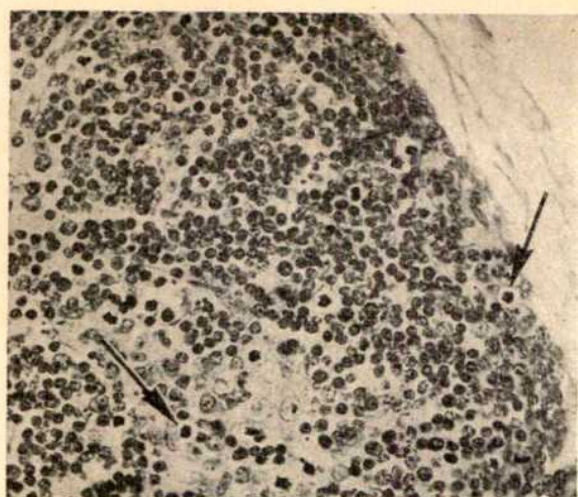


Fig. 2. Thymus of the same embryo showing metaphases (arrowed) distributed throughout thymocytes. ($\times c. 900$)

embryogenesis. The rapidity with which chimaerism is established in both organs indicates that there is a considerable inflow of cells into them. That the spleen, but not the marrow, is highly chimaeric in the embryos derived from double-yolked eggs probably reflects the fact that at sampling (12 days' incubation) haemopoiesis is firmly established in the spleen but there are few blood-forming cells in the marrow. There is evidence that an inflow of cells to the spleen and marrow occurs in the adult animal. Thus chromosome marker investigations have shown that there is an afferent stream of cells to both organs in the adult mouse^{12,13}.

Similarly, investigations on the repopulation of thymic grafts¹⁴ and on the movement of haemopoietic cells in adult parabiosed mice¹⁵ using the T6 chromosome marker system have demonstrated an afferent stream of blood-borne cells to the thymus. The chimaerism found in the thymus of some embryos in the present investigation shows that there are cells entering the avian thymus during embryogenesis.

Considerable interest has centred lately around the bursa of Fabricius as a site of production of antibody-forming cells¹⁶ and possibly of a hormone necessary for the maturation of such cells¹⁷. Lymphoid development begins within the organ at 14–15 days' incubation with the formation of follicular structures closely associated with epithelium, and most subsequent cell proliferation occurs

within these lymphoid follicles (Fig. 1). The fact that up to 50 per cent of the dividing cells in the bursas of parabiosed embryos are derived from the opposite partner suggests that the lymphoid follicles are largely populated by blood-borne cells. The absence of chimaerism in the 11- and 12-day embryos derived from the double-yolked eggs and in one of the two parabionts sampled at 15 days' incubation is not surprising in view of the fact that at these times very little follicular development has occurred.

In summary, it may be stated that, although there is some variability both between and within parabiosed pairs, the large number of dividing cells of opposite sex found proliferating in the spleen, marrow and bursa of Fabricius of most parabionts after various periods of incubation indicates that these organs are largely populated by blood-borne cells. Chimaerism was found less frequently and to a lesser degree in the thymus. The nature and origin of the blood-borne cells remain unknown, but they could be derived from many embryonic tissues in addition to the organs studied. Thus the pancreas, kidneys and gonads are all sites of intense granulopoiesis in the late embryo¹⁷ although the avian liver, unlike its mammalian counterpart, is not a site of haemopoiesis. The yolk sac produces various types of blood cells from a very early stage of embryogenesis and is a possible source of progenitor cells. Further experiments using grafting and chromosome marker techniques are being carried out in an attempt to clarify some of these problems.

We thank E. M. McDermid, Thornber Bros., Ltd., near Halifax, for supplying the double-yolked eggs.

¹ Owen, R. D., Davis, H. P., and Morgan, R. F., *J. Hered.*, **37**, 290 (1946).

² Ohno, S., Trujillo, J. M., Stenius, C., Christian, L. C., and Teplitz, R. L., *Cytogenetics*, **1**, 258 (1962).

³ Benirschke, K., and Brownhill, L. E., *Cytogenetics*, **1**, 245 (1962).

⁴ Woodruff, M. F. A., Fox, M., Buckton, K. A., and Jacobs, P. A., *Lancet*, **i**, 192 (1962).

⁵ Gengozian, N., Batson, J. S., and Eide, P., *Cytogenetics*, **3**, 384 (1964).

⁶ Billingham, R. E., Brent, L., and Medawar, P. B., *Phil. Trans. Roy. Soc.*, **B**, 357 (1956).

⁷ Hrabá, T., *Folia Biol. (Prague)*, **2**, 165 (1956).

⁸ Owen, J. J. T., *Chromosoma (Berl.)*, **16**, 601 (1965).

⁹ Owen, J. J. T., Moore, M. A. S., and Harrison, G. A., *Nature*, **207**, 313 (1965).

¹⁰ Hasek, M., *Ceskoslov. Biol.*, **2**, 25 (1953).

¹¹ Hamilton, H. L., *Lillie's Development of the Chick* (Henry Holt and Co., New York, 1952).

¹² Metcalf, D., and Wakonis-Vaartaja, R., *Lancet*, **i**, 1012 (1964).

¹³ Harris, J. E., Ford, C. E., Barnes, D. W. H., and Evans, E. P., *Nature*, **201**, 884 (1964).

¹⁴ Miller, J. F. A. P., *Lancet*, **i**, 43 (1963).

¹⁵ Cooper, M. D., Peterson, R. D. A., and Good, R. A., *Nature*, **205**, 143 (1965).

¹⁶ St. Pierre, R. L., and Ackerman, G. A., *Science*, **147**, 1307 (1965).

¹⁷ Lucas, A. M., and Jamroz, C., *Atlas of Avian Haematology* (U.S. Dept. of Agric., Washington, 1961).

ORIGIN OF THE GEOELECTRIC EFFECT IN PLANTS

By PROF. MALCOLM B. WILKINS and A. E. R. WOODCOCK

School of Biological Sciences, University of East Anglia, Norwich

WHEN a plant shoot, such as the coleoptile of *Avena sativa* and *Zea mays* or the hypocotyl of *Helianthus annuus*, is placed in the horizontal position, a potential difference develops between the upper and lower surfaces, the lower becoming positively charged with respect to the upper. This phenomenon, first reported by Bose¹ and afterwards investigated in a number of laboratories^{2–10}, is known as the geoelectric effect. In addition, shoots which have been placed in the horizontal position develop an upward curvature and this geotropic response continues until they have once again attained the vertical position. The relationship between the geoelectric effect and the geotropic response is obscure, but it has been suggested^{11–13} that the former may be an essential link between the perception of a gravitational stimulus by the shoot and the induction of the upward curvature.

Recent investigations of the geotropic response of shoots have clearly shown that the increased growth rate of the lower half and the decreased growth rate of the upper half are due to the development of different concentrations of auxin in the two halves of the organ. This concentration gradient is brought about by lateral movement of auxin from the upper to the lower half of the horizontal shoot^{11,14–17}.

The present investigation was begun to determine the relationship between the geotropic response and the geoelectric effect since, from a review of the literature, it seemed to us that there was a large body of evidence which pointed to the geoelectric effect being due to the establishment of an auxin concentration gradient across geotropically stimulated shoots. This evidence can be briefly summarized: (1) The geoelectric effect has little or

nothing to do with the primary gravi-perception mechanism since it does not develop in de-tipped *Helianthus* hypocotyls⁹ which can, nevertheless, perceive a geotropic stimulus¹⁸. It must therefore be connected, if it is connected at all, to a later stage in the geotropic response system. (2) The geoelectric effect and geotropic curvature begin to develop at a similar time (about 15 min) after the onset of geotropic stimulation^{10,19}. (3) The characteristics of the induction of a geotropic response are closely similar to those for the induction of the geoelectric effect in a variety of different experimental procedures^{10,20}. The response in both phenomena is proportional to the logarithm of the stimulation time, the presentation time for each is about 2.5 min and the angle at which the maximum response is achieved for both the geotropic response and the geoelectric effect is about 130° from the vertical. Furthermore, in experimental procedures such as those of Zimmermann²¹ the magnitude of the geotropic response and the geoelectric effect undergo closely similar changes^{10,21}. These facts clearly point to the geoelectric effect and the geotropic response being released by stimulation of the same gravi-perception system. Since the geoelectric effect cannot be connected to the primary gravi-perception system for reasons outlined in (1), the evidence in this section strongly suggests that it is associated with an intermediate stage in the geotropic response system. (4) The geoelectric effect does not develop in plant tissues in which no lateral movement of auxin occurs. De-tipped hypocotyls of *Helianthus* and other plants do not develop a geoelectric potential⁹, nor do they develop a geotropic response even if they are left in the horizontal position for 14 h (ref. 18). That the lack of geotropic response is due only to the lack of auxin in the tissue is shown by the fact that a curvature develops in response to the original gravitational stimulus when the hypocotyls are restored to the vertical position and then supplied at their apical ends with indolyl-3-acetic acid¹⁸. A similar finding has been reported for the perception of a gravitational stimulus in coleoptiles of *Avena* and *Zea*²². In the case of *Avena* and *Zea* coleoptiles, however, Grahm and Hertz^{9,10} have shown that the de-tipped organs do develop a geoelectric potential. Examination of the methods of Grahm and Hertz^{9,10} reveals that they removed only 1 mm of the apex of the coleoptile about 5 h prior to beginning the electrical measurements. In this time a physiological tip would have developed^{14,15,22,23} and this would produce auxin which would undergo lateral re-distribution on geotropic stimulation of the coleoptiles, thus giving rise to the observed geoelectric potential. (5) Neither the geoelectric potential⁹ nor the geotropic response develops in *Zea* coleoptiles under anaerobic conditions. These observations agree with the view that both phenomena are due to the occurrence of lateral movement of auxin in horizontal shoots since both the longitudinal and lateral transport of auxin are totally inhibited under anaerobic conditions²⁴.

Our hypothesis that the geoelectric effect is due to the different concentrations of auxin in the upper and lower halves of the geotropically stimulated shoot is amenable to direct test by establishing in vertical shoots a lateral auxin gradient and determining whether or not this gives rise to a potential difference between the two sides of the shoot. A lateral gradient of auxin has therefore been set up in vertical sections of *Zea mays* coleoptiles by supplying the coleoptiles at their apical ends with an asymmetric source of indolyl-3-acetic acid in a manner similar to that previously used by Goldsmith and Wilkins¹⁵. This procedure has been shown to set up in the section an auxin gradient which is proportional to the concentration of indolyl-3-acetic acid in the asymmetric source¹⁵.

Seeds of *Zea mays* L. var. 'Giant White Horse Tooth' were soaked in water for about 8 h, planted in washed vermiculite and grown in darkness. Five days after planting, the coleoptiles were decapitated twice under dim red light of wave-lengths above 595 nm. A de-tipped coleoptile was then detached from its mesocotyl

by a cut made 2.5–3.5 cm below the top of the coleoptile, and transferred to the measuring device which provided support and electrical contact at the base of the coleoptile by means of agar-calcium chloride gel.

The electrode system provided a base-reference electrode in contact with the agar-calcium chloride gel. On either side of the coleoptile, contact was made by means of a flowing 5 mM calcium chloride solution to two Calomel electrodes (Pye pH reference electrodes, No. 11161). The potential of each electrode with respect to the base was measured with a 'Vibron 33B' electrometer and the output recorded on a Kipp-Zonen, '2B-Microvolt' chart recorder. The base electrode could be varied about the earth potential by a potentiometer incorporated in the circuit. The whole electrode assembly was screened at earth potential by a zinc metal cage and illuminated from above the coleoptile segment with red light of wave-length greater than 595 nm.

At the beginning and end of an experiment the two electrodes were placed with their tips in contact so that by earthing one of the electrodes the potential across the whole system could be measured. The contact potential was never found to be more than 1 mV and was usually 0.2 mV. After the initial test of the electrode contact potential a coleoptile was placed vertically in the system and the electrodes placed in contact with either side and adjusted until both were at the same distance (usually about 3–4 mm) from the top of the coleoptile segment. This contact was maintained for 10–15 min before the experiment was begun to ensure that the system had attained electrical stability. The potential difference across the coleoptile at time zero, that is the moment before the agar block or lanolin paste is applied, was taken as zero. In practice this potential difference was never more than 2 mV. Fig. 1 shows the changes in this potential with time both before and after time zero. The potential across the coleoptile was measured at 6–12 sec intervals.

An auxin concentration gradient in the coleoptile segment was established by supplying indolyl-3-acetic acid to one-half of the apical end of the segment¹⁵. For this, two carrying media were used: (1) 1.5 per cent Difco 'Bacto-agar' which had previously been dialysed for 7 days, and (2) anhydrous lanolin. Owing to the low

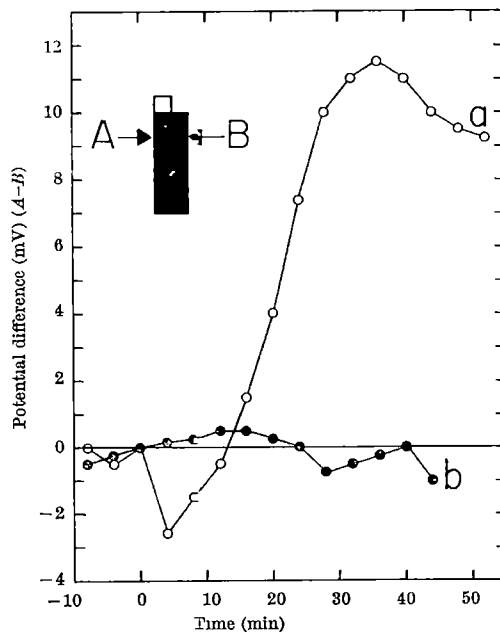


Fig. 1. The changes in the potential difference across vertical coleoptile segments of *Zea mays* after the application at time zero of an asymmetric supply of indolyl-3-acetic acid (0.02 per cent) in lanolin paste (curve *a*) and of lanolin paste without indolyl-3-acetic acid (curve *b*). Ordinate, potential difference across the coleoptile in mV (*A-B*). Abscissa, time in minutes before and after the application of the paste. The arrangement of the coleoptile, electrodes *A* and *B* and lanolin paste is shown in the inset diagram. Curves (*a*) and (*b*) are respectively the means of 7 and 5 individual experiments

humidity desirable for the satisfactory working of the electrode system the agar block (1 mm²) tended to dry out rapidly. This was undesirable, since the concentration of indolyl-3-acetic acid in the source would thus increase with time. In order to overcome this difficulty a paste of aqueous indolyl-3-acetic acid solution was made with anhydrous lanolin which proved to be a more satisfactory carrying medium. The concentrations of indolyl-3-acetic acid used were 10⁻⁶ M in the agar blocks and 0.02 per cent in the lanolin. Control experiments were carried out by placing the carrying medium without indolyl-3-acetic acid asymmetrically on the top of the coleoptile segment.

The development of a lateral potential with time after the asymmetric application of indolyl-3-acetic acid-lanolin paste to the top of a *Zea* coleoptile segment is shown in Fig. 1. The curve labelled (a) is the mean of 7 separate runs each carried out on a different coleoptile. During the first 8–10 min after the application of indolyl-3-acetic acid, the side of the coleoptile under the source tended to become electronegative with respect to the other side. In the mean curve (a in Fig. 1) this difference was not significant although it did occur in 4 of the 7 individual experiments. It is particularly marked in curve (a) of Fig. 2, which indicates changes in the potential difference observed in one of the experiments which contributed to the mean data presented in Fig. 1. About 15 min after the application of the indolyl-3-acetic acid, the side of the coleoptile under the source becomes electropositive with respect to the other side and the potential difference continues to increase for about 20 min. A difference of approximately 10 mV is maintained for at least a further 20 min, but by the end of this time the potential difference appears to be gradually decreasing. When lanolin paste without indolyl-3-acetic acid is applied asymmetrically to the apical end of the coleoptile segment, no significant potential difference develops between the two opposite sides of the organ. This is shown by curve (b) of Fig. 1, which is a mean of 5 individual experiments. The mean potential difference developed by the coleoptiles treated with indolyl-3-acetic acid and shown in curve (a) of Fig. 1 is significant at the 0.05 level after 28 min.

The changes in the potential difference across an individual coleoptile treated with indolyl-3-acetic acid paste and in one treated only with the carrying medium are shown in Fig. 2. These data are presented to show the stability of the potential difference, if any, across single coleoptiles.

Earlier experiments using dialysed agar as a carrying medium provided similar results, except that the agar

alone tended to cause the side of the coleoptile under the block to become about 6 mV electronegative to the other side. When indolyl-3-acetic acid was present in the block, however, the side under the block became electropositive to the other side. This potential developed gradually, beginning about 6–8 min after the application of the indolyl-3-acetic acid and becoming as much as 16 mV after 40–50 min.

The experiments described in this article show that when a lateral concentration gradient of indolyl-3-acetic acid is set up across a vertical coleoptile segment of *Zea mays*, the half with the highest concentration develops a surface potential at least 10 mV positive to that on the surface of the half with the lower concentration.

When a coleoptile is placed in the horizontal position a lateral movement of indolyl-3-acetic acid takes place from the upper to the lower half^{14–16}. This results in different concentrations of indolyl-3-acetic acid in the upper and lower halves of the tissue, and it is this difference in concentration that gives rise to the upward curvature of the coleoptile. It is now clear that the different concentrations of indolyl-3-acetic acid in the upper and lower halves of the coleoptile also give rise to the geoelectric effect. Our results confirm the large body of circumstantial evidence summarized in the introduction that the development of the geoelectric effect in shoots is due to the lateral movement of auxin in the tissue during geotropic stimulation. The geoelectric effect in shoots must therefore be regarded as a side-effect arising as a consequence of a critical intermediate stage in the geotropic response system.

The pattern of the change in the potential difference across the coleoptile with time is similar regardless of whether the auxin gradient occurs as the result of geotropic stimulation⁹ or as the result of the procedure used in this investigation. Graham and Hertz⁹ found the lower side to become slightly electronegative to the upper side during the first 10–15 min after the onset of geotropic stimulation. The close similarity in the time required for the positive charge to develop on the lower side of the coleoptiles in the experiments of Graham and Hertz⁹ and in the side under the source of indolyl-3-acetic acid in our experiments is probably fortuitous, since in our experiments this time is almost certainly a function of the distance between the apical end of the coleoptile segment and the electrodes.

Further investigation of the mechanism of the geoelectric effect in plant shoots is now in progress.

This investigation was carried out while one of us (A. E. R. W.) was supported by a Science Research Council studentship. We thank Dr. Alan Walker for many helpful discussions.

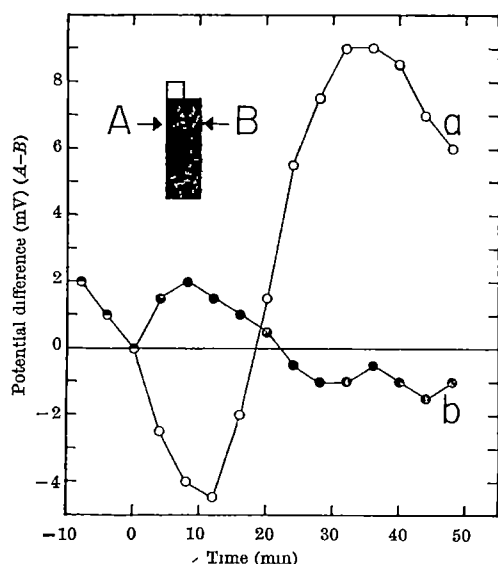


Fig. 2. Same as Fig. 1 except that the curves show the change in potential difference across individual coleoptiles supplied asymmetrically either with indolyl-3-acetic acid (curve a) or with the carrying medium without the indolyl-3-acetic acid (curve b)

¹ Bose, J. C., *Comparative Electrophysiology*, 434 (Longmans Green and Co., London, 1907).

² Brauner, L., *Jahrb. Wiss. Bot.*, **66**, 381 (1927).

³ Brauner, L., *Planta*, **53**, 449 (1959).

⁴ Brauner, L., von Dellingshausen, M., and Bock, A., *Planta*, **62**, 195 (1964).

⁵ Clark, W. G., *Plant Physiol.*, **12**, 409 (1937).

⁶ Schrank, A. R., in *Bioelectric Fields and Growth*, edit. by Lund, E. J. (Univ. of Texas Press, Austin, 1947).

⁷ Jantsch, B., *Zeit. Bot.*, **47**, 336 (1959).

⁸ Hertz, C. H., *Nature*, **137**, 320 (1960).

⁹ Graham, L., and Hertz, C. H., *Physiol. Plant*, **15**, 95 (1962).

¹⁰ Graham, L., and Hertz, C. H., *Physiol. Plant*, **17**, 186 (1964).

¹¹ Dolik, H., *Geotropie en Groeistof*, Dissert., Univ. of Utrecht, Netherlands (1930).

¹² Cholodny, N., *Planta*, **13**, 665 (1931).

¹³ Amlog, H. U., *Planta*, **21**, 211 (1933).

¹⁴ Goldsmith, M. H. M., and Wilkins, M. B., *Plant Physiol.*, Suppl., **37**, xvii (1962).

¹⁵ Goldsmith, M. H. M., and Wilkins, M. B., *Plant Physiol.*, **39**, 151 (1964).

¹⁶ Gillespie, B., and Thimann, K. V., *Plant Physiol.*, **38**, 214 (1963).

¹⁷ Gillespie, B., and Briggs, W. R., *Plant Physiol.*, **36**, 364 (1961).

¹⁸ Brauner, L., and Hager, A., *Planta*, **51**, 115 (1958).

¹⁹ Parkinson, K., Ph.D. thesis, Dunselm (1963).

²⁰ Larsen, P., *Handbuch der Pflanzenphysiologie*, **17** (2), 153 (Springer-Verlag, Berlin, 1962).

²¹ Zimmermann, W., *Jahrb. Wiss. Bot.*, **66**, 631 (1927).

²² Hahe, I., *Planta*, **57**, 557 (1961).

²³ Went, F. W., and Thimann, K. V., *Phytohormones*, 25 (Macmillan and Co., New York, 1937).

²⁴ Goldsmith, M. H. M. (personal communication).

LETTERS TO THE EDITOR

ASTRONOMY

Hydrogen Content of Virgo Cluster Galaxies

INVESTIGATIONS with small radio telescopes¹ have been unable to detect 21-cm radiation from galaxies much beyond those in the *M* 81 group, at a distance of 3 megaparsecs. The uncertainties in the distances of individual galaxies, and thus in their masses, have made it difficult to compare the hydrogen content of different morphological types. There are considerable advantages in studying galaxies in one of the large clusters, for the members can be assumed to be at approximately the same distance. In the nearby groups there are no isolated elliptical or other early-type galaxies. A knowledge of the hydrogen content of these systems would be important for an understanding of their evolution, and in the large clusters there are numbers of such early-type galaxies suitable for investigation at 21 cm.

The Virgo cluster of galaxies is the nearest of the large clusters, at a distance of about 12 megaparsecs. A preliminary investigation of 18 member galaxies has been made during 1964 and 1965 with the Australian 210-ft. telescope. 21 cm radiation has been detected from eight of these, including the giant elliptical galaxy *NGC* 4472.

The most significant datum yielded by extra-galactic 21-cm observations is the total mass of hydrogen in a galaxy. When the galaxy is much smaller than the telescope beam the hydrogen mass M_H (in solar masses) is:

$$M_H/M_\odot = 3.08 D^2 (\lambda^2/A_0) \int_0^\infty T_a(\nu) d\nu \quad (1)$$

where $T_a(\nu)$ is the antenna temperature at frequency ν , D is the distance in kiloparsecs, λ is the wave-length, A_0 is the effective collecting area of the telescope.

The integration over frequency can be performed by using a band-width B wide enough to receive all the 21-cm radiation from the galaxy. If the observed antenna temperature is T_a :

$$\int T_a(\nu) d\nu = T_a \cdot B$$

In the investigation reported here, a band-width of 1.5 megacycles was used, corresponding to a velocity spread of 320 km/sec. This may not be wide enough to receive all the radiation from edge-on systems, so that the observations then yield lower limits to the hydrogen masses.

For the 210-ft. telescope an antenna temperature T_a of 0.1° K in a band-width of 1.5 Mc/s would be produced by a hydrogen mass of $2 \times 10^9 M_\odot$ at the distance of the Virgo cluster. A low-noise parametric receiver² was used, with a noise temperature of 160° K. An analogue integrator averaged the receiver output for periods of 100 sec, reducing the root mean square fluctuation to 0.035° K. The telescope was set alternately on the galaxy and on a nearby reference region. This sequence was usually repeated for 40 min or more, the errors then being reduced to below 0.016° K. In some cases the measurement was repeated with another reference region.

In the preliminary investigations the receiver has been switched between two frequencies—one corresponding to the optical redshift³ of each galaxy, the other either a band-width higher or a band-width lower. If the redshift of a galaxy differs from that adopted, the measured antenna temperatures will be different for the two reference frequencies. The higher value has been adopted in calculating the hydrogen content.

The results are listed in Table 1. The line radiation from eight galaxies is above the limit of detection, the hydrogen masses ranging from 3×10^8 to 2.4×10^9 solar

Table 1. 21-CM OBSERVATIONS OF VIRGO CLUSTER GALAXIES

NGC	Type	Magnitude	Optical redshift (km/sec)	Signal velocity (km/sec)	Reference velocity (km/sec)	Antenna temperature (°K)	Hydrogen mass (M_\odot) ($10^9 M_\odot$)	Luminosity (L) ($10^9 L_\odot$)	M_H/L	Assumed total mass (M_\odot) ($10^{11} M_\odot$)	M_H/M_T	Notes
4116	<i>Sdm</i>	12.3	1304	1325 1300	1010 1695	0.070 ± 0.020 0.051 ± 0.010	1.4	2.6	0.53	0.1	0.14	<i>a</i>
4303	<i>Sbc</i>	10.0	1671	1670 1670	1350 2040	0.087 ± 0.020 0.120 ± 0.015	2.4	23	0.10	0.5	0.048	<i>a</i>
4321	<i>Sbc</i>	10.0	1617	1630 1630	1320 2040	0.034 ± 0.020 0.053 ± 0.017	1.1	23	0.05	1	0.01	<i>a</i>
4382	<i>SO</i>	10.0	773	770	1100	0.008 ± 0.018	< 0.5	23	< 0.02	4	< 0.001	<i>a</i>
4450	<i>Sab</i>	10.8	2048	2050	1730	0.002 ± 0.018	< 0.4	10.6	< 0.04	1	< 0.004	<i>a</i>
4472	<i>E2</i>	9.3	1013	1010 1010	650 1325	0.016 ± 0.010 0.015 ± 0.008	0.3	41	0.007	15	0.0002	<i>a, b, e</i>
4501	<i>Sb</i>	10.1	2120	2120 2120	1790 2520	0.018 ± 0.017 0.008 ± 0.012	< 0.7	21	< 0.033	1.5	< 0.005	<i>a</i>
4527	<i>Sbc</i>	11.3	1727	1670 1730	1350 2100	0.078 ± 0.012 0.110 ± 0.014	2.2	6.6	0.33	1.0	0.022	<i>a</i>
4535	<i>Sc</i>	10.4	1930	1950 1950 1045 1040	1650 2275 1335 1600	0.061 ± 0.020 0.071 ± 0.014 -0.003 ± 0.011 -0.013 ± 0.016	1.4	16	0.09	0.7	0.02	<i>a</i>
4536	<i>Sbc</i>	10.9	1927	1930	2240	0.060 ± 0.017	1.2	9.6	0.13	0.3	0.04	<i>a</i>
4569	<i>Sab</i>	10.1	960	960	650	-0.001 ± 0.016	< 0.2	20	< 0.01	1	< 0.002	<i>a</i>
4579	<i>Sb</i>	10.3	1752	1010 1750	1325 1450	-0.009 ± 0.014 0.008 ± 0.015	< 0.6	17	< 0.04	1.0	< 0.006	<i>a</i>
4594	<i>Sa</i>	9.0	1207	1750 1320	2150 1010	0.018 ± 0.013 0.008 ± 0.012	< 0.5	58	< 0.009	12	< 0.0004	<i>a</i>
4636	<i>E1</i>	10.4	973	1210 970	1690 650	0.008 ± 0.019 0.005 ± 0.012	< 0.3	13	< 0.02	5	< 0.0006	<i>a</i>
4649	<i>E2</i>	9.9	1389	970 1320	1350 950	0.000 ± 0.017 -0.012 ± 0.017	< 0.3	24	< 0.015	10	< 0.0003	<i>a</i>
4697	<i>E6</i>	10.1	1308	1320 1310	1660 950	-0.002 ± 0.018 0.006 ± 0.017	< 0.5	20	< 0.025	8	< 0.0006	<i>a</i>
4699	<i>Sb</i>	10.2	1511	1320 1510	1630 1100	0.020 ± 0.016 0.036 ± 0.014	1.0	18	0.05	1	0.01	<i>a</i>
4753	<i>SO_p</i>	10.2	1364	1510 1360	1820 950	0.052 ± 0.014 -0.005 ± 0.015	< 0.4	18	< 0.02	4	< 0.001	<i>a</i>
				1360	1660	-0.02 ± 0.022						

a, Reference point 30' east
b, " " 30' west
c, " " 1° east
d, " " 2° east
e, " " 1° south

masses. The error quoted is the standard deviation of the measurement. The distance modulus of the Virgo cluster⁴ is assumed to be 30.5. The ratio of hydrogen mass to luminosity, M_H/L , is the one quantity which is independent of errors in the distance. For NGC 4116 the ratio of 0.53 (in solar units) agrees well with that found for other irregulars². For the late-type spiral systems M_H/L lies within a narrower range than found for members of nearby groups¹, with an average value of 0.14. The ratio decreases further for the early-type systems, being below 0.01 in several cases.

The total masses M_T of the galaxies have been taken from Holmberg's catalogue⁴, or estimated by assuming that the mass-luminosity ratio varies from 4 for *Sc* to 40 for *E*. For the late-type spirals the ratio M_H/M_T agrees with that found for nearby galaxies. For the giant elliptical NGC 4472 M_H/M_T is 0.0002, well below the limit for ellipticals set by the observations of M 32 (refs. 5 and 6). The other ellipticals examined are less massive and no hydrogen could be detected in them; in each case M_H/M_T is less than 0.0006.

Davies *et al.*⁶ observed the spiral galaxy NGC 4535 with the Jodrell Bank 250-ft. telescope. They found no emission at the expected velocity (1950 km/sec), but reported a signal of 0.2° K for a velocity of 1038 km/sec. They suggested that this represented emission from an intergalactic cloud. In the present investigation a search was made for emission at this velocity, taking reference points both 1° and 2° east of NGC 4535. The mean measured antenna temperature is $-0.01^\circ \pm 0.01^\circ$ K. It thus appears unlikely that there is any emission from this direction other than that associated with NGC 4535 itself.

For some of the galaxies in Table 1 it would not be difficult to measure detailed 21-cm line profiles with narrow-band equipment, and then to determine redshifts with an accuracy higher than that available from optical measurements.

B. J. ROBINSON

C.S.I.R.O. Radiophysics Laboratory,
Sydney, Australia.

J. A. KOEHLER*

Mt. Stromlo Observatory,
Canberra, Australia.

* Present address: Arecibo Ionospheric Observatory, Puerto Rico.

¹ Epstein, E. E., *Astron. J.*, **69**, 490 (1964).

² Robinson, B. J., and van Damme, K. J., *Austral. J. Phys.* (in the press).

³ Humason, M. L., Mayall, N. U., and Sandage, A. R., *Astron. J.*, **61**, 97 (1956).

⁴ Holmberg, E., *Arkiv for Astronomi*, **3**, No. 30 (1964).

⁵ Wentzel, D. G., and van Woerden, H., *Bull. Astron. Inst. Netherlands*, **14**, 335 (1959).

⁶ Davies, R. D., Gottesman, S. T., Reddish, V. C., and Verschuur, G. L., *Observatory*, **83**, 245 (1963).

PHYSICS

Townsend's First Ionization Coefficient for Compressed Nitrogen

THERE is a growing interest in the use of compressed gases, especially nitrogen and sulphur hexafluoride, for high-voltage insulation. However, there have been few measurements of the fundamental parameters, such as the Townsend α -coefficient, in gases at pressures above atmospheric. Allen¹ has made measurements at pressures up to 4 atmospheres absolute in nitrogen and has shown that, for values of E/p (electric stress/pressure) greater than 40 (V/cm)/mm mercury, the value of α agrees well with results from work at pressures below atmospheric. In the work recorded here it has been possible to extend measurements in high-pressure nitrogen down to $E/p_0 = 31.5$ (V/cm)/mm mercury (p_0 is the pressure corrected to 0° C) with pressures up to 10 atmospheres.

The pre-breakdown current was measured as a function of electrode spacing d , and α was derived from the Townsend relation:

$$i = i_0 \exp \alpha d$$

The initial current i_0 was 6×10^{-13} amp and was induced from a nickel cathode by directing ultra-violet light through a perforated anode of stainless steel. The total current i was restricted to less than 2×10^{-10} amp and electrode spacings up to 5 mm were used. The electrode had a 2.5-cm diameter plane area with 1.3-cm radius edges. Before assembling the test cell the plane areas of the electrodes were polished on a felt disk impregnated with 1 μ diameter diamond dust and were then washed in *n*-hexane. The test cell was evacuated to a pressure of about 0.3 mm mercury for several hours before measurements were made. The nitrogen used was 99.9 per cent purity (British Oxygen Co., Ltd.).

The values of α/p_0 are shown in Fig. 1 and it can be seen that they agree remarkably well with previous values taken from work at low pressures²⁻⁴. The higher α values found by Heylen⁵ may be due to his use of a very high-purity gas sample (99.998 per cent). In this investigation the values for E/p_0 greater than 38.6 (V/cm)/mm mercury were derived from measurements at 2 atmospheres absolute, but work involving the examination of individual avalanche growth⁶ has shown that the same values are obtained at 7 atmospheres absolute. At the lowest value of E/p_0 , 31.5 (V/cm)/mm mercury, measurements were made at 6, 8 and 10 atmospheres absolute.

As might be expected, these results confirm that α/p_0 is a function of E/p_0 and independent of pressure. Measurements at different pressures and constant E/p_0 yielded results of α/p_0 which agreed to within 4 per cent. Both E and p_0 could usually be measured with an error of less than ± 1 per cent, although an error in pressure of ± 2 per cent may be possible at the lowest pressure of 2 atmospheres absolute.

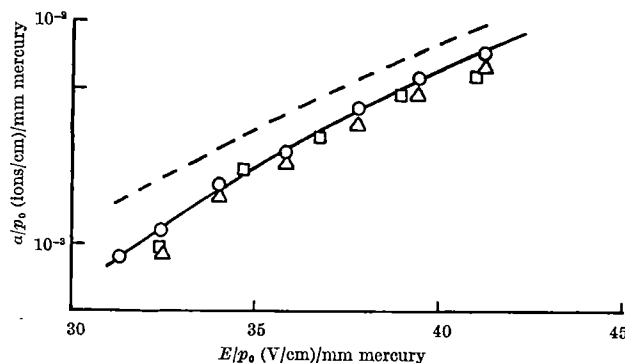


Fig. 1. α/p_0 versus E/p_0 in nitrogen. —, Present work, ---, ref. 5; O, ref. 3; □, ref. 4, Δ, ref. 2.

In view of the need for a better understanding of the breakdown process in compressed gases, it would be useful to extend measurements of α to much lower values of E/p_0 . However, such measurements may prove difficult. At pressures above 10 atmospheres Paschen's law is no longer valid^{7,8} and current measurements are hampered by low and erratic electric strengths. For example⁹, with stainless-steel electrodes and a 1-mm gap in nitrogen at 26 atmospheres absolute, breakdown was found to occur at a stress of about 150 kV/cm, when $E/p_0 = 8.3$ (V/cm)/mm mercury. Also, care has to be taken at higher pressures because the high electrical stresses involved lead to field emission. At pressures above 10 atmospheres the electric strength as well as field emission becomes dependent on the nature of the electrode surfaces. Consequently, a careful choice of electrode material and method of electrode preparation will be necessary if Townsend's α coefficient is to be determined for E/p_0 less than 30 (V/cm)/mm mercury and p_0 greater than 7,600 mm

mercury. In this work it was found important to keep the electrode surfaces reasonably dust-free, and with a nickel cathode it was possible to reach a stress of 220 V/cm without appreciable field emission. Work in this laboratory on compressed methane⁶ has also shown that high electric strength and low field emission can be obtained with a heavily oxidized copper cathode.

This work was supported by the Central Electricity Generating Board. I thank Prof. M. W. Humphrey Davies for the facilities provided.

B. W. WARD*

Materials Laboratory,
Electrical Engineering Department,
Queen Mary College,
London, E.1.

* Present address: Electrical and Control Engineering Department, City and Guilds College of Technology, London, S.W.11.

Allen, N. L., *Brit. J. Appl. Phys.*, **14**, 589 (1963)

De Bitteto, D. L., and Fisher, L. H., *Phys. Rev.*, **104**, 1213 (1956)

Masch, K., *Arch. Elektrotech.*, **26**, 587 (1932).

Posin, D. Q., *Phys. Rev.*, **50**, 650 (1936).

Heylen, A. E. D., *Nature*, **183**, 1545 (1959).

Jackson, A. H. (personal communication).

Llewellyn-Jones, F., *Ionization and Breakdown in Gases*, 61 (Methuen, 1957).

Llewellyn-Jones, F., and Morgan, C. G., *Phys. Rev.*, **82**, 970 (1951).

Goldspink, G. F. (personal communication).

CHEMISTRY

Phosphorus-31 Chemical Shifts in Secondary and Tertiary Phosphines

ALTHOUGH several theoretical and empirical attempts¹⁻⁴ have been made to predict the ³¹P chemical shifts of phosphorus compounds, these have seldom achieved an accuracy of better than 10–20 p.p.m. For example, Van Vazer *et al.*³ assigned to alkyl groups the 'group shift' of +20 p.p.m. in trivalent phosphorus compounds. On this basis a tertiary phosphine should have a ³¹P chemical shift of about +60 p.p.m. However, the known values for twenty tertiary phosphines (Table 1) vary widely from his prediction, ranging from –19.3 to +62 p.p.m.

Table 1. CALCULATED AND OBSERVED CHEMICAL SHIFTS OF TWENTY-NINE PHOSPHINES

Phosphine	Calcd. Δ (p.p.m.)	Obs. Δ (p.p.m.)	Reference
Me ₃ P	+62	+62	3
Et ₃ P	+20	+20.4	2, 5
<i>n</i> -Pr ₃ P	+32	+33	6
<i>n</i> -Bu ₃ P	+32	+32.3	5
<i>i</i> -Pr ₃ P	–19	–19.4	*
<i>i</i> -Bu ₃ P	+41	+40	6
<i>cy</i> -Hx ₃ P	–7	–7	6
Ph ₃ P	+8	+8	3
Ph ₂ MeP	+26	+28	7
Ph ₂ EtP	+12	+12	6
Ph ₂ (<i>i</i> -Pr)P	–1	–0.2	*
Ph ₂ (<i>n</i> -Bu)P	+16	+17.1	*
Ph ₂ (<i>cy</i> -Hx)P	+3	+4.4	*
PhMe ₂ P	+44	+46	6
PhEt ₂ P	+16	+16	6
Ph(<i>i</i> -Pr) ₂ P	–10	–10	*
Ph(<i>n</i> -Bu) ₂ P	+24	+26.2	*
Ph(<i>cy</i> -Hx) ₂ P	–2	–2.5	*
Me ₂ EtP	+48	+48.5	4
Et ₂ MeP	+34	+34	6
Me ₂ PhP	+99	+99.5	7
MeEtPhP	+78	+77	8
Me(<i>n</i> -Pr)PH	+84	+87	8
Me(<i>n</i> -Bu)PH	+84	+86	8
MePhPH	+72	+72.3	8
Et ₂ PH	+57	+55.5	7
(<i>n</i> -Bu) ₂ PH	+69	+69.5	6
(<i>i</i> -Bu) ₂ PH	+78	+82	6
Ph ₂ PH	+45	+41.1	7

* Our measurements.

Others⁴ have noted a regular variation in the chemical shift of a tertiary, secondary, or halo-phosphine on stepwise substitution of the alkyl groups by other alkyl groups.

In the course of studies with a variety of phosphines and their derivatives, we have found that the ³¹P chemical shift (Δ p.p.m., relative to 85 per cent phosphoric acid) of a tertiary phosphine is given by equation (1) with a standard deviation of 1.0 for twenty compounds

$$\Delta (\text{p.p.m.}) = 62 - \sum_{n=1}^3 \sigma_n^P \quad (1)$$

of which the shifts have been measured. Further, the ³¹P chemical shift of a secondary phosphine is given by equation (2) with a

$$\Delta (\text{p.p.m.}) = 99 - 1.5 \sum_{n=1}^2 \sigma_n^P \quad (2)$$

standard deviation of 2.3 for the nine compounds for which data are available. More accurate estimation of the chemical shift by use of fractional σ^P values was not attempted since the accuracy of experimental chemical shifts is only about ± 1 p.p.m.

Insufficient data on primary phosphines are available to give a similar generalization for them.

The values for the constant σ^P for several organic groups are given in column 2 of Table 2.

Table 2. COMPARISON OF σ^P CONSTANTS WITH TAFT AND HAMMETT CONSTANTS

R Group	σ^P	σ^*	σ_m	σ_p
Me	0	0.0	–0.07	–0.17
Et	+14	–0.10	–0.07	–0.15
<i>n</i> -Pr	+10	–0.115	–0.05	–0.13
<i>i</i> -Pr	+27	–0.19	–0.07	–0.15
<i>n</i> -Bu	+10	–0.13	–0.07	–0.16
<i>i</i> -Bu	+7	–0.125	–	–0.12
<i>cy</i> -Hx	+23	–0.15	–	–
Ph	+18	+0.60	+0.06	+0.01

Fig. 1 shows the observed chemical shifts of the tertiary and secondary phosphines plotted against $\sum \sigma^P$, and Fig. 2 shows the observed chemical shifts of all twenty-nine phosphines plotted against the values obtained from equations (1) and (2). The numerical values are listed in Table 1.

The basicities of primary, secondary, and tertiary phosphines have been shown by Henderson and Streuli⁹ (with the use of Taft¹⁰ σ^* constants) to display a similar additive substituent effect, but inspection of the σ^* constants for various groups given in column 3 of Table 2 reveals little correlation between them and the corresponding σ^* constants. The *meta* and *para* Hammett σ constants likewise fail to display correlation with σ^P .

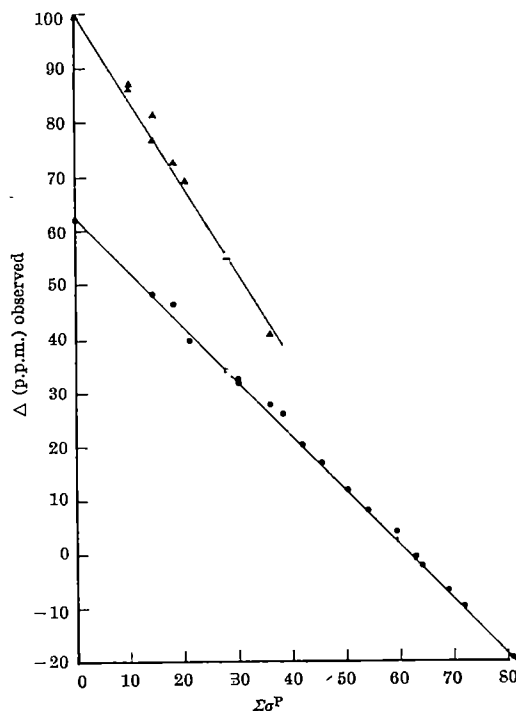


Fig. 1. Observed chemical shifts in relation to $\sum \sigma^P$. Δ , Secondary phosphines; \bullet , tertiary phosphines

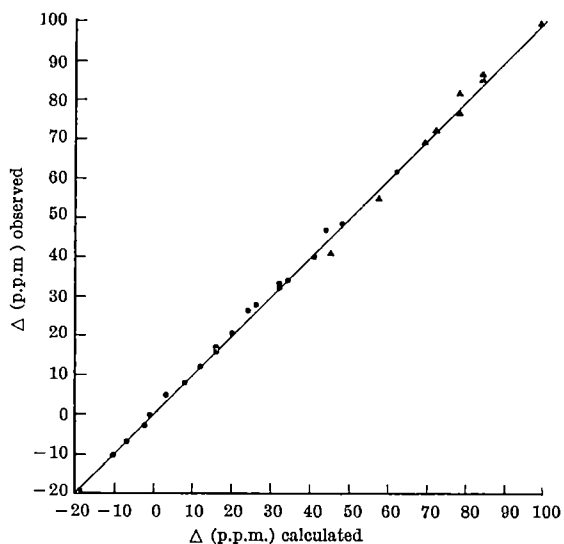


Fig. 2. Observed chemical shifts in relation to chemical shifts calculated from equations (1) and (2). Δ , Secondary phosphines; \bullet , tertiary phosphines

The σ^* constants are essentially measures of inductive effect and thus it follows that the inductive effect is not the only factor influencing the ^{31}P chemical shift. Indeed, the order of the σ^* constants indicates that the inductive effect is not the major factor in determining ^{31}P chemical shift. Resonance effects, such as hyperconjugation involving the phosphorus $3d$ orbitals¹¹, may contribute and, in addition, steric effects which alter the bond angles and thus the hybridization of phosphorus are important². The resulting different anisotropies about phosphorus would markedly affect the chemical shift, but sufficient data are not yet available to determine the relative magnitudes of the different effects.

Work is continuing on derivatives of these and other phosphines with the aim of establishing similar relationships.

This work was supported in part by a National Science Foundation grant G-24443. The Advanced Research Projects Agency, Department of Defence, purchased the phosphorus nuclear magnetic resonance equipment. Both of us are recipients of the University of Maryland General Research Board awards, 1964.

SAMUEL O. GRIM
WILLIAM MCFARLANE

Department of Chemistry,
University of Maryland, College Park, Maryland.

- ¹ Gutowsky, H. S., and McCall, D. W., *J. Chem. Phys.*, **22**, 162 (1954).
- ² Muller, N., Lauterbur, P. C., and Goldenson, J., *J. Amer. Chem. Soc.*, **78**, 3557 (1956).
- ³ Van Wazer, J. R., Callis, C. F., Shoolery, J. N., and Jones, R. C., *J. Amer. Chem. Soc.*, **78**, 5715 (1956).
- ⁴ Groenweghe, L. C. D., Maier, L., and Moedritzer, K., *J. Phys. Chem.*, **66**, 901 (1962).
- ⁵ Finegold, H., *Ann. N.Y. Acad. Sci.*, **70**, 875 (1958).
- ⁶ Henderson, W. A., and Buckler, S. A., *J. Amer. Chem. Soc.*, **82**, 5794 (1960).
- ⁷ Moedritzer, K., Maier, L., and Groenweghe, L. C. D., *J. Chem. Eng. Data*, **7**, 307 (1962).
- ⁸ Maier, L., *Chem. Ber.*, **94**, 3056 (1961).
- ⁹ Henderson, W. A., and Streuli, C. A., *J. Amer. Chem. Soc.*, **82**, 5791 (1960).
- ¹⁰ Taft, jun., R. W., in *Steric Effects in Organic Chemistry*, edit by Newman, M. S., 619 (Wiley, New York, 1956).
- ¹¹ McDaniel, D. H., *Science*, **125**, 545 (1957).

Relative Ionization Efficiencies of Hydrocarbon Molecules as a Function of Bombarding Electron Energy

It has recently been shown that ions play an important part in the radiation chemistry of alkanes¹. Since the ionizations that occur during γ - or fast electron-radiolyses are caused by interactions of molecules with electrons of a wide range of energies, it is desirable to obtain information about the relative ionization efficiencies of different types

of hydrocarbons as a function of bombarding electron energy.

Recent results of Schram *et al.*² can be used to show that the relative ionization cross-sections (RICS) of saturated and unsaturated hydrocarbons are constant over the electron energy-range of 12,000 eV–600 eV and are approximately (within ± 15 per cent from methanol to benzene) proportional to the total number of electrons in the molecule. Results of Kebabian and Godbole³ can be used to show that the RICS of hydrocarbons are constant within an experimental uncertainty of about 20 per cent over the electron energy-range of 5,000 eV–70 eV. The work recorded here extends these investigations from 70 eV to 15 eV.

Cross-sections for the ionization of molecules by 75 eV electrons are proportional to the polarizabilities of the molecules⁴. For a given type of molecule, the cross-sections for 75 eV electrons are also proportional to the number of valence electrons in the molecules⁵. The excitation potential of the K -shell electrons in a carbon atom is 280 eV (ref. 6) so these electrons would not be affected by bombardment with 75 eV electrons.

In this communication the RICS of a compound will be expressed as the ionization cross-section (ICS) per valence electron in the compound relative to that per valence electron in *n*-butane:

$$\text{RICS of molecule} = \frac{\text{ICS of molecule}/\text{No. of valence electrons in molecule}}{\text{ICS of } n\text{-butane}/26}$$

Butane was chosen as a standard for comparison because it is common to the investigations of Schram² and Kebabian³.

The RICS of cyclohexane, cyclohexene and benzene are shown in Fig. 1 as a function of bombarding electron energy. The results were obtained with the aid of an Associated Electrical Industries, Ltd., MS2H mass spectrometer. The values of RICS are unity, within experimental error, for all the compounds down to bombarding electron energy of 20 eV. The ionization potentials of these compounds are: butane, 10.8 eV; cyclohexane, 10.3 eV; cyclohexene, 9.2 eV; benzene 9.2 eV (ref. 7). The absolute ICS of a molecule decrease rapidly as the energy of the bombarding electron approaches the ionization potential of the molecule^{7,8}. The absolute ICS of *n*-butane decreases more rapidly than those of cyclohexane, cyclohexene and benzene at energies below 20 eV, so the RICS of the latter compound increase.

The values of RICS are constant down to an electron bombarding energy that is only double the ionization potential of the molecules.

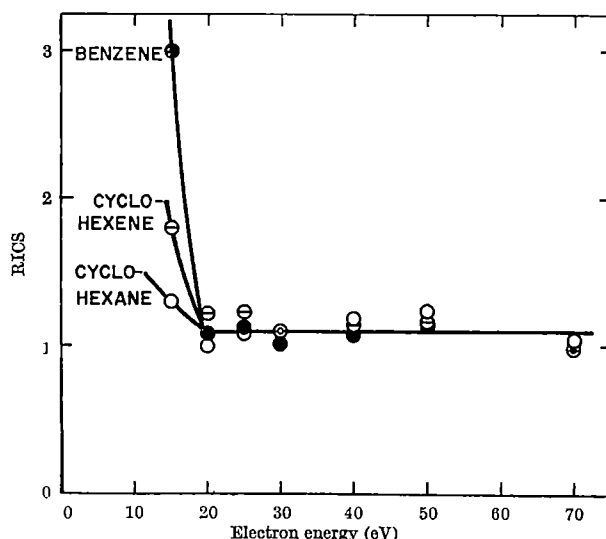


Fig. 1. Relative ionization cross-sections (RICS) of benzene, cyclohexene, and cyclohexane as a function of bombarding electron energy. The RICS values were determined relative to the ionization cross-sections of *n*-butane at the respective electron energies

The ICS of a hydrocarbon is proportional to the total number of electrons in its molecule for electron bombarding energies above 600 eV (ref. 2) and is proportional to the number of valence electrons in its molecule for bombarding electron energies between about 300 eV (the ionization potential of a *K* electron in carbon) and 20 eV. In mixtures of hydrocarbons, 'valence electron' fractions do not differ by more than 10 per cent from the 'total electron' fractions. In view of the wide energy spectrum of the electrons that cause ionization during the radiolysis of a system, the assumption that the initial distribution of ionizations among the components of a mixture of hydrocarbons is according to the respective 'total electron' fractions of the components is adequate. Furthermore, the fact that total electron fractions emphasize unsaturated compounds slightly more than do valence electron fractions tends to compensate for the fact that the RICS of unsaturated compounds is greater than that of saturated compounds at low bombarding energies.

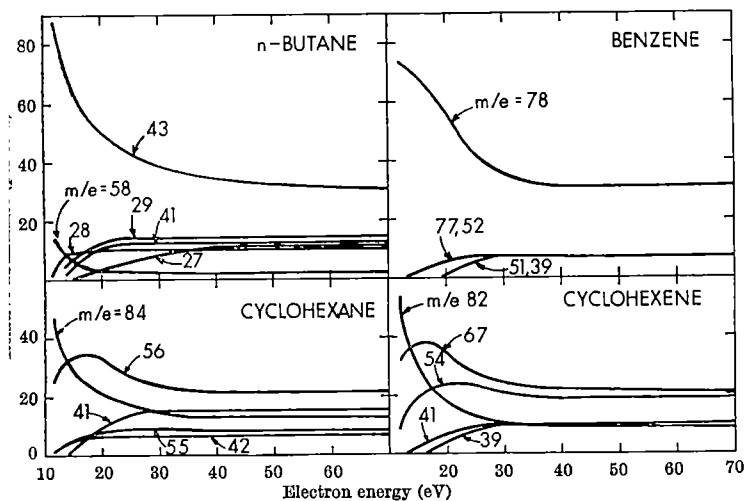


Fig. 2. Fragmentation patterns of *n*-butane, benzene, cyclohexane, and cyclohexene as a function of bombarding electron energy. The ion currents due to the individual ions are expressed as fractions of the total ion current. Only the major ions are represented.

An additional piece of information is available from the present work. Kebarle and Godbole³ determined that the relative abundances of the major ions in the mass spectrum of a hydrocarbon were nearly constant over the bombarding electron energy-range of 10,000 eV–70 eV. The present results project this investigation to lower energies. The relative abundances of the major ions in the cyclohexane, cyclohexene, benzene and *n*-butane mass spectra are shown in Fig. 2 for bombarding electron energies in the range of 70 eV–12 eV. The relative abundances of the major ions from all the compounds are essentially independent of electron energies down to 40 eV. This means that, neglecting minor processes such as inner-shell excitation, the energy distribution of excited hydrocarbon ions is independent of bombarding electron energy above about 40 eV (about four times the binding energy of the electrons being perturbed).

J. A. TAKACS
G. R. FREEMAN

Department of Chemistry,
University of Alberta, Edmonton, Canada.

For example: (a) Williams, F., *J. Amer. Chem. Soc.*, **86**, 3954 (1964). (b) Freeman, G. R., *J. Chem. Phys.*, **43**, 93 (1965).
Schram, B. L., v.d. Wiel, M. J., de Heer, F. J., and Moustafa, H. R., *J. Chem. Phys.* (in the press).
Kebarle, P., and Godbole, E. W., *J. Chem. Phys.*, **36**, 302 (1962).
Lampe, F. W., Franklin, J. L., and Field, F. H., *J. Amer. Chem. Soc.*, **79**, 6129 (1957).
Otvos, J. W., and Stevenson, D. P., *J. Amer. Chem. Soc.*, **78**, 546 (1956).
Tables of X-ray Emission Spectra, compiled by North American Phillips Co., Inc., Mount Vernon, N.Y.
Field, F. H., and Franklin, J. L., *Electron Impact Phenomena*, 263 (Academic Press Inc., New York, 1957).
Tate, J. T., and Smith, P. T., *Phys. Rev.*, **39**, 270 (1932).

Androgenic Steroid Complexes with *p*-Bromophenol

ONE of the major problems encountered in using the heavy atom X-ray method¹ to determine the structure of steroids^{2,3} (or any other molecule) is the possibility that the direct attachment of a heavy atom to the molecule will cause distortion. Theoretically, the amount of distortion is determined by steric factors and the stability of the electronic configurations adjacent to the site of attachment. For example, when a bromine atom is covalently attached to carbon four of an aromatic A-ring of the steroid nucleus, it is reasonable to expect that the resulting distortion would be insignificant, because of the stability imparted to the A-ring by the aromatic system. Experimental evidence supporting this expectation was obtained in the molecular-structure determinations of 4-bromoestrone² and 4-bromoestradiol³. In these brominated steroids, the A-rings are extremely planar (non-planarity coefficients 0.02 Å in both cases) despite the covalently attached bromine.

On the other hand, when a heavy atom is attached to non-aromatic rings (or to various side groups) which lack the stability of the electronically imposed planarity of aromatic rings, considerable distortion should be expected, especially in the immediate vicinity of the molecular perturbations caused by the substitution. Evidence supporting this is suggested by the fact that the absorption spectra of some of the nuclear-substituted bromo-derivatives of steroids (such as the α -bromo ketones) are distinctly different from the absorption spectra of the unbrominated compounds. This is so because the equatorial halogen, which is co-planar with the carbonyl group, interacts inductively with the carbonyl group, shortening and strengthening the C=O double bond. This results in a small hypsochromic shift in the ultra-violet and in a larger change in the infra-red region where the frequency of the carbonyl stretching mode is increased as much as 20 cm⁻¹. Axially attached bromine, on the other hand, produces a pronounced bathochromic shift in the ultra-violet of about 20–30 m μ but no changes in the infra-red. In extreme cases, such as the 6 β (a)-bromo- Δ^4 -3-oxosteroids, the compounds are quite labile and can be de-hydrobrominated with comparatively mild reagents⁴.

Papers reporting the amount of distortion in quantitative terms are scarce in the literature. We have been unable to find examples of X-ray structure determinations of compounds approximately the same size as steroids with and without a heavy atom. It is hoped that when the work has been completed, the structure determinations of 16- and 21-bromo isomers of 5 α -pregnan-3 β ,17 α -diol-11,20-dione, at present being made in our laboratory, will shed some light on the problem. In the meantime, we have turned our attention to another approach.

The whole idea of the heavy atom method is to add a few heavy atoms to the unit cell which are crystallographically arranged and which have high enough atomic scattering factors to dominate the phases of the waves scattered by the total contents of the unit cell. Thus, from the point of view of crystal structure determination, it is completely unimportant whether or not the heavy atoms are bound to the molecule the structure of which is being determined. The whole problem of molecular distortion by the covalent binding of heavy atoms may be circumvented by the co-crystallization of the steroid molecule with a smaller molecule containing an appropriate heavy atom. With this idea in mind, one of us (C. E.) undertook to prepare such complexes. The purpose of this communication is to report the preparation, heavy atom analysis, spectroscopic data, and single crystal X-ray diffraction

data for a series of molecular complexes between some of the androgenic steroids and *p*-bromophenol.

The steroids and the *p*-bromophenol were mixed in a 1:1 molar ratio, dissolved in *n*-hexane, and left in stoppered vials for crystallization. In an effort to obtain crystals suitable for X-ray analysis (diameter ≈ 0.03 mm), the vials were kept in vacuum flasks heated by a thermoregulated electrical resistance coil and the temperature reduced gradually from 60°C to room temperature over a period of approximately one week. The mother liquor was decanted and the crystals washed thoroughly with *n*-hexane and afterwards dried at 40°C in a vacuum.

The products were subjected to combustion in a Schöniger oxygen flask⁵ and afterwards analysed for bromine by potentiometric titration of the bromide formed with a calibrated silver nitrate solution. The results of these analyses are given in Table 1.

Infra-red absorption spectroscopy is one of the powerful tools for the detection and quantitative evaluation of the hydrogen bond. It has been well established in a surprisingly large number of papers that molecular association between phenols and ketones is stabilized by hydrogen bonding, the phenol acting as a proton donor and the carbonyl group as the acceptor⁶⁻¹³. In general, the inter-

molecular association involving hydrogen bonding is characterized by a number of changes in the specific absorption bands of the participating functional groups.

The OH stretching mode of the alcohol or phenol is shifted to lower frequencies because of the increase of the O—H bond length. A similar decrease in frequency is observed for the C=O stretching mode of a carbonyl group participating in hydrogen bonding as a proton acceptor, while the C—O—H deformation mode (that is the in-plane bending of the alcoholic or phenolic O—H) is shifted to higher frequencies. In all these cases the amount of shift and absorptivity is related to the strength of the hydrogen bond involved¹⁴.

Our preliminary infra-red spectroscopic measurement support the assumption that intermolecular complexes between androgenic steroids and *p*-bromophenol are hydrogen bonded (Table 2). This is indicated by a shift of the C=O stretching of the steroid carbonyl groups to lower frequencies: 1,744–1,728 cm⁻¹ for androsterone and 1,680–1,658 cm⁻¹ for testosterone. A similar change of the OH stretching can be observed for the phenolic OH group. Thus, when steroid is added to the *p*-bromophenol solution, the intensity of the monomeric OH band at 3,610 cm⁻¹ is decreased, while a new broad band between 3,400 cm⁻¹ and 3,340 cm⁻¹ appears, due to the hydrogen bonded OH group. A self-association of the *p*-bromophenol can be detected at higher concentrations in the 3,400–3,520 cm⁻¹ range with a peak at 3,480 cm⁻¹. There is considerable overlap of the association bands in this region, presumably because of different hydrogen bonded molecular species formed by the *p*-bromophenol. Additional evidence of association by hydrogen binding is the frequency shift of the OH deformation modes of the *p*-bromophenol. Phenol and some of its substituted derivatives show two bands in the 1,180 cm⁻¹ and 1,330 cm⁻¹ regions, the first with a more pronounced OH character demonstrated by a larger hydrogen bond induced frequency shift. It has been concluded that at least one of these modes is the coupled vibration between the OH bending and the C—O stretching mode; the latter, coupled with the benzene ring vibration¹⁵, absorbs in the 1,250 cm⁻¹ region. Although definite assignments of these two bands are still questioned, it has been shown that both respond to hydrogen bond formation. In our measurements we observed a definite weakening of the 1,176, 1,168 cm⁻¹ 'doublet' of the *p*-bromophenol and the appearance of a new band at 1,215 cm⁻¹, which is a shift of approximately 40 cm⁻¹. On the other hand, we were unable to identify with certainty a corresponding shift in the 1,300 cm⁻¹ region. Results similar to those described here were obtained with cyclohexanone and cyclopentanone, the ketonic ring system found in the steroids, and they are included here for comparison. The C=O stretching band of cyclohexanone is shifted from 1,718 cm⁻¹ to 1,703 cm⁻¹, and the one for cyclopentanone from 1,749 cm⁻¹ to 1,728 cm⁻¹, while the OH stretching and the deformation modes of the *p*-bromophenol show the changes already mentioned.

Table 1. BROMINE ANALYSES

Complexes of <i>p</i> -bromophenol and	Steroid- <i>p</i> -bromophenol molar ratio	% Bromine (calculated)*	% Bromine (measured)†
5 α -Androstan-3 α -ol-17-one (androsterone)	1:1	17.24	16.92
4 Δ^5 -Androsten-3 β -ol-17-one (dehydroepiandrosterone)	3:2	13.20	13.17
5 β -Androstan-3 α -ol-17-one (etiocolanolone)	2:1	10.60	10.54
4 Δ^5 -Androsten-17 β -ol-3-one (testosterone)	1:1	17.32	17.34
5 α -Androstan-17 β -ol-3-one	1:1	17.24	17.03
1,4-Androstadien-17 β -ol-3-one	1:1	17.41	17.24
5 β -Androstan-17 β -ol-3-one	1:1	17.24	17.00

* For the specified ratios.

† The experimental error is estimated to be ± 1 per cent or better.

Table 2. INFRA-RED ABSORPTION SPECTRA (CM⁻¹)*

Frequencies†	<i>p</i> -Bromophenol	Androsterone	Testosterone	Cyclopentanone	Cyclohexanone
ν_s C=O	—	1,744	1,680	1,749	1,718
ν_s O—H (monomer)	3,610	3,628	3,635	—	—
ν_s O—H (self-associated)	3,480	—	—	—	—
ν_b O—H (phenol)	1,176 1,168	—	—	—	—
Frequencies†	Androsterone + <i>p</i> -bromophenol	Testosterone + <i>p</i> -bromophenol	Cyclopentanone + <i>p</i> -bromophenol	Cyclohexanone + <i>p</i> -bromophenol	
ν_s C=O	1,728	1,658	1,728	1,703	
ν_s O—H (monomer)	3,610	3,610	3,609	3,614	
ν_s O—H (associated)	3,360	3,360	3,400	3,340	
ν_b O—H (phenol)	1,176 1,168 1,215	1,176 1,168 1,210	1,176 1,168 1,210	1,176 1,168 1,210	

* All measurements were made in carbon tetrachloride.

† ν_s , stretching mode; ν_b , 'in plane' bending mode.

p-Bromophenol concentration, 0.125 M. Steroid and ketone concentration, 0.02 M.

Table 3. SINGLE CRYSTAL DATA

Formula	(1) C ₁₈ H ₃₀ O ₂ C ₈ H ₇ OBr	(2) (C ₁₈ H ₃₀ O ₂) ₂ C ₈ H ₇ OBr ₂	(3) (C ₁₈ H ₃₀ O ₂) ₂ C ₈ H ₇ OBr	(4) C ₁₈ H ₃₀ O ₂ C ₈ H ₇ OBr	(5) C ₁₈ H ₃₀ O ₂ C ₈ H ₇ OBr	(6) C ₁₈ H ₃₀ O ₂ C ₈ H ₇ OBr	(7) C ₁₈ H ₃₀ O ₂ C ₈ H ₇ OBr
Mol. wt. (g.cm ⁻³ , meas.)	463.45	1,211.27	753.88	461.43	926.90	459.42	463.47
(g.cm ⁻³ , calc.)	1.815	1.263	1.222	1.325	1.274	1.366	1.322
<i>Z</i>	1	1	1	1	1	1	1
Space group	<i>P</i> ₁	<i>P</i> ₁	<i>P</i> ₁	<i>P</i> ₂ , <i>P</i> ₂ , <i>P</i> ₂	<i>P</i> ₁	<i>P</i> ₁	<i>P</i> ₁
<i>a</i> (Å)*	9.584	11.599	10.763	13.113	13.318	10.342	11.794
<i>b</i> (Å)*	11.272	12.412	13.645	22.753	13.488	10.566	13.739
<i>c</i> (Å)*	6.525	11.041	7.073	7.667	7.533	6.151	7.255
α	62.06°	87.64°	93.90°	—	91.60°	113.53°	85.64°
β	78.09°	84.19°	96.34°	—	90.83°	103.54°	89.18°
γ	71.54°	88.77°	105.80°	—	120.41°	116.36°	84.62°
<i>V</i> (Å ³)	589	1,580	993	2,288	1,167	551	1,167

* ± 0.004 Å.

(1) 5 α -androstan-3 α -ol-17-one : *p*-bromophenol (androsterone). (2) 4 Δ^5 -androsten-3 β -ol-17-one : *p*-bromophenol (dehydroepiandrosterone). (3) 5 β -androstan-3 α -ol-17-one : *p*-bromophenol (etiocolanolone). (4) 4 Δ^5 -androsten-17 β -ol-3-one : *p*-bromophenol (testosterone). (5) 5 α -androstan-17 β -ol-3-one : *p*-bromophenol. (6) 1,4-androstadien-17 β -ol-3-one : *p*-bromophenol. (7) 5 β -androstan-17 β -ol-3-one : *p*-bromophenol.

At the present time, we do not have enough evidence to identify the interaction between the bromine and the teroidal alcohol group which is suggested in the two cases in which the molar ratios of steroid and bromophenol are 3:2 and 2:1 respectively. From our data, however, it is evident that the intensity of the alcoholic absorption bands (3,628 and 3,635 cm^{-1}) decreases with the concentration increase of *p*-bromophenol, indicating intermolecular hydrogen bonding.

Unit cell constants were measured on a General Electric XRD-5 X-ray diffraction unit equipped with a single crystal orienter, using copper $K\alpha$ radiation. Space groups were determined from considerations of Friedel symmetry and optical activity. Densities were determined by flotation. All the crystals examined appear to be good enough to be used in structure determinations, although some of them may have to be mounted in sealed glass capillary tubes to protect them from atmospheric moisture or possible loss of volatile components. The single crystal data are given in Table 3.

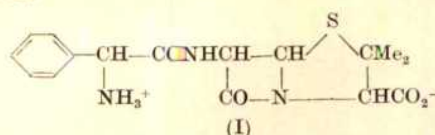
In addition to offering a useful tool in the X-ray crystallographic determination of steroids, the molecular complexes between androgenic steroids and *p*-bromophenol described in this communication are of considerable biological interest. Since phenol groups are often present in proteins, perhaps the type (or types) of binding responsible for the formation of the complexes reported here are also important in steroid-protein interactions. It is interesting to note that similar complexes between other androgenic steroids (also progestational steroids) and β -naphthol, resorcinol, phloroglucinol, and naphthalene have been reported in the recent literature¹⁶.

We thank Chia Tang Lu for obtaining the single crystal data and Carl Reggie for helping with some of the chemical procedures. This investigation was supported in part by U.S. Public Health Service research grant (CA-06183) from the National Cancer Institute and in part by research grant (UHF-G-64-RP-7) from the United Health Foundation of Western New York.

CHAIM EGER
DORITA A. NORTON

Biophysics Department,
Roswell Park Memorial Institute,
Buffalo, New York.

salts in moist organic solvents with amines of high molecular weight⁴. We wish to report further details of the various solid forms of ampicillin, both anhydrous and hydrated, and the means whereby they may be inter-converted.



Ampicillin is normally isolated from aqueous solution by adjusting the pH to 4-6.5, followed, if necessary, by concentration at low temperature and pressure, whereupon, provided the solution is reasonably pure, needle-like crystals separate readily⁵. However, if much impurity is present, which may happen with some preparative methods, the initial product may consist of heavily hydrated agglomerates, although slurring with water to which sufficient alkali has been added to bring the pH to between 7 and 8 readily converts such solid into the crystalline form⁶. The first crystals obtained in this laboratory were dried *in vacuo* over phosphorus pentoxide prior to analysis, and then had the elemental composition of a monohydrate⁵. However, when the crystals from subsequent runs were dried in air at temperatures up to about 65° C they proved to be the trihydrate. Further drying *in vacuo*, even at room temperature, readily reduced the water content to the monohydrate level, but dehydration beyond this stage was slow.

Grant and Alburn² heated an aqueous suspension or slurry of the monohydrate at 40°-100° C for a few minutes to give crystalline anhydrous ampicillin. In this laboratory the anhydrate was similarly prepared from any form of ampicillin⁷, although reasonably rapid conversion of trihydrate required a temperature of 80°-100° C. Hydrated ampicillin was also converted into the anhydrate by heating a suspension in nitromethane or other nitrohydrocarbon⁸.

The various forms of ampicillin may be distinguished by their infra-red spectra in the solid state (Fig. 1). The broad-banded spectrum attributed by Grant and Alburn²

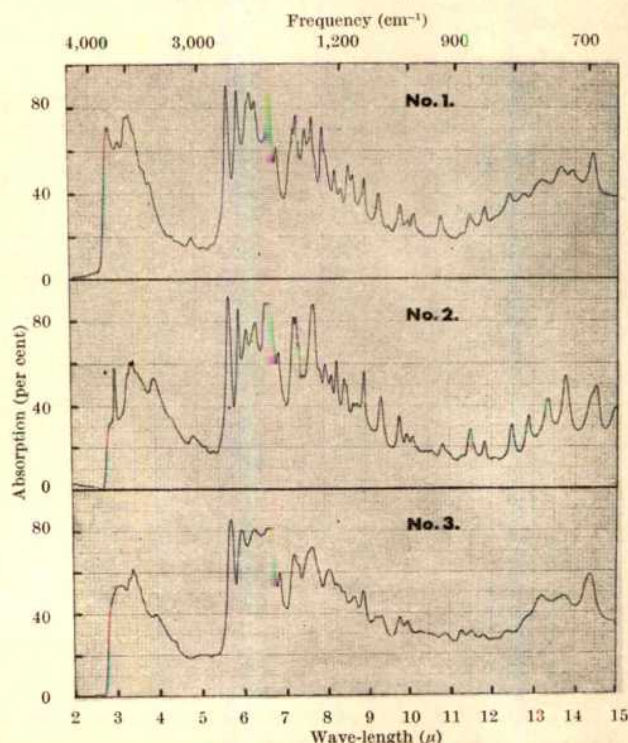


Fig. 1. Infra-red spectra of ampicillin forms (0.4 per cent in potassium bromide). (1) Trihydrate; (2) anhydrate; (3) other forms

Crystalline Modifications of Ampicillin

SINCE the introduction in 1961 of 6[D(-) α -amino-phenyl-acetamido]penicillanic acid¹ (I; ampicillin), its synthesis, bacteriology, pharmacology, and clinical use as a broad-spectrum penicillin have been extensively reported. Recently, attention has been turned to the physical properties of the solid. Thus, Grant and Alburn² have distinguished between a crystalline anhydrous form and a monohydrate, which differ in solid-state infra-red absorption spectra, density, solubility, and thermal stability. They make no mention, however, of the highly crystalline ampicillin trihydrate which may be obtained by crystallization from water³ or by treating acid addition

- ¹ Robertson, J. M., *Organic Crystals and Molecules*, chap. 6 (Cornell University Press, Ithaca, 1953).
- ² Norton, D. A., Kartha, G., and Lu, C. T., *Acta Cryst.*, **17**, 77 (1964).
- ³ Norton, D. A., Kartha, G., and Lu, C. T., *Acta Cryst.*, **16**, 89 (1964).
- ⁴ Collins, D. J., and Hobbs, J. J., *Austral. J. Chem.*, **17**, 661 (1964).
- ⁵ Schöniger, W., *Mikrochimica Acta*, 123 (1955); 869 (1956).
- ⁶ Mizushima, S., Tsuboi, M., Shimanouchi, T., and Tsuda, Y., *Spectrochim. Acta*, **7**, 100 (1955).
- ⁷ Gray, E., *Ann. de Chim.*, **12**, 355 (1948).
- ⁸ Taboury, F. J., and Queuille, J., *Mem. Soc. Chim.*, 772 (1947).
- ⁹ Lecompte, J., Gray, E., and Taboury, F. J., *Mem. Soc. Chim.*, 774 (1947).
- ¹⁰ Lecompte, J., Taboury, F., and Gray, E., *Mem. Soc. Chim.*, 779 (1947).
- ¹¹ Whetsel, K. B., and Kagase, R. E., *Spectrochim. Acta*, **18**, 315 (1962).
- ¹² Fritzsche, H., *Acta Chim. Hung.*, **40**, 31 (1964).
- ¹³ Pimental, G. C., and McClellan, A. L., *The Hydrogen Bond* (W. H. Freeman and Co., San Francisco, London, 1960).
- ¹⁴ Tsubomura, H., *J. Chem. Phys.*, **24**, 927 (1956).
- ¹⁵ Mecke, R., and Rossmly, G., *Z. Elektrochemie*, **59**, 866 (1955).
- ¹⁶ Kadarné, P. J., *Magyar Ke'miai Folyóirat*, **70**, 325 (1964).

to the monohydrate is, in our experience, typical of trihydrate which has been kept *in vacuo* to reduce the water content to any level below about 10 per cent, and also of amorphous or freeze-dried specimens containing up to about 20 per cent water. This type of spectrum (No. 3) may be contrasted with those of the anhydrate and trihydrate, in both of which the bands are sharp and well resolved. Spectra of the trihydrate (No. 1) and anhydrate (No. 2) differ chiefly in the NH and OH stretching region, where a sharp isolated band at 3.00μ is characteristic of the anhydrate, and in a group of bands between 6.05 and 6.7μ which include the carbonyl stretching band of the ionized carboxyl group, the 'amide II' (NH/CN combination) band, and possibly an NH_3^+ deformation band. In the latter region the trihydrate shows a shoulder at 6.14μ and bands at 6.21 , 6.34 and 6.66μ , whereas the anhydrate shows distinct bands at 6.08 and 6.32μ as well as a compound band centred at about 6.6μ . An unassigned medium-intensity band at 7.48μ appears to be characteristic of the trihydrate.

Grant and Alburn² attribute the diffuseness of their monohydrate spectrum to a low degree of order in the crystal; by the same token the trihydrate and anhydrate crystals should both be highly ordered. The spectrum of the trihydrate suggests that the three water molecules may be intimately associated with the two ionized groups and with the amide NH group. With all the water molecules thus rigidly bound, the trihydrate is as stable as the anhydrate to normal storage, while its lower stability above 70°C is probably due to break-down of the crystal lattice and rupture of the sensitive β -lactam by the liberated water. When part of the crystal water is removed *in vacuo* stability is decreased, possibly due to greater freedom of movement in the impaired lattice.

The solubility of the crystalline anhydrate in water is relatively little affected by temperature, being approximately 1.1 per cent at 30°C and 1.3 per cent at 87°C , whereas that of the trihydrate is only 0.7 per cent at 30°C but increases fairly sharply with temperature so that the two solubility curves intersect at about 50°C . Solutions of ampicillin in one equivalent of N hydrochloric acid were stirred at various temperatures while the calculated quantity of 2 N sodium hydroxide was added and crystallization occurred. Crystals separating below 50°C proved to be pure trihydrate, above 60°C they were the anhydrate, while at intermediate temperatures the solid was shown spectroscopically to contain both forms. The trihydrate and anhydrate, each in their appropriate temperature ranges, thus appear to be the only crystalline forms capable of existing in equilibrium with the saturated solution. We therefore believe that samples of ampicillin which have been recorded as the monohydrate^{2,5,9}, dihydrate⁴ or sesquihydrate¹⁰ were either amorphous or consisted of partially dehydrated trihydrate.

We thank Mr. A. E. Bird, Dr. H. D. C. Rapson and Dr. J. H. C. Naylor for their advice.

K. W. B. AUSTIN
A. C. MARSHALL
H. SMITH

Chemistry Research Department,
Beecham Research Laboratories,
Brockham Park,
Betchworth, Surrey.

¹ Doyle, F. P., Naylor, J. H. C., Smith, H., and Stove, E. R., *Nature*, **191**, 1091 (1961).

² Grant, N. H., and Alburn, H. E., *Nature*, **207**, 645 (1965).

³ Fosker, G. R., Naylor, J. H. C., and Wilcox, J. A., *Brit. Pat. Spec.*, 991,586 (1965).

⁴ Johnson, D. A., and Hardcastle, G. A., *U.S. Pat. Spec.*, 3,157,640 (1964).

⁵ Doyle, F. P., Fosker, G. R., Naylor, J. H. C., and Smith, H., *J. Chem. Soc.*, 1440 (1962). Doyle, F. P., Naylor, J. H. C., and Smith, H., *U.S. Pat. Spec.*, 2,985,648 (1961).

⁶ Soulat, M. J., and Woodford, M. C. (unpublished observations).

⁷ Rapson, H. D. C., *Brit. Pat. applied for*, No. 24919 (1963).

⁸ Stove, E. R., *Brit. Pat. applied for*, No. 35626 (1963).

⁹ Grant, N. H., and Alburn, H. E., *J. Amer. Chem. Soc.*, **86**, 3870 (1964).

¹⁰ Dane, E., and Dockner, T., *Chem. Ber.*, **98**, 789 (1965).

BIOCHEMISTRY

Photochemical Synthesis of Condensed Phosphates

In the course of unsuccessful attempts to use *metanitrophenylphosphate* as a photochemical phosphorylating agent¹, we noted the appearance of pyrophosphate in a number of our control tubes. Further experiments have shown that ultra-violet irradiation of solutions of phosphoric acid or *tetrabutylammonium dihydrogen phosphate* in certain pyridine derivatives or in aliphatic ketone leads to the formation of condensed phosphates. In this communication we describe our semi-quantitative observations on these systems. We examined 0.01–0.05 M solutions of phosphate in the following solvents: pyridine 2-, 3-, and 4-picoline, acetone, methyl ethyl ketone, pyruvic acid, acetylacetone, benzaldehyde, acetophenone, anisole, *n*-butyraldehyde and propionaldehyde. Only those listed in Table 1 were we able to detect pyrophosphate. (The sensitivity of our method was ≈ 0.5 per cent conversion.) Irradiations were carried out in a RPR-10 'Rayonet' photochemical chamber using RPR-2537 lamps for the ketone solution and RPR-3500 Å lamps for the pyridine solutions. The solutions were contained in quartz tubes 10 mm in diameter. The temperature of the solutions was $45^\circ \pm 5^\circ\text{C}$.

Table 1. PERCENTAGE CONVERSION OF ORTHOPHOSPHATE TO PYROPHOSPHATE IN ULTRA-VIOLET-IRRADIATED SOLUTIONS. THE RESULTS ARE EXPRESSED IN PERCENTAGE OF PHOSPHORUS PRESENT IN PYROPHOSPHATE

Solvent	Solute	Conc. (M)	Time of irradiation (h)				
			16	32	48	80	170
Pyridine	(Bu ₄ N)H ₂ PO ₄	0.05	2	5–7		15	15
	H ₃ PO ₄	0.05	2.5	2.5		2.5	
2-Picoline	(Bu ₄ N)H ₂ PO ₄	0.05	1.5	5		5	
3-Picoline	(Bu ₄ N)H ₂ PO ₄	0.05	1.5	5		10	8–10
4-Picoline	(Bu ₄ N)H ₂ PO ₄	0.05		1		3	5–8
Acetone	(Bu ₄ N)H ₂ PO ₄	0.025	7				
	H ₃ PO ₄	0.05	8	8	8		
Methyl ethyl ketone	(Bu ₄ N)H ₂ PO ₄	0.01	20				
	H ₃ PO ₄	0.05	7				

Rough quantitative estimates of the extent of conversion of phosphate to pyrophosphate under a variety of conditions are given in Table 1 (ref. 2). The consequences of prolonged irradiation are more complex and less reproducible, probably because the organic solvents undergo extensive degradation. However, two phenomena of considerable interest were observed repeatedly; namely the formation of tripolyphosphate in solution and the formation of precipitates containing much of the phosphorus as pyrophosphate, tripolyphosphate, and trimetaphosphate.

We first became interested in these novel reactions in the context of 'origins of life' investigations. However we now doubt that our findings have any relevance. While the reaction proceeds equally well in damp pyridine and in pyridine dried over calcium hydride, the addition of 50 per cent water completely quenches the condensation. Until a condensing agent can be found which brings about pyrophosphate formation in aqueous solution, the involvement of reactions of this type in prebiological synthesis remains without experimental support. Our experimental findings do not permit any conclusions concerning the mechanism of the reactions.

We understand that Dr. C. Zioudrou and Prof. G. Stein have made related observations.

A. BECK
L. E. ORGHEL

Salk Institute for Biological Studies,
La Jolla,
California.

¹ Havinga, E., DeJongh, R. O., and Dorst, W., *Rec. Trav. Chim.*, **75**, 878 (1956).

² The phosphates were analysed by paper chromatography (Kolloff, R. H., *Anal. Chem.*, **33**, 373 (1961), and Fuchs, R. J., and Czech, F. W., *Anal. Chem.*, **35**, 769 (1963)).

Effect of Hydrolysed Glucose Cycloacetoacetate in the Prevention of Fatty Infiltration in the Liver

GLUCOSE cycloacetoacetate (GCA) while hydrolysed with dilute hydrochloric acid has been found to have beneficial effects in experimental diabetes¹⁻³. It has also recently been observed in this laboratory to facilitate transmethylation reactions in *E. coli*⁴. It was reported earlier by Saikia, Brahmanekar and Nath⁵ that hydrolysed glucose cycloacetoacetate in a daily dose of 80 mg/100 g body-wt can cause considerable desaturation of the liver fat, which, according to Schoenheimer and Rittenberg⁶, is an indication of increased fat metabolism. Nath and Saikia^{7,8} have also observed earlier the prevention of atherosclerotic and hyperlipaemic conditions in animals by the hydrolysed product of glucose cycloacetoacetate (GCAH). Investigations were therefore undertaken to observe the lipotropic effect of GCAH in experimental animals. Because vitamin B₁₂ is known to have a lipotropic effect it was also thought desirable to study the effect of very small doses of vitamin B₁₂ in partially hepatectomized animals.

Table 1

No. of animals	Treatment Substance	Period	Fat in liver just after partial hepatectomy (per cent)	Fat 48 h after partial hepatectomy (per cent)	Increase of fat (per cent)
6	Nil	—	5.18 ± 0.25	8.95 ± 0.32	72
6	GCAH inject	From 3 days before partial hepatectomy	5.08 ± 0.15	5.31 ± 0.14	2.5
4	20 mg/kg GCAH inject	"	4.86 ± 0.14	4.90 ± 0.12	0.8
6	25 mg/kg Vlt. B ₁₂ inject	"	5.10 ± 0.11	5.96 ± 0.15	16.8
	10 µg/kg				

Montini and Pontremoli⁹ have reported that, 48 h after partial hepatectomy of the rat, the remaining portion of the liver undergoes fatty degeneration with a very large increase in the percentage of fat. This technique was used in this investigation and the experiment was made with twenty-two rats each weighing about 200 g. Sixteen animals were used for the experiment and six as control. GCAH and vitamin B₁₂ were injected intraperitoneally for three consecutive days before partial hepatectomy and for 2 days more thereafter before killing. The results are shown in Table 1.

It is thus evident from the results that GCAH, which has been found to help formation of methionine¹⁰ as well as choline¹¹ in liver, is also lipotropic in nature.

Further investigations are in progress.

N. NATH
V. D. YAWALKAR
M. C. NATH

Department of Biochemistry,
University of Nagpur, India.

- ¹ Nath, M. C., and Bhattathiry, E. P. M., *Metabolism*, **5**, 11 (1956).
² Nath, M. C., and Behki, R. M., *Metabolism*, **6**, 127 (1957).
³ Nath, M. C., and Behki, R. M., *Arch. Biochem. Biophys.*, **75**, 65 (1958).
⁴ Nath, M. C., and Nath, N. (paper communicated).
⁵ Saikia, A., Brahmanekar, D. M., and Nath, M. C., *Ann. Biochem. and Exp. Med.*, **19**, 275 (1959).
⁶ Schoenheimer, R., and Rittenberg, D., *J. Biol. Chem.*, **113**, 505 (1936).
⁷ Nath, M. C., and Saikia, A., *Ind. J. Physiol. and All. Sci.*, **13**, 102 (1959).
⁸ Nath, M. C., and Saikia, A., *Arch. Biochem. Biophys.*, **84**, 162 (1959).
⁹ Montini, T., and Pontremoli, S., *Boll. Soc. Ital. Biol. Sper.*, **26**, 8 (1950).
¹⁰ Nath, M. C., and Saikia, A., *Arch. Biochem. and Biophys.*, **79**, 216 (1959).
¹¹ Brahmanekar, D. M., and Nath, M. C., *Ann. Biochem. and Exp. Med.*, **23**, 65 (1963).

Rapid Method for Selenium Assay of Plant Material

WE have found the following to be a particularly rapid and reproducible means of assaying for selenium in leafy plant material. It is based on the use of perchloric acid and H₂O₂ to facilitate digestion and clarification of the sample and on the spectrophotometric determination of

the elemental selenium produced on reduction of the combined forms of the element. Our method represents, in part, a combination of techniques recently found useful for higher plant and microbial material¹⁻³.

We used the selenium accumulator *Astragalus bisulcatus* L. in soil watered once daily with 1 p.p.m. of selenium as Na₂SeO₃·5H₂O and the closely related non-accumulators *A. canadensis* L. and *A. succulentus* L., grown in the same way except that no selenium was added to the soil in which the latter species were grown.

Approximately 0.5 g fresh weight of the plant sample containing Se is placed in a 250-ml. Erlenmeyer flask, to which is added 10 ml. of the digesting mixture consisting of 3 parts conc. HNO₃ and 1 part of HClO₄ (60 per cent). The mixture is heated gently on a hotplate until all the HNO₃ is driven off and the fumes of HClO₄ begin to fill the flask. After the material is completely digested, the flask is cooled thoroughly in cold water. 2–3 ml. H₂O₂ is added, and after 5 min or more the flask is reheated until fumes of HClO₄ appear again, and all the H₂O₂ has been expelled. The clear and colourless sample is again cooled and the flask is then placed into a boiling-water bath for 2–3 min. A mixture of 10 ml. of a 3 per cent hydrazine sulphate solution plus 3 ml. of a 2.5 per cent solution of gum arabic is added. It is extremely important to add the mixture in aliquots of 2 ml. or less and to allow a short period of time between addition. The flask is then placed in the boiling-water bath for 10 min, by which time the characteristic orange-red colour of elemental Se should be fully developed. The suspension is transferred to a volumetric flask and made up to 25 ml. with distilled water. The absorbancy of the samples at 420µ is measured in a spectrophotometer (Beckman DU) and the Se concentration is calculated using the straight-line calibration curve obtained from known concentrations of elemental Se. To prepare the standards, 1.0 g elemental Se is added to 10 ml. concentrated HNO₃ and the mixture heated to dryness on a hotplate. 10 ml. distilled water is added, and the suspension is again dried by heating. The addition of water and heating is repeated twice. The Se is now treated as in the preparation of the unknown sample, except that the H₂O₂ can be omitted, as has been shown by preliminary tests. The final dilutions can be varied according to the expected range of Se concentrations in the unknown samples. The Se concentration of *A. bisulcatus* L. averaged 1,100 p.p.m., while the non-accumulators averaged 0.03 p.p.m., all on a fresh-weight basis.

This work was supported by U.S. Public Health Service grant A100596-C. We thank L. Sonsteli for technical assistance, and Dr. O. A. Beath of the University of Wyoming for seed of the *Astragalus* species.

R. DUFF
M. CHESIN

Department of Botany,
Montana State University,
Missoula, Montana.

- ¹ Cousins, F. B., *Austral. J. Exp. Biol. and Med. Sci.*, **38**, 11 (1960).
² Falcone, G., and Nickerson, W. J., *Giorn. Microbiol.*, **8**, 129 (1960).
³ Watkins, J. H., *Anal. Chem.*, **32**, 981 (1960).

Disruption of Protozoa by Indole and Related Compounds

THE disruption of protozoa in saturated aqueous indole solutions, first described by Eadie and Oxford¹, has been used in the preparation of enzyme extracts from rumen protozoa² and ribosomes from *Tetrahymena*³. This type of biochemical disruption is of interest not only because of its spectacular nature but also because it is selective for rumen protozoa and any associated bacteria are not broken². In examining the disruptive effect of other compounds Eadie and Oxford¹ confined themselves largely to those closely related to indole and skatole (see Table 1). They concluded that, while a fairly close relationship to the tryptophan structure might be important, the

Table 1. DISRUPTION OF PROTOZOA BY SOME ORGANIC COMPOUNDS

	<i>Tetrahymena</i>		<i>Rumen protozoa</i>	
	Killed	Disrupted	Killed	Disrupted
(a) Present work				
Indole, indoline, quinoline, 1,2,3,4-tetrahydroquinoline, isoquinoline, 1,2,3,4-tetrahydro-isoquinoline, benzene, <i>N</i> -ethyl aniline	+	+	+	+
Piperidine	+	+	+	+
Pyrrolidine	+	+	+	+
Pyrrole, pyridine, aniline, carbazole, naphthalene, <i>n</i> -hexane, diethyl ether, indole-3-acetate	-	-	-	-
(b) Eadie and Oxford ¹				
Indole, skatole, isatin, 5-, and 7-methyl indole			+	+
Aniline, tryptamine, tryptophan, indolyl-3-acetate, -propionate, -butyrate	-	-	-	-

- = non-toxic or not disrupted.

primary requisites for disruption were "slight water solubility with ready lipid solubility and the absence of any strongly polar centre in the molecule". We have now examined the disruptive action of a much wider range of compounds on both rumen protozoa and *Tetrahymena* in order to test these conclusions.

Mixed rumen protozoa were freshly isolated⁴ from the rumen contents of cattle fasted overnight; *Tetrahymena* were grown for seven days³. In each case the protozoa were collected by centrifuging (200*g*) and portions (0.1 g wet weight) suspended in 2 ml. solution (water at 20° C for *Tetrahymena*, buffer⁴ at 37° C for rumen protozoa) containing water-insoluble compounds at saturation and water-soluble compounds at 0.1 per cent w/v (equivalent to the indole concentration at saturation). The organisms were examined microscopically at intervals during 1 h and the following effects were distinguished: (a) no apparent effect, (b) loss of motility without disruption (killed only) and (c) loss of motility followed by complete disruption. In earlier work², rumen protozoa which had been isolated and incubated for 24 h were used and disruption with indole was slow; in contrast to this, *Tetrahymena* always burst instantly on contact with indole. The freshly isolated rumen protozoa used in the present work were more active organisms and when disruption occurred after addition to the test solution it was almost always instantaneous. The results obtained with various substances are summarized in Table 1.

These results show that protozoal disruption is not solely associated with indole and its derivatives but that the other conclusions of Eadie and Oxford¹ concerning

solubility and the absence of polar groups are substantiated. Thus, in general, all the disruptive compounds are only slightly soluble in water but show ready lipid solubility and have no strongly polar centre in the molecule. The strongly basic and/or water-soluble, single-ring heterocyclic compounds are largely non-disruptive under the conditions used, but the two-ring heterocyclic compounds are very active, because the presence of the fused benzenic ring increases the lipid solubility and decreases the basicity (except for tetrahydro-isoquinoline). Not all the lipid soluble compounds tested, however, are active. While in some cases this may be related to the fact that they are too insoluble in water (for example, naphthalene, carbazole, *n*-hexane), in other cases it could be due to the presence of a polar centre in the molecule (for example, aniline, ether). Our results suggest that solutions saturated with benzene rather than indole may be more convenient for disrupting protozoa. Control experiments have shown that the benzene in such extracts does not interfere with carboxylase activity and it has the advantage of not giving a false colour with the Folin-Ciocalteu proteolytic reagent as does indole².

R. W. BAILEY
G. B. RUSSELL

Plant Chemistry Division,
Department of Scientific and Industrial Research,
Palmerston North,
New Zealand.

¹ Eadie, J. M., and Oxford, A. E., *Nature*, **174**, 973 (1954).

² Bailey, R. W., and Howard, B. H., *Arch. Biochem. Biophys.*, **99**, 299 (1962).

³ Lyttleton, J. W., *Exp. Cell Res.*, **31**, 385 (1963).

⁴ Oxford, A. E., *N.Z. J. Agric. Res.*, **1**, 809 (1958).

PHYSIOLOGY

Cerebello-rubral Connexions in the Cat

SMALL stereotaxic lesions have been made unilaterally in each of the three cerebellar nuclei in a series of cats. After a survival period of 8-15 days, the animals were killed by intracarotid perfusion of 10 per cent formalin under anaesthesia. In each case, the cerebellum was cut in serial frozen section at 100μ and stained by the Nissl method. Projection drawings from these sections made it possible to localize and reconstruct the lesion.

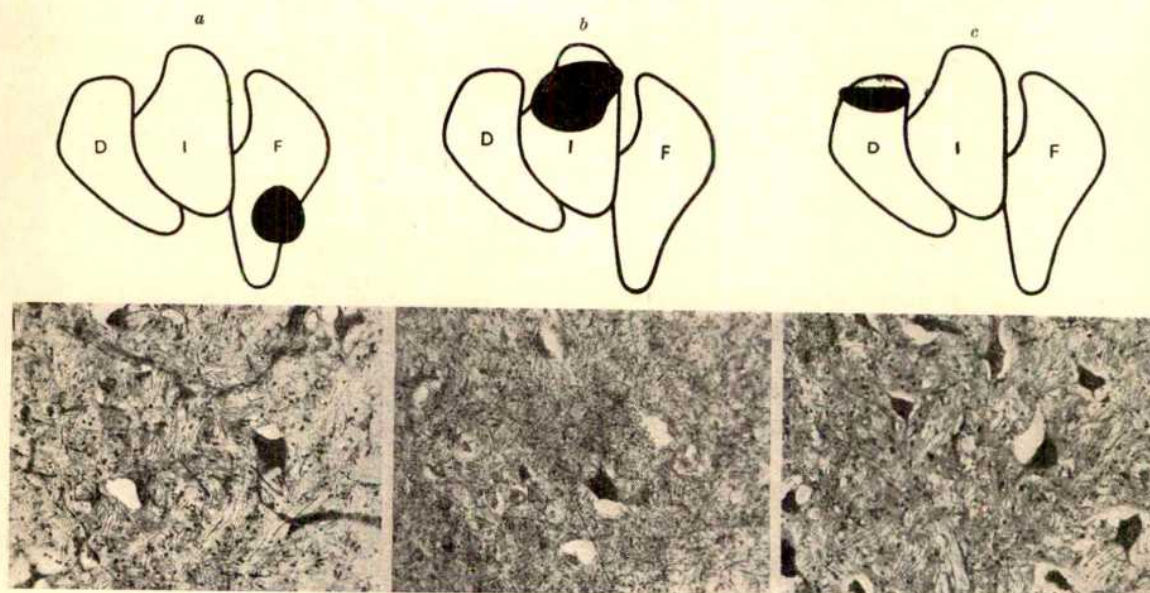


Fig. 1. The upper row shows representative lesions in each of the three cerebellar nuclei. Below each diagram is a photomicrograph ($\times 310$) of the appearance of the contralateral red nucleus, impregnated by the method of Nauta, in the case of the lesion illustrated. Lesions of the fastigial (a) and lateral (c) nuclei produce no degeneration in the red nucleus, while a lesion of the n. interpositus (b) provokes intense pre-terminal degeneration.

The rest of the brain, apart from the cerebellum, was cut in serial frozen section at 30 μ , and every tenth section was impregnated by Nauta's modification¹ of the Nauta-Gygax technique². Examination of these sections for degenerating axons and pre-terminals enabled us to establish that no fibres from either n. fastigii or n. lateralis terminate in the magnocellular red nucleus (Fig. 1, a and c), though there is a small amount of pre-terminal degeneration in the pre-rubral region of the zona incerta following lesions of n. lateralis. In contrast to this, lesions of n. interpositus cause massive pre-terminal degeneration in the contralateral red nucleus (Fig. 1b). So far as the fastigial nucleus is concerned, these findings are in agreement with those of previous authors^{3,4}; but we cannot agree with Cohen *et al.*⁴ that there is any projection from the lateral cerebellar (dentate) nucleus to the red nucleus. The discrepancy may be due to the fact that we have been able to produce small coagulations which were confined to single cerebellar nuclei. It is interesting that only the nucleus interpositus appears to receive reciprocal afferent innervation from the red nucleus in the cat⁵.

Like Cohen *et al.*⁴, in the cat, and in contradistinction to the findings of Carrea and Mettler⁶ in the monkey and the electrophysiological study of Orioli⁷ in the cat, we were unable to find any evidence suggesting that fibres of the descending limb of the brachium conjunctivum extend below the level of the superior olive.

PIERRE ANGAUT

Centre d'Etudes de Physiologie Nerveuse,
Paris.

DAVID BOWSER

Department of Anatomy,
University of Liverpool.

¹ Nauta, W. J. H., in *New Research Techniques in Neuroanatomy*, edit. by Windle, W. F., 17 (Charles C. Thomas, Springfield, Ill., 1957).

² Nauta, W. J. H., and Gygax, P. A., *Stain Technol.*, **29**, 91 (1954).

³ Carpenter, M. B., Brittin, G. M., and Pines, J., *J. Comp. Neurol.*, **109**, 67 (1958).

⁴ Cohen, D., Chambers, W. E., and Sprague, J. M., *J. Comp. Neurol.*, **109**, 233 (1958).

⁵ Rinvik, E., and Brodal, A., *Acta Neurol. Scand.* (in the press).

⁶ Carrea, R. M. E., and Mettler, F. A., *J. Comp. Neurol.*, **101**, 565 (1964).

⁷ Orioli, F. L., *J. Neurophysiol.*, **24**, 583 (1961).

Delayed Mortality in Adult Chickens exposed to 1 Atmosphere Oxygen

THE growing chick (2-7-week-old White Leghorn) is markedly resistant to the usually lethal effects of 100 per cent oxygen at one atmosphere (OAP), exhibiting only a depressed growth rate and feed intake¹. It has been theorized that the different anatomy of the avian respiratory system might enhance resistance to a toxic agent believed to act primarily on the lungs. However, the young of several mammalian species are more resistant to the toxic action of OAP than the adults². The question of whether the chicks' mild reaction to OAP was a function of its young age or unique anatomy could not be decided from the literature, which, so far as the domestic fowl is concerned, appears to be limited to a report on three adults³. Therefore, further tests involving the exposures of mature White Leghorns to OAP were undertaken.

Continuous exposure to OAP took place in sealed plastic isolators similar to those used with chicks¹, except for the substitution of cages capable of holding one to two adult birds. Isolator conditions were controlled and physiological variables determined essentially as for the chicks, with the addition that body temperature (T_B) was measured by a rectal thermistor inserted 4-6 cm via the cloaca. Isolator conditions averaged: oxygen = 98 ± 2 per cent; carbon dioxide = less than 0.5 per cent; $T =$ between 23° and 28° C; relative humidity = mostly 100 per cent, except 42-65 per cent in two early trials. The birds used were single-comb White Leghorns ranging in age from 8 months (six males and three females) to

2 years (the remaining 10 females). About midway through the tests, adult white mice were introduced into the isolators to serve as biological indicators of the toxicity of the atmosphere.

Initial examination of mortality data showed no difference between 8-month-old and 2-year-old birds, hence survival time is presented in Table 1 without regard to age. The overall average survival time was 10 days, with no statistically significant difference due to sex. The range was 5-16 days, with one male still alive, but barely so, on day 16. These results are in general agreement with Soulie's⁴ results on three chickens, two of which survived beyond 11 days. By contrast, adult white mice exposed simultaneously to the same atmosphere lived only half as long, 5 days on the average, with a range of 4-8 days (Table 1).

A uniform observation in all trials was a complete cessation of both feed and water intake within 2 days, generally after 24 h, which was associated with a progressive lethargy. Because of the adipsia the relative humidity in the isolator was permitted to rise to saturation, for, although high humidity is reported to shorten survival in OAP⁴, we thought that dehydration in these extended exposures might be even more detrimental. The effect of the anorexia is seen in the 31-33 per cent loss in body-weight at time of death (Table 1).

Although feed intake ceased early, a dark, watery faecal material continued to be deposited throughout the stay in OAP. Post-mortem examination of the digestive system showed that in every case the tract from gizzard to cloaca was empty and free of overt lesions; however, in a number of animals the upper tract-crop, proventriculus and gizzard contained feed material in various stages of decomposition. It would seem that OAP inhibited motility anterior of the gizzard but not posteriorly, and the dark faecal deposits may have been desquamated intestinal debris mixed with bile segments.

Though primary interest in this work centred on survival time, a number of ancillary measurements were made at random during the course of the exposures (Table 1). The finding of a pO_2 of more than 400 mm mercury after an average of 8 days in OAP indicates that there was no barrier preventing contact between blood and the hyperoxic atmosphere. It suggests further that if hypoxaemia develops in these birds it must be quite late in the exposure. This was visually confirmed by maintenance of red colour in comb and wattles until near death. The haematocrit of 40 is high for adult hens⁵, probably reflecting dehydration resulting from the adipsia. Body temperature remained normal over most of the exposure, making it unlikely that the prolonged survival is due to any drastic depression in metabolism. In one or two birds a possible neural symptom was observed in the form of a twisting of head and neck as terminal events approached.

A marked effect of OAP was noted on respiration. Breathing rate decreased progressively with exposure finally degenerating into short gasps alternating with long periods of apnoea. Nevertheless, on post-mortem, lungs and thorax appeared for the most part grossly normal—certainly showing nothing comparable to the congestion, hyperaemia and oedema observed in the mice which died in OAP.

Table 1. EFFECT OF 100 PER CENT OXYGEN AT 1 ATMOSPHERE ON THE ADULT WHITE LEGHORN

		Male	Female
		(No.) av. \pm S.E.	(No.) av. \pm S.E.
Survival-time (days)	Chicken	(6) 9.5 \pm 1.5	(13) 10.7 \pm 0.8
		(19) 10.3 \pm 0.7	
Body-weight (kg)	Mice	(8) 5.0 \pm 0.5	
	Initial	(4) 1.74 \pm 0.12	(8) 1.45 \pm 0.23
	Final	(4) 1.17 \pm 0.04	(8) 1.00 \pm 0.16
	Per cent loss	31.2	32.7
Arterial O_2 tension (mm Hg) (av. exposure—8 days)			(4) 432 \pm 41
Venous O_2 tension (mm Hg) (av. exposure—8 days)			(3) 54 \pm 15
Haematocrit (per cent) (av. exposure—8 days)			(3) 40.0 \pm 3.8
Body temperature ($^{\circ}$ C) (av. exposure—7 days)			(4) 41.0 \pm 0.05
Respiratory rate (min) (av. exposure—10 days)			(4) 9.2 \pm 1.2

This investigation did not supply an unequivocal answer to our original question of whether the resistance of the growing chick to OAP was a function of its youth or of the different anatomy of the avian respiratory system⁵. The average survival time of 10 days for the adult is a far cry from the absence of mortality and morbidity after 4 weeks in the chick¹, and indicates that there must indeed be greater resistance with decrease in age in the chicken, as in the rat and possibly other species².

On the other hand, despite some similar reactions to OAP, for example, the anorexia and dyspnoea, there are definite differences in response between chickens and other species. Thus, simultaneously exposed mice survived on the average only half as long, 5 days, an interval which appears to be reasonably typical for most small- and medium-sized mammals, and perhaps even other birds^{2,3,6}, and, on post-mortem, the chicken shows little of the pulmonary congestion, hyperaemia and oedema typical of the mammal succumbing to OAP. It seems quite possible, in fact, that the extended period without eating or drinking could have contributed considerably to the death of the chickens.

There seems to be good reason to assume, therefore, that the chicken does have an inherently high resistance to OAP, and that it may be related to its specialized respiratory system. Pursuit of this problem could provide further understanding of the mechanism of oxygen toxicity—such as the role of atelectasis⁷, presumably less likely in the semi-rigid avian lung with its continuous air capillaries and air sacs, or the role of surfactant⁸, possibly deficient in the avian lung⁹.

This work was supported by grants from the National Aeronautics and Space Administration and the National Institutes of Health. We thank Dr. C. W. Smith for help with the blood gas analyses and J. F. Pitt for his assistance.

HAROLD S. WEISS
DAVID L. BECKMAN
RONALD A. WRIGHT

Environmental Physiology Laboratory,
Department of Physiology,
College of Medicine,
Ohio State University,
Columbus, Ohio.

¹ Weiss, H. S., Wright, R. A., and Hiatt, E. P., *J. App. Physiol.* (in the press).

² Bean, J. W., *Physiol. Rev.*, **25**, 1 (1945).

³ Soulie, P., *C.R. Soc. Biol.*, **130**, 539 (1939).

⁴ Huppie, H. R., and Cole, V. V., *J. Lab. Clin. Med.*, **29**, 1134 (1944).

⁵ Sturkie, P. D., *Avian Physiology* (Comstock Publishing Associates, Ithaca, New York, 1954).

⁶ Weir, F. W., Bath, D. W., Yevich, P., and Oberst, F. W., *Aerospace Med.*, **36**, 117 (1965).

⁷ Penrod, K. E., *J. App. Physiol.*, **9**, 1 (1956).

⁸ Hackney, J. P., Collier, C. R., Conrad, D., and Coggin, J., *Clin. Res.*, **11**, 91 (1963).

⁹ Miller, D. A., and Bondurant, S., *J. App. Physiol.*, **16**, 1075 (1961).

Nerve Potential Recordings during electrically and mechanically evoked Monosynaptic Reflexes in Man

A MONOSYNAPTIC reflex can frequently be evoked in the calf muscles on electrical stimulation of the tibial nerve (*H*-reflex) at a stimulus intensity below threshold for the motor fibres. A centripetal nerve volley can sometimes be recorded below threshold for the α -motor fibres on stimulation of various peripheral nerves in the upper and lower extremity^{1,2}, in the distribution of which an *H*-reflex is an unusual occurrence. Yet Magladery *et al.*³, recording from the spinal roots with intrathecal electrodes, found, on stimulation of the tibial nerve, that an afferent volley did not occur until after the reflex volley had developed. One purpose of the work reported here was to investigate the relationship between centripetal nerve volleys and the threshold for the monosynaptic reflex in the calf muscles.

The *H*-reflex in the calf muscles has been identified as the electrically evoked counterpart of the ankle jerk^{4,5}. However, the tendon jerk depends additionally on the sensitivity of the muscle spindle organs the activity of which is controlled by fusimotor fibres. The identity of the mechanically and electrically evoked monosynaptic reflexes forms the basis of a system for analysing changes in fusimotor function⁶⁻¹⁰. Another aim was to investigate the relation of the *H*-reflex and ankle jerk by recording nerve potentials during these reflexes.

Fifteen normal subjects were examined. Nerve action potentials were recorded with bipolar non-insulated needle electrodes placed in proximity to the tibial nerve in the upper popliteal fossa and the sciatic nerve in the upper thigh, on stimulation of the posterior tibial nerve low in the popliteal fossa. Simultaneous recordings of the muscle action potential from the calf muscles were made with bipolar subcutaneous needle electrodes fixed in position over the thickest part of the muscle and tendon. The stimulus intensity was increased in stages from that sub-threshold for the reflex to that supramaximal for the direct motor response. The recording electrodes were kept in position and an angle jerk elicited using a reflex hammer with a built-in micro-switch which closed a circuit on percussion and triggered the sweep of the oscilloscope.

Centripetal nerve volleys were frequently recorded at a stimulus intensity sub-threshold for the reflex response (Figs. 1a, 2a). When the afferent volley reached a critical magnitude, an *H*-reflex could be evoked by the Jendrassik manoeuvre, demonstrating that the low-threshold proprioceptive afferents engaged in the two-neurone reflex were contributing to the volley. With increasing stimulus intensity the *H*-reflex first increased in size, and then decreased in size with the development of the direct motor response. The simultaneously recorded nerve potentials showed a progressive increase in amplitude. It is likely that the increase in size of the nerve potential with

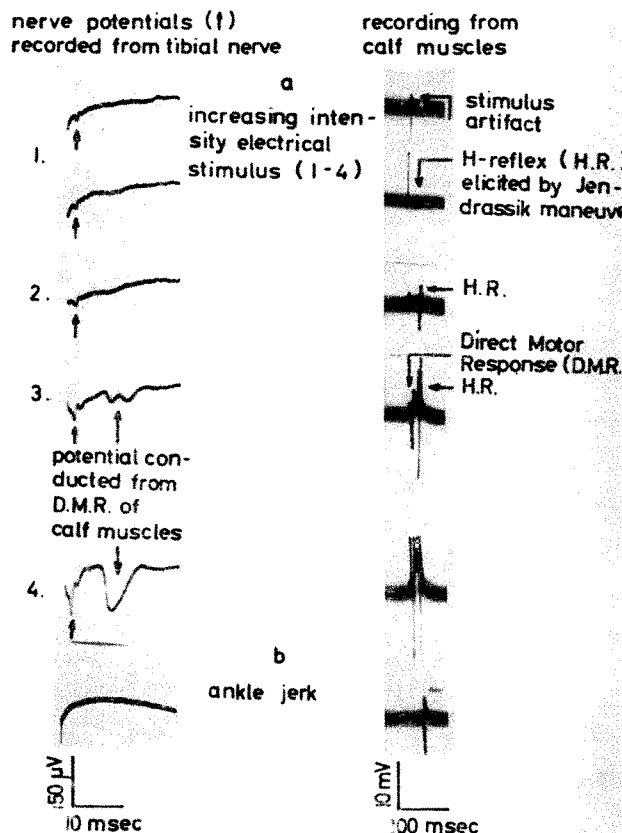


Fig. 1. Recording for nerve potentials in the high popliteal region on electrical stimulation of the posterior tibial nerve in the popliteal fossa (a), and on evoking the ankle jerk by mechanical tap of the Achilles tendon (b). The response from the calf muscle is recorded simultaneously.

increasing stimulus intensity is due to excitation of additional afferent fibres and to motor fibres antidromically stimulated. At high-intensity stimulation additional nerve potentials of longer latency were sometimes recorded. These later nerve potentials were not related to the reflex *per se* and were probably due to stimulation of slower conducting fibres.

In no instance when the same electrodes were in position was a nerve potential recorded on tendon tap when a maximal ankle jerk was being evoked (Figs. 1b and 2b).

There is thus a basic difference in the character of the afferent volleys subserving the two reflexes; and the most apparent explanation is that the proprioceptive volley on tendon tap is dispersed, whereas that on *H*-reflex is summated. A summated nerve volley has been recorded from the dorsal root or mixed nerve in cats on single-shock stimulation of low-threshold afferents in the peripheral nerve¹¹, whereas briefly stretching the tendon of a muscle by means of a solenoid resulted in a succession of imperfectly synchronized afferent discharges¹². The occurrence of a centripetal nerve volley below threshold for the electrically evoked reflex indicates the need for spatial summation before the monosynaptic reflex discharge develops; while on tendon tap the motoneurons might be facilitated by the afferent volleys early in the stretch and fired by later components of the afferent stream¹². Thus, on one hand, spatial summation is involved primarily; whereas, on the other, temporal summation is also complexly influenced by firing from other muscle afferents (group 1b, 2 fibres). It is therefore possible that the relatively integrated response of a given population of motoneurons may be different in the two instances. Such a difference would complicate the inference regarding

fusimotor function by the relationship of changes in the electrically and mechanically evoked reflexes.

M. M. GASSEL

E. DIAMANTOPOULOS

Department of Clinical Neurophysiology,
University of Freiburg,
West Germany.

¹ Dawson, G. D., and Scott, L. W., *J. Neurol. Neurosurg. Psychiat.*, **12**, 259 (1949).

² Gilliat, R. W., Goodman, H. V., and Willison, R. G., *J. Neurol. Neurosurg. Psychiat.*, **24**, 305 (1961).

³ Maglader, J. W., Teasdale, R. D., Park, A. M., and Porter, W. E., *Bull. Johns Hopkins Hosp.*, **88**, 520 (1951).

⁴ Hoffmann, P., *Z. Biol.*, **68**, 351 (1918).

⁵ Maglader, J. W., Park, A. M., Porter, W. E., and Teasdale, R. D., *Res. Publ. Assoc. Nerv. Ment. Dis.*, **30**, 118 (1952).

⁶ Sommer, J., *Dtsch. Z. Nervenzheilk.*, **150**, 249 (1940).

⁷ Paillard, J., *Arnette*, Paris (1955).

⁸ Buller, A. J., *Lancet*, **273**, 1262 (1957).

⁹ Weaver, R. A., Landau, W. M., and Higgins, J. F., *Arch. Neurol.*, **9**, 127 (1963).

¹⁰ Gassel, M. M., and Diamantopoulos, E., *Brain*, **87**, 729 (1964).

¹¹ Lloyd, D. P. C., *J. Neurophysiol.*, **6**, 293 (1943).

¹² Lloyd, D. P. C., *J. Neurophysiol.*, **6**, 317 (1943).

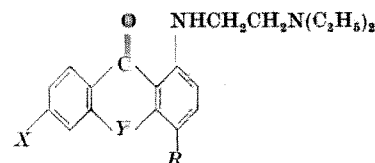
PHARMACOLOGY

A New, Active Metabolite of 'Miracil D'

'MIRACIL D' ('Lucanthone', I)¹ is an orally active schistosomicidal drug which is of limited value in the treatment of human *S. haematobium* and *S. mansoni* infections². For a variety of reasons it was thought that the effects of this agent were mediated through a therapeutically more active metabolite. A number of abortive attempts have been made to identify and isolate this substance. The latest and most thorough investigation was carried out by Strufe³, who found that the drug underwent extensive metabolic transformation in all species studied. The major urinary metabolite in the mouse proved to be the sulphone (II), while the monkey furnished the sulfoxide (III) and man excreted a 'chromopeptide', a complex of III with polypeptide. It was concluded that none of the above 'Miracil D' derivatives qualified as the elusive active agent.

We have found that the organism *Aspergillus sclerotiorum* converted 'Miracil D' to a mixture of new substances, the structures of which were established by appropriate chemical and spectroscopic methods⁴. The major transformation product was the hydroxymethyl compound IV (hycanthone) accompanied by smaller amounts of the aldehyde V.

The outstanding chemical property of IV is its extreme sensitivity to acid. When exposed to the action of mineral acid at moderate temperature it is largely converted to a water-soluble neutral material which is probably a polymeric quaternary ammonium salt.



- I $R = \text{CH}_3$, $Y = \text{S}$, $X = \text{H}$
 II $R = \text{CH}_3$, $Y = \text{SO}_2$, $X = \text{H}$
 III $R = \text{CH}_3$, $Y = \text{SO}$, $X = \text{H}$
 IV $R = \text{CH}_2\text{OH}$, $Y = \text{S}$, $X = \text{H}$
 V $R = \text{CHO}$, $Y = \text{S}$, $X = \text{H}$
 VI $R = \text{CH}_3$, $Y = \text{S}$, $X = \text{Cl}$
 VII $R = \text{CH}_2\text{OH}$, $Y = \text{S}$, $X = \text{Cl}$

The outstanding bio-logical property of IV is its high schistosomicidal activity when administered *per os* or intraperitoneally to experimentally infected hamsters. When tested according to the procedure of Berberian and

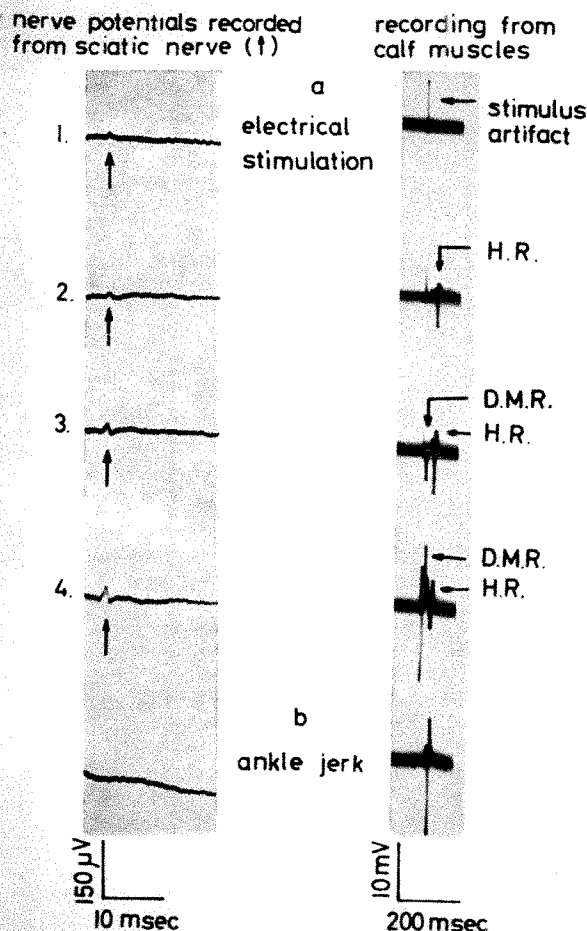


Fig. 2. Recording for nerve potentials in the upper thigh. *a* and *b* as in Fig. 1

Freele⁵ the five-day ED_{50} of hycanthone (IV) was 0.78 mg/kg (*per os*). The corresponding value for 'Miracil D' was 8.0 mg/kg (*per os*). Intraperitoneally the single dose ED_{50} of IV was 0.8 mg/kg.

The high therapeutic activity of IV when given by either route led us to suspect that it may be the active metabolite of 'Miracil D'. The acid sensitivity of IV could explain why it had escaped detection by previous investigators. Accordingly, monkeys were medicated with a total dose of 150 mg of I and the urines were collected over a 48-h period. These were carefully extracted, avoiding acid treatment at every stage and the extracts were examined by means of thin-layer chromatography. In agreement with Strufe's findings the sulphoxide (III) was the major metabolite; only traces of IV were detected. However, when the urine extracts were incubated with glucuronidase prior to thin-layer chromatography, a substance identical with IV (R_F value in several systems, ultra-violet spectra, sensitivity to acid, etc.) proved to be a major component of the mixture.

Kikuth and Gönner⁶ reported that 'Miracil D' was relatively less active in mice than in monkeys infected with the same strain of *S. mansoni*. After glucuronidase treatment the presence of IV was observed in the urine of mice medicated with I, but to a much lesser extent than in the case of the monkey. The same authors also stated that the 6-chloro derivative, VI, was active in mice but not in monkeys. In keeping with this observation it was found that after medication with VI, the glucuronide of VII was present as a major metabolite in mouse urine but only in trace amounts in monkey urine.

These findings support the hypothesis that the hydroxymethyl derivative IV is the important biologically active metabolite of 'Miracil D'. In a discussion of structure-activity relationships in the 'Miracil' series, Gönner⁷ noted that the presence of a 4-methyl group is an absolute requirement for biological activity. The reason for this now becomes clear.

DAVID ROSI
GEORGE PERUZZOTTI
E. W. DENNIS
D. A. BERBERIAN
H. FREELE
S. ARCHER

Sterling-Winthrop Research Institute,
Rensselaer, New York.

¹ Mauss, H., *Ber.*, **81**, 19 (1948).

² Blair, D. M., *Bull. World Health Org.*, **18**, 989 (1958).

³ Strufe, R., *Med. Chemie*, **7**, 337 (1963).

⁴ Analyses for all new compounds were satisfactory. These were carried out under the supervision of Mr. K. D. Fleischer. Nuclear magnetic resonance and infra-red spectra were performed under the supervision of Dr. F. C. Nachod. We thank Drs. H. P. Drobeck and F. J. Rosenberg for their assistance.

⁵ Berberian, D. A., and Freele, H., *J. Parasitol.*, **50**, 435 (1964).

⁶ Kikuth, W., and Gönner, R., *Ann. Trop. Med. Parasit.*, **42**, 256 (1948).

⁷ Gönner, R., *Bull. World Health Org.*, **25**, 702 (1961).

Pronethalol-induced Reversal of Adrenergic Vasodepression

SEVERAL substances have been described as causing reversal of the adrenergic vasodepression observed after blockade of α -receptors, adrenaline reacquiring its usual pressor action. However, when tested in animals treated with long-acting, potent and almost irreversible adrenergic blocking drugs like the β -haloalkylamines dibenamine and phenoxybenzamine, only some pressor agents as, for example, ephedrine, vasopressin, methoxamine, phenylephrine and barium chloride¹⁻³ provoke with regularity negation of adrenergic blockade. In an attempt to investigate the mechanism of this anti-adrenolytic action further, we used the β -adrenergic blocking drug, pronethalol ('Nethalide', 'Alderlin', ref. 4) and made the unexpected finding that it also causes restoration of the pressor action of adrenaline, when injected after blockade of α -receptors.

Cats weighing 2.7 to 3.8 kg and dogs weighing 6.0 to 13.8 kg were anaesthetized with pentobarbitone sodium (30 mg/kg) or chloralose (0.1 g/kg); blood pressure was recorded with mercury manometers, contractions of the nictitating membrane with a frontal writing lever and heart rate with a direct writing electrocardiograph (lead II); plasma potassium was measured in samples of arterial blood using a flame photometer. Suitable doses of adrenaline, noradrenaline and isoprenaline (2.5 to 10 μ g/kg), graded in geometric progression, were injected before adrenergic blockade, 30 min after phenoxybenzamine (5 or 10 mg/kg) and again after pronethalol (5 mg/kg); the latter drug was given by slow intravenous infusion, in 20 min, in order to minimize the fall in blood pressure caused by its rapid injection.

After adrenergic blockade by phenoxybenzamine, adrenaline and isoprenaline had purely depressor effects and the pressor response to noradrenaline was greatly reduced, abolished or reversed to a slight hypotension. Pronethalol caused reversal of adrenaline, augmentation of the residual pressor effect of noradrenaline (or reversal, in the cases in which noradrenaline caused, after phenoxybenzamine, a hypotensive response) and reduction or abolition of the depressor effect caused by isoprenaline; in a cat, isoprenaline even produced, after pronethalol, a monophasic rise in the blood pressure. This anti-adrenolytic action, exhibited by pronethalol, is clearly shown in Fig. 1; it was entirely reversible, its duration never exceeding 2 h, and could be restored by repeating the dose of pronethalol.

Dose-effect curves show that, after pronethalol, adrenaline never re-acquired the pressor potency it exhibited before establishment of adrenergic blockade of α -receptors and that the slopes of the curves are significantly different: before phenoxybenzamine the curves are steeper than after pronethalol. Much the same applies to noradrenaline, which behaved in a parallel fashion. Partial restoration of the pressor action of adrenaline, as already described, was accompanied by a return of the contractions of the nictitating membrane, which had been abolished by phenoxybenzamine; however, the effects of adrenaline on the membrane were much smaller than prior to adrenergic blockade and the restorative effect of pronethalol on the blood pressure responses to adrenaline was disproportionately more marked. During the pressor responses to adrenaline, observed after pronethalol, there was neither increase in heart rate nor a return of the increase in plasma potassium, which had been abolished by phenoxybenzamine.

Recently, Karim⁵, reporting results obtained with an experimental design similar to our own (anaesthetized cats, treated with phentolamine or phenoxybenzamine and pronethalol), mentioned that the depressor effect of noradrenaline injected after blockade of the α -receptors was abolished by pronethalol; however, he used very low doses of noradrenaline (and adrenaline) and could therefore not observe the reappearance of the pressor response, which, in our experience, is seen when doses of the order of some μ g/kg are used.

Drug-induced return of adrenaline hypertension after blockade of adrenergic α -receptors has been explained by assuming that adrenaline causes the heart to beat more frequently and more forcefully, ejecting a greater volume of blood into a constricted vascular bed. Reversal of adrenergic vasodepression would be due to an increased cardiac action working against an increased peripheral resistance (Levy and Ahlquist¹, Sutherland, Ahlquist and Ogden⁶, Levy⁷), and the same explanation has been offered for isoprenaline reversal, which often occurs under the same experimental circumstances. However, this assumption has been challenged by other work, showing that the postulated increase in cardiac action does not occur and that isoprenaline is not devoid of vasoconstrictor effects; the hypothesis of a vascular point of action fits the facts better (Osswald and Guimarães¹¹, Butterworth⁸). The

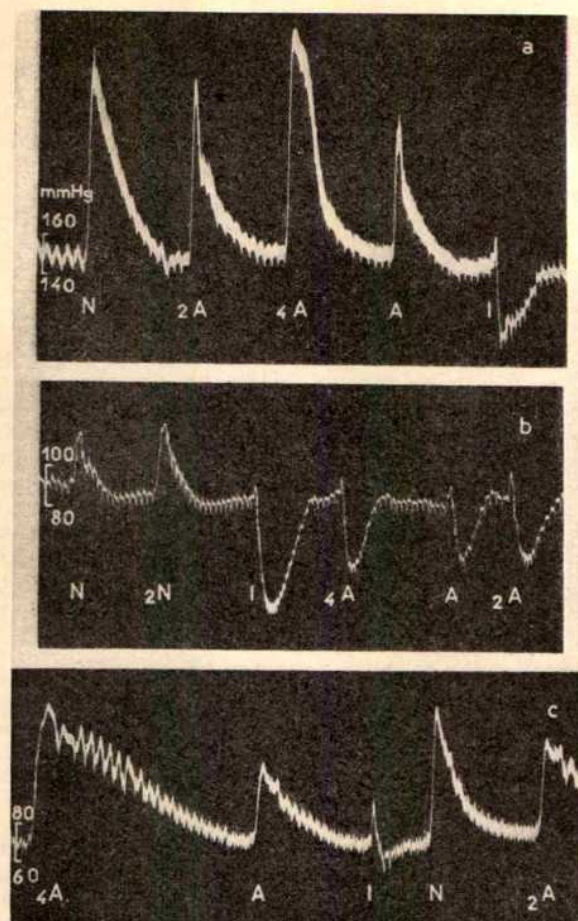


Fig. 1. Blood pressure of dog, 6 kg, pentobarbitone anaesthesia. N = noradrenaline 2.5 μ g/kg; 2N = noradrenaline 5.0 μ g/kg; A = adrenaline 1.25 μ g/kg; 2A = adrenaline 2.5 μ g/kg; 4A = adrenaline 5.0 μ g/kg; I = isoprenaline 5.0 μ g/kg. Between upper (a) and middle record (b), phenoxybenzamine 10 mg/kg was injected. The lower record (c) was obtained after injection of pronethalol 5 mg/kg.

occupied by the blocking drug. Further experimental evidence for this view has been described⁹. If this hypothesis is correct, pronethalol causes reversal of adrenergic vasodepression by blocking the receptors the activation of which causes the vessels to dilate (β -receptors) and therefore makes the free α -receptors available for interaction with catecholamines, that is, those not already blocked by phenoxybenzamine. The following facts seem to fit this explanation well. First, adrenaline never reacquires its full pressor effect; secondly, the return of adrenaline hypertension is accompanied by the reappearance of evident although rather small contractions of the nictitating membrane; thirdly, dose-effect curves of adrenaline and noradrenaline were significantly different from those observed before adrenergic blockade, being displaced to the right and flatter. If the suggested mechanism of action is true, these modifications should be expected to happen, as they actually did. Further work is necessary, however, to confirm the validity of the present hypothesis.

M. GONÇALVES MOREIRA
W. OSSWALD

Laboratório de Farmacologia,
Faculdade de Medicina,
Porto,
Portugal.

¹ Levy, B., and Ahlquist, R. P., *J. Pharmacol. Exper. Therap.*, **121**, 414 (1957).

² Osswald, W., *Inversao Vasomotora* (thesis, Porto, 1958).

³ Osswald, W., *Arch. Intern. Pharmacodyn.*, **126**, 346 (1960).

⁴ Black, J. W., and Stephenson, J. S., *Lancet*, **ii**, 311 (1962).

⁵ Karim, S., *Brit. J. Pharmacol.*, **23**, 594 (1964).

⁶ Sutherland, J. H. R., Ahlquist, R. P., and Ogden, W. S., *Fed. Proc.*, **23**, 124 (1964).

⁷ Levy, B., *J. Pharmacol. Exper. Therap.*, **146**, 129 (1964).

⁸ Butterworth, K. R., *Brit. J. Pharmacol.*, **21**, 378 (1963).

⁹ Koch-Weser, E., *J. Pharmacol. Exper. Therap.*, **146**, 318 (1964).

¹⁰ Nickerson, M., Henry, J. W., and Nomaguchi, G. M., *J. Pharmacol. Exper. Therap.*, **107**, 300 (1953).

¹¹ Osswald, W., and Guimarães, S., *Naunyn-Schmiedeberg's Arch. exp. Path. u. Pharmacol.*, **243**, 1 (1962).

Inhibition of Anaphylactic Release of Vascular Permeability Factor or Histamine by Specific Protease Inhibitor in Tissue Culture

OUR knowledge of the cellular mechanism of histamine release in anaphylaxis is imperfect; there are several controversial hypotheses^{1,2}. The difficulty is due partly to the lack of a specific metabolic inhibitor for the enzymatic reactions activated by the antigen-antibody reaction. By the use of cultured cells, we obtained the rapid activation of a heat-labile, specific SH-protease (Arthus protease) in the early stage of the reaction and the release of a polypeptide inhibitor of this enzyme at a later stage³⁻⁵. The inhibitor was also isolated from skin lesions during Arthus reactions, and it was highly purified as a homogeneous substance paper-electrophoretically and by ultracentrifugation⁶. Its molecular weight was approximately 12,500 (ref. 7). It inactivated the cellular SH-protease and papain but had no effect on trypsin and α -chymotrypsin. The inactivation of the proteases by this inhibitor occurred in parallel with a decrease in the titrable SH-groups of the enzyme molecules⁸. We further obtained the release of an immediate antihistamine susceptible permeability factor (PF) and a delayed antihistamine insusceptible PF (Arthus PF)^{9,10} during the anaphylactic reaction. This purified inhibitor has been used to characterize further the enzymatic mechanism of the anaphylactic release of immediate PF and histamine.

Albino rabbits (2-2.3 kg) were immunized with five subcutaneous injections of 3 ml. bovine serum albumin (BSA) (25 mg/ml.; Armcur)⁹. Explants 1 mm in diameter from the omentum of the animals were cultivated in our culture chamber which allowed simultaneous microscopic and chemical observation⁹. After 5-6 days, almost every cell in each culture was a fibroblast-like cell. The cell

former hypothesis, although theoretically acceptable in the case of reversal of adrenergic vasodepression caused by pressor drugs like ergotamine or ephedrine, cannot explain the results reported in this paper. As a matter of fact, pronethalol does not exhibit any pressor effects and, on the contrary, regularly causes a fall of blood pressure; on the other hand, it effectively blocks the positive chronotropic and inotropic effects of catecholamines, as was well demonstrated in previous investigations^{4,9} and also is apparent from our results.

An alternative explanation would be a direct deblocking action of pronethalol on α -receptors occupied by phenoxybenzamine (or its active metabolites) with removal of the α -adrenergic blocking drug from reactive sites, making them once more available to catecholamines. However, this hypothesis is neither in accordance with the mechanism of action of the β -haloalkylamines (which cause a long-lasting, non-competitive, insurmountable blockade) nor with the facts reported in this work: if such a deblocking action existed, catecholamines would reacquire their full pressor action (or even be potentiated, since β -blockade by pronethalol does potentiate adrenaline in animals not treated with adrenergic blocking drugs) and contractions of the nictitating membrane would be restored to their original magnitude. Since experimental evidence does not support this assumption, the following explanation is offered:

Some years ago, Nickerson, Henry and Nomaguchi¹⁰ suggested that adrenergic blockade by β -haloalkylamines causes adrenaline reversal when only a part (up to 50 per cent) of the receptors subserving vasoconstriction is

numbers were $30,000 \pm 4,000$ per culture. The cells were incubated with 1 ml. inhibitor (in Gey's buffered solution, pH 7.2) or 1 ml. Gey's for 1 min, and after sufficient washing they were incubated with BSA (20 mg/ml. in Gey's) or 1 ml. BSA containing the inhibitor at the same concentrations for 14 min. Each culture fluid sample taken was tested for its histamine content¹¹ and PF effects¹². PF effects were shown by the amount of pontamine blue extracted; immediately after intradermal injections of 0.1 ml. samples the animals were given intravenous dye, 60 mg/ml. as a 5 per cent solution in 0.425 per cent saline, and killed 30 min later. The same type of experiment was performed with *N*-ethylmaleimide, di-isopropyl fluorophosphate (DFP) or soybean trypsin inhibitor (SBTI; Novo).

Table 1. INHIBITION OF ANAPHYLACTIC RELEASE OF IMMEDIATE PF AND HISTAMINE BY PROTEASE INHIBITOR

	Present throughout	Dye leakage (μ g)	Histamine release (ng/ml.)
Inhibitor	None	14.3 (0)	43.0 (0)
"	2 mg	5.6 (61)	14.0 (67)
"	0.5	6.7 (53)	15.0 (65)
"	0.1	10.0 (30)	28.9 (32)
Pretreatment inhibitor	None	14.3 (0)	43.0 (0)
"	2 mg	nt	nt
"	0.5	7.4 (48)	14.8 (65)
"	0.1	10.4 (27)	25.5 (39)

The values in parentheses present each inhibition per cent.

Table 1 shows that the release of immediate PF (as indicated by dye leakage) or histamine by the specific antigen was greatly diminished by our inhibitor. Its inhibitory effect seemed maximal at a concentration of 0.5 mg/ml. It was noteworthy that this inhibitor itself induced no morphological change in the cells when tested by phase-contrast microphotography; and characteristic early morphological changes by the antigen occurred independently of the presence of the inhibitor. The changes were characterized by decreased motion of cytoplasmic processes, decreased pinocytosis, and decreased cytoplasmic flow, but no morphological change in the mitochondria, Golgi bodies and nuclei of the cells was observed at the time of release of immediate PF and histamine. By a pinocytotic mechanism, the inhibitor was probably taken up by the cells prior to adding antigen, but its uptake was suppressed by apparently decreased pinocytosis immediately after antigen application. Accordingly, it seemed reasonable to assume that the inhibitor, which had been taken up by the cells, inactivated specially the intracellular antigen-antibody reaction activated SH-protease (Arthus protease) and resulted in the prevention of release. It did not affect the action of immediate PF and histamine⁹. Little difference in inhibition was observed when the inhibitor was present at the time of addition of antigen or when it was washed before adding antigen. Mongar and Schild¹ suggested the possible involvement of an intracellular cathepsin in histamine release.

In view of the inhibitory effect of *N*-ethylmaleimide on anaphylactic release of histamine in chopped guinea-pig lung¹³, this SH-blocking agent was assayed for histamine release from the cells (PF effect of culture samples was not examined because of permeability-inducing activity of this agent itself). The inhibition by this agent was 95 per cent complete at 10^{-3} M, 51 per cent at 10^{-4} M and 0 per cent at 10^{-5} M. No difference in the inhibition was seen when this agent was present at the time of antigen addition or when it was washed before adding antigen. In spite of such inhibitory effects, *N*-ethylmaleimide seemed unsuitable for the present experiments; it induced rapidly marked morphological changes in the cells even in low concentration (10^{-5} M); there was seen a complete stopping of the motion of cytoplasmic processes and mitochondria and of cytoplasmic flow. Characteristic early morphological changes by the antigen, therefore, could not be detected even when *N*-ethylmaleimide was washed before antigen addition, because of the irreversible changes produced.

DFP was also effective in preventing histamine release in chopped guinea-pig lung, suggesting an involvement of a chymotrypsin-like enzyme in the reaction¹⁴. The inhibition by DFP of histamine release from the cells was 67 per cent complete at 10^{-2} M, 40 per cent at 10^{-3} M and 16 per cent at 10^{-4} M. The inhibition was less marked when DFP was washed before antigen addition; 56 per cent, 21 per cent and 7 per cent, respectively. This agent seemed more toxic to the cells; it induced rapidly marked degenerative changes in the cells. In addition to the changes described above, there occurred an irreversible granulation and vesiculation of mitochondria, retraction of cytoplasmic processes and blister formation from the cell surface even at low concentration (10^{-5} M).

Accordingly, the characteristic features induced by the antigen could not be ascertained even when DFP was washed before antigen addition. DFP also seemed unsuitable for the present experiment. No inhibition of histamine release was obtained with SBTI.

In conclusion, our Arthus protease may play an integral part in the cellular mechanism of immediate PF and histamine release. However, the steps leading to the anaphylactic release are presumably multiple and there is no reason to assume that the characteristics of this reaction are due to the properties of a single enzyme.

We thank Prof. H. O. Schild, University College, London, for his advice and Dr. K. Udaoka for his assistance. This work was supported by grants from the Japanese Ministry of Education and U.S. Army Research and Development Group Far East (DA-92-557-FEC-35779).

H. HAYASHI
Y. KINUWAKI
M. YOSHINAGA

Department of Pathology, Medical School,
Kumamoto University, Kumamoto,
Japan.

- ¹ Mongar, J. L., and Schild, H. O., *Physiol. Revs.*, **42**, 226 (1962).
- ² Austen, K. F., and Humphrey, J. H., *Adv. Immunology*, **3**, 3 (Academic Press, New York, 1963).
- ³ Hayashi, H., Tokuda, A., and Udaoka, K., *J. Exp. Med.*, **112**, 237 (1960).
- ⁴ Tokuda, A., Hayashi, H., and Matsuba, K., *J. Exp. Med.*, **112**, 249 (1960).
- ⁵ Kuze, T., *Mie-Igaku*, **4**, 1613 (1960) (in Japanese).
- ⁶ Udaoka, K., and Hayashi, H., *Biochim. Biophys. Acta*, **97**, 251 (1965).
- ⁷ Udaoka, K., and Hayashi, H., *Biochim. Biophys. Acta*, **104**, 600 (1965).
- ⁸ Udaoka, K., and Hayashi, H., *Symp. Enzyme Chem.*, **16**, 71 (1964) (in Japanese).
- ⁹ Hayashi, H., Kinuwaki, Y., Kono, M., and Takaba, Y., *Lab. Invest.*, **13**, 1124 (1964).
- ¹⁰ Hayashi, H., Yoshinaga, M., Kono, M., Miyoshi, H., and Matsumura, Y., *Brit. J. Exp. Path.*, **45**, 419 (1964).
- ¹¹ Shore, P. A., Burkhalter, A., and Cohn, V. H., *J. Pharmacol. Exp. Ther.*, **127**, 182 (1959).
- ¹² Nitta, R., Hayashi, H., and Norimatsu, K., *Proc. Soc. Exp. Biol., N.Y.*, **113**, 185 (1963).
- ¹³ Edman, K. A. P., and Mongar, J. L., *J. Physiol.*, **157**, 40P (1961).
- ¹⁴ Austen, K. F., and Brocklehurst, W. E., *J. Exp. Med.*, **113**, 521 (1961).

PATHOLOGY

A Screening Test for Substances inhibiting the Cancer Coagulative Factor

MALIGNANT tissues produce a thermolabile coagulative factor which diffuses out of cells and lays down fibrin¹⁻³. Tumours have little fibrinolytic activity and the unresolved fibrin around them leads to the growth of connective tissue, blood vessels, and lymph vessels, which form the stroma of the tumour and help the invasion of healthy tissues². Four fractions of the cancer coagulative factor have been identified⁴. Three of these are also present in placental chorion, but only very little of one fraction is found in a few normal tissues⁴. It has been known for some time that watery extracts of placenta have a thromboplastic activity⁵⁻⁷ and that placental extracts cause multiple blood clots when they are injected into animals^{6,7}. It seemed probable that the coagulative effects of cancer and chorion are due to similar factors.

The cancer coagulative factor can be inhibited by protamine sulphate *in vitro*⁸ and the growth of experimental mouse tumours can be slowed by protamine sulphate or its derivatives⁹. There is evidence also that pharmaceutical preparations of protamine can sometimes slow down the growth of malignant tumours in man¹⁰⁻¹³. This makes it seem possible that substances inhibiting the cancer coagulative factor could have practical value. Thus it was of interest to find out whether protamine could counteract the toxicity of placental extracts, and whether this could be the basis of a test.

Two types of watery extracts of fresh placentae were obtained. One was produced at room temperature after mashing up the tissue, and it was heat-stable. It was present mostly in amnion, and when injected into the tail vein of mice it caused dyspnoea within 15 sec and death within 1 min, but it had no delayed effects. The other was made as described by O'Meara and Thornes¹⁴. It was extracted under ice from strips of chorion, and its activity was quickly destroyed by heating. Its potency diminished if it stood at room temperature for 5-10 min and for that reason it was always kept on ice and injected cold. It produced dyspnoea 15-60 sec after injection into a tail vein and death within 5 min, though there were some later deaths. This latter extract resembles the cancer coagulative factor⁴ and it is the one used throughout the tests. Examinations after death showed that both extracts caused multiple blood clots in the lungs.

Table 1. NUMBERS OF MICE PROTECTED FROM LETHAL EFFECTS OF CHORION EXTRACTS

Substance	Dose (mg/kg body-wt.)	Test substance Survived	Test substance Died	Controls Survived	Controls Died	Significance <i>P</i> (2 α)
Protamine sulphate	33	11	3	0	15	0.00002
'Prolothan G'	33	5	0	0	5	0.008
Dextran sulphate	667	14	2	3	16	0.00005
'Eblimar'	1,000	12	1	2	13	0.00007
Plasma ultrafiltrate	100	4	10	0	15	0.08

Experiments were carried out on male and female white mice bred at the Evans Medical Research Laboratories; but tests with white mice from Glaxo Laboratories did not show any differences between breeds, nor was there any evidence of sex difference. All the animals weighed 16-18 g. Each mouse was accurately weighed immediately before the injections. The LD_{50} of chorion extracts injected into tail veins was estimated on the basis of deaths within 5 min. Extracts causing any death in less than 15 sec or with an LD_{50} of more than 3 ml./100 g body-wt. were rejected. Test substances were given in doses of $1/3 LD_{50}$, as experience had shown that higher doses could cause confusion between the ill effects of the test substance and those of the chorion extract. The test substances were made up in 0.9 per cent NaCl solution and given in volumes of 0.5-2 ml./100 g body-wt. The test solution, or an equal volume of 0.9 per cent sodium chloride solution, was injected into tail veins of alternate mice 60 sec before injecting an LD_{50} of chorion extract. The numbers of survivals and deaths were expressed in 2×2 tables and the exact probability of the difference between treated and control mice was calculated, taking $P < 0.05$ (2 α) as significant.

Table 1 shows that there was significant protection with protamine sulphate and with a neutral solution of protamine in glucose ('Prolothan G') suitable for use in man, both from clupein. Significant protection was also obtained with large doses of high-molecular-weight dextran sulphate (Glaxo), and a degraded carrageenan ('Eblimar', Evans Medical)¹⁵. Ultrafiltrate of lamb plasma obtained through a 50 Å filter gave some protection; but this was not significant. Among substances that were ineffective were histone from fowl erythrocytes and from calf thymus, spermine and some of its derivatives, low-molecular-weight dextran ('Dextran 40'), and several synthetic guanidine derivatives, all in doses of $1/3 LD_{50}$.

These results support the view that the effect of protamine on malignant tumours could be due to an inhibi-

tion of the cancer coagulative factor^{10,11}. The test can be used as a screen for inhibitors of the cancer coagulative factor, though, of course, it cannot provide proof of anti-tumour activity.

E. M. GLASER

P. SPINK

Evans Medical Research Laboratories,

Speke

Liverpool 24.

R. A. Q. O'MEARA

Trinity College,

Dublin.

¹ O'Meara, R. A. Q., *Irish J. Med. Sci.*, **394**, 474 (1958).

² O'Meara, R. A. Q., *Arch. D. Vecchi*, **31**, 365 (1960).

³ O'Meara, R. A. Q., and Jackson, R. D., *Irish J. Med. Sci.*, **391**, 327 (1958).

⁴ Boggust, W. A., O'Brien, J. D., O'Meara, R. A. Q., and Thornes, R. D., *Irish J. Med. Sci.*, **447**, 231 (1963).

⁵ Finkelstein, M., *Nature*, **155**, 202 (1945).

⁶ Schneider, C. L., *Proc. Soc. Exp. Biol. and Med.*, **N.Y.**, **62**, 322 (1946).

⁷ Schneider, C. L., *Amer. J. Physiol.*, **149**, 123 (1946).

⁸ Thornes, R. D., and O'Meara, R. A. Q., *Irish J. Med. Sci.*, **428**, 361 (1961).

⁹ Muggleton, P. W., MacLennan, J. G., and Dyke, W. J. C., *Lancet*, **i**, 409 (1964).

¹⁰ O'Meara, R. A. Q., and O'Halloran, M. J., *Lancet*, **ii**, 613 (1963).

¹¹ O'Halloran, M. J., and O'Meara, R. A. Q., *Bull. Soc. Int. Chir.*, **13**, 30 (1964).

¹² Hughes, L. E., *Lancet*, **i**, 458 (1964).

¹³ Lutton, A., *Lancet*, **i**, 768 (1964).

¹⁴ O'Meara, R. A. Q., and Thornes, R. D., *Irish J. Med. Sci.*, **423**, 106 (1961).

¹⁵ Anderson, W., *J. Pharm.*, **15**, 139 (1961).

Distribution of the Intestinal Factor of Normal Mice which inactivates Murine Virus Hepatitis

THE ability of the intestinal content and intestine of normal mice to inactivate murine hepatitis virus (MHV-3) was recently reported¹. The present paper deals with the localization of this activity in different sections of the intestine and in separate strata of the intestinal wall. The distribution of the inhibitor has also been studied in mice of varying ages, and in various tissues of mice, as well as in the intestine of several other species. The interaction between the inhibitor and the virus has also been studied *in vitro* at 37° C and at 2° C.

In order to test the effect of the different materials on virus infectivity, the following procedure was used: the virus², stored at -70° C as a 10 per cent suspension ($LD_{50} = 10^{-8.1}$), was used in 10-fold dilution from 10^{-1} to 10^{-9} . Depending on the experiment, several series of these dilutions were prepared to contain a homogenate of the different materials in final concentration of 10 per cent. One more series was used as a control (no homogenate added). All samples, after addition of antibiotics, were incubated at 37° C for 90 min and frequently shaken. Virus levels were then determined in each sample according to the method of Reed and Muench³.

Table 1 shows that the inhibitory activity is predominantly present in the first section of the small intestine, and decreases in the middle and still further in the lower sections. The large intestine possesses a certain ability to inactivate the virus, although to a lesser extent than the small intestine. Table 2 shows that the intestinal mucosa is the most effective tissue in inactivating the virus, whereas the rest of the intestine (without the mucosa) displays a lower activity. The inactivating power varies with the age of the mouse, reaching a maximum in adult-

Table 1. EFFECT OF DIFFERENT SECTIONS OF INTESTINE OF ADULT MICE* ON MHV-3 VIRUS INFECTIVITY

Materials	-Log LD_{50}
Virus	7.3
Virus + first portion (13 cm) of small intestine	1.9
Virus + middle portion (13 cm) of small intestine	3.0
Virus + last portion (13 cm) of small intestine	4.1
Virus + large intestine	5.1

* N.M.R.I. strain.

Soon after killing the animals the intestine was thoroughly washed to remove any faecal debris. Mice were injected intraperitoneally (0.1 ml.) with 10-fold dilution of each material from 10^{-1} to 10^{-9} (10 mice for each dilution). The experiment reported in Table 1 is one of three which gave similar results.

hood (Table 3). As far as the localization in other tissues is concerned, it was found that, besides the intestine, only the stomach, of all the tissues studied, possesses a certain activity (Table 4). In the experiments summarized in Table 5 the intestine of various species of animals, and different intestinal sections from man, were tested. It is evident that, besides the mouse, only the rat possesses, albeit to a lesser extent, the ability to inactivate the virus. No activity was found in horse intestine mucosa. Fig. 1 shows that, at 37° C, the activity increases with incubation time, whereas at 2° C no activity at all could be detected.

From the data presented here, it appears that the principle which inactivates MHV-3 virus is mainly present in the intestinal tract of the adult mouse, and particularly in the first section of the mucosa of the small intestine. Furthermore, it is evident that interaction between the inhibitor and the virus *in vitro* is dependent on temperature.

Work is in progress to determine the chemical nature of the inhibitor and to attain its purification. Preliminary experiments have already revealed the difference between

Table 2. EFFECT OF VARIOUS INTESTINAL TISSUE STRATA OF ADULT MICE* ON MHV-3 VIRUS INFECTIVITY

Materials	-Log LD ₅₀
Virus	7.4
Virus + intestinal mucosa	2.0
Virus + mucosa-free intestine	4.7
Virus + whole intestine	3.1

* N.M.R.I. strain.

The mucosa was removed with a piece of glass.
For details see text and Table 1.

Table 3. EFFECT OF THE INTESTINE OF MICE* OF VARIOUS AGES ON MHV-3 VIRUS INFECTIVITY

Materials	-Log LD ₅₀
Virus	7.3
Virus + intestine, 2-day-old mice	5.9
Virus + intestine, 7-day-old mice	5.0
Virus + intestine, 20-day-old mice	4.6
Virus + intestine, 40-day-old mice	3.5
Virus + intestine, 100-day-old mice	2.4

* N.M.R.I. strain.

For details see text and Table 1.

Table 4. EFFECT OF VARIOUS ADULT MOUSE* TISSUES ON MHV-3 VIRUS INFECTIVITY

Exp. No.	Materials	-Log LD ₅₀
1	Virus	7.4
	Virus + intestine	3.0
	Virus + stomach	5.0
	Virus + liver	7.4
	Virus + brain	7.3
2	Virus	7.3
	Virus + kidney	7.2
	Virus + spleen	7.0
	Virus + lung	6.9
	Virus + heart	7.4
3	Virus	7.5
	Virus + muscle	7.0
	Virus + pancreas	7.4
	Virus + blood	6.9

* N.M.R.I. strain.

Mice were injected intraperitoneally (0.1 ml.) with 10-fold dilution of each material from 10⁻¹ to 10⁻⁴ (10 mice for each dilution). The experiment reported in Table 4 is one of three which gave similar results.

Table 5. EFFECT OF INTESTINE OF VARIOUS ANIMAL SPECIES ON MHV-3 VIRUS INFECTIVITY

Exp. No.	Materials	-Log LD ₅₀
1	Virus	7.5
	Virus + mouse intestine*	3.3
	Virus + rat intestine*	4.0
	Virus + guinea-pig intestine*	7.4
	Virus + rabbit intestine*	7.5
2	Virus + sheep intestine*	7.3
	Virus	7.7
	Virus + human duodenum (first portion)	7.7
	Virus + human ileum (last portion)	7.3
	Virus + human vermiform appendix	7.5
3	Virus + human colon (first portion)	7.6
	Virus + human rectum (last portion)	7.2
	Virus	7.4
	Virus + ox intestine*	7.4
	Virus + horse intestine* mucosa	7.5
4	Virus + mucosa-free horse intestine*	7.2
	Virus + pig intestine*	6.8
	Virus	7.5
	Virus + dog intestine*	7.4
	Virus + cat intestine*	7.5
	Virus + chicken intestine*	6.9
	Virus + pigeon intestine*	7.0

* First portion of small intestine.

For details see text and Table 1.

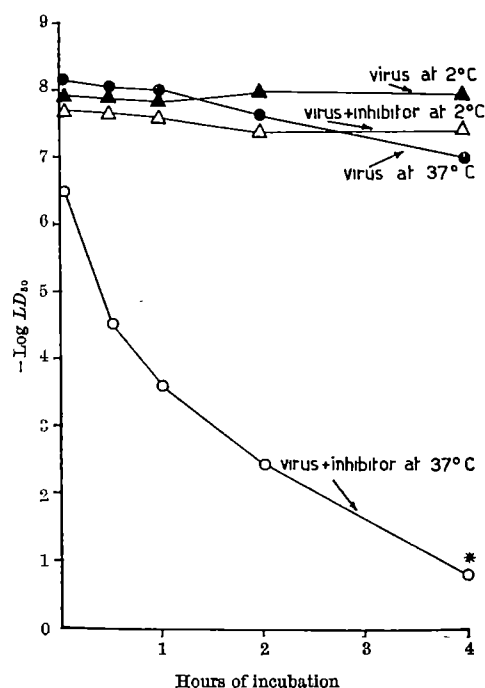


Fig. 1 Interaction between inhibitor and virus at 2° C and at 37° C. Before the addition of the inhibitor to the virus dilutions, the whole system had been equilibrated at 37° C and at 2° C respectively

* LD₅₀ < 10⁻¹ (at 10⁻¹ dilution 2 mice of ten inoculated died).
For details see text and Table 1.

our substance (inactivated at 70° C for 10 min) and the one isolated from the intestine of mice by Mandel and Racker⁴ (resistant at 100° C for 10 min).

M. PIAZZA
A. AMODIO
G. PANE

Institute of Infectious Diseases,
"Gesù e Maria",
University of Naples.

¹ Piazza, M., *Nature*, **203**, 1196 (1964).

² Dick, G. W. A., Niven, J. S. F., and Gledhill, A. W., *Brit. J. Exp. Path.*, **37**, 90 (1956).

³ Reed, L. S., and Muench, H., *Amer. J. Hyg.*, **27**, 493 (1938).

⁴ Mandel, B., and Racker, E., *J. Exp. Med.*, **98**, 399 (1953).

IMMUNOLOGY

Reaction of Adenine-specific Antibodies with Denatured Deoxyribonucleic Acid

At the present time there is no doubt regarding the role of nucleic acids in carrying genetic information, and their participation in protein synthesis and other biological events is now well known¹. There is therefore considerable biochemical and genetical importance in the availability of specific antibodies capable of reacting with nucleic acids. The production of such antibodies has been described recently as the result of immunization with DNA that had been complexed with methylated bovine serum albumin², by coupling periodate-oxidized nucleotides or nucleosides to proteins³, or by binding uridine-5'-carboxylic acid to a synthetic polypeptide⁴. Such data promise to make the specificity and sensitivity of immunochemical procedures, which have been of great value in the study of other polymers such as polysaccharides and proteins⁵, similarly available for an analysis of nucleic acids.

This communication will report on further observations that support the feasibility of producing antibodies that can react with nucleic acids. It is known that periodate ions are able to attack adjacent hydroxyl groups, cleaving the carbon-carbon bond, yielding aldehydes⁶ which can

react with several other groups such as amines^{7,8}. In our studies adenosine-specific antibodies were obtained by immunizing rabbits with conjugates of periodate-oxidized adenosine coupled to human gamma globulin and albumin, which also had been oxidized by periodate (see Fig. 1).

The conjugates were prepared as follows: 735 mg sodium periodate was dissolved in 20 ml. water; to this solution 184 mg adenosine was added and incubated at 4° C for 12 h. The solution was divided in two 10-ml. portions. To one portion 250 mg of human gamma globulin, dissolved in 5 ml. saline, was added. To the other portion, 250 mg of human albumin dissolved in 5 ml. water was added. Both solutions were incubated at 4° C with stirring for 24 h. During this time a precipitate formed in both solutions. The suspensions were then lyophilized, resuspended and dialysed, and the final volume was adjusted to 10 ml. Since the conjugates were

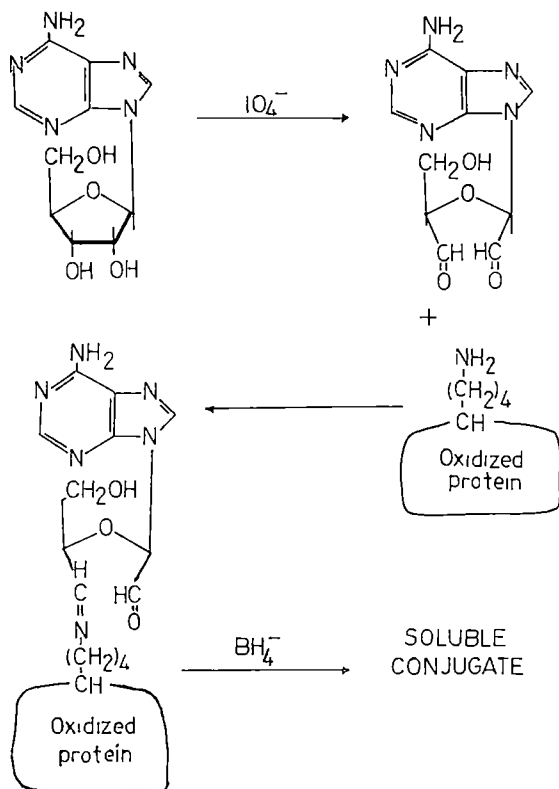


Fig. 1. Periodate oxidation of adenosine, and conjugation

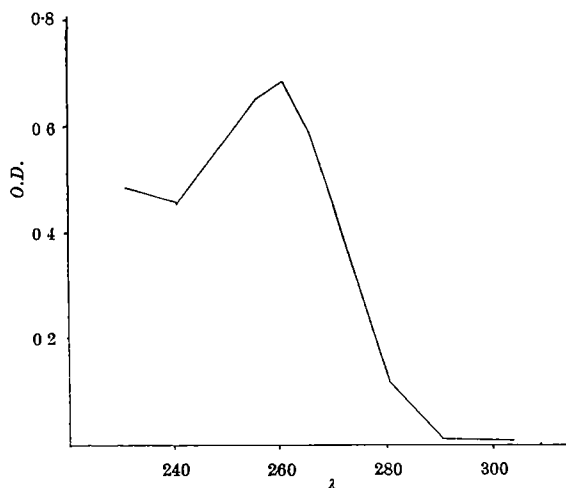


Fig. 2. Periodate oxidized adenosine

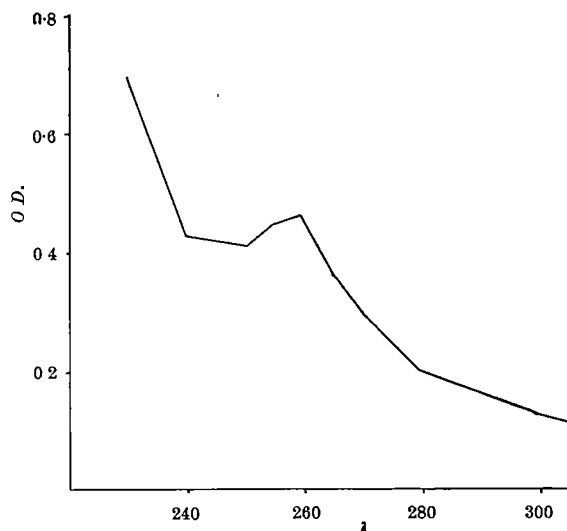


Fig. 3. Conjugate γ -globulin-adenosine (IO_4^- , BH_4^-)

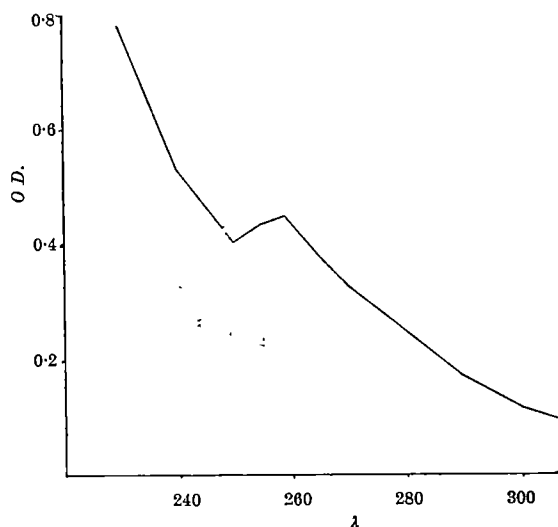


Fig. 4. Conjugate albumin-adenosine (IO_4^- , BH_4^-)

insoluble, they could be washed with water or saline and, when desired, rendered soluble by the addition of sodium borohydride (amyl alcohol was added to avoid foaming). Soluble preparations were used for precipitation reaction, insoluble preparations were employed in immunization. When conjugates were made soluble the excess of borohydride was eliminated by dialysis.

Figs. 2, 3 and 4 show the ultra-violet absorption spectra of periodate-oxidized adenosine and of the solubilized conjugates. According to the absorption spectra of conjugates and oxidized adenosine, the approximate ratio of adenosine to protein was 20–25:1 for gamma globulin and 10–12:1 for albumin.

For immunization, 0.5 ml. of the suspended insoluble conjugates was mixed with 0.5 ml. of adjuvant (Arlacel-Drakeol 1:9 + 2 mg of *Mycobacterium bovis* per ml.) and injected into the foot pads of rabbits. Two rabbits were used for each conjugate. The animals received five additional intradermal injections at weekly intervals and were bled six days after the last injection.

The sera from the four immunized rabbits precipitated both types (albumin and globulin) of soluble conjugates, but did not react with the heterologous oxidized protein free of coupled adenosine. The four antisera precipitated heat-denatured calf thymus and salmon sperm DNA as shown in Table 1. As shown in Table 2, the amount of antibody that is precipitated will vary with the source of

Table 1. PRECIPITIN REACTION INVOLVING RABBIT ANTI-ADENINE SERA* AND DEOXYRIBONUCLEIC ACID GLOBULIN (10_2 , BH $_2$), ALBUMIN (10_2), OR CONJUGATES THEREOF WITH ADENOSINE

Rabbits	Immunized with:	Reaction with:			
		GLOB-ADEN (10_2 , BH $_2$)	ALB-ADEN (10_2 , BH $_2$)	Calf thymus DNA	GLOB (10_2 , BH $_2$)†
Rabbit 1	GLOB-ADEN (10_2)	+	+	+	+
Rabbit 2	GLOB-ADEN (10_2)	+	+	+	+
Rabbit 3	ALB-ADEN (10_2)	+	+	+	(-)
Rabbit 4	ALB-ADEN (10_2)	+	+	+	+

* Produced after immunization of rabbits with conjugates of periodate-oxidized adenosine and either albumin (ALB) or globulin (GLOB).

† Periodate-oxidation of gamma globulin yields an insoluble product, but it could be solubilized by addition of sodium borohydride.

Table 2. QUANTITATIVE PRECIPITIN REACTION WITH ANTI-ADENINE SERUM FROM RABBIT 1 INJECTED WITH A GLOB-ADEN (10_2) CONJUGATE

Source of DNA	Amount added (μ g)	Antibody precipitated (as N, ml. 0-2° C) (μ g)
Calf thymus (Nutritional Biochemical Corporation)	20	10*
	50	14
	150	28
	300	27
Calf thymus (Sigma)	20	28
Salmon sperm (California Biochemical Research)	20	45

* After addition of 10 mg of adenosine only 3 μ g antibody N/ml. is precipitated.

DNA and this may be due to differences in the degree of polymerization of different preparations or, possibly, to differences in the content and location of adenosine in the various heat-denatured DNA preparations.

SERGIO ESTRADA-PARRA

AMANDA SCHMILL

ROSA-MARÍA MARTÍNEZ

Departamento de Microbiología,
Escuela Nacional de Ciencias Biológicas,
Instituto Politécnico Nacional,
México 17, D.F.

¹ Vogel, H. J., Bryson, V., and Lampen, J. O., *Informational Macromolecules* (Academic Press, New York, 1963).

² Plescia, O. J., Braun, W., and Palczuk, N. C., *Proc. U.S. Nat. Acad. Sci.*, **52**, 279 (1964).

³ Erlanger, B. F., and Belser, S. M., *Proc. U.S. Nat. Acad. Sci.*, **52**, 68 (1964).

⁴ Sela, M., Ungar-Waron, H., and Schechter, Y., *Proc. U.S. Nat. Acad. Sci.*, **52**, 285 (1964).

⁵ Heidelberger, M., *Fortsch. der Chem. Org. Naturstoffe*, **18**, 503 (1960).

⁶ Dyer, J. R., *Methods of Biochemical Analysis*, **3** (Interscience Publ. Co., New York, 1956).

⁷ Rebers, P. A., Hurwitz, E., Heidelberger, M., and Estrada-Parra, S., *J. Bacteriol.*, **83**, 335 (1962).

⁸ Rebers, P. A., Estrada-Parra, S., and Heidelberger, M., *J. Bacteriol.*, **86**, 882 (1963).

BIOLOGY

Experimentation with Plants at Sub-atmospheric Oxygen-levels: Effects of Oxygen Pressure and Salts on Germination of Winter Rye

In a series of recent investigations, it has been shown that plants can be grown in atmospheres with reduced oxygen pressures or in air at reduced total pressure. Germination and seedling growth are in some cases accelerated at sub-atmospheric oxygen levels, and are commonly comparable with these processes in air.

In spite of their essentially normal growth and gross morphology, a number of pronounced changes in biochemical composition^{2,3} and metabolism^{4,5} have been demonstrated. Some experiments have assessed relationships between pO_2 and specific physiological processes such as abscission⁶ or other environmental factors such as heat or cold.

One of the many factors influencing seed germination and seedling growth is salinity. The present paper reports on experiments relating pO_2 and calcium-ion to the effect of saline water on winter rye.

Commercial winter rye seed (*Secale cereale*) (1962 crop) were germinated routinely on filter paper in sterile Petri dishes, containing 25-30 seeds and 10 ml. distilled water.

Seeds were incubated at $25^\circ \pm 1^\circ$ C in constant light (cool white fluorescent, 100-ft. candles). Gas mixtures consisted initially of oxygen plus nitrogen.

Rye is a facultative anaerobe, and a highly competent microaerobe, which germinates somewhat more slowly at $pO_2 = 15$ mm than at higher levels when in distilled water (Table 1). In sodium chloride at 0.25 M, germination at all oxygen levels except 150 mm (air) was reduced to one-half or less relative to distilled water. In 0.75 M, sodium chloride inhibition of germination was complete at $pO_2 = 15$ mm, and higher oxygen pressures permitted only a few seeds to germinate poorly. At 0.1 M, sodium chloride does not inhibit germination. When $Ca(NO_3)_2$ at 0.02 M is added to this level of sodium chloride, germination at $pO_2 = 15$ mm is unaffected. In contrast, at all higher oxygen levels in 0.1 M sodium chloride, the presence of calcium nitrate results in significantly elevated germination. When salinity is raised to 0.25 M, $Ca(NO_3)_2$ has a beneficial effect at all oxygen levels, but is relatively more stimulatory at pO_2 50-100 mm.

The most striking response, however, was obtained at 0.75 M sodium chloride, where germination was completely arrested at $pO_2 = 15$ mm, but proceeds to a slight degree at higher oxygen pressures. At this salinity the calcium effect is seen to be quite large, but quantitatively dependent on the pO_2 .

The response of primary root growth (Table 2) post-germination follows the general pattern seen in germination but differs in some significant details.

(a) An optimum pO_2 of 50 mm in distilled water is indicated, but disappears in sodium chloride solutions.

(b) Marked salt inhibition appears between 0.025 and 0.10 M sodium chloride, which is less than required for inhibition of germination.

Again, increasing pO_2 and $Ca(NO_3)_2$ enhances root elongation, and the calcium-effect is aerobic in character.

Table 1. EFFECT OF OXYGEN PRESSURE AND CALCIUM-ION ON THE GERMINATION OF WINTER RYE IN SALT SOLUTIONS

NaCl (M)	pO_2 (mm Hg)					
	150		100		50	
	-Ca	+Ca†	-Ca	+Ca	-Ca	+Ca
0	83*	79	79	60	60	58
0.025	83	91	70	76	58	58
0.100	73	88	71	83	59	50
0.250	62	83	22	80	23	46
0.750	(10)†	88	(7)	59	(7)	18

* Mean per cent germination after 3 days at 25° C based on replicates totalling 150 seeds. L.S.D._{5%} = 9%.

† As $Ca(NO_3)_2$, supplied at one-fifth corresponding molarity of NaCl.

‡ (10) denotes emergence = 0.1 cm.

Table 2. EFFECT OF OXYGEN PRESSURE AND CALCIUM-ION ON THE ROOT-LENGTH OF WINTER RYE IN SALT SOLUTIONS

NaCl (M)	pO_2 (mm Hg)					
	150		100		50	
	-Ca	+Ca†	-Ca	+Ca	-Ca	+Ca
0	2.8*	2.8	2.9	2.9	3.6	3.6
0.025	2.4	1.8	2.8	1.7	2.5	1.8
0.100	0.8	2.3	0.7	2.7	0.9	2.4
0.250	0.3	2.9	0.4	3.0	0.5	2.5
0.750	0.1	0.3	0.1	0.4	0.1	0.4

* Mean length (cm) of roots after 3 days at 25° C, based on aggregate populations of 35-50 seedlings; L.S.D._{5%} = 0.7 cm.

† As $Ca(NO_3)_2$, supplied at one-fifth corresponding molarity of NaCl.

Table 3. RELATIVE EFFECTS OF CALCIUM AND NITRATE-IONS ON ROOT GROWTH IN SALT SOLUTIONS AT VARIOUS OXYGEN PRESSURES

	pO_2 (mm Hg)			
	150	50	15	
Water	2.8*	3.6	1.3	
0.1 M NaCl	1.0	0.8	0.3	
+ 0.02 M $Ca(NO_3)_2$	3.0	2.8	1.1	
+ 0.02 M $CaCl_2$	3.7	1.0	0.4	
+ 0.02 M KNO_3	1.1	1.4	0.3	

* Mean length (cm) of roots after 3 days at 25° C based on 35-50 seedlings. L.S.D._{5%} = 0.7 cm.

Table 4. GERMINATION OF RYE SUBMERGED IN AERATED SOLUTIONS

Solution	Ionic strength μ	Germination after 10 days (%)
H $_2$ O	—	89
0.7 M sucrose	—	100
0.5 M NaCl	0.50	15
0.5 M KCl	0.50	21
0.18 M $MgCl_2$	0.54	4
0.18 M $CaCl_2$	0.54	87
0.4 M $CaCl_2$	1.2	28

The effect of calcium nitrate was examined further, assuming that either constituent ion, or the two in concert, could be responsible for reversal of salt inhibition. Accordingly, the effects of several appropriate salts on the extension of roots in 0.10 M sodium chloride were compared (Table 3). At $pO_2 = 15$ mm and 50 mm (optimum for root elongation), only $Ca(NO_3)_2$ had a significant effect, whereas at the oxygen-level of air, both calcium salts were active, the chloride being somewhat more so than the nitrate. Thus, if at lower oxygen levels NO_3^- is serving as an electron acceptor, it apparently can do so only in the presence of Ca^{++} . Conversely, if Ca^{++} acts, conceivably at the membrane, to counteract the effects of excess Na^+ , it also can only do so when NO_3^- is present. Under strictly anaerobic conditions, KNO_3 at concentrations of 10^{-3} to 5×10^{-2} M can stimulate rye germination¹, whereas calcium salts (other than nitrate) ordinarily have no effect.

Calcium salts other than the nitrate can have unique effects in certain rather unusual cases of oxygen-limited germination (Table 4). When rye seeds are submerged in 15 cm of water or 0.7 M sucrose, their germination is slow but otherwise normal and essentially complete. In 0.5 M sodium and potassium chloride, germination is severely suppressed, although these solutions are approximately isosmotic with the sugar. Further, magnesium and calcium chloride are comparable in ionic strength to potassium chloride, yet the magnesium solution is highly inhibiting whereas the calcium solution permits nearly normal germination.

This work was carried out under National Aeronautics and Space Administration contract NASw-767.

S. M. SIEGEL
OLIVE DALY
CONSTANCE GIUMARRO

Union Carbide Research Institute,
Tarrytown, New York.

¹ Siegel, S., Renwick, G., Daly, O., Giumarro, C., Davis, G., and Halpern, L., in *Current Aspects of Exobiology*, edit. by Mamikunian and Briggs (Pergamon Press, 1965).

² Siegel, S., Rosen, L., and Giumarro, C., *Nature*, **198**, 1288 (1963).

³ Siegel, S., Rosen, L., and Renwick, G., *Physiol. Plant.*, **15**, 304 (1962).

⁴ Siegel, S., Renwick, G., and Rosen, L., *Science*, **137**, 683 (1962).

⁵ Renwick, G., Giumarro, C., and Siegel, S., *Plant Physiol.*, **39**, 303 (1964).

⁶ Rosen, L., and Siegel, S., *Plant Physiol.*, **38**, 189 (1963).

Morphactins, a Novel Group of Plant-growth Regulators

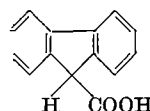
IN 1960 some interesting effects of fluorene-9-carboxylic acids on plants were observed at the biological research laboratories of E. Merck AG, Darmstadt, Germany. An intensified research programme on these compounds and on a great many derivatives was initiated by this company, which, during the following years, led to the discovery of a large-numbered group of compounds of substantial interest, some of which show an extremely strong action on the growth and development of plants¹. This particular action has novel and somewhat unique features, and was reported first in 1964 by Schneider².

Compared with the known types of plant-growth substances³, the new fluorene-regulators do not fit into the existing scheme of such growth-regulating activities and, therefore, they really constitute a novel class of plant-growth substances.

Their overall activity is growth retardation, resulting in stunted and often malformed dwarf plants: bushy-stunted growth type or otherwise peculiar habits. Typical symptoms were already briefly summarized². Out of the new regulators, some have a remarkably broad spectrum of activity over a very wide range of concentrations (corresponding to that of the gibberellins), essentially free from phytotoxic side effects. Treated plants often become gradually darker green in colour².

These and other outstanding features established the new group's name, namely *morphactins* (*morphogenetic*

active substances)². A more detailed account on basic work with these compounds will be presented elsewhere⁴.



Fluorene-9-carboxylic acid, basic structure of the morphactins

The broad activity spectrum combined with low phytotoxic side effects to the plants treated, even at high dosage-levels, makes certain members of the morphactins interesting for slowing down the growth of mixed vegetations, where to a certain extent plant coverage of soil is essential for prevention of soil erosion by wind and/or rainfall. These substances, for example, methyl-2-chloro-9-hydroxy-fluorene-(9)-carboxylate, seem to be useful agents for growth retardation and suppression, respectively, for example, along highways, railroads, ditch banks, sporting areas, and even in special perennial crops such as orchards and vineyards, too. In this connexion, it should be mentioned that residual activity of the new compounds in soils is strongly limited; these will be broken down in most soil types within a few weeks after application, mainly by microbial attack^{5,6}.

Besides growth retardation, other members of the group offer new possibilities for broad-spectrum control of weeds in cereals and grassland (pastures and meadows), because of synergistic action with known herbicides of different kinds¹, such as phenoxy compounds^{5,7}. For example, suitable formulations containing the morphactins *n*-butyl-9-hydroxy-fluorene-(9)-carboxylate or salts of 9-hydroxy-fluorene-(9)-carboxylic acid together with MCPA and/or 2,4-D and/or another phenoxy compound in optimal proportions give good control of a variety of weeds which are difficult to control, such as *Galeopsis*, *Galium*, *Stellaria*, *Polygonum*, *Matricaria*, *Chrysanthemum*, *Lamium*, *Veronica*. Proper timing of application, that is to say, spraying at early developmental stages of the weeds, is essential for complete success^{5,7}.

Moreover, besides growth retardation and broad-spectrum weed control, morphactins offer some other features and advantages, which make this new experimental group an attractive one. Toxicity in mammals and fish is very low, for example, in rats (Wistar) LD_{50} oral acute is greater than 5,000 mg/kg body-wt.⁷

In the meantime research on a variety of fundamentals in plant growth and development and also on a range of practical aspects is in progress. Basic information on the morphactin group is now available⁷.

GERHART SCHNEIDER SIGMUND LUST
DIETRICH ERDIANN GÜNTHER MOHR
KONRAD NIETHAMMER

Research Laboratories of E. Merck AG,
Darmstadt, Germany.

¹ Patents applied for in Germany and abroad by E. Merck AG, Darmstadt, Germany.

² Schneider, G., *Naturwiss.*, **51**, 416 (1964).

³ Jonsson, A., in *Encyclopedia of Plant Physiology*, **14**, 959 (1961).

⁴ Schneider, G. (in preparation).

⁵ Schneider, G., and Flemming, H., paper at the "6. Deutsche Arbeitstagung über Fragen der Unkrautbiologie und -bekämpfung", Hohenheim, Germany, March 4-5, 1965. *Z. Pflanzenkrankh.*, Supplementum III (1965, in the press).

⁶ Schneider, G. (unpublished results).

⁷ *Morphactins, Technical Data Sheet*, 1965 (E. Merck AG).

Winter Scale Rings in Lates niloticus (Pisces: Centropomidae) from Lake Chad

THE formation of scale rings in tropical fishes has usually been ascribed to some form of physiological stress producing a temporary check in the growth-rate. Holden¹ thus proved that in *Tilapia* spp. from Lake Victoria ring

Table 1. STATE OF SCALE MARGIN IN IMMATURE MALES*

Month (1963)	No. of fish	Width of marginal zone			Mean lake temperature (°C)
		Narrow	Medium	Wide	
Jan.	18	22.0	5.5	71.5	—
Feb.	29	41.3	17.2	41.3	23.9
Mar.	7	85.2	14.2	—	26.4
Apr.	26	46.0	42.2	11.5	27.8
May	No sample				
June	11	9.1	45.4	45.4	29.0
July	No sample				28.5
Aug.	53	5.4	5.4	84.6	28.1
Sept.	29	6.8	—	93.2	29.0
Oct.	17	5.8	—	94.2	28.2
Nov.	31	6.4	3.2	90.1	25.1
Dec.	18	5.5	5.5	88.8	21.2

* Showing percentage occurrence according to the width of the zone between the most recently formed ring and the margin of the scale.

formation is associated with spawning; Johnels² and Daget³ both correlated the annual rings which occur in many West African savannah species of fish with adverse environmental conditions, particularly food shortage, occurring during seasonal drying-up of the rivers. It appears that scale rings usually formed under tropical conditions are not strictly comparable with those of temperate species, which are laid down as a consequence of differences between summer and winter growth-rates, and where change in temperature is the most important factor.

It is therefore interesting to record that during a recent examination of *Lates niloticus* (Nile perch) in Lake Chad (lat. 13° 30' north) genuine 'winter' rings were laid down in immature fish. The annulations consist of breaks in an otherwise regular series of concentric circles on the anterior half of each scale traceable posteriorly to marked lines of discontinuity near the etenoid sector of the scale. An analysis of scale margin data for immature males (Table 1) shows that in a high proportion of fish the scale ring was formed between December and March. Water temperatures fell during this period to as much as 12° C below the maximum temperatures recorded during June and September, the warmest months of the year. It seems reasonable to correlate the formation of scale rings with a temperature drop of this magnitude.

Results indicate that rings are usually formed in three successive winters before males of *L. niloticus* mature at a total length of approximately 50 cm. Preliminary investigations suggest that age determination in mature fish may be complicated by the formation of spawning rings in addition to the annual winter rings.

A. J. HOPSON

Federal Fisheries Service,
Lake Chad Research Station,
Maiduguri, Northern Nigeria.

¹ Holden, M. J., *Ann. Rep. E. Afr. Fish. Res. Org.*, 1954/1955, 36 (1955).

² Johnels, A. G., *Arkiv. zool. Stockholm*, (2), 6, 327 (1954).

³ Daget, J., *Hydrobiologia*, 9 (4), 321 (1957).

Technique for the Removal of the Brain in Bats

In public health investigations it is essential that animal hosts and reservoirs be correctly identified. Specialists, particularly mammalogists, are usually willing to identify specimens collected in return for retaining all or a portion of the mammals submitted, especially if the specimens, such as bats, come from a zoologically interesting area, are rare in collections, or might present some challenging taxonomic problem. Since there is an increasing interest in the public health importance of bats (such as their involvement with rabies), countries which have not yet made surveys of their bat populations for the incidence of rabies are being encouraged to do so (Expert Committee on Rabies—Fourth Report, W.H.O. Tech. Rep. Ser. No. 201, 1960). For the purposes of accurate identification, the head is the most important part of the body to the mammalogist, while its contents are of paramount importance to the epidemiologist. The conventional laboratory techniques used for brain tissue removal in rabies diagnosis

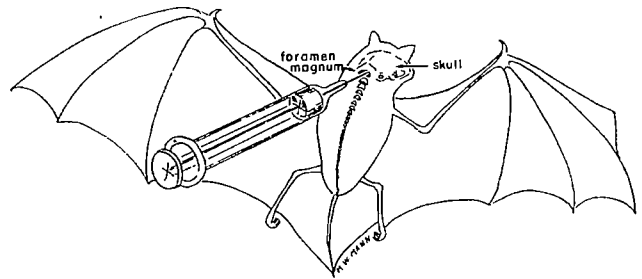


Fig. 1 Brain tissue may be hypodermically withdrawn through foramen magnum

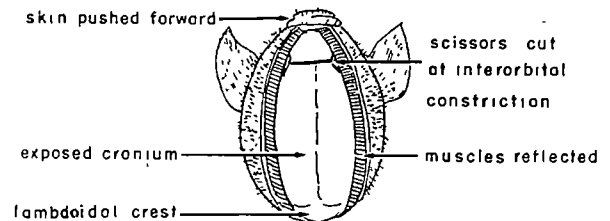


Fig. 2 Cranium exposed after longitudinal incision made along head midline

(W.H.O. Monogr. Ser. No. 23, 1954, and U.S. Public Health Service Pub. No. 568, 1957) frequently mutilate the skin and skull and not only make identification difficult but ruin the specimen for museum purposes.

While investigating bat ecology and rabies in Trinidad, we devised methods satisfactory to both mammalogist and virologist, removing brain tissue with little or no head damage. We found it unnecessary to open the calvaria, or brain case, either on very small bats or on those with specialized attachments between the ears, or unusual glandular structures on the top of the head, since sufficient brain tissue may be hypodermically withdrawn through a needle inserted into the foramen magnum without skull damage (Fig. 1). The optimum needle size can be determined with a little practice. It is sometimes necessary to agitate the needle or even inject a drop of physiological saline solution within the brain case to loosen the brain tissue and permit easier suction into the syringe. A needle with a dull tip is safer to use than a sharp one; for it makes it possible to probe the cranial interior without danger of penetrating the bone with the sharp point. In our experience, freshly killed warm specimens, or those that have been allowed to warm up after refrigeration, are easiest to manipulate.

If a study calls for the removal of the entire brain, our alternative method does damage the skull, but not necessarily beyond repair, if care is taken in the process and cutting instruments are kept sharp. The bat is held in the palm of the hand with its back exposed. With the ball of the thumb, the skin at the base of the head and close to the cervical and interscapular fossae is pushed forward over the top of the head toward the snout, and held taut. It may be necessary to limber the skin by moving it back and forth. Then as much skin as possible is moved forward and tightly held. With a sterile scalpel, a longitudinal incision is made along the midline of the head, beginning anteriorly just in front of the eyes and extending posteriorly to the base of the skull, cutting through the skin and fasciae, finally exposing the cranium. In some of the larger phyllostomid bats such as *Phyllostomus*, *Vampyrus* and *Artibeus*, it is necessary to dissect the temporal muscles away from both sides of the cranium and reflect them laterally before exposing the bone (Fig. 2). With sterile pointed-tipped scissors, preferably with curved blades, a transverse cut is made just above the eyes through the frontal area at the interorbital constriction. The bone of the skull is then incised on both sides, cutting posteriorly through the squamous portion of the temporal bone (taking care not to destroy the zygomatic arch) above the

auditory bulla, through the base of the parietal bone to the base of the lambdoidal crest (Fig. 3). Do not cut through the crest. The calvaria may be then lifted from the frontal cut and turned back, using the lambdoidal crest as a hinge, exposing the brain. With fresh sterile scissors, the brain may be severed from its site and lifted out entire, using the partially opened curved blades as a rest, and placed in a container (Fig. 4).

In order to assist the mammalogist in his task of identification, and to preserve a good museum specimen, it is important that the calvaria be carefully replaced along the incisions, the temporal muscles neatly returned to both sides of the cranium, and the integument gently eased backward over the head and returned to its original placement so that the initial incision lies close to or over the cervical or interscapular fossae. If necessary, this incision may be used to remove the brown adipose tissue which lies within the two fossae (Fig. 5).

Extreme caution should be taken by persons opening bat heads. Rubber gloves should be worn, and careful operative technique employed. Whether the procedure is carried out in the laboratory or in the field, sterile instru-

ments should be used to reduce the chances of bacterial contamination.

ARTHUR M. GREENHALL

Bird and Mammal Laboratories,
U.S. Fish and Wildlife Service,
Washington.

MICROBIOLOGY

Micro-organisms associated with Dead Insect Larvae in Nigeria

SPECIMENS of dead soil-inhabiting insect larvae (*Anomala* sp. and *Heteroligus* sp.) and noctuid larvae were supplied to me or collected by me for microbial isolations. Larvae of *Anomala* and noctuids were collected from fields on Moor Plantation, Ibadan, while yam-beetle larvae (*Heteroligus* sp.) were collected around Benin and Onitsha. *Anomala* and *Heteroligus* larvae were either dead on being dug up in the field or died while under storage in moist soil in the laboratory. Stored larvae were adequately provided with food (for example, pieces of yam) and aeration.

When a visible fungus growth appeared on the larvae, a pair of fine flame-sterilized forceps was used in picking out spores and/or hyphae and incubating them at 30° C on glucose-yeast extract agar (GYA) containing 2 g NaNO₃, 1 g KH₂PO₄, 0.5 g MgSO₄, 0.5 g NaCl, 5 g yeast extract (Difco), 10 g glucose, and 15 g agar in a litre of water. After picking out surface fungal propagules and in cases where no surface-growth was evident, the larvae were surface-sterilized with 0.1 per cent HgCl₂ in 70 per cent alcohol followed by thorough washing in sterile water¹. The larvae were then cut into small pieces under sterile conditions and incubated on GYA or nutrient agar at 30° C. The micro-organisms isolated, the source and the number of larvae from which they were isolated are shown in Table 1.

It might be expected that the micro-organisms would 'clear' chitin² (that is, cause a disappearance of finely ground chitin particles incorporated into an agar medium), since chitin makes up 20–30 per cent of the insect exoskeleton³. Only *Aspergillus giganteus* and the bacterium, however, cleared chitin after 12 and 13 days incubation respectively on chitin agar² at 30° C. Similar observations of the inability of insect-pathogenic fungi to clear chitin have been made with respect to *Beauveria*⁴. When a washed suspension of the bacterium was mixed with soil in which larvae of *Anomala* sp. were placed, as many larvae died each fortnight in the control as in the soil with the bacterium. When, however, the bacterium was introduced with a micro-injector through the integument into the body cavity of ten larvae, all ten died within 24 h, whereas all the controls (injected with equivalent

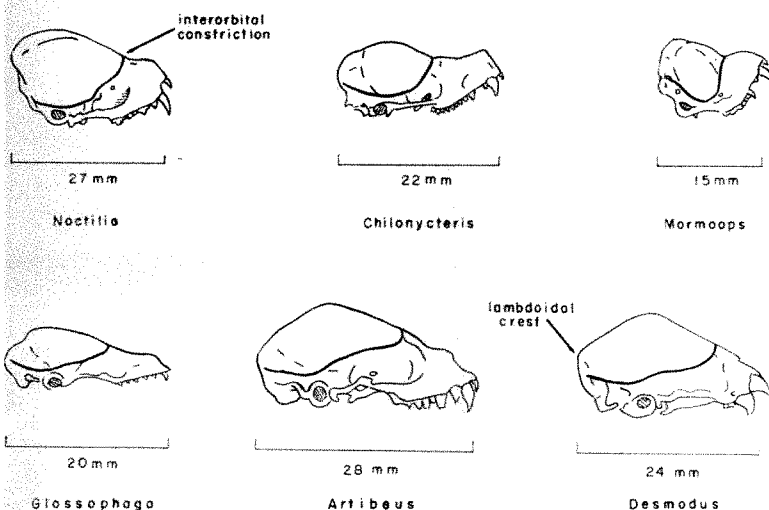


Fig. 3. Skulls of several neotropical bats showing line of cut along side of cranium

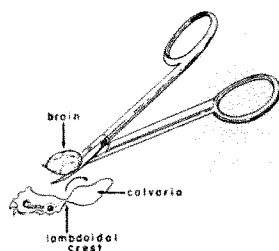


Fig. 4. Calvaria turned back and brain removed

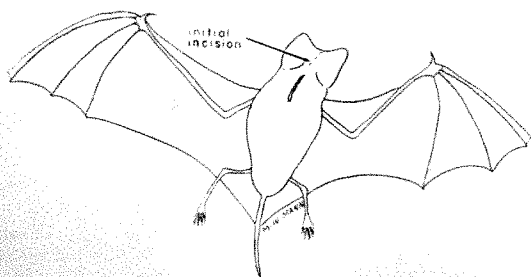


Fig. 5. With calvaria and integument replaced, initial head incision (see Fig. 2) now lies over cervical or interscapular fossae

Table 1. MICRO-ORGANISMS ISOLATED FROM DEAD INSECT LARVAE

Fungi	Source (larvae)	No. of specimens from which isolated
<i>Aspergillus awamori</i> Wehm. (IMI 112343)	<i>Anomala</i> sp.	8
<i>Fusarium solani</i> (Mart.) Sacc. (IMI 112344)	<i>Anomala</i> sp.	27
<i>Fusarium semitectum</i> Berk. and Rav. (IMI 112345)	<i>Anomala</i> sp.	30
<i>Geotrichum candidum</i> Link ex. Pers.	<i>Anomala</i> sp.	9
<i>Humicola fuscoatra</i> Traaen (IMI 112347)	<i>Anomala</i> sp.	6
<i>Metarrhizium anisopliae</i> (Metsch) Sorok (IMI 112348)	<i>Anomala</i> sp.	25
<i>Aspergillus giganteus</i> Wehm. (IMI 112341)	<i>Heteroligus</i> sp.	23
<i>Geotrichum candidum</i> Link ex. Pers.	<i>Heteroligus</i> sp.	18
? <i>Mortierella</i> sp. (IMI 112349)	<i>Heteroligus</i> sp.	15
<i>Aspergillus sclerotiorum</i> Huker (IMI 112342)	Noctuid	20
Bacterium		
<i>Arthrobacter</i> sp. (NCIB 973C). (Gram-variable, branched, non-motile rod, breaking-up into cocci in 2–7 days.)	<i>Anomala</i> sp.	17

volumes of sterile distilled water) survived. Similar results were obtained when the experiment was repeated. Insects were kept in moist soil in the dark at room temperature during the tests. The bacterial inoculum was prepared by scraping off bacteria from a 6-day slope, and making it up in 10 ml. of sterile distilled water; 0.005 ml. of the suspension was used.

Except for the bacterium, no valid suggestion can be made that the micro-organisms recorded in this experiment were responsible for the death of the larvae, although some of the fungi (for example, *Metarrhizium*) may have been pathogenic, symbiotic or saprophytic in or on the living insect; they may, on the other hand, have developed as saprophytes on larvae which have died from other causes.

The value of this communication appears to me to lie in the reporting of some of the fungi for the first time in Nigeria, in the unusual ecological niches provided for the micro-organisms by the larvae and in the possibility that some of the isolates may prove useful as agents in the biological control of insect pests.

I thank Dr. M. L. Jerath and Mr. S. A. Adeyemi for providing respectively the soil larvae and some of the noctuid larvae, Miss Evelyn Page for assistance, and the staff of the National Collection of Industrial Bacteria for examining the bacterium.

N. OKAFOR*

Federal Department of Agricultural Research,
Moor Plantation, Ibadan, Nigeria.

* Present address: Department of Microbiology, University of Nigeria, Nsukka.

¹ Steinhilber, E. A., *Principles of Insect Pathology* (McGraw-Hill Book Co. Inc., New York, 1949).

² Okafor, M. N., Ph.D. thesis, Univ. Cambridge (1964).

³ Wigglesworth, V. B., *Biol. Rev.*, **23**, 408 (1948).

⁴ Gray, T. R. G., and Bell, T. F., in *Soil Organisms*, edit. by Doeksen, J., and van der Drift, J. (Amsterdam, 1963).

A Rapid Screening Test for Transfer Factors in Drug-sensitive Enterobacteriaceae

In a recent article¹ a technique was described for screening drug-sensitive strains of Enterobacteriaceae for the presence of transfer factors. This entails the incorporation of each strain under test in a mixture consisting of an intermediate recipient, containing a non-transferring resistance determinant (*R*-determinant)^{1,2} and a final recipient which is drug-sensitive and devoid of a transfer factor. If a test strain carries a transfer factor, this migrates into the intermediate recipient and unites with the *R*-determinant to form an *R*-factor which then transfers into the final recipient.

The intermediate strain used in these tests was *Salmonella typhimurium* type 36 carrying *S'*, an *R*-determinant for streptomycin resistance, but unable to transfer it because of the absence of the transfer factor Δ with which the *R*-determinant was originally associated¹; this strain is referred to as *36S'* Δ -. The final recipient was *Escherichia coli* K12F-. The test strains and the intermediate and final recipients were inoculated separately into nutrient broth, and incubated with agitation for 5-6 h at 37° C. 3 ml. of the culture of each test strain was then mixed with an equal volume of *36S'* Δ - and incubated without agitation for 2 h at 37° C. This provided any transfer factor present in the test strains with an opportunity to transfer to *36S'* Δ - to form a streptomycin *R*-factor. 3 ml. of the K12F- broth culture was then added and the mating mixtures were incubated overnight at 37° C. The following morning, they were streaked in decimal dilutions on streptomycin-MacConkey agar plates on which 0.3 ml. of 01 phage (titre > 10¹¹ particles/ml.) had been spread. These plates were incubated overnight at 37° C. The drug-sensitive donor strains were eliminated by the streptomycin in the medium, and the intermediate *S. typhimurium* recipient by the 01 phage. Thus, only K12F- cells that had

received mobilized streptomycin resistance could grow freely, to produce red, lactose-fermenting colonies.

This screening test may be speeded up by spotting the undiluted mating mixtures, with a loop delivering c. 0.01 ml., on streptomycin-MacConkey agar on which phage 01 has been spread. After incubation, the presence of a transfer factor in a test strain is shown by a growth of lactose-fermenting colonies of K12F- which has received the newly formed streptomycin *R*-factor. A number of drug-sensitive strains may be screened for transfer factors on a single plate in this way. The mating mixtures are stored at 4° C until the results of the spot test are read. Those containing drug-sensitive donor strains giving positive results are then spread in decimal dilutions on streptomycin-MacConkey agar as described earlier, for more precise examination and clonal selection of K12 colonies that have received streptomycin *R*-factors. The characteristics of the respective transfer factors may then be investigated further.

Fig. 1 shows a plate on which a spot screening test for transfer factors has been performed on wild, drug-sensitive strains of *S. typhimurium*.

The contents of the various sectors of the plate shown in Fig. 1 are as follows (phage-types of wild *S. typhimurium* are referred to as types, and K12F- as K12): (1) Type 29 \times *36S'* Δ - \times K12. (2) Type 29 \times *36S'* Δ - \times K12. (3) Type 44 \times *36S'* Δ - \times K12. (4) Type 29 \times *36S'* Δ - \times K12. (5) Type 29 \times *36S'* Δ - \times K12. (6) Type U194 \times *36S'* Δ - \times K12. (7) *36S'* Δ - \times *36S'* Δ - \times K12 (= positive control). (8) *36S'* Δ - \times K12 (= negative control). (9) K12 alone (= negative control).

All the wild strains of *S. typhimurium* were of independent origin and were isolated in 1965.

Mating mixtures 1, 5, 6 and 7 show positive results, that is, the conversion of K12 to streptomycin resistance by transfer of a newly formed streptomycin *R*-factor. The positive control contained as the donor a strain of phage-type 36 of *S. typhimurium* into which the Δ transfer factor alone had been introduced¹. As the test spots receive the undiluted mating mixtures, colonies of the intermediate *S. typhimurium* recipient resistant to phage 01 usually appear. However, when transfer has occurred, the red, lactose-fermenting K12 colonies are easily distinguishable from the yellow, non-lactose-fermenting *S. typhimurium* colonies as Fig. 1 shows.

Controls were also set up of each wild donor strain crossed with K12, to exclude the possibility that streptomycin *R*-factors might be phenotypically masked in the

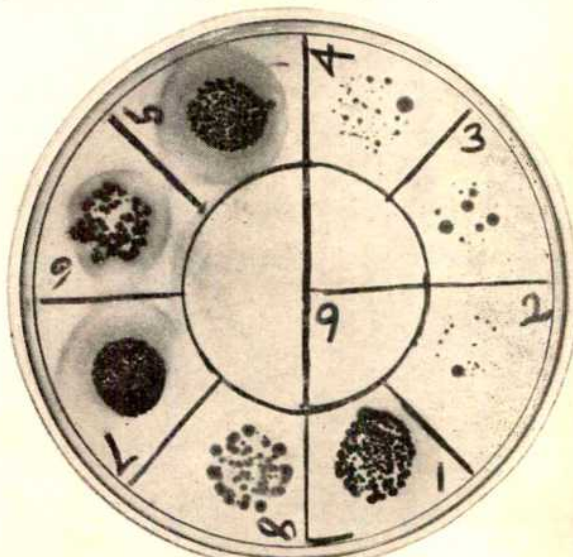


Fig. 1. Streptomycin-MacConkey agar plate showing the presence of transfer factors in wild, drug-sensitive strains of *S. typhimurium*. The strains represented in sectors 1, 5 and 6 carry transfer factors. Sector 7 contains the positive control (\times c. 0.8).

drug-sensitive donors; these controls were uniformly negative.

The origins of the wild drug-sensitive *S. typhimurium* strains carrying *R*-factors were as follows: (1) phage-type 29; isolated from a turkey (Mundford, Norfolk); (5) phage-type 29; isolated from a chick (Gloucester); (6) phage-type U194; isolated from man (Oldham, Lancashire).

Our tests show that transfer factors unattached to *R*-determinants may be widely distributed in the Enterobacteriaceae. If such factors gain access to strains carrying non-transferring *R*-determinants to which they can become attached, transfer of drug resistance is initiated. This may be the process by which *R*-factors are initially formed. The presence of antibiotics and synthetic antibacterial drugs in the animal hosts parasitized by Enterobacteriaceae possessing these potentialities may accelerate both the initiation and the spread of this type of resistance.

E. S. ANDERSON

Enteric Reference Laboratory,
Public Health Laboratory Service,
Colindale Avenue, London, N.W.9.

¹ Anderson, E. S., and Lewis, M. J., *Nature*, **208**, 843 (1965).

² Anderson, E. S., and Lewis, M. J., *Nature*, **206**, 579 (1965).

Effect of Incubation Temperature on the Flagellation of Gram-negative Rods

THE primary factor used in *Bergey's Manual of Determinative Bacteriology*¹ for the division of Gram-negative rods into families is the flagellation of the cells. The method has always proved a source of difficulty and has led to much confusion as to the taxonomic position of cultures. This point was well illustrated by Dye², who produced micrographs of cells of a culture of *Xanthomonas trifolii* in which polar or lateral monotrichous and peritrichous flagellation could all be demonstrated.

Coetzee and de Klerk³ have shown incubation temperature to be a factor that could affect the type of flagellation of *Proteus* and *Providencia* cultures. These authors used incubation temperatures of 43°, 37° and 30° C. When re-defining mono- and lophotrichous in terms of a flagella index, Lautrop and Jessen⁴ arrived at their conclusions after examining a collection of pseudomonad cultures, mainly *Pseudomonas aeruginosa*, which were grown at 'room temperature', at 30° or at 10° C. Although they recommended that cultures with intermediate flagella indices should be studied after growth at 10° C, Lautrop and Jessen did not emphasize the influence of incubation temperature on flagellation.

While attempting to classify a collection of 100 cultures of Gram-negative rods which had been isolated from milk, flagella preparations were made from cultures which had been incubated on Oxoid 'Lab-lemco' agar slopes at 30° C. These cultures were found to be lopho-, monotrichous or aflagellate. A number of polar or sub-polar monotrichous cultures were encountered that from their biochemical reactions appeared to belong to the Enterobacteriaceae. The cultures were, therefore, examined for flagellation after incubation at 30°, 22° and 15° C and a few cultures also at 5° C.

The results showed that the use of only one incubation temperature could give rise to errors in assessing both the numbers of flagella and the type of flagellation. Among the pseudomonad group, the non-pigmented culture 369 showed monotrichous polar flagellation at all four incubation temperatures, culture 149 (*Pseudomonas fluorescens* type) was predominantly lophotrichous only at 15° C, while the biochemically similar culture 131 showed lophotrichous cells only at 22° C. Culture 143, a biochemically rather inactive pseudomonad, gave the same proportions of mono- and lophotrichous cells at the four incubation temperatures as did culture 149.

Cultures 119 and 533, which oxidized glucose and gave a positive oxidase reaction, both showed polar monotrichous flagellation at 30° C but peritrichous flagellation at 22° C. The same flagellation effect was shown by culture 466, which failed to ferment glucose and was oxidase-negative.

In the same way, apparently similar fermentative, oxidase-negative cultures showed different types of flagellation after growth at different incubation temperatures. Culture 460, which was monotrichous at 30° C, was peritrichous at 22°, while culture 373 became peritrichous at 15° C. Another fermentative culture (495), which was aflagellate at 30° C, showed predominantly monoflagellate cells at 22° and 15° C but was peritrichous at 5° C. This latter culture showed motility after incubation at 30° C on soft nutrient agar (0.2 per cent agar).

If flagellation is to be retained as the main criterion for the classification of Gram-negative rods, then more emphasis should be placed on the examination of cultures grown under varying conditions before assigning a taxonomic position based on flagellation.

DOROTHY A. ORR

MARGARET M. TAYLOR

Hannah Dairy Research Institute, Ayr.

¹ *Bergey's Manual of Determinative Bacteriology*, seventh ed. (Baillière, Tindall and Cox Ltd., London, 1957).

² Dye, D. W., *N.Z. J. Sci.*, **7**, 261 (1964).

³ Coetzee, J. N., and de Klerk, H. C., *Nature*, **202**, 211 (1964).

⁴ Lautrop, H., and Jessen, O., *Acta Path. Microbiol. Scand.*, **60**, 588 (1964).

VIROLOGY

Mechanical Transmission of Hop Mosaic Virus

HOP MOSAIC VIRUS (HMV), the cause of a severe disease of the Golding hop varieties, was shown by Paine¹ to be transmitted by the winged form of the damson-hop aphid, *Phorodon humuli* Schr., but transmission by sap extracts has not been reported. This has been accomplished with both concentrated and crude extracts of leaves from plants of sensitive and tolerant hop varieties, by inoculating single-node hop cuttings a few days after removal from the mist chamber where they had formed roots; similar cuttings were used as test plants throughout this investigation. 30-g young leaves from recently infected Golding hop plants were macerated in 150 ml. 0.05 M phosphate buffer, pH 8.3. The sap was squeezed through cotton cloth and clarified by centrifugation for 10 min at 10,000g. The supernatant was centrifuged for 180 min at 70,000g (Spinco L No. 40 rotor at 32,500 r.p.m.). The pellets were resuspended in 3 ml. distilled water and, after the addition of 'Celite', the suspension was used to inoculate young leaves on twenty cuttings of a Golding hop, nine of which became infected.

Later, HMV was transmitted from crude extracts of young hop leaves. Leaf tissue was macerated in phosphate buffer (5 ml. per g); the extract, separated from the fibrous residue by squeezing through cotton cloth and after the addition of 'Carborundum' (400 mesh), was rubbed on leaves of Golding hop, *Chenopodium quinoa*, *Nicotiana clevelandii*, *N. tabacum* var. White Burley and *N. glutinosa*. Three of five inoculated hop plants were infected with mosaic virus. Pale green spots and mottle symptoms developed on some of the inoculated leaves of the *Nicotiana* spp. and *C. quinoa* plants, one of which also developed pale green vein-banding and leaf distortion. However, HMV was recovered only from the *N. clevelandii* plants, all eight of which developed in 5-9 days pale green spots (1-3 mm diameter) with necrotic centres on the inoculated leaves. These plants gradually deteriorated, their leaves became chlorotic and necrotic and many of their roots died. Eighteen days after inoculation leaves from pairs of *N. clevelandii* plants were bulked, macerated in an equal volume of phosphate buffer and sap extracts from each pair inoculated to two Golding hop plants; one extract

Table 1. ISOLATION OF HOP MOSAIC VIRUS FROM *N. clelandii*

Source leaves of <i>N. clelandii</i>		Test plants infected/inoculated	
		Golding hop	<i>N. clelandii</i>
Inoculated	With symptoms	5/6	2/2
	Without symptoms	0/14	0/7
Uninoculated	With symptoms	3/4	4/4
	From plants without systemic symptoms	2/4	4/4

infected both test plants and each of the other three inocula infected one.

The distribution of HMV in *N. clelandii* was examined in ten plants that were inoculated with crude extract from Golding hop leaves. All plants developed primary lesions on some inoculated leaves; systemic symptoms in the form of chlorotic spotting occurred on the later-formed leaves of two plants. Inoculated leaves were harvested after 7 days and uninoculated younger leaves after 6 weeks. Leaves with and without symptoms were macerated separately and the extracts used to inoculate Golding hop and *N. clelandii* plants. HMV was transmitted from inoculated leaves with symptoms whether or not the plant developed systemic symptoms, but not from symptomless inoculated leaves (Table 1).

J. T. LEGG

East Malling Research Station,
Maidstone, Kent.

¹ Paine, J., *Rep. E. Malling Res. Sta.*, 120 (1952).

CYTOLOGY

Cytoplasmic Labelling with Uridine-5-³H in Human Lymphocytes cultured with Phytohaemagglutinin

Hayhoe and Quaglino¹ have reported investigations of RNA metabolism in normal human leucocytes when cultured with phytohaemagglutinin, using uridine-5-³H as a specific precursor of RNA. They found little evidence of cytoplasmic labelling after terminal incubation for 1 h with tritiated uridine (0.5 μ c./ml.), either at 24, 48 or 72 h of culture.

We have been conducting similar investigations with this nucleoside specific activity; 22.4 c./mmole added at a somewhat higher dosage (5.0 μ c./ml.), followed by mixing for 2 min on a cell suspension mixer, and incubation at 37° C for 1 h immediately preceding termination of the culture. Smears were made of the centrifuged cell deposit. Slides were coated with Eastman 'Kodak NTB 3' emulsion, exposed for 7 days and stained through the fixed emulsion with a modified MacNeal's tetrachrome². At the dosage of tritiated uridine used, distinction between nuclear and cytoplasmic labelling is difficult if the nuclear-cytoplasmic (*N:C*) ratio is high, especially when heavy labelling is present. However, there are two types of transforming lymphocyte equally distributed in these cultures³. They make their appearance in increasing numbers during the first 24 h before any evidence of DNA synthesis occurs³. The first of these types, the transitional lymphocyte, has a very high *N:C* ratio, with a leptochromatic nucleus occupying almost all the cell (Fig. 1*a*), and in this cell it is difficult to be sure whether labelling is cytoplasmic or not. The other type, the so-called atypical transitional lymphocyte (Fig. 1*b*), has a much lower *N:C* ratio, and, in our experience, it is only this cell which really provides suitable material for the investigation of cytoplasmic labelling in its earliest stages.

Immediately after setting up the culture, there was nuclear labelling, but virtually no evidence of cytoplasmic labelling in lymphocytes at 0 h, that is after incubating for 1 h with tritiated uridine (Fig. 2, *a* and *b*). At 3 and 6 h there was a significant increase in the proportion of labelled cells and their mean grain counts, but the labelling was still confined to the nucleus in the overwhelming majority of cells. At 12 h there was weak cytoplasmic labelling, but from 18 h onwards this was more marked (Fig. 3, *a* and *b*), together with further progressive increases

both in the proportion of labelled cells and their mean grain counts.

At 48 h there was also significant cytoplasmic labelling of blast cells after 1 h terminal incubation with uridine-5-³H. Here again blast cells with a high *N:C* ratio were

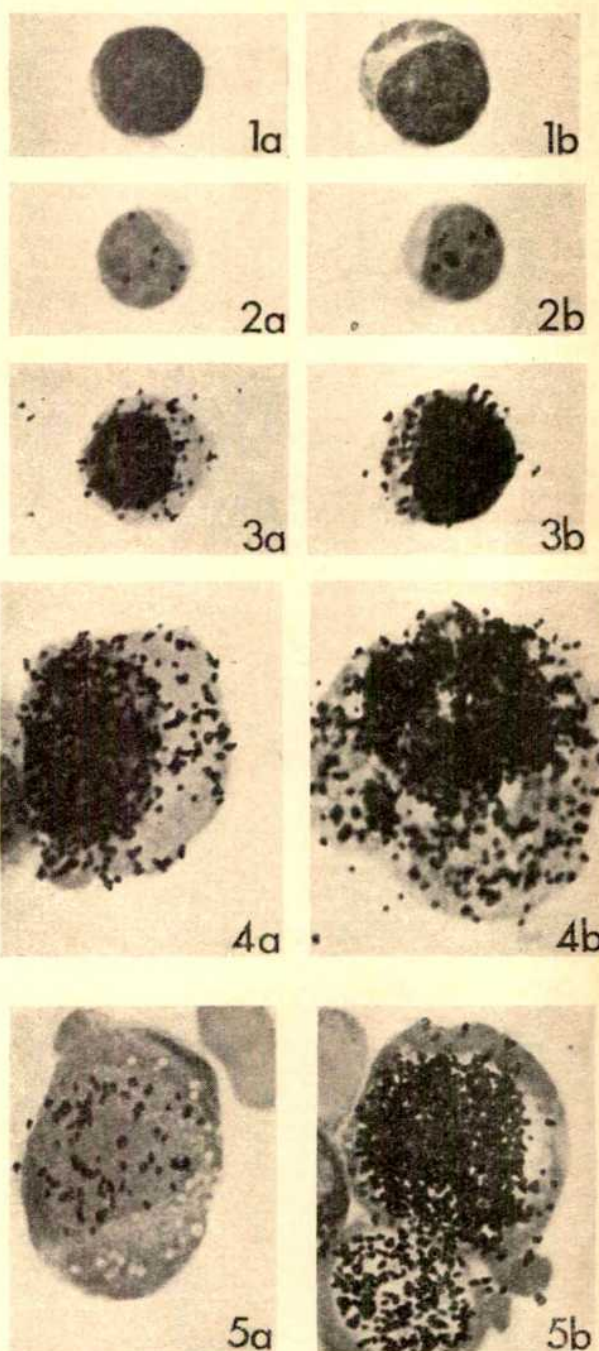


Fig. 1. 24-h culture. *a*, Transitional lymphocyte with very high *N:C* ratio ($\times 1,500$). *b*, Atypical transitional lymphocyte with medium *N:C* ratio ($\times 1,500$).

Fig. 2. 0-h culture. *a* and *b*, Medium-sized lymphocytes showing nuclear labelling only (³H-uridine added for 1 h—see text) ($\times 1,500$).

Fig. 3. 24-h culture. *a* and *b*, Atypical transitional lymphocytes showing nuclear and cytoplasmic labelling (³H-uridine added for 1 h at term—see text) ($\times 1,500$).

Fig. 4. 48-h culture. *a* and *b*, Blast cells with low *N:C* ratio showing nuclear and cytoplasmic labelling (³H-uridine added for 1 h at term—see text) ($\times 1,500$).

Fig. 5. 48-h culture. *a*, Blast cell showing nuclear labelling only (³H-uridine added immediately prior to the making of smears—see text) ($\times 1,500$). *b*, Blast cell showing nuclear and cytoplasmic labelling. Large atypical transitional lymphocyte (below) showing nuclear labelling only (³H-uridine added for 15 min at term—see text) ($\times 1,500$).

difficult to interpret, but in blast cells with a low $N:C$ ratio³ one invariably found cytoplasmic labelling markedly in excess of background labelling (Fig. 4, *a* and *b*).

In 48-h cultures when smears were made as soon as possible after the addition of uridine-5-³H to the cell suspension without further incubation, nuclear labelling only was observed both in atypical transitionals and blast cells (Fig. 5*a*). The time between the addition of tritiated uridine and preparation of the smear was of the order of 7–8 min. If the suspension was incubated for 7.5 min after the addition of this nucleoside, similar results were obtained, but after incubation for 15 min cytoplasmic labelling was present in some cells, though the number of cells and intensity of labelling were appreciably less than with the usual period of 60 min (Fig. 5*b*).

Harris⁴ has shown that in the nuclei of mammalian cells there are mechanisms both for the degradation of much of the rapidly labelled RNA, and also for the production of a more stable form of this compound. He has suggested that there may be overproduction of the more rapidly labelled RNA, a fraction of which is used to produce more stable RNA and the remainder degraded. Whether this cytoplasmic labelling represents a relatively rapid transfer of the more stable nuclear RNA to cytoplasm, once the process of cell growth initiated by PHA is under way, or whether it represents diffusion of acid-soluble end products outwards from the nucleus⁴ or both, remains uncertain.

We thank Mr. A. K. Sykes for taking the photomicrographs and Mr. C. L. Chen for technical assistance. One of us (G. C. B. W.) thanks the K. G. Laurence Group of Limited Companies for generous supplementary financial assistance.

G. C. B. WINTER
J. M. YOFFEY

Department of Anatomy,
The Medical School,
University of Bristol.

¹ Hayhoe, F. G. J., and Quaglino, D., *Nature*, **205**, 151 (1965).

² MacNeal, W. J., *J. Amer. Med. Assoc.*, **78**, 1122 (1922).

³ Yoffey, J. M., Winter, G. C. B., Osmond, D. G., and Meek, E. S., *Brit. J. Haematol.*, **11**, 488 (1965).

⁴ Harris, H., *Nature*, **201**, 863 (1964).

GENETICS

Differential Deoxyribonucleic Acid Replication in the Giant Foot-pad Cells of *Sarcophaga bullata*

GIANT polytene chromosomes from the salivary glands of various fly larvae have been extensively examined; because of their enormous size they have become one of the most important tools in investigations of gene activity and nuclear-cytoplasmic relationships. Malpighian tubules and certain other tissues of flies also possess polytene chromosomes, but they show a lower degree of polyteny. It is generally accepted that in most animal and plant cells there is a constant relation between the DNA content and the number of chromosome 'sets' it contains. Synthesis of DNA results in a doubling of this pre-existing amount; a dipteran polytene chromosome may result from more than a thousand such replications¹. Recently it has been suggested² that differentiation in a multicellular organism involves the progressive inactivation of DNA, and that, in the polytene chromosome, three conditions characterize these inactivated regions: first, inactivity with respect to RNA synthesis; secondly, late replication, replication being slower and continuing longer than over the rest of the chromosome; thirdly, the appearance of dense packing. Heavy bands and heterochromatic regions are given as typical examples of such regions.

Recently, giant cells containing giant polytene chromosomes have been described for the pupal foot-pads of developing adult flies^{3,4}. Continued work with this

material, particularly with the fly *Sarcophaga bullata*, shows that, whereas the highly polytene condition of the giant chromosomes is reached by DNA replication along the whole chromosome length, as typically occurs in salivary gland chromosomes, there is also seen here striking differential replication of specific chromosomal regions, at specific times, resulting in the 'budding off' of dozens of 'granules', each containing DNA. The granules lie between the chromosomes and the nuclear membrane, most commonly immediately within the nuclear membrane.

Two of these giant cells occur to each foot-pad. In the first-day pupa (reared at 25° C) the cells are little larger than those surrounding tenent hair cells⁴ that have just completed mitosis (cf also Figs. 1*A* and 1*B*). In a two-day pupa the nucleus of the future giant cell is still very small in comparison with the whole developing foot-pad, whereas in a four-day pupa the nucleus is approximately 70 μ in diameter, and the cell itself extends over half (the second cell covers the other half) of the entire dorsal surface of the pad. This extremely rapid growth in a relatively short time is accompanied by correspondingly rapid DNA synthesis resulting in the very highly polytene, distinctly banded chromosomes (Fig. 1*A*). Throughout the growth phase the nucleolus is very large relative to the size of the nucleus, but in the mature cell the greater growth of the chromosomes results in a proportionately smaller nucleolus. It contains strands of DNA, but these occur in the core of the nucleolus and do not extend to, and beyond, the periphery (Fig. 2*E*). The DNA granules are not associated with the nucleolus. A micro-nucleolus is also present in the growing cells (located at the position *X* on chromosome *B* Fig. 1). It is not present in this day-7 preparation, as it disappears in a late day-4 pupa, by which time growth has ceased. Characteristic puffing occurs in the chromosomes during growth and at the same time as DNA replication. These puffs are very probably involved in the growth processes. However, it is immediately before and during the extensive secretion and the darkening of the adult cuticle that the most striking changes in the puffing sequences occur. Fig. 2*A* is of a whole nucleus contained within the foot of a ten-day pupa in which darkening of the cuticle is proceeding. The two puffs seen on the nucleolus-bearing chromosome (*A'*, Fig. 1—at arrows—does not have these puffs in the day-7 pupa) characteristically appear only at this time of cuticle darkening. A very large puff is also present (slightly out of the plane of focus) at the third arrow. This also is characteristically large at this stage of development (puffs are present along this chromosome: *C'*, Fig. 1, but at day 7 the very large puff is not present).

Such chromosomal RNA puffs are much-studied features of dipteran salivary gland chromosomes. DNA puffs, on the other hand, are recorded from relatively few forms, for example, in the sciarids, *Rhynchosciara* and *Sciara coprophila*⁵, and in the chironomid *Glyptotendipes*⁶. In these cuticle-secreting cells of *Sarcophaga*, the shorter arm of the nucleolus bearing chromosome (Fig. 1: *A'*) possesses a very characteristic DNA puff. Throughout the time of cuticle secretion this has a highly granular appearance, but is most noticeably puffed in a day 7–8 pupa (Fig. 2*C*).

The chromosomes in the fully grown cell, such as that shown in Fig. 2*A*, are aligned, very strikingly, immediately within the nuclear membrane. They present a beautiful appearance, being so arranged that at no point do two chromosomes or two chromosome regions overlap, with the result that each and every chromosome band lies at right angles and close to the nuclear membrane. They are connected to the nuclear membrane by numerous strands, clearly seen in isolated nuclei, gently rolled between slide and coverslip and viewed with phase optics (similar attachment to the nuclear membrane has been described for the salivary gland of *Chironomus dorsalis* by Kimoto⁷).

At all stages of cuticle secretion and darkening the DNA granules are present, and their distribution can be determined even in whole nuclei. Particular groups of granules arise from the chromosomes at specific developmental stages. At these times the size and appearance of the granules are similar to those of the granules of the chromosomal region from which they appear to be 'budding off'. The granules maintain contact with one another, with the chromosomes, and with the nuclear membrane, by strands which can be seen with RNA staining procedures. They do not appear to be connected by DNA strands, although these could be present, but beyond the resolution of the light microscope. The granules show up with equal clarity in Feulgen/fast green preparations, in azure B at pH 4, in orcein/light green, and in methyl green/pyronin preparations; they are digested with DNase. Each granule is surrounded by a peripheral region the staining reaction of which is similar to that of the many conspicuous chromosomal RNA puffs. In many instances the region of the chromosome from which the granules arise is unexpanded; in others, the regions of the centromeres are involved. The DNA puff on the nucleolus-bearing chromosome is also suspected of having given rise to granules which have been found to lie between it and the nuclear membrane, as is seen in Fig. 2D. Likewise a puff occurring near the end of the longer arm of the longest chromosome (E'', Fig. 1), in a stage 3-4 pupa, appears as a DNA puff, and at this stage numerous granules are in close proximity, so that it, too, is suspected of being involved in the replication of DNA granules.

This puff, in Fig. 1 (arrow), which is of a later day-7 pupa, has resumed a normal banded appearance.

The DNA granules are of relatively large dimension. For comparison of size, Figs. 1A and B show a group of about five granules at the lower left of A which approach the overall dimensions of the whole chromosomes of B. The invariable presence of the granules, their origin at different times and their location immediately within the nuclear membrane tend to suggest that they are performing some function vital to the cell's activity. That such granules are present in these cells and not apparently in the better-known salivary gland cells may find an explanation in some developmental differences. Whereas a salivary gland cell grows slowly and takes several instars to attain maximum size, growth here is extremely rapid, and increase in size is tremendous. Furthermore, the cell is called into action to produce cuticle when seemingly immediately cell growth has ceased. Also, the volume of secretion is large and produced at a rapid rate, which presumably means that turnover must be rapid. It would seem that the ribosomal elements involved in the formation of mucoproteins and other cuticular elements are required in very large numbers within a short time-span. It is not impossible that these extra-chromosomal DNA granules may be performing the function of 'sub-factories' (of the specific gene loci from which they have arisen) for the production of messenger RNA, a need which may not otherwise be met, even by the extensive DNA replication resulting in the high degree of polyteny attained along the rest of the chromosome complement.

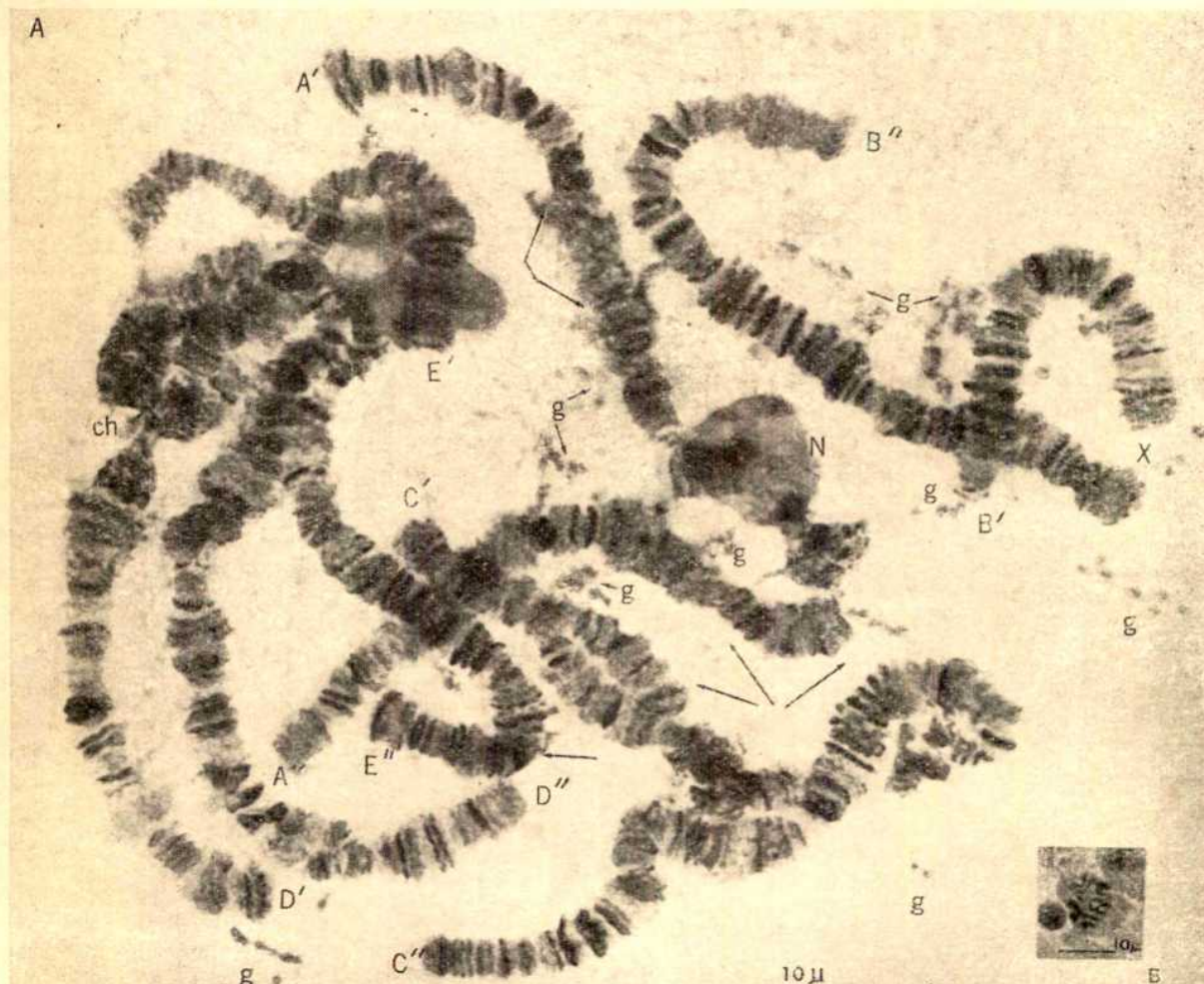


Fig. 1. A, Chromosome squash of giant foot-pad cell nucleus from a day-7 pupa of *Sarcophaga bullata*, reared at 25° C. ch, Chromocentre; g, DNA granules; N, nucleolus; X, position of micro-nucleolus at earlier stage. Arrows: position of puffs referred to in text. A' A'', B' B'', C' C'', D' D'', E' E'': ends of short and long arms respectively of chromosomes. Stained: orcein/light green. B, Metaphase chromosome squash from testis of *Sarcophaga* for comparison of chromosome size with A. Lacto-acetic-orcein squash

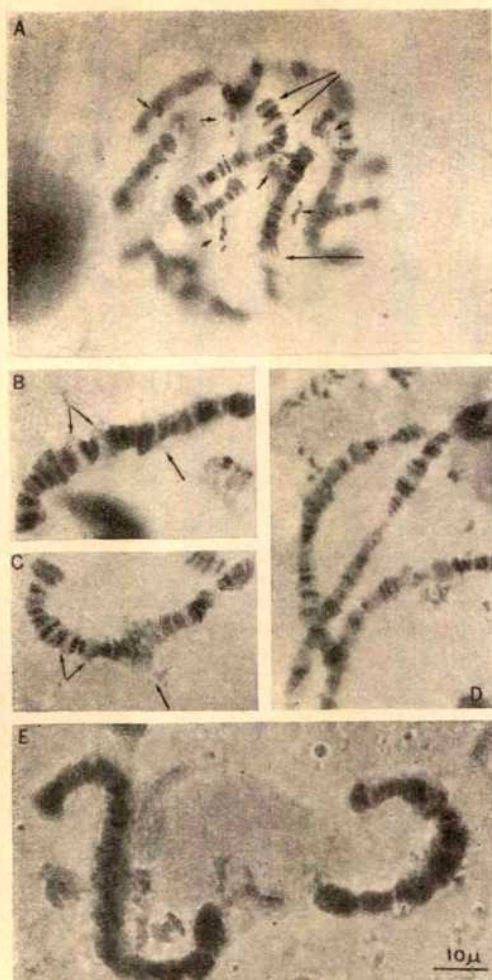


Fig. 2. A, Whole nucleus from a day-10 pupa of *Sarcophaga*, Feulgen stained. The chromosomes are aligned immediately within the nuclear membrane, and this micrograph is focused on those chromosomes lining the upper border of the spherical nucleus. Note the DNA granules at the shorter arrows, and the chromosomal puffs at the longer arrows (referred to more specifically in text). B, C, Two views of the shorter arm of the nucleolus-bearing chromosome. In B the region at the single arrow is granular, but not puffed; day-5 pupa. In C the same region is distinctly puffed; day-8 pupa. Feulgen/fast green. D, Chromosome squash of a day-8 foot showing granules of DNA lining the inner border of the nuclear membrane (top left), and near the DNA puff on the shorter arm of the nucleolus bearing chromosome. Feulgen/fast green, lightly squashed. E, Lacto-acetic-orcein squash to show the distribution of DNA within the nucleolus; focus is on the nucleolus and not the chromosome.

It has been suggested² that the presence of early and late replicating DNA along the same polytene chromosome is "indicative of the presence of multiple sub-units arranged tandemly in the metazoan chromosomes". The present example of DNA synthesis at specific locations would lend additional support to this suggestion. Here, however, it would seem that it is not only a question of late or early replication but of repeated 'extra' replication occurring at certain regions along the chromosomes. Since the number, position of origin, and hence the composition of these particular granules is likely to be specific to these cells, they would seem, at first sight, to be visible evidence of seemingly irreversible nuclear differentiation.

The exact function of these granules in relation to other chromosomal activities, such as that of the nucleolus, of the micro-nucleolus, of the DNA puffs and of the complex sequences of RNA puffs, pose some new and intriguing questions. Kaufman and Gay⁶ described, at the electron microscope level, 'extra chromosomal' material, associated with the phenomenon of nuclear-membrane 'blebbing', in *Drosophila*. Gay also described⁹ DNA as 'secreted' by certain bands of the late third instar salivary gland chromosome of *Drosophila*. Extra-chromosomal DNA has

also been described by Beyreuther¹⁰ during oogenesis in Tipulidae, where it is produced from heterochromatic regions of the chromosomes. These observations and the findings recorded here may be of comparable phenomena; the very considerable size and large numbers of the present granules make the foot-pads particularly suited for further investigation at both light- and electron-microscope levels. Work is continuing using histochemical, autoradiographic and electron-microscope techniques. Details of the general development of the feet, of the banding pattern of each of the chromosomes, and the striking puffing sequences at the various developmental stages are being given elsewhere.

This work has been supported by a grant from the National Science Foundation.

JOAN M. WHITTEN

Department of Biological Sciences,
Northwestern University,
Evanston, Illinois.

¹ Swift, H., in *The Molecular Control of Cellular Activity*, 73 (McGraw-Hill, New York, 1962).

² Fujita, S., *Nature*, **206**, 1742 (1965).

³ Whitten, J. M., *Proc. Sixteenth Intern. Zool. Congress, Washington*, 276 (1963).

⁴ Whitten, J. M., *Science*, **143**, 1437 (1964).

⁵ Breuer, M. E. and Pavan, C., *Chromosoma*, **7**, 371 (1955).

⁶ Stich, H. F., and Naylor, J. M., *Exp. Cell. Res.*, **14**, 442 (1958).

⁷ Kimoto, Y., *Cytologia*, **23**, 478 (1958).

⁸ Kaufman, B. P., and Gay, H., *The Nucleus*, **1**, 57 (1958).

⁹ Gay, H., *Cold Spring Harb. Symp. Quant. Biol.*, **21**, 257 (1956).

¹⁰ Beyreuther, K., *Chromosoma*, **7**, 508 (1956).

PSYCHOLOGY

Behavioural Deficit in the Rat induced by feeding Phenylalanine

PHENYLKETONURIA, high serum levels of phenylalanine and its metabolites, and elevated levels of free phenylalanine in the milk can be induced in pregnant and lactating animals by the addition of phenylalanine to the diet¹. The effects on the learned behaviour of offspring born and raised by mothers on such diets are of interest because the rate of incorporation of phenylalanine into brain tissue is higher before weaning than later^{2,3}. High levels of phenylalanine produce behavioural impairment in mice only if fed prior to weaning⁴. Furthermore, inability to metabolize phenylalanine normally may have no effect on the intelligence of human phenylketonurics if dietary treatment allows early normal mental development⁵. High levels of phenylalanine may produce behavioural impairment only when present during an early critical period of development.

This report describes a behavioural deficit due to adding 7 per cent D,L-phenylalanine (Phe) to the maternal diet of rats prior to weaning of their pups. In addition, we report evidence suggesting partial attenuation of the observed deficit by maintenance on normal diets after weaning.

Seven pregnant Holtzman rats were placed on special diets 2 days before parturition. A basal diet of Rockland ground mouse chow contained 7 per cent Phe for four of these; the basal diet for the remainder contained 7 per cent 'CellufLOUR' (C). Live pups were born to all mothers. The pups were left with their mothers until weaning at the age of 21 days. Seventeen pups from litters of the mothers on C and seventeen pups from the litters of the mothers on Phe had survived. After weaning, nine randomly chosen pups from each group of seventeen were fed the Phe diet and eight from each group were fed the C diet. Three pups from the maternal Phe group that had been placed on post-weaning C diet died prior to behavioural testing.

At 10 weeks of age all pups, after 24 h of water deprivation, were trained in an LVE 1316 operant behaviour chamber (Lehigh Valley Electronics) to approach a dipper to obtain a drop of water. Afterwards, they were deprived

of water once each week for the 24 h preceding behavioural testing in the chamber.

During a second 1-h session, each pup was trained to depress a small lever in order to obtain each drop of water. During the third session only the first five responses were reinforced with water, and all subsequent responses during 1 h were unreinforced. After this exposure to extinction, all pups were given three weekly 0.5-h sessions of retraining during which every lever press was reinforced. No experimental differences between groups were evident during these six lever-pressing sessions. All pups learned, extinguished, and relearned quite rapidly.

During sessions 7-28 (16-37 weeks of age) responses were reinforced on a DRL (Differential Reinforcement of Low rate of responding) 12-sec schedule⁶. Only responses that occurred after a 12-sec period of non-responding were reinforced. Responses that followed a pause of less than 12 sec were not reinforced. Twenty reinforcements were obtained during each DRL session.

Group differences were immediately apparent on the DRL schedule, and persisted throughout the 5-month test, as shown in Fig. 1 (ref. 7). Pups with pre- and post-weaning Phe (group Phe-Phe) made significantly (all probability values reported are based on two-tailed 'U' tests) more responses ($P < 0.001$) than pups fed Phe only after weaning (group C-Phe). Pups fed pre-weaning Phe and post-weaning C (group Phe-C) made significantly more responses ($P < 0.05$) than pups with pre- and post-weaning C (groups C-C). Thus, in both comparisons, pre-weaning Phe increased responding in adulthood.

There was very little overlap between the response-levels of pups from the two pre-weaning conditions. Only four pups from C-diet mothers had mean responses per session higher than the lowest mean of any pup from Phe mothers. The observed differences were therefore not due to a selective effect of different mortality rates of the two

groups. If the groups were actually the same, and if mortality had selectively eliminated pups that would have shown high or low response levels, the range of one remaining group of subjects would have subtended that of the other. The response levels of a majority of pups from Phe mothers were consistently higher than those of any pups from C mothers.

The adult behaviour of both groups of pups from Phe mothers was less efficient than that of those from C mothers. Pre-weaning pups made many more unnecessary responses than pre-weaning C pups and thus prolonged the amount of time it took to obtain their reinforcements ($P < 0.001$). This inefficiency persisted during adulthood.

Initiation of post-weaning Phe had no significant effect on pups which had previously had pre-weaning C diet (group C-Phe versus group C-C). However, the opposite shift in diet tended to have an effect; pups in group Phe-Phe made more responses than those in group Phe-C ($P < 0.06$). Post-weaning dietary treatment with Phe affected pups that already had a behavioural deficit due to pre-weaning Phe; but it had no effect on those with pre-weaning C diets. Two interpretations of these results are possible: initiation of post-weaning C diet may have produced a partial attenuation of the pre-weaning deficit; or continuance of the Phe diet could have made worse the deficit already started.

One major problem in interpreting our data concerns the question of whether the adult deficit was due to a change in metabolism or to other factors. For example, if Phe mothers behaved differently toward their pups than did C mothers, this differential behaviour could have caused adult behavioural differences in offspring not attributable to Phe metabolism. Conclusive evidence bearing on this problem will require further research.

The interaction of pre- and post-weaning dietary effects on the deficit, however, tends to favour a metabolic interpretation of its cause. With our behavioural test, a deficit due to post-weaning Phe was apparent only after a history of pre-weaning Phe.

If one were to assume that the cause of the deficit was indeed metabolic, a second problem of interpretation would concern the nature of this deficit. Was it due to changes in learning ability, motivation, sensory capacity, simple debilitation, or to still other factors?

The higher response-levels observed during DRL testing weigh against the likelihood that the deficit was one of gross debilitation or of lack of motivation. On the other hand, the higher response-levels could have been due to heightened motivation or increased motor activity; we did not, however, note any apparent changes in level of activity or amount of water consumed by the different groups.

Clearly, further research is necessary to distinguish between the remaining interpretations in order to establish whether or not the performance deficit is, in fact, due to impaired learning ability.

This investigation was supported in part by Public Health Service research grant MH 7663 from the National Institutes of Health.

RUSSELL C. LEAF

The Squibb Institute for Medical Research,
New Brunswick, New Jersey.

PETER L. CARLTON

Rutgers—The State University,
New Brunswick, New Jersey.

SIDNEY M. HESS

The Squibb Institute for Medical Research,
and Rutgers—The State University,
New Brunswick, New Jersey.

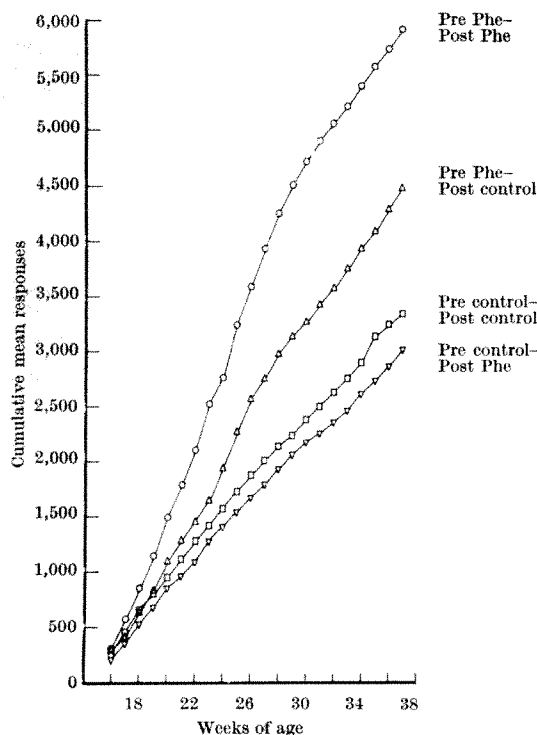


Fig. 1. Mean cumulative lever-pressing responses emitted by four groups of water-deprived rats. Only responses that occurred after a 12-sec period of non-responding were reinforced. High response levels indicate inefficient behaviour. Young rats from mothers fed a basal diet of Rockland mouse diet containing 7 per cent D,L-phenylalanine or containing 7 per cent 'Cellufloor' were used. After weaning the group previously fed phenylalanine was divided into two sub-groups; nine pups were continued on 7 per cent D,L-phenylalanine (Phe-Phe) and the diet of five pups was changed to 7 per cent 'Cellufloor' (Phe-C). After weaning the group previously fed 'Cellufloor' was also divided into two groups; the diet of nine pups was changed to 7 per cent D,L-phenylalanine (C-Phe) and eight pups were continued on 7 per cent 'Cellufloor' (C-C).

¹ Boggs, D. E., and Waisman, H. A., *Life Sciences*, **1**, 373 (1962).

² Wender, M., and Waligora, Z., *J. Neurochem.*, **7**, 259 (1961).

³ Roberts, R. B., Flexner, J. B., and Flexner, L. B., *J. Neurochem.*, **4**, 78 (1959).

⁴ Woolley, D. W., and van der Hoeven, T., *Science*, **144**, 883 (1964).

⁵ Horner, F. A., Streamer, C. W., Alejandrino, C. L., Reed, L. H., and Ibbott, F., *New England J. Med.*, **266**, 79 (1962).

⁶ Ferster, C. B., and Skinner, B. F., *Schedules of Reinforcement* (Appleton Century Crofts, New York, 1957).

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, December 6

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 4 p.m.—Dr. J. C. Gilsen: "Asbestos, Recent Work on the Biological Importance of Fibre Type".*

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Acoustic-Wave Amplification" opened by Dr. E. G. S. Paige and Mr. C. P. Sandbank.

INSTITUTION OF MECHANICAL ENGINEERS, THERMODYNAMICS AND FLUID MECHANICS GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Is Heat Transfer Theory of Any Use?"

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 6 p.m.—"The Underwater Search" (Colour Film).

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (joint meeting with the Heavy Organic Chemicals Group, at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. H. L. Riley: "Recent Developments in the Tonnage Production of Aromatic Chemicals".

Tuesday, December 7

UNIVERSITY OF LONDON (at the Westminster Medical School, Horseferry Road, London, S.W.1), at 5.15 p.m.—Dr. J. F. Zilva: "Physiological Variations in Serum Iron Levels".*

INSTITUTION OF ELECTRICAL ENGINEERS, CONTROL AND AUTOMATION DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Human Factors in the Control of Road Vehicles", opened by Mr. I. D. Brown and Mr. R. L. Moore.

UNIVERSITY OF LONDON (at Imperial College of Science and Technology, London, S.W.7), at 5.30 p.m.—Prof. H. R. Hower: "The Length and Breadth of Zoology" (Inaugural Lecture).*

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. P. J. Lachmann: "Allergic Reactions, Connective Tissue and Disease". (Twelfth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation).*

INSTITUTION OF MECHANICAL ENGINEERS, LUBRICATION AND WEAR GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Compressor Fires and Explosions".

PLASTICS INSTITUTE, LONDON SECTION (at the Wellcome Building, Euston Road, London, N.W.1), at 6.30 p.m.—Dr. H. Ebneth (Farbenfabriken Bayer AG): "A General Survey of ABS Materials".

Wednesday, December 8

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 2 p.m.—Dr. F. C. Greenwood: "Assay of Protein Hormones".*

INSTITUTE OF NAVIGATION (at the Royal Institution of Naval Architects, 10 Upper Belgrave Street, London, S.W.1), at 3 p.m.—Meeting on "The Training of Navigators".

SIR THOMAS MIDDLETON MEMORIAL TRUST (in the Auditorium, The Wellcome Building, 183 Euston Road, London, N.W.1), at 3 p.m.—Dr. J. L. Monteith: "Physical Limitations to Crop Growth" (Tenth Memorial Lecture).

INSTITUTION OF ELECTRICAL ENGINEERS, CONTROL AND AUTOMATION DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. P. L. Moreton: "Simple New Power-Factor Meter Utilizing Dry Reed Relays"; Mr. J. Dean and Mr. C. E. Lyth: "A Wattmetric Transducer".

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. J. Kutina (Charles University, Prague): "Genetic Interpretation of Zoning Phenomena in Ore Deposits".*

ENVIRONMENTAL GROUP (in the Department of Electrical Engineering, Imperial College of Science and Technology, London, S.W.7), at 6 p.m.—Discussion Meeting on "Information for Architects on Environmental Matters".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 6 p.m.—Discussion on "A Review of the Present Situation of Electronic Switching", opened by Mr. J. A. Lawrence and Mr. J. R. Pollard.

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, TELEVISION GROUP (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Symposium on "Semi-conductor Scanning Circuits".

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (at Shell-Mex House, Strand, London, W.C.2), at 6 p.m.—Annual General Meeting, followed by Dr. R. E. Parker: "The Work of the R.I.C.".*

SOCIETY OF INSTRUMENT TECHNOLOGY (at Manson House, 26 Portland Place, London, W.1), at 6 p.m.—Mr. E. Duncombe: "Some Transducer Techniques for Use at Elevated Temperatures".

INSTITUTE OF INFORMATION SCIENTISTS (at the Whitehall Hotel, Bloomsbury Square, London, W.C.1), at 6.15 p.m.—Mr. F. Liebesny: "Information from Patents".

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, Westminster, London, S.W.1), at 6.15 p.m.—Mr. D. G. McBeth: "The Victoria Line Survey".

SOCIETY OF CHEMICAL INDUSTRY FOOD GROUP—NUTRITION PANEL (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Mr. A. B. E. De Jong: "Survival Rations".

INSTITUTE OF SCIENCE TECHNOLOGY, LONDON BRANCH (at King's College, Strand, London, W.C.2), at 6.30 p.m.—Mr. S. Lenton: "Brazilian Adventure".

OIL AND COLOUR CHEMISTS' ASSOCIATION, LONDON SECTION (in the Small Physics Lecture Theatre, Imperial College of Science and Technology, London, S.W.7), at 7 p.m.—Mr. T. B. M. Rybak: "The Use of Computers in Process Control".

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (at Hatfield College of Technology, Roe Green, Hatfield), at 7 p.m.—Dr. A. E. A. Werner: "The Scientific Examination of Paintings and Antiquities" (Ladies' Evening).

ASSOCIATION OF THE WILLIAM PENGELLAY CAVE RESEARCH CENTRE, WITH IMPERIAL COLLEGE CATING CLUB (in the Department of Physics, Imperial College of Science and Technology, Prince Consort Road, London, S.W.7), at 7.30 p.m.—Dr. Harold Lord: "Cave Communications".

Thursday, December 9

INSTITUTION OF MECHANICAL ENGINEERS, MANIPULATIVE AND MECHANICAL HANDLING MACHINERY GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 4.30 p.m.—Discussion on "Bulk Handling and Storage of Iron Ore and Coal".

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Dr. W. Hayes, F.R.S.: "Some Controversial Aspects of Bacterial Sexuality" (The Leeuwenhoek Lecture).

INSTITUTE OF PETROLEUM, ECONOMICS AND OPERATIONS GROUP (at 6 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. Bal Wagle: "Some Recent Developments in Forecasting Techniques".

INSTITUTION OF CIVIL ENGINEERS, TRANSPORTATION ENGINEERING GROUP (at Great George Street, Westminster, London, S.W.1), at 5.30 p.m.—Mr. D. B. Sampson: "Public Relations and Its Effect on Traffic Behaviour in North America".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. D. E. N. Davies and Dr. B. S. McCartney: "Cylindrical Arrays with Electronic Beam Scanning"; Dr. D. E. N. Davies: "Beam-Positioning Radar Systems Utilizing Continuous Scanning Techniques".

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Prof. G. H. Rawcliffe and Dr. W. Fong: "Two-speed Induction Motors Using Fractional-Slot Windings"; Dr. W. Fong: "Wide-ratio Two-speed-Single-winding Induction Motors".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. F. T. G. Prunty: "Androgen Metabolism in Man—Some Current Concepts". (Thirteenth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation).*

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. J. Kutina (Charles University, Prague): "Selection Replacement in Hypogene Ore Deposition".*

SOCIETY FOR ANALYTICAL CHEMISTRY, BIOLOGICAL METHODS GROUP (at "The Feathers", Tudor Street, London, E.C.4), at 6.30 p.m.—Annual General Meeting, followed by a Discussion Meeting on "Problems and Dilemmas in Microbiological Assay" opened by Mr. S. A. Price.

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (at Manson House, 26 Portland Place, London, W.1), at 7.30 p.m.—Dr. Cicely Williams: "Population Problems in Developing Countries".

Friday, December 10

UNIVERSITY OF LONDON (at the Institute of Obstetrics and Gynaecology, Queen Charlotte's Hospital, London, W.6), at 11.15 a.m.—Mr. C. J. Dewhurst: "The Determination of Sex".

INSTITUTION OF ELECTRICAL ENGINEERS, CONTROL AND AUTOMATION DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "The ONC: Better Opportunities for Failure?" opened by Mr. E. Toner.

SOCIETY FOR ANALYTICAL CHEMISTRY, MICROCHEMICAL METHODS GROUP (at the Chemical Society, Burlington House, Piccadilly, London, W.1), at 6.45 p.m.—Annual General Meeting, followed by Mr. A. K. Soper: "Practical Experience in Statistics Applied to Chemistry".

Saturday, December 11

INNER LONDON EDUCATION AUTHORITY (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Dr. John Waechter: "Underwater Archaeology".*

Monday, December 13

OPERATIONAL RESEARCH SOCIETY (at the Royal Aeronautical Society, 4 Hamilton Place, London, W.1), at 4.30 p.m.—Mr. D. W. Trigg: "Logic of Stock Replenishment Subject to Production Constraints".

SOCIETY OF CHEMICAL INDUSTRY, COLLOID AND SURFACE CHEMISTRY GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Mr. S. A. Mitchell: "The Surface Properties of Amorphous Silicas".

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS (at 9 Bedford Square, London, W.C.1), at 6 p.m.—Annual General Meeting.

INSTITUTION OF MECHANICAL ENGINEERS, HYDRAULIC PLANT AND MACHINERY GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Application of Control Theory to Control of Surges".

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, W.C.2), at 6.30 p.m.—Mr. L. Drucquer—Presidential Address.

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Dr. R. B. Sarjeant: "Background to the Yemen War".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER (with qualifications and research interests preferably in some branch of mathematical statistics, but candidates with interests in applied statistics will also receive consideration) IN THE DEPARTMENT OF PURE MATHEMATICS AND MATHEMATICAL STATISTICS—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (December 10).

RESEARCH ASSISTANT (graduate in physiology, biochemistry or medicine) IN THE PHYSIOLOGY DEPARTMENT (current research is on pituitary tissue culture, and on the reaction between the pituitary and cancer)—The Professor of Physiology, University College, Galway, Republic of Ireland (December 10).

ASSISTANT LIBRARIAN (qualified librarian, preferably with some experience in a college library)—The Clerk to the Governors, Woolwich Polytechnic, Wellington Street, London, S.E.18 (December 13).

LECTURER IN THE DEPARTMENT OF PURE MATHEMATICS or the DEPARTMENT OF STATISTICS, School of Mathematics, University of New South Wales—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, December 15).

SENIOR LECTURER/LECTURER IN PURE MATHEMATICS at the University of New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, December 15).

COMPUTER MANAGER (with previous experience with large-scale electronic computers) for the KDF 9 installation in the Electronic Computing Laboratory—The Registrar, The University, Leeds, 2 (December 17).

CHAIR OF MICROBIOLOGY—The Secretary of the University Court, University of Glasgow, Glasgow (December 18).

LECTURER IN ECOLOGY IN THE DEPARTMENT OF ZOOLOGY—The Registrar, University of Leicester, Leicester (December 18).

LECTURERS (2) IN APPLIED MATHEMATICS—The Registrar, University of Leicester, Leicester (December 18).

PROFESSOR AND HEAD (with an appropriate combination of academic research and industrial experience) of the DEPARTMENT OF MATERIALS SCIENCE AND TECHNOLOGY—The Principal, Brunel College, Woodlands Avenue, London, W.3 (December 20).

CHAIR OF ELECTRICAL ENGINEERING SCIENCE—The Registrar, University of Essex, Wivenhoe Park, Colchester, Essex (December 21).

SECOND CHAIR OF ZOOLOGY AT QUEEN MARY COLLEGE—The Academic Registrar, University of London, Senate House, London, W.C.1 (December 22).

CHAIR OF BIOCHEMISTRY AND AGRICULTURAL BIOCHEMISTRY—The Registrar, University College of Wales, Aberystwyth (December 31).

LECTURER IN ORGANIC CHEMISTRY—The Secretary, The Queen's University, Belfast, Northern Ireland (December 31).

PROFESSOR IN EDUCATION at the School of Education—The Registrar, The Chinese University of Hong Kong, Hang Seng Bank Building, 677 Nathan Road, Kowloon, Hong Kong (December 31).

SENIOR LECTURER or LECTURER IN BOTANY; and a SENIOR LECTURER or LECTURER IN ZOOLOGY at the University of Natal, South Africa—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, December 31).

SENIOR LECTURER or LECTURER (preferably with qualifications in one of the following fields: topology, applied mathematics or algebra) IN THE DEPARTMENT OF MATHEMATICS, University of Ghana—The Assistant Registrar, Ghana High Commission, Higher Education Section, 15 Gordon Square, London, W.C.1; or The Registrar, University of Ghana, P.O. Box 25, Legon, Accra, Ghana (December 31).

SENIOR LECTURER or LECTURER (with special qualifications and interests in economic geography) IN GEOGRAPHY—The Secretary, The Queen's University, Belfast, Northern Ireland (December 31).

SENIOR SCIENTIFIC OFFICER/SCIENTIFIC OFFICER-CHEMIST (experienced in water chemistry and preferably a knowledge of the chemistry of submerged soils)—The Director, Tropical Fish Culture Research Institute, Batu Berendam, Malacca, Malaysia (December 31).

TEMPORARY LECTURER IN ELECTRICAL ENGINEERING at the University of Western Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 8).

CHAIR OF PHYSICAL CHEMISTRY—The Secretary, The Queen's University, Belfast, Northern Ireland (January 15).

SENIOR LECTURER (with suitable academic qualifications in physics or electronics and preferably experience in university teaching) IN ELECTRONIC PHYSICS IN THE DEPARTMENT OF ELECTRICAL ENGINEERING, Faculty of Applied Science—The Secretary, University of St. Andrews, Queen's College, Dundee, Scotland (January 15).

SENIOR LECTURER IN PHYSICAL OCEANOGRAPHY at the University of Cape Town—The Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1; and The Registrar, University of Cape Town, Private Bag, Rondebosch, Cape Town, South Africa (January 31).

CHAIR OF APPLIED GEOLOGY IN THE DEPARTMENT OF MINING ENGINEERING—The Registrar, University of Strathclyde, George Street, Glasgow, G.1.

LECTURER (with experience in experimental research with special reference to learning theory, and in teaching at degree level) IN PSYCHOLOGY—The Registrar, Hatfield College of Technology, Hatfield, Herts.

PROFESSOR and an ASSOCIATE PROFESSOR IN APPLIED MATHEMATICS—The Senior Professor, Applied Mathematics, University of Western Ontario, London, Ontario, Canada.

RESEARCH ASSISTANT (preferably with previous experience in electrophysiological techniques) IN ELECTROPHYSIOLOGY IN THE DEPARTMENT OF ZOOLOGY, to join a small group working on the trophic properties of the insect neuromuscular system—The Secretary of the University Court, University of Glasgow, Glasgow, W.2.

SENIOR GEOLOGIST (male, with a first- or upper second-class honours degree in geology, and at least three years' post-graduate experience in igneous and/or metamorphic rocks, structural geology and prospecting, as well as training in economic geology) IN BRITISH GUIANA, to organize a team of geologists for geological mapping and prospecting and in the investigation of mineral deposits—The Appointments Officer, Ministry of Overseas Development, Room 301, Eland House, Stag Place, London, S.W.1, quoting Ref. RO 218/25/05.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Business Education. Pp. 7. (London: Federation of British Industries, 1965.) 9d. [1210]

United Kingdom Atomic Energy Authority. Eleventh Annual Report for the period 1st April, 1964–31st March, 1965. Pp. x+102+8 photographs. 8s. net. Atom 1965. (An illustrated summary of the Eleventh Annual Report, 1st April, 1964–31st March, 1965.) Pp. 40. 2s. 6d. (London: H.M. Stationery Office, 1965.) [2010]

Ministry of Power. Fuel Policy. (Cmd. 2798.) Pp. iii+35. (London: H.M. Stationery Office, 1965.) 3s. net. [2510]

Committee on Manpower Resources for Science and Technology. A Review of the Scope and Problems of Scientific and Technological Manpower Policy. (Cmd. 2800.) Pp. ii+17. (London: H.M. Stationery Office, 1965.) 1s. 6d. net. [311]

Freshwater Biological Association. Scientific Publication No. 16: A Revised Key to the British Water Bugs (Hemiptera-Heteroptera), with Notes on their Ecology. By Dr. T. T. Macan. Pp. 78. (Ambleside: Freshwater Biological Association, 1965.) 5s. 6d. [311]

Office of Health Economics. The Local Health Services. Pp. 40. (London: Office of Health Economics, 1965.) 2s. 6d. [311]

The Brain and the Unity of Conscious Experience. By Sir John Eccles. (The Nineteenth Arthur Stanley Eddington Memorial Lecture, 15 October 1965.) Pp. 45. (London: Cambridge University Press, 1965.) 4s. 6d. net; 95 cents. [311]

Imperial College of Science and Technology. Research Report of the Royal College of Science, 1962–65. Pp. 87. (London: Imperial College of Science and Technology, University of London, 1965.) [311]

Year Book of the Royal Society of Edinburgh 1965. (Session 1963–1964.) Pp. 183. (Edinburgh: Royal Society of Edinburgh, 1965.) 20s.; 8.50 dollars. [311]

Ministry of Overseas Development. Overseas Research Publication No. 11: Termite Infestation of Pines in British Honduras—Termite Research in British Honduras under Research Scheme R.1048. By R. M. C. Williams. Pp. iv+31+4 plates. (London: H.M. Stationery Office, 1965.) 9s. net. [311]

Bulletin of the British Museum (Natural History). Botany. Vol. 3, No. 6: The Ceylon Species of *Asplenium*. By W. A. Sledge. Pp. 233–277+plate 20. 15s. Entomology. Vol. 16, No. 4: On Some Coccidae (Homoptera), Chiefly from Africa. By G. De Lotto. Pp. 175–239. 25s. Zoology. Vol. 13, No. 4: *Barbus* (Pisces, Cyprinidae) of the Volta Region. By A. J. and J. Hopson. Pp. 99–149. 22s. Vol. 13, No. 5: Notes on the Cupuladriidae (Polychaeta, Anasca). By Patricia L. Cook. Pp. 151–187+plates 1–3. 17s. Vol. 13, No. 6: Polychaeta from West Africa, The Cupuladriidae, (Chelostomata, Anasca). By Patricia L. Cook. Pp. 189–227+plates 1–3. 18s. (London: British Museum (Natural History), 1965.) [311]

University Grants Committee. Returns from Universities and University Colleges in receipt of Exchequer Grant, Academic Year 1963–1964. (Cmd. 2778.) Pp. v+46. (London: H.M. Stationery Office, 1965.) 6s. 6d. net. [311]

Ambassade de France, Service de Presse et d'Information. The Election of the President of the Republic and the Political Forces in France. Pp. 25. (London: Ambassade de France, Service de Presse et d'Information, 1965.) [311]

The Empire Cotton Growing Corporation. Progress Reports from Experiment Stations, Season 1963–64. Malawi. By B. E. Costelloe. Pp. 12. 2s. 6d. Swaziland. By M. E. Cornish-Bowden. Pp. 13. 2s. 6d. Tanzania—Costal Regions. By T. Bradley and J. S. Watson. Pp. 9. 2s. 6d. (London: The Empire Cotton Growing Corporation, 1965.) [311]

Ministry of Technology. Forest Products Research. Special Report No. 21: The Compressive Strength of Home-Grown Pit-Props. By J. G. Sunley. Pp. iv+15+2 plates. (London: H.M. Stationery Office, 1965.) 2s. 3d. net. [311]

Proceedings of the Royal Irish Academy. Vol. 64, Section B, No. 8: The Effect of Constant and Varying Temperatures on the Development of *Acanthocyclops virdis* (Jurine). By M. Faheem Khan. Pp. 117–130. 3s. 6d. Vol. 64, Section B, No. 9: Late-Pleistocene Shorelines and Drift Limits in North Donegal. By N. Stephens and F. M. Syngé. Pp. 131–158+plate 8. 5s. (Dublin: Royal Irish Academy, 1965.) [311]

Other Countries

Transactions of the Royal Society of New Zealand. Geology. Vol. 3, No. 5 (April 27, 1965): A Permian Fauna from North-West Nelson, New Zealand. By J. B. Waterhouse and Paul Vella. Pp. 57–84+5 plates. Vol. 3, No. 6 (April 14, 1965): New Invertebrates from the Lower Miocene Pakaurangi Beds, Kaipara Harbour, with a Redescription of the Gastropod *Clidonia* Laws. By J. A. Grant-Mackie. Pp. 85–94+2 plates. Zoology. Vol. 6, No. 9 (April 14, 1965): The Alimentary Canal of *Anisobatis littorea* (White) (Dermaptora: Labiduridae), with Special Reference to the Peritrophic Membrane. By E. T. Giles. Pp. 87–101+4 plates. Vol. 6, No. 11 (April 14, 1965): A Common but Hitherto Undescribed Species of *Orchomenella* (Crustacea Amphipoda: Family Lysianassidae) from the Ross Sea. By D. E. Hurley. Pp. 107–113. Vol. 6, No. 10 (May 19, 1965): A New Species of *Periplexis* (Sphynridae, Copepoda) from the Southern Ocean. By G. C. Hewitt. Pp. 103–106. (Wellington: The Royal Society of New Zealand, 1965.) [410]

United States Department of the Interior: Geological Survey. Bulletin 1195: Bibliography of North American Geology, 1950–1959. By Ruth Reece King, Elisabeth S. Loud, Mildred C. Mead, Virginia M. Jussen and Georgianna D. Conant. Part 1: Bibliography. Vol. 1: A–L. Pp. iii+1–1012. Vol. 2: M–Z. Pp. 1013–1790. Part 2: Index. Vol. 3: A–L. Pp. 1791–2796. Vol. 4: M–Z. Pp. 2797–4025. (Washington, D.C.: Government Printing Office, 1965.) 10.75 dollars per set of four volumes. [410]

Carbohydrate Research, Vol. 1, No. 1 (July/August 1965). Pp. 1–96. Subscription rate: 126s.; 63 d.f.; 17.50 dollars per volume (6 issues). (Amsterdam: Elsevier Publishing Company, 1965.) [411]

New Zealand. Report of the Director-General of Forests for the year ended 31 March 1965. Pp. 59. (Wellington: Government Printer, 1965.) 2s. [411]

Conseil National de la Politique Scientifique. Rapport Annuel 1964. Pp. 200. (Bruxelles: Conseil National de la Politique Scientifique, 1965.) [411]

Australia: Commonwealth Scientific and Industrial Research Organization. Seventeenth Annual Report of the Division of Coal Research, 1964–1965. Pp. 29. (Chatswood, N.S.W.: Commonwealth Scientific and Industrial Research Organization, 1965.) [411]

United States Department of Agriculture. Leaflet No. 274: Control of Apple Tree Borers. Pp. 8. (Washington, D.C.: Government Printing Office, 1965.) [411]

Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Tropical Pastures, 1964–65. Pp. 87. (Brisbane: Commonwealth Scientific and Industrial Research Organization, 1965.) [411]

Editorial and Publishing Offices of "NATURE"

MACMILLAN (JOURNALS) LIMITED

4 LITTLE ESSEX STREET, LONDON, W.C.2

Telephone Number: Temple Bar 6633. Telegrams: Phusis London W.C.2

Annual subscription: Inland £14, Overseas £13 15s., payable in advance, postage paid to any part of the world.

Advertisements only should be addressed to

T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2

Telephone Number: Holborn 4743

Registered as a newspaper at the General Post Office

Copyright © Macmillan (Journals) Limited, December 4, 1965

NATURE

No. 5015

SATURDAY, DECEMBER 11, 1965

Vol. 208

Published by
MACMILLAN (JOURNALS) LTD.

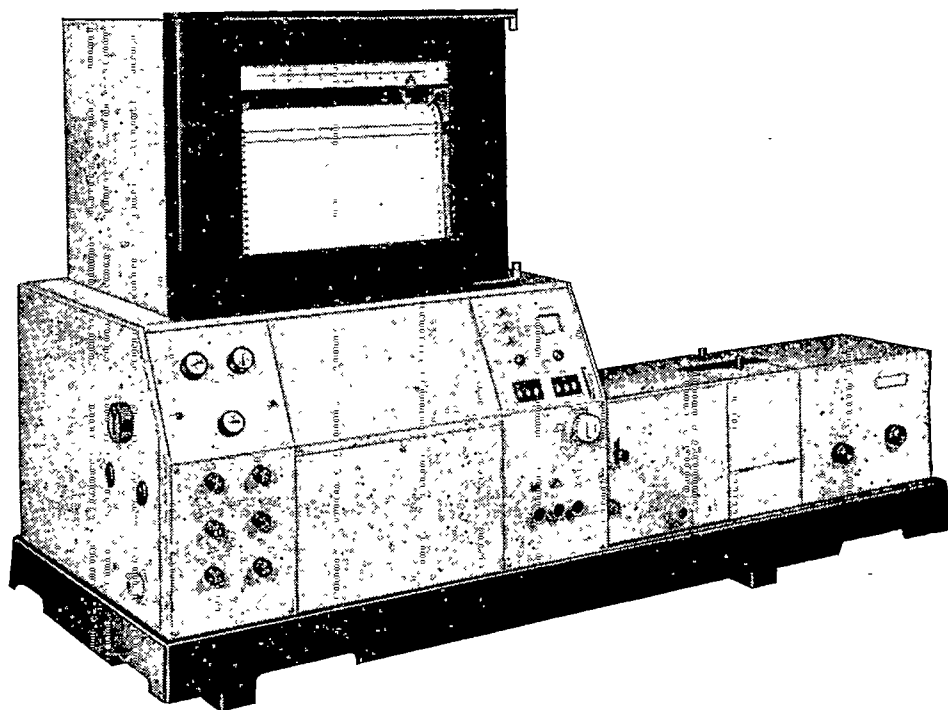
CONTENTS

	Page
THE CASE FOR APPLIED RESEARCH.	1025
BEHAVIOUR THERAPY. By Dr. Boris Semeonoff	1027
DRUG DOSAGE IN LABORATORY ANIMALS. By Dr. J. A. Parsons	1028
TEACHING IMMUNOLOGY. By Dr. P. D'Arcy Hart	1028
DIAGNOSIS OF PARASITES. By Dr. G. Lapage	1029
AN INTRODUCTION TO MOLECULAR GENETICS. By Dr. S. W. Glover	1029
GEOLOGY OF GRANITE. By Dr. P. E. Brown	1030
A BROAD MAGNETIC FIELD. By Dr. J. H. Davis	1030
OPTICAL ANALOGUE METHODS IN DIFFRACTION STUDIES. By Dr. C. W. Bunn	1031
BIOLOGICAL AND MENTAL EVOLUTION: AN EXERCISE IN ANALOGY. By Arthur Koestler	1033
HISTORY AS THE ORGANIZATION OF MAN'S MEMORY. By Prof. Herbert Butterfield	1036
MAN-MADE LAKES. By Dr. Rosemary L. McConnell and Dr. E. B. Warthington	1039
OBITUARIES:	
PROF. JOCELYN PATTERSON. By Prof. W. J. Hamilton	1042
PROF. WILFRED MERCHANT. By Dr. S. S. Gill	1043
DR. PAUL MÜLLER	1043
NEWS AND VIEWS	1044
BIOLOGICAL SYSTEMS AT THE MOLECULAR LEVEL. By Dr. B. Askonas, Prof. J. A. V. Butler, F.R.S., Prof. F. Jacob, Dr. D. C. Phillips, Dr. L. Sachs and Dr. G. V. Sherbet	1048
LIVINGSTONE'S CONTRIBUTIONS TO MEDICINE.	1050
CHEMISTRY OF PLANT PIGMENTS. By Dr. J. B. Harborne.	1051
DESIGN METHOD	1052
VALIDITY OF RADIOCARBON DATES ON BONE. By Dr. M. A. Tamers and F. J. Pearson, jun.	1053
REGIONAL GEOCHEMICAL RECONNAISSANCE APPLIED TO SOME AGRICULTURAL PROBLEMS IN CO. LIMERICK, EIRE. By Prof. John S. Webb and W. J. Atkinson	1056
A NEW HAEMOGLOBIN, J TORONTO ($\alpha 5$ ALANINE \rightarrow ASPARTIC ACID). By Dr. J. H. Crookston, D. Beale, D. Irvine and Dr. H. Lehmann	1059
GLOBIN SYNTHESIS IN THALASSAEMIA: AN <i>in vitro</i> STUDY. By Dr. D. J. Weatherall, Dr. J. B. Clegg and Dr. M. A. Naughton.	1061
PROMOTION BY KINETIN OF THE POLAR TRANSPORT OF TWO AUXINS. By Dr. C. C. McCready, Dr. Daphne J. Osborne and M. Kay Black	1065
ENZYMATIC ADAPTATION TO CONTRACTILE ACTIVITY IN SKELETAL MUSCLE. By J. Kendrick-Jones and Prof. S. V. Perry	1068
LIFE-SHORTENING IN MICE EXPOSED TO RADIATION: EFFECTS OF AGE AND OF HYPOXIA. By Dr. Patricia J. Lindop and Prof. J. Rotblat, C.B.E.	1070
ALTERATION OF HAMSTER CELLS BY NUCLEIC ACID <i>in vitro</i> . By Kate Shepley, Dr. E. J. Ambrose and Dr. K. S. Kirby	1072
DETERMINATION OF THE TOTAL ALPHA ACTIVITY OF DRINKING WATER USING A NUCLEAR EMULSION. By M. W. Tytell, I. C. Demetsopoulos and A. J. L. Collinson	1074
RELATIVE INSTABILITY OF CEPHALORIDINE TO STAPHYLOCOCCAL PENICILLINASE. By Dr. Mark Ridley and Dr. Ian Phillips	1076
MEASUREMENTS OF SPONTANEOUS BIOLUMINESCENCE IN THE SEA. By Dr. Brian P. Boden, Dr. Elizabeth M. Kampa and James M. Snodgrass	1078

(Continued overleaf)

NEW UNICAM SP700

recording
spectrophotometer



now choose **WAVELENGTH** (SP700A)
or **WAVENUMBER** (SP700C)

plus these other advantages

HIGH performance over wide spectral range $186\text{m}\mu - 3.6\mu$

FIVE linear transmittance and three linear absorbance scales

FIVE scanning speeds and three chart speeds

BEAM balance adjustment for base line stability

IMPROVED layout of controls

SAMPLING accessories include diffuse reflectance attachment

send for further details today

UNICAM

Precision Spectrophotometers

LETTERS TO THE EDITOR

	Page		Page
PHYSICS		PATHOLOGY	
Laser-induced Damage in Diamond.—Dr. D. J. Bradley, M. Engwell and H. Komatsu	1081	Inactivation of T-2 Bacteriophage by Sensitized Leucocytes <i>in vitro</i> .—Robert Young and Dr. Frank H. Ruddle	1105
Acoustic Velocity Dispersion in some Non-associated Organic Liquids.—Dr. S. E. A. Hakim and W. J. Comley	1082	Electrophoretic Mobility of an Immune Globulin from Rainbow Trout Serum.—Harold O. Hodgins, Dr. George J. Ridgway and Fred M. Jtter	1106
Fall-out in London Rain-water during 1964.—Dr. R. Wood, J. R. W. Kerr and L. A. Richards	1083	PATHOLOGY	
GEOCHEMISTRY		Lactic Dehydrogenase Isoenzymes in Muscle from Patients with Duchenne Muscular Dystrophy.—Prof. Thomas H. Shepard, Dr. Lawrence H. Gordon and Dr. John E. Wollenweber	
Strontium-90 and Caesium-137 in Columbia River Plume, July 1964.—Prof. Kilho Park, Marilyn J. George, Yasuo Miyake, Katsuko Saruhashi, Yukio Katsuragi and Teruko Kanazawa	1084	1107	
Metal Grains in Chondritic Meteorites.—J. A. Wood	1085	RADIOBIOLOGY	
RADIOCHEMISTRY		A Reduced Oxygen Enhancement Ratio for X-ray Survival of HeLa Cells <i>in vitro</i> , after Treatment with 'Methotrexate'.—Dr. Roger J. Berry	
Site of Attack in the Radiolysis of Pyrimidine Compounds.—Prof. L. S. Myers, jun., J. F. Ward, W. T. Tsukamoto and D. E. Holmes	1086	Effects of Dietary Levels of Phosphorus and Calcium on the Comparative Behaviour of Strontium and Calcium.—K. Kostial, S. Vojvodin and Prof. C. L. Comar	
CHEMISTRY		1110	
Advances in Liquid Chromatography.—Dr. B. Makin and F. P. Speakman	1087	BIOLOGY	
Microstructure of Fibrous Sulphur.—A. Jaskolska and A. E. B. Presland	1088	Occurrence of <i>Salvinia auriculata</i> Aublet on the Congo River.—Dr. E. C. S. Little, N.B.E.	
Infra-red Emission Spectra of Minerals.—Prof. M. J. D. Low	1089	Air Ion Effects on the Oxygen Consumption of Barley Seedlings.—Sadao Kotaka, Prof. Albert P. Krueger, Paul C. Andriese, Kazutoshi Nishizawa, Takeshi Ohuchi, Mitsuko Takenobu and Yasuo Kozure	
Nitroso Formaldehyde.—I. M. Napier and Prof. R. G. W. Norrish, F.R.S.	1090	Presence of Plant Growth Substances in Earthworms demonstrated by Paper Chromatography and the Went Pea Test.—R. L. Nielson	
Use of 'Sephadex' Gel for the Estimation of Molecular Weight of Humic Substances in Natural Water.—E. T. Gjessing	1091	Promotion of Germination in Light-requiring Seed by Chloramphenicol.—Dr. M. Flack and M. Richardson	
Errors in the Calculation of Surface Area and Average Pore Radius.—Dr. D. Dollimore and Dr. G. R. Heal	1092	Oxidative and Biosynthetic Utilization by Human Spermatozoa of a Metabolite of the Female Reproductive Tract.—Prof. Charles Turner	
BIOCHEMISTRY		Electromagnetic Recording Diver Balance.—Søren Lovtrup and Sune Larsson	
Correlation of the Binding to DNA Loops or to DNA Helices with the Effect on RNA Synthesis.—Prof. John H. Frenter	1093	Photo-induced Hydroxylation of Cinnamic Acid in Gherkin Hypocotyls.—Dr. G. Engelsma	
Isolation and Amino-acid Sequence of β -LPH from Sheep Pituitary Glands.—Prof. Choh Hao Li, Livio Barnafi, Michel Chrétien and David Chung	1093	Histological Investigations of the Mechanism of Sterility induced by Deuterium Oxide in Mice.—Ann M. Hughes and Dr. Laurel E. Glas	
Reactivity of the ϵ -Amino Group of Collagen.—J. H. Bowes, A. S. Raistrick and Dr. J. A. Moss	1094	MICROBIOLOGY	
Antiviral Action of Oxidized Spermine—Inactivation of Plant Viruses.—U. Bachrach, Shlomit Rabina, G. Loebenstein and G. Eilon	1095	Simplified Technique for Preparative Disc Electrophoresis.—Prof. D. Sulitzanu and W. F. Goldman	
Maintenance of <i>Escherichia coli</i> and the Assimilation of Glucose.—Sarah B. McGrew and Prof. M. F. Mallette	1098	1120	
PHYSIOLOGY		ENTOMOLOGY	
Hydrolysis of Disaccharides during Absorption by the Perfused Small Intestine of Amphibia.—Dr. D. S. Parsons and J. S. Prichard	1097	An Inhibitory Effect of Allatectomized Males and Females on the Sexual Maturation of Young Male Adults of <i>Schistocerca gregaria</i> (Forsk.) (Orthoptera: Acrididae).—M. J. Norris and M. P. Pender	
Protective Effect of Oestrogens against the Toxic Decomposition Products of Tribromoethanol.—Prof. T. Nicol, Dr. B. Vernon-Roberts and Dr. D. C. Quantock	1098	1122	
Aldosterone Secretion: Differences in Direct Effects of Rubidium and Potassium Perfusion into Dog Adrenals.—Prof. James G. Hilton, Dr. Sheldon Alter and Dr. Paul Cushman	1099	VIROLOGY	
A New Property of the Early Receptor Potential of Rat Retina.—Dr. G. B. Arden and Hisako Ikeda	1100	Nuclear Surface <i>N</i> -Acetyl Neuraminic Acid Terminating Receptors for Myxovirus Attachment.—Prof. Philip I. Marcus, Jesse M. Salb and Vera Galinovic Schwartz	
PHARMACOLOGY		Effect of β -Propiolactone and Thiomersal on Growth of <i>B. H. K.</i> Cells in the Hamster Cheek Pouch.—S. A. Keeble and C. S. Heymann	
Inhibition of Conjugation by Anti-inflammatory Drugs.—Dr. T. Hargreaves	1101	1125	
Preferential Release of Adrenaline from the Adrenal Medulla by Muscarine and Pilocarpine.—Prof. W. W. Douglas and Dr. A. M. Poisner	1102	GENETICS	
HAEMATOLOGY		Linkage in Man: the <i>Inr</i> and the <i>Lp</i> Serum Type Systems.—Dr. T. Gedde-Dahl, jun., and Dr. K. Berg	
Quantitative Determination of Haemoglobin A ₁ by Acrylamide-gel Electrophoresis.—Lieut.-Commander Thomas G. Ferris, Robert E. Easterling and Richard E. Budd	1103	Artificial Insemination of Dystrophic Mice with Mixtures of Spermatozoa.—Janice L. Southard, H. Glenn Wolfe and Dr. Elizabeth S. Russell	
IMMUNOLOGY		1126	
Time-course Studies on Antibody Response in Thymectomized and Sham-thymectomized Mice.—Dr. Nicholas R. Sinclair	1104	PSYCHOLOGY	
		Shift Working: the Arrangement of Hours on Night Work.—Mrs. Gwynneth de la Mare and Dr. J. Walker	
		Effect of Infantile Treadmill Experience on Body-weight and Resistance to Exhaustion in the Rat.—Robert A. Levitt and Prof. Wilis B. Webb	
		Form-similarity between Phosphenes of Adults and pre-School Children's Scribbles.—Rhoda Kellogg, Prof. M. Knoll and Dr. J. Kugler	
		1129	



Gas Chromatograph Test Set & Reference Signal Source



enables the Chemist or Instrument Engineer to check quickly the operation and performance of his complete Gas Chromatograph equipment. If the equipment is not operating correctly, it will quickly localise the faulty section. No electrical knowledge is necessary to use this Test Set, which is suitable for Flame Ionisation, Thermistor type and Katharometer Gas Chromatographs.

Please write for details now:-



A. M. Lock & Co. Ltd.
Prudential Buildings,
79, Union Street, Oldham, Lancs.
Telephone: MAIn 0333

CW11804

STOP WATCHES

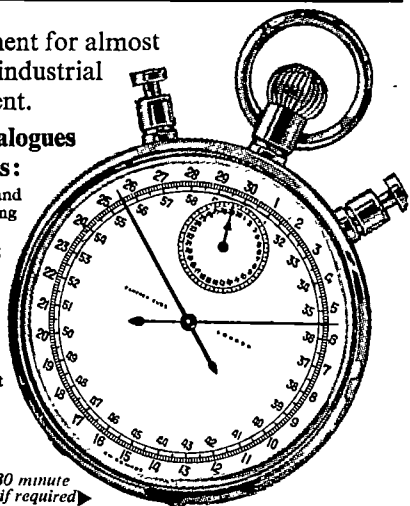
or timing equipment for almost any scientific or industrial timing requirement.

Our range of catalogues and leaflets covers:

Watches, stop watches and chronographs; Recording apparatus; Clocks, domestic and industrial; Process timers and time interval meters; Electrically controlled stop watches, tachometers, revolution and impulse counters. Any of these will be sent on request.

Repair Service

Model 603 1 10th second escapement 'split hands' 30 minute recorder. NPL certificate if required ▶



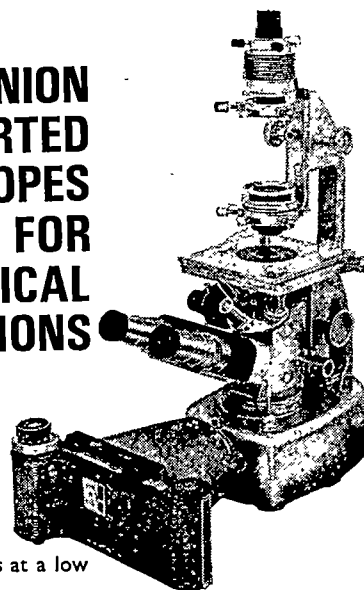
CAMERER CUSS

TIMING AND INSTRUMENT DIVISION

54/56, NEW OXFORD STREET,
LONDON W.C.1 Tel: MUSEum 4861

also at 5 New Cavendish Street W.1

UNION INVERTED MICROSCOPES FOR BIOLOGICAL APPLICATIONS



- Good quality optics at a low price.
- Long working distance condenser to accommodate thick containers.
- Long working distance 40x phase objective for observation through thick bottomed containers.
- Uses 35 mm. roll or polaroid, retaining binocular whilst photographing.
- Transformer built-in to base.

Full details of this and other UNION microscopes available from:

**POLARON
EQUIPMENT LTD.**

4 Shakespeare Road,
Finchley, London, N.3.
Telephone: VIRginia 9241.

THE CASE FOR APPLIED RESEARCH

MR. J. C. DANCY, in his presidential address to Section L (Education) of the British Association for the Advancement of Science at its meeting in Cambridge on September 2, discussed the place of technology in a liberal education. This was, in fact, the main theme of the Section's programme, which sought to explore the implications, more particularly for education, of the idea that, instead of the two cultures made familiar by C. P. Snow (now Lord Snow), there is a single unified twentieth-century culture of which technology is an integral part. The programme, moreover, sought to show that many aspects of technology have important educational values and should be studied in their own right, apart from such important considerations as the need for more technologists. To some extent the programme overlapped with discussions in Section X, particularly in so far as the relation between the creative process as understood by the artist, the engineer and the scientist was concerned, or the measures which could be taken to give the technologist a more liberal education.

Mr. A. Koestler addressed himself to the former question in a lecture, "Science—the Dominant Philosophy", to Section X, as did, to some extent, Dr. Magnus Pyke in his presidential address, "Scientific Understanding and the Chief End of Man", while the latter question was discussed by Mr. A. C. D. Peterson and Mr. F. Metcalfe in their contributions to a joint session of the two Sections on "The Cultured Technologist". Despite an aim that was essentially pragmatic, the programme entered into the philosophical, as well as the psychological, aspects to a considerable extent. Nor is this surprising, in view of the extent to which the several addresses also reflected other recent discussions of the broad theme. Both Sir Edward Boyle and Mr. Dancy quoted Sir Peter Medawar's Tizard Memorial Lecture, and the former and Mr. A. Koestler referred at length to Prof. T. S. Kuhn's *The Structure of Scientific Revolutions*.

The Master of Marlborough College argued that technology had a true and proper place as an integral part of a liberal education at the secondary level, and he had in mind, above all, the experience at school of making things which work—the direct and active experience of creative technology. Mr. Dancy maintained that at any level technology required a three-fold creative activity: of intellect, of hand and of eye. Accordingly, it required the skills of the scientist, the artist and the craftsman, and he suggested that the opportunities for creative intellectual work are probably greater to-day in applied than in pure science. He thought that the Nuffield Science Project would help to restore this critical and creative element to science in the schools, but he also emphasized that the technologist was inevitably immersed in human problems, and he suggested that we might find in the arts sixth forms some who would help to make up the present shortage of engineers and technologists.

When, on the following day, Dr. D. G. Christopherson looked at education, he began with a warning that pro-

jects designed to give sixth-formers some insight into the intellectual approach and activity of the technologist should beware of transferring to the schools work which was really the responsibility of the universities and colleges of technology. A good general education was absolutely essential for the technologist, and it would be highly undesirable to disturb the present balance in the sixth-form programmes, which are really much closer to the ideal specifications of departments of engineering and applied science than they are to the contemporary view of those of science and mathematics. He was more disturbed at the tendency of young people to turn away from science as a whole than at the failure to attract a sufficient proportion of the ablest to technology, and he noted the very marked trend for universities to accept a lower standard of entry from their science candidates than from their arts candidates.

Here Dr. Christopherson touched more generally on a point which Prof. R. F. Jones elaborated earlier in the year, with reference to physicists, in his Joseph Payne Memorial Lecture, "The Advancement of Learning, A.D. 1605–2005" (*Nature*, 206, 129; 1965). Like Prof. Jones, he referred to the failure to recruit science teachers of adequate quality in the schools, to which he attributes the trend against science in the sixth form. The task at hand is thus to give science as a whole more immediate impact, interest and relevance to ordinary experience, especially in the middle school, before the choice is made. It is also a matter of helping all young people to feel at home on unfamiliar ground, in a society which is bound to be shaped largely by technology. Dr. Christopherson reminds us that technology cannot function as a closed shop: it works only in a society in which a basic understanding of how it works is widespread.

The intellectual approach of the technologist, he points out, is essentially pragmatic—a characteristic which he shares more thoroughly with the pure scientist than is often realized. This approach is essentially exploratory and experimental, and the thinking required is almost always basically quantitative. Any technological advance involves an element of the creative, and in each of these characteristic attitudes of mind there is an element which is active rather than reflective. Any project to interest and stimulate the imagination of school-children in technology must take account of these characteristics as well as the needs and capacity of the pupils, and, like Mr. Dancy, Dr. Christopherson emphasizes that people and the relations between peoples form an essential part of the technological world. To an increasing extent, moreover, the technologist will derive his information from the social and biological sciences, from economics and psychology, as well as from the physical sciences.

It should thus be apparent that the contribution by Dr. Christopherson has some points in common with the following paper, in which Sir Edward Boyle discussed the philosophical aspects of the differentiation between pure and applied science, and whether, in fact, the differentiation was tenable. At the outset, Sir Edward made

the welcome admission that the concern with the supply of scientists and technologists in Britain's economy extended also to an adequate supply of technicians and craftsmen, and of educated men and women generally who were competent to take their place in patterns of production which were far more complex than in the past. He suggested that a clear distinction could no longer be drawn between pure and applied research, and that usually such a distinction simply reflected the motives of those who sponsored the research.

Accordingly, the question of the relations between research institutions and industry became an important aspect of the organization of civil research, and Sir Edward referred to the development in the United States of the research company as an intermediary between academic institutions and manufacturing industry. One such organization, developed by Columbia University and other universities on the east coast of the United States, is Associated Universities Incorporated. This is a non-profit corporation in the State of New York which acts as a buffer between the universities and the Federal Government, operating, for example, the Brooklyn National Laboratory on contract from the Atomic Energy Commission, and a national radio-astronomy observatory on contract from the National Science Foundation, and specializing in projects involving large facilities and staff. Sir Edward thought that this was a development that might be pursued in Britain.

It was at this stage, after remarking that the boundary between pure and applied science appeared to be subject to erosion from both sides, that Sir Edward quoted the comment by Sir Peter Medawar in his Tizard Memorial Lecture that the reverence for pure science in Britain was rather parochial and could not be sustained on philosophical grounds. The scientist, Sir Peter urged, measured the value of research by the size of its contribution to that huge, logically articulated structure of ideas, which is already, though not yet half built, the most glorious accomplishment of mankind. Admittedly, Sir Peter accepts Bacon's distinction between research that increases our power over Nature and research that increases our understanding of Nature. However, he was arguing for a critical investigation of the organization of research—not the allocation of administrative responsibilities, or the economics and logistics of science, but an investigation of the behavioural and intellectual structure of all that contributes to the enlargement of our knowledge and understanding of Nature. He was appealing for more critical and constructive thinking about all the factors that contribute to the productivity of scientists or are involved in the relations between research and its administration.

Sir Edward did not himself pursue this question, any more than he examined in further detail the organization of civil science, though in passing he emphasized the importance of working technologists remaining in close touch with the academic institutions where they were trained. Moreover, after suggesting that the relation between the pure and applied sciences was subtle and complex, he directed particular attention to the criteria used by scientists when judging the discoveries of their colleagues and the interpretations put on them—just as Sir Peter had done. Foremost among these criteria is their explanatory value—their generality, span of relevance or rank in the general hierarchy of explanation. Next is their clarifying power—the degree to which they can resolve what has hitherto been perplexing. Thirdly is

the degree of originality involved in the research—the novelty of the solution to which it led. Some weight is given to the elegance of the solution, the economy of thought and work by which it was achieved, or the magnitude of the obstacles that had to be surmounted. In Sir Peter's experience, while one scientist might say of the work of another, "How elegant", "How ingenious" or "How illuminating", its purity or usefulness was never mentioned.

Sir Edward Boyle thought the implications of this needed consideration. He believed that there were real differences between science and technology, although they had become more closely linked, but he suggested that the progress of modern technology should be seen in the perspective of the revolutionary changes in scientific thought during the early years of this century. It was in this context that he referred to the account of scientific revolutions which Prof. Kuhn gives in his book, *The Structure of Scientific Revolutions*, in which he challenges the idea that the advance of science is necessarily cumulative. On the contrary, Prof. Kuhn argues that from time to time it is necessary to change the meaning of established and familiar concepts as, for example, was necessary under the impact of Einstein's theory.

Sir Edward regarded three points made by Kuhn as being of special significance. First, we seem to be confronting frontiers of language and communication as well as of science. Kuhn considers that what occurs during a scientific revolution is not fully reducible to a misinterpretation of individual data, if only because these data are not unequivocally stable.

Secondly, he referred to Kuhn's emphasis on the role of youth in fundamental research, and also on the role of the 'invader'. The second point regarding youth was also emphasized by Prof. R. V. Jones in his Payne Memorial Lecture, while Sir Edward made the pertinent suggestion that it would be worth while examining in some detail the extent to which the 'invader' from another discipline had contributed to the development of science—including social science and industry.

Thirdly, Sir Edward directed attention to Kuhn's emphasis on "the unparalleled insulation of Nature's scientific communication from the demands of the laity and of everyday life". He agreed that it is important not to underrate the type of scientific activity that Prof. Kuhn describes and that it is a notable aspect of the growth of science in the modern world. However, Sir Edward pointed out that this kind of work at the outer frontiers of knowledge shifting over concepts or providing new constitutive principles is limited in extent. It involves only a few men of genius and perhaps only a dozen or two institutions in the world as a whole. While the balance of the research effort should always allow for such work, he challenged the idea that research should to any extent be isolated from the community. Sir Edward considered that there should be a wider understanding of research and of scientific activity both inside and outside Parliament, and that universities as a whole should be more responsive to the needs and activities of the community. It may be neither possible nor desirable to preserve rigid boundaries between pure and applied science, but although the boundary is being rapidly eroded it is not obliterated, nor is it likely to be. This should apply no less to the distinction between the scientist and technologist, and if Sir Edward dealt rather superficially with Prof. Kuhn's main argument, his address should at least contribute to a clear understanding

of the present-day relation between pure and applied science, between scientist and technologist, and the directions in which we might seek, in practice, to improve our organization.

BEHAVIOUR THERAPY

Experiments in Behaviour Therapy

Readings in Modern Methods of Treatment of Mental Disorders derived from Learning Theory. Edited by H. J. Eysenck. Pp. x+558. (Oxford, London and New York: Pergamon Press, 1964.) 100s.

The Causes and Cures of Neurosis

An Introduction to Modern Behaviour Therapy Based on Learning Theory and the Principles of Conditioning. By H. J. Eysenck and Dr. S. Rachman. Pp. xii+318. (London: Routledge and Kegan Paul, 1965.) 28s. net.

BEHAVIOUR therapy is a term for which one will look in vain in the psychological dictionaries—or the general text-books. It is also a term which carries considerable emotional overtones to many psychologists and, still more perhaps, to the average informed layman. Part of the disquiet is probably due to an at least partial misapprehension—the narrowing of the concept to cover only its most dramatic and in some ways most characteristic form, although it is by no means the most frequently used method among those that are subsumed under the general heading of behaviour therapy. I refer to aversive conditioning, or, as it is more commonly called, aversion therapy, the most widely publicized application of which has been in the treatment of alcoholism through the use of emetic drugs. 'Punishment', in the non-technical sense of the term, is a word often invoked by the hostile critic. Up to a point the imputation is just, but, as Prof. Eysenck points out in one of his own contributions to the present readings (p. 180), there are fundamental differences. In common with other varieties of behaviour therapy, aversive conditioning is based on the principles of learning theory; in other words, behaviour therapy represents a scientific approach to the problem of altering human behaviour "in the direction of greater sanity, greater happiness, and greater social usefulness" (foreword, p. ix).

Much of the basic literature on behaviour therapy has already been reprinted in Eysenck's *Behaviour Therapy and the Neuroses* (1960), to which *Experiments* is a sequel and companion volume. Understandably, therefore, the present emphasis is now on experimental studies and case reports, but there are also important papers of a general character, notably by the editor himself and by Joseph Wolpe, of the University of the Witwatersrand, to whom, incidentally, the book is dedicated. Wolpe's technique of 'systematic desensitization' is central to the first of the four parts into which the book is divided, although this section, in fact, carries the title "Reciprocal Inhibition"; the other three are devoted, respectively, to "Operant Conditioning", "Other Methods" and "Behaviour Therapy with Children". The essence of desensitization consists of, to quote the author, "presenting to the imagination of the deeply relaxed patient the feeblest item in a list of anxiety-provoking stimuli—repeatedly, until no more anxiety is evoked. The next item of the list is then presented, and so on, until, eventually, even the strongest of the anxiety-evoking stimuli fails to evoke any stir of anxiety in the patient". Wolpe claims that "... consistently ... a stimulus that evokes no anxiety when imagined in a state of relaxation will also evoke no anxiety when encountered in reality".

This is clearly a less tough-minded procedure than that of aversion therapy, and less open to criticism on grounds of callousness. It would, in fact, appear to tie up closely with the 'rational psychotherapy' of Albert Ellis which constitutes the major contribution under "Other Methods".

Rational psychotherapy is based on the view that, as Ellis puts it, "neurosis consists of stupid behaviour by an un-stupid person". Treatment, to quote again, consists of "teaching clients to organize and discipline their thinking". To the sceptic this may seem to amount to little more than the adjuration to take a grip on oneself which centuries of experience have shown to be ineffectual. Nevertheless, a high measure of success is claimed for the technique, as indeed for all the other methods described in the book. One feels that for therapy of this kind to be successful the patient must have a high level not only of motivation but also of sophistication. The same is probably true of the relaxation preparatory to desensitization, and of the imaginal processes involved in the actual desensitization. The work of Natadze and the Tbilisi school on the effect of imagined experience on perception of illusions has suggested that there are very large individual differences in the ability to evolve a 'set' on this basis, and one would welcome information on whether this might not also be true of receptiveness to desensitization.

Unlike some of Prof. Eysenck's previous volumes with similar titles, which were based entirely on work carried out at the Institute of Psychiatry or under its inspiration, the present collection of readings is culled from a wide variety of sources. In particular, the papers on operant conditioning are all of United States origin, chiefly from Indiana. The main difference from reciprocal inhibition is that while the latter claims to "cure the patient", operant conditioning usually "focuses on one specific symptom". This would appear to be more closely in line with Eysenck's often reiterated statement of his own theoretical position: that "there is no neurosis underlying the symptom, but merely the symptom itself". On this view, if the symptom is removed, the patient is cured. It is manipulation of behaviour along these lines that is the behaviour therapist's main concern. That this can be done is abundantly clear, and it is perhaps the individual role of the psychologist, *qua* psychologist and distinct from the psychiatrist, to devise and validate techniques of doing it.

The foregoing was written before *The Causes and Cures of Neurosis* came to hand, and most of what has just been said could stand equally as comment on the latter book: the authors, in fact, say many of these things in almost the same words. The book carries the sub-title "An Introduction to Modern Behaviour Therapy Based on Learning Theory and the Principles of Conditioning", and covers the same ground as the *Experiments*, with only a slight redistribution of emphasis. It may be said to stand midway between the *Experiments* and Prof. Eysenck's recent 'Pelican', and as such no doubt fulfils a different function. Nevertheless, reading the book, one has a strong feeling of *déjà vu*—the case material is familiar, and even the same photographs turn up again. Furthermore, despite its title, the book is still predominantly research-centred, and in no sense more patient-centred than Prof. Eysenck's other books. Writing in the *Experiments* (p. 3) of some work by Yates on the treatment of tics, Eysenck states: "It would be quite wrong to regard its main purpose as the alleviation of human suffering". In the next sentence but one he alludes to "the preoccupation of many psychiatrists with curing patients". It is perhaps unfair to wrench these phrases from their context, but, be that as it may, it is easy to understand why Eysenck attracts criticism as bitter as some of his own polemics against psychoanalysis. It is a pity that advocacy of behaviour therapy should so frequently be associated with attacks on other disciplines; equally it is a pity that behaviour therapy should, in some quarters at least, be equated with brain-washing, or worse. There is ample evidence in these books that it can be beneficial. Whether its theoretical foundations are unassailable is a matter for dispassionate examination. **BORIS SEMONOFF**

¹ *Fact and Fiction in Psychology* (Harmondsworth: Penguin Books, 1965). 5s.

DRUG DOSAGE IN LABORATORY ANIMALS

• Drug Dosage in Laboratory Animals

A Handbook. By C. D. Barnes and L. G. Eltherington. Pp. 302. (Berkeley and Los Angeles: University of California Press; London: Cambridge University Press, 1964.) 64s.

DRUG *Dosage in Laboratory Animals* is an attempt to summarize doses which have been used in the common laboratory species, collected from a great many published papers in the pharmacological literature. Although costly, it is unsatisfactory in a number of ways.

The only parts of the book which are intended to be read are a two-page introduction and a nine-page account of some factors which determine drug dosage and its variability. The remainder consists of more than 200 tables, each devoted to a single drug and each divided to give columns for mouse, rat, guinea-pig, rabbit, cat, dog and monkey.

Doses are given simply as figures, one for each route of administration of which a record has been found. The tables are subdivided into horizontal sections, of which the top one in each case presents lethal doses and the others are devoted, when appropriate, to different effects of the drug in question.

The information thus presented is so highly abbreviated and so incomplete that it is hard to imagine what type of reader might find it useful. For example, anyone wishing to inject nicotine into a cat will learn only that 0.02 mg/kg intravenously is a "behavioural" dose and that 0.2 mg/kg by the same route is a "cardiovascular" one. Much of the classical work of Langley on indirect pressor and ganglion-blocking actions of nicotine was performed in cats and the doses he used would surely be appropriate here, but the only references are to a paper on "a nicotinic receptor in the CNS related to EEG arousal" (1961) and to another entitled "Homatropine Methyl-bromide: a Pharmacological Re-evaluation" (1952). Again, the only information recorded about the action of histamine in the cat is that 0.005 mg/kg intravenously is a "cardiovascular" dose. No references at all are given in this case, and one is left wondering whether the work of Dale and his colleagues on vasodilatation due to histamine and on its secondary pressor effect has been omitted from the text-books in California. Let it be added that no national bias can be detected in the matter of omissions of classical work. Doses used in the important papers of Eggleston and Hatcher on apomorphine, of Cattell and Gold on digitoxin and ouabain, and of V. E. Henderson on atropine are also absent and no references to their work are to be found.

It is, perhaps, unnecessary to point out that information does not exist to fill most of the empty spaces which the terse tabular presentation adopted creates; about 90 per cent of the table area consists of vacant duplicated rectangles. Yet, even when published dose figures do exist and are reasonably accessible from reviews and bibliographies, they have often not been included. Thus there are glaring omissions from the tables for angiotensin, digoxin, histamine, ouabain and noradrenaline (which is indexed under four names, but not as nor-adrenaline).

Most of the doses which are given carry a reference number, and one of the more valuable features of the book is its bibliography, which has 819 references, often to the recent literature. However, for a few drugs no literature references are given, all doses listed having been obtained from manufacturers (for example, digoxin and chlorothiazide). The selection of drugs in the 200 tables is comprehensive and includes unfamiliar recent compounds such as dibozane, hexocyclium, mebutamate, mepenzolate and proflidil. It is, therefore, surprising that oxytocin and vasopressin are omitted.

Further criticism must be made of the sections which list doses for use of some drugs *in vitro*. These give scarcely any information, even in the case of compounds which have been much investigated using isolated tissues, such as angiotensin, calcium chloride, carbachol, decamethonium, hexamethonium, methacholine, morphine, hyosine and tetramethyl ammonium. When doses are given, they are often uncritically high and unsubstantiated by references. If these tables could not be made more complete and more critical, they would have been better omitted. A table in the appendix which sets out the composition of physiological saline solutions for isolated organ work also has important omissions, containing no mention of Krebs-Henseleit solution, of Ringer's solutions for frog tissues, or of de Jalon's solution.

A comprehensive and critical collection of drug doses for use in the laboratory when performing animal experiments would meet a real practical need and it is a pity that this handbook falls short in many respects of what is required.

J. A. PARSONS

TEACHING IMMUNOLOGY

Immunology for Students of Medicine

By J. H. Humphrey and Prof. R. G. White. Second edition. Pp. ix + 498. (Oxford: Blackwell Scientific Publications, 1964.) 47s. 6d. net.

THE present-day medical student is fortunate in having available some informative and stimulating, and reasonably short, text-books on the pathological sciences, which not only set out basic principles but also indicate growing edges.

Immunology for Students of Medicine by two leaders in the immunological field (first published in 1963 and already now in its second edition) provides an excellent example.

The book has twelve chapters, starting with a succinct historical introduction. An account of innate immunity is followed by descriptions of the nature of circulating antibodies and how they are produced, and of the nature of antigens (with a useful amount of chemistry in both cases). Then comes a chapter on detection and measurement of antigen and antibody, followed by examples of practical uses of immunization in man. The scene then changes to hypersensitivity of the immediate and delayed types, succeeded by an account of immunological tolerance, and the final chapter on auto-immunity and its relation to human disease. Perhaps the most illuminating parts are those concerning the immunoglobulins; the mechanism of antibody production; delayed hypersensitivity; immunological tolerance; and auto-antibodies.

The style of writing is light and attractive, the production excellent, the headings and sub-headings well arranged. The inclusion of a glossary (a little expansion might be considered) is most considerate, indicating incidentally that immunologists are not averse to poaching where definitions are concerned.

The authors have wisely used detail mainly to illustrate principles (though allowing themselves more scope in their examples of prophylactic immunization and in their descriptions of diseases in which auto-immune phenomena are evident), thus avoiding a text-book on immunological diseases, and making their book very readable. Yet the human aspects are constantly emphasized. Recognizing the difficulties of the student (not to forget those of the writer) in this rapidly expanding subject, and his tendency to be scared away, they present him with conclusions where they are justified, but tell him at the same time what is not known; thus the student has stable platforms on which to rest his faith, though warned that these may be jumping-off stages for new advances. By this policy the authors should interest and instruct most of their

readers, inspire the more enquiring, and even make a few future immunologists.

In this outstanding book it is difficult to pick on faults or give suggestions for improvement. But with an eye to the next edition, the section on cellular immunity might be expanded somewhat with advantage; the allusions to the immunological aspects of tuberculosis need certain changes of emphasis in the light of recent knowledge; the general definitions of allergy, etc. (on p. 287) could be tidied up. One wonders whether the commendable desire not to clutter up the book with references (restricted at present to a list of suggested further reading), merely giving names in the text in the manner of some encyclopaedias, has gone too far.

Although Humphrey and White have had medical students primarily in mind, there is no doubt that their book should have a much broader appeal; on the one hand to practising clinicians and on the other to biochemists, microbiologists, geneticists and general biologists. With the expansion and continual re-tooling of immunological research, the number of investigators requiring at least to know the language of this branch of science must be increasing.

P. D'ARCY HART

DIAGNOSIS OF PARASITES

Microscopic Diagnosis of the Parasites of Man

By Dr. Robert B. Burrows. Pp. xii+328. (New Haven and London: Yale University Press, 1965.) 15 dollars; 105s. net.

THE author of *Microscopic Diagnosis of the Parasites of Man* is head of the Parasitology Section of the Wellcome Research Laboratories in Tuckahoe, New York, and the text of the book certainly demonstrates his wide experience of the difficulties of diagnosis of parasitic infections. The book has, he says, been written to help the inexperienced technician or the student to broaden his knowledge and it can be said at once that the text of the book will do this. Part 1 of the book deals with infections that are diagnosed by examination of the faeces, urine and sputum. It contains a valuable chapter on routine examinations and staining, and another on techniques for concentrating the eggs or larvae of helminths or the cysts or trophozoites of Protozoa, in such a manner that they are not altered by the treatments given to them. A useful chapter on the artificial cultivation of Protozoa precedes chapters on miscellaneous helminthological techniques, on the oral and genital flagellates, the oral and intestinal amoebae and other intestinal Protozoa. The intestinal helminths, Trematoda, Cestoda and Nematoda, are next dealt with, before Part 2 considers infections diagnosed by examination of blood and tissues and the techniques used to deal with these. An appendix deals with the parasites of dogs and cats and the book concludes with an author and a subject index. Each chapter concludes with a valuable bibliography, and these references to the literature will enable the reader to extend his knowledge still further.

While the text will be found invaluable, it cannot be said that the illustrations reach the high standard attained by other publications of this kind. The author rightly says that many technicians find it difficult to compare a line drawing with a specimen seen through the microscope and he has endeavoured to make this easier by using photographs of helminths throughout and elsewhere by using drawings. Unfortunately, in several examples, the material supplied by the author has not been clearly reproduced. Most of the figures bring out the very different sizes of the objects figured, and the magnifications of these are given. This is a valuable feature of the book, because it largely overcomes another difficulty that many students have—that of appreciating how big or, often, how very small are the objects they are looking for. Fig. 1,

for example, assembles in one illustration the comparative sizes of the eggs, larvae and cysts of some representative parasites, and this figure is clearly reproduced. Some other figures, on the other hand, are not. Fig. 8, for example, shows only pale ghosts of the unstained trophozoites of such important species as *Entamoeba coli* and *Entamoeba histolytica* and other intestinal amoebae. Unsatisfactory also is the photograph of *Demodex canis* in Fig. 100. With these and a few other exceptions, the illustrations are effective and useful and the failure of those mentioned is clearly not the fault of the author, to whom the sympathy of the reader will surely be extended. In other respects the book is attractively produced. The author is to be congratulated on its text, which will make the book a useful addition to the library of any laboratory concerned with the diagnosis of parasitic infections of man.

G. LAPAGE

AN INTRODUCTION TO MOLECULAR GENETICS

Microbial and Molecular Genetics

By Prof. J. R. S. Fincham. (Modern Biology.) Pp. x+149. (London: English Universities Press, 1965.) 15s. net.

DURING the past few decades many major advances have been made in the new science of molecular genetics, and the flood of original contributions in this field continues unabated. Apart from one or two advanced texts and symposia on this subject there is, to my knowledge, no introductory account. *Microbial and Molecular Genetics*, the most recent addition to the "Modern Biology" series, is intended to provide just such an introduction for biology students.

There are two obvious ways of treating the material dealt with in this book. The first takes as its starting point the physico-chemical structure of the genetic material (DNA) and from this develops the concept of the gene as a unit of structure, mutation and function. The second is the historic approach leading from genetic analysis to DNA. In choosing a path somewhere between these two, Prof. Fincham has had to sacrifice a certain degree of continuity in his text so that the student may find little to connect the material of one chapter with the next.

A short introduction which describes briefly the basic cellular processes of mitosis and meiosis is followed by a clear account of the structure and replication of DNA. It is perhaps just a little unfortunate that the manner of presentation does not lay greater emphasis on the evidence on which the identification of DNA as the genetic material rests, since historically the significance of genetic transformation with DNA was only slowly recognized, yet this and other evidence provide one of the cornerstones of molecular biology. In a chapter on mapping the genetic material, the methods of genetic analysis in a micro-organism with a sexual cycle are clearly explained and illustrated with data from *Neurospora*. The three principal mechanisms of genetic exchange between bacteria; conjugation, transduction and transformation are described and the way they can be used to construct genetic maps is explained. Finally, a brief and non-technical account is given of the genetics of bacteriophage. The chapter on mutation contains a good account of the molecular basis of chemical mutagenesis. In the chapters on gene action and its regulation, the principal concepts are clearly presented and here, as elsewhere, the author makes frequent and well-chosen use of experimental data from the literature to clarify each step in his argument. A final chapter is devoted to an account of present-day knowledge of episomes.

The text is written without reference to source material, but each chapter is provided with a carefully selected list

of references for further reading which the student will find extremely valuable.

To provide an account of microbial and molecular genetics within the compass of a slim volume which is both popular and up to date without, on one hand, oversimplifying, or, on the other hand, condensing to the point of incomprehensibility, is a difficult task. Prof. Fincham has come very close to achieving this task. But the uninitiated student would do well to follow the advice of the general editor of this series when he says, in the foreword, "This is a book that deserves and indeed requires careful reading more than once".

S. W. GLOVER

GEOLOGY OF GRANITE

Geology of Granite

By Prof. E. Raguin. Translated from the second French edition by E. H. Kranok and P. R. Eakins, with Jean M. Eakins. Pp. xxi+314. (London and New York: Interscience Publishers, a Division of John Wiley and Sons. 1965.) 68s.

ENGLISH-speaking students of geology whose knowledge of continental points of view on the granite problem has largely been derived from review articles, such as Prof. Read's "Meditations", now have the advantage of this translation of E. Raguin's often quoted text-book.

Geology of Granite, as implied by the title, aims at providing a comprehensive account of the geology of granite, including mineralogy, petrography and occurrence. The main interest, however, for most readers, will be in following the position of Prof. Raguin on the subject of granite petrogenesis, backed by many illustrative examples and an international reference list. The author bases his thesis on the recognition of two great categories of granite, circumscribed massifs (*massifs circonscrits*) and anatectic granites with migmatites; the former have sharp limits and appear to be replacements or displacements of their country rock, while the latter are vast diffuse granites which impregnate immense volumes of the Earth's crust. These diffuse granites are the result of advanced mobilization of deep-seated zones, a mobilization which resembles a partial fusion. The existence of connecting links between these two main divisions is recognized but not discussed in detail. This is unfortunate, as these connecting links are important to the concept of a granite series, a concept which evidently has the support of Prof. Raguin, but which is not developed. Indeed, the difficulty of linking the phenomena described together to form a series is well evident, and in my opinion stems from the fact that much granite has originated at depths beneath those at present exposed by erosion, and thus the connecting links of the granite series, if present, are not readily observable. It is a pity that in these matters the reader, after being introduced to many of the fundamental facts and ideas, is left to draw his own conclusions and infer those of Prof. Raguin, whose attitude throughout is one of extreme caution.

An interesting account is given of the relations between salic volcanic rocks and granitic plutons. The sub-volcanic granites are regarded as exceptional and though the relation of volcanism to plutonism is regarded as an unsolved problem, the hypothesis that there is no direct relationship of volcanic rocks and major plutons in the Earth's crust seems to be preferred. No reference is made to W. Q. Kennedy's concept of volcanic and plutonic associations or the more fruitful one of orogenic and anorogenic associations, though the common coincidence of granite and orogenesis is emphasized.

The translation is of the second French edition and there are some references quoted up to 1961. It is unfortunate that little reference is made to experimental evidence on the origin of granite, and the early work of Tuttle and

Bowen on the granite system is but briefly mentioned. The fact that this is a translation of a text originally written some years ago is also evident in various other ways. Thus, in the section dealing with the mineralogy of granite, the feldspars receive only summary treatment and the amphiboles are dealt with in five lines. Also, in recent years, there has been something of a revolution in thinking on the relation of the Earth's mantle to many problematical aspects of geology, and the incorporation of this new information, together with such other modern developments as radiometric dating and isotope geology, would completely alter many aspects of the book.

These criticisms aside, we must be grateful to the translators and publishers for making this text readily available. The granite controversy has often been bedevilled by the volume of words contributed by the disputants; this, really, is symptomatic of the fact that the concepts involved are often as nebulous as the rocks themselves. This difficulty is perhaps even greater in a translation, but nevertheless the translators are to be congratulated on producing a readable and accurate text.

P. E. BROWN

A BROAD MAGNETIC FIELD

Proceedings of the International Conference on Magnetism, Nottingham, September 1964

Pp. xvi+878. (London: The Institute of Physics and The Physical Society, 1965.) 168s.

ALTHOUGH this, the sixth international conference on 'Magnetism', attracted fewer participants than the fifth at Kyoto (1961), and the same number of papers was presented at each, yet the Nottingham conference was undoubtedly extremely successful. This fact and the greatly increased realization of the importance of magnetism as a branch of solid-state physics are clearly shown in the volume under review. Of the 228 papers delivered, the book gives 225, with one paper (by Lowde) promised, and two by other authors given in title only.

The titles of the sessions of the conference have been retained for the chapter headings, and, disregarding repetitions, these are: theory, transition metals, critical phenomena, Fermi surface, metals and alloys, rare-earth metals, neutron diffraction, spin waves, covalency and exchange effects, ferro- and antiferro-magnetism, nuclear magnetic resonance, resonance and relaxation, non-metallic ferro- and antiferro-magnetics, oxides and compounds, miscellaneous oxides, haematite, ferrites, garnets, magnetization processes, anisotropy, hard magnetic materials, thin films, electron microscopy.

Most papers, as published, show some expansion relatively to the matter actually delivered *viva voce*, but a few authors have merely re-submitted the summary circulated to all members before the first meeting. In many cases a more detailed paper is promised in a footnote. The discussions following each paper at the conference have been reported in, regrettably, very few cases, regrettably briefly, and often without the author's reply.

Now, I heard only one paper which excited neither question nor comment. Therefore, the inadequate reporting of the discussions seems to be attributable to a weakness in the recording system used, or the lack of circulated sheets for submission of comments in writing. This last was, in fact, a noticeable omission at the meeting.

Comparing the subjects of the papers with those of the two relevant volumes which appeared after the Kyoto conference reveals no world-shaking advance in magnetism, but, instead, steady progress throughout nearly the whole range of the subject. Papers appear on some techniques for the first time at these conferences; for example, the use of electron-microscopy and of metallic whiskers for domain investigation. On the other hand, interest in magnetism of rocks seems to have subsided.

The range of topics coming within the scope of 'magnetism' as a subject is now very wide, and this is fully represented in this book. Readers will, therefore, make many different selections of the most interesting or most useful paper. In spite of the much-increased availability of Russian literature these days, many readers will regard the review of "Recent Work by Soviet Physicists on the Theory of Magnetism" by Vonsovskii as particularly valuable, not least because of the 188 references given. Following a similar paper by the same author at Kyoto, this would appear to be becoming a regular feature of these conferences. The question arises whether similar reviews from elsewhere might not also be useful.

Those of us who saw the remarkable film of spin-waves in chromium tribromide shown by Dillon, Walker and Remeika will be a little disappointed by the presentation of the photographs in their paper, but one must accept the inevitable limitations.

I feel compelled, however, to criticize the presentation of the book. The enormous size (30.7 × 22.5 × 6.0 cm) and weight (2.75 kg) make it most inconvenient to read. This is a book to be studied, not referred to as is a dictionary. Dimensions and weight are, therefore, important. If each successive paper had been begun on the same page as its predecessor, and if two whole pages had not been used for each chapter heading, the bulk of the book would have been much reduced. A count of the first 100 pages reveals the equivalent of more than 20 blank pages, indicating a possible reduction of 20 per cent in size and weight. This might also have affected the price, which is nearly 50 per cent higher than that of the Kyoto proceedings, in spite of the fact that the latter also contained 400 pages on the simultaneous crystallography conference. Finally, was a cloth binding (not paper) essential, and was one volume (not three) inevitable?

Ziman¹ has cast doubt on the value of conference reports, particularly beyond the first year or two after publication. This report, however, justifies itself as well as may be. Because of the wide range of the papers given, it provides an introduction to the latest activity in many fields, and to the immediately preceding papers leading up to it. Workers compelled by the trends of their own research to cross for a time the boundary into other fields should thus find the book providing a valuable short cut to the most recent work.

J. H. DAVIS

¹ Ziman, J. M., *Bull. Inst. Phys.*, 13, No. 7, 196 (1962).

OPTICAL ANALOGUE METHODS IN DIFFRACTION STUDIES

Optical Transforms

Their Preparation and Application to X-ray Diffraction Problems. By Dr. C. A. Taylor and Prof. H. Lipson. Pp. x+182+54 plates. (London: G. Bell and Sons, Ltd., 1964.) 45s. net.

OPTICAL transform is the name given to the pattern of diffracted light given by an object such as a group of diffracting centres, a name based on the fact that the optical diffraction pattern corresponds to the Fourier transform calculated mathematically. In short, *Optical Transforms* is about the use of optical diffraction methods in place of mathematical methods for demonstrating the basic principles of diffraction and for solving diffraction problems. The experience of the authors has been mainly in crystal structure determination from X-ray diffraction patterns, but optical analogue methods are quite generally valid for all diffraction problems.

The book starts with a historical survey of the development of these methods, from their introduction by W. L. Bragg in 1939, through their application to the solution of fully ordered crystal structures (notably sodium benzyl penicillin), to recent applications to disordered or distorted structures. There follow chapters on basic

concepts and the mathematical treatment of diffraction, on the optical apparatus necessary for observing and recording diffraction patterns (culminating in the large diffractometers used by the authors in Manchester), and on the preparation of 'masks'—sets of holes in opaque screens, representing atomic arrangements.

In the main part of the book the characteristics of optical transforms and their use in interpreting the X-ray diffraction patterns of crystals are considered in detail. The exposition here (and, indeed, throughout the book) is admirably clear, and all the points made are lavishly illustrated by many beautifully reproduced diffraction patterns. Authors and publisher deserve the highest praise for putting all this beautiful experimental material so clearly before the reader.

The educational value of this section of the book can hardly be exaggerated. By studying the examples and contemplating the diffraction patterns, the reader acquires a valuable appreciation of the reciprocal relation between the characteristics of an arrangement or pattern and those of its diffraction pattern; one grows, so to speak, a Fourier eye, and acquires some facility in the art of mentally turning things inside out—a facility which can scarcely fail to play a useful part in solving the practical problems of interpretation which confront the X-ray crystallographer. The authors justly say that the optical transform approach to diffraction emphasizes the basic physics, and observe that "this is particularly important in X-ray applications where there has been a tendency to over-emphasize the mathematical aspects". The choice of methods of approach to problems is to some extent a matter of personal taste; certainly, to those who prefer a physical rather than a mathematical approach it is refreshing to appreciate, for example, the broad principles of intensity statistics in terms of the characteristics of diffraction patterns rather than as the outcome of equations, or to see in a diffraction pattern a visual demonstration of the presence and orientation of a benzene ring or a chain of atoms.

In the latter part of the book the lessons learnt from optical analogues are applied to the solution of crystal structures from X-ray diffraction patterns; there is much useful information on practical procedures together with some detailed examples of structure determination. One of the more striking fruits of this approach is the recognition of two distinct features, such as a ring and a chain, in the same molecule, since the two features stamp their hall-marks as if independently on the diffraction pattern. The authors might have added even more striking examples: the transform concept has played an important part in revealing the helical conformation of chain polymers, notably synthetic hydrocarbons and nucleic acids—though optical analogues have been little used.

Crystallographers will ask whether optical analogue methods can play a useful part in their work, apart from the undoubted educational value. One of the reasons for the introduction of these methods—to relieve the crystallographer of the heavy burden of computation—is (for fully ordered structures) no longer operative in this age of electronic computers; and for solving the initial structure-puzzle the limitations of the methods tend to restrict their usefulness to molecules with marked regularities; nevertheless, wherever trial and error methods have to be used, the testing and above all the rational modification of trial models can be done rapidly and effectively by optical analogue methods. This book leaves one with the impression that the optical methods may be of most value in the study of distorted or partly disordered structures such as certain alloys and semi-crystalline polymers, where mathematical treatment is daunting even in the computer age. But all crystallographers, and indeed all diffractionists, whether or not they are likely to use optical analogue methods themselves, are strongly advised to read this book; they will get much pleasure and profit from it.

C. W. BUNN

Advances in Radiation Biology

Vol. 1. Edited by Leroy G. Augenstein, R. Mason and H. Quastler. Pp. x+285. (New York and London: Academic Press, 1964.) 88s.

ADVANCES in *Radiation Biology* cannot be recommended as light holiday reading. The editors say "the need is clear for a series of review articles designed to keep more of us in contact with branches of the subject other than our own", but there is a danger that what they offer will have the opposite effect, of convincing the average radiation biologist that he has no hope of grasping the physico-chemical fundamentals of his subject and of driving him to seek a soft option in some corner where such knowledge is unnecessary. For the brave, however, and none but the brave, this book is just right, though the title is an over-simplification and it should be called "Advances in Radio-bio-physico-chemistry".

It starts with a comparatively easy and clear account of the radiolysis products of water, with valuable tables of rate constants for reactions of the electron, the hydrogen atom and the hydroxyl radical, which should help to keep speculators on the rails; but even in this first chapter it foreshadows the horrors that are to come by saying, "a knowledge of rate constants . . . is only a small part of the problem. The reaction of a radical with a molecule produces a second radical. The nature of the new radical is not even known for most of the reactions given in Tables I, II and III". This prepares us, somewhat inadequately, for shocks such as the table on p. 180 showing the nineteen irradiation products of such an unreactive substance as α -aminobutyric acid.

The second chapter, on photosynthesis, though excellent, should not be included unless the title is further expanded to "Radio- and Photo- . . . etc.", because the irrational but useful convention is fully established that 'radio-' refers to 'ionizing' radiation and 'photo-' to visible light. Nevertheless, biologists who wish to get a glimmering of applied quantum mechanics, beautifully expounded, would do well to read it. After this it is restful to read that tritium has an atomic weight of three and to meet the nice compact table on pp. 110-112 summarizing the depressed growth and suicide that it causes in all sorts of biological material. The next chapter goes to the heart of the radiobiological problem, collecting all those puzzling cases where effects are produced by far smaller doses of radiation than can possibly be explained. The next chapter, on amino-acids, will break the back of all but the toughest reader, not with high-brow physics, but with its mass of empirical chemistry. The final chapter uses all the previously enunciated concepts to discuss the inactivation of enzymes, after which the reader goes on holiday with some nice non-controversial light reading such as Beilstein. J. ST. L. PHILPOT

The Systematic Identification of Organic Compounds

A Laboratory Manual. Fifth edition. By R. L. Shriner, R. C. Fuson and D. Y. Curtin. Pp. ix+458. (New York, London and Sydney: John Wiley and Sons, Inc., 1964.) 59s.

THE fifth edition of this well-known publication on the qualitative analysis of organic compounds will continue to be warmly welcomed by the student and research worker as a supreme laboratory vade-mecum.

The fundamental chemical and physical tests leading to the identification of organic compounds are lucidly described. Much attention is directed to the manner in which laboratory reports on unknown substances should be represented and there are a number of most useful examples of the final sequential system of deductive reasoning which leads to the positive identification of the unknown compound.

Excellent chapters are provided on instrumental applications, the methods of infra-red and ultra-violet

spectroscopy being succinctly demonstrated. Nuclear magnetic resonance phenomena are concisely explained with most adequate references to more advanced textbooks.

The book concludes with nearly a hundred pages of physico-chemical data associated with derivatives of hydrocarbons, amines, nitriles, etc.; a most valuable compilation, providing confirmatory evidence of identification of unequivocal character. The "Sets" of problems for students which the authors have provided must prove a most valuable educational aid. Like its predecessors, this is a first-class book for teachers and students in schools, technical colleges and universities.

D. T. LEWIS

Proceedings of the International Symposium on Chemotherapy of Cancer

Lugano, 28th April to 1st May, 1964. Edited by Prof. Placidus A. Plattner. Pp. ix+324. (Amsterdam, London and New York: Elsevier Publishing Company, 1964.) 75s.

IN the *Proceedings of the International Symposium on the Chemotherapy of Cancer*, organized by the Swiss Academy of Medical Sciences, the editorial hand of Prof. P. A. Plattner has rested lightly on his expert contributors. This results in a volume in which the individual styles of the authors are retained, as is the sense of occasion, so that, provided the reader approaches the book in the spirit in which it has been compiled, he is carried easily through it.

The subject of the symposium is well and authoritatively covered. There are chapters on the biochemistry and modes of action of alkylating agents and antimetabolites, on clinical experience with established drugs, laboratory and clinical reports on a number of newer ones, and a section entitled (perhaps unhappily) "Selective Toxicity of Anti-Tumor Compounds", in which the toxic effects due to non-specificity of action are clearly and frankly set out. It is hoped that the decision of the organizers to include in this symposium a paper on polyoma virus and its oncogenic action will not result in an interesting review article being missed by some who would profit from it.

This, clearly, is a book to be read by all those engaged with problems of the treatment of cancer, whether in the laboratory or in the ward. It is also to be commended to workers in the basic sciences who, by reading what is written, noting what is not, and examining critically each assumption (both stated and implied) made throughout the book, may well find pointers to help them to provide the fundamental information on which a rational and successful therapy may be based. D. C. ROBERTS

Elements of the Natural Movement of Population

By Egon Vielrose. Translated from the Polish by I. Dobosz. Translation edited by H. Infeld and P. F. Knightsfield. Pp. 288. (London and New York: Pergamon Press; Warszawa: Panstwowe Wydawnictwo Ekonomiczne, 1965.) 70s. net.

ELEMENTS of the *Natural Movement of Population* is neither an introduction to demographic analysis nor an outline of the development of world population nor an attempt to explain differential rates of growth. It consists of an extensive commentary on selected vital statistics, dealing with population structure, fertility, nuptiality and mortality. It is largely derivative and little attempt is made to take account of post-war advances in demography. Moreover, the vital statistics discussed are used rather uncritically and with little attempt to assess the adequacy of the system by which they are collected. To give but one random example, on p. 146 the average numbers of live births per female in Angola and Australia are given side by side without any indication of the relative reliability of the data. E. GREBENIK

BIOLOGICAL AND MENTAL EVOLUTION: AN EXERCISE IN ANALOGY*

By ARTHUR KOESTLER

ALLOW me to take you on a ride on the treacherous wings of analogy, starting with an excursion into genetics. Creativity is a concept notoriously difficult to define; and it is sometimes useful to approach a difficult subject by way of contrast. The opposite of the creative individual is the pedant, the slave of habit, whose thinking and behaviour move in rigid grooves. His biological equivalent is the over-specialized animal. Take, for example, that charming and pathetic creature, the koala bear, which specializes in feeding on the leaves of a particular variety of eucalyptus tree and on nothing else; and which, in lieu of fingers, has hook-like claws, ideally suited for clinging to the bark of the tree—and for nothing else. Some of our departments of higher learning seem expressly designed for breeding koala bears.

Sir Julian Huxley has described over-specialization as the principal cause why evolution in all branches of the animal kingdom—except man's—seems to have ended either in stagnation or in extinction. But, having made his point, he drew a conclusion which you may find less convincing. "Evolution," he concluded, "is thus seen as an enormous number of blind alleys with a very occasional path to progress. It is like a maze in which almost all turnings are wrong turnings¹." With due respect, I think this metaphor is suspiciously close to the old-fashioned behaviourist's views of the rat in the maze as a paradigm of human learning. In both cases the explicit or tacit assumption is that progress results from a kind of blind man's buff—random mutations preserved by natural selection, or random tries preserved by reinforcement—and that that is all there is to it. However, it is possible to dissent from this view without invoking a *deus ex machina*, or a Socratic *daimon*, by making the simple assumption that, while random events no doubt play an important part in the picture, that is not all there is to it.

One line of escape from the maze is indicated by a phenomenon known to students of evolution by the ugly name of paedomorphism, a term coined by Garstang² some forty years ago. The existence of the phenomenon is well established; but there is little mention of it in the text-books, perhaps because it runs against the *Zeitgeist*. It indicates that in certain circumstances evolution can re-trace its steps, as it were, along the path which led to the dead-end and make a fresh start in a more promising direction. To put it simply, paedomorphism means the appearance of some evolutionary novelty in the larval or embryonic stage of the ancestral animal, a novelty which may disappear before the adult stage is reached, but which reappears in the adult descendant. This bit of evolutionary magic is made possible by the well-known mechanism of neoteny, that is to say, the gradual retardation of bodily development beyond the age of sexual maturity, with the result that breeding takes place while the animal still displays larval or juvenile features. Hardy³, de Beer⁴ and others have pointed out that if this tendency toward 'prolonged childhood' were accompanied by a corresponding squeezing out of the later adult stages of ontogeny, the result would be a rejuvenation and de-specialization of the race which would thus regain some of its lost adaptive plasticity. But of even greater importance than this re-winding of the biological clock is the fact that in the paedomorphic type of evolution selective pressure operates on the early, malleable stages of ontogeny. In contrast to this, gerontomorphism—the

appearance of novel characters in the late-adult stages—can only modify structures which are already highly specialized. One is accordingly led to expect that the major evolutionary advances were due to paedomorphism and not to gerontomorphism—to changes in the larval or embryonic, and not in the adult, stage.

Let me give an example, which will make clearer what I am driving at. There is now strong evidence in favour of the theory, proposed by Garstang² in 1922, that the chordates, and thus we, the vertebrates, descended from the larval state of some primitive echinoderm, perhaps rather like the sea-urchin or sea-cucumber. Now an adult sea-cucumber would not be a very inspiring ancestor—it is a sluggish creature which looks like an ill-stuffed sausage, lying on the sea-bottom. But its free-floating larva is a much more promising proposition: unlike the adult, it has bilateral symmetry, a ciliary band presumed to be the forerunner of the neural fold, and other sophisticated features not found in the adult animal. We must assume that the sedentary adult residing on the sea-bottom had to rely on mobile larvae to spread the species far and wide in the ocean, as plants scatter their seeds in the wind; and that the larvae, which had to fend for themselves, exposed to much stronger selective pressures than the adults, gradually became more fish-like; and lastly became sexually mature while still in the free-swimming, larval state—thus giving rise to a new type of animal which never settled on the bottom at all and altogether eliminated the senile, sessile cucumber stage from its life-history.

It seems that the same re-tracing of steps to escape the dead-ends of the maze was repeated at each decisive evolutionary turning-point—the last time, so far as we know, when the line which bore our own species branched off from some ancestral primate. It is now generally recognized that the human adult resembles more the embryo of an ape than an adult ape. In both, the ratio of brain-weight to body-weight is disproportionately high; in both, the closing of the sutures of the skull is retarded to allow for further brain growth. The back to front axis through man's head—the direction of his line of sight—forms an angle of ninety degrees with his spinal column; a condition which, in apes and other mammals, is only found in the embryonic stage. The same applies to the angle between the uro-genital canal and the backbone, which accounts for the singularity of the human way of mating. Other embryonic—or, to use Bolck's⁵ term, foetalized—features are the absence of brow-ridges, scantiness of body-hair, retarded development of the teeth, and so on. As Haldane⁶ has said: "If human evolution is to continue along the same lines as in the past, it will probably involve a still greater prolongation of childhood and retardation of maturity. Some of the characters distinguishing adult man will be lost." But there is a reverse to the medal, which Aldous Huxley gleefully showed us in *After Many A Summer*: artificial prolongation of the absolute life-span of man might provide an opportunity for features of the adult ape to re-appear in Methuselah. But this only by the way.

The essence of the process which I have described is a retreat from highly specialized adult forms of bodily structure and behaviour to an earlier, more plastic and less committed stage—followed by a sudden advance in a new direction. It is as if the stream of life had momentarily reversed its course, flowing uphill for a while, then opened up a new stream-bed—leaving the koala bear stranded on its tree like a discarded hypothesis. We have now reached the crucial point in our excursion,

* Substance of an address delivered on September 18 at the Bicentennial Celebration commemorating the birth of James Smithson, held in Washington during September 16–18 (see *Nature*, 208, 320; 1965).

because it seems to me that this process of *reculer pour mieux sauter*—of drawing back to leap, of undoing and re-doing—is a basic feature of all significant progress, both in biological and mental evolution.

It can be shown, I think, that these two types of progress—the emergence of biological novelties and the creation of mental novelties—are analogous processes on different levels of the developmental hierarchy. But to demonstrate the connexion we must proceed stepwise from lower to higher organisms. One of the fundamental properties of living organisms is their power of self-repair, and the most dramatic manifestations of this power are the phenomena of regeneration (which Needham⁷ called “one of the more spectacular pieces of magic in the repertoire of living organisms”). Primitive creatures, like flatworms, when cut into slices, can regenerate a whole animal from a tiny fragment; Amphibia can regenerate limbs and organs; and once more the ‘magic’ is performed by *reculer pour mieux sauter*—the regression of specialized tissues to a genetically less committed, quasi-embryonic stage, a de-differentiation or de-specialization followed by a re-differentiation.

Now the replacement of a lost limb or lost eye is a phenomenon of a quite different order from the adaptive processes in a normal environment. Regeneration could be called a meta-adaptation to traumatizing challenges. The power to perform such meta-adaptations manifests itself only when the challenge exceeds a critical limit and can only be met by having recourse to the genetic plasticity of the embryonic stage. We have just seen that the major phylogenetic changes were brought about by a similar retreat from adult to embryonic forms. Indeed, the main line of development which led up to our species could be described as a series of operations of phylogenetic self-repair: of escapes from blind alleys by the undoing and re-moulding of maladapted structures.

Evidently, self-repair by the individual produces no evolutionary novelty, it merely restores the *status quo ante*. But that is all the individual needs in order to regain its normal adaptive balance in a static environment (assuming that the traumatizing disturbance was only a momentary one). Phylogenetic ‘self-repair’, on the other hand, implies changes in the genotype to restore the adaptive balance in a changing environment.

As we move toward the higher animals, the power of regenerating physical structures is superseded by the equally remarkable power of the nervous system to re-organize its mode of function. (Ultimately, of course, these reorganizations must also involve structural changes of a fine-grained nature in terms of circuitry, molecular chemistry or both, and so we are still moving along a continuous line.) Lashley⁸ taught his rats certain visual discrimination skills; when he removed their optical cortex, the learning was gone, as one would expect; but, contrary to what one would expect, the mutilated rats were able to learn the same tasks again. Some other brain area, not normally specializing in visual learning, must have taken over this function, deputizing for the lost area.

Similar feats of meta-adaptation have been reported in insects, birds, chimpanzees and so on. But let us get on to man, and to those lofty forms of self-repair which we call self-realization, and which include creativity in its broadest sense. Psycho-therapy, ancient and modern, from shamanism down to contemporary forms of abreaction therapy, has always relied on what Ernst Kris⁹ has called “regression in the service of the ego”. The neurotic with his compulsions, phobias and elaborate defence-mechanisms is a victim of maladaptive specialization—a koala bear hanging on for dear life to a barren telegraph pole. The therapist’s aim is to regress the patient to an infantile or primitive condition; to make him retrace his steps to the point where they went wrong, and to come up again, metamorphosed, re-born. Goethe’s *Stirb und Werde*, the inexhaustible variations of the

archetype of death and resurrection, dark night and spiritual rebirth, all revolve around this basic paradigm—Joseph in the well, Jesus in the tomb, Buddha in the desert, Jonah in the belly of the whale.

There is no sharp dividing line between self-repair and self-realization. All creative activity is a kind of do-it-yourself therapy, an attempt to come to terms with traumatizing experiences. In the scientist’s case the trauma is some apparent paradox of Nature, some anomaly in the motion of the planets, the sting of data which contradict each other, disrupt an established theory, and make nonsense of his cherished beliefs. In the artist’s case, challenge and response are manifested in his tantalizing struggle to express the inexpressible, to conquer the resistance of his medium, to escape from the distortions and restraints imposed by the conventional styles and techniques of his time.

In other words, the so-called revolutions in the history of both science and art are successful escapes from blind alleys. The evolution of science is neither continuous nor strictly cumulative except for those periods of consolidation and elaboration which follow immediately after a major breakthrough. Sooner or later, however, the process of consolidation leads to increasing rigidity and orthodoxy, and so into the dead-end of over-specialization. The proliferation of esoteric jargons which seems to characterize this phase reminds one sometimes of the monstrous antlers of the Irish elk, and sometimes of the neurotic’s elaborate defence-mechanisms against the threats of reality. Eventually, the process leads to a crisis, and thus to a new revolutionary break-through—followed by another period of consolidation, a new orthodoxy, and so the cycle starts again.

In the history of art, this cyclic process is even more obvious: periods of cumulative progress within a given school and technique end inevitably in stagnation, mannerism or decadence, until the crisis is resolved by a revolutionary shift in sensibility, emphasis, style.

Every revolution has a destructive and a constructive aspect. In science the destruction is wrought by jettisoning previously unassailable doctrines, including some seemingly self-evident axioms of thought. In art, it involves an equally agonizing re-appraisal of accepted values, criteria of relevance, frames of perception. When we discuss the evolution of art and science from the historian’s detached point of view, this un-doing and re-doing process appears as a normal and inevitable part of the whole story. But when we focus our attention on any concrete individual who initiated a revolutionary change, we are immediately made to realize the immense intellectual and emotional obstacles he had to overcome. I mean not only the inertial forces of society; the primary locus of resistance against heretical novelty is inside the skull of the individual who conceives of it. It reverberates in Kepler’s agonized cry when he discovered that the planets move in elliptical pathways: “who am I, Johannes Kepler, to destroy the divine symmetry of the circular orbits!”. On a more down-to-earth level the same agony is reflected in Jerome Bruner’s¹⁰ experimental subjects who, when shown for a split second a playing card with a black queen of hearts, saw it as red, as it should be; and when the card was shown again, reacted with nausea at such a perversion of the laws of Nature. To unlearn is more difficult than to learn; and it seems that the task of breaking up rigid cognitive structures and reassembling them into a new synthesis cannot, as a rule, be performed in the full daylight of the conscious, rational mind. It can only be done by reverting to those more fluid, less committed and specialized forms of ideation which normally operate in the twilight below the level of focal awareness. Such intervention of unconscious processes in the creative act is now generally, if sometimes reluctantly, accepted even by behaviourists with a strong positivist bias. Allow me, therefore, to take it for granted that in the period of incubation—to use Graham Wallis’s¹¹

term—the creative individual experiences a temporary regression to patterns of thinking which are normally inhibited in the rational adult.

But it would be a gross over-simplification to identify—as is sometimes done—these patterns with Freud's so-called "Primary Process". The primary process is supposedly devoid of logic, governed by the pleasure principle, apt to confuse perception and hallucination, expressed in spontaneous action, and accompanied by massive affective discharge. I believe that between this very primary process, and the so-called secondary process governed by the reality principle, we must interpolate a whole hierarchy of cognitive structures which are not simply mixtures of primary and secondary processes, but are autonomous systems in their own right, each governed by a distinct set of rules. The paranoid delusion, the dream, the daydream, free association, the mentality of children at various ages and of primitives at various stages, should not be lumped together, for each has its own logic or rules of the game. But while clearly different in many respects, all these forms of ideation have certain features in common, since they are ontogenetically, and perhaps phylogenetically, older than those of the civilized adult. I have elsewhere¹² called them "games of the underground", because if not kept under restraint they would play havoc with the routines of disciplined thinking. But under exceptional conditions, when disciplined thinking is at the end of its tether, a temporary indulgence in these underground games may suddenly produce a solution which was beyond the reach of the conscious, rational mind—that new synthesis which Poincaré¹³ called the happy combination of ideas, and which I like to call 'bisociation' (as distinct from associative routine). I have discussed this process in some detail in a recent book¹² and shall not dwell on its intricate details. The point I want to make here is that the creation of novelty in mental evolution follows the same pattern of *reculer pour mieux sauter*, of a temporary regression to a naive or juvenile level, followed by a forward leap, which we have found in biological evolution. We can carry the analogy further and interpret the Aha reaction, or 'Eureka' cry, as the signal of a happy escape from a blind alley—an act of mental self-repair, achieved by the de-differentiation of cognitive structures to a more plastic state, and the resulting liberation of creative potentials—the equivalent of the release of genetic growth-potentials in regenerating tissues.

It is a truism to say that in mental evolution social inheritance replaces genetic inheritance. But there is a less trivial parallel between phylogenesis and the evolution of ideas: neither of them proceeds along a continuous curve in a strictly cumulative manner. Newton said that if he saw farther than others it was because he stood on the shoulders of giants. But did he really stand on their shoulders or some other part of their anatomy? He adopted Galileo's laws of free fall, but rejected Galileo's astronomy. He adopted Kepler's planetary laws, but demolished the rest of the Keplerian edifice. He did not take as his point of departure their completed 'adult' theories, but retraced their development to the point where it had gone wrong. Nor was the Keplerian edifice built on top of the Copernican structure. That ramshackle structure of epicycles he tore down and kept only its foundations. Nor did Copernicus continue to build where Ptolemy had left off. He went back two thousand years to Aristarchus. The great revolutionary turns in the evolution of ideas have a decidedly paedomorphic character. The new paradigm, to use Thomas Kuhn's¹⁴ term, which emerges from the revolution is not derived from a previous adult paradigm; not from the aged sea-urchin but from its mobile larva, floating in the currents of the ocean. Only in the relatively peaceful periods of consolidation and elaboration do we find gerontomorphism—small improvements to a fully mature body of knowledge. In the history of art the process is again all too obvious; there is no need to elaborate on it.

I began with a wiseful remark about the treacherous wings of analogy, aware of the fact that those who trust these waxen wings usually share the fate of Icarus. But it is one thing to argue from analogy, and quite another to point to an apparent similarity which has perhaps not been paid sufficient attention, and then to ask whether that similarity has some significance or whether it is trivial and deceptive. I believe that the parallel between certain processes underlying biological and mental evolution has some significance. Biological evolution could be described as a history of escapes from overspecialization, the evolution of ideas as a series of escapes from the bondage of mental habit; and the escape-mechanism in both cases is based on the same principles. We get an inkling of them through the phenomena of regeneration—the remoulding of structures and reorganization of functions—which only enter into action when the challenge exceeds a critical limit. They point to the existence of unsuspected 'meta-adaptive' potentials which are inhibited or dormant in the normal routines of existence, and, when revealed, make us sometimes feel that we move like sleepwalkers in a world of untapped resources and unexplored possibilities.

It could be objected that I have presented a reductionist view; that it is sacrilegious to call the creation of a Brahms symphony or of Maxwell's field equations an act of self-repair, and to compare it with the mutation of a sea-squirt larva, the regeneration of a newt-tail, the re-learning process in the rat or the rehabilitation of patients by psycho-therapy. But I think that such a view is the opposite of sacrilegious. It points, however tentatively, at a common denominator, a factor of purposiveness, without invoking a *deus ex machina*. It does not deny that trial and error are inherent in all progressive development. But there is a world of difference between the random tries of the monkey at the typewriter, and the process which I called, for lack of a better name, *reculer pour mieux sauter*. The first means reeling off all possible responses in the organism's repertory until the correct one is hit on by chance and stamped in by reinforcement. The second may still be called trial and error, but of a purposive kind, using more complex, sophisticated methods: a groping and searching, retreating and advancing towards a goal. "Purpose," to quote Herbert J. Muller¹⁵, "is not imported into Nature and need not be puzzled over as a strange or divine something. . . . It is simply implicit in the fact or organisation." This directiveness of vital processes is present all along the line, from conscious behaviour down to what Needham⁷ called "the striving of the blastula to grow into a chicken". How tenacious and resourceful that striving has been demonstrated by experimental embryology, from Speeman to Paul Weiss—though its lessons have not yet been fully digested.

Thus to talk of goal-directedness or purpose in ontogeny has become respectable again. In phylogeny the monkey still seems to be hammering away at the typewriter, perhaps because the crude alternatives that had been offered—amorphous entelechies, or the Lysenko brand of Lamarckism—were even more repellent to the scientific mind. On the other hand, some evolutionary geneticists are beginning to discover that the typewriter is structured and organized in such a way as to defeat the monkey, because it will print only meaningful words and sentences. In recent years the rigid, atomistic concepts of Mendelian genetics have undergone a softening process and have been supplemented by a whole series of new terms with an almost holistic ring. Thus we learn that the genetic system represents a "micro-hierarchy" which exercises its selective and regulative control on the molecular, chromosomal and cellular level; that development is "canalized", stabilized by "developmental homeostasis" or "evolutionary homeostasis"¹⁶ so that mutations affect not a single unit character but a "whole organ in a harmonious way"¹⁷, and, finally, that these various forms of 'internal

selection" create a restricted "mutation spectrum"¹⁸ or may even have a "direct, moulding influence guiding evolutionary change along certain avenues"¹⁹—and all this happens long before external, Darwinian selection gets to work. But if this is the case, then the part played by a lucky chance mutation is reduced to that of the trigger which releases the co-ordinated action of the system; and to maintain that evolution is the product of blind chance means to confuse the simple action of the trigger, governed by the laws of statistics, with the complex, purposive processes which it sets off. Their purposiveness is manifested in different ways on different levels of the hierarchy, from the self-regulating properties of the genetic system through internal and external selection, culminating perhaps in the phenomena of phylogenetic self-repair: escapes from blind alleys and departures in new directions. On each level there is trial and error, but on each level it takes a more sophisticated form. Some twenty years ago, Tolman and Krechevsky²⁰ created a stir by proclaiming that the rat learns to run a maze by forming hypotheses; soon it may be permissible to extend the metaphor and to say that evolution progresses by making and discarding hypotheses.

Any directive process, whether you call it selective, adaptive or expectative, implies a reference to the future. The equifinality of developmental processes, the striving of the blastula to grow into an embryo, regardless of the obstacles and hazards to which it is exposed, might lead the unprejudiced observer to the conclusion that the pull of the future is as real and sometimes more important

than the pressure of the past. The pressure may be compared to the action of a compressed spring, the pull to that of an extended spring, threaded on the axis of time. Neither of them is more or less mechanistic than the other. If the future is completely determined in the Laplacian sense, then there is nothing to choose between the actions of the two springs. If it is indeterminate in the Heisenbergian sense, then indeterminacy works in both directions, and the distant past is as blurred and unknowable as the future; and if there is something like a free choice operating within the air-bubbles in the stream of causality, then it must be directed towards the future and oriented by feed-back from the past.

¹ Huxley, J., *Man in the Modern World*, 13 (New York, 1948).

² Garstang, W., *J. Linnean Soc. Lond. (Zoology)*, 85, 81 (1922).

³ Hardy, A. C., in *Evolution as a Process* (New York, 1954).

⁴ de Beer, G. R., *Embryos and Ancestors* (Oxford, 1940).

⁵ Bolk, L., *Das Problem der Menschwerdung* (Jena, 1926).

⁶ Haldane, J. B. S., *The Causes of Evolution*, 150 (London, 1932).

⁷ Needham, A. E., *New Scientist*, London, November 2, 1961.

⁸ Lashley, K. S., *Brain Mechanisms and Intelligence* (Chicago, 1929).

⁹ Kris, E., *Psychoanalytic Explorations in Art* (New York, 1952).

¹⁰ Bruner, J. S., and Postman, L., *J. of Personality*, XVIII (1949).

¹¹ Wallas, G., *The Art of Thought* (London, 1954).

¹² Koestler, A., *The Act of Creation* (New York, 1964).

¹³ Poincaré, H., in *The Creative Process* (Berkeley, Calif., 1952).

¹⁴ Kuhn, T. H., *The Structure of Scientific Revolutions* (Chicago, 1962).

¹⁵ Muller, H. J., *Science and Criticism* (New Haven, Conn., 1943).

¹⁶ Cannon, H. G., *The Evolution of Living Things* (Manchester, 1958).

¹⁷ Waddington, C. H., *The Listener*, London (Nov. 13, 1952).

¹⁸ Spurway, H., in *Supplemento. La Ricerca Scientifica* (Pallanza Symp.), 18 (Cons. Naz. delle Ricerche, Rome, 1940).

¹⁹ For a survey of literature in this field see Whyte, L. L., *Internal Factors in Evolution* (London, 1965).

²⁰ Krechevsky, I., *Psychol. Rev.*, 39 (1932).

HISTORY AS THE ORGANIZATION OF MAN'S MEMORY*

By PROF. HERBERT BUTTERFIELD

Master of Peterhouse, University of Cambridge

WE often think of Western civilization as scientific, and we do not always remember that it is equally remarkable for being so historically minded. In both respects the only parallel to it is ancient China—so wonderful in its science and technology, but possessing also a historical literature of almost incredible vastness. Even in China there did not develop those modern techniques which, in our section of the globe, led to the scientific revolution of the seventeenth century and the somewhat parallel historiographical revolution in the nineteenth. In both fields the developments in Europe were unique; and the Chinese have had to become pupils of the West.

Some civilizations, like that of ancient India, seem to have been governed by religions or philosophies which deny significance to the facts of history as such, and the sequence of events in time. Yet, between a culture which has soaked itself in historical memories and one for which the past is only chance and change—only bubble and froth—there must develop great differences in general mentality, in intellectual habits, and in the degree of control that can be acquired over the course of events: and the differences must extend to still deeper things that affect the very nature of the human consciousness.

Our interest in the past—our very sense for the past—(like our prowess in the natural sciences) is therefore a thing that requires to be explained. Even the case that we to-day might make for the study of history would have no meaning for those earlier generations of mankind that gave the start to the whole endeavour. We of the twentieth century might say that a society is going to be

very constricted in its development unless it looks behind itself, organizes its memory, reflects on its larger and longer experiences, learns to measure the direction in which it is moving, and gets some notion of long-term tendencies which have been observed. But this kind of diagnosis—this way of learning where we stand in the processes of time—is a thing that comes only late in the day, when civilization and scholarship itself have progressed very far. Nobody could have known in advance that by the study of the past we should be able to examine the processes of things in time. Indeed, until the world was fairly mature, nobody could even have guessed that there existed such things as historical processes which might call for analysis.

It is possible that, in every age and society, children will love to listen to the tales of a grandfather and will look back at least to the time just before they were born. All the world seems to love a story, and, even if there were no inferences to be drawn from it, we are all likely to be interested in the account by Arthur M. Schlesinger, jun., of the expedition to the Bay of Pigs—interested in it purely as the narrative of something that actually happened. I suspect that, however scientific and analytical and statistical historical scholarship may become in the latter half of the twentieth century, a great mass of people will go on loving this narrative history—the history that tells of men and their vicissitudes. Perhaps these are the people who will keep the subject sane—keep history an important humanistic factor in our civilization. But, though the telling of stories may awaken an interest in the past, it is not likely in itself to alter the structure of our mentality. Also, it is scarcely enough in itself to drive the mind to research and criticism and the passionate quest for truth. The *raconteur* knows

* Substance of an address delivered at the Bicentennial Celebration commemorating the birth of James Smithson, held in Washington during September 16–18 (see *Nature*, 208, 320; 1965).

that if he investigates the truth of the matter, he is only too likely to lose a good story.

Before writing began, the past would seem to have been just a rag-bag of old myths and stories—on the whole, a jumble of things that happened 'once upon a time'. An essential progress was achieved when the mind learned to project itself into that forest of data, and began to acquire some sense of the distances involved. It was necessary that time should be turned into something like a long tape-measure, with markings that roughly indicated succession and duration and degrees of remoteness. This is not easily achieved by peoples who have not learned to serialize or particularize the years by numbering them in the way which we are accustomed to doing. Fortunately, our distant ancestors appear to have had a mania for making lists. Some of these lists are in a sense the beginnings of history, just as others, which perform a preliminary work of classification, are in a sense the beginning of the natural sciences. It was when they put together their lists of successive kings or priests that these ancient peoples acquired their first impressions of the tremendous stretch of time behind them. The ancient Greeks had very defective lists and thought that only a comparatively short period separated them from the age when the gods had walked and sported on the Earth. But some of them learned about the huge lists of Egyptian priests and came to realize that there had been thousands of years of human history before their day. If the whole of this is taken together, it involves a change in what it means to have an existence in time—in a time which stretches behind and before.

It is comparatively easy for an individual to keep some recollection of his own personal past; but the difficulties are great if the human race or the body politic wants to achieve and organize and refine its collective memory. In the civilization of ancient Mesopotamia the arts had reached a remarkable level, and technology had done wonderful things before there existed any serious history. Even in the modern centuries the development of historical scholarship has proceeded at a slower pace than that of the natural sciences; and the intellectual revolution—with the modern techniques of discovery—comes in the nineteenth rather than the seventeenth century. In generations which were capable of the most profound philosophy and the most abstruse mathematics there would flourish a remarkable credulity about the records and stories of the past. In the middle of the nineteenth century, when the archives of the European capitals were being opened, a great stimulus was given to the subject—men felt that now, at least, they could really get down to the study of history. For centuries before that, they had more or less accepted the history provided for them, but it is not clear that they committed their souls to it, except where it was guaranteed by revealed religion. They would scarcely realize how many of the facts of history were going to be capable of more secure establishment in the future.

So it seems that Homer was accepted as history at first—the only available account—though the Greeks had not yet scrutinized very carefully their notion of historicity. For the knowledge of these early periods it was perhaps a handicap that in Homer they possessed such an impressive substitute for history. The epics were so regarded as the genuine article that the first serious attempts at historical criticism were directed at their contents. There was no possibility of discovering dates, but family trees were important, and some of the Greek noble families claimed descent from epic heroes. Here legend and history seem to meet. It would appear to be genealogy which first called for investigation.

The remoter periods always presented special difficulties. It was no easy matter to recover recollections that had once been lost. A short-term memory was a more practicable affair, however; and the more serious kind of history developed through the recording of recent

events. It is possible that in every age and society men refer to the past—if only to the recent past—for the understanding of present-day problems and even for their very formulation. Because we ourselves have become so historically minded, and because we have come to be so aware of the continuity of processes in history, we carry this tendency almost infinitely further than our distant forefathers took it. Whether we are discussing Vietnam or the incidence of prostitution, we slip into a kind of historical retrospect before we are aware of what we are doing. With us at least, it seems that talk about the present slides almost insensibly into talk about the past. But even thousands of years ago—and even before the existence of historical study—the same tendency is visible on occasion and it operated as a stimulus to historiography. One of the earliest pieces of historical narrative that we possess is a royal account of a quarrel over a frontier. The victor in the conflict, as he announced its settlement and laid out his case, referred back to the original fixing of the boundary and the successive infringements of it by his enemy. Some of the most remarkable examples of historical writing in the ancient world come from the Hittites, who, centuries before any part of the Old Testament had been written, provided their peace-treaties with historical preambles, and would even produce a similar résumé to explain an act of governmental policy. The very considerable historical literature of ancient Egypt and Assyria was the work of people who showed no sign of having any real interest in the past. It came from monarchs who desired to place on record their own building achievements, their prowess in the hunt and their victories in battle. What they intended was either to overawe their contemporaries or to make sure of their future fame or to express their gratitude to the gods. There developed, therefore, the history of the type of commemorative tablet, and it was produced on a massive scale, sometimes with elaborate literary ornament, but it was bound to lead to a dead end. At the same time, it remained true for thousands of years that the history which most gripped the rails and came closest to authenticity was that which men wrote about the events in which they had taken part or which they had more or less observed. The attempt to reconstruct a distant past from raw materials—from literary and archaeological survivals—is, on the whole, a remarkably modern enterprise. The best history was like that of Thucydides—like that of Winston Churchill, giving his account of the great wars in which he had taken part. Those who undertook to write about remoter periods relied to a considerable degree on the men who in those periods had narrated what we call contemporary history. It was in the year 1824 that Ranke produced quite a milestone in the history of historiography by demonstrating that it was not a satisfactory thing to take one's account of a given period from the writer of that period, the men who had written "histories of their own times".

The Old Testament brought things to a new stage. It envisaged the whole of mankind from the Creation and then presented a full-length national history. The influence of its presiding idea helped to give shape to what became the Western view of the time-process.

The ancient Hebrews had been a semi-nomadic people, and it was characteristic of such clans that they yearned to enter the territory of the sedentary populations and share the benefits of settled agricultural life. They expected their god to vindicate himself by procuring this; and the most vivid of the Hebrew folk-memories concerned the way in which they had been brought out of the land of Egypt to the country of the Promise. By all the rules of the game they ought to have then proceeded to adopt the Nature gods and the fertility rites of settled agricultural peoples; but their semi-nomadic beliefs prevailed—they turned from the gods of Nature to the God of history. When others had ceremonies for the cycle of the seasons, they preferred to celebrate

historical events. If young people asked why they should obey the commandments, the answer was "Because God brought us up out of the land of Egypt". They stressed the fact that their God had kept his Promise; and the Promise was a developing thing, always turning out to be something higher than had previously been thought. Also their nation was to be blessed by heaven at the finish. History pointed to a grand consummation some time.

History, then, was not merely cyclic—mere aimless revolving and vain repetition. The ancient Hebrews would insist on occasion that God, besides being the original Creator, could go on doing new things, as time proceeded. Christianity itself emerged as a 'historical' religion in a peculiarly technical sense of the words. We can see the way in which St. Augustine decided that the cyclic view of history was incompatible with Christianity. The West developed under the presidency of these Jewish ideas and for more than fifteen hundred years the men of Europe could learn about their religion only through a Bible that was packed with history.

Later ages have seen in the Old Testament almost an adumbration of the future idea of progress. Towards the end of the seventeenth and in the eighteenth centuries this idea was superseding Greek cyclic views and the belief that the natural processes of time bring decadence. The change was partly due to the realization of the accumulating achievements of the sciences and technology. But the general idea of progress may have involved an element of faith—a belief in the future, almost the notion of history as based on the Promise. Once the idea of progress had emerged it gave a further stimulus to the study of history itself. Now it was possible to organize the memory a little more, to give shape to the human story, and to see the whole in terms of development. It was possible to put to history a further range of questions. One could ask it to show how mankind, from its primitive beginnings, had come to its present civilized state.

There was a sense in which men had always gone to history for answers to questions; and almost before they had any history they would investigate genealogies, as we have seen. At the next stage, certain miscellaneous problems tended to attract them. We meet these in ancient Mesopotamia, in the Old Testament, among the ancient Greeks, in Renaissance Europe, and elsewhere. Once the initial curiosity had been aroused, the same type of question seems to have been presented everywhere. People wanted to know why mankind had been divided into nations and why, among the nations, there was such a confusion of tongues. They asked how tribes and cities and rivers had got their names. They wondered why a certain place—a well or a mountain perhaps—had come to be considered sacred. At this stage of their development, the question would be answered by the telling of a story. In ancient Babylonia the priests rather than the monarchs were responsible for the records and they tended to bring history closer to the problems of human destiny. Here one particularly observes the device of explaining things by giving an account of their origins—in other words, producing a myth.

It was in ancient Greece that questions began to be answered more effectively: and the advance was connected with a wider rationalizing movement, a great development in what we should call the sciences. It was assisted by the fact that an interest in neighbouring countries had led to geographical and ethnographical studies; and history emerged in Greece as almost the by-product of these. It made its appearance in a more scientific context; and there was a disposition to account for both geographical and historical phenomena on more naturalistic lines. The belief that the gods constantly interfered in the course of events must hitherto have obstructed the discovery of laws and processes in history.

The leadership of the Greeks became most important, however, in the scientific use of the facts that the historian

establishes; and this came to Europe at the Renaissance. They learned to extract from all the complexity of material certain types of data that could be usefully collated with one another. A famous example of this was followed also by Machiavelli when he was setting out to show that politics should be handled in a scientific manner. It involved a comparative study of all the conspiracies of history, from which it was possible, for example, to discover maxims for men who wanted to assassinate their king. Perhaps the most famous and successful use of this method is the case of the Marxists studying all the revolutions of history for the purpose of arriving at a proper technique of revolution; Richelieu and Napoleon are among the men who claimed to have studied historical examples in order to learn maxims of policies. For centuries, from the time of the Renaissance, the promoters of historical study insisted that it provided a training in statesmanship.

But the more scientific treatment which the Greeks tried to give to established historical data—the hunt for correspondences and correlations—led in the long run to a different way of thinking and talking about man's activity in the world, a different sense of the part that he plays in the unrolling of time. It brought into existence the consciousness of the processes that take place in the course of human history. For the political analyst, the City-States of Greece were excellent laboratory specimens, where the processes (the transition from monarchy to democracy, for example) took place on a comparatively small scale—not too complicated by other things, fairly visible to the naked eye, and telescoped into a reasonably short period of time. The processes could be examined and, for example, it was not the modern Marxists but the world of classical antiquity that first brought out the significance of the class-conflict in the whole development of things. The coming of the modern idea of progress greatly increased the disposition to look for deeper movements underlying the whole human drama. Whereas history had once been written as though it involved mere acts of will on the part of men and gods—acts of will taking place in the free air—there emerged the view that human beings are involved in processes, entangled in systems of conditioning circumstance. Whereas one's first impulse is to blame entirely the wills of a few people for the outbreak of the First World War of 1914, Prof. Temperley pointed out that one must go back at least as far as Bismarck in 1871 for the origin of that War. From that earlier date sprang the tendencies and developed the predicaments which set the terms for human action later. Over the course of the centuries the actual writing of history has changed its character, therefore, and its texture is no longer that of the mere story—the story of men doing just this and then that. What we call historical narrative in a man like Ranke or even Macaulay has a more analytical quality—the story wrapped up in expositions of processes and conditioning circumstance.

Furthermore, from the quasi-scientific use of the kind of data that the historian establishes there sprang a sort of political science, but later also political economy, various types of social study and other specializations like military strategy. They became autonomous and separated themselves from history; and in the course of time some of them were able to emerge as rivals to it. They have occasionally tried to make the return journey—their results are fed back into history and they may end by trying to secure dominion over it. We may be told that, on the last analysis, it is the study of the economic substructure of history that really matters. In the twentieth century—and not least in the United States—the relations between history and the social sciences have formed a crucial issue, for some have wanted to see history interpreted as really a branch of social science. On the other hand, the question might arise as to whether, after all the associated kinds of science have been

abstracted from it, there is anything left for the residual study—mere history.

We can see, therefore, how the memory of the human race has tended to acquire a certain form and organization. In general, the study of the past has affected at different levels our consciousness of life in time, and our feeling for the processes of time. It alters our vision of the present day, just as a piece of landscape must look different when the observer has the eye of a geologist. It affects our everyday habits of mind: and somebody has said that in the nineteenth century all thought ran to history—a fact very noticeable in politics, theology, philosophy and even natural science. Between this last and the study of history there have been interesting interactions, particularly at the birth of rationalism in early Greece and after the scientific revolution of the seventeenth century. From the eighteenth century even the scientists were showing a consciousness of time, as though the ultimate objective was to lay out the whole story of the physical universe. It should be remembered that, though we think of the historian as recovering the truth about the past, he is often revealing things never previously known to any man. We no longer hold the view (which until comparatively recently was sufficient for our predecessors) that somebody in the past always knew the truth about what had happened, and all that was necessary was to find this man. In the crisis of July 1914, every Foreign Office in Europe would have internal knowledge of only its own policy and its own reactions to the conduct of other Powers. The historian, using the papers of all the Foreign Offices concerned, can reach a kind of knowledge not possessed by any of the statesmen of the time. He can therefore give a higher organization to the whole story.

It is proper, moreover, that history should hold its ground as a thing possessing its own function and nature. Those who try to show from psychoanalysis what 'must' have happened may be liable to contradiction by new documentary evidence. Events are now always historically established if the case in their favour depends on some economic theory that is fashionable at the moment. There is need for a form of study that, in a sense, keeps close to earth, merely establishing what it was that

actually 'happened'. The need is all the greater, and the point becomes fundamental if we remember how much, in the workings of time, must still depend on the wills of human beings. We cannot exempt the men of July 1914 from all responsibility or pretend that everything was predetermined, the present simply the direct product of the past. Between the past and the future there always lie the decisions that human beings make in the living present. Because these can never be simply inferred, we must go to the evidence for them, and history must remain an empirical study.

Men may be entangled in a network of conditioning circumstance, but it is live men who are so entangled—men liable to make one decision at breakfast-time and a different one at dinner-time. It is important not to lose sight of these live human beings and not to eliminate their role by saying that they are mere products of their age. They are individual fountains of life and action, and all the factors that play on their minds are churned and recombined inside each of them, so that we can still never quite predict their action. Sometimes the historical processes which seem to paralyse their activity can bring a colossal leverage to the force that a single one of them exerts. At the same time, the view that recognizes this importance of the personality in the machinery of time must always allow a similar force to the operation of contingency. A short story may tell about hundreds of tiny actions, but one single coincidence among these may transform the entire development. Once again, we require, not merely a science of general laws, but an empirical study of detailed happenings, and a search for the ones that are pivotal. There is something in history to which justice cannot be done save by the kind of narrative in which we do not quite know what is going to happen next. It is true, then, that we need to study all that system of necessity which conditions human action, and interpretations of history tend to be commentaries on just that system. But we examine it rather to increase our control—we learn about it so that we may know better where our freedom lies. Because history so involves the awareness of this play of personality, it may be the safeguard for humanism in a technological civilization.

MAN-MADE LAKES

MAN-MADE lakes are coming to occupy a substantial area of former land. In Africa, for example, the 1,700 square miles of Lake Kariba on the Zambesi, the 3,200 square miles of Lake Volta in Ghana, the 1,800 square miles of Lake Nasser on the Nile, and the 500-square-mile lake due to form behind the Niger River dam at Kamji, will flood more than 7,000 square miles, and some 200,000 people are having to be resettled from the flooded areas.

The magnitude of these lakes demands that the fullest use be made of them. In addition to hydroelectric power, for which most of the dams are primarily constructed, their creation provides innumerable new opportunities, for irrigated farming, for fisheries development to supply much-needed protein, for transport, for the creation of nature reserves, National Parks and tourism. Some of the lakes are probably large enough to affect the local climate. The resettlement of people provides exceptional opportunities for inducing social and economic change, and for improving health. But to achieve all these things, each new lake must be considered as part of an integrated lake-basin development programme, and multi-disciplinary research is essential. Soil and vegetation surveys should be carried out to find suitable agricultural and for the displaced people. Biologists should be

concerned with problems of flooding, bush clearing, weed control and fishery development; anthropologists and sociologists with resettlement. Geographers and archaeologists and many others should come together with the engineers and hydrologists.

The United Kingdom also has its problems of man-made lakes, essential in a civilization whose demands for water increase at 4–5 per cent per annum. The old idea of a reservoir for water-supply only is giving way, under pressure, to the new idea of multi-purpose use, including recreation. Moreover, new lakes may encroach on the sea as well as on the land. Reclamation of estuaries, for freshwater supply as well as for agricultural land, has long been carried out in Holland, and the feasibility of doing something similar in Britain is now being considered in relation to Morecambe Bay and the Solway Firth. The Wash and the Thames have been suggested as other possibilities; while in Hong Kong an arm of the sea is to become a freshwater reservoir very soon.

The integrated investigation of such man-made lakes is comparatively new, and information is widely scattered. Therefore the successful symposium organized by the Institute of Biology during September 30–October 1 on this subject was very welcome. The meetings, which were held in the Royal Geographical Society hall, were attended

by about 200 delegates, including biologists, hydrologists, engineers and sociologists. It was a little surprising that there were present very few geographers, though they might have been expected to be specially attracted by this synthesis. The representation of disciplines stimulated lively discussions at each of the four sessions. As the study of man-made lakes is still in the data-gathering stage, each new lake is helped by the information derived from its predecessors, which adds to the importance of collating information as soon as it is available.

The first day was devoted to man-made lakes in the tropics; the second to those of the temperate zone. In each case the morning session was concerned primarily with aspects of the natural history of such lakes, and the afternoon sessions with problems arising from making and using them. As all the papers had been circulated before the meeting, contributors spoke briefly, summarizing their papers, adding up-to-date points, and illustrating them with photographs and slides. This allowed the maximum time for discussion.

The meeting was opened by Dr. Barton Worthington, scientific director of the International Biological Programme, who, as chairman of the first session, gave an introductory survey of the major schemes in progress and prospect in the tropics. He emphasized the beneficial influences of the great natural lakes of tropical Africa on those countries which are lucky enough to possess them, and pointed to the advantages of planning the new man-made lakes in such a way that they would bring similar benefits. Some biological research on the productivity of these lakes is already being done under the aegis of the International Biological Programme which has an integrated plan for expanded research. Next, followed papers on the hydrology and fisheries of Lake Kariba by D. Harding, formerly of the Zambian Fisheries Department; on the biological investigations in progress on the Volta Lake by Prof. D. W. Ewer, University of Ghana; and on the Brokopondo research project in Surinam, another 500-square-mile lake formed by closing the Suriname River dam in February, 1964, by Dr. P. Leentvaar. From data given in these three papers, a pattern emerged, of aquatic deoxygenation after the dams are closed and of the invasion of the new lakes by floating plants. At Kariba, there was also a huge burst of fish production. Supplementary data were provided on the hydrology and zooplankton of the artificial lakes on the Nile, such as the Jebel Aulia reservoir, by Dr. J. Rzoska and Dr. J. F. Talling, and by Dr. D. J. Lewis on the problems of mosquitoes and malaria which were associated with their formation. The research in progress on the River Niger in anticipation of the lake to be formed above the Kainji dam was described by Dr. E. White, who had just returned after a survey conducted by fourteen scientists drawn mainly from the Universities of Liverpool and Ife.

In Africa, where the man-made lakes are all impounded from large rivers, they have a much larger through-put of water than the natural lakes. The Nile impoundments at Sennar and Roseires fill and empty every year; Kariba with its much greater volume took four and a half years to fill. But R. S. A. Beauchamp mentioned that Lake Tanganyika, if emptied, would take 1,500 years to fill from its inflows. These differences are reflected in the water chemistry of the 'young' water of the new lakes and the 'old', almost fossilized, water of the old lakes. As a contrast, Dr. C. M. Yonge spoke of the problems of turning an arm of the sea into fresh water in Hong Kong, with an abrupt change from salt water to fresh water. A lively discussion was stimulated by the question how much notice and research are needed for biologists and hydrologists to make useful prognostications of happenings in the stored water, for engineers to take the requisite remedial measures.

The meeting was fortunate in having Sir Robert Jackson, special adviser to the United Nations Special

Fund, as its chairman for the afternoon session concerned with the problems arising from making lakes in the tropics, for Sir Robert has been intimately involved with the Volta and other schemes for a long time. Two papers presented were on the establishment of fisheries in the new lakes, by P. B. N. Jackson of Jinja and the Food and Agriculture Organization, and by Dr. L. Joeris of the Food and Agriculture Organization Kariba Research Institute; Dr. E. C. S. Little of the Weed Research Organization spoke on the invasion of man-made lakes by plants, and Dr. B. B. Waddy of the London School of Hygiene and Tropical Medicine on medical problems arising from making lakes in the tropics. The social and economic problems of the indigenous peoples were discussed by Dr. T. Scudder of the University of California, who had studied the displaced Tonga at Kariba and been concerned with the plans for the resettlement of the Nubians from Lake Nasser and of those people to be displaced by the Kainji Lake. Additional contributions came from Prof. W. E. Kershaw on the *Simulium* problems at Kainji, from Dr. C. F. Hickling on weed control and barriers to fish migration. Dr. C. G. L. Bertram commented on the idea of using manatees to control aquatic weeds.

Sir Robert Jackson pointed out that the budgets of these large schemes may be limited by outside factors, in the case of Volta by the price of aluminium. The budgets of the consultants have to be framed to fit and this may mean that not enough money can be allowed for research, for resettlement or for clearing bush. The Government concerned will then have to try and find other funds, and until the research is done it is difficult to convince Governments of such needs. In developing countries the sheer weight of problems needing solution, falling perhaps on relatively few trained personnel, may lead to serious delays.

Clearing the bush from land to be flooded is costly. How much clearing is really necessary to enable the lakes to be profitably fished? Experience has shown that the main species of fish in the man-made lakes of Africa, *Tilapia* and *Labeo* in particular, can best be caught in bottom-set gillnets which are impossible to use unless the flooded land has been cleared. Furthermore, rotting vegetation, if the bush is not cleared, causes extensive de-oxygenation, so that the bottom waters become barren of fish and other life, and strongly sulphurated waters affect the turbines. How long these situations will persist depends on the shape, size and position of the lake in relation to temperature changes and prevailing winds. Lake Kariba, lying more than 1,500 ft. above sea-level and some distance from the equator, has a seasonal overturn which carries oxygenated water to the bottom. Lakes Volta and Brokopondo, lying nearer the equator and at a lower altitude, have less marked seasonal changes, and de-oxygenated bottom waters have persisted throughout the year. But, as the last-mentioned two lakes only started to fill in spring 1964, it is too soon to say how they will continue to behave. Lakes Kariba and Volta are both in savanna bush country. At Kariba a sizeable sum was spent on clearing, and a fishery developed almost immediately. On the Volta site very little clearing was done; there is as yet no significant fishery. Brokopondo lake lies in high rain forest and no clearing was attempted. Lake Nasser lies in desert so the problem of clearing does not arise. But for the Niger lake, which is to be filled in 1967 and which lies in savanna bush, the question of how much bush needs to be cleared is now an urgent issue.

Uncleared bush also aggravates the weed problem. Dramatic photographs of Kariba showed the mats of floating weed, *Salvinia auriculata* and *Pistia stratiotes*, accumulated around the tops of submerged trees. Such weed impedes access to the shore, interferes with lake transport and fishery development, and provides a

stratum on which larger sudd-forming vegetation is able to establish itself. But in time the Kariba weed has died down, and is now confined to bays and inlets. Volta Lake, where an anxious watch and chemical sprayings of possible foci have been used to prevent weed growth, was at first free of weed, but it now also has the problem of *Pistia* accumulation. In Brokopondo in South America the water hyacinth, *Eichhornia crassipes*, has invaded large areas of the lake, and a water fern, *Ceratopteris thalictroides*, has established itself also. The Congo and Nile are among the many waters to have suffered from extensive *Eichhornia* outbreaks, and the high cost of weed control, necessary to keep channels open for navigation and to stop the weed mats blocking sluices and turbines, makes any research which is designed to prevent outbreaks well worth while. There is a further important problem concerning the influencing of floating vegetation on the hydrology, arising from the evidence that transpiration from the plants is higher than evaporation from an equivalent area of free water surface.

The great new tropical lakes around which whole populations have to be resettled provide a wide range of human as well as biological problems. Health measures are essential in connexion both with dam building and resettlement. The labour force is relatively easy to care for, but there is likely to be a most unhealthy focus of concentrated humanity with heavy parasite loads at some boom-village near the dam site, composed of those who came there hoping for jobs but were found unfit.

Several illnesses associated with water require study. Oncocerciasis carried by *Simulium*, the larvae of which live in running water, may become less of a problem as the river becomes a lake, but malaria and schistosomiasis are both associated with still water and may present greater dangers. There are also disease hazards when people are moved to their new homes. The mental stress of adopting a new way of life away from familiar and sometimes sacred things like graves of ancestors and shrines is not the least of the problems. Land has to be cleared for cultivation in the new areas, and after moving, the people have to await the rains before the new gardens can be planted. Anthropological and archaeological studies in the lake area before, during and after flooding may help to understand and mitigate such problems.

Sociologists have found that the removal period provides opportunities which should not be missed. The resettled people tend initially to be in a state of cultural shock, and at this time are receptive to new ideas; for example, new fishing methods can then be introduced. But in a few years' time a period of resistance to new ideas tends to set in, so the opportunity is lost if not seized at once. Resettlement calls for much advance planning, land surveys to determine suitable agricultural land and alternative means of employment if land is short, as well as building new villages. If the fishery is to be fully developed, fishing schools will have to be established to teach the people to use the new types of gear necessary to crop a lake. Moreover, the best gear must be decided and made available, including boats with engines to cover the larger area of water. Experience shows that not enough time and resources are generally allowed for these complicated operations.

The Dutch have unrivalled experience in impounding waters from the sea, and the meeting was fortunate in having Dr. K. F. Vass, Director of the Hydrobiological Institute, Yerseke, Holland, to speak on lakes in Dutch reclamation schemes. This contribution opened the discussion on the natural history of man-made lakes in the temperate zone under the chairmanship of Dr. J. W. G. Lund. Papers followed on the biological implications of the proposed barrages across Morecambe Bay and the Solway Firth by Mr. H. C. Gilson, of the Freshwater Biological Association, and on the hydroelectric schemes in Scotland, especially their effects on the salmonid fisheries, by Dr. K. A. Pyefinch, from Pitlochry.

In addition to water supply, an area such as Morecambe Bay impounded by a barrage could provide water for cooling power stations, a road to the north-west, and recreational facilities for sailing, fishing, etc. Additional information was contributed on weed and plankton growth in Loch Leven by Mr. N. C. Morgan, as this lake was thought to be one which the impounded Morecambe Bay would come to resemble. Mr. Gilson predicted that Morecambe Bay could support a good trout fishery, but management would be necessary because it would tend to become rich in pike and eels. The problems of the descent of salmon smolts to the sea could perhaps be overcome by trapping them in fresh water, acclimatizing them to brackish water and then releasing them in the sea.

The behaviour of salmonids, which support valuable sport fisheries, and the survival of smolts as they pass through turbines, were discussed with reference to the Scottish experience of the use of louvres to deflect smolts from turbines. Experience showed that biologists need extensive knowledge of the engineering details of such schemes and the physiology and behaviour of the fish to give proper advice.

The extensive investigations carried out in the U.S.S.R. on man-made lakes, which were described by Dr. J. Rzoska, have been made mainly in the temperate zone. The pattern common to most new lakes is of the original stock of river food being demolished and fish spawning grounds disturbed in the first year, often followed by a period of accelerated fish growth and increased yields associated with eutropication and leaching of soils. It takes at least 10 years before the zooplankton becomes stabilized, and up to 18 years before the benthic fauna settles down. The fish fauna changes enormously, often in ways not foreseen, so that it may take in all more than 20 years before the new lake is stabilized. Even then it may be found that some ecological niches are not filled. Where the emphasis is on the production of fish for food, should gaps in the food chain be closed by introductions of benthic fauna, or of fish to utilize the productivity of the zooplankton? Russian experience has also shown how reservoirs create conditions for the spread of animals: the Caspian fauna is spreading northwards and the northern fauna southwards. The study of man-made lakes is considered so important in the U.S.S.R. that a new institute and a journal for publication of results have been especially established.

At the final session, under the chairmanship of Prof. O. E. Lowenstein, other problems associated with the use of man-made lakes in the temperate zone were considered. Papers were presented on engineering and economic aspects of large-scale water supply by F. Law of the Fylde Water Board; on maintaining the safety and quality of water supplies by Dr. G. U. Houghton of the South Essex Waterworks Co.; and on the multi-purpose use of reservoirs by L. H. Brown, Mid-Northamptonshire Water Board.

Mr. Law compared the costs of water storage by conventional means (some £400 per million gallons) with estimated costs for storage behind a sea barrier, which he thought would be about eight times as great. Differences in water supply in the north-west and south-east of England were commented on. In the north-west there is more rain, but in the south-east larger storage reservoirs are necessary to guard against a series of dry years. In the north-west the water is used once only, but in the south-east it may be used several times, being circulated through sewage works, back into the rivers and then used again farther down-stream before going to sea. The health aspects and types of filters and treatment plant were described by Dr. Houghton. Would it be cheaper for people to have their own filters and use untreated water for purposes such as washing cars? And how does metering affect water costs? Another contributor to the discussion pointed out that desalinization

costs were falling, whereas conventional storage costs were rising.

The final paper, on experience gained in the United States in recreational use of artificial waters, was given by R. H. Stroud of the Sport Fishing Institute, Washington, D.C. After considering fishery management in reservoirs, Mr. Stroud ended with a very useful commentary on some of the work described in earlier papers, including the tropical work. A main question in the discussion which followed was the extent to which water supply can be considered in isolation from other uses of reservoirs. With the development of motor-ways opening up the country, there is increased demand for access to reservoirs for recreational purposes. Since the water is treated anyway, there may be little harm in allowing access to reservoirs for fishing, sailing and bird watching, though access must be controlled to prevent abuse. Some recreational interests may conflict: reservoirs which are wild fowl reserves would suffer if opened to the general public, and water-skiing is not popular with anglers. Experience in the United States strongly supports the multi-purpose use of reservoirs. In the United States 95 per cent of the waters are publicly owned, in contrast with many waters in private hands in the United Kingdom, and the surprisingly high figure of 40 per cent of the commercial food fish eaten in the United States is caught by anglers.

The information required from biologists by engineers was discussed. It was pointed out that engineers have to reach decisions whether or not all the facts are known; therefore, biologists should, perhaps, be more concerned with orders of magnitude than with seeking answers in too precise quantitative terms, when trying to assist engineers. Biologists need a data-gathering stage, and experience suggests that five or six years is necessary if the biologist is to advise soundly on large and complex schemes. Each study helps later investigations, and there is a great need to record results fully and to co-ordinate the experience now gleaned from the various projects. Turning a river into a lake, as was pointed out by F. T. K. Pentelov, is bound to lead to an unstable situation. The period needed to attain stability in each case can, at present, only be guessed at, but will be a function of the generation time of the plants and animals surviving or introduced. Thus it is probably longer in higher latitudes than in the tropics. Some problems arising, such as weed growth, may solve themselves, but there is a need to define the long-term and persistent problems before tackling them. A general pattern which emerges is that there is likely to be very high productivity in the first year or so after the lake fills, due to leaching of the soils and decomposition of drowned plant and animal matter. Then there may be a rather abrupt fall in productivity, followed by a period of many years before the lake stabilizes. Estimates from experience gained in the U.S.S.R. suggest at least 6–10 years in lakes below latitude 50° N. and 25–30 years in higher latitudes.

At every session discussions were vigorous and many questions remained unasked and unanswered. Since biologists have to predict results, such as the yields of fish likely from certain bodies of water, before the research

is done, in order to justify and obtain funds for research, one would have liked more information on how actual yields have borne out predictions—and on the reasons for any major discrepancies. Moreover, there is still need for data from natural lakes which guide to predictions for man-made lakes. There was no mention of the fact that Lake Victoria, nearly the size of Ireland, is now the largest reservoir in the world by reason of the Owen Falls dam at its outlet. One would have liked, too, to have heard what happens to the accumulative chemical pesticides when water is purified for re-circulation. As Mr. Stroud pointed out, the enormous amount of work on the 1,200 reservoirs in the United States was scarcely considered; these cover a wide geographical range, and data from them, now being processed by computer, might help predictions in other areas.

The symposium brought out clearly the opportunities presented by the making of new lakes if the problems are tackled in a multi-disciplinary manner. There is always a need for a strong focal point to bring the biologists and sociologists into touch with the engineers at an early stage. The time factor is vital: not only must the research start early enough, but it is also necessary to beware of the hiatus which often occurs between the initial scientific work on a scheme and its implementation. This gap may last years while finance is being sought and it often causes a loss of impetus in the scientific programme. As a result a hastily organized crash programme becomes necessary.

In addition to the formal papers presented and discussed, this symposium was particularly valuable in bringing together representatives of varied disciplines. Many of those attending had never before had the opportunity to meet their colleagues working on similar problems in other parts of the world. Thanks are due to the Institute of Biology for organizing the meeting, and to the Royal Society, the Ford Foundation and the American Conservation Association, who provided travel funds to enable some of the overseas delegates to attend.

The desire was expressed, especially by the tropical workers, for a meeting a few years hence, to bring the working scientists and engineers together again, preferably on one of the new great lakes in the tropics. Air fares may seem expensive, but exchange of experience and the proper planning of these great developments can prove a sound investment. Another follow-up to the symposium may be expected in the field of information and bibliography. Stimulated by the special interest of ICSU in the subject, the African Science Board of the National Academy of Sciences, Washington, D.C., has arranged for the preparation of a selected guide to the literature on man-made lakes, and copies were available at the symposium. Another bibliography, on reservoir fishery biology in North America, was issued this year by the Department of the Interior, Washington, D.C. Perhaps there is a case now for establishing a small bureau of scientific information on all aspects of man-made lakes, so that data can be collected and distributed without the inevitable delays associated with the normal routine of publication.

ROSEMARY L. McCONNELL
E. B. WORTHINGTON

OBITUARIES

Prof. Jocelyn Patterson

PROF. JOCELYN PATTERSON, head of chemical pathology at Charing Cross Hospital Medical School, University of London, since 1948, died suddenly at his home in Chorley Wood on September 6 within a few days of his sixty-fifth birthday.

He was educated at Armstrong College, University of Durham, and graduated with first-class honours in

chemistry in 1920; the following year he was awarded a Master's degree. His first research work was on the halogenation of unsaturated hydrocarbons and on organic sulphur compounds. Moving to St. Salvator's College, University of St. Andrews, he gained his Ph.D. in 1923, and worked under the direction of the late principal, Sir James Irvine, much of this time acting as the latter's private research assistant. Here he gained wide experience

in the chemistry of carbohydrates, especially with regard to fructose and its derivatives.

In January 1924 Charing Cross Hospital and Medical School were fortunate and wise to secure his services as their first biochemist and lecturer in chemical pathology. His research activities were chiefly on quantitative aspects of chemical pathology.

In 1938, collaborating with H. W. C. Vines (later professor of pathology at Charing Cross Hospital) and others, he assisted in writing *The Adrenal Cortex and Intersexuality*, and in 1952 he contributed a section to *Recent Advances in Chemical Pathology*. Apart from his main interests, many papers published by other Charing Cross staff acknowledge the assistance he gave to them in their work; indeed, his wise and balanced judgement was widely sought and appreciated by all who came into contact with him.

After the Second World War in 1947, when the pre-clinical school of Charing Cross was re-established, Prof. Patterson became responsible for the teaching not only of chemical pathology but also of biochemistry. In 1948 the University of London conferred on him the title of professor of chemical pathology. Just previous to his death the University of London conferred on him the title of emeritus professor. He served as vice-dean of the Medical School from 1954 until 1957, served on the School Council, and for many years he was on the Board of Governors of the Hospital. He was a member of the Boards of Studies in Biochemistry and in Pathology and also of the Board of Pre-Clinical Studies of the University of London. After Charing Cross Hospital became associated (in 1959) with the West London and Fulham Hospitals, Prof. Patterson took on the additional responsibility of supervising their chemical laboratories.

Prof. Patterson's hobbies included gardening, and at his home in Chorley Wood he had a beautiful well-kept garden.

In 1926 he married Kathleen (*née* Thompson), who survives him. To her and his son we extend our sympathy.

W. J. HAMILTON

Prof. Wilfred Merchant

PROF. WILFRED MERCHANT, professor of structural engineering in the Faculty of Technology, University of Manchester, since 1957, died on October 12 at the age of fifty-three after a long illness. He was educated at Manchester Grammar School and graduated with first-class honours in engineering science at Oxford in 1933. He then spent a number of years with a large firm of structural engineers.

He held a Commonwealth Fellowship at the Massachusetts Institute of Technology and obtained the degree of M.S. in 1939 with a thesis in soil mechanics. This work was the first rational advance of the general theory of consolidation of clay since its formulation in 1923, and it has recently been recognized that modern treatments of the subject are mathematically equivalent to that in Merchant's 1939 thesis.

During the Second World War, Prof. Merchant was employed by Metropolitan Vickers, Ltd., on the design of jet engines. He made major contributions to the aerodynamic theory of flow through compressor blading. There was very little knowledge or experience of axial flow compressors at that time and his contributions played an important part in the design of jet engines.

Prof. Merchant joined the staff of the Manchester College of Science and Technology, Faculty of Technology in the University of Manchester, in 1946, and was appointed reader in applied mechanics in 1951. After he joined the College his main interests were in the prediction of the failure load of structures taking account of the interaction of plasticity and stability. His intuition led him to propose an interaction formula which is now accepted and known by his name in the literature of the

subject. During the past few years he developed computer techniques to carry out the erection calculations for the new Forth and Severn suspension bridges.

He was a member of the Institution of Structural Engineers, a member of the Institution of Civil Engineers and an associate member of the Institution of Mechanical Engineers and of the American Society of Civil Engineers. He served his professional institutions in many ways and was chairman of the Lancashire and Cheshire branch of the Institution of Structural Engineers during 1960-61.

As a teacher he always found time to discuss problems individually with his students. His advice and guidance both to his students and to his colleagues were of great value. His contributions in three major branches of engineering are significant of his wide interests and keen analytical mind.

He leaves a wife, a son and a daughter. S. S. GILL

Dr. Paul Müller

PAUL MÜLLER, who was awarded the 1948 Nobel Prize for Physiology and Medicine, died in Basle on October 13, 1965, after a short illness.

Paul Müller was born on January 12, 1899, in Olten. His father, who was on the staff of the Swiss Railways (SBB), moved soon afterwards to Basle, and Basle became his home. There, after a spell as laboratory technician, he completed his studies at what was then the Obere Realschule (Modern High School) and then read chemistry at the University under Profs. Fichter and Rupe. In 1925 he took his doctorate with the highest honours, with a thesis on "The Chemical and Electrochemical Oxidation of Asymmetrical *m*-Xylidene and its Mono- and Di-methyl Derivatives". In the same year he joined Geigy as a research chemist, and he stayed with the firm for the rest of his life.

At first he worked on tanning, and developed a number of new synthetic tans. The problem of the preservation and disinfection of animal skins soon turned his attention to biology; and from 1935 he became more and more interested in pest control, a field with which organic chemists at that time had little concern. In the difficult years of the Second World War, when the battle for production was on, he developed and made available to Swiss farmers a mercury-free seed dressing. While still engaged on this development work his interest was turning to insecticides, and with characteristic doggedness he tested large numbers of chemical compounds, at first without success. But in 1939 his perseverance, coupled with his acute perception and methodical approach to all his experimental work, led him to the discovery of the insecticidal properties of dichloro-diphenyl trichloroethane, the active ingredient of DDT, which he developed for practical use. His achievement was to have discovered, at a critical time in the War, an insecticide with a contact and persistent action far and away superior to any other known product. His discovery was used both during and immediately after the War to protect millions of service-men and civilian populations in all countries against epidemic diseases. Apart from their use in hygiene and agriculture, DDT insecticides are still extensively used to-day with success in many parts of the world against the *Anopheles* mosquito, the carrier of malaria. The significance of this great discovery was fittingly acknowledged by the award of the Nobel Prize for Physiology and Medicine in 1948.

In 1963 Müller received an honorary doctorate in the Faculty of Medicine at the University of Thessalonika, in recognition of the beneficial effect of DDT products in the Mediterranean area. He also became an honorary member of the Swiss Nature Research Society and of the Paris Society of Industrial Chemistry.

For all these many distinctions, Paul Müller remained what he had always been: a modest man, outwardly reserved, but with a passionate devotion to science. Even

as deputy head of pest control research at Geigy, he preferred above all else the work of a research chemist. After his retirement in 1961 he continued to devote his energy and enthusiasm to work in his private laboratory at his home in Oberwil, near Basle. He found relaxation

in his beloved countryside, in the Jura mountains and the Alps, and in family life. By his death, Basle and its chemical industry, and indeed the world of science, have lost a scientist whose personality and attainments will not soon be forgotten.

NEWS and VIEWS

The Royal Society:

PROF. P. M. S. BLACKETT, emeritus professor of physics, University of London, Senior Research Fellow, Imperial College of Science and Technology, London, and part-time scientific adviser to the Ministry of Technology, has been elected president of the Royal Society in succession to Lord Florey. The new physical secretary is Prof. M. J. Lighthill, Royal Society research professor at the Imperial College of Science and Technology. The new foreign secretary is Prof. H. W. Thompson, professor of chemistry in the Physical Chemistry Laboratory, Oxford. The officers re-elected for the ensuing year were: *Treasurer*, Lord Fleck, formerly chairman of Imperial Chemical Industries, Ltd.; *Biological Secretary*, Prof. A. A. Miles, director of the Lister Institute and professor of experimental pathology in the University of London. Other members of Council elected (or re-elected, marked *) were: Prof. M. S. Bartlett, professor of statistics at University College, London; *Prof. D. H. R. Barton, professor of organic chemistry at the Imperial College of Science and Technology, London; Lord Brain of Eynsham, consulting neurologist to the London Hospital and consulting physician to Maida Vale Hospital; *Dr. F. S. Dainton, vice-chancellor of the University of Nottingham and honorary director of the Cookridge High Energy Radiation Research Centre, University of Leeds; *Prof. K. C. Dunham, professor of geology in the University of Durham; *Prof. G. W. Harris, Dr. Lee's professor of anatomy at the University of Oxford; Dr. S. G. Hooker, technical director (aero), Bristol Siddeley Engines, Ltd.; *Prof. W. O. James, professor of botany at the Imperial College of Science and Technology, London; Dr. J. C. Kendrew, deputy chairman of the Medical Research Council Laboratory of Molecular Biology, Cambridge; Dr. R. D. Keynes, director of the Institute of Animal Physiology, Babraham, Cambridge; *Sir Hans Krebs, Whitley professor of biochemistry at the University of Oxford; *Dr. N. Kurti, reader in physics, University of Oxford, and Senior Research Fellow, Brasenose College; *Dr. K. Mather, vice-chancellor of the University of Southampton; Prof. P. T. Matthews, professor of theoretical physics at the Imperial College of Science and Technology, London; Dr. L. Rotherham, member for research, Central Electricity Generating Board; Sir Solly Zuckerman, chief scientific adviser to the Secretary of State for Defence, scientific adviser, Cabinet Office, and Sands Cox professor of anatomy in the University of Birmingham.

Prof. P. M. S. Blackett, C.H., P.R.S.

THE attainment of the highest scientific honour in the land crowns a distinguished career of more than forty years. Patrick Maynard Stuart Blackett has received many honours. A range of physical research, particularly an intensive study of cosmic rays by the cloud chamber method, led to his election as Fellow of the Royal Society in 1933, the award of a Royal Society Medal in 1940 and a Nobel Prize for Physics in 1948. He has three times served on the Council of the Royal Society—during 1940–42, during 1944–46, when he held the office of vice-

Officers

president for a year, and during 1963–65. In 1946 he was awarded the American Medal of Merit for his work in operational research, and 10 years later he was again honoured by the Royal Society, receiving the Copley Medal for his work in the fields of cosmic rays and palaeomagnetism.

Blackett began his scientific career at Cambridge in the great days of the late Lord Rutherford. He had achieved an international reputation before he was thirty—a more remarkable achievement in that he started late, for he began life as a sailor. Born on November 18, 1897, Blackett was educated at the Royal Naval Colleges at Osborne and Dartmouth. He served in the Royal Navy throughout the First World War, taking part in the battles of Jutland and the Falkland Islands. His keen interest in science led him after the War to Cambridge, where he studied physics under Prof. Rutherford (as he then was). Blackett graduated in 1921, and 2 years later was made a Fellow of King's College. He worked in the Cavendish Laboratory from 1921 until 1933 when he became professor of physics at Birkbeck College. In 1937 he went to the University of Manchester as Langworthy professor of physics, succeeding Sir Lawrence Bragg who himself had succeeded Rutherford. During his 16 years at Manchester, Blackett presided over and greatly expanded a department which already had a fine tradition. This achievement was repeated by Blackett at the Imperial College of Science and Technology, which he joined in 1953 as professor of physics and head of the physics department, and was responsible for the planning and supervision of the College's fine new Physics Building which was opened in 1960. Perhaps Blackett's most important contribution to the growth of British academic physics during this period was his persuasive support for the College's campaign for a very substantial increase in the number of professors. This trend away from the old European concept of one professor towards the multi-professorial pattern forming a department with a broad field of study is now widely accepted. Blackett has served the Imperial College in many capacities. He was dean of the Royal College of Science from 1954 until 1960 and pro-rector from 1961 until 1963. On his retirement in September 1965, he was appointed a Senior Research Fellow and will continue to have a research laboratory in the physics department.

Blackett and those working with him have made significant contributions to three main fields of physical discovery: the interaction with matter of fast particles from radioactive sources, the nature of the particles in the cosmic rays, and the magnetism of the Earth. During 1921–31 he was chiefly occupied with the development and operation of automatic Wilson cloud chambers and their application to the precise measurement of the parameters involved in collisions between α -particles and atomic nuclei. He photographed for the first time the disintegration of a nucleus. From 1931 onwards he applied and extended the cloud-chamber technique to the study of the collisions involved in the cosmic rays, establishing with Occhialini the existence of showers of nearly equal numbers of positive and negative electrons. From 1947 onwards, Blackett revived interest in the old

theories of Schuster and others of the possibility of a 'fundamental' connexion between the angular momentum of a large rotating body such as the Earth, and its magnetic field. Further astronomical and geophysical evidence made the theory untenable. As a by-product, a special magnetometer was designed for this work and has been widely used in the rapidly growing subject of palaeomagnetism. Out of this work, which is being energetically pursued in many countries, is emerging the beginnings of a history of both the wandering of the continents and of the Earth's magnetic field. During recent years Blackett has directed a small group working on palaeomagnetic problems, doing much of the experimental research himself.

Blackett has made many valuable contributions in the wider field of British science. Always an enthusiastic supporter of and advocate for the applied sciences he became an active member of the National Research and Development Corporation in 1949, and is now deputy chairman of the Advisory Council in the Ministry of Technology. From 1956 until 1960 he was a member of the council of the Department of Scientific and Industrial Research during a period when a phenomenal growth occurred in grants to university departments for scientific and technological research. In January 1965 he was appointed by the Secretary of State for Education and Science to the Council for Scientific Policy. Blackett has played an active part in many aspects of the impact of science on public affairs. His contributions to the study of problems of disarmament are well known, as also is his interest in scientific and technological aid to emergent countries—the subject of his Presidential Address to the British Association for the Advancement of Science in 1957.

Biochemistry and Organic Chemistry at Charing Cross Hospital Medical School : Prof. A. N. Davison

DR. A. N. DAVISON has been appointed to succeed the late Prof. J. Patterson (see p. 1042 of this issue of *Nature*) in the chair of biochemistry and organic chemistry at Charing Cross Hospital Medical School. Dr. Davison was born in 1925 and was educated at Westcliff High School, University College, Nottingham, and Birkbeck College, London. He holds the following degrees of London University: B.Pharm. (1946), B.Sc. (1950), Ph.D. (1954) and D.Sc. (1963). He served in the Royal Army Medical Corps from 1946 until 1948, and was afterwards appointed a group manager with Messrs. Potter and Clark, Ltd. In 1950 he joined the staff of the Medical Research Council Unit for Research in Toxicology to work on cholinesterase inhibitors. A year (1954–55) at the Sorbonne was followed by his appointment as biochemist to Roche Products, Ltd., and then (1957) by his becoming a member of the Medical Research Council external staff in pathology at Guy's Hospital Medical School. In 1960, Dr. Davison was appointed lecturer and then reader in the Biochemistry and Chemistry Department at Guy's Hospital Medical School. He is particularly well known for his work on the biochemistry of brain lipids and on aspects of brain metabolism generally. His demonstration (with the late Prof. G. Payling Wright and Dr. J. Dobbing) in 1958 of the persistence of radioactive cholesterol, once deposited in the brains of young animals, contradicted generally held biochemical opinion, which was dominated by Schoenheimer's conception of a dynamic equilibrium of constituents of the living body.

Science in Belgium

THE National Council for Scientific Policy, Belgium, has published a study of the scientific potential of Belgium in 1961. It covers the whole range of scientific activity in Belgium, including manpower, organization, institutions, equipment, financial resources and distribution (Conseil

National de la Politique Scientifique. *Inventaire du Potentiel Scientifique 1964 de la Belgique—Année 1961*. Pp. 303. Bruxelles: Conseil National de la Politique Scientifique, 1964). Of a total of 22,504 full-time scientific workers, 6,703 had university degrees and 2,451 diplomas or some higher technical qualification, the remaining 13,350 being technicians or other workers. These were distributed among 1,644 scientific units with an expenditure of 4,312 million francs, excluding that in the educational field, inter-university centres and learned societies; 810 units employing 3,623 of these with a university degree were in the educational sector; 204 (1,100) were in the public sector; 431 (1,437) in private enterprise; 57 (328) in research associations; 4 (143) in inter-university centres; and 84 (11) in learned societies. The expenditure in the public sector was 1,465 million francs; in private enterprise, 2,404 million francs; and in research associations, 400 million francs. Of the scientific units and persons with a university qualification, 1,284 and 5,433, respectively, were engaged in the exact sciences; biology (174 and 764), medicine (182 and 1,064), and technology (620 and 2,136) taking the most, with physics (99 and 604) and chemistry (85 and 496) coming next; 180 units and 821 of the qualified personnel were in the social sciences, but expenditure was only 264 million francs, compared with 3,902 million francs on the exact sciences. In the field of education, in 8.5 per cent of the scientific units employing 85.5 per cent of the staff, research was an important activity; in the public sector the corresponding figures were 71.6 per cent and 73.9 per cent; in private enterprise, 55.7 per cent and 86.6 per cent; and in research associations, 61.4 per cent and 71 per cent. 80 per cent of the scientific units and 88 per cent of qualified staff in the educational sector are in the four universities, and 56 per cent of this staff is in the faculties of science and medicine. Only 10 per cent of the private firms employed more than 1,000 people, but these firms employed 49 per cent of the university-qualified staff engaged in this sector. Almost half the scientific units and a little more than half the staff with a university degree are employed in the provinces of Brabant, Liège and East Flanders, with some 15 per cent of the scientific units, and corresponding proportions of qualified staff, but in the latter province some 85 per cent are employed in the educational sector. In the exact sciences, 25.9 per cent of the scientific units and 33.7 per cent of the qualified staff were engaged exclusively or mainly in fundamental research, and 62.1 per cent and 54.1 per cent, respectively, exclusively or mainly in applied research.

Training of Public Health Officials

UNDER the lengthy title *Special Courses for National Staff with Higher Administrative Responsibilities in the Health Services*, a report has been published by the World Health Organization (Technical Report Series, No. 311. Report of a WHO Study Group. Pp. 31. Geneva: World Health Organization; London: H.M.S.O., 1965. 2 Sw. francs; 3s. 6d.; 0.60 dollars). The document attempts to emphasize the need for well-informed public health officials and to outline the general principles underlying the training they require. The report points out that many public health administrators, whether medically qualified or not, are not conversant with the knowledge and techniques required for the efficient administration of public health services. In order to meet the pressing demand for competent health administrators, particularly in the developing countries, the World Health Organization Study Group has advocated the establishment of intensified two-month courses for health administrators with or without medical qualifications. Such courses, the report concludes, should be sponsored at Government level or by official health organizations. The content of such courses should cover three principal aspects of public health: (1) historical development and philosophical concepts;

(2) scientific and technical subjects involving the study of such matters as the implementation of public health programmes and the nature of environmental and social health problems; (3) administration. Under this last heading such topics as the evaluation of public health programmes and the economic aspects of health and disease would be considered. Full-time courses, fellowships, and seminars covering similar schemes of study are also advocated for personnel who are likely to be more suited to these forms of training. Details of the types of programmes in public health administration already adopted or advocated by universities in Yugoslavia, Canada and the United States, and by Government organizations in Mexico and India, are given in appendixes to the report.

South Australian Museum

THE annual report of the South Australian Museum for the year ended June 30, 1964, states that the most noteworthy aspect of the year's progress is the increased efficiency resulting from the appointment of the Museum's first information officer (South Australia. Report of the Museum Board, 1 July, 1963, to 30 June, 1964. Pp. 11. Adelaide: Government Printer, 1965). This has relieved the research staff of an immense volume of enquiries and is of special benefit to trainee-teachers and senior students. A registry of speech and sound collections has been prepared. This includes wax cylinders, acetate disks and tape-recordings of data on the Australian Aborigines. It has been of especial value to the Australian Institute of Aboriginal Studies. The staff has been active in assisting cognate organizations such as National Parks and Wild Life Reserves and the Flora and Fauna Advisory Committee of the Zoological Society. More than thirty major papers have been published by the staff during the period under review.

Pesticide-Wildlife Studies

SUMMARIES of Federal pesticide-wildlife investigations in the United States have been prepared from time to time. In 1963, in order to integrate Federal studies with those of other agencies, the United States Fish and Wildlife Service requested the co-operation of the Canadian Government and Provinces, each State, and the universities in listing and describing their pesticide-wildlife investigations. These investigations have been summarized, giving the identification of the State, Province, or university, research worker in charge, title and description of the work, and a brief abstract of findings and progress. The tabulation is alphabetical by States, and separately by Provinces, where the work was done or administered. Some of the projects may now have been terminated or may not be active. Much of the work is unpublished, but a list of addresses should facilitate direct communication with investigators. The survey was part of an international listing of research co-ordinated by the Committee on Ecological Effects of Chemical Controls of the International Union for the Conservation of Nature and Natural Resources, and prepared by John L. George, co-operator, Division of Wildlife Research, Bureau of Sport Fisheries and Wildlife. The international listing is arranged alphabetically by author under various subjects such as analytical techniques for chemicals, ecological effects on organisms of different major habitats or of captive animals, and vegetation control of chemicals (U.S. Department of the Interior: Fish and Wildlife Service. Circular No. 224: *Pesticide-Wildlife Studies by States, Provinces and Universities—An Annotated List of Investigations through 1964*. Pp. i+30. Washington, D.C.: Government Printing Office, 1965).

Laboratory Animals for Rheumatism Research

THE Nuffield Foundation, as part of its programme of research into possible underlying causes of rheumatic

disease, is anxious to intensify the search for naturally occurring forms of rheumatoid, or rheumatoid-like, lesions in animals suitable for laboratory investigation. A naturally occurring rheumatoid arthritis in monkeys, for example, would have important implications for the controlled study of the disease. The fact that no evidence—or very little—has so far been obtained that this affliction of man is shared by other primates may simply be due to the almost total preponderance of young animals among those which have been available for study. The Foundation would now like to extend the search to cover a reasonably large sample of older monkeys, and to submit to expert examination the hands and feet (to include wrist and ankle joints) of elderly monkeys, preferably of known age. Centres already concerned in primate investigations, in the laboratory or in the field, and where colonies of monkeys are maintained throughout their natural life span, would evidently be in the best position to help. Offers of assistance should be addressed to Mr. Brian Young, the Nuffield Foundation, Nuffield Lodge, Regent's Park, London, N.W.1, stating the numbers, species and age-groups of monkeys available for examination. In some cases it might be possible to arrange for on-the-spot X-radiography. In other cases, for example, after death, it might be possible to send specimens, suitably preserved, to the rheumatologist responsible for examining the joints for the Foundation. Suitable instructions about this, and any other help required, which may include defrayment of expenses, can then be given according to circumstances.

African Mammals

THE first number of *Zoologica Africana* reports the proceedings of a symposium on African mammals held at Salisbury, Rhodesia, during September 26–28, 1963, under the auspices of the Zoological Society of Southern Africa, when it and a number of visiting delegates were the guests of the University College of Rhodesia and Nyasaland (*Zoologica Africana*, 1, No. 1. Edit. by M. K. Rowan. Pp. 265. Cape Town: Zoological Society of Southern Africa, 1965). The twenty-five papers are gathered into sections on physiology and behaviour, systematics, geographical distribution, population ecology, and wildlife diseases. There are, in addition, opening and closing addresses, a note on latitude-longitude grid maps of Africa, and a record of the resolutions from the Plenary Session of the Congress. The papers covering this very wide field are, as would be expected, of unequal weight, but none is without value; the delegates who attended the symposium returned home with the feeling that it marked the beginning of a new drive in the study of African mammals. The problems to be investigated are almost infinite, and the near-certainty of the drastic reduction and perhaps extinction of the populations of some species emphasizes the urgency of pressing on with the biological study of the African fauna before it is too late. African zoologists are fully aware of the danger to the wildlife of Africa; isolated and handicapped by lack of facilities (as many of them are), they are making the most of their opportunities and, as the results of this symposium show, accumulating a valuable body of knowledge. It is much to be hoped that they will receive the encouragement and backing that they deserve from the various authorities controlling the territories concerned and will be granted the financial aid without which such work cannot be sustained.

Zoological Society of London

THE modernization plans of the Zoological Society of London have made significant progress in 1965. In March, the new Elephant and Rhinoceros Pavilion was opened; in April, the Nuffield Institute of Comparative Medicine was inaugurated and is now fully operative; in May, the first group of birds was installed in the new Snowdon Aviary; in June, the building of the Smal

Mammal House in the Middle Gardens began. In October the Snowdon Aviary was opened to visitors. Among the births in the Collection in 1965 were two red deer and four blackbuck; on the Mappin Terraces wild boars were born and the seven piglets trotting around the enclosure with their mother have been a great attraction. In the Monkey House a tufted capuchin monkey, the first since 1935, and a mandrill were born; and in the Chimpanzee Breeding House, established in May 1964, two female chimpanzees were born but, unfortunately, one has since died. While chimpanzees are perhaps one of the most common of zoo animals, comparatively few are bred in captivity. In recent months, a lion, a puma, a Chinese leopard, two Caracal lynxes, Arctic foxes, a Syrian bear, an American bison and a wallaroo have been born. Among the birds hatched were four Kenya eagle owls, two spotted eagle owls and a barn owl. One of the most exciting additions to the bird collection was a magnificent group of 24 birds of paradise presented by Sir Edward Hallström to mark the visit of Earl Mountbatten of Burma, accompanied by Sir Solly Zuckerman, to Taronga Zoological Park, Sydney. These birds are rarely seen outside Australasia. This superb collection includes the ribbon tail, the sickle bill and lesser superb birds of paradise, the magnificent bird of paradise and the raggi bird of paradise. As part of the same collection, Sir Edward Hallström presented six green pythons, which are some 3-5 ft. long. Lord Chaplin, a former secretary of the Society, brought back from Trinidad an interesting collection of reptiles and amphibians, including four species of frogs new to the London Zoo—the spotted tree frog, the Trinidad golden tree frog, Fitzgerald's marsupial frog and the giant tree frog. The number of visitors to the Gardens in 1965 was considerably higher than in 1964.

Vegetation of Uganda

I. Langdale-Brown, J. A. Osmaston and J. G. Wilson have produced a detailed and authoritative account of the vegetation of Uganda (*The Vegetation of Uganda and Its Bearing on Land-Use*. Pp. 159+24 plates. Entebbe: Government Printer; London: The Uganda High Commission, 1964. 30s.). This will be invaluable for all students of the ecology of the country and for all those engaged in the improvement of land use both for agriculture and livestock production and for forestry. The book is well produced by the Government of Uganda, well illustrated and supported by a substantial folder of maps. It is to be recommended to all those concerned with development in Uganda and to botanists interested in the vegetation of the Equatorial Great Lakes Region.

American Geographical Society: Awards

THE following awards of the American Geographical Society have been presented: *David Livingstone Centenary Medal*, to Dr. Bassett Maguire, for his scientific achievement in the geography of the Southern hemisphere; *Cullum Geographical Medal*, to Prof. K. F. Mather, for distinguished service in the advancement of geographical science; *Charles P. Daly Medal*, to Prof. W. S. Cooper, for distinguished geographical services as a plant ecologist and geomorphologist; *Honorary Fellowships*, to Prof. M. J. Dunbar, for his outstanding contributions to geography through the disciplines of marine science, and to Dr. P. Meigs, in recognition of his work in the problems of water resources, arid lands, climatology and historical geography.

University News:

Belfast

H. R. MARTIN has been appointed to a lectureship in mechanical engineering.

Bristol

THE following lecturers have been appointed: Dr. E. G. Ellison (mechanical engineering); Dr. J. Hindley (bio-

chemistry); Dr. M. S. Knapp (medicine); Dr. F. P. Sayer (theoretical mechanics). The title of professor emeritus has been conferred on the following: Prof. W. Baker (formerly professor of organic chemistry); Prof. R. J. Brocklehurst (formerly professor of physiology).

Leeds

PROF. P. GRAY has been appointed professor of physical chemistry and head of the Department of Physical Chemistry. Dr. F. G. Holliman has been appointed to an additional chair of organic chemistry. Dr. D. R. Baulch has been appointed lecturer in the Department of Physical Chemistry. The title of reader has been conferred on the following: Dr. K. S. Zinnemann (bacteriology); Dr. B. E. C. Nordén (in respect of his post as director of the Mineral Metabolism Research Unit of the Medical Research Council).

London

THE following readers have been appointed: Dr. D. C. Watts (biochemistry), tenable at Guy's Hospital Medical School; Dr. D. W. Allan (mathematics), tenable at King's College. The following title has also been conferred: H. Billett, professor of mechanical engineering, in respect of his post at University College.

Manchester

THE title of professor emeritus has been conferred on the following: Prof. E. W. Anderson (formerly professor of psychiatry and director of the Department); Sir Robert Platt (formerly professor of medicine); Prof. W. Schlapp (formerly Brackenbury professor of physiology and director of the physiological laboratories).

Announcements

Prof. Marcel Nicoler, Dr. Sydney Chapman and Dr. Joseph Kaplan have been awarded the Hodgkins Gold Medal of the American Meteorological Society, for their outstanding contributions to the field of atmospheric research.

THE fourth Matscience Anniversary Symposium will be held at the Institute of Mathematical Sciences, Madras, during January 3-14. Further information can be obtained from the Institute of Mathematical Sciences, Central Polytechnical Buildings, Adyar, Madras 20.

A MEETING of the Biochemical Society will be held in the University of Sheffield during January 5-6. The programme will include a colloquium on "Control of Enzyme Patterns in Tissues". Further information can be obtained from the Administrative Secretary, the Biochemical Society, 20 Park Crescent, London, W.1.

A SYMPOSIUM on "Cutting Costs through the Control of Quality", arranged by the Institution of Engineering Inspection, will be held in the University of Strathclyde on January 6. Further information can be obtained from the Secretary, Institution of Engineering Inspection, 616 Grand Buildings, Trafalgar Square, London, W.C.2.

AN evening meeting of Aslib will be held in London on January 27. The programme will include a lecture by H. A. Chesshyre (Haxfield College of Technology) on "Local Co-operation: a Positive Force". Further information can be obtained from the Meetings Organizer, Aslib, 3 Belgrave Square, London, S.W.1.

THE Royal Institution Christmas Lectures for Young People will be delivered by Sir Bernard Lovell, Prof. F. Graham Smith, Prof. Martin Ryle and Dr. A. Hewish on "The Exploration of the Universe". The lectures will include: introduction and techniques (December 28); the solar system (December 30); the Earth as a planet (January 1); the Milky Way (January 4); radio galaxies (January 6); cosmology (January 8). Further information can be obtained from the Royal Institution, 21 Albemarle Street, London, W.1.

BIOLOGICAL SYSTEMS AT THE MOLECULAR LEVEL

A SYMPOSIUM was organized by the Commission of Molecular Biophysics of the International Union of Pure and Applied Biophysics during September 8-11, under the auspices of the International Laboratory of Genetics and Biophysics, Naples—the local arrangements being made by Prof. A. Buzatti-Traverso.

The first day's discussions, under the chairmanship of Prof. H. A. Scheraga (Cornell), were concerned fundamentally with the problem of predicting the conformations of proteins from their primary structure.

In the first paper Dr. S. Lifson (Rehovoth) gave an account of his recent statistical-mechanical investigations of conformational changes in polypeptides. His method consisted in defining sequences, sequence partition functions and sequence-generating functions and in using them to derive an equation for the contribution of each chain element to the partition function of the whole molecule. The results make possible, for example, an assessment of the relative importance of hydrogen bonding and hydrophobic interactions in determining the conformation of poly-L-alanine at different temperatures.

The remaining papers were more closely concerned with the investigation of specific conformations and the limitations imposed on them by interactions between non-bonded atoms. Given that the conformation of the peptide group is generally *trans* and planar, the most important variables determining the conformation of a polypeptide chain are the angles ψ and ϕ for rotation about the N—C α and C α —C β bonds respectively. Prof. G. N. Ramachandran (Madras) reviewed the earlier work in which he and his colleagues had studied the values of ψ and ϕ that are allowed when fixed minimum distances of approach are set for the atoms of a dipeptide unit comprising the two peptide groups and the β -carbon joined to an α -carbon atom. This had shown that only certain regions in a ψ , ϕ -diagram are sterically allowed and that these regions enclose, or nearly enclose, most of the conformations that have been proposed for polypeptides or observed in analyses of relevant structures. He then described an investigation of the effect of allowing the angle NC α C β to vary by $\pm 5^\circ$ from the tetrahedral value (while the deviations from 110° of the other angles at C α were minimized), which showed that the allowed regions in the ψ , ϕ -diagram are increased, by the introduction of this flexibility, to embrace most of the observed conformations that were previously just forbidden.

Prof. Ramachandran went on to consider the properties of helical polypeptide chains, in which the angles ψ and ϕ are kept constant at each α -carbon, with particular reference to the main chain —NH . . . O hydrogen bonding. He showed that families of helices of the right- and left-handed 3·6 $_{13}$ or α -type and of the 3·0 $_{10}$ type (with hydrogen bonds from peptides 1 to 4, and 1 to 3, respectively) are sterically allowed, with hydrogen bonds of acceptable length that depart from colinearity by less than 30° , provided that the bond angle at C α is close to 110° . On the other hand, right- and left-handed π -helices (1·5 hydrogen bonded) are allowed only when the angle at C α is 115° , and the 2·2 $_{7/2}$ ribbon structure (1·2 bonded) is permitted only with hydrogen-bond-angles greater than 20° . The triple helix structure for collagen with two inter-chain hydrogen bonds (angles of 27° and 30°) for every three residues, as proposed in Madras, falls within a fully allowed region in the ψ , ϕ -diagram, and a new suggestion was put forward that this structure also includes additional CH . . . O hydrogen bonds between neighbouring chains. When collagen has the sequence gly-pro-hyp in all three chains, however, it appears that only one hydrogen bond can occur for every three residues and the preferred structural parameters closely follow those given by Rich and Crick.

Finally, Prof. Ramachandran discussed the conformations of amino-acid side-chains that have been observed in crystal structures, showing (in keeping with earlier reviews) that they usually adopt fully staggered conformations, but that any one of the possible variants may be adopted in response to a particular environment.

Developments of the limiting-contact approach to the analysis of polypeptide conformations were also described by Dr. G. Némethy. In these studies the influence of various amino-acid side-chains on the conformation of the dipeptide unit was investigated, the results being presented again in terms of the allowed regions in the ψ , ϕ -diagram. It was shown that the addition of a γ -carbon atom reduces the percentage of allowed conformations from 16 per cent for C β alone to 14 per cent, even though there are three rotational positions giving staggered conformations of the side-chain from which to choose. The addition of atoms beyond C γ in an unbranched side-chain does not limit the range of allowed conformations any more, but the presence of more than one δ -atom, as in leu, reduces the allowed conformations to 11 per cent of the possible, and branching at C β , as in val, ile or thr, reduces this total to only 4·5 per cent.

From all these analyses by the limiting-contact method it is clear, therefore, that local steric restrictions, quite apart from the interaction of groups widely separated in the primary structure, severely restrict the number of conformations accessible to a polypeptide chain and go some way (though not yet far enough) towards making practicable the calculation of conformation from amino-acid sequence. A general method for handling such calculations has been pioneered by Prof. A. M. Liquori (Naples), and his colleagues, who described his preliminary attempts to calculate the helical conformations of minimum potential energy, taking into account the forces between non-covalently-bonded atoms. These calculations have led to diagrams of the potential energy as functions of ψ and ϕ which indicate, for example, that the right-handed α -helix with the original Pauling and Corey parameters is a very stable conformation even without consideration of the hydrogen-bonding that stabilizes it still further. There are four other potential-energy minima, including one corresponding to the left-handed α -helix which is shown, encouragingly, to be less stable than its right-handed counterpart.

Analysis of the structure of myoglobin has shown that these stable helical values of ψ and ϕ also occur frequently in non-helical regions of the molecule. Prof. Liquori suggested, therefore, that it might be interesting to investigate an idealized structure for myoglobin in which all the dihedral angles were constrained to take the values at the closest of these favoured pairs. Here again, of course, the aim was to reduce the number of conformations that has to be considered to manageable proportions.

Unfortunately there are very few reliable data from which the conformational potential energy of a polypeptide can be calculated. Prof. Liquori presented some evidence to suggest that the general features of the ψ , ϕ potential energy diagram for helices do not depend very sensitively on the exact shape of the interaction potential curves used in the calculations. He emphasized his belief that while precise energy values are not yet available, proper use of the relatively reliable van der Waals's radii may enable progress to be made towards the prediction of conformations.

In a final short paper in this session, Dr. D. C. Phillips (London) described the structure of lysozyme, recently determined at the Royal Institution, remarking particularly on the occurrence in it of some residues forming an anti-parallel pleated sheet and of some others in the conformation of the 3·0 $_{10}$ helix with corresponding hydro-

gen bonding. Nearly all the helices in the molecule appear to be distorted to some extent from the standard α -structure and there is evidence that the hydrogen bonds in them sometimes depart from linearity by 20° or more. He described how comparison of the main features of the structure with the varying hydrophobicity of the amino-acid residues in the primary structure had suggested that the polypeptide chain folds itself from the terminal amino-end, forming first a compact unit with a hydrophobic core, then an extended arm of hydrophilic residues, partly in the β -conformation, and finally a coil that nearly closes the gap between the two parts, leaving a cleft that appears to be the active region, before winding itself around the terminal amino-end. Following ideas developed in collaboration with Dr. P. Dunnill, he noted that analysis of the distribution of hydrophobicity along a polypeptide chain might be useful in predicting conformations if enough guiding principles could be established.

A preliminary calculation of the dihedral angles ψ , ϕ in lysozyme had shown them to be nearly all in allowed regions of a potential energy diagram calculated for a peptide unit by the use of Lennard-Jones type interaction potentials. This diagram, which was similar to that calculated by Brant and Flory, differed from the original limiting-contact diagram mainly in allowing a greater range of conformations near those appropriate to the $3\cdot0_{10}$ and the left-handed α -helices. This was presumably because in these regions the occurrence of marginally short contacts, which are forbidden in the limiting-contact analysis, are outweighed, in the calculation of potential energies, by the presence of a large number of favourable contacts. The effect on the ψ , ϕ -diagram is very similar to that reported by Prof. Ramachandran to result from allowing the $\text{NC}_\alpha\text{C}^\beta$ angle to depart from 110° .

These papers provoked a lively discussion in the course of which Dr. F. H. C. Crick (Cambridge) remarked that the study of allowed helical conformations has really progressed very little beyond the results obtained by Donohue from the careful measurement of models. He urged strongly that rigorous attempts should be made to distinguish the important conformational variables, by comparison of the relative energies involved, from among covalent bond-lengths and bond angles, hydrogen bond lengths and angles, van der Waals's contact distances and rotations about bonds, suggesting that calculations based on simple crystal structures might provide necessary criteria for the establishment of valid energy parameters. Dr. J. C. Kendrew (Cambridge) described the helices found in myoglobin, noting that they are less regular than was first supposed and that some residues, particularly at the carboxyl ends of α -helices, are in the $3\cdot0_{10}$ conformation. There was general agreement that models based only on α -helices and 'random coils' must be considered inadequate. In view of the present very large number of investigations in which peptide conformations are described in terms of ψ , ϕ -diagrams, a plea was made for the general adoption of the standard conventions for labelling rotations that have been drawn up by Dr. G. Némethy. These are shortly to be published in the leading journals.

The second day, under the chairmanship of Dr. G. M. Edelman (New York), was devoted to the structural basis of the immune response. Dr. A. Nisonoff (Urbana) gave an extensive review of work done on structure of immunoglobulins over many years, leading up to the present-day concept of a divalent multi-chain structure consisting of two heavy chains, and two light chains with two antigen-binding sites per molecule. Even specific antibody directed to a single antigen is highly heterogeneous. Four classes of immunoglobulins are known, which share light chains but differ in heavy chains. Although the main antigen-binding site is on the heavy chain, a consensus of opinion is that both light and heavy chains contribute to the configuration of the antigen-binding site. Recent studies suggested that the two heavy chains are held together by only one S—S bond. In closing, Dr. Nisonoff

discussed recent work by Drs. Hilschmann and Craig and Dr. Putnam and his colleagues, who have determined a partial amino-acid sequence of three Bence Jones proteins of type I (equivalent or similar to light chains of type I immunoglobulins). The C-terminal half of the molecule is constant in the three Bence Jones proteins, while the N-terminal half shows wide variation. Dr. Milstein (Cambridge) gave further data on the structures of several antigen proteins and suggested that the results were incompatible with the concept that the variations are due to single point mutations. We all await further sequence studies to throw light on the constant and variable regions of the antibody molecules.

Next, Prof. Jerne (Pittsburg) discussed the cellular kinetics of the antibody response when antigen is injected into the whole animal. Studying haemolytic antibody formation by individual spleen cells, using his plaque assay technique, he observed the events following the injection of sheep red blood cells. There is a rapid rise and fall in the number of antibody-forming cells. Subsequent to the injection of sheep red blood cells one is dealing with a cell population multiplying for a few days, after which time the cells reach an end-stage and do not divide into further antibody-forming cells. Dosage of sheep red blood cells affects the slope of the increase in antibody-forming cells as well as the time (in days) taken to reach a peak number of antibody-forming cells. At low doses of sheep red blood cells only two-thirds of the animals tend to respond and the slope of the increase of antibody-forming cells is much flatter. This is not easily explained by a population of reactive cells multiplying at a certain rate. Prof. Jerne discussed various possibilities. He suggested that perhaps the most likely explanation would be that lymph gland has several compartments, and that contact of antigen with reactive cells can only occur in certain parts of the lymph gland, perhaps the germinal follicles.

In the third paper in this section, Dr. M. Cohn (La Jolla) gave a lucid discussion of the potentiality of single cells. Since it has not been possible to clone antibody-forming cells in tissue culture, three approaches have been used. Different investigators have studied: (1) antibodies formed by single cells; (2) immunoglobulins formed by clones of transplantable murine plasma cell tumours; (3) the use of fluorescent staining techniques to detect different types of immunoglobulins in individual cells. The various studies agreed in finding that at least 90–95 per cent of the cells make only one antibody, one class of immunoglobulin and one type of light or heavy chain. In an investigation testing for genetic markers on heavy chains less than 5 per cent of the cells had a potential to express both alleles. Therefore, only one structural gene appears to be expressed by the majority of cells. The significance of the low percentage of apparently multipotential cells is not clear at present. No two myeloma proteins have been found to be alike. Therefore, the number of possible light and heavy chains must be very large.

In closing, Dr. Cohn discussed theories of antibody formation, germ-line versus soma. Soma would rely on mutation during the life of the cell and be less useful than the germ-line, which would follow Mendelian genetics.

Dr. B. Askonas (London) discussed the processing of antigens and the role of information at macromolecules in the immune response. She stated that the problem of control of antibody synthesis by antigen runs far behind the other problems. The fate of antigen was discussed at the cellular and biochemical level. Radioactive antigen is taken up by phagocytic cells throughout the lymph gland; in the secondary response it is particularly concentrated in the dendritic cells in the germinal centre. Although a major part of the antigen taken up is degraded very rapidly, the remaining antigen persists for weeks and is lost only gradually from the cells. The failure to detect radioactively labelled antigen in antibody-forming cells by G. J. V. Nossal and J. H. Humphrey and their

collaborators has shown that there can be only very few antigenic determinants present in the antibody-forming cell.

The question of how antigen stimulates the potential antibody-forming cell is still a vital problem. Whether it does so by direct interaction with the reactive cell or has to go through an intermediary cell needs further clarification. Since the phagocytic cells, the macrophages, take up the antigen they have been implicated as possible intermediary cells. RNA preparations containing antigen can be extracted from macrophages and they are highly active in inducing antibody, but whether this is an essential step in the induction of antibody is not clear. Suggestions that antigen-free informational molecules are transferred from macrophages to the reactive cells have also been made, but this has not been shown convincingly. Further experimentation is required to throw light on this problem.

On the third day a discussion on allosteric enzymes was held with Dr. F. Jacob (Paris) as chairman. The fact that a combination of one molecule of ligand with a macromolecule can influence the combination of another, the same or different, has been known for a long time. The term 'allosteric' proteins was introduced by Monod and Jacob to describe proteins in which such interactions occur. Although it is to be expected that many proteins are in some degree allosteric, the introduction of this term has been especially useful in directing attention to a particularly important class of phenomena involving enzymes which, potentially at least, provide an explanation of the regulation of metabolic processes in the organism.

The original observations of such interactions were on haemoglobin which may be considered as a type case of an allosteric protein. This was the subject of Dr. Wyman's talk. Dr. Wyman discussed mainly the haem-haem interaction, considered as a model of interaction between sites for the same ligand.

The second speaker, Dr. J. Monod (Paris), described a model which aims at explaining both the interactions between similar and between different ligands, in terms of quaternary structures of proteins. In this model an allosteric protein is considered to be a polymer with an axis of symmetry, which can exist under at least two different states which are assumed to differ between them by the degree of association between the sub-units. The two states are supposed to differ in their affinity for the ligands which the protein can bind so that the presence of a given ligand can push the equilibrium towards a given state.

The third speaker was Dr. H. K. Schachman (Berkeley, California), who gave a physico-chemical description of the enzyme aspartyltranscarbamylase of *E. coli*. Dr. Schachman showed that the enzyme is made of different sub-units, some of which possess a site specific for one of the substrates, aspartate, and others a site specific for CTP which inhibits the reaction catalysed by the enzyme. Isolated sub-units still exhibit affinity for their respective ligand but without co-operative effects, those being restricted to the complex polymer.

In the discussion, many other enzymes were discussed which exhibit similar behaviour. This is the case, for example, of the enzyme dCMP amino hydrolase which has been extensively investigated by Dr. Scarano. In

contrast, other complex enzymes appear to operate on a different scheme. This is the case, for example, of the enzyme glutamine synthetase of *E. coli*, investigated by Dr. E. Stadtman, an enzyme the activity of which is susceptible to partial inhibition by eight different compounds of widely different structures.

In the final session on "Molecular Aspects of Differentiation", with Prof. J. Brachet (Brussels) as chairman, the main topic discussed was the synthesis of nucleic acids and proteins during early development. Prof. Monroy (Palermo) described in detail the significance of the events following the process of fertilization in sea-urchin eggs. Ribosomes from unfertilized eggs are not capable of incorporating amino-acids, while those obtained from fertilized eggs are capable of doing so. Prof. Monroy presented the following simple experiment. When RNA from unfertilized eggs was added to liver ribosomes the latter incorporated amino-acids. But no incorporation was detected when RNA from unfertilized eggs was added to ribosomes from unfertilized eggs. He has therefore suggested that some inhibitor is present on the ribosomes which prevents them from synthesizing protein. Though the unfertilized egg has a store of messenger RNA, the ribosomes become active only after fertilization. Prof. Monroy further indicated that inhibition on the inactive ribosomes could be lifted considerably by treating them with trypsin and removing the trypsin by washing through a sucrose layer.

Dr. D. Brown (Baltimore) examined the synthesis of ribosomal, soluble and DNA-like RNA during development of *Xenopus*. The kind of RNA synthesized varies conspicuously as development proceeds. Dr. Brown has been able to show that DNA-like RNA and soluble RNA are synthesized during late cleavage phase and the synthesis continues after gastrulation. The synthesis of ribosomal RNA starts only at the onset of gastrulation and increases as development proceeds.

The final paper was one on cell interactions and carcinogenesis by Dr. L. Sachs (Rehovoth), who discussed *in vitro* studies on the mechanism of carcinogenesis by polyoma virus and by carcinogenic hydrocarbons. In the experiments with polyoma it was shown that virus infection can induce the synthesis of cellular DNA after normal cell DNA synthesis has been repressed by contact inhibition or by X-irradiation, that each cell is induced to synthesize about double its DNA content, and that this induction is not dependent on the replication of viral DNA, but is a function of the viral genome. It was suggested that all the known experimental findings on cell-virus interactions with the small DNA tumour viruses can be explained by the synthesis of a messenger RNA early after virus infection that mediates the induction of cellular enzymes required for DNA synthesis by way of alteration of the cell surface. In the experiments with carcinogenic hydrocarbons it was shown that these chemicals can directly and rapidly induce *in vitro* a high frequency of transformation of normal cells to tumour cells. Such *in vitro* investigations provide evidence on the similarities between the two types of carcinogenesis.

B. ASKONAS	D. C. PHILLIPS
J. A. V. BUTLER	L. SACHS
F. JACOB	G. V. SHERBET

LIVINGSTONE'S CONTRIBUTIONS TO MEDICINE

DAVID LIVINGSTONE has usually been looked on as a missionary and explorer, yet his contributions to medicine were numerous and varied. A review of them is given by M. Gelfand in a recent issue of *The Central African Journal of Medicine* (11, No. 7, 192; July, 1965).

He qualified as a licentiate of the Royal Faculty of Physicians and Surgeons (Glasgow) in 1840, and throughout

his life, so far as circumstances would permit, he did all he could to keep himself fully informed and up to date. For the greater part of his time in Africa he was tolerant of native practices in medicine, seeking to understand their materials and methods. Patients flocked to him in hundreds, and by the standards of the day he was particularly successful with eye complaints and obstetrics. He was probably the first to describe trypanosomiasis in

cattle, and as early as 1841 he gave arsenic to a mare infected with the disease.

When in the Tete district in 1856 the people were afflicted with a severe epidemic of smallpox, he tried to produce a vaccine by inoculating a heifer, but the inoculation failed to take; instead, he had to send to the Cape for a supply of vaccine. In the same year the Governor of Quelimane requested that he report on the causes of unhealthiness of Senna, a town on the Lower Zambesi. He attributed the ill-health to the low-lying nature of the land, polluted expanses of water, and decomposing vegetable matter.

Among other experiments, he carried out tests on the most suitable colours for clothing in the tropics; he reported on a disease called 'maculo', now no longer occurring in Africa, characterized by a spreading gangrene of the rectum; he described the earth-eating habits of natives in Northern Rhodesia; he wrote a vivid account of tropical ulcer among slaves; and he noticed that Africans living on a manioc diet, high in carbohydrates, suffered from disturbances of vision.

Livingstone was the first to record that the tick (*Ornithodoros moubata*) was the vector for relapsing fever; and he may have also been the first man of medicine to associate mosquitoes with malaria. He was aware of the great British expeditions to the Niger in 1832 and 1841, both of which ended in disaster, and after studying the account by M'William of the second expedition he came to the con-

clusion that quinine was effective against malaria if it was given at an early stage and in sufficient quantity. That led him to devise his pill, consisting of quinine and purgatives, which he was able to try out in a dramatic manner on two English hunters who were dangerously ill with fever at Lake Ngami one companion of theirs having already died. At this time his wife and children also became infected with malaria; he treated them successfully in the same kind of way. It was because of this treatment that he was able to travel right across Africa from west to east in 1856, and maintain activities in the unhealthy areas of the Zambesi and Shire Rivers for more than four years.

Livingstone also deserves credit for some attempts which he failed to carry through. When near the East Coast, for example, he came across trees in the forests of Senna which he thought were cinchonas, and he sent samples to England, only to learn from Kew that he had been mistaken. At another time he tried to prepare chloroform for his wife when her baby was due; he had obtained some chloral hydrate, having read of Simpson's discovery, but lacked a suitable retort to prepare the anaesthetic. On a further occasion he made use of electricity in treating a Mr. Hamilton in Bechuanaland who was suffering from paralysis.

In all these endeavours and discoveries Livingstone was moved by a pure missionary spirit, never seeking to make use of his medical knowledge for pecuniary gain.

CHEMISTRY OF PLANT PIGMENTS

A SYMPOSIUM on the chemistry of plant pigments, organized by the Phytochemical Group, was held in the University of Aberdeen on September 23-24. Attention in recent years has been given increasingly to pigments which are not readily analysed by conventional techniques either because of their lability or their intractability, and most of the substances discussed during the first day of the meeting fell into one or other of these two classes. Progress in the chemistry of the betacyanins and betaxanthins, for example, has been delayed because of their susceptibility to aerial oxidation during purification. In the first paper, Prof. M. Piattelli (Naples) described how he was able to separate these elusive nitrogenous colouring matters by fractionating plant extracts on polyamide columns. He found them occurring in plants of the Centrospermae as glycosides. Betanidin was the only aglycone of the many betacyanins, but he had discovered as many as eight betaxanthin aglycones. These yellow pigments were found to be related in structure to betanidin and differ in that the leucodopachrome moiety of betanidin is replaced by proline, 4-hydroxyproline, glutamine, glutamic acid, aspartic acid, tyramine, dopamine, or methionine sulfoxide.

In the second lecture, Dr. F. G. Holliman (Leeds) summed up the results of several years work on the red phenazine pigments present in *Pseudomonas aeruginosa*. He presented convincing structural and synthetic evidence that they are 2-amino-10-methylphenazinium betaines. One of them, aeruginosin B, is unique as a natural product in having a sulphonic acid group on a heterocyclic system, and furthermore is only the second natural sulphonic acid to be discovered, the first being the sulpholipid sugar moiety, sulphoquinovose. In the third paper, Prof. W. B. Whalley (London) also dealt with a *Pseudomonas* pigment, this time a blue pigment in *P. lemmonieri*. The compound, which analysed for CHON and the metal calcium, proved to be extremely intractable and did not respond to any of the usual physical methods of structure determination. After many attempts, Prof. Whalley eventually isolated a degradation product from chromium trioxide oxidation, a

pyridone quinone with an *n*-octyl side-chain, but the full structure of the pigment remains to be elucidated.

The afternoon session was devoted to the natural black pigments and began with an account of the melanins by Prof. R. A. Nicolaus (Naples). After discussing the chemical structure of sepiamelanin from cuttlefish ink, he went on to point out that whereas all animal melanins examined (19 sources) were derived from 5,6-dihydroxyindole, plant melanins, such as that in *Ustilago maydis* spores and in nine other sources, were derived from catechol. The question was raised, in discussion of this paper, whether plant melanins could justly be called melanins, since they did not contain nitrogen, and should instead be called humic acids. However, humic acids, as were described by Prof. R. D. Haworth (Sheffield) in the following paper, are organic pigments present in the soil, formed from decaying vegetation over the course of centuries. After a brief survey of the chemistry of humic acids, Prof. Haworth went on to outline his own recent investigations, which were based on the discovery that these compounds give electron spin resonance signals. He thought that humic acid consisted of an organic polycyclic 'core', which was responsible for electron spin resonance signals, to which metals, carbohydrates, phenolic and amino-acids are attached. He supported this view with the results from zinc dust distillation of humic acids, which had produced xanthone, anthraquinone and a series of alkylnaphthalenes. Dr. H. M. Hurst (Liverpool), in a paper on the phenolic moieties in humic acid, described how he had obtained more than 30 phenolic compounds, all being lignin and flavonoid derived units, from mild reduction of humic acids with sodium amalgam in weak alkali. He suggested that humic acid was a polymer derived mainly from lignins and flavonoids, which, after microbial degradation, were leached by rain-water from decaying leaves and deposited in the podzol. In the final paper on humic acids, Dr. R. I. Morrison (Macaulay Institute, Aberdeen) reported that, by oxidizing methylated humic acids with permanganate and identifying the products by gas chromatography, he had also obtained lignin-derived fragments. The products

he identified included anisic, veratric, 3,4,5-trimethoxybenzoic, 4-methoxysophthalic and isohemipinic acids.

The first paper presented on the second day was by Dr. J. C. Hughes (Norwich) and carried on the theme of humic acids, since it dealt with black and brown pigments formed in the potato after cooking or as a result of enzyme action. Production of the black pigment, an iron-chlorogenic acid complex, in cooked potatoes was greatly affected both by biochemical factors in the potato and by the environmental conditions in which the potato was grown; it could largely be prevented by adding citric acid to the cooking water. The level of browning, on the other hand, is determined principally by the amount of substrate (tyrosine) and not the amount of enzyme (phenolase) in the potato. Turning from vegetable to flower pigments, Prof. E. Bayer (Tübingen) then described his work on naturally occurring anthocyanin metal complexes. Protoecyanin, the natural chelate in cornflowers, he showed, can be split to cyanin, polygalacturonic acid, iron and aluminium. He pointed out that the reason why cyanin, present in red roses, would not complex with metal to give a blue colour was because chelating metals in these petals are not freely available, as they are bound to other substances of higher complex stability.

Anthocyanin pigments occur widely in leaves as well as flowers of higher plants and are abundantly present, for example, in leaves of the Asiatic *Coleus* species from which the popular ornamental plants are derived. However, Prof. H. Eugster (Zurich), in examining the red pigments on leaves of East African *Coleus* spp., found, not anthocyanins, but some new types of quinonoid pigments. The structures of two, coleon A and B from *Coleus ignarius* (fam. Labiatae), proved relatively easy to determine; A is a 2,5-hydroxynaphthaquinone with an isoprenoid attachment and B is a related hydroquinone. Fuestion, from *Fuerstia africana* (also Labiatae), proved to be unusually labile, being sensitive to acid bases and heat, but, by very skilful experimentation, Prof. Eugster had been able to show that it was a methylene-quinone, somewhat related in structure to pristimerine.

Yellow xanthone pigments were the subject of the next paper by Dr. J. C. Roberts (Nottingham), who surveyed their occurrence in flowering plants (nine families), in fungi (three genera) and in lichens (one genus). He described methods of structural determination, and dealt specifically with the identification of sterigmatocystin from *Aspergillus versicolor* and mangiferin from *Mangifera indica*. The latter xanthone has a *O*-glucosyl attachment and it thus formed a natural bridge to the next paper on naturally occurring *O*-glycosyl compounds by Prof. L. J. Haynes (Jamaica). He described the nuclear magnetic resonance evidence which supported *O*-glycosyl structures for mangiferin and related compounds, and outlined recent research on a new *O*-glucosylchromone in bitter aloes. The final paper by Dr. R. M. Horowitz (Pasadena) was on *O*-glycosylflavones, and in it he presented nuclear magnetic resonance evidence which proved that vitexin was 8-*O*-glucosylapigenin, isovitexin 6-*O*-glucosylapigenin and the lucenins 6,8-di-*O*-glucosyl-luteolins. He used similar data in very elegant fashion to show that the xylose in a *D*-xylosylvitexin (isolated from oranges) was attached by a $\beta 1 \rightarrow 2$ link to the 8-glucosyl residue, and that a *p*-hydroxybenzoic acid derivative of vitexin was similarly constituted.

The meeting ended with short reports on the xanthenes in *Kielmeyera* spp. (M. T. Magalhaes, Brazil) and in *Calophyllum* spp. (F. Scheinmann, Salford), on the quinones in *Tabebuia ipé* (A. R. Burnett, Aberdeen), on a novel yellow fungus pigment from *Paxillus involutus* (R. L. Edwards, Bradford), on umbelliferone biosynthesis (D. J. Austin, Glasgow), and on the *Aspergillus* hydroxylating system (S. M. Bocks, Oxford). It was clear from this symposium that plant pigments continue to provide a challenge to the skill and ingenuity of the organic chemist and that problems remain which will take much effort to solve, unless dramatically new techniques become available. The attraction of their colour will always ensure abundant interest in the chemistry of these fascinating substances.

J. B. HARBORNE

DESIGN METHOD

THE OXFORD DICTIONARY lists many different interpretations of the word 'design' used either as noun or verb, but it now has a new meaning in its own right: a subject to be studied for its own sake, regardless of any primary discipline involved. Design as a fundamental concept is on its way to becoming a science. In the sense of producing new and useful commodities of all kinds, it is seen as a major challenge to human ability. "The better understanding of the problems involved provides a task for the behavioural scientists. The better development of designers requires careful thought by management." The theoretical and practical values of pioneering inter-disciplinary thinking in the broad field of design had to be tested. That the task has proved more than successful is attested by the symposium sponsored by the Design and Innovation Group of the University of Aston in Birmingham (designate), convened in Birmingham during September 21-23, of which a copy of the preprints of the papers presented is now available*.

This volume undoubtedly brings together, in a substantial way and for the first time in Great Britain, the general trend of thought about the nature and problems of design by many different people in many walks of life having wide experience in this field. Thirty papers dealing with design from inception to realization and utilization are included. Breadth of coverage is shown by the wide range of technologies and sciences represented; these are

architecture, behavioural sciences, chemical engineering, electrical engineering, electronics, ergonomics, fuel technology, information science, instrument technology, mechanical engineering, metallurgy, philosophy, physics, production engineering, psychology and sociology.

As if to emphasize the concept of the building and ultimate evolution of design science, the symposium is appropriately divided into six successive sections: (1) the design process; (2) human needs and satisfaction; (3) stages of design; (4) practical design and techniques; (5) realization; (6) design and management.

The design process is concerned with overviews of the total process of design. It is illustrated by five papers: "Design Methods Reviewed", by J. C. Jones; "Some American Views on the Design Process", by R. J. McCrory; "A Survey of Design Methodology", by W. E. Eder; "Preparatory Work for Research into Electronics Design", by H. V. Beck; and "Design Science", by S. A. Gregory. On the bases of experience in these fields, it is deduced that the time is ripe for research into design and the establishment of design science as an aid to the practical designer.

"All technological design attempts to fulfil some human need or provide some satisfaction." The section on human needs and satisfaction is concerned with establishing the concepts involved and exploring ways of finding out or measuring human needs. Herein, to guide constructive thinking, are a further five papers: "Design and Human Satisfaction", by W. H. Mayall; "Invention, Design and Market Research", by A. F. Stobart; "Sociology and

* *Design Method Symposium*, University of Aston in Birmingham Designate (College of Advanced Technology), September 21-23, 1965—Preprints (Organized by the Design and Innovation Group.) Pp. 322. (Butterworths, London, 1965.)

Design", by A. M. Penny; "Ergonomics and Design", by B. Shackel; and "Choosing and Evaluating", by A. G. Pleydell-Pearce.

The foreword to the section on stages of design reads: "From the perception of need it is possible to set down the design process in successive stages. These stages are not intrinsically isolated but may be conveniently separated for the purpose of description and study. In practice the operations involved in the sequence continually re-define each other until a solution satisfactory for the particular circumstances arrives." In support of this dictum are again five papers: "The Elements of Design", by R. D. Watts; "Information for Design", by J. E. L. Farradane; "Patterns", by A. D. Newman; "Creativity", by G. H. Broadbent; and "Design and Decision", by S. A. Gregory.

The theme of practical design and techniques is an analysis of the methods that designers are finding useful and consideration of progress in application of new ideas. In this section we get down to what may be termed the day-to-day function of the designer in certain fields, rather than the more philosophical aspects of his work. "Designers, in reporting their work, tend to concentrate upon the more technical aspects of the task." But there is more to it than this, as is apparent from these papers: "A Practical Design—An Oil Burner for Large Water-tube Boilers", by A. M. Needham; "The Implications of Economics in Engineering Design", by A. P. Shahbenderian; "Models", by C. Hulse; "The Use of a Digital Computer in Design Offices", by K. C. Parton; and "Fundamental Design Method", by E. Matchett and A. H. Briggs, an interesting case of how a combination of systematic method and psychological stimulation contributed to the production of a useful design.

In the section on realization, the underlying motive is that eventually all practical designs, if successful, must come to a material embodiment. This demands study of the interaction of function, fabrication techniques, number and complexity of products. In this concept such interaction affects assembly, reliability, maintenance, best use of space, and overall cost. All decisions made in this category must be communicated to everybody involved, to make this goal of realization truly effective. To show how this can be done, F. L. Ellis contributes a paper on

"Design Realization"; "Selection of Materials" is discussed by A. L. Davies; a paper by C. T. Corney entitled "Reliability and Maintenance" is followed by one on "Design of Materials", by S. A. Gregory, while a welcome note on "New Ideas in the Drawing Office" is struck by P. McMullen.

The final section on design and management probably goes to the root of the whole project of a design science, in that it seeks to equate formulation of policy, operation and motivation of working groups, effect of major organization on design, and methods of evaluating the effectiveness of design organizations as a whole, as a matter of fundamental concern. The exponents in this argument are B. T. Turner, in his paper on "Design Policy Formulation"; R. Davis in his "Innovative Design is a Policy Function of Directorship", wherein it is emphasized that relationship between design and the whole enterprise of which it forms part must be carefully established and the overriding policy laid down for all concerned to appreciate and act on; I. M. Ross discusses "Effect of Organizational Procedures on Design"; M. E. Peplow, "Design Acceptance"; and finally C. H. Buck, who has written an admirable summary of the proceedings at the symposium, enclosed with this volume, contributes his share on the vital subject of "Communication".

There is no doubt that this symposium, and what will follow from it, breaks new and profitable ground. Pressure towards a rational analysis of activities involved in the process of design has, of recent years, steadily increased. Previously in Great Britain, stimuli in this direction were the outcome of the Feilden Report (*Nature*, 206, 643, 1965), the 1962 Conference on Design Methods, and the Scarborough Conference on Teaching of Engineering Design in 1964. This volume shows that we have gone a long way from the impetus of those deliberations and in a comparatively short time. It is the intention of Butterworth and Co. (Publishers), Ltd., to issue a version of the *Proceedings*, together with discussion, suitably edited by S. A. Gregory, on behalf of the Design and Innovation Group, which will make available in durable form the wealth of new information and ideas implicit in and realized by this important symposium: thus is a new science born.

VALIDITY OF RADIOCARBON DATES ON BONE

By DR. M. A. TAMERS and F. J. PEARSON, jun.

Department of Chemistry, Instituto Venezolano de Investigaciones Científicas, Caracas, Venezuela, and Radiocarbon Dating Laboratory, University of Texas, Austin

A SURVEY of the scientific literature reveals the fact that carbon-14 dates on bone often do not accord with independent estimates of the site ages. Bone dates are generally regarded as considerably less reliable than determinations using charcoal, wood, or other materials with large carbon contents (except shell). Most laboratories are in agreement that, whenever possible, bone should not be used for radiocarbon dating.

However, in many instances important finds are made where the only datable material available is bone. A problem illustrating this is the well-known palaeo-indian site, Muaco, in Venezuela¹, which is a kill site. No charcoal was found associated with the animal bones and human artefacts. Two specimens of the bones have given carbon-14 ages of $16,375 \pm 444$ B.P. and $14,300 \pm 500$ B.P.², which are the oldest dates associated with human habitation in South America. Furthermore, these dates fall within a period (between 12,000 and at least 25,000 years ago) in which there was little or no human activity in North America, as evidenced by the scarcity of radiocarbon dates of these ages. A serious controversy has

arisen as to whether the Muaco site is really as ancient as the radiocarbon dates indicate or whether it is possible that radiocarbon dating of bone can give falsely old ages.

The validity of radiocarbon dates on bone is investigated by determining the natural carbon-14 concentrations of samples of known age, that is, historically dated specimens or those found associated with charcoal or other materials known to give reliable radiocarbon dates. Several radiocarbon dating laboratories have measured known-age bone samples with varying results. Recently, Berger, Horney and Libby³ have concluded that, with an acid-dissolving preliminary treatment, bones will be capable of reliable radiocarbon ages. Dates on bone not so treated can give incorrect ages, but there is no indication of evidence that the errors could be such as to make the bone samples appear older than they really are (which is the question in the Muaco site) and insufficient data to permit conclusions as to the extent of the errors involved. Furthermore, it seems reasonable to investigate in more detail the reliability of the special preliminary treatment, used to

remove carbonates completely, with more samples in order to improve the knowledge of the limits of confidence of these bone dates. This article presents the results of a series of measurements on known-age samples and allows the errors connected with bone dating to be defined.

Carbon in bone is present as organic material and as carbonate. It might be expected that the carbonate portion would undergo isotope exchange with carbon dioxide from the atmosphere or soil atmosphere, or with carbonate dissolved in ground water. Thus, a radiocarbon date based on total bone carbon could be falsely young if the bone carbonate had exchanged with atmospheric carbon dioxide or falsely old if the exchange had been with dead limestone-derived carbonate in ground water. However, independent considerations indicate that the carbonate portion of bones can give carbon-14 dates that are incorrectly young, but not too old. This is seen by examining what is known about carbonate exchange processes in general. Laboratory experiments⁴ have shown that carbon dioxide gas exchanges readily with wet, solid carbonates, but that exchange between solid carbonate and dissolved HCO_3^- or CO_3^{2-} was not observed. Recent work on natural systems^{5,6} shows that no isotope exchange between carbonate species dissolved in ground water and the solid carbonate of the aquifer can be detected. Therefore, it is to be expected that the carbonate of bones could be made falsely young by isotope exchange with atmospheric carbon dioxide, but they should not appear falsely old owing to exchange with limestone-derived dissolved carbonate, since this type of exchange does not seem to take place.

In the work described here, the bone as submitted was scrubbed and washed thoroughly and, if necessary, broken into pieces of a few centimetres length. After this, the following treatment methods were investigated:

A-Fraction. The cleaned bone pieces were placed in a large beaker, dilute hydrochloric acid added and the solution heated to boiling. Enough acid was used to dissolve the bones, thus assuring the complete removal of carbonates. Prolonged heating evaporated the solution to dryness. The residue was burned in a stream of oxygen in the usual manner and the carbon dioxide collected for analysis.

B-Fraction. A weighed amount of bone was treated in an enclosed system with an excess of hydrochloric acid. The procedure was carried out in an apparatus similar to that used for shell and other carbonate samples, so that the evolved carbon dioxide could be collected quantitatively. This fraction represents the carbon present in the bone as acid soluble carbonates.

C-Fraction. For some samples, clean bone pieces were burned in oxygen directly, after only a superficial washing with dilute hydrochloric acid (about 5 min at room temperature). It has been demonstrated several times in the course of these experiments that this treatment is not sufficient to completely remove the carbonates present.

Fractions *A* and *B* will provide the most useful data since they represent separate portions of the bone carbon. Fraction *C* gives ambiguous results; it is carbon that comes from a more or less quantitative collection of that present as what could be extracted as fraction *A*, but only part of the carbonates of fraction *B*, since the combustion temperatures are not high enough to completely decompose the calcium carbonate in the bone. This is seen by the fact that the amount of carbon extracted as fraction *C* on a particular bone sample is almost always a little higher than fraction *A*. Furthermore, the carbon-14 dates on the fraction *C* usually differ from those obtained on fraction *A* and in a direction that can be explained by considering the carbon-14 age observed for fraction *B*. A good illustration of this is our sample *Tx-164*, which was a collection of pieces of the shell of a large turtle, now extinct⁷.

Tx-164-A. Fraction *A* was examined in two separate lots which gave 0.45 and 0.64 per cent as the organic

carbon contents of the bones. The combined sample dated $15,800 \pm 1,600$ B.P.

Tx-164-B. There was 1.2 per cent carbon as fraction *B* in the single lot of bones investigated. The carbon-14 date was $8,780 \pm 250$ B.P.

Tx-164-C. This fraction *C*, from bones washed for 5 min in dilute hydrochloric acid, showed a bone carbon content of 0.95 per cent and dated $10,250 \pm 250$ B.P.

If it is assumed that the fraction *A* carbon content of the bones used for the *Tx-164-C* analysis was the average of that found for *Tx-164-A*, that is, 0.55 per cent, and that the organic fraction of *Tx-164-C* was completely collected, but the carbonate fraction only partially (the 0.95 per cent carbon content was made up of a 55 per cent fraction *A* carbon content and 40 per cent fraction *B* content), the weighted averages of the ages of *A* and *B* would indicate that *Tx-164-C* should date 12,800 B.P. The difference between this and the observed age can be accounted for by the quoted statistical errors of the countings.

The same sort of analyses were made with the different fractions of samples *Tx-162*, *Tx-163*, *Tx-229*, and *Tx-231* shown in Tables 1-3. Assuming an uncertainty of 15 per cent in the carbon concentrations, the observed ages of the fractions *C* could be accounted for by considering those given by the fractions *A* and *B*, as was already described here for the turtle bones sample *Tx-164*.

The results of analyses of known-age bone samples are shown in Tables 1-3. The specimen numbers with *Tx*-indicate measurements done at the University of Texas, and those with *IVIC*- at the Instituto Venezolano de Investigaciones Cientificas. Both radiocarbon dating laboratories convert the samples into benzene^{8,9} and count with liquid scintillation techniques. The errors quoted with the dates are the standard errors calculated from the random nature of the disintegration process. A previous investigation has verified that all other laboratory errors, such as isotope effect, amount to less than 0.5 per cent and are negligible for our purposes¹⁰. The dates in the tables, except where indicated, are in years B.P., that is, years before 1950, and are calculated using a carbon-14 half-life of 5,568 years. A description of the sites, conditions of the finds, and references to the associated charcoal ages can be found in the bone sample references quoted in Tables 1-3.

The deviations of the observed carbon-14 bones ages from the presumably correct associated charcoal ages are given in the last columns of Tables 1-3. A negative deviation indicates that the bone age is falsely young. In all measurements the bones dated either the same age (statistically indistinguishable) as the associated charcoal or were younger. The most common method of bone sample treatment, fraction *C*, is the least successful and has given an incorrect date in every case measured. There are also samples quoted in the literature using treatments similar to fraction *C* (refs. 11-16), all of which show bones with falsely recent dates. This is of interest in the case of the Muaco site, mentioned in the introduction of this paper, whose bones were dated with this type of treatment. The indications are strong that the Muaco specimens are at least as old as the controversial carbon-14 dates claim and not younger as has been suggested.

Bone dates on the acid-soluble carbonate portion (fraction *B*) hold no promise of accuracy. Samples of this sort appearing in the literature also show bone carbonate ages incorrectly young¹⁵⁻¹⁷. Radiocarbon dating laboratories are in general agreement that carbonates are particularly suspect materials for dating owing to their ease of exchange with contemporary carbon dioxide.

The most interesting of the methods investigated is the acid-dissolving pretreatment which produces fraction *A*. Table 1 shows that one-third of the samples gave correct dates, but that the others all have negative deviations indicating bone ages that are too young. The reason for this is probably contamination with more recent or

Table 1. BONE DATES WITH ACID-DISSOLVING PRETREATMENT (FRACTION A)

Site	Charred	Bone specimen No.	Bone specimen reference	% Carbon	Bone age	Associated charcoal age	Deviation
Fort St. Louis	no	<i>Tx-162-A</i>	(7)	4.2	Modern	195 ± 105	-2.5 ± 2.1% excess
San Lorenzo	no	<i>Tx-163-A</i>	(7)	4.5	450 ± 100	380 ± 100	+70 ± 140
Candelaria	no	<i>Tx-51</i>	(21)	9.0	745 ± 85	745 ± 110	0 ± 120
El Cerro	yes	<i>IVIC-21</i>	(22)	1.9	500 ± 150	790 ± 100	-290 ± 180
El Chao	yes	<i>IVIC-70</i>	(22)	5.2	850 ± 220	690 ± 110	+160 ± 250
El Chao	no	<i>IVIC-55</i>	(22)	5.1	0 ± 130	680 ± 110	-680 ± 170
La Betania	no	<i>IVIC-121</i>	(22)	2.2	1,160 ± 150	2,180 ± 120	-1,020 ± 190
La Betania	no	<i>IVIC-119</i>	(22)	0.4	250 ± 240	2,180 ± 120	-1,930 ± 270
Bonfire Shelter	yes	<i>Tx-47</i>	(21)	1.3	2,810 ± 110	2,645 ± 75	+165 ± 130
Bonfire Shelter	yes	<i>Tx-229-A</i>	(7)	1.2	2,660 ± 640	2,645 ± 75	-15 ± 645
Bonfire Shelter	no	<i>Tx-230-A</i>	(7)	0.8	7,470 ± 160	10,230 ± 160	-2,760 ± 225
Bonfire Shelter	yes	<i>Tx-118</i>	(7)	4.5	8,380 ± 180	10,230 ± 160	-1,850 ± 240
Bonfire Shelter	yes	<i>Tx-231-A</i>	(7)	1.5	9,120 ± 200	10,230 ± 160	-1,110 ± 225
Bonfire Shelter	no	<i>Tx-232-A</i>	(7)	0.7	9,080 ± 210	10,230 ± 160	-1,150 ± 265

Table 2. BONE DATES ON ACID-SOLUBLE CARBONATE PORTIONS (FRACTION B)

Site	Charred	Bone specimen No.	Bone specimen reference	% Carbon	Bone age	Associated charcoal age	Deviation
Fort St. Louis	no	<i>Tx-162-B</i>	(7)	0.8	Modern	195 ± 105	-5.9 ± 1.8% excess
San Lorenzo	no	<i>Tx-163-B</i>	(7)	1.3	Modern	380 ± 100	-8.2 ± 1.7% excess
Bonfire Shelter	yes	<i>Tx-229-B</i>	(7)	7.2	1,560 ± 100	2,645 ± 75	-1,085 ± 125
Bonfire Shelter	no	<i>Tx-230-B</i>	(7)	7.3	7,110 ± 160	10,230 ± 160	-3,120 ± 225
Bonfire Shelter	yes	<i>Tx-231-B</i>	(7)	7.0	7,230 ± 160	10,230 ± 160	-3,000 ± 225
Bonfire Shelter	no	<i>Tx-232-B</i>	(7)	7.1	7,230 ± 380	10,230 ± 160	-3,000 ± 410

Table 3. BONE DATES WITH ACID-WASHING PRETREATMENT (FRACTION C)

Site	Charred	Bone specimen No.	Bone specimen reference	% Carbon	Bone age	Associated charcoal age	Deviation
Fort St. Louis	no	<i>Tx-162-C</i>	(7)	6.0	Modern	195 ± 105	-2.0 ± 2.2% excess
San Lorenzo	no	<i>Tx-163-C</i>	(7)	6.5	Modern	380 ± 100	-6.4 ± 1.6% excess
Bonfire Shelter	yes	<i>Tx-46</i>	(21)	0.7	2,310 ± 210	2,645 ± 75	-335 ± 220
Bonfire Shelter	yes	<i>Tx-229-C</i>	(7)	1.5	2,130 ± 110	2,645 ± 75	-415 ± 135
Bonfire Shelter	no	<i>Tx-231-C</i>	(7)	1.8	9,210 ± 200	10,230 ± 160	-1,020 ± 225

contemporary organic material in the ground. Bones, being very porous, are difficult to clean thoroughly and this is the major origin of the difficulties. The known-age bone samples of Berger, Horney, and Libby³ show the same phenomenon. Their Chimney Cave mummy was presumably not exposed to ground contamination and gives a correct age. The other two samples both date too recent, but the bones from Santa Rosa Island are not in error enough to be statistically distinguishable from the associated charcoal. There are other examples of bones dated with treatments similar to fraction A; several specimens give correct ages¹⁵⁻¹⁸ but others have dates clearly too recent^{15, 16, 19, 20}.

Contrary to widespread opinion, bone samples which were charred do not give more reliable dates than those that were not. However, there seems to be a tendency for bones with low organic carbon concentrations to produce worse results than the others. Charred bones generally have higher organic carbon contents than those that were not burned.

In conclusion, it can be seen that the majority of radiocarbon dates on bone are in error. Most of the bone dates appearing in the literature were obtained with acid-washing pretreatment methods similar to that described as our fraction C. Very few of these values should be correct, except possibly in those situations where the bones were not buried or for relatively recent sites (less than a few hundred years old). The use of the carbon present as acid-soluble carbonates for the dating is not advisable in any case. The best method is clearly that involving the acid dissolving of the bones, in this way ensuring the complete removal of the carbonate fraction. However, even this treatment gives incorrect dates in more than half the samples and the results should be used with caution.

A very important factor in the errors connected with bone dates is that in all cases of measurements on known-age bones, both those presented in this paper and samples described in the literature, the observed errors are in the same direction, that is, giving falsely young ages. It can

be suggested, on the basis of this evidence, that a more realistic use of bone dates would be to take them as only lower limits and to precede the dates with a sign indicating 'greater than or equal to'.

The extent of the errors is not such as to make bone entirely unsuitable in cases where an upper limit to the age of the material is of principal interest. As expected, the absolute values of the deviations tend to increase with the sample ages. For the bones described in this paper, which range from 200 to 10,000 years old, the maximum error is 3,000 years. In the best of the treatment methods (fraction A), only one sample out of the fourteen measured has an error greater than 2,000 years. With scrupulous physical cleaning of the bone specimens, it might be hoped that the errors of unknown-age samples, up to at least 10,000 years old, will not exceed a few thousand years.

¹ Rouse, I., and Cruxent, J. M., *Venezuelan Archaeology* (Yale Univ. Press, New Haven, 1963).

² Crane, H. R., and Griffin, J. B., *Radiocarbon*, **4**, 183 (1962).

³ Berger, R., Horney, A. G., and Libby, W. F., *Science*, **144**, 999 (1964).

⁴ Samos, G., *Science*, **110**, 663 (1949).

⁵ Pearson, jun., F. J. (in preparation).

⁶ Hanshaw, B. B. (personal communication).

⁷ Pearson, jun., F. J., and Davis, E. M., *Radiocarbon* (to be published in 1966).

⁸ Tamers, M. A., Stipp, J. J., and Collier, J., *Geochim. Cosmochim. Acta*, **24**, 266 (1961).

⁹ Noakes, J. E., Isbell, A. F., Stipp, J. J., and Hood, D. W., *Geochim. Cosmochim. Acta*, **27**, 797 (1963).

¹⁰ Tamers, M. A., and Pearson, jun., F. J., *Nature*, **205**, 1205 (1965).

¹¹ Ralph, E. K., *Amer. J. Sci., Radiocarbon Suppl.*, **1**, 45 (1959).

¹² Libby, W. F., *Science*, **114**, 291 (1951).

¹³ Olson, E. A., and Broecker, W. S., *Amer. J. Sci., Radiocarbon Suppl.*, **1**, 1 (1959).

¹⁴ Walton, A., Trautman, M. A., and Friend, J. P., *Radiocarbon*, **3**, 47 (1961).

¹⁵ Olson, I., *Amer. J. Sci., Radiocarbon Suppl.*, **1**, 87 (1959).

¹⁶ Olson, E. A., and Broecker, W. S., *Radiocarbon*, **3**, 141 (1961).

¹⁷ Vogel, J. C., and Waterbolk, H. T., *Radiocarbon*, **5**, 163 (1963).

¹⁸ Agrawal, D. P., Kusumgar, S., Lal, D., and Sarna, R. P., *Radiocarbon*, **6**, 226 (1964).

¹⁹ Grant-Taylor, T. L., and Rafter, T. A., *Radiocarbon*, **5**, 127 (1963).

²⁰ Vogel, J. C., and Waterbolk, H. T., *Radiocarbon*, **6**, 349 (1964).

²¹ Tamers, M. A., Pearson, jun., F. J., and Davis, E. M., *Radiocarbon*, **6**, 138 (1964).

²² Tamers, M. A., *Radiocarbon*, **7**, 54 (1965).

REGIONAL GEOCHEMICAL RECONNAISSANCE APPLIED TO SOME AGRICULTURAL PROBLEMS IN CO. LIMERICK, EIRE

By PROF. JOHN S. WEBB and W. J. ATKINSON

Applied Geochemistry Research Group, Department of Geology, Imperial College of Science and Technology, London

TOXIC levels of selenium and molybdenum in soils and herbage have been reported by the Irish Agricultural Institute at a number of localities, notably in Counties Limerick, Tipperary and Meath¹⁻⁵. The clinical symptoms exhibited by cattle and horses in the affected areas, and the composition of the local rocks, soils and plants, are described in detail in the references cited. In consultation with the Agricultural Institute, these areas were included in a preliminary investigation of the application to agricultural problems of regional geochemical reconnaissance techniques, originally developed for purposes of mineral exploration. The results of these trial reconnaissance surveys, based on stream sediment sampling at a density of 1 sample per sq. mile, disclosed anomalous patterns for molybdenum in each of the suspect areas where this metal had been recorded in the soils; patterns of anomalously low values for cobalt were also observed in a cobalt-deficient area in Co. Wicklow⁶. Since then, our investigations have been extended to include selenium, with special reference to the toxic area in Co. Limerick. Here, more detailed sampling has shown an encouraging degree of correlation between the regional distribution patterns of selenium and molybdenum in the drainage on the one hand and in the soils and herbage on the other. The following description is concerned solely with the empirical results of the systematic surveys that have been carried out in the Limerick area and which are now providing the basis for further investigations of more fundamental aspects of this biogeochemical problem.

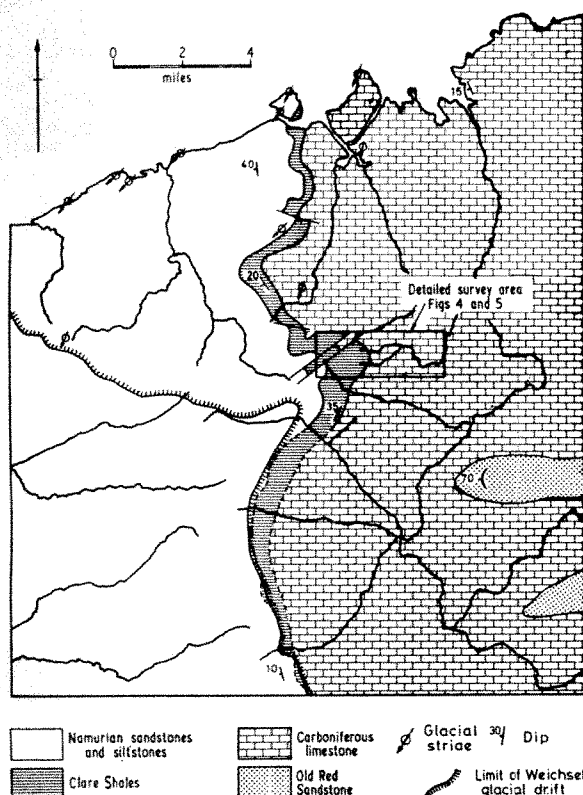


Fig. 1. Simplified geology of the area covered by the regional geochemical survey, Co. Limerick. (After Hodson and Le Warne⁷ and Irish Geological Survey)

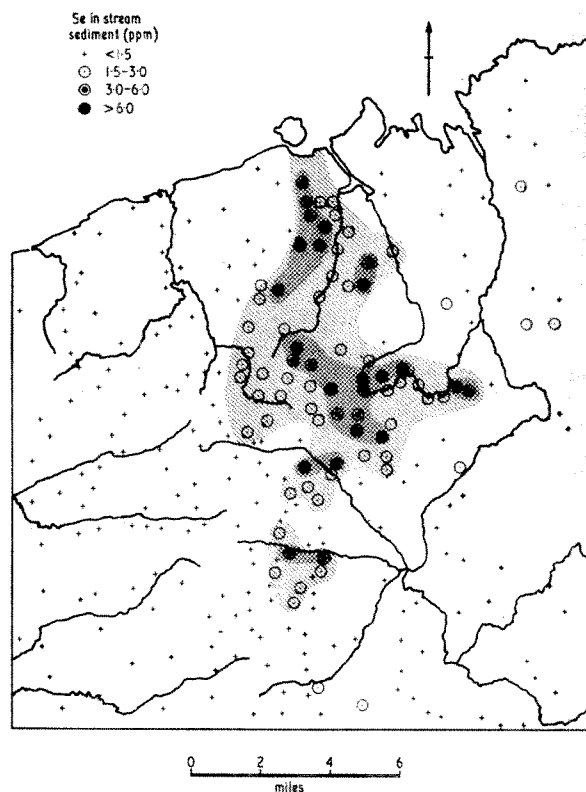


Fig. 2. Distribution of selenium in the stream sediment of tributary drainage (data refer to -80-mesh fraction)

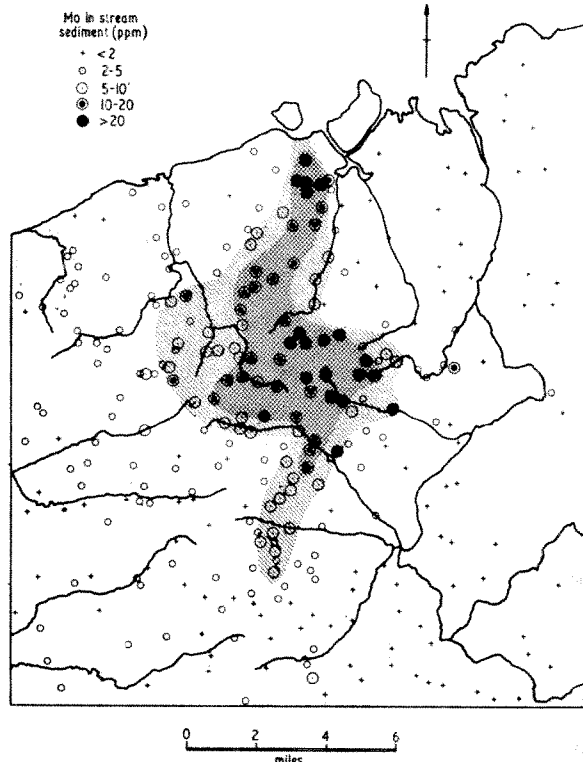


Fig. 3. Distribution of molybdenum in stream sediment of tributary drainage (data refer to -80-mesh fraction)

Description of the study area in Co. Limerick. The geology of the area (Fig. 1) is composed of Carboniferous limestone overlain in the west by a Namurian sequence of sandstones and siltstones at the base of which occurs a black marine shale horizon, defined as the Clare Shales⁷. Cross-sections of these shales, previously noted as the source of the selenium and molybdenum in the affected soils^{3,4}, have been sampled systematically in detail and show bands ranging in content from 5 to 30 p.p.m. selenium and 5 to 150 p.p.m. molybdenum; the higher values usually being recorded in the more carbonaceous and pyritic horizons. By comparison, the limestones, sandstones and siltstones generally contain less than 1 p.p.m. selenium and 2 p.p.m. molybdenum.

Geomorphologically, the boundary of the arenaceous rocks and the Clare Shales marks a more or less well-defined scarp overlooking the lower ground underlain by the limestone. Boulder-clay with local patches of fluvio-glacial gravels cover much of the area, except in the south-west where the overburden is predominantly residual in origin (Fig. 1). Peat is commonly developed on the higher ground and in boggy depressions at the base of the scarp.

Drainage is to the east and west of the watershed, which follows approximately the outcrop of the sandstones immediately overlying the shales. Streams mostly rise in peaty areas. In the upper reaches, the banks are usually composed of colluvium where the streams are incised, but on the flatter ground variable widths of flanking alluvium are developed. As is common in many parts of Eire, appreciable stretches of the stream channels have been modified by dredging.

The agriculture of the area is based on dairying, with small units ranging from about 20 to 40 acres at the base of the scarp to larger farms of up to 200 acres on the limestone in the east. Unfenced hill land to the west is associated with poorly drained peaty soils of low nutritional status and supports low-yielding cross-bred cattle.

The geochemical patterns. For reconnaissance purposes, samples of the active stream sediment were taken from tributary drainage at a mean density of 1 sample per sq. mile. A total area of 250 sq. miles was covered, centred on the known seleniferous localities. The unground minus 80-mesh fraction was analysed spectrographically for 15 elements including molybdenum⁹, and colorimetrically for selenium using a rapid method based on 3,3'-diaminobenzidine capable of 25 determinations per man-day¹⁰.

The resulting patterns show broadly coincident anomalies for molybdenum and selenium covering an area of about 30 sq. miles. Peak values up to 200 p.p.m. molybdenum and 110 p.p.m. selenium occur on or near the Clare Shale sub-outcrop (Figs. 2 and 3). The extension of the anomaly on to the Namurian rocks to the west is related to the glacial dispersion of Clare Shale material in a south-westerly direction up to the limit of the drift deposited during the last (Weichsel) glaciation, as mapped by the Irish Geological Survey (Fig. 1). The apparent extension eastwards on to the limestones is largely due to alluvial transport along the present-day surface drainage system. Copper and vanadium also show a tendency to follow molybdenum and selen-

ium, though contrast between anomaly and background is much less marked. Dredging operations in the stream channels appear to have had little influence on the regional patterns of metal distribution in the stream sediments.

In order to determine the significance of the stream sediment patterns in terms of the metal content of the local soils and vegetation, more detailed sampling was undertaken in the area centred on two known toxic selenium localities (Figs. 1 and 4). In addition to close-spaced stream sediment samples, the soil and pasture herbage were collected systematically on an 880-ft. square-grid giving a total of 220 sampling points over 5 sq. miles. The patterns obtained by analysis of the stream sediment, the minus 80-mesh fraction of the soil at 18-24 in. and the oven-dried mixed herbage are shown in Figs. 4 and 5.

Focal points of the stream sediment anomalies are clearly related to extensive areas of molybdenum- and selenium-rich soils. The drainage trains extend downstream for 1-2 miles and are believed to have developed largely by erosion of metal-rich soil in the headwaters. The stream waters draining the Clare Shales are also anomalous and carry up to 60 p.p.b. (parts per billion,

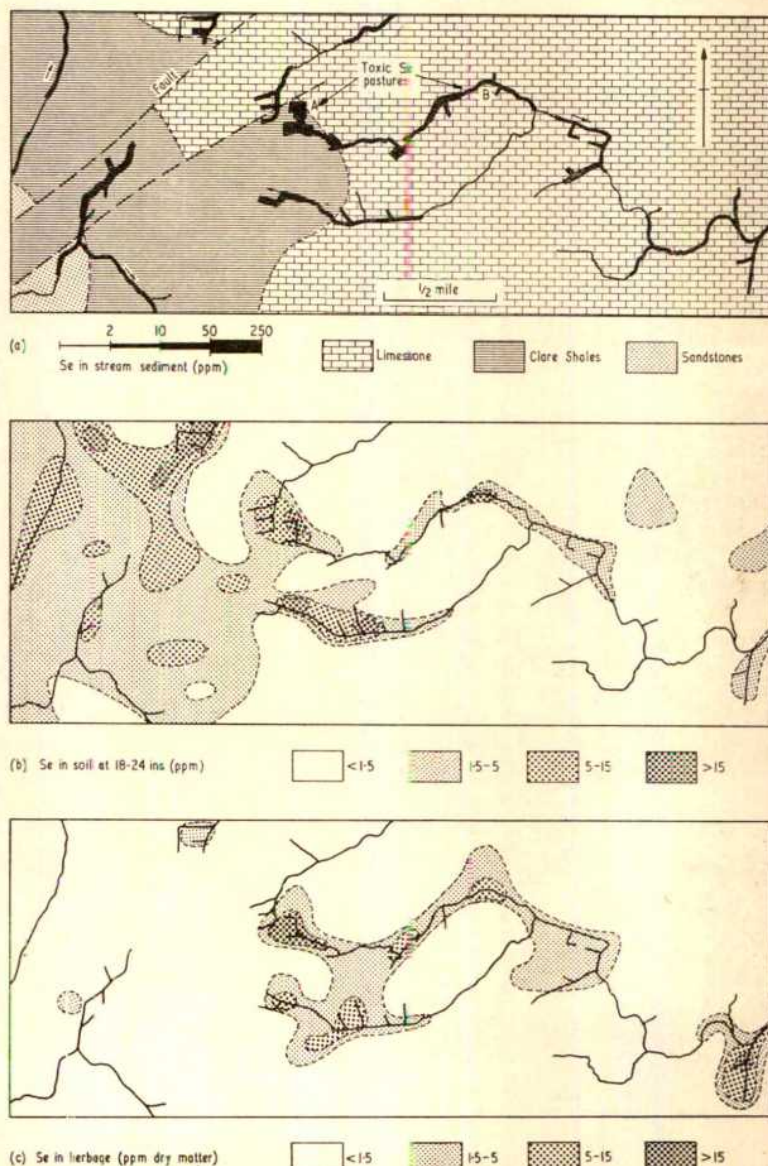


Fig. 4. Distribution of selenium in stream sediments, soil and mixed herbage (data based on 220 samples collected on an 880-ft. square-grid); geology (after Hodson and Le Warne⁷) also shown in 4(a)

10⁻⁹) molybdenum and 17 p.p.b. selenium compared with background values of less than 0.5 p.p.b. The possibility that precipitation of metal from the stream waters may contribute to the sediment anomaly cannot, therefore, be discounted at this stage.

The greater part of the extensive anomalous soil patterns in the west is related to the metal-rich glacial overburden and patches of residuum derived from the Clare Shales. Peak soil values ranging up to 3,000 p.p.m. molybdenum and 500 p.p.m. selenium occur mainly in boggy seepage areas in the stream headwaters where the metals have accumulated by precipitation in the organic-rich reducing environment (as reported by the Irish Agricultural Institute). This view has been confirmed by analyses of ground-water draining into these areas from the Clare Shales, which have been found to contain up to 7.0 p.p.b. selenium and 15 p.p.b. molybdenum compared with background values of less than 0.5 p.p.b. Recent analysis has also shown an additional trend, particularly evident in the case of molybdenum, for the metals to follow iron in seepage soils and spring sediments. The extensions of the soil anomalies down the

valleys are confined to the metal-rich alluvium deposited alongside anomalous streams and which overlies essentially barren limestone drift.

Analysis of grab samples representative of the mixed herbage at each soil sample point shows an interesting contrast in the soil-plant relationships for molybdenum and selenium. Broadly speaking, there is some degree of correlation between the total molybdenum content of the soil and herbage irrespective of local variations in soil-type. In contrast, selenium-rich herbage is found only on the more alkaline, organic-rich poorly drained soils within the seleniferous area. Without denying their influence, local variations in soil type would, therefore, seem to be less restrictive in controlling the ratio of total to available molybdenum as compared with their effect on the selenium ratio.

While the foregoing establishes the existence of a useful broad relationship between metal content of the drainage and the rocks, soils and herbage of the catchment area, detailed examination indicates a number of interesting features which undoubtedly reflect the modifying influence of geological, geochemical and biochemical factors. Work in progress is therefore primarily directed towards those aspects of the fundamental problem bearing on interpretation of the geochemical data, including: (1) the distribution and dispersion of molybdenum and selenium in both the bedrock and the zone of weathering; (2) the relative mobilities of those elements and the relationships between total and available metal; (3) the influence of relevant environmental factors, such as pH, Eh, drainage and soil type. Attention is also being given to the distribution of certain other constituents, notably copper, vanadium, phosphorus and sulphate.

Relationship between the geochemical patterns and animal health. Horses and cattle grazing herbage containing more than 5 p.p.m. selenium in the dried sample have shown symptoms of chronic selenium poisoning, including unthriftiness, loss of hair and abnormal growth of the hooves^{1,2,4}. At the known toxic locality A (Fig. 4) the maximum recorded value in the herbage is 100 p.p.m. selenium, while at B the content is 5–10 p.p.m. On the basis of a threshold level of 5 p.p.m.⁴, the results given in Fig. 4(c) point to the possibility of other suspect pastures in the immediate vicinity. Considering the region as a whole, four other toxic seleniferous localities are now known, all of which lie in the peak anomalous area indicated by the regional geochemical data (Fig. 2). Assuming similar sediment-plant relationships as exist in the detailed investigation area, other toxic localities—at the clinical or sub-clinical level—might be found elsewhere within the anomalous region where conditions of drainage and soil type are locally propitious for accumulation of selenium in a form available to the herbage.

Turning to molybdenum, some symptoms of molybdenum toxicity—notably fading of the coat and scouring—have been reported from Co. Limerick⁴. The provisional threshold level for toxic herbage is given as 5 p.p.m. in dry matter³, and soils with more than 10 p.p.m. total molybdenum are considered to be suspect⁴. On this basis, molybdenum toxicity or molybdenum-induced copper deficiency could exist in much of the western half of the detailed investigation area (Fig. 5). Again assuming comparable sediment-plant relationships in similar en-

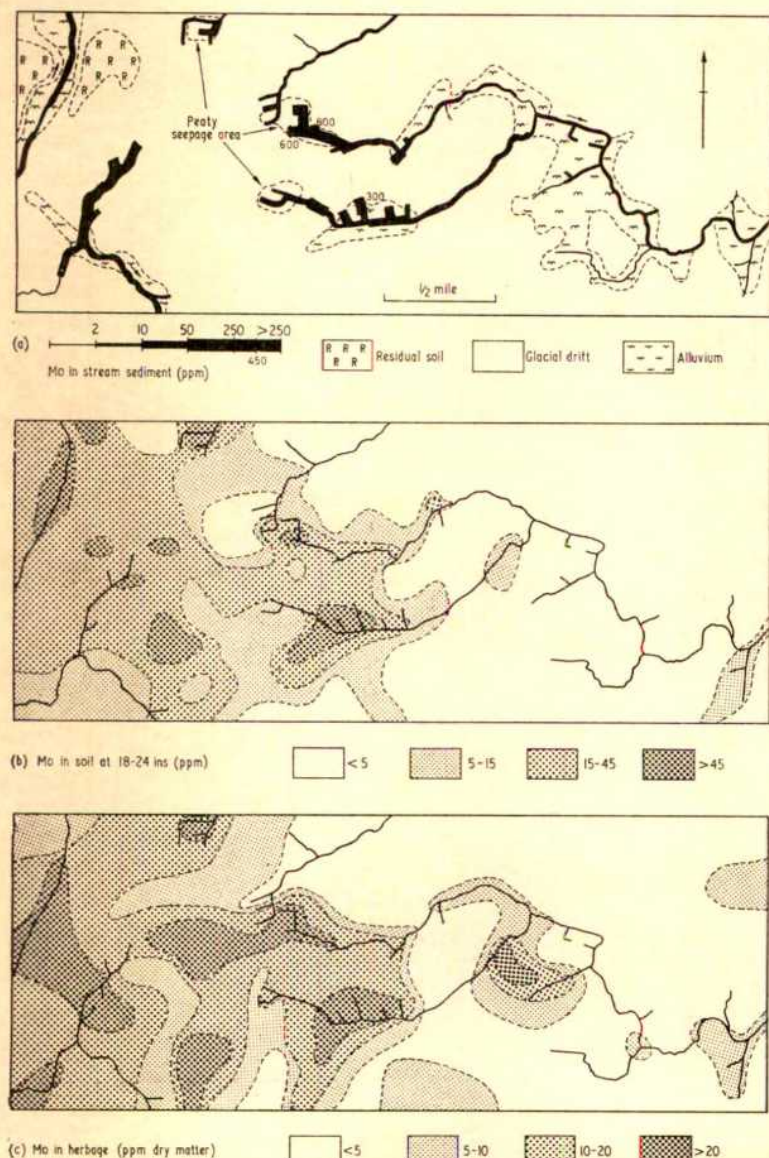


Fig. 5. Distribution of molybdenum in stream sediments, soil and mixed herbage (same samples as Fig. 4); simplified composition of the overburden (after O'Meara⁵) also shown in 5(a).

vironments in the region as a whole, the stream sediment pattern shown in Fig. 3 might well outline a considerable area wherein clinical or sub-clinical molybdenum toxicity (including molybdenum-induced copper deficiency) could be suspected. Since uptake of molybdenum from metal-rich soils appears to occur over a wider range of conditions than does selenium, excess molybdenum could well constitute the more extensive agricultural problem.

Conclusions. Though as yet incomplete, the results recorded here, taken in conjunction with earlier trials in Eire and the United Kingdom⁶, support the view that geochemical drainage reconnaissance has a useful part to play in the agricultural field. In this context, regional stream sediment surveys could provide an economical means of rapidly delineating suspect problem areas wherein to concentrate detailed investigation by more conventional methods not so well suited to regional application. Of considerable significance is the possibility that the data may also serve to outline areas of sub-clinical or latent¹¹ excesses or deficiencies, which can be the cause of great economic loss through sub-optimum yield or may only become apparent when the land is subjected to more intensive agriculture. On the research side, stream sediment surveys may provide a source of information concerning the regional distribution of a wide range of constituents of interest in the study of trace element interactions and nutritional imbalances in plants, animals and man.

Considerable work clearly remains to be done before the scope and limitations of the method can be fully appreciated. Nevertheless, there already seems good reason for believing that regional geochemical reconnaissance, properly used as an ancillary tool in conjunction

with geological, soil survey and other relevant techniques, can aid in agricultural appraisal and, possibly, eventually in epidemiology.

The fact that, with little additional effort, the same results may be used for mineral prospecting purposes could contribute a further incentive for undertaking regional geochemical surveys in appropriate areas.

We thank the Irish Agricultural Institute and the Irish Geological Survey for their advice. Considerable assistance was also given by our analytical colleagues, especially J. Henderson-Hamilton, Ann Cole, D. Williams and T. Forward.

The work forms part of the Department's continuing programme in applied biogeochemistry which latterly has been aided by a special research grant originally awarded by the Department of Scientific and Industrial Research and now received from the National Economic Research Council. One of us (W. J. A.) holds a Rio Tinto Zinc Corporation research bursary.

¹ Walsh, T., Fleming, G. A., O'Connor, R., and Sweeney, A., *Nature*, **168**, 881 (1951).

² Walsh, T., and Fleming, G. A., *Trans. Fifth Intern. Congr. Soil Sci.*, Dublin, 2, Sect. A, 178 (1952).

³ Walsh, T., Neenan, M., and O'Moore, L. B., *J. Dept. Agric., Eire*, **48**, 3 (1952).

⁴ Fleming, G., and Walsh, T., *Roy. Irish Acad. Proc.*, **58**, Sect. B, No. 7, 151 (1957).

⁵ Fleming, G., *Soil Sci.*, **94**, 28 (1962).

⁶ Webb, J. S., *New Sci.*, **23**, 504 (1964).

⁷ Hodson, F. C., and Le Ware, G. C., *Quart. J. Geol. Soc., Lond.*, **107**, 307 (1961).

⁸ O'Meara, M. (Irish Geol. Survey) (personal communication).

⁹ Nichol, I., and Henderson-Hamilton, J. C., *Trans. Inst. Min. Metall. (Lond.)*, **74**, 955 (1965).

¹⁰ Stanton, R. E., and McDonald, Alison J., *Analyst*, **90**, 497 (1965).

¹¹ Schütte, K. H., *The Biology of the Trace Elements: Their Role in Nutrition* (Crosby Lockwood and Son Ltd., London, 1964).

A NEW HAEMOGLOBIN, J TORONTO ($\alpha 5$ ALANINE \rightarrow ASPARTIC ACID)

By DR. J. H. CROOKSTON

Departments of Medicine and Pathology, University of Toronto, and the Toronto General Hospital, Toronto, Canada
AND

D. BEALE, D. IRVINE and DR. H. LEHMANN

Medical Research Council Abnormal Haemoglobin Research Unit, University Department of Biochemistry, Cambridge

DURING a deliberate search for abnormal haemoglobins among patients at the Toronto General Hospital, a 17-year-old girl of English ancestry was found to have a fast-moving abnormal haemoglobin band on electrophoresis at alkaline pH. The band was also present in the blood of her mother (Mrs. F. M.) (Fig. 1) but not in that of the mother's sister.

The mother was English, 48 years old, and in good health. Physical examination showed no abnormalities. Her haemoglobin level was 12 g/100 ml. with 2 per cent reticulocytes. The blood film showed very slight anisocytosis, but no target cells were seen. Incubation of a blood haemolysate at 48° C for 3 h with phosphate buffer, pH 7.2, failed to produce any precipitate. Incubation of her fresh red cells with acetylphenyl-hydrazine and with brilliant cresyl blue gave normal results.

An electrophoretically fast-moving haemoglobin was seen in the position of haemoglobin J, indicating that it carried one more negative charge per half molecule than haemoglobin A. The ratio of the haemoglobins A : J was 4 : 1. There were two haemoglobin A₂ fractions, one in the position of normal A₂ and another moving in front of it on paper and starch-gel electrophoresis at alkaline pH. The distance between the abnormal and the normal haemoglobin A₂ was the same as the distance between haemoglobin J and haemoglobin A. The 'double' haemoglobin A₂ suggested that the abnormality in the haemoglobin J resided in the α -chains, since haemoglobin A₂ possesses the same α -chains as haemoglobin A. An abnormality

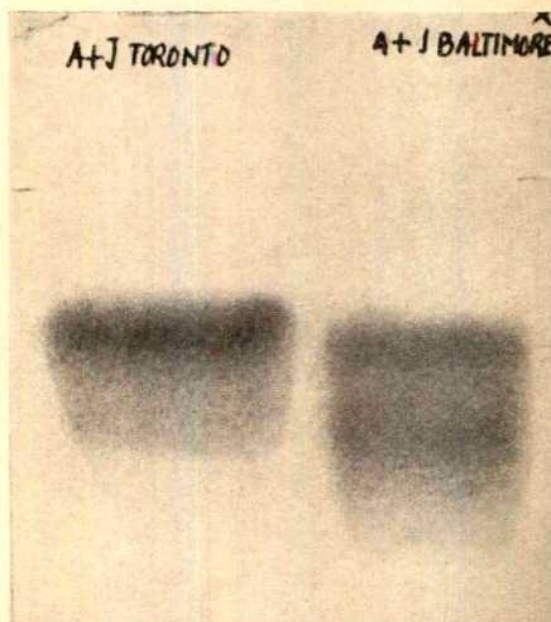


Fig. 1. Paper electrophoresis (pH 8.9) of the haemoglobin of Mrs. F. M. (left) showing haemoglobin A, and a band moving in the position of haemoglobin J. On the right are haemoglobins A+J β Baltimore for comparison. Note that the concentration of the new haemoglobin J is not equal to that of A as it is in the haemoglobin A+J Baltimore sample.

of the α -chains in haemoglobin J ($\alpha^J\beta_2$) would be accompanied by an additional (abnormal) haemoglobin A₂ ($\alpha^J\delta_2$). Hybridization of the isolated haemoglobin J with canine haemoglobin, followed by electrophoresis at alkaline pH, showed that the β^J -subunits migrated like β^A , but the α^J -chains moved faster than α^A polypeptide chains. These findings confirmed that the haemoglobin was indeed a haemoglobin J α .

Examination of the amino-acid substitution. 'Fingerprints' of the haemoglobin J α , prepared according to Ingram¹ and Baglioni², were compared with those from haemoglobin A. References to these and other procedures involved have recently been listed³. They include the preparation of globin from haemoglobin, followed by tryptic digestion which splits the α -chain and the β -chain into small peptides wherever there are lysyl or arginyl bonds. These peptides are afterwards separated by electrophoresis in one direction (horizontal in Figs. 2 and 3) and then at right angles by chromatography (vertical in Figs. 2 and 3). This two-dimensional separation of the peptides results in a characteristic map of the peptides—the fingerprint. The peptides are numbered in the sequence in which they occur along α - and β -chains, respectively. α TpI (the α -chain's tryptic peptide No. 1) corresponds to residues α 1–7, and α TpII to residues 8–11 of the α -chain. Some free peptides α TpI and α TpII are formed on tryptic digestion, but the greater part of the sequence of residues 1–11 (the two peptides α TpI and α TpII) remains unhydrolysed under the conventional conditions of tryptic hydrolysis. The explanation is that

1	2	3	4	5	6	7	8	9	10	11
Val-Leu-Ser-Pro-Ala-Asp-Lys-Thr-Asn-Val-Lys										

Tryptic peptides α TpI (residues 1–7) and α TpII (residues 8–11) of the α -chain of haemoglobin A.
The arrow indicates where the two peptides are separated by tryptic hydrolysis. This separation is partially inhibited by the acidic aspartyl at position 6.

Table 2. AMINO-ACID ANALYSIS OF α TpI+II FROM HAEMOGLOBIN J AND HAEMOGLOBIN A

	Haemoglobin J μ moles	Residues	Haemoglobin A μ moles	Residues
Asp	0.222	3.1	0.221	2.1
Thr	0.077	1.1	0.114	1.1
Ser	0.075	1.0	0.117	1.1
Pro	0.079	1.1	0.124	1.2
Ala	0.007	< 0.1	0.122	1.2
Val	0.126	1.8	0.190	1.8
Leu	0.069	1.0	0.100	1.0
Lys	0.141	2.0	0.211	2.0

the acidic residue of aspartic acid in the position $\alpha 6$ inhibits the tryptic hydrolysis of the neighbouring bond of the basic lysine residue (Table 1).

Figs. 2 and 3 show peptide maps of haemoglobin A and haemoglobin J respectively. It will be seen that the spot representing α TpI+II in haemoglobin A is missing in the fingerprint of haemoglobin J. α TpI is also missing, but a faint spot representing α TpII is present. Two new spots are present in the fingerprint of haemoglobin J, one a neutral peptide close to β TpI, and another below β TpV (Figs. 2 and 3).

It was assumed that the more strongly staining spot in the neutral area represented the α TpI+II of haemoglobin J, and that the new acidic peptide was α TpI. These observations suggested a replacement of a neutral by an acidic residue in α TpI, a mutation which would result in a haemoglobin variant migrating electrophoretically as haemoglobin J.

To identify the amino-acid substitution, both α TpI+II and the presumed α TpI+II were separated by paper electrophoresis at pH 6.4 followed by chromatography for 24 h. At this stage the haemoglobin J peptide gave a positive staining reaction for histidine, indicating contamination by the histidyl-containing β TpI. Complete resolution from β TpI was obtained by a second electrophoresis at pH 6.4 with a potential gradient of 60 V/cm for 2 h.

Table 2 shows the results of the amino-acid analysis of α TpI+II and of the neutral peptide obtained from haemoglobin J. It will be seen that the haemoglobin J peptide contains all the amino-acids found after acid hydrolysis of α TpI+II except that alanine is missing and an additional aspartic acid is present. This finding suggests that the alanyl in position 5 of the α -chain of haemoglobin A has been replaced by a residue of aspartic acid and that this haemoglobin J is α_5^J Ala \rightarrow Asp β_2 . Such a variant has not been described before. It is proposed that this variant be named haemoglobin J α Toronto.

In the fingerprint of haemoglobin J Toronto the new faintly staining acidic peptide below β TpV almost certainly represented a trace of α TpI. However, because of the small amount present, attempts to isolate this peptide were unsuccessful.

Although no less than five mutations are known for the first peptide of the β -chain of human haemoglobin, none has previously been described for the α -chain (Table 3). It is thought that in human evolution the α -chain gave rise to the γ -chain and that

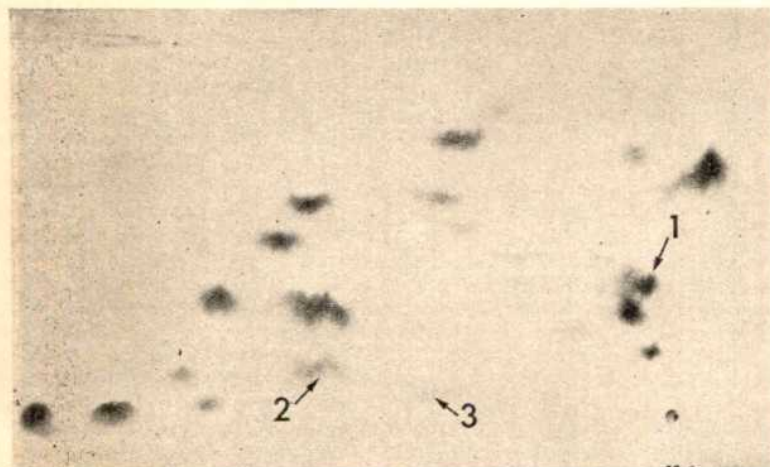


Fig. 2. Fingerprint of haemoglobin A. 1, α TpI (residues 1–7 of α -chain); 2, α TpII (residues 8–11); 3, α TpI+II

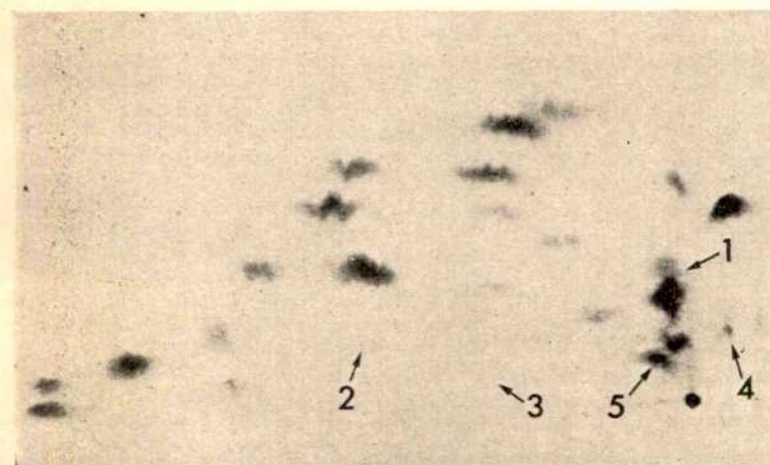


Fig. 3. Fingerprint of haemoglobin J α Toronto. 1, α TpI is missing; 2, α TpII is present but faint; 3, α TpI+II is missing; 4, a new peptide— α TpI; 5, a new peptide— α TpI+II

the β -chain arose afterwards from the γ -chain, and the δ -chain from the β -chain. It is of interest that the mutation α 5 Ala \rightarrow Asp in haemoglobin J Toronto represents a change, that is, from a neutral to an acidic residue similar to that (Ala \rightarrow Glu) which must have occurred in the non- α -chains (Table 4).

Twenty-seven types of amino-acid substitutions observed in human haemoglobins (including four differences between β - and δ -chains) have recently been summarized⁴. Since then the substitution Lys \rightarrow Gln has been observed in haemoglobin K Woolwich⁵; the present observation adds a

Table 3. β TpI and α TpI. A COMPARISON OF THE SUBSTITUTIONS KNOWN

						Lys	Lys
						Val	Gly
β TpI	Val	His	Leu	Thr	Pro	Glu	Glu
	1	2	3	4	5	6	7

α TpI	Val	Leu	Ser	Pro	Ala	Asp	Lys
	1	2	3	4	5	6	7

Although many amino-acid substitutions are known for the first peptide of the β -chain (above) none has previously been described for the corresponding peptide of the α -chain (below)

Table 4. THE FIRST PEPTIDES OF THE α , γ , β AND δ CHAINS OF HUMAN HAEMOGLOBIN

The gap between α 1 and α 2 does not exist in Nature; but by its introduction, maximum correspondence is permitted between the α -chain and the three other chains

	1	2	3	4	5	6	7
α	VAL	—	LEU	SER	PRO	ALA	ASP
γ	GLY	HIS	PHE	THR	GLU	GLU	ASP
β	VAL	HIS	LEU	THR	PRO	GLU	GLU
δ	VAL	HIS	LEU	THR	PRO	GLU	GLU
	1	2	3	4	5	6	7

further substitution Ala \rightarrow Asp. All these substitutions are compatible with single point mutations according to the Triplet Code elaborated by Nirenberg *et al.*⁶.

We thank Mrs. K. Burnie, Miss P. Kynoch and Mrs. T. Vanderheyden for their assistance.

¹ Ingram, V. M., *Biochim. Biophys. Acta*, **28**, 539 (1958).

² Baglioni, C., *Biochim. Biophys. Acta*, **48**, 392 (1961).

³ Watson-Williams, E. J., Beale, D., Irvine, D., and Lehmann, H., *Nature*, **205**, 1273 (1965).

⁴ Beale, D., and Lehmann, H., *Nature*, **207**, 259 (1965).

⁵ Allan, N., Beale, D., Irvine, D., and Lehmann, H., *Nature*, **208**, 658 (1965).

⁶ Nirenberg, M., Leder, P., Bernfield, M., Brimacombe, R., Trupin, J., Rottman, F., and O'Neal, C., *Proc. U.S. Nat. Acad. Sci.*, **53**, 1161 (1965).

GLOBIN SYNTHESIS IN THALASSAEMIA: AN *IN VITRO* STUDY

By DR. D. J. WEATHERALL*, DR. J. B. CLEGG† and DR. M. A. NAUGHTON

Departments of Medicine and Biophysics, The John Hopkins University School of Medicine, Baltimore, Maryland

THE inherited haemoglobinopathies are of two main types. There are those, like sickle-cell disease, in which a structural change in one of the peptide chains of the globin fraction can be demonstrated, and there are others in which, despite evidence of an inherited defect in globin synthesis, no structural change in the globin moiety can be found. Disorders of haemoglobin synthesis of the second type are often found in patients with the clinical and haematological findings of thalassaemia and are, therefore, designated the 'thalassaemia syndromes'¹.

Human adult haemoglobin has a major and a minor component called haemoglobins A and A₂ respectively². Haemoglobin A has two α - and two β -peptide chains ($\alpha_2\beta_2$) and haemoglobin A₂ has two α - and two δ -chains ($\alpha_2\delta_2$) (ref. 3). Foetal haemoglobin, which has usually disappeared by the age of one year, has two α - and two γ -chains ($\alpha_2\gamma_2$) (ref. 4). There is good evidence that the structures of the α -, β -, γ -, and δ -chains are determined by separate pairs of genes⁵.

In some patients with the clinical picture of thalassaemia, foetal haemoglobin synthesis persists beyond the first year of life and haemoglobin A₂-levels exceed the normal range of 1.5–3.5 per cent of the total haemoglobin³. Furthermore, the genetic determinant for this type of thalassaemia behaves as though it were allelic or closely linked to the β -chain locus and also interacts with the sickle-cell (β^S -chain) gene⁶. These observations suggest that this form of thalassaemia results from an inherited defect in β -chain synthesis, the increased amounts of haemoglobins F and A₂ reflecting an attempt at compensation for the deficit in β -chains. The disorder is therefore designated β -thalassaemia.

Some patients with the clinical picture of thalassaemia do not have increased levels of haemoglobin A₂ and F, however, and the genetic determinant segregates separately from the β -chain gene⁶. Some, but not all, individuals in this group carry variable quantities of haemoglobins H and Bart's. Haemoglobin H is a tetramer of normal

β -chains (β_4), while haemoglobin Bart's is composed of four normal γ -chains (γ_4) (refs. 7, 8). It has been suggested, therefore, that this form of thalassaemia results from an inherited defect in α -chain synthesis, α -thalassaemia⁷, the resulting excess of β -chains or γ -chains aggregating to form haemoglobins H or Bart's respectively. This concept of the genetic basis of the thalassaemias, while serving as a useful model for their further investigation, is incomplete since there is increasing evidence for the existence of several types of β -thalassaemia and, probably, of α -thalassaemia¹.

Little is known about the mechanisms involved in the control of normal α - and β -chain synthesis in man, or about the factors which maintain δ -chain synthesis at about 1/40 of the level of β -chain synthesis. Furthermore, although there is evidence that the reticulocyte ribosomes of thalassaemic individuals have a reduced capacity for globin synthesis¹⁰, there has been no direct evidence for defective α - and β -chain synthesis in these disorders. The chemical structure of these chains appears to be normal¹¹ and the nature of the apparent defect in synthesis remains quite obscure. The experiments to be described here were carried out in an attempt to clarify some of these problems by comparing the *in vitro* incorporation of radioactive amino-acids into α -, β -, and δ -chains in the reticulocytes of thalassaemic and non-thalassaemic persons.

Reticulocyte-rich blood was obtained from 19 persons with the conditions listed in Tables 1, 2 and 3. 2–8 ml. of washed red cells was suspended in an amino-acid mixture¹² containing 25–100 μ c. of uniformly labelled ¹⁴C-leucine, and incubated in a Dubnoff metabolic shaker at 37° C, aliquots being removed at various times and immediately frozen. Haemoglobin fractions were purified by dialysis, column chromatography, and starch-block electrophoresis as previously described¹³.

In some experiments ribosomes were prepared¹⁴ before fractionation of the haemoglobins. Globin was prepared¹⁵ from either purified haemoglobin fractions or, in some cases, directly from the washed whole-cell lysates without further purification. The α - and β -chains were separated by gradient elution chromatography on carboxymethyl-

* Present address: Department of Medicine, University of Liverpool.

† Present address: Medical Research Council Laboratory of Molecular Biology, Hill Road, Cambridge.

cellulose columns in urea/mercaptoethanol buffers as recently described¹⁶. In some experiments, chain separation was also achieved by hybridization with canine haemoglobin or by counter-current distribution¹⁷. The purity of each chain was checked by fingerprinting¹⁸ and the radioactivity measured, after plating out about 0.5 mg of protein on aluminium planchets, using a low-background gas-flow counter¹⁹. In order to determine the distribution of radioactivity in finished or partly finished chains on the ribosomes, 5–10 mg of unlabelled carrier globin was added to the ribosomal pellets and the α - and β -chains of the mixture separated by carboxymethyl-cellulose chromatography¹⁶.

Non-thalassaemic Reticulocytes

The specific activities of the separated α - and β -chains of purified haemoglobin A from a variety of non-thalassaemic conditions are summarized in Table 1 and the distribution of radioactivity in a typical chain separation, after 30 min incubation, is shown in Fig. 1. Under all conditions investigated the specific activities of the α - and β -chains were very similar at incubation times of 5 min to 5 h. At shorter times, however, the specific activity of the β -chain was always significantly greater than that of the α -chain. The pattern of radioactivity in separated α - and β -chains obtained from addition of carrier globin to ribosomal pellets was always the same in this group. Thus, the radioactivity under the α -chain peak always exceeded that under the β -chain peak, the ratio α -chain/ β -chain ranging from 1.5 to 2.1/1. Clear peaks of radioactivity were seen for both chains in each case, the radioactivity peak usually being slightly displaced from the

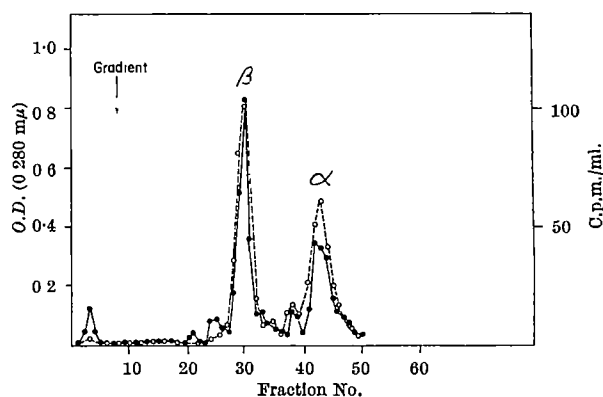


Fig. 1. Incorporation of radioactivity into the α - and β -chains of haemoglobin A prepared from the red cells of a patient with hereditary spherocytosis. Incubation period 45 min. ●—●, O.D. (0.280 μ); ○—○, radioactivity

protein peak. These results indicate that since the number of leucine residues in α - and β -chains is the same, α - and β -chain synthesis is normally synchronous. The fact that the specific activity of the β -chain is greater than that of the α -chain at short times of incubation, and the presence of an excess of radioactivity under the α -chain peak from the ribosomes after long periods of incubation, implies that a small pool of α -chain exists on the ribosomes. The existence of only a small peak of radioactivity associated with the β -chain from the ribosomes indicates that release of β -chains from the ribosomes is much more rapid than that of α -chains, but is not instantaneous. These observations suggest that finished β -chains are probably necessary for the removal of α -chains from the ribosomes. A similar situation has been described in the rabbit reticulocyte¹⁸ where the discrepancy in chromatographic behaviour between radioactivity (newly made chain) and carrier chain was also noted. Whether this means that the newly made chain, while still on the ribosome, is chemically different from that found in finished haemoglobin is not clear, but this would seem very likely.

In order to follow the incorporation of radioactive amino-acids into the α - and δ -chains of haemoglobin A₂, reticulocytes from an individual with hereditary spherocytosis were incubated with ¹⁴C-leucine for 60 min and haemoglobins A and A₂ isolated¹³. The α - and β -chains of haemoglobin A and the α - and δ -chains of haemoglobin A₂ were then separated and the specific activities of the whole haemoglobin fractions and separated chains determined (Table 3). As in previous experiments¹³ the specific activity of haemoglobin A was more than twice that of haemoglobin A₂. The specific activities of the α - and β -chains of haemoglobin A were very similar. The specific activity of the α -chain of haemoglobin A₂ was less than that of haemoglobin A—this finding being compatible with the finding¹³ that, in the *in vitro* system, haemoglobin A₂ synthesis in reticulocytes is retarded before that of haemoglobin A. This observation cannot, however, explain the ratio of specific activity of α -chain to δ -chain of 5/1 in haemoglobin A₂. It has recently been suggested that one reason for the relatively slow rate of δ -chain synthesis might lie in the presence of one or more 'slow points' during assembly of the δ -chain²⁰. In such an event, δ -chain clearance from the ribosomes would be slow relative to α -chain clearance. Thus, in investigations of radioactive incorporation, the time taken to achieve uniform δ -chain labelling would be longer than that required for uniform α -chain labelling, resulting in the marked difference in specific activity between α -chain and δ -chain observed in these experiments. These findings are thus compatible with the recent observation that uniform labelling of the δ -chain does in fact take a long period of incubation²⁰. Another explanation would be the exchange of α -chains between newly made haemoglobin A and haemoglobin A₂, the synthetic rate of which falls

Table 1. RELATIVE SPECIFIC ACTIVITIES OF α - AND β -CHAINS OF HAEMOGLOBIN A PREPARED FROM RED CELLS OF PATIENTS WITH A VARIETY OF HAEMATOLOGICAL STATES. ¹⁴C-LEUCINE WAS USED IN EACH CASE

Clinical disorder	Incubation time (min)	Specific activity of α -chain (c.p.m./mg)	Specific activity of β -chain (c.p.m./mg)
Hereditary spherocytosis	4	15	28
	15	88	77
	45	138	163
	240	422	439
Hereditary spherocytosis	2	39	93
	4	105	210
	200	1,617	1,652
Hereditary spherocytosis	2	189	616
	4	691	1,470
	300	8,960	9,296
Pyruvate kinase deficiency	4	418	595
	15	2,240	2,030
	60	3,780	4,116
	240	6,930	6,370

Table 2. RELATIVE SPECIFIC ACTIVITIES OF α - AND β -CHAINS OF HAEMOGLOBIN A PREPARED FROM THE RED CELLS OF PATIENTS WITH β -THALASSAEMIA AND HAEMOGLOBIN H DISEASE (α -THALASSAEMIA). ¹⁴C-LEUCINE WAS USED IN EACH CASE

Clinical disorder	Incubation time (min)	Specific activity of α -chain (c.p.m./mg)	Specific activity of β -chain (c.p.m./mg)
Thalassaemia major	30	494	266
	30	840	470
	30	1,638	1,043
	30	242	61
	30	39	24
	60	126	180
	30	494	266
	180	3,064	1,904
	12 h	4,718	3,924
	3	627	373
	10	945	441
	30	1,638	1,043
Haemoglobin H disease	180	4,382	2,618
	30	1,660	277
	30	260	65
	30	80	10
	30	267	19
	240	67	13
	20 h	100	43
	10	35	2
	30	127	11
	180	189	32

Table 3. DISTRIBUTION OF RADIOACTIVITY IN THE PEPTIDE CHAINS OF HAEMOGLOBINS A AND A₂ AFTER 60-MIN INCUBATION OF THE RED CELLS WITH ¹⁴C-LEUCINE

Haemoglobin type	Specific activity of α -chain (c.p.m./mg)	Specific activity of β -chain (c.p.m./mg)	Specific activity of δ -chain (c.p.m./mg)
A	59.8	52.7	—
A ₂	32.1	—	6.9

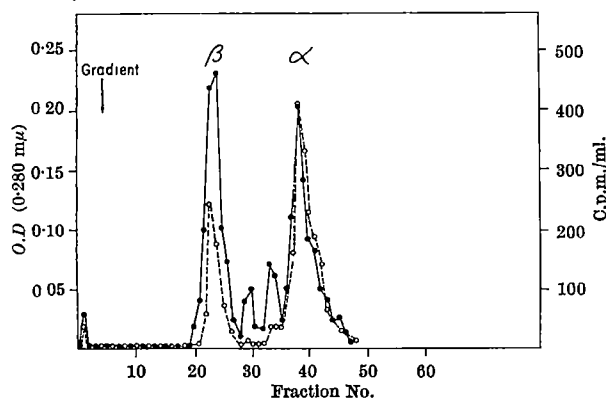


Fig. 2. Incorporation of radioactivity into the α - and β -chains of haemoglobin A prepared from the cells of a patient with β -thalassaemia major. Incubation time 60 min. \bullet — \bullet , O.D. (0.280 $m\mu$); \circ — \circ , radioactivity.

off so rapidly in peripheral blood, but this mechanism seems very unlikely.

These results suggest, therefore, that there are 'slow points' in δ -chain synthesis giving rise to a relatively slow rate of clearance from the ribosomes. It is unlikely that the slow rate of δ -chain synthesis is associated with a quantitative reduction in messenger RNA since, although this would result in the production of fewer δ -chains, their specific activity would be similar to that of α -chains, if the rates of assembly of the two chains were comparable.

β -Thalassaemia Reticulocytes

The findings in the experiments utilizing cells from persons with β -thalassaemia, which were quite different from those in non-thalassaemic samples, are summarized in Table 2. The specific activity of the α -chain of purified haemoglobin A always exceeded that of the β -chain, the ratios α/β ranging from 1.5/1 to 7/1. These values were obtained at incubation times of 30 min–5 h, the differences being less marked after longer periods of incubation (Fig. 2). These ratios were similar to those recently reported¹⁰. In order to rule out the possibility of variation of leucine pool size for the two chains in this disorder, experiments were also performed with ^{14}C -lysine and ^{14}C -valine, similar differences in specific activity being noted. Such differences between the specific activities of the α - and β -chains in finished haemoglobin A could occur if a large intracellular pool of β -chain existed at any given time. After introduction of the radioisotope, newly made (and therefore labelled) β -chain would be diluted out by pre-existing unlabelled β -chains present in the pool. To examine this possibility, two experiments were carried out in which a chain separation was performed on a washed, whole-cell lysate without prior purification of haemoglobin fractions (Fig. 3). The recovery of protein and radioactivity exceeded 90 per cent in each case. Fingerprinting of each peak showed only α - and β -chain peptides, no new spots being observed. In both experiments the amount of radioactivity under the β -chain peak was similar to that found in the β -chain from purified haemoglobin A, which had been prepared from an equivalent amount of the original red-cell lysate. In one experiment a large peak of protein and radioactivity was eluted before the β -chain peak, this being the γ -chain of haemoglobin F, while in another too little haemoglobin F was present for clear separation of β - and γ -chains. These results thus excluded the presence of a large intracellular pool of β -chain, with subsequent dilution of newly labelled chains, as a basis for the observed differences in specific activity in α - and β -chains.

Such differences in specific activity could occur, however, if there were a large block (that is, rate-limiting step) at some point during the assembly of the β -chain or its release from the ribosome, associated with the presence on the ribosomes at any given time of many completed or

partially completed chains. As in the case of normal δ -chains already discussed, relatively long periods of time would then be required to achieve uniform labelling of newly synthesized β -chain. At long times, each newly synthesized chain would then have the same specific activity as an α -chain synthesized from the same amino-acid pool. The time required to achieve uniform labelling would thus depend on the severity of the block, that is, of the ease with which unlabelled β -chains present at the time of introduction of the radioactive amino-acid were cleared from the ribosomes.

One consequence of a rate-limiting step in synthesis, of the sort already discussed, would be the absence of radioactivity associated with β -chain on the ribosomes, in contrast to normal ribosomes where both labelled α - and β -chains can be shown to be present after a few minutes incubation (see earlier). Prolonged periods of incubation would be required to clear pre-existing unlabelled β -chains from the ribosomes and replace them with labelled chains. Ribosomes were therefore prepared from the red cells of two patients, homozygous for β -thalassaemia, after incubation of the cells with ^{14}C -leucine for 45 min. After addition of carrier globin to the ribosome pellet, the chains were separated by column chromatography. No peak of radioactivity corresponding to the β -chain was observed, while a large peak was seen in the α -chain region in each case (Fig. 4).

These findings are thus compatible with the concept of a gross defect in β -chain synthesis at the ribosomal level in β -thalassaemia. Whether, since α -chains appear to require finished β -chains for their release from the ribosomes, this results in a secondary accumulation of finished α -chain on the ribosomes is uncertain, although this seems likely since the relative amount of radioactivity in the α -chain fraction was several times that observed in

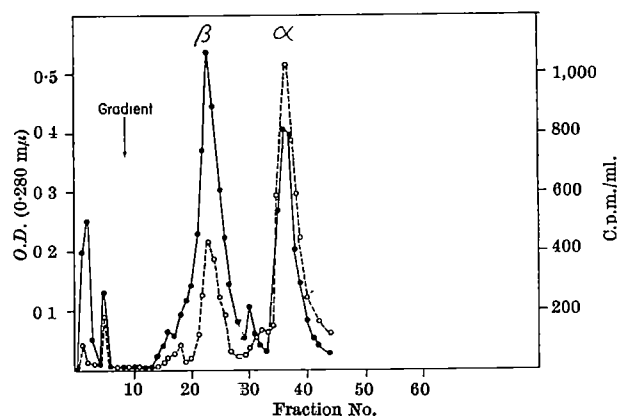


Fig. 3. Distribution of radioactivity compared with protein in a washed whole-cell lysate prepared from the red cells of a patient with β -thalassaemia major. Incubation time 60 min. \bullet — \bullet , O.D. (0.280 $m\mu$); \circ — \circ , radioactivity.

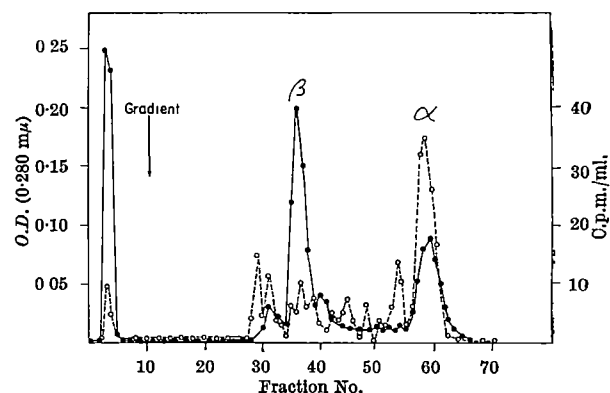


Fig. 4. Distribution of radioactivity after addition of 10 mg unlabelled carrier globin to the ribosomal pellet prepared from the cells of a patient with β -thalassaemia major. Incubation time 45 min. \bullet — \bullet , O.D. (0.280 $m\mu$); \circ — \circ , radioactivity.

ribosomes obtained from non-thalassaemic reticulocytes. Further attempts to demonstrate the site of this proposed block in β -chain synthesis have been made by comparing the ratios of the specific activities of α - and β -chains of purified haemoglobin A prepared from four parallel incubations of aliquots of the same red cell sample using ^{14}C -labelled leucine, arginine, histidine, and tyrosine. Since these amino-acids are not distributed evenly along the peptide chain, the specific activities of non-uniformly labelled β -chains might be expected to differ with each amino-acid. The specific activities of individual peptides¹⁴ of thalassaemic β -chain were also measured. No definite site of delay in synthesis has yet been demonstrated, although the results have not excluded a 'slow point' either at, or near, the carboxyl-terminal end of the β -chain.

α -Thalassaemia (Haemoglobin H Disease) Reticulocytes

The results of six ^{14}C -leucine incubation experiments performed on the cells of persons with haemoglobin H thalassaemia are shown in Table 2. At incubation times of 30 min–5 h the specific activity of the α -chain of purified haemoglobin A was 1.5–15 times that of the β -chain (Fig. 5). This difference became less marked with more prolonged incubation of the red cells. Because of these findings, which were surprisingly like those in β -thalassaemia, the possibility of a large intracellular pool of β -chain diluting out newly made and, therefore, labelled β -chains again had to be examined. Washed whole-cell lysates from two persons with haemoglobin H disease were converted to globin without prior purification of the haemoglobins and the α - and β -chains separated (Fig. 6). The radioactivity in the β -chain fraction exceeded that in the α -chain fraction by a factor of 2.3/1 in one case and 3.0/1 in the second, while there was an associated increase in the optical density values for the β -chain peak. In a

separate experiment using identical amounts of the same two samples of washed whole-cell lysates, the amounts of haemoglobin A and H were determined and the radioactivity in each fraction measured. The excess of both radioactivity and optical density associated with the β -chain (over that present in the α -chain) of the whole-cell lysate could all be accounted for by the amount of protein and radioactivity found in the purified haemoglobin H fraction in the second experiment. Similar results were observed with ^{14}C -lysine. These results suggest that in haemoglobin H disease β -chain synthesis occurs at a rate of about 2–3 times that of α -chain synthesis, and that β -chains are freely released into the red cell where they form a large pool from which they are capable of uniting with newly made α -chains as these become available. When the cell is haemolysed, most of these β -chains appear in the haemoglobin H fraction. The presence of this large pool of β -chains at any one time probably explains the striking difference between the specific activities of the α - and β -chains of haemoglobin A in haemoglobin H disease. Newly-made labelled α -chains will combine with unlabelled β -chains which were already present in this pool. Whether the excess β -chains exist as haemoglobin H (β_4) in the red cell is uncertain, but at least in an *in vitro* system haemoglobin H readily combines with α -chains to form haemoglobin A²¹.

These experimental results must, of course, be interpreted with caution since the behaviour of cells in an *in vitro* system may not fully reflect their *in vivo* properties. Furthermore, this type of experiment utilizing peripheral blood only measures the last vestiges of protein synthesis in reticulocytes. However, certain tentative conclusions can be drawn. It appears that in non-thalassaemic individuals, α - and β -chain synthesis is synchronous and that, as previously suggested¹⁸, completed β -chains are required for the release of α -chains from the ribosomes. This probably results in a small number of finished α -chains being present on the ribosomes at any given time. Whether the peaks of radioactivity seen on ribosomal chain separations represent completed or partially completed chains is uncertain, although the high resolution of the chromatographic system used would have resulted in the separation of chains differing by as little as one charged residue¹⁸.

From the results of experiments on the kinetics of α - and δ -chain synthesis of haemoglobin A₂, it seems likely that at least one mechanism whereby δ -chain synthesis occurs at a slower rate than α - and β -chain synthesis is the slow ribosomal release of δ -chains. This slow release may well be due to the presence of one or more 'slow points' during assembly of the δ -chain.

In both forms of thalassaemia the specific activity of the α -chain of haemoglobin A exceeded that of the β -chain, even after long periods of cell incubation.

For α -thalassaemia, it has been clearly shown that α -chain synthesis occurs at about half the rate of β -chain synthesis, giving rise to a pool of free β -chain in the cell. Thus, although the rate of β -chain synthesis is greater than that of α -chain, the effect of the pre-existing pool of free β -chain is to dilute newly synthesized labelled β -chains. The specific activity of the β -chain is thus considerably lower than that of the α -chain even though synthesis of the latter is impaired.

In β -thalassaemia, no such pool of β -chains could be demonstrated, nor was a large pool of free α -chain detected. However, excess α -chains do occur. Some are bound to γ -chain (as haemoglobin F), others appear to be attached to ribosomes, and some excess α -chain has been observed in chain separations of globin prepared from red cell stroma. These observations are in keeping with the suggestion²² that excess α -chain may be insoluble and precipitates in the cell to form the inclusion bodies characteristic of β -thalassaemia.

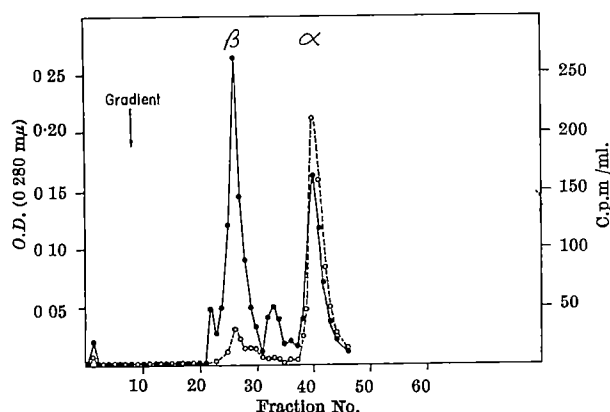


Fig. 5. Distribution of radioactivity in the α - and β -chains of haemoglobin A from the cells of a patient with haemoglobin H disease. Incubation time 45 min. ●—●, O.D. (0.280 μ); ○---○, radioactivity

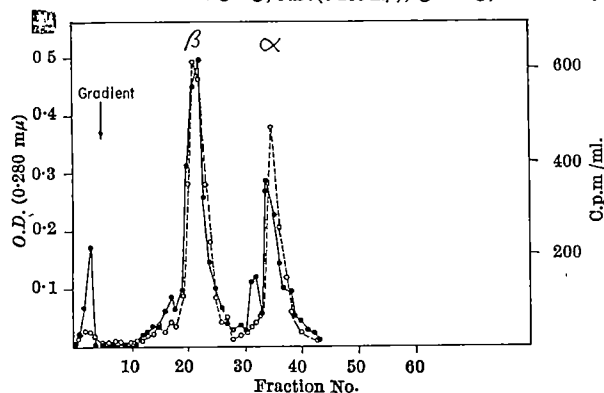


Fig. 6. Distribution of radioactivity compared with protein in a washed whole cell lysate prepared from the red cells of a patient with haemoglobin H disease. Incubation time 45 min. ●—●, O.D. (0.280 μ); ○---○, radioactivity

From the data presented, the most probable explanation of the marked difference of specific activity between α - and β -chains in β -thalassaemia is a block or 'slow point' during the assembly of the β -chain, resulting in an accumulation of completed or partially completed β -chains on the ribosomes. It seems likely that since α -chains require β -chains for their release, there is also a secondary accumulation of α -chains on the ribosomes. It is difficult to imagine a mechanism which could give rise to the differences in the specific activities of the α - and β -chains noted here, and yet at the same time leave the rate of assembly of the β -chain unaltered. If, for example, the defect were simply in the number of ribosomes active in β -chain synthesis, there would be a deficit in β -chains but those which were made would have the same specific activity as α -chains. Similarly, a net reduction of messenger RNA for β -chains would lead to a deficit in β -chains, but would not be expected to give rise to different specific activities of the α - and β -chains in completed haemoglobin A unless the rates of assembly of the two chains were also different. Experiments using multiple ^{14}C -labelled amino-acids have tentatively ruled out the N-terminal and central region of the chain as a site of a block in synthesis, but it could occur at or near the carboxyl-terminal end of the molecule. Whichever is true, it seems quite probable that in different instances of β -thalassaemia the site of the defect may not always be the same. The heterogeneity of β -thalassaemias already recognized¹ may be, in some part, a reflexion of this. The similarity of the kinetics of peptide chain synthesis in thalassaemic β -chains and the δ -chains of normal haemoglobin A₂ suggests that a rewarding approach to the β -thalassaemia problem might be in the search for rate-limiting steps in synthesis. A reduction in the rate of α -chain synthesis in haemoglobin H disease has been shown and a similar search for the presence of 'slow points' in α -chain synthesis in this disorder is indicated.

In the absence of evidence indicating structural changes in the α - and β -chains of haemoglobin A in thalassaemia, it seems likely that explanations for these disorders may be found in changes in one or more of the factors which affect the process of assembly of the protein on the ribosomes. Mutations leading to changes in messenger RNA codewords, for example, while producing no change in the sequence of amino-acids, might, nevertheless, alter the rates of translation by requiring recognition of the altered codons by other sRNA anti-codons. It is conceivable that if synthesis of the α - or β -chains is dependent on a polycistronic messenger RNA, then changes in messenger RNA at a site remote from the α - or β -chain cistron might in some way influence the rate of assembly

or the α - or β -peptide chain²³. However, this mechanism seems less probable than one directly involving the α - or β -chain messenger RNA. Alternatively, it is possible that mutations leading to changes in specific sRNAs or ribosomal RNA could also influence the rate of assembly of one peptide chain relative to another. An instance where a leucine-specific sRNA is involved in the coding of only one out of some 30 leucine residues in the rabbit haemoglobin molecule is already known²⁴. The recent advances in the techniques for isolating and studying messenger RNA and sRNA from mammalian cells may eventually provide the necessary means of investigating the problem at this level.

We thank Dr. Marion Erlandson and Dr. Carl Smith for their help in obtaining blood samples for these investigations. We also thank Dr. S. H. Boyer, Dr. C. L. Conley, Dr. H. M. Dintzis and Dr. G. Von Ehrenstein for their advice.

This investigation was supported in part by grant HE-02799, U.S. Public Health Service, the National Heart Institute; grant T1-AM-5260, the National Institute of Arthritis and Metabolic Diseases; grant AM-06006-03, National Institutes of Health; and grant CB-2630, National Science Foundation.

¹ Weatherall, D. J., *The Thalassaemia Syndromes* (Blackwell Scientific Publications, Oxford, 1965).

² Kunkel, H. G., Ceppellini, R., Muller-Eberhard, U., and Wolf, J., *J. Clin. Invest.*, **38**, 1615 (1957).

³ Baglioni, C., in *Molecular Biology*, edit. by Taylor, J. H., part I, 405 (Academic Press, New York, 1963).

⁴ Schroeder, W. A., and Matsuda, G., *J. Amer. Chem. Soc.*, **80**, 1521 (1958).

⁵ Ceppellini, R., in *Ciba Found. Symp. Biochem. Genet.*, edit. by Wolstenholme, G. E. W., and O'Connor, C. M., 133 (Churchill, London, 1959).

⁶ Cohen, F., Zuelzer, W. W., Neel, J. V., and Robinson, A. R., *Blood*, **14**, 816 (1959).

⁷ Jones, R. T., Schroeder, W. A., Balog, J. E., and Vinograd, J. R., *J. Amer. Chem. Soc.*, **81**, 3161 (1959).

⁸ Hunt, J. A., and Lehmann, H., *Nature*, **184**, 872 (1959).

⁹ Ingram, V. M., and Stretton, A. O. W., *Nature*, **184**, 1903 (1959).

¹⁰ Burka, E. R., and Marks, P. A., *Nature*, **199**, 706 (1963).

¹¹ Guldotti, G., cited by Ingram, V. M., *Medicine*, **43**, 759 (1964).

¹² Vinograd, J., and Hutchison, W. D., *Nature*, **187**, 216 (1960).

¹³ Rieder, R. F., and Weatherall, D. J., *J. Clin. Invest.*, **44**, 42 (1965).

¹⁴ Dintzis, H. M., *Proc. U.S. Nat. Acad. Sci.*, **47**, 247 (1961).

¹⁵ Anson, M. C., and Mirsky, A. E., *J. Gen. Physiol.*, **13**, 469 (1930).

¹⁶ Clegg, J. B., Naughton, M. A., and Weatherall, D. J., *Nature*, **207**, 945 (1965).

¹⁷ Ingram, V. M., and Stretton, A. O. W., *Biochim. Biophys. Acta*, **62**, 456 (1962).

¹⁸ Baglioni, C., and Colombo, B., *Cold Spring Harb. Symp. Quant. Biol.*, **29**, 347 (1964).

¹⁹ Heywood, J. D., Karon, M., and Weissman, S., *Science*, **146**, 530 (1964).

²⁰ Ingram, V. M., *Medicine*, **43**, 759 (1964).

²¹ Huehns, E. R., Beaven, G. H., and Stevens, B. C., *Biochem. J.*, **52**, 444 (1964).

²² Fessas, P., and Loukopoulou, D., *Science*, **143**, 500 (1964).

²³ Stent, G. S., *Science*, **144**, 813 (1964).

²⁴ Weissblum, B., Gonano, F., Von Ehrenstein, G., and Benzer, S., *Proc. U.S. Nat. Acad. Sci.*, **53**, 328 (1965).

PROMOTION BY KINETIN OF THE POLAR TRANSPORT OF TWO AUXINS

By DR. C. C. MCCREADY, DR. DAPHNE J. OSBORNE and M. KAY BLACK*

Agricultural Research Council Unit of Experimental Agronomy, Department of Agriculture, University of Oxford

IN higher plants, auxin is synthesized chiefly in certain limited regions. The amounts of auxin reaching cells remote from these regions must depend in part on the ability of the intervening tissues to transport auxin. At present, little is known of the ways in which the movement of auxin is regulated. We describe here experiments on the transport of a natural and a synthetic auxin through segments cut from bean petioles. In both cases auxin transport was greatly enhanced by treatment of the segments with a growth regulator of another type, the cytokinin¹ kinetin (6-furfurylaminopurine). This effect was best observed in segments which had been excised from the plant some time before the measurement of auxin transport was begun.

Seedlings of *Phaseolus vulgaris*, var. Canadian Wonder, were raised in a greenhouse and used when the petioles of the young primary leaves were elongating rapidly. Each experiment was conducted in two stages. Segments cut from these petioles were first treated either with kinetin or with water, as described in detail later. Afterwards the basipetal movement of each auxin through the segments was determined by the following method, which in principle resembles that of Went². A donor block of 1.5 per cent agar gel, 23 μl . in volume, incorporating the auxin labelled with carbon-14, was applied to the distal end of each segment. A receiver block of plain agar gel was at once applied to the proximal end of the segment, and transport was allowed to proceed at $25^\circ \pm 0.5^\circ \text{C}$ in a humid atmosphere in darkness. After a suitable interval the donor and the receiver blocks were removed and

* Permanent address: Department of Biochemistry, University of California, Riverside, California.

assayed for carbon-14. Standard sets of unused donor blocks were similarly assayed. All results were corrected for background. The materials and methods used have been more fully described by McCready³. To indicate the effect of kinetin on the transport of carbon-14, the counts per minute (c.p.m.) in receivers from kinetin-treated segments are shown in Tables 1-3 as a percentage of the c.p.m. in receivers from control segments which had been treated with water instead of kinetin. The auxins used were indolyl-3-acetic acid-methylene-¹⁴C (IAA) and 2,4,5-trichlorophenoxyacetic acid-carboxyl-¹⁴C (2,4,5-T). The latter was chosen because in these segments it is transported with high polarity in larger amounts than IAA and is less subject to breakdown (McCready, unpublished results).

In experiments designed to compare freshly excised tissue with aged tissue, treatments were applied in the following ways. If the tissue was to be used fresh, a segment 5.4 mm in length was cut from the middle of the petiole of each of the paired primary leaves of a seedling. The segments were laid horizontally on grooved racks which left their ends free. One segment of each pair was at once treated with kinetin, 1 μ l. of a 233 μ M solution being placed on each cut end with a micrometer syringe. One

μ l. of water was similarly applied to each end of the segment from the opposite petiole, which served as a control. After this treatment the segments were left in a humid atmosphere in darkness at 25° C for 40-45 min to allow absorption of the droplets before donor and receiver blocks were applied.

If the tissue was to be used after a period of ageing, segments 8.0 mm in length were cut and laid on racks in the same way. Kinetin was applied to some segments before ageing, and to others after ageing. In either case, the segments and their paired controls were stored for 13 or 20 h in the conditions already described. From the middle of each 8-mm segment a 5.4-mm portion was then cut, and donor and receiver blocks were applied to its ends for the measurement of transport. Unless the agar blocks were applied to surfaces freshly cut in this way, auxin transport through stored segments was much reduced, especially with IAA. This reduction was probably due in part to a gradual distortion of the ends of the segments during storage which prevented proper contact between tissue and blocks, and in part to the development of IAA-destroying enzymes at the exposed cut surfaces.

Table 1 shows the results of an experiment in which the transport of IAA was measured with and without kinetin, the treatment being applied in three different ways to segments cut from the same batch of plants. In the first type of treatment, fresh 5.4-mm segments were treated with kinetin or with water and left for 40-45 min, after which auxin transport was measured over a period of 4 h. Kinetin had no effect on the amount of carbon-14 appearing in the receiver blocks. In the second type of treatment, 8-mm segments were treated with kinetin or water and stored for 20 h. The segments were then cut to 5.4 mm, 1 μ l. of water was added to each end, and after 40-60 min transport was measured as before. In the receivers from stored control segments which had been given no kinetin, only 1/5-1/3 as much carbon-14 appeared as in receivers from fresh control segments. However, the stored tissue which had been treated with kinetin transported about 1.7 times as much carbon-14 as the stored controls. Although transport through the segments treated before storage was still only about half that through fresh segments, the effect of kinetin on transport through the stored tissue was clearly substantial.

The fact that kinetin enhanced the transport of auxin only in the stored segments suggested a possible explanation of its mode of action. It has been reported that kinetin retards or prevents some of the changes typical of ageing in detached leaves⁴⁻⁶. To test whether its function here was of a similar nature, the third type of treatment was included: 8-mm segments were treated with water alone, stored for 20 h, cut to a length of 5.4 mm and then given kinetin or water 20-45 min before auxin transport began. Kinetin applied in this way, after the ageing period, was just as effective in promoting auxin transport as kinetin applied before ageing. It was therefore concluded that the action of kinetin was not simply to retard the fall in the ability of the segments to transport auxin. After the transport capacity of the tissues had fallen during storage without kinetin, they responded to kinetin by increased auxin transport. In the stored segments some change had occurred which caused auxin transport to become sensitive to kinetin.

Table 2 shows the results of an experiment, similar in principle, in which 2,4,5-T was the auxin transported. Only the timing was altered, to allow for the fact that 2,4,5-T, like 2,4-dichlorophenoxyacetic acid^{3,7}, moves with a much lower velocity than IAA. It was therefore convenient to measure 2,4,5-T transport over a longer period, in this case 12 h. To avoid using excessively old tissues, the period of storage was reduced to 13 h. In stored segments kinetin caused an even larger promotion of the transport of 2,4,5-T than of IAA and again it was immaterial whether kinetin was applied before or after the period of storage. A small effect was seen even with

Table 1. BASIPETAL TRANSPORT OF INDOLYL-3-ACETIC ACID-¹⁴C (IAA) THROUGH PETIOLE SEGMENTS OF *P. vulgaris* WITH OR WITHOUT KINETIN

Pretreatment	C.p.m. from receivers		+ Kinetin - Kinetin Duplicates	$\times 100$ Mean
	- Kinetin	+ Kinetin		
Fresh segments	43 36	47 34	109 94	102
Segments stored for 20 h after kinetin treatment	9.4 12.2	15.0 22.8	160 187	174
Segments stored for 20 h before kinetin treatment	7.5 11.0	13.5 18.0	180 164	172

Pretreatments: 1 μ l. kinetin, 233 μ M, or water to each end of segment (for details see text). Initial concentration of IAA in donors: 5 μ M. Initial radioactivity in donors: 852 c.p.m. Transport 4 h through 5.4 mm segments. Duplicate treatments, 10 segments in each.

Table 2. BASIPETAL TRANSPORT OF 2,4,5-TRICHLOROPHENOXYACETIC ACID-¹⁴C (2,4,5-T) THROUGH PETIOLE SEGMENTS OF *P. vulgaris* WITH OR WITHOUT KINETIN

Pretreatment	C.p.m. from receivers		+ Kinetin - Kinetin Duplicates	$\times 100$ Mean
	- Kinetin	+ Kinetin		
Fresh segments	356 412	416 490	117 119	118
Segments stored for 13 h after kinetin treatment	111 121	297 326	267 269	268
Segments stored for 13 h before kinetin treatment	103 (112)*	262 313	254 280	267

* Calculated mean of preceding three controls, to replace missing sample.

Pretreatments: 1 μ l. kinetin, 233 μ M, or water to each end of segment (for details see text). Initial concentration of 2,4,5-T in donors: 5 μ M. Initial radioactivity in donors: 2,362 c.p.m. Transport 12 h through 5.4 mm segments. Duplicate treatments, 11 segments in each.

Table 3. CHROMATOGRAPHY OF EXTRACTS FROM RECEIVERS AFTER BASIPETAL TRANSPORT OF ¹⁴C-LABELLED AUXINS THROUGH PETIOLE SEGMENTS OF *P. vulgaris* STORED WITH OR WITHOUT KINETIN

Auxin:		Indolyl-3-acetic acid (IAA)		2,4,5-Trichlorophenoxyacetic acid (2,4,5-T)	
C.p.m. from receivers, + kinetin/-kinetin $\times 100$:					
Duplicates	Mean	280	244	141	143
		262		142	
Chromatographic solvent:		Acid		Alkaline	
R_F of auxin zone:		0.75-0.90		0.20-0.35	
C.p.m. from auxin zone as percentage of total c.p.m. from chromatogram:		Acid		Alkaline	
		0.75-0.90		0.55-0.70	
- Kinetin		76		88	
+ Kinetin		80		87	

Pretreatments: 1 μ l. kinetin, 233 μ M, or water to each end of segment. For IAA, 10.5 mm segments \pm kinetin were stored for 14-16 h at 25° C. For 2,4,5-T, 10.5 mm segments \pm kinetin were stored for 14-16 h at 20° C. All segments were cut to 5.4 mm for transport. Initial concentration of IAA in donors: 20 μ M. Initial radioactivity in donors: 8,914 c.p.m. Transport 5 h. 16 segments per treatment for assay, 92 for chromatography. Initial concentration of 2,4,5-T in donors: 20 μ M. Initial radioactivity in donors: 3,678 c.p.m. Transport 14 h. 16 segments per treatment for assay, 94 for chromatography. For chromatographic solvents see text.

fresh tissue, perhaps because the duration of the transport experiment was long enough to allow the development of some sensitivity to kinetin.

We next considered the possibility that in these experiments kinetin was promoting not the transport of the auxins themselves but the production or movement of labelled metabolites formed from them. It seemed unlikely that this could be the explanation of such similar effects on two chemically dissimilar auxins, labelled in positions which were not analogous. Nevertheless, the suggestion was tested by examining chromatographically the labelled substances extracted from receiver blocks. Transport experiments were set up with each auxin using segments from tissue pretreated with kinetin or water as previously described and later stored as shown in Table 3. At the end of the transport period, duplicate sets of receivers from both treated and control segments were assayed for carbon-14 to estimate the magnitude of the kinetin effect. The remaining treated and control receivers were extracted three times with ether slightly acidified with acetic acid (0.05 per cent v/v for IAA, 0.2 per cent for 2,4,5-T). Samples of 16 receivers assayed after extraction contained 15–17 per cent (IAA) or 0.5–1.1 per cent (2,4,5-T) of the carbon-14 in receivers assayed before extraction. Each extract was concentrated to a small volume in a stream of nitrogen and spotted on to two paper strips for development by ascending chromatography, one in an acid solvent (*n*-butanol/acetic acid/water, 5:2:2 v/v) and the other in an alkaline solvent (isopropanol/ammonia/water, 10:1:1 v/v). These and similar solvents have been widely used for the separation of metabolites of both IAA and chloro-substituted phenoxyacetic acids. After running for 10 h each chromatogram was dried and divided into 20 sections equal in size except for that next to the origin, which was extended by 5 mm to include the whole area of the initial spot. The sections were assayed for carbon-14 by means of a liquid scintillation counter⁸.

In Table 3 the c.p.m. from a zone 0.15 R_F units in extent including the auxin peak are shown as a percentage of the total c.p.m. from each chromatogram. When extracts of receivers from kinetin-treated and control segments were compared, the proportion of the carbon-14 appearing in the auxin peak in the two cases never differed by more than 4 per cent of the total on the chromatogram. Therefore the greater radioactivity of the receivers from kinetin-treated tissue—262 per cent of the controls with IAA, 142 per cent with 2,4,5-T—could not to any important extent be due to an increase in the proportion of the carbon-14 found in substances separable from the auxins by chromatography in the two solvents used.

We conclude that when segments of *Phaseolus* petioles are stored as described, those treated with kinetin later transport auxins in markedly larger amounts than do controls. Although the promotion of auxin transport was reproducible within each experiment, as may be seen from the agreement of duplicate treatments, the magnitude of the effect differed from occasion to occasion. We attribute this variability to differences between successive batches of our plants, which were not grown in rigidly controlled conditions. Nevertheless in every one of our series of 34 experiments, some of which are to be reported in another communication, kinetin increased auxin transport.

The amount of auxin entering the segments was estimated from the difference in carbon-14 content between unused donor blocks and donors removed at the end of an experiment. The effects of kinetin on auxin uptake were small and variable but usually positive. In the great majority of cases—111 out of 128 individual treatments—kinetin caused an increase in the amount of carbon-14 entering the receivers which was greater than any increase in the amount leaving the corresponding donors. It follows that the increased transport into the receivers from treated segments could not have been caused solely by a promotion of uptake by kinetin. Indeed, any increase in uptake could

have been simply a consequence of increased transport of auxin away from the distal ends of the kinetin-treated segments.

Mothes, Engelbrecht and Kulajewa⁵ have described and others have confirmed⁹ a "directed transport" of various metabolites into kinetin-treated areas of the laminae of detached leaves. Conrad¹⁰ has reported a similar effect of local applications of kinetin on the distribution of both endogenous auxin and applied IAA in the laminae of detached leaves of *Brassica napus*. Auxin was accumulated and apparently retained in the treated areas. This phenomenon seems to be quite distinct from that revealed by our experiments, in which an increase occurred, not in the amounts of auxins accumulated in the kinetin-treated petiole segments, but in the amounts transported through and out of them.

The difference in response to added kinetin between fresh and aged segments may be simply explained by the hypothesis that the polar transport of auxin can proceed at its maximum rate only in the presence of a sufficient concentration of a cytokinin. It may be supposed that fresh tissue initially contains adequate supplies of an endogenous cytokinin but that this is so depleted during storage of the segments that lack of it becomes a limiting factor for auxin transport. Added kinetin can then to some extent substitute for the natural cytokinin and partly restore auxin transport. The persistence of the effect of kinetin applied to segments before storage is probably due to the use in the present experiments of amounts which were later found to be greatly in excess of the level which sufficed to produce the maximum response.

It has recently been shown^{11,12} that another synthetic cytokinin, *N*₆-benzyladenine, is itself transported in a polar fashion through segments of *Phaseolus* petioles. If the supposed endogenous cytokinin moves similarly, its depletion in the stored segments might well be the result of basipetal movement out of the parts of the segments later used for the measurement of auxin transport.

We end with a speculation. It has been reported elsewhere^{11,12} that the transport of benzyladenine is greatly increased by IAA. The experiments described here show that IAA transport is markedly increased by kinetin, and Black and Osborne¹² find that benzyladenine has a similar effect. It is well established that the balance between kinetin and IAA can profoundly affect plant growth: see, for example, the discussions by Miller¹³ and Setterfield¹⁴. It seems reasonable to suppose that in the intact plant an endogenous cytokinin may behave similarly to kinetin and benzyladenine in all these ways. If so, the mutual promotion of cytokinin and auxin transport might serve to control the ratio of the amounts of the two substances reaching a particular tissue from regions of synthesis elsewhere. The interdependent transport of the two hormones would then constitute a regulatory mechanism of a novel kind.

We thank Prof. G. E. Blackman for advice, Mr. R. G. Powell for preparing the radioactive solutions and for much helpful discussion, and Dr. M. A. Foster for the use of a liquid scintillation counter.

¹ Skoog, F., Strong, F. M., and Miller, C. O., *Science*, **148**, 532 (1965).

² Went, F. W., *Recl. Trav. bot. néerl.*, **25**, 1 (1928).

³ McCready, C. C., *New Phytol.*, **62**, 3 (1963).

⁴ Richmond, A. E., and Lang, A., *Science*, **125**, 650 (1957).

⁵ Mothes, K., Engelbrecht, L., and Kulajewa, O., *Flora (Jena)*, **147**, 445 (1959).

⁶ Osborne, D. J., *Plant Physiol.*, **37**, 595 (1962).

⁷ McCready, C. C., and Jacobs, W. P., *New Phytol.*, **62**, 19 (1963).

⁸ Wang, C. H., and Jones, D. E., *Biochem. Biophys. Res. Commun.*, **1**, 203 (1959). Gelger, J. W., and Wright, L. D., *Biochem. Biophys. Res. Commun.*, **2**, 282 (1960).

⁹ Gunning, B. E. S., and Backley, W. K., *Nature*, **199**, 262 (1963) and references cited therein.

¹⁰ Conrad, K., *Flora (Jena)*, **151**, 345 (1961).

¹¹ Osborne, D. J., and Black, M. K., *Nature*, **201**, 97 (1964). Osborne, D. J., and McCready, C. C., *Nature*, **206**, 697 (1965).

¹² Black, M. K. and Osborne, D. J., *Plant Physiol.* (in the press).

¹³ Miller, C. O., *Ann. Rev. Plant Physiol.*, **12**, 395 (1961).

¹⁴ Setterfield, G., *Symp. Soc. Exp. Biol.*, **17**, 98 (1963).

ENZYMATIC ADAPTATION TO CONTRACTILE ACTIVITY IN SKELETAL MUSCLE

By J. KENDRICK-JONES and PROF. S. V. PERRY

Department of Biochemistry, University of Birmingham

IF skeletal muscle is made to contract in a regular and repeated fashion in excess of normal use, as in training, the tissue appears to adapt to the increased activity by improved performance and often marked hypertrophy. Nevertheless, attempts to relate the marked changes in performance brought about by exercise to persistent biochemical changes in the skeletal muscle cell have led to somewhat contradictory results.

There is some evidence¹ that exercise can increase the size and number of myofibrils in the sartorius muscle of the rat, an observation which would correlate well with Helander's² finding that exercise increases, and restriction in activity decreases, the proportion of myofibrillar to sarcoplasmic protein.

Unequivocal demonstrations of appreciable changes in the activity of skeletal muscle enzymes as a result of exercise which are widely accepted by all workers are hard to find. Lawrie³ has, however, reported an increase in the myoglobin content of the muscles of rats subjected to strenuous daily exercise for an extended period. So far as can be determined by a search of the literature no *in vitro* investigations of enzymatic adaptation to muscle activity have been published.

Much of the early work on the biochemical adaptation of muscle to activity was carried out by Russian workers⁴, who reported increases in α -glycerophosphate dehydrogenase, hexokinase, lactic dehydrogenase and succinic dehydrogenase in both heart and skeletal muscle of rats subjected to a programme of regular strenuous exercise. Yakovlev⁵ has also claimed that exercise increases the myosin ATPase activity by increasing the content of thiol groups which bind ATP. More recent investigations have failed to confirm increases in succinic⁶ and lactic dehydrogenases^{7,8} and have indicated that the levels of aldolase⁹, malic dehydrogenase⁸, phosphorylase, creatine phosphokinase⁸, and calcium-activated adenosine triphosphatase^{8,10} do not increase, when related to total nitrogen or to non-collagen protein nitrogen, in skeletal muscle after exercise. Hearn and Gollnick^{7,10} have, however, reported increases of a few per cent in the activity of heart-ventricle lactic dehydrogenase and adenosine triphosphatase after exercise, although the activity of the skeletal muscle enzymes of the same animals were unchanged. Apart from the early Russian reports, work of most investigators suggests, in the main, that after long or short periods of exercise little change can be demonstrated in the specific activities of the muscle enzymes.

Our interest in this problem arose out of an investigation of the biochemical changes occurring during development in skeletal muscle and the finding¹¹ that in several species there was a good correlation between the onset of regular use of a skeletal muscle and the rapid increases in the specific activities of creatine phosphokinase and 5'-adenylyc deaminase which occur in early neonatal life. The findings suggested that the rise in activity of these enzymes was closely associated with increased contractile function. In the case of the new-born rabbit it was possible to bring forward by several days the increase in creatine phosphokinase in the thigh muscles of the back legs by encouraging the young rabbit to move about earlier than was normal by disturbing the mother's nest. Similar although less clear-cut results were obtained with 5'-adenylyc deaminase. The latter findings are in line with evidence¹² for the adaptive formation of this enzyme

in skeletal muscle of the rat exercised in the Noble-Collip drum, briefly reported by Ninomya *et al.*¹², the results of which we became aware after this work was completed.

Extension of the findings with young rabbits to other species showed that exercising young rats in a treadmill for relatively short periods (4–24 h) produced a significant increase in the creatine phosphokinase-levels of leg muscles compared with controls permitted normal or restricted activity. Some of the results obtained are summarized in Table 1 where the enzymatic activity is related to the total protein nitrogen, extracted under a procedure (Table 1) which removed all the enzyme from the tissue. The values can be directly related to the wet weight or the total nitrogen content of whole muscle, as control experiments showed that the procedure extracted a constant fraction of the total nitrogen of the muscle and that this fraction was not changed by exercise. It is significant that the percentage increases in specific activity obtained were often more marked if the animal was restricted in activity for a period before the experimental period.

In the case of restricted controls the creatine phosphokinase-levels fell to lower values than those found in the same muscles of litter-mate controls permitted normal activity. The results suggested that the creatine phosphokinase level is maintained at fairly high values by normal use of the muscles and only when the use is restricted appreciably does the enzymatic level fall. In these cases a subsequent period of intense activity caused a marked increase in creatine phosphokinase activity. The failure of other workers to demonstrate increases in enzymatic activity after exercise may, in part, have been due to the fact that the activity of muscles of the control animals was sufficiently great to maintain the creatine phosphokinase at an adequate level which was not readily increased by further exercise. The age of the experimental animals may also be important in so far as the ability of skeletal muscle to adapt is probably more highly developed in young animals.

Similar significant increases in aldolase and myokinase¹⁴ activity were also found in homogenates of whole flight muscle of the desert locust, *Schistocerca gregaria*, made to fly in a current of air by the method of Weis-Fogh¹⁵ for six periods of 30 min, each period interspersed with 30 min rest. These locusts, which had been reared from the larval stage in captivity, had not previously flown. Creatine phosphokinase and 5'-adenylyc deaminase could not be detected in these muscles.

As all the foregoing results were obtained *in vitro*, it was difficult to decide whether the response in enzymatic levels was controlled entirely by intracellular mechanisms or was a response to a more systemic stimulus. The endogenous nature of the response was indicated in experiments with the isolated sartorius of *Rana temporaria*.

In these experiments isotonic twitches were elicited once every 8 sec by stimuli of supramaximal intensity using electrodes placed close to the muscle and immersed in oxygenated Ringer. The twitches were recorded with a simple unloaded lever-drum kymograph system and the corresponding unstimulated muscle from the other limb of the same frog was stretched to a similar rest length either alongside the contracting muscle in the same bath, or, in some experiments, in a separate identical chamber.

Table 1. EFFECT OF EXERCISE ON THE CREATINE PHOSPHOKINASE AND ALDOLASE ACTIVITIES OF RAT HIND-LEG MUSCLE

Exp. No.		Age (days)	Body-wt. (g)	Creatine phosphokinase (μ g creatine/mg N of extract/min)	Aldolase (μ g triose P/mg N of extract/15 min)
1	Controls	100	121.3 ± 4.1	497 = 12.9 (3)	
	20 h exercise immediately before death		126.4 ± 5.2	659 = 6.5 (3)	
2	Controls, 100 days normal activity, 21 days confined	121	178 ± 6.2	283 = 7.0 (3)	
	100 days normal, 20 days confined, 1 day exercise		179 ± 5.7	410 = 13.9 (3)	
3	Controls	21	34.8 ± 0.7	406 392 387	100 98 94
	4 h exercise, 4 h normal		33.5 32.0	439 445	108 111
	8 h exercise, 4 h normal		31.5 33.1	490 502	139 142
4	Controls	28	38.4 39.1	441 439	87.2 85.0
	12 h exercise immediately before death		37.5 38.8	570 640	106 107
5	Controls	21	35.8 36.5	421 437	
	12 h exercise immediately before death		34.2 34.9	568 587	
6	Controls	21	35.7 36.2	434 439	
	12 h exercise, 72 h normal		35.8 35.7	460 446	

Control and exercised animals were litter-mates of comparable weight. Animals exercised in a treadmill usually performed 5,000–6,000 revolutions in a 12-h period. The animals used in experiments 1 and 2 were all from the same litter. Likewise for experiments 5 and 6. The specific activities represent the mean of duplicate assays on the combined right and left leg muscles of single rats except in experiments 1 and 2 where the results from a number of animals, indicated in parentheses, are averaged. Enzymatic assays¹³ carried out for convenience in sampling on extract obtained as follows. 1–2 gm thigh muscle from back legs was homogenized in 5–10 vol. 0.25 M sucrose and centrifuged for 20 min at 20,000g. Supernatant was removed and the residue rehomogenized in 5 vol. sucrose, recentrifuged and the supernatant again removed. The residue was once again washed with 5 vol. sucrose and supernatants combined and made up to volume.

The results in Table 2 indicate that when related to total nitrogen content of the muscle the creatine phosphokinase activity in all cases was greater in stimulated muscles than in the resting controls. The greatest increases were obtained when muscles were allowed to equilibrate for 30–60 min in oxygenated Ringer after dissection, stimulated continuously for 4–5 h and then assayed immediately. If after stimulation the muscle and the appropriate control were kept in Ringer overnight at 2° before assay, the difference between enzymatic levels in the rested and stimulated control, although still significant, was somewhat lower. In this respect it is of interest that in the *in vivo* experiments with the rat the largest increases in creatine phosphokinase activities were obtained when assays were made immediately after the exercise period (Table 1). When the exercised animals were allowed to return to normal activity for some hours after the exercise period before removal of the muscle

and enzymatic assay, the differences in level between control and exercised animals were less. This is another factor which may not have been given sufficient attention by investigators who have been unable to demonstrate increases in enzymatic activity after exercise.

The results obtained with mammalian and insect skeletal muscles *in vivo* and *in vitro* with amphibian tissue clearly indicate that continued contractile activity results in an increase in activity of enzymes of special significance for muscle metabolism. This applies particularly to creatine phosphokinase and also to aldolase, 5'-adenylic deaminase and myokinase, although these systems have been studied in less detail. The increases represent a net increase in enzymatic activity when related either to the wet weight or to the total nitrogen content of the tissue. No significant change in nitrogen content due to the periods of activity used either in the *in vitro* or the *in vivo* investigations could be detected.

The increases in creatine phosphokinase could arise in either from activation of an inactive precursor, as in the case of phosphorylase¹⁶, or could be due to the synthesis of new enzyme stimulated by continued contractile activity. As yet there is no evidence of the existence in normal muscle of creatine phosphokinase as an inactive or less active precursor, but this possibility cannot be excluded. If formation of new enzyme occurred, it would be an example of adaptive enzyme synthesis in response to contractile activity and could be explained as a response to the changed metabolic levels in the cell resulting from the continued contractile activity. Although it is not yet possible to decide between these two explanations, the observations that a number of enzymes respond in the same way and that the enzymatic activity of frog sartorius increased with longer periods of activity would support a mechanism involving synthesis of new protein. Such a mechanism would also be supported by the reports¹⁷ that muscle activity causes a stimulation of

Table 2. EFFECT OF REPEATED CONTRACTION ON THE CREATINE PHOSPHOKINASE ACTIVITY OF THE ISOLATED FROG SARTORIUS MUSCLE

Exp. No.	Duration of stimulation (h)	Creatine phosphokinase activity (mg creatine/mg T.N./min)	
		Resting	Stimulated
1	1.0	1.01	1.11
		1.07	1.20
2	1.5	1.60	1.87
		1.06	1.25
3	2.0	1.14	1.38
		1.46	2.23
4	5.0	1.27	2.06
		1.73	2.75
5	5.0	1.72	2.62
		1.53	2.40
6	5.5	1.65	2.35
		0.92	1.60
7	12.0	0.87	1.49

Muscles dissected from frogs kept at +2° and equilibrated for 1 h in Ringer saturated with 95 per cent oxygen; 5 per cent carbon dioxide. Creatine phosphokinase assays¹³ carried out on homogenates of whole muscle and total nitrogen determined by Kjeldahl technique. In experiments 4 to 7 duplicate assays on the homogenates of the right and left muscles, one stimulated, the other the resting control, were carried out; in all other cases results are means of duplicate assays on separate pairs of muscles.

protein and RNA metabolism. Investigations are now in progress to enable a decision to be made between these two possible mechanisms.

- ¹ Holmes, R., and Rasch, P. J., *Amer. J. Physiol.*, **195**, 50 (1958). Molbert, E., and Jijima, S., *Verh. Deutsch. Path. Ges.*, **42**, 349 (1959).
- ² Helander, E. A. S., *Biochem. J.*, **78**, 478 (1961).
- ³ Lawrie, R. A., *Nature*, **171**, 1069 (1953).
- ⁴ Chepinoga, O., *Biokhem. Zhur.*, **14**, 5 (1939); **15**, 45 (1940). Yakovlev, N. N., *Sechenov J. Physiol.*, **36**, 744 (1950). Yampolskaya, L. I., and Yakovlev, N. N., *Sechenov J. Physiol.*, **37**, 110 (1951). Yampolskaya, L. I., *Sechenov J. Physiol.*, **38**, 91 (1952).
- ⁵ Yakovlev, N. N., Krasnova, A. F., and Chagovets, N. R., in *The Effect of Use and Disuse on Neuromuscular Functions*, edit. by Gutmann, E., and Hnik, P., 461 (Czech. Acad. Sci., Prague, 1963).
- ⁶ Hearn, G. R., and Wainio, W. W., *Amer. J. Physiol.*, **185**, 348 (1956).

- ⁷ Gollnick, P. D., and Hearn, G. R., *Amer. J. Physiol.*, **201**, 694 (1961).
- ⁸ Gould, M. K., and Rawlinson, W. A., *Biochem. J.*, **73**, 41 (1959). Rawlinson, W. A., and Gould, M. K., *Biochem. J.*, **73**, 44 (1959).
- ⁹ Hearn, G. R., and Wainio, W. W., *Amer. J. Physiol.*, **190**, 206 (1957).
- ¹⁰ Hearn, G. R., and Gollnick, P. D., *Inter. Z. Angew. Physiol.*, **19**, 23 (1961).
- ¹¹ Kendrick-Jones, J., and Perry, S. V., *Biochem. J.*, **95**, 48P (1965).
- ¹² Ninomiya, H., Buxton, R. W., and Michaelis, M., *Bull. Sch. of Med. Univ. Maryland*, **46**, 61 (1961).
- ¹³ Chappell, J. B., and Perry, S. V., *Biochem. J.*, **57**, 421 (1954). Sibley, J. A., and Lehninger, A. L., *J. Biol. Chem.*, **177**, 859 (1949).
- ¹⁴ Colowick, S. P., in *Methods in Enzymology*, **2**, 598 (Academic Press, New York, 1955).
- ¹⁵ Weis-Fogh, T., *Phil. Trans. Roy. Soc., B.*, **239**, 459 (1956).
- ¹⁶ Cori, G. T., and Green, A. A., *J. Biol. Chem.*, **151**, 31 (1945).
- ¹⁷ Zak, R., Gutmann, E., and Vrbova, G., *Experientia*, **13**, 80 (1957). Gutmann, E., and Zak, R., *Physiol. Behav.*, **10**, 501 (1961).

LIFE-SHORTENING IN MICE EXPOSED TO RADIATION: EFFECTS OF AGE AND OF HYPOXIA

By DR. PATRICIA J. LINDOP and PROF. J. ROTBLAT, C.B.E.
St. Bartholomew's Hospital Medical College, London

IN an investigation of radiation-induced life-shortening described previously^{1,2}, mice were exposed, while breathing air, to single whole-body doses of high-energy X-rays at the age of 4 weeks. Over the range of doses examined (50 to about 800 rads) it was found that the amount of life-shortening was proportional to the dose of radiation, the reduction in life-span amounting to 5.8 weeks per 100 rads. The X-rays used were of an energy of 15 MeV and the dose-rate was about 500 rads/min.

In subsequent experiments³, in which mice were exposed to radiation at different ages, it was found that the life-shortening effect was markedly dependent on age at exposure. It was also found that exposure of mice which were breathing nitrogen for a short time before and during the irradiation resulted in considerable reduction of the life-shortening effect, the protection factor being apparently dependent on age. These were pilot experiments, with small numbers of animals; but the results appeared to justify a full-scale investigation of the effects of age at irradiation and of hypoxia on life-shortening.

The experiment designed for this purpose was on a large scale; it included five age-groups with six dose-levels in each, and the mice were breathing either air, or nitrogen, or oxygen during the irradiation. The total number of mice involved was more than 10,000. Unfortunately, when the investigation was already well advanced, an epidemic of ectromelia hit the colony, and most of the mice had to be destroyed. Further irradiations were carried out afterwards, but on a much-reduced scale, and the results from only a few dose-groups are available. Since it is unlikely that the full-scale investigation will be repeated in the near future, it seems worth while to publish the results obtained so far.

Variation of life-shortening with age at exposure. The experimental conditions were the same as in the investigation of the 4-week age group^{1,2}. SAS/4 albino mice were used, and they breathed air at atmospheric pressure before and during the irradiation. In the older age-groups four mice were exposed at the same time, each in its own container. For the 1-day- and 1-week-old groups, the whole litter from each mother was put in one container and irradiated together. The young mice were returned to their mother immediately after the exposure. At the age of 3 weeks they were weaned and placed in cages, eight mice per cage, like the older age-groups. There they were kept until death; the age at death was noted and a post-mortem examination carried out for pathological lesions. The analysis of the incidence of diseases found at death will be reported elsewhere. This article is concerned only with the effect of radiation on the life-span.

Owing to the small number of mice, the results from both sexes were pooled. It has been shown previously⁴ that for this strain of mice the difference in life-span between males and females is very small. For each dose-group, the mean age at death, and the standard error of the mean, were calculated.

The results are presented in Table 1, and those for the 1-day-old mice are also shown on curve A of Fig. 1. It is seen that for this age-group the experimental points fall quite well on a straight line. Since the lowest dose in this group was about 400 rads, one cannot exclude the possibility of a deviation from linearity at small doses, but this deviation cannot be large. The same applies to the 1-week-old group. For the 8- and 30-week groups results are available for about 100 and 200 rads as well, and in these cases the linearity is much better established.

Assuming this linearity to hold for all age-groups, we can calculate the life-shortening per unit dose. This is given in Table 2 and expressed in weeks per 100 rads; Table 2 also includes the results obtained previously for the 4-weeks group. It is seen that the life-shortening effect decreases with increasing age at exposure. This has

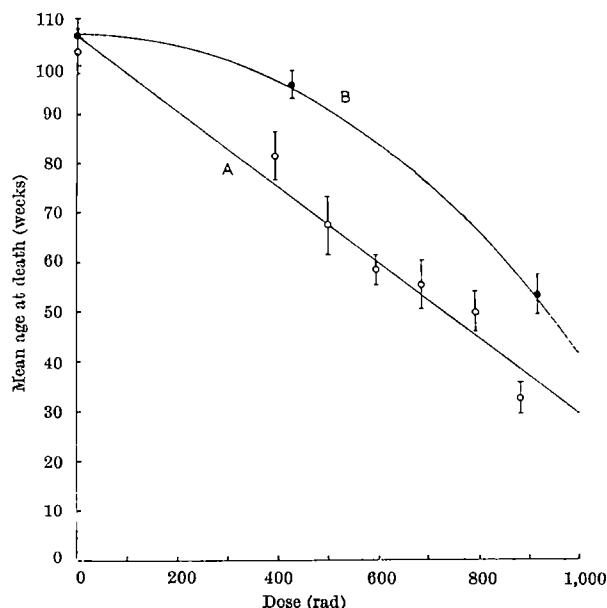


Fig. 1. Mean age at death of mice exposed at 1 day old. A, While breathing air; B, while breathing nitrogen

Table 1. MEAN AGE AT DEATH OF MICE EXPOSED TO A SINGLE WHOLE-BODY DOSE OF RADIATION AT VARIOUS AGES WHILE BREATHING AIR

1 day		1 week		8 weeks		30 weeks	
Dose (rads)	Mean age (weeks)	Dose (rads)	Mean age (weeks)	Dose (rads)	Mean age (weeks)	Dose (rads)	Mean age (weeks)
0	103.0 ± 4.5	0	104.5 ± 4.0	0	108.2 ± 2.0	0	103.7 ± 1.8
396	81.6 ± 5.0	202	99.5 ± 6.0	103	99.4 ± 1.9	101	105.6 ± 1.7
500	67.5 ± 6.0	403	77.3 ± 3.5	206	94.7 ± 2.1	203	98.7 ± 1.7
594	58.5 ± 3.0	592	65.3 ± 3.0	412	84.6 ± 1.6	408	94.3 ± 1.6
686	55.5 ± 5.0						
794	50.0 ± 4.0						
882	32.5 ± 3.0						

Table 2. LIFE-SHORTENING OF MICE EXPOSED AT DIFFERENT AGES WHILE BREATHING AIR

Age at irradiation	Life-shortening (weeks per 100 rad)
1 day	7.63 ± 0.64
1 week	7.08 ± 1.14
4 weeks	5.77 ± 0.19
8 weeks	5.55 ± 0.56
30 weeks	2.66 ± 0.83

been observed by others for mice at older ages⁵. It has been suggested⁶ that the percentage life-shortening is proportional to the remaining life expectation, and this agrees, within the experimental error, with the results for the older mice. At very young ages, 1 day and 1 week, this does not appear to be the case, the life-shortening effect being too large.

Effect of hypoxia on life-shortening. Mice of the same age-groups as already mentioned were exposed under hypoxic conditions. The mice were anaesthetized with 20–60 mg/kg 'Nembutal' according to age, to avoid convulsions, and were then put into a container through which a stream of nitrogen at body temperature flowed at the rate of 8 l./min. The mice were kept in this nitrogen atmosphere for periods varying from 50 sec for the 1-day-old group to 30 sec for the 30-week group. These times were found by experiment to be the longest which the mice of a given age-group could tolerate without obvious ill-effects. The immediate mortality rate due to nitrogen was less than 10 per cent. Just before the end of the period of nitrogen breathing the mice were exposed to a beam of 15 MeV electrons at a dose-rate of about 40,000 rad/min (1.6 rads/pulse from the linear accelerator, at a repetition frequency of 400 pulses/sec). Immediately after the exposure oxygen was flushed through the container for about a minute to facilitate the revival of the mice.

Owing to limitation in the animal house space, only two dose-levels, about 425 and 900 rads, were used. For each age-group there was also a control group of mice, which went through the same procedure of nitrogen breathing but without receiving any irradiation. To make possible the comparison with the previous results, at each age-group there was also a group of mice exposed under the same irradiation conditions to about 425 rads while breathing air.

The results obtained are summarized in Table 3. A comparison with Table 1 shows that in each age-group exposure while breathing nitrogen considerably reduced the life-shortening effect. The protection against radiation damage by the absence of oxygen, which has been previously found for acute effects^{3,7}, has thus been confirmed for long-term effects as well. However, there are marked differences in this protective action of hypoxia between the older and younger age-groups.

Table 3. EFFECT OF HYPOXIA ON MEAN AGE AT DEATH

Gas	1 day		1 week		8 weeks		30 weeks	
	Dose (rads)	Mean age (weeks)	Dose (rads)	Mean age (weeks)	Dose (rads)	Mean age (weeks)	Dose (rads)	Mean age (weeks)
N ₂	0	106.0 ± 3.4	0	109.5 ± 2.5	0	101.4 ± 2.6	0	99.4 ± 3.4
Air	434	83.4 ± 3.2	411	81.3 ± 3.5	405	84.1 ± 2.5	453	91.8 ± 5.0
N ₂	430	96.3 ± 2.8	419	100.5 ± 3.2	405	97.1 ± 2.6	454	96.9 ± 3.4
N ₂	920	58.5 ± 4.0	878	65.4 ± 3.9	845	89.9 ± 2.8	937	95.9 ± 3.2

For the 8- and 30-week groups, the life-shortening, although much smaller, is still proportional to the dose. The life-shortening per 100 rad is 1.3 weeks and 0.5 weeks for the two age-groups respectively. A comparison with the life-shortening effect produced by the same age-groups exposed to 425 rads while breathing air shows that breath-

ing nitrogen reduced the effect by about a factor of 3. This is about the maximum which has been found for nitrogen protection in other systems and it shows that the procedure adopted reduced the oxygen tension in the tissues of the mice to very low values.

However, for the two younger age-groups, 1 day and 1 week, exposure under hypoxic conditions appears to have changed the dose response relationship, which is no longer linear. This can be seen for the 1-day-old group on curve B of Fig. 1. It comes out more dramatically when one inspects the survival curves for the mice exposed to different doses at the age of 1 day (Fig. 2). It is seen that the percentage survival of mice exposed to 434 rads while breathing nitrogen differs very little from that of the control mice, thus indicating a large protection effect; whereas the 920-rad group in nitrogen is shifted well to the left.

Because of the curvilinear nature of the life-shortening effect in nitrogen, one cannot quote a specific protection factor for the young mice; all one can say is that the protection is large at small doses, but very small at high doses.

In the absence of a theory of the life-shortening effect, it is not easy to explain the transition from a curvilinear to a linear relation with increasing age. However, several possibilities suggest themselves. For example, one may assume that life-shortening is the result of a number of 'faults' produced in some definite targets, and that such 'faults' may accumulate in the course of time from causes other than radiation. Thus, in the older animal, one hit by a quantum of radiation may be sufficient to produce life-shortening; this would result in a linear relation between the effect and dose. On the other hand, in the young animal, more than one hit is required, hence the curvilinear relation.

Another possibility is to relate life-shortening to specific somatic damage, say to a chromosome break, and to the probability of repair of such damage. It is known that radiation damage may be repaired spontaneously, even if it is in the form of a single chromosome break⁸. If we assume that the probability of such repair is very high in young mice, when irradiated under hypoxic conditions, then little life-shortening would be experienced at exposure to low doses at which only one break is likely to occur. On the other hand, at high doses two breaks may occur; these have a much lower probability of repair, hence the life-shortening would be large. For such a double event the variation of the effect with dose should follow a square law. In fact, in curve B of Fig. 1 the life-shortening is approximately proportional to the square of the dose.

With both hypotheses the effect of age would be more apparent under hypoxic conditions, since in the presence of oxygen the amount of damage by a given quantum of radiation is larger, and one hit may be sufficient even at a young age.

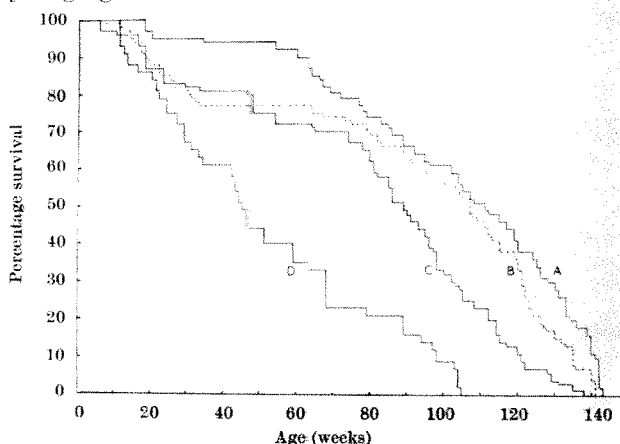


Fig. 2. Survival curves for mice exposed at 1 day old: A, Control; B, 434 rad in nitrogen; C, 430 rad in air; D, 920 rad in nitrogen.

This work was supported by the British Empire Cancer Campaign for Research and the U.S. National Institutes of Health.

¹ Lindop, P. J., and Rotblat, J., *Nature*, **189**, 645 (1961).

² Lindop, P. J., and Rotblat, J., *Proc. Roy. Soc.*, **154**, 332 (1961).

³ Lindop, P. J., and Rotblat, J., *Brit. J. Radiol.*, **35**, 23 (1962).

⁴ Lindop, P. J., *Gerontologia*, **5**, 193 (1961).

⁵ Kohn, H. I., and Guttman, P. H., *Rad. Res.*, **18**, 348 (1963).

⁶ Lindop, P. J., *Scientific Basis of Medicine Ann. Rev.*, **91** (1965).

⁷ Lindop, P. J., and Rotblat, J., *Nature*, **185**, 593 (1960).

⁸ Howard-Flanders, P., and Boyce, R. P., *Cellular Radiation Biology*, 52 (Williams and Wilkins, 1965).

ALTERATION OF HAMSTER CELLS BY NUCLEIC ACID IN VITRO

By KATE SHEPLEY, DR. E. J. AMBROSE and DR. K. S. KIRBY

Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital, Fulham Road, London, S.W.3

THERE have been occasional reports in the literature of changes in cell properties brought about by treatment with nucleic acid preparations made from foreign cells. For example, Jones¹ has reported morphological changes brought about by treatment with RNA preparations. Of particular interest are the recent observations of Cohen and Parks² which have demonstrated the transfer of immunological sensitivity to non-immune spleen cells by supplying RNA from immune cells. The RNA was added to a cell suspension of non-immune spleen cells and incubated for 30 min at 37° C. In the experiments described in this article, a few DNA (but mainly RNA) preparations were supplied to the medium of C13 hamster fibroblasts grown in monolayer culture. Stable changes in cell morphology and clonal appearance have been obtained, which persist through subsequent cell generations after removal of the nucleic acid.

Culture of hamster cells. Baby hamster kidney fibroblasts (BHK 21/C13) used in these experiments were between 40 and 50 generations after cloning.

The medium used throughout consisted of 10 per cent calf serum and 10 per cent tryptose phosphate broth (Difco) in Eagle's HeLa medium, as modified by Stoker to contain twice the normal concentration of vitamins and amino-acids. For sub-culture of the cells 0.05 per cent trypsin (Difco) was used in 0.0002 per cent 'Versene' in phosphate buffered saline.

Nucleic acids. RNA and DNA were prepared by one of us (K. S. K.). Nucleic acid was dissolved in sterile Hanks's saline and added to the medium in which the cells were already growing, to give a final concentration of 100 µg/ml. The culture was then allowed to grow for a further 4 days before sub-culture into fresh medium which did not contain nucleic acid. When the cells had been propagated for at least 3 generations, they were trypsinized and planted in Petri dishes in numbers appropriate to colony formation (usually 5×10^3 cells in a 6-cm dish). After 7 days the cultures were fixed in 10 per cent formol saline and stained with a Jenner-Giemsa stain.

Effects of various types of foreign RNA. In the presence of any foreign RNA, cells grew much faster than a control culture. The whole monolayer became thick and tended to strip, and the medium became acid. On sub-culture, the rapid growth-rate was not so noticeable, but if a small number of cells were planted in a Petri dish, a number of the colonies formed in 7 days were large, piled up and deeply staining, compared with the relatively small, flat colonies formed by normal cells (Fig. 1b). In contrast to the piled-up colonies formed by polyoma virus transformed cells, which grow in an irregular criss-cross pattern (Fig. 1a), these colonies retained the regular parallel growth of the normal colonies (Fig. 1c).

RNA isolated from hamster kidney, rat liver, *B. subtilis* and polyoma virus-transformed hamster kidney fibroblasts all produced large colonies, the greatest effect being with the RNA isolated from a stilboestrol-induced kidney tumour of the hamster, where up to 20 per cent of the colonies formed were of the altered morphology. The RNA isolated from the BHK 21/C13 themselves had no effect.

It made no difference to the effect of the RNA whether it was added directly to the Petri dish when the cells were planted for colony growth, or whether several generations were allowed to elapse between treatment and planting as has already been described.

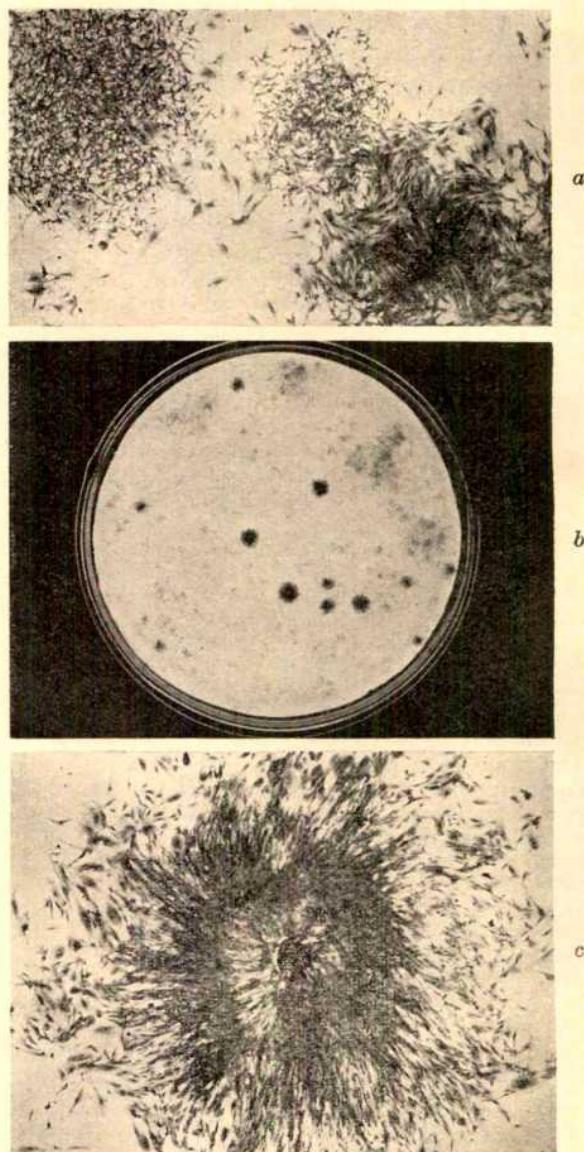


Fig. 1. a, Bottom right: colony of C13 hamster fibroblasts showing parallel alignment of groups of spindle-shaped cells. Centre and top left: colonies of polyoma virus transformed cells showing more random pattern of growth. b, Petri dish showing plating out in a new medium in the absence of RNA of cells previously treated with tumour RNA. Faint and diffuse normal colonies can be seen, also dense colonies of stable variants arising from RNA treatment. c, Higher power view of RNA variant. Reasonably parallel alignment of denser regions. More random pattern of growth in the outer regions.

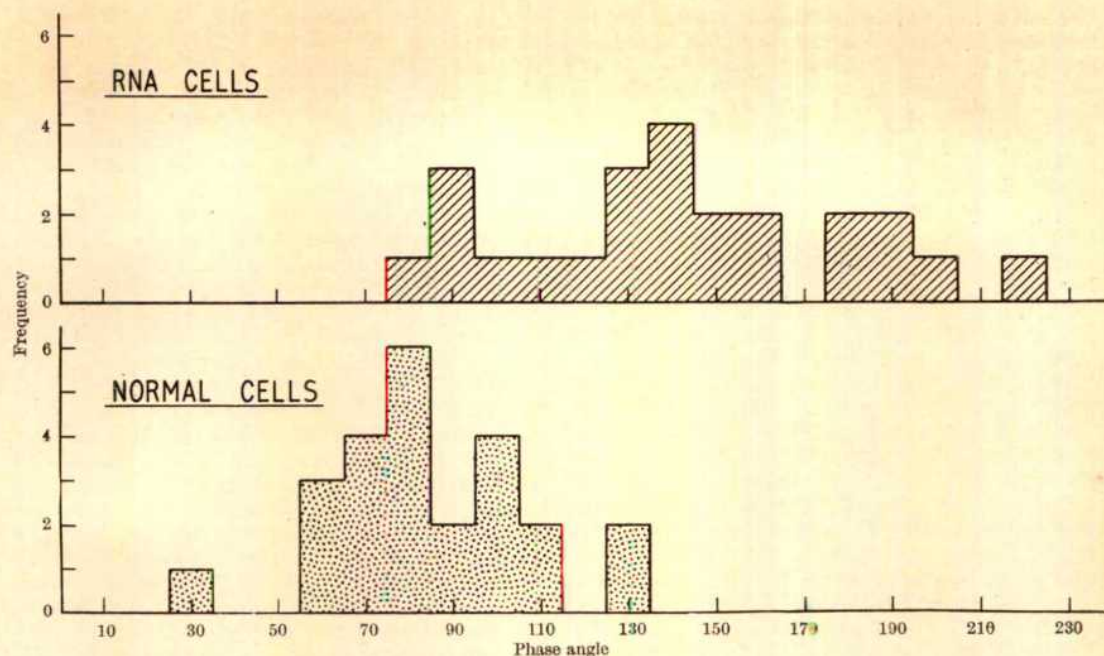


Fig. 2. Interference microscopy of normal and treated clones

Effect of ribonuclease, nucleosides and nucleic acid bases. If, in place of the RNA, the cells were incubated with a mixture of ribonucleosides, or a mixture of adenine,

guanine, cytosine and uracil, no large colonies formed. However, if any of the foreign RNAs mentioned were pre-treated with ribonuclease and the cells incubated as usual with the product of digestion, the same large colonies formed as with whole RNA. This indicated that the oligonucleotide core of the RNA molecule is the part which effects the alteration of the cells.

Interference measurements. By means of an interference microscope, the difference in optical path between a cell and air at a space on the glass near the cell has been measured and the difference taken as a measure of the dry mass per unit area. In this way, a comparison has been made between the dry mass per unit area of cells in an RNA colony and a normal colony. Fig. 2 shows that the average reading of cells in an RNA colony is greater than that of a normal colony. The large scatter of readings within a group is presumably due to the cells being of a different age after division.

Comparison of normal and RNA cells. Normal BHK 21/C13 cells occasionally do form large piled-up colonies. However, the difference between these and the ones produced by treatment with RNA can be seen by examina-

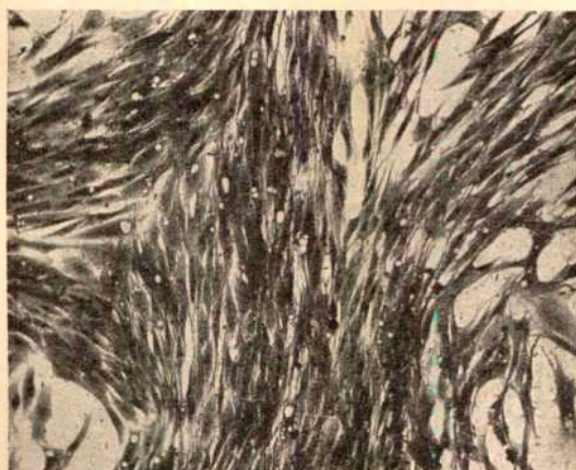
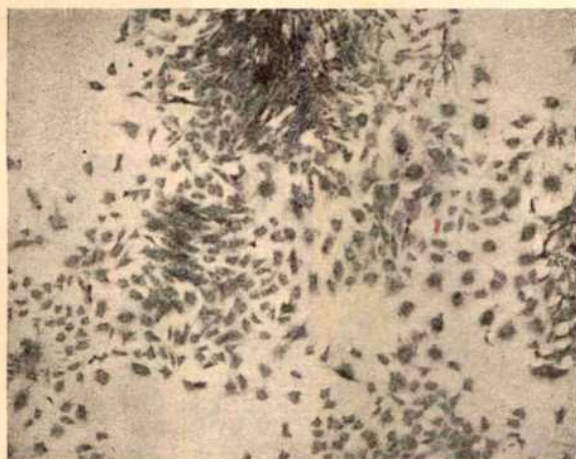


Fig. 3. *a*, Secondary RNA colony showing absence of spindle-shaped cells. *b*, High power of C13 fibroblasts showing high proportion of spindle-shaped cells. *c*, High power of secondary RNA colony showing characteristic appearance of the cells with numerous independent pseudopodia and absence of spindle-shaped cells at low cell density

tion of the cells at higher magnification. Although the cells in the centre of each colony are thin and spindle-shaped, those on the edge of the RNA colony are large, spread out, often multi-polar, and have large nuclei (Fig. 3c). Presumably those in the centre have been squashed thin by the rapid growth and pressure of adjacent cells. On the edge of the normal colony the cells are as thin and spindle-shaped as in the centre (Figs. 1a and b). When one of the RNA colonies was isolated and grown further, the culture consisted entirely of the large spread-out cells, and this characteristic was retained in the colonies which were grown from single cells (see Fig. 3a). The large piled-up colonies did not form again.

Cultures of these cells have undergone five sub-cultures (approximately 15 generations) since isolation of the original large colony, and the cells still retain these characteristics. Attempts have been made to re-alter the RNA cells back to their original fibroblastic shape by treatment with normal C13 RNA, but without success.

From earlier investigations of cell movements and cell contacts we have good grounds for believing that the appearance of cell colonies in monolayer culture is determined almost entirely by three factors: (1) the polarity of the cell, that is, its tendency to produce one dominant pseudopodium, several independent pseudopodia or numerous microvillae; (2) adhesiveness of the cell to the solid substrate; (3) intercellular adhesions. The polarity appears to depend on local structural or temporal changes in the properties of the cell membrane. The adhesiveness to the solid substrate depends on comparatively non-specific adhesive properties of the cell surface. The intercellular adhesiveness may be partly specific and partly non-specific. Changes in clonal morphology therefore reflect some change which has arisen in the properties of the cell surface.

It may be inferred, therefore, that the alterations after treatment with RNA preparations described in this

chapter have almost certainly produced an altered property of the cell surface, which is transmitted through subsequent cell generations after removal of RNA from the medium. This would suggest that the synthetic mechanism of the cell is disturbed, leading to the production of altered surface material. It is interesting to note that rather similar changes can be brought about by RNA preparations from several sources, though with most frequency by tumour RNA. They are not produced by RNA from the same cell type as the recipients. It is not claimed that the changes described in this article are malignant transformations. When close packed at high sensitivity they show parallel alignment of cells and contact inhibition rather similar to the C13 cells. At low cell density, however, the multiple pseudopodia, more characteristic of malignant cells in culture, appear reproducibly in serial transfer. Qualitatively, the changes in behaviour are rather like those seen in cells transformed with polyoma virus or Rous sarcoma virus, but to a less marked degree. There are several similarities to the malignant change, that is, a number of different agents, viral or chemical, giving rise to a similar end effect in biological terms. The production of a great variety of abnormal surface proteins or mucoproteins may disturb the normal specific interactions which occur in normal tissues and hence may give rise to malignant properties.

We thank Prof. A. Haddow for his advice and Prof. M. Stoker for supplies of C13 and TCY cells. This work has been supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital) from the Medical Research Council and the British Empire Cancer Campaign for Research, and by U.S. Public Health Service research grant CA-03188-08 from the National Cancer Institute.

¹ Jones, P. C. T., *Nature*, **202**, 1226 (1964).

² Cohen, E. P., and Parks, J. P., *Science*, **144**, 1012 (1964).

DETERMINATION OF THE TOTAL ALPHA ACTIVITY OF DRINKING WATER USING A NUCLEAR EMULSION

By M. W. TYRRELL, I. C. DEMETSOPOULLOS and A. J. L. COLLINSON

Borough Polytechnic, London, S.E.1

A METHOD is described for estimating the total α -activity of solutions, using 50 μ 'Ilford G5' nuclear emulsions. A tentative value of approximately 10^{-13} c.i.-¹, with a statistical accuracy of 15 per cent, was obtained for the α -activity of a sample of drinking-water from the London area.

Several authors have investigated the use of nuclear emulsions for detecting low-level activities of α emitters in solution. The main methods described are:

(a) Total immersion of the emulsion in the liquid concerned¹; (b) the capillary tube method². This involves the use of thin-wall capillary tubes filled with the liquid sample and placed in contact with the emulsion; (c) mixing the active solution with gelatine and coating the emulsion with the mixture³; (d) sandwiching a drop of the active solution between two emulsions⁴.

All the foregoing methods had a sensitivity below 10^{-10} c.i.-¹. Values for the activity of drinking-water obtained by Turner⁵ indicated that a method was required with a sensitivity in excess of these methods. The technique adopted for this investigation was that of concentrating the activity of the solution, a sample of which was subsequently absorbed in a 50- μ thick emulsion, and counting the number of tracks after suitable exposure and development.

It was assumed that the measured α -activity of the drinking-water was due mainly to radium and its decay products. The daughter element radon (half-life 3.82

days) is a gas at N.T.P. with a freezing-point of -71° C. A subsidiary experiment with a 'clean' emulsion in contact with an emulsion containing absorbed active solution, established that a significant fraction of radon was lost through diffusion. As a consequence of this, it was decided to trap the radon by lowering the temperature to -82° C during exposure, by means of a freezing mixture of solid carbon dioxide and acetone. Dissolved radon was expelled by boiling the solution for 1 h before depositing the sample on the emulsion, thus eliminating the uncertainty as to the initial amount of dissolved radon, the concentration of which could be as high as 1,000 times⁶ the equilibrium value.

The pH of the solution was adjusted to pH 7 to avoid eradication of tracks caused by weakly acidic solutions as found by Ackermann and Faissner⁷.

A small volume of the boiled solution was spread uniformly over a 2 in. \times 2 in. emulsion, the mass deposited being measured by weighing using a sensitive chemical balance. The emulsion was then placed under a bell jar and dried as quickly as possible employing a desiccant and a reduced pressure of 25 torr. The dried emulsion was then transferred to a container kept at -82° C and left for approximately four days before removing and developing using 1D19 developer.

Difficulties were encountered with cracking and peeling of the emulsion after removal from the container. It was found that this effect could be avoided by carefully con-

trolling the rise of temperature of the emulsion. This is contrary to the experience of M. Debearais-Wack⁴, who found that, in order to preserve the emulsion intact, the glass substrate had to be replaced by a cellulose nitrate base.

The emulsions were scanned, using a Cook M40503 nuclear emulsion microscope, by making parallel traverses using a $\times 10$ objective, equipped with a graticule. Each traverse was scanned once, using the fine focus control to include the total thickness of the emulsion, the number of α -tracks being recorded.

With activities in the order of $\mu\mu$ c.l.⁻¹ it was essential to use emulsions not older than three days at the time of exposure. Emulsions older than this were associated with a background which was too high to permit statistically significant measurements without unduly long exposure times. Plastics were chosen for the construction of the emulsion supports since they have a low α contamination, as reported by Yagoda⁵. In order to determine the background a second identical emulsion, but without the active deposit, was introduced into the low-temperature container and both emulsions developed together after exposure.

The activity equivalent background was 0.6×10^{-13} c.l.⁻¹. A solution containing 1.6×10^{-13} g radium l.⁻¹ was used as a standard. Volumes of solution ranging from 0.25 to 1.5 c.c. were deposited on emulsions and exposed. Assuming that only radium was present the number of α -particles emitted for various exposure times from 1 c.c. of standard solution was calculated⁶. The results are plotted in Fig. 1. From the curve it can be seen that for exposure times in excess of 70 h, the increase in the number of α -particles emitted is linear. Since all the exposure times were greater than 70 h, a linear standardization of the results was carried out to find the number of α -particles emitted from the various amounts of standard solution for a set exposure time. The results are plotted against the calculated number of α -particles emitted in Fig. 2.

Comparison of activities of drinking-water obtained by Turner⁵ with that of the standard solution indicated that the standard solution was approximately 400 times more active. Bearing these figures in mind, the drinking water sample was concentrated by a factor of 400 by boiling. This allowed approximately equal exposure times to be used for both the water sample and the standard solution.

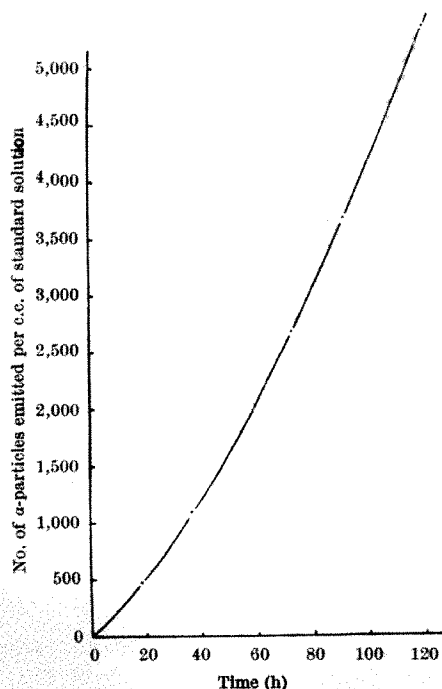


Fig. 1. Theoretical activity versus exposure time

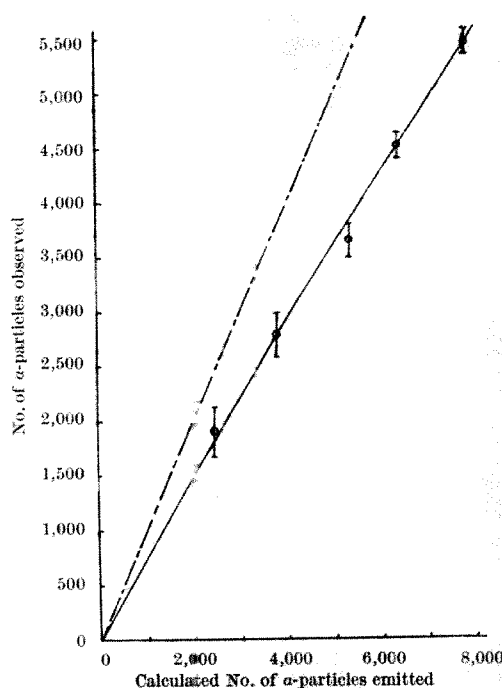


Fig. 2. Observed activity versus calculated activity. — · —, calculated; —, measured

It was found that boiling the sample produced a precipitate. This with the solution was deposited on the emulsion. An attempt was made to measure the activity of this precipitate separately and it was found that the activity was not measurable above background.

An analysis of the plate was carried out and the total number of α -particle tracks observed normalized to an exposure time equal to that of the standard solutions. From Fig. 2 the corrected number of α -particles emitted from the sample was extrapolated and found to equal $0.17 \pm 0.02 \mu\mu$ c.l.⁻¹.

In applying statistics to the events, two assumptions were made: the active solution was distributed uniformly over the emulsion surface, and the nuclear events were random. Measurements made indicated the first assumption to be correct. The second assumption is an inherent characteristic of nuclear decay.

Let the plate plus a source be termed A and the plate used for background determination be B . If for plate A the number of traverses was η_A and the number of tracks was N_A and, for plate B , they were η_B and N_B respectively, then the relative standard deviation of the number of disintegrations from the source per traverse equals:

$$\frac{\Delta S}{S} = \frac{\left(\frac{N_A}{\eta_A} + \frac{N_B}{\eta_B}\right)^{\frac{1}{2}}}{N_A - N_B}$$

where S is the number of disintegrations from the source per traverse.

In a paper by Y. K. Lim¹⁰, a method to determine the efficiency in area scanning is described. Although detailed measurements as suggested in the paper were not made, sections of the various emulsions were rescanned. The number of tracks observed in a traverse were found to vary up to 6 per cent on recounting.

It was found that the efficiency depended on several factors. The state of the emulsion in terms of surface scratches was very important: in certain cases, small areas of the emulsion had to be left unscanned as the efficiency dropped enormously. Decrease in efficiency was also observed with increase in time of continuous scanning. Barkas¹¹ discusses most of these points. Less sensitive emulsions, namely $K6$, $K1$ and $K2$ plates, were used in an attempt to reduce the general background, but it was

found that at -82°C the α tracks became tenuous and were difficult to count.

We thank Dr. A. K. M. M. Haque for his assistance, and the Rutherford Laboratory of the Science Research Council for allowing one of us (M. W. T.) time to complete this work.

¹ Broda, E., *J. Sci. Instrum.*, **24**, 136 (1947).

² Bonetti, A., and Occhialini, G. P. S., *Nuovo cim.*, **8**, 9, 725 (1951).

³ Nicoli, M., *Revue de Physique Academie L. Republique Polopulaire Roumaine*, **6**, No. 1 (1961).

⁴ Debearais-Wack, M., *Inst. J. App. Rad. and Isotopes*, **13**, Sept., 483 (1962).

⁵ Turner, R. C., *Nature*, **189**, 348 (1961).

⁶ Mayneord, W. V., *M.R.C. Rep. App. E. Comnd.* 1225 (1960).

⁷ Ackermann, H., and Faissner, H., *Nuclear Instruments and Methods*, **10**, No. 4 (1961).

⁸ Yagoda, H., *Radioactive Measurements with Nuclear Emulsions* (J. Wiley and Sons, Inc., New York).

⁹ Evans, R. D., *The Atomic Nucleus*, 490 (McGraw-Hill).

¹⁰ Lim, Y. K., *Nuovo Cimento*, **15**, No. 3, 382 (1960).

¹¹ Barkas, W. H., *Nuclear Research Emulsions*, 1 (Academic Press, New York).

RELATIVE INSTABILITY OF CEPHALORIDINE TO STAPHYLOCOCCAL PENICILLINASE

By DR. MARK RIDLEY and DR. IAN PHILLIPS

Department of Clinical Microbiology, St. Thomas's Hospital, London

CEPHALORIDINE¹ 7-[(2-thienyl)acetamido]-3-(1-pyridyl-methyl)-3-cephem-4-carboxylic acid betaine) is a semisynthetic antibiotic derived from the nucleus 7-amino-cephalosporanic acid (7-ACA). It shares and combines many of the properties of the semisynthetic penicillins, methicillin, cloxacillin and ampicillin which are derived from the 6-amino-penicillanic acid nucleus (6-APA). Three outstanding properties of cephaloridine are: its relative insusceptibility to staphylococcal penicillinase (β -lactamase), a property which it therefore shares with methicillin and cloxacillin; its broad antibacterial spectrum, which is similar but not identical to that of ampicillin; its lack of cross-allergenicity with antibiotics based on the nucleus 6-APA, which means that it may be given safely to patients hypersensitive to penicillins.

In an attempt to define the place of cephaloridine in current antibiotic therapy a comparison was made of the antistaphylococcal activity of cephaloridine, methicillin and cloxacillin. In preliminary work the minimal inhibitory concentrations of each of these antibiotics for 169 strains of *Staph. aureus*, which had been recently isolated from patients, were determined using large inocula (0.003 ml. of undiluted overnight broth cultures). The minimal inhibitory concentrations were measured by making doubling or closer dilutions of each antibiotic in nutrient agar (Oxoid 'D.S.T.') and recording for each strain the lowest concentration of each antibiotic that caused complete inhibition of visible growth after incubation for

24 and 48 h. The results at 24 h are given in Table 1 and show that with cephaloridine, but not with the other antibiotics, there is a marked gradation of response of different staphylococcal strains. Penicillin-sensitive *Staphylococci* were inhibited by concentrations of cephaloridine ranging from 0.025 $\mu\text{g}/\text{ml.}$ to 0.1 $\mu\text{g}/\text{ml.}$; strains resistant only to penicillin appeared more resistant, requiring 0.25–5 $\mu\text{g}/\text{ml.}$ for inhibition; while multiple antibiotic-resistant *Staphylococci* (resistant to 3 or more antibiotics) were inhibited by 2–20 $\mu\text{g}/\text{ml.}$ Finally, the methicillin-resistant strains shown in Table 1, which were also resistant to multiple antibiotics, were inhibited by concentrations of cephaloridine ranging from 5–50 $\mu\text{g}/\text{ml.}$

It seemed probable that these initial results were due to relative instability of cephaloridine to staphylococcal β -lactamase, compared with methicillin and cloxacillin. The cephaloridine minimal inhibitory concentrations were repeated for each strain on several occasions and revealed that the results were variable for the multiple antibiotic-resistant and to a lesser extent for the strains resistant to penicillin only. Furthermore, the minimal inhibitory concentrations of cephaloridine tended to increase from the figure recorded at 24 h on further incubation to 48 h. No such great variation or increase in minimal inhibitory concentration was observed with methicillin or cloxacillin. The variability was shown to be due to slight differences in inoculum size which cause marked differences in the cephaloridine minimal inhibitory concentrations of penicillinase-

Table 1. MINIMAL INHIBITORY CONCENTRATIONS FOR 169 STRAINS OF *Staph. aureus*

Antibiotic	Sensitivity pattern	No. of strains	No. of strains inhibited after 24 h by $\mu\text{g}/\text{ml.}$:														
			0.025	0.05	0.075	0.1	0.25	0.5	1	2	3	4	5	8	10	15	20
I. Cephaloridine	A	31	1	9	14	7											
	B	31					1	6	8	8	2	3	3				
	C	60								1	3	4	5	13	11	16	7
	D	47											3		1	21	15
II. Cloxacillin	A	31				1	25	5									
	B	31						31									
	C	60						30	29	1							
	D	47												1	2	1	6
III. Methicillin	A	31							12	13	6						
	B	31							5	17	9						
	C	60							3	25	32						
	D	47														3	44

Key to sensitivity patterns: A, penicillin-sensitive; B, resistant to penicillin only; C, multiple-resistant; D, methicillin-resistant.

producing strains. Even a small change in inoculum size (six-fold) may cause a marked difference in the minimal inhibitory concentration result, and it is therefore apparent that testing the sensitivity of penicillinase-producing *Staphylococci* to cephaloridine is influenced by inoculum size, a situation which is well known with benzyl penicillin. This effect of inoculum size with cephaloridine has been noted by Barber².

As the effect of inoculum size cannot be taken as *prima facie* evidence for a penicillinase type of resistance³, further experiments to assess this probable penicillinase effect were carried out. A membrane technique described by Knox⁴ as a modification of the Gots⁵ and Haight-Finland⁶ methods was used. In the first series of these experiments large assay plates were poured to contain 300 ml. of nutrient agar in which was incorporated cephaloridine in a final concentration of 1 µg/ml., and 1.5 ml. of an overnight broth culture of a penicillin-sensitive *Staphylococcus* (minimal inhibitory concentration of cephaloridine for this indicator strain was 0.025 µg/ml).

Ninety-one Oxoid membranes were sterilized and placed aseptically on the surface of the medium; each membrane was inoculated, using a wire loop, with a very large inoculum of a single strain of *Staph. aureus* previously grown up on blood agar plates. In this way the 31 *Staph. aureus* strains resistant to penicillin only and the 60 strains resistant to multiple antibiotics were compared simultaneously on the same plate. All these strains appeared to grow well on the membranes, because of the very large inoculum used, although the concentration of cephaloridine (1 µg/ml.) in the plate was close to or even exceeded the minimal inhibitory concentration values previously obtained for some of these same strains. The assay plates were incubated and examined for growth of the indicator *Staphylococcus* after 24 and 48 h. Growth of the indicator strain incorporated in the agar was taken to indicate removal or destruction of the cephaloridine from the medium immediately beneath the membranes. The results of a typical experiment are given in Table 2 (Exp. I).

A modification of these first experiments was introduced in order to avoid any difference between strains due to possible variations in degree of inhibition of growth at the concentration of cephaloridine used. The strains were inoculated on to membranes placed on nutrient agar plates containing no antibiotic. Heavy and approximately equal growth of all strains was obtained after overnight incubation. The membranes were then removed and the agar plugs beneath them were cut out with a cork borer and transferred aseptically to plates containing cephaloridine and the sensitive indicator *Staphylococcus*. The agar plugs were sterile and contained only the diffusible products of the *Staphylococci* which had previously grown on membranes above them. These secondary plates were examined for growth of the indicator bacteria after incubation for 24 and 48 h. Fig. 1 shows this transfer method in use with small standard Petri dishes poured to contain cephaloridine 1 µg/ml. and the indicator strain. It is clear from this experiment that cephaloridine is destroyed by a diffusible product present in the agar and produced by multiple antibiotic resistant *Staphylococci* but not by penicillinase negative *Staphylococci* (including a strain of the Oxford *Staphylococcus* rendered resistant to methicillin *in vitro*), nor in this particular experiment by

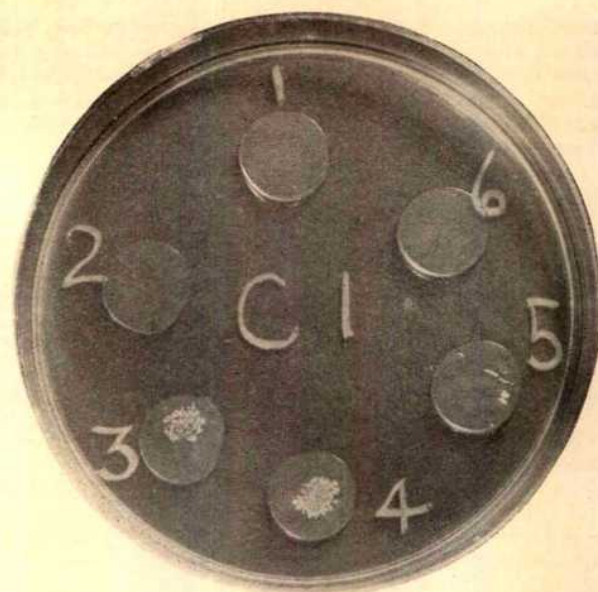


Fig. 1. Agar-plug transfer method for detection of drug-destroying enzymes. 1, Penicillin-sensitive *Staph. aureus* strain; 2, penicillin-resistant *Staph. aureus* strain; 3, multiple antibiotic-resistant *Staph. aureus* strain; 4, natural methicillin-resistant *Staph. aureus* strain; 5, Oxford *Staph.* (methicillin-resistant strain); 6, agar plug from beneath uniaeroculated membrane

the penicillin-resistant strain 2, which is a poor penicillinase producer. It seems virtually certain that this diffusible product is a β -lactamase although this has not been formally proved. It is produced in larger amounts by the multiple antibiotic-resistant *Staphylococci*, which tend to produce more penicillinase, as described by Richmond *et al.*⁷, than by the strains resistant to penicillin only. No destruction of methicillin was detected using either the direct membrane or agar plug transfer test. Some slight destruction of cloxacillin was, however, found by the direct technique at 48 h but not at 24 h nor at all by the transfer technique. It is well known that cloxacillin is less stable to staphylococcal penicillinase than methicillin⁸.

Using large assay plates the agar-plug transfer method was compared with the direct membrane method as a means of assessing β -lactamase production by the same ninety-one strains examined in Exp. I. The results of a single experiment are shown in Table 2, Exp. II. The results with the agar plug transfer technique are almost the same as those obtained by the direct membrane technique for the 60 multiple antibiotic-resistant strains, most of which produced a large enough amount of exo-penicillinase in the plugs to destroy the cephaloridine. Different results were, however, obtained with these two techniques for the strains resistant to penicillin only. With the direct technique 10 strains permitted growth of the indicator after 24 h, a further 10 strains after 48 h incubation, and 11 strains appeared to cause no destruction of the antibiotic. It is interesting that these eleven strains included all the phage group II strains in this series of 31 penicillin-resistant *Staphylococci*. Richmond⁹ has shown that of three types of staphylococcal penicillinase,

Table 2. ASSESSMENT OF β -LACTAMASE PRODUCTION BY 91 STRAINS OF *Staph. aureus*

Sensitivity pattern	No. of strains	Exp. I. Membrane			Exp. II. Agar-plug transfer		
		Growth of indicator organisms			Growth of indicator organisms		
		Appearing after 24 h	Appearing after 48 h	None	Appearing after 24 h	Appearing after 48 h	None
B	31	10	10	11	5	0	26
C	60	57	3	0	51	5	4

Key to sensitivity pattern: B, resistant to penicillin only; C, multiple resistant.

distinguishable on chemical, enzymological and immunological grounds, that with the weakest enzyme activity appears to be produced only by Group II *Staphylococci*. In the transfer technique it will be seen from Table 2 that only five of the penicillin-resistant *Staphylococci* produced destruction of the cephaloridine. These five strains were all among the ten strains that produced growth of the indicator in 24 h in the first experiment. There are several possible reasons for the difference in results obtained by the direct and transfer methods. The transfer method can only measure diffusible (exopenicillinase) and does not therefore take account of cell-bound penicillinase; it also involves an inevitable dilution of the exopenicillinase in the agar plug before transfer; however, the transfer method has the advantage that it avoids any induction of penicillinase in the *Staphylococci*, which do not come into contact with the antibiotic.

In conclusion, it seems clear that cephaloridine is destroyed by staphylococcal penicillinase in circumstances in which no significant destruction of methicillin or cloxacillin could be detected. Three points of interest arise from this finding. First, its possible importance in the treatment of infections due to penicillinase-producing

Staphylococci needs further investigation. Secondly, it makes difficult the assessment of the sensitivity to cephaloridine of penicillinase-producing *Staphylococci*. Thirdly, cephaloridine is a useful tool for simple microbiological assessment of penicillinase production by bacteria because it is more resistant to staphylococcal penicillinase than benzyl penicillin, which is too easily destroyed by all penicillinase-producing *Staphylococci*, but it is less resistant than methicillin, which is scarcely inactivated at all by the staphylococcal enzyme. It may also prove useful in investigations of the inducing capability of penicillins and cephalosporins on different strains of *Staphylococci*.

¹ Muggleton, P. W., O'Callaghan, C. H., and Stevens, W. K., *Brit. Med. J.*, ii, 1234 (1964).

² Barber, M., and Waterworth, P. M., *Brit. Med. J.*, ii, 344 (1964).

³ Knox, R., and Smith, J. T., *Brit. Med. J.*, ii, 205 (1963).

⁴ Knox, R., and Smith, J. T., *Nature*, **191**, 926 (1961).

⁵ Gots, J. J., *Science*, **102**, 309 (1945).

⁶ Haight, T. H., and Finland, M., *Amer. J. Clin. Path.*, **22**, 806 (1952).

⁷ Richmond, M. H., Parker, M. T., Jevons, M. P., and John, M., *Lancet*, i, 293 (1964).

⁸ Smith, J. T., Hamilton-Miller, J. M. T., and Knox, R., *Nature*, **195**, 1300 (1962).

⁹ Richmond, H. M., *Biochem. J.*, **94**, 584 (1965).

MEASUREMENTS OF SPONTANEOUS BIOLUMINESCENCE IN THE SEA

By DR. BRIAN P. BODEN, DR. ELIZABETH M. KAMPA and JAMES M. SNODGRASS

Scripps Institution of Oceanography, University of California, San Diego

IN the literature there are numerous reports of strata in the ocean in which the intensity of the ambient light is too high to be accounted for simply by the transmission of sun- and sky-light¹⁻³. Superimposed on this apparently high background level are flashes of considerable magnitude. These strata are generally detected initially by acoustic means, such as sonar, and form the sonic-scattering layers or 'deep-scattering layers'.

Collections made in these layers have revealed concentrations of planktonic organisms associated with concentrations of larger organisms. Many representatives of both the plankton and nekton in such layers are known to be capable of brilliant bioluminescence, and the hypothesis has been that the sometimes rather dramatic increases of light with depth have been due to such bioluminescence. The animals in many of these layers migrate toward and from the surface at dusk and dawn, and experiments made at sea have indicated an increase in flashing rate and intensity at scattering layer levels during such migrations². There has been some implication that this increase may be due to an increased metabolic rate and that it is possibly a means of keeping members of a rapidly moving community in sight of each other.

In common with other workers (for example, ref. 4) we have been uneasy for some time that such apparent increases of light with depth may be artefacts created by agitation caused by the passage of the sensor through the water.

Such reservations about our previous results have led us to design experiments to provide further insight into the question of *in situ* spontaneous bioluminescence, and some very preliminary results are reported here.

Several procedures are open to an investigator of this problem, and they are all unsatisfactory in some respects. Numerous laboratory experiments have demonstrated unequivocally that there is an inherent diurnal luminescent activity in unicellular planktonic organisms such as the dinoflagellates⁵⁻⁷. In most of the experiments, reliance is placed at some time on artificial stimulation—mechanical agitation, light flashes or chemicals. Morris⁴ used a constant-speed propeller to agitate concentrations of

dinoflagellates. Presumably, this could be adapted to undersea observations; but the objection always arises that an artificial stimulus is being applied. This is always true of laboratory investigations.

The same objection can be raised against direct visual observation, either by self-contained under-water breathing apparatus (which has resulted in several tragic deaths) or from submersible craft such as the mesoscapha. Such techniques result in an undesirable perturbation in the natural environment, for a large foreign body, generally equipped with lights, is intruding on it.

We have chosen to take an instrumental approach in which we employ small, dual radiance meters with a coincident circuitry and a common, remote purview. By this means we hope to observe animals in an undisturbed state, for only flashes in a common, approximately spherical field, some distance from the instrument, would be considered spontaneous. Some objections to this system will be discussed later.

The radiance meters. A detailed description of the radiance meters will be published after more definitive experiments have been undertaken. However, a brief description is necessary here.

Each meter is self-contained. The sensor (a model 6472 multiplier phototube similar, electrically, to a model 931-A), power-pack, amplifier circuit, and recorder are contained in one package. The recorder is a 'Minifon' (type L) wire-recorder with a recording time of 4 h.

In the preliminary experiments from which the records described here were obtained the instruments were lowered on single-conductor cable, a sea-return was used, and the signal was monitored and recorded on an Ampex (model No. 403-2) tape-recorder on deck. In routine use, the coincident meters will be lowered on non-electrical, hydrographic or towing cable.

The two undersea units were inter-connected; the signal from the one containing the 'Minifon' was frequency modulated, and that from the other was amplitude modulated. Fig. 1 shows the read-back system.

In front of each phototube was a collimating tube which restricted the incident light to a 10° solid cone. The instruments were mounted on a crude rack and oriented

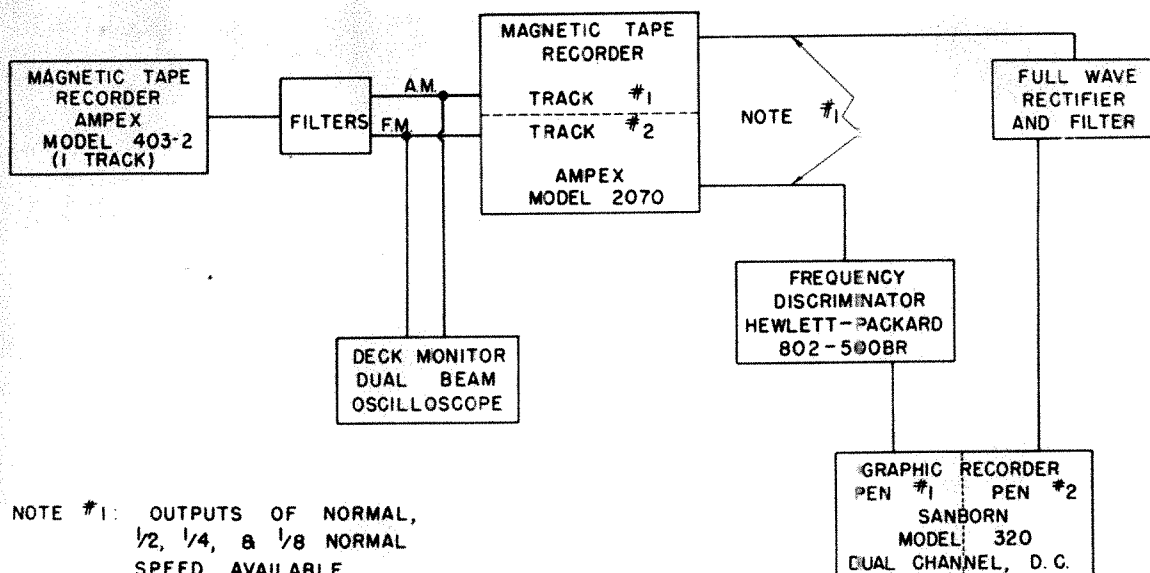


Fig. 1

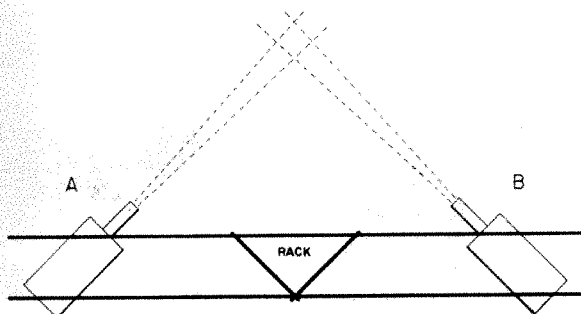


Fig. 2. Diagram of rack mounting of dual radiance meters, showing 10° cones and region of common purview

so that they observed a common volume of about 1 litre at a distance of 1 metre (Fig. 2). In future use, narrow-band interference filters will be placed between the collimator and the sensor surface.

The original tape records obtained at sea were re-recorded in the laboratory and graphic records were made on a Speedomax recorder (not in Fig. 1) and a Sanborn (model No. 320) dual-channel recorder.

Magnetic tape and the modern magnetic tape recorders and reproducers together constitute an exceedingly versatile and powerful research tool. It is quite practical to 'play back' tapes either faster or slower than originally recorded. We have used this technique to 'slow-down' the light flashes by a factor of eight, so that they may be observed with increased fidelity by mechanical graphic recorders such as the Sanborn model No. 320.

The records. The records shown here were obtained when the dual radiance meters were looking downward. Fig. 3A depicts recorded luminescence above the scattering layer and Fig. 3B shows the increased activity within the layer. The instruments were relatively stationary during each recording. These records were made on the Sanborn recorder at 10 sec/cm chart speed and show what we think may be nearly natural conditions. We enjoyed a zero sea, zero wind and pitch or roll of the ship was minimal.

As we have stated, we consider that only flashes observed coincidentally by the two meters may be spontaneous. For example, Fig. 4 shows what we have considered as a coincident flash (A,A) as well as a non-coincident flash (B). Conceivably, each could have been caused by the

same organism if, for example, it had collided with the frequency-modulated meter outside the common field of vision and an artificially induced flash (B) had resulted. However, this would demand a swimming speed of about 10 msec. Alternatively, the animal could have moved

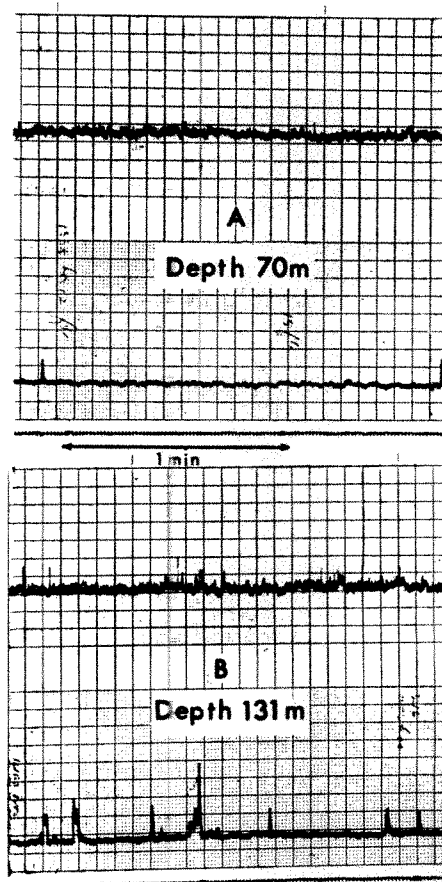


Fig. 3. A, luminescent activity recorded at 70 m. The sonic-scattering layer was visible on the precision depth recorded at between 125 and 135 metres; B, luminescent activity recorded at 131 m. The radiance meters were stationary in both A and B. The record is expanded by a factor of 8.

out of the field of vision of one of the instruments and flashed again. This is an improbably short flash interval and the likelihood that flash *B* was caused by another organism is high. Objectively, such non-coincident flashes must be disregarded.

During the experiment at sea the output of the frequency-modulated instrument was recorded simultaneously on a Speedomax recorder and on magnetic tape. The Speedomax record resembles closely a record of the same type as those we have obtained previously with a single instrument^{2,3}. We lowered the apparatus to a depth below that of the scattering layer and raised it through the layer. Significantly, during the lowering little change was noticed in the baseline of ambient light. However, during the hoisting of the apparatus through the scattering layer a considerable increase in background light occurred, and the flashes increased in both amplitude and frequency (Fig. 5A). Such activity decreased after the hoisting. The cusps on the base line of the record at the left of Fig. 4A are typical of the records obtained from all such hoists. It is intriguing to contemplate that these cusps may be indicative of inhomogeneities within the layer that are not evinced by acoustic techniques.

The magnetic tape referred to here, both tracks of which were utilized (one by each of the photometers), was re-recorded on the dual-track Sanborn recorder, and a part of this record is depicted in Fig. 5B.

Coincident flashes, which we hope are spontaneous and not artificially caused except during hoistings, were counted, and Table 1 was constructed from the counts.

Table 1. FREQUENCY OF FLASHING IN THE SCATTERING LAYER OBSERVED WITH THE INSTRUMENT IN MOTION AND AT REST

Flashes/2.5 min	Winch activity	Flashes/2.5 min	Winch activity
1	Still	2	Still
27	Hoist 25 m	5	Still
21	Still	8	Still
10	Still	25	Hoist 25 m
6	Still	12	Still
7	Still	5	Still
2	Still	4	Still
3	Still	3	Still
7	Still	3	Still
3	Lowering 25 m	4	Still
6	Still	0	Still
1	Still	4	Still

Discussion. The foregoing results reveal an apparent increase in bioluminescent activity at scattering-layer depths. They show also, however, that our previous claims, possibly in common with those of other workers, of the magnitude of the increase in activity may have been exaggerated.

Any instrument, subject to surge or being dragged through the water, must cause a physical agitation of the organisms and a possible resultant increase in luminescence which would be recorded uncritically. A serious objection to records we have made previously by means of our irradiance meter is that it is possible for a single organism, such as *Pyrosoma*, to become entangled in the rigging and cause a spurious record. Dual photometers,

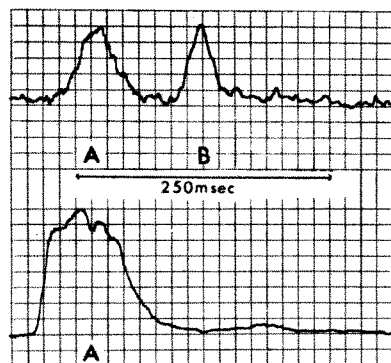


Fig. 4. Time-expanded view of a coincident (A-A) and non-coincident (B) flash. The record is expanded by a factor of 8. Only coincident flashes are considered as spontaneous.

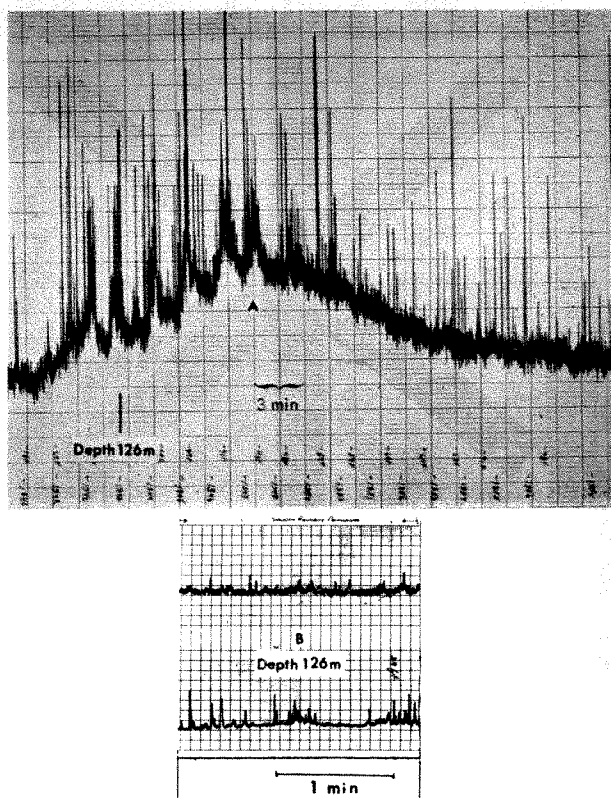


Fig. 5. A, luminescence recorded on the Speedomax during a hoisting of the recorders through the scattering layer. The apparatus was hoisted abruptly through about 30 m and then held stationary. It appears that luminescent activity lessens after a period of adjustment; B, a portion of the same record as shown on the Sanborn at an expanded chart speed.

mounted on a single rack and subject to the same forces do of course cause similar agitation. However, since only flashes mutually observed in a distant, undisturbed volume are considered it is probable that the observations system has been purified.

Cogent arguments can be raised that we are not, in fact, actually observing completely spontaneous luminescence in an undisturbed environment.

One of the most plausible arguments would be that the apparatus is causing flashing by agitation and that such flashing is causing sympathetic flashing by other organisms⁶. The design of the instrument precludes its observation of the primary flashes. However, it may conceivably observe flash-induced flashes.

In our opinion these results invalidate the statistics scrutiny to which previous records have been subjected.

These are extremely tentative results obtained on a cruise taken merely to test the instruments.

It is planned that more definite experiments be made soon, and the problem of the amount of sympathetic flashing that occurs in Nature is obviously one of many that will exercise us considerably.

This investigation was supported by U.S. Public Health Service Research grant NB 02841, from the National Institute of Neurological Diseases and Blindness. It was also undertaken under contract between the U.S. Office of Naval Research and the University of California.

¹ Clarke, G. L., and Wertheim, G. K., *Deep-Sea Res.*, **3**, 189 (1956).

² Kampa, E. M., and Boden, B. P., *Deep-Sea Res.*, **4** (2), 73 (1956).

³ Boden, B. P., and Kampa, E. M., *Pacific Sci.*, **11**, 229 (1957).

⁴ Hardy, A. C., and Kay, E. H., *J. Mar. Biol. Assoc. U.K.*, **44** (2), 435 (1964).

⁵ Haxo, F. T., and Sweeney, B. M., in *The Luminescence of Biological Systems*, edit. by Johnson, F. H. (Amer. Assoc. Adv. Sci., Washington, D.C., 41 (1955)).

⁶ Hastings, J. W., and Sweeney, B. M., *Proc. U.S. Nat. Acad. Sci.*, **43** (4), 804 (1957b).

⁷ Hastings, J. W., and Sweeney, B. M., *Biol. Bull., Woods Hole*, **115** (3), 44 (1958).

⁸ Mauchline, J., *Proc. Roy. Soc. Edin.*, **B**, **67**, Part II, No. 9 (1960).

LETTERS TO THE EDITOR

PHYSICS

Laser-induced Damage in Diamond

AN observation of laser-induced damage in clear diamond has recently been reported¹ and the conclusions were drawn are that damage occurred on the exit face only and that small holes cannot be drilled because of damage by acoustic phonons. This is contrary to our experience. Using a ruby laser operating in relaxation oscillations with an output of only 0.2 joule and a beam divergence of 3 milliradians, we can produce a hole with 10 laser shots. Fig. 1 shows such a hole of 40 μ diameter through a diamond plate 0.5 mm thick cut parallel to the (10) plane. The laser beam was focused on to the front (10) surface by a plano-convex lens of 17 mm focal length, creating a triangular depression in this surface. Blackening of the walls of the hole also occurred, probably due to graphitization. This diamond plate had also been hit by the same laser operating with a rotating prism Q-switch to give a 5 MW pulse, beam divergence 1 milliradian, focused by an 8-mm microscope objective. Under these conditions blackening and damage occurred on the exit surface only and can be seen on the right of Fig. 1. The microscope objective was wrecked in the process.



Fig. 1. Reflexion two-beam interferogram of diamond plate exit surface showing relaxation-oscillation laser hole on left and giant-pulse laser exit damage on right

Fig. 2 shows an interferogram of another diamond plate which was shattered into two pieces by a single relaxation oscillation beam of 0.2 joule energy. This was a colourless, water-clear diamond plate cut parallel to the (110) plane to form a flat rhombus 3 mm long and 1 mm thick. The surfaces were polished to a fine finish and before laser irradiation the plate was examined with a polarizing microscope which showed it to be free from flaws or serious strain. Focusing was again made on to the front surface with a 17 mm focal length plano-convex lens.

Blackened depressions were observed on both the rear and front surfaces. The front surface depression is 120 μ deep and the exit depression is 30 μ deep, and there was also created a narrow blackened channel going through the crystal along the direction of the beam, and connecting the two depressions. The rest of the diamond plate remained undamaged and colourless. The specimen was cleaved by the laser beam along the (111) plane and the two cleaved surfaces were flat enough to be examined by two-beam interferometry. Fig. 2 shows the two cleaved surfaces set in a matched position with the rear surfaces together. The two cleaved surfaces did not match

exactly; clearly small fragments must have been lost, but in general good matching agreement can be seen in the cleavage features. The fringe patterns are not identical in appearance because fringe direction and dispersion are accidentally determined by the arbitrary inclination between object surface and the optical flat on which it rests.

Four different types of damage have been noted.

(1) Irregular conchoidal fractures along the blackened channel beginning at the bottom of the front surface depression and ending at the bottom of the rear surface depression.

(2) Fine cleavage lines and steps in a large triangular cross-sectioned damage region surrounding the front surface depression.

(3) 'River system' cleavage lines and steps radiating from a starting point on the laser beam path 90 μ from the bottom of the smaller rear surface depression. These are the dominating cleavage features of the damage, and interferometry shows the edges of the cleavage steps to be sharp and reasonably straight.

(4) Conchoidal fractures at the exit point of the laser beam.

It is interesting to note that the directions of the cleavage lines or steps in regions 2 and 3 are not concordant with each other. Also after irradiation some polarization strain was observed around the blackened channel. As with the holed diamond the diameter of the damage channel is smallest near the exit and it would be reasonable to attribute the front surface damage to thermal burning and shock and the rear surface conchoidal fractures and

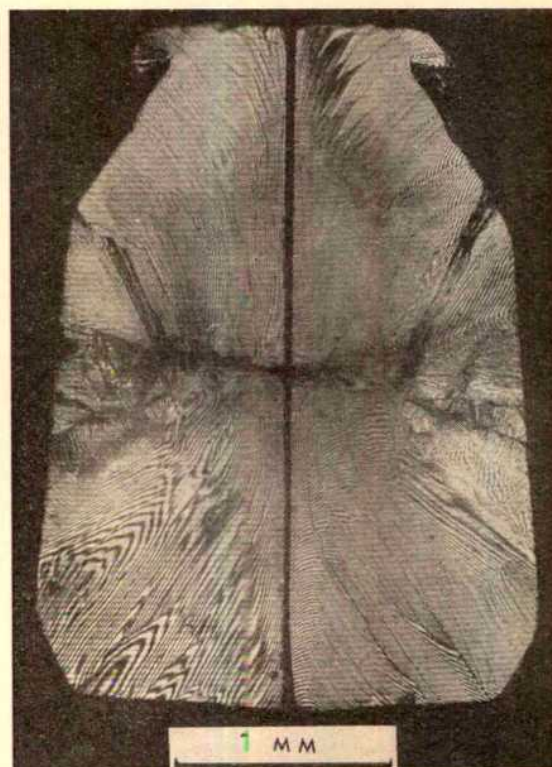


Fig. 2. Reflexion two-beam interferograms of laser cleaved diamond surfaces. Exit depression is at the centre of the figure

the 'river system' features to acoustic phonons travelling in the direction of the laser beam².

We have drilled a similar hole in a silicon carbide crystal with a single 0.2 joule relaxation-oscillation beam. We also find that with glass slides laser-induced damage can be produced on the front or back surfaces depending on the position of focus. This is contrary to a previous report³ of damage occurring only at the exit face.

This work forms part of a general investigation into laser-induced damage in dielectric materials. We are indebted to Prof. S. Tolansky for suggesting the problem and for valuable advice.

D. J. BRADLEY
M. ENGWELL
H. KOMATSU

Department of Physics,
Royal Holloway College,
Englefield Green, Surrey.

¹ Whiteman, P., and Wilson, G. W., *Nature*, **208**, 66 (1965).

² Chiao, R. Y., and Townes, C. H., *Phys. Rev. Letters*, **13**, 592 (1964).

³ Giuliano, C. R., *App. Phys. Letters*, **7**, 137 (1964).

Acoustic Velocity Dispersion in some Non-associated Organic Liquids

OPTICAL-SCATTERING techniques depending on the Brillouin effect¹ enable measurements of acoustic velocity to be made at frequencies above the conventional ultrasonic range, namely, above 10^8 c/s.

When a beam of monochromatic light is incident on a transparent liquid, the scattered light contains two displaced (Brillouin) components, $\nu \pm \Delta\nu$, where $\Delta\nu$ is given by:

$$\frac{\Delta\nu}{\nu} = \frac{2nV}{c} \sin \frac{\theta}{2} \quad (1)$$

with V = velocity of the ultra-high-frequency Debye waves responsible for this part of the scattering, ν = frequency of the incident light, n = refractive index of the liquid, c = velocity of light in *vacuo* and θ = angle of scattering.

Measurement of the Brillouin shift $\Delta\nu$ enables the velocity V to be determined²⁻⁴.

In the work described here, a beam of monochromatic light of $\lambda = 6328 \text{ \AA}$ from a helium-neon laser (of line-width 0.047 cm^{-1}) was passed through the dust-free test liquid contained in a quartz cubical cell. The light scattered at 90° from the liquid fell normally on a Fabry-Perot etalon and fringes produced by the etalon were photographed.

The effects of stray reflexions were reduced to a minimum by placing a collimator between the cell and the etalon. Continuous background scattering that appeared in the fringes was reduced by putting a polaroid filter, suitably oriented, between the scattering cell and the etalon.

A front-reflecting mirror with a dielectric coating of reflectivity 0.99 at $\lambda = 6328 \text{ \AA}$ was mounted in a levelling

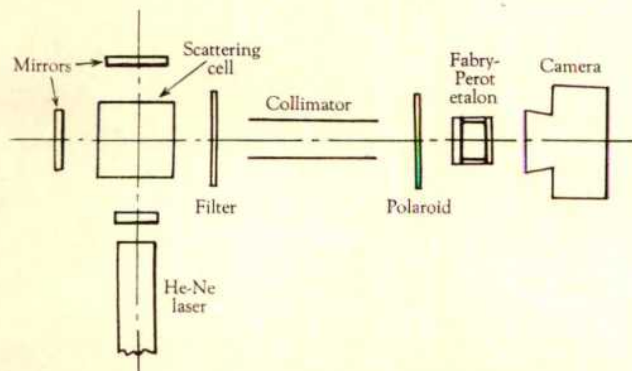


Fig. 1

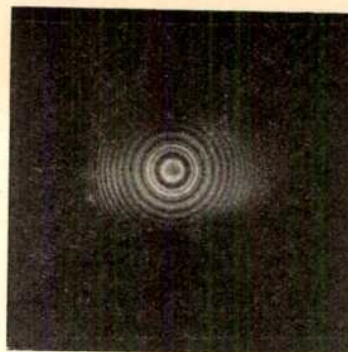


Fig. 2

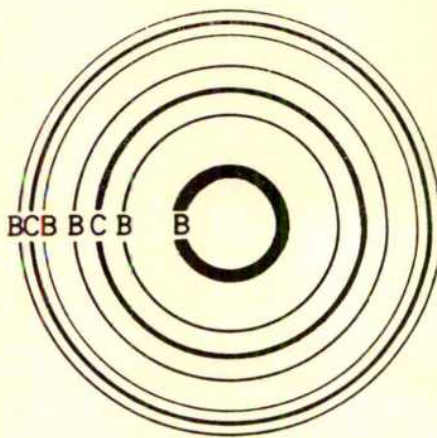


Fig. 3. B, Brillouin component; C, central component

holder and was placed on the remote side of the cell to reflect back the transmitted laser beam. Another front reflecting mirror was placed behind the cell, facing the collimator, as shown in Fig. 1. This technique has greatly reduced the time of exposure. For some of the photographs taken, an exposure of 30 sec was quite adequate (output power of the laser used was about 2 mW).

Fig. 2 is a photograph showing the Brillouin component for one of the liquids investigated (1-4 dioxan). The two displaced components appear as faint fringes located near the brighter undisplaced ('central') component of the incident laser beam (Fig. 3).

The radii of the fringes were determined and the average shift in frequency, $\Delta\nu$, of the Brillouin components was calculated. The velocity of Debye waves (that is, the acoustic velocity) at ultra-high frequency was then obtained from equation (1). The frequency of these waves is equal to the Brillouin shift, $\Delta\nu$.

The results of the measurements are shown in Table 1.

For benzene, velocity dispersion is evident and confirms the observations of Fabelinskii⁵ and Benedek *et al.*⁶ In *m*-xylene and toluene, no velocity dispersion is shown for 90° scattering. Again, no dispersion is given by 1-4 dioxan.

On the other hand, benzoyl chloride shows dispersion of about 5 per cent, which is outside the range of experimental error; this liquid also shows considerable broadening of the Brillouin components.

Further work is proceeding on a range of liquids. It is worth noting that the upper frequency limit of this technique for a given liquid can be determined from the relationship:

$$f = \frac{2nV}{\lambda} \sin \frac{\theta}{2} \quad (2)$$

where f = frequency of acoustic (Debye) waves, and λ = wave-length of incident light. For some polyatomic

Table 1

Liquid	Temperature (°C)	Brillouin shift (cm ⁻¹)	Frequency ($\times 10^9$ c/s)	Velocity V_{CHF} (m/s)	Ultrasonic velocity ² V_{US} (m/s)	Dispersion $\frac{V_{CHF}-V_{US}}{V_{US}}$
Benzene	22.5	0.164	4.92	1,470 \pm 1%	1,313	12%
m-Xylene	24.1	0.149	4.47	1,330 \pm 1%	1,330	—
Toluene	22.7	0.146	4.38	1,310 \pm 1%	1,321	—
1-4 Dioxan	23.8	0.1465	4.40	1,384 \pm 0.5%	1,379	—
Benzoyl chloride	23.7	0.162	4.86	1,400 \pm 2%	1,335	5%
Ethyl bromide	23.8	0.097	2.91	920 \pm 2%	906	2%

liquids, with 90° scattering and $\lambda = 6328 \text{ \AA}$, a frequency as high as 9×10^9 c/s may be reached. Still higher frequencies may be attained by increasing the angle of scattering or by using shorter light wave-lengths.

We thank Dr. R. W. B. Stephens for his advice.

S. E. A. HAKIM*

W. J. COMLEY†

Department of Physics,
Imperial College of Science and Technology,
London, S.W.7.

* Present address: Department of Physics, Faculty of Engineering,
Sairo University, Guiza, Egypt.

† Present address: Department of Physics, Northern Polytechnic, London,
N.7.

Brillouin, L., *Ann. Phys.*, **17**, 88 (1922).

Chiao, R. Y., and Stoicheff, B. P., *J. Opt. Soc. Amer.*, **54**, 1286 (1964).

Fabelinskii, I. L., *Soviet Phys. Uspekhi* (U.S.A.), **5**, 667 (1963).

Comley, W. J., *Contemp. Phys.*, **4**, 15 (1962).

Parthasarathy, S., *Proc. Indian Acad. Sci.*, **2**, 497 (1935); **3**, 519 (1936).

Benedek, G. B., Lastovka, J. B., Fritsch, K., and Greytak, T., *J. Opt. Soc. Amer.*, **54**, 1284 (1964).

Fall-out in London Rain-water during 1964

DURING 1964, monthly samples of rain-water have been collected on the roof of the Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, S.E.1, and analysed for the presence of various fission products. This programme was a continuation of the rain-water sampling and radiochemical analysis previously performed at Clement's Inn Passage, Strand, London, W.C.2, during September 1961–December 1963 (ref. 1). The new sampling site is approximately 0.6 miles 15° E. of S. away from the old one. Analytical results for samples taken at both sites during November–December 1963 showed a good measurement of agreement¹. This agreement indicated that the continuity of the radiochemical analyses of London rain-water was undisturbed by the change of site.

The method of sampling has not been altered and the radiochemical procedures employed for the determination of the individual radionuclides have already been described². Regular measurements were made during the year of caesium-137, cerium-144, manganese-54, promethium-147, strontium-90, yttrium-91 and zirconium-95 radionuclides. The levels of these radionuclides are plotted in Fig. 1 on a logarithmic scale, the individual activities being corrected to the mid-point of the sampling period. Throughout the year the changes in the levels of the shorter-lived yttrium-91 (half-life 57 days) and zirconium-95 (half-life 65 days) followed each other closely, as do those of the long-lived nuclides.

Strontium-89 due to the American and Russian tests of 1962 was last positively identified in April 1964 when the level was 0.8 pc./l. Also the levels of yttrium-91 and zirconium-95 were approaching the limits of detection towards the end of the year. The peak levels of radioactivity occurred in May when 33 pc./l. and 59 pc./l. of strontium-90 and caesium-137 respectively were recorded. This compares with the corresponding peaks of 33 pc./l. and 64 pc./l. found during the period June–August 1963. Concentrations of the various radionuclides found in the September sample were relatively high due to an abnormally low rainfall. Thereafter, towards the end of the year, there was a rapid drop, the levels of caesium-137 and

strontium-90 during November–December being the lowest in London rain since October 1962.

Fall-out debris from the low-yield Chinese nuclear test on October 16 did not substantially affect the concentrations of radioactivity in London rain-water during the last three months of the year. After the test, separate sampling was immediately commenced for the determination of iodine-131 (half-life 8.05 days). However, the rainfall was light and the samples correspondingly small (less than 500 ml.) in the fortnight following the test. The first positive identification of iodine-131 was made in rain collected between October 21, p.m. and October 22, a.m. This, and succeeding similarly small samples collected until the end of the month, contained concentrations of 10–60 pc./l. of iodine-131. A modified¹ Glendenin and Metcalf³ procedure was used to isolate the radio-iodine and all counting was performed in low-background anti-coincidence β -counting assemblies.

The other short-lived radionuclides from the Chinese test detected once more in October–December rain samples were barium-140 (half-life 12.8 days) and strontium-89 (half-life 51 days). The results are listed in Table 1. Although they might be expected in fresh fission debris on account of their short half-lives and relatively high fission yields neither cerium-141 (half-life 33.1 days) nor other rare earth activity such as praseodymium-143 (half-life 13.8 days) and neodymium (half-life 11.6 days) were found. The failure to detect cerium-141 was probably due to the inability of absorber measurement to distinguish the β -emission of a small amount of cerium-141 from that of a large preponderance of cerium-144 in a cerium source. Also, the relatively large amount of

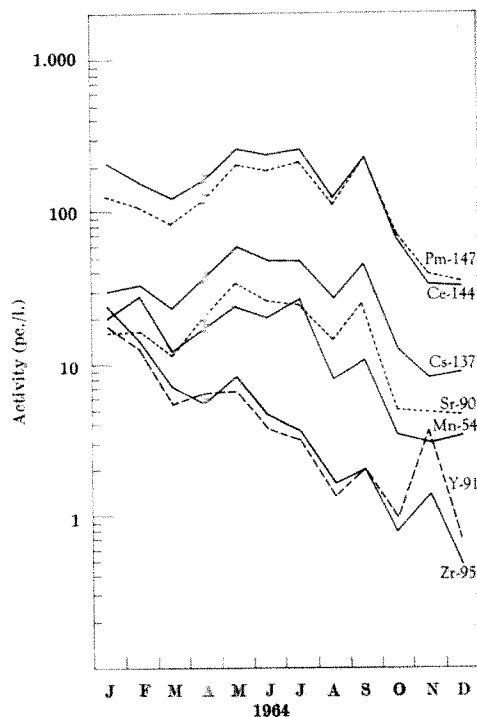


Fig. 1. Radionuclides in London rain

Table 1. CONCENTRATION OF SHORT-LIVED RADIONUCLIDES IN LONDON RAIN, OCTOBER TO DECEMBER, 1964

Nuclide	October*	November†	December†
Sr-89	14	6.5	1.10
Ba-140	168	15.5	0.85

* Corrected for decay to the time of the nuclear test (Oct. 16, 1964) and calculated on the rainfall between October 21 and 31, 1964, inclusive.
† Corrected for decay to the mid-point of sampling.

promethium-147 in the rare earth source would mask any small amount of praseodymium-143 and neodymium-147 present.

The successive decreases in the concentration of barium-140 and strontium-89 in Table 1 are larger than would be expected by decay and can be accounted for by dilution of the initial radioactive cloud by dispersal in the troposphere.

Fig. 1 shows that the concentrations of zirconium-95 and yttrium-91 were enhanced during the period of the Chinese test while any small increases in the concentration of the long-lived nuclides were unnoticed graphically.

R. WOOD
J. R. W. KERR
L. A. RICHARDS

Laboratory of the Government Chemist,
Ministry of Technology,
Stamford Street, London, S.E.1.

¹ Wood, R., Metson, P., and Richards, L. A., *Nature*, **203**, 617 (1964).

² Wood, R., and Richards, L. A., *Analysis*, **90**, 606 (1965).

³ Glendenin, L. E., and Metcalf, R. P., *Nat. Nuclear Energy Ser.*, **9** (3), 1625.

GEOCHEMISTRY

Strontium-90 and Caesium-137 in Columbia River Plume, July 1964

OCEANOGRAPHERS informally term the extension of Columbia River outflow into the Pacific Ocean as the 'Columbia River plume'. The plume can be identified by its low salinity even at a distance of several hundred kilometres from the mouth of the river (Fig. 1). The plume distributes dissolved and suspended substances in the river water over a wide surface area of the ocean and introduces annually to the euphotic zone over a billion moles of phosphorus and nitrogen fertilizers, as phosphate and nitrate. Equally significantly, the radionuclides contained in the river water are diffused over a wide region¹.

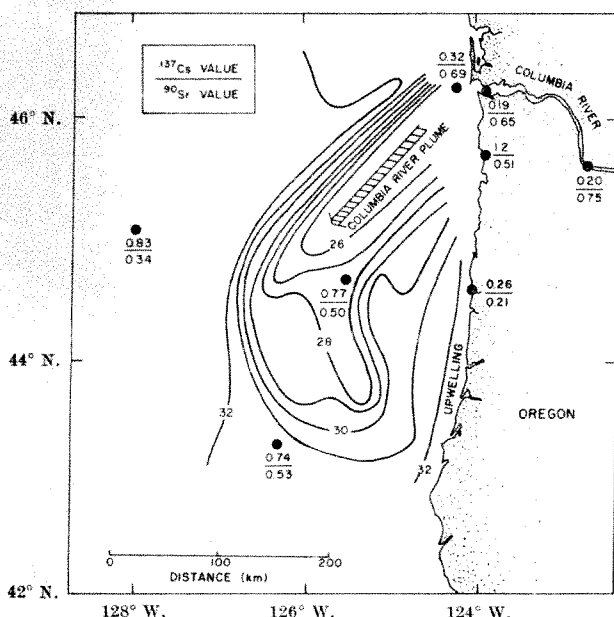


Fig. 1. Surface salinity, ⁹⁰Sr and ¹³⁷Cs distributions in the Columbia River plume, July 1964. Contours are surface salinity in parts per thousand. ⁹⁰Sr and ¹³⁷Cs concentrations are in μCi/l. with a relative standard deviation of ± 10 per cent.

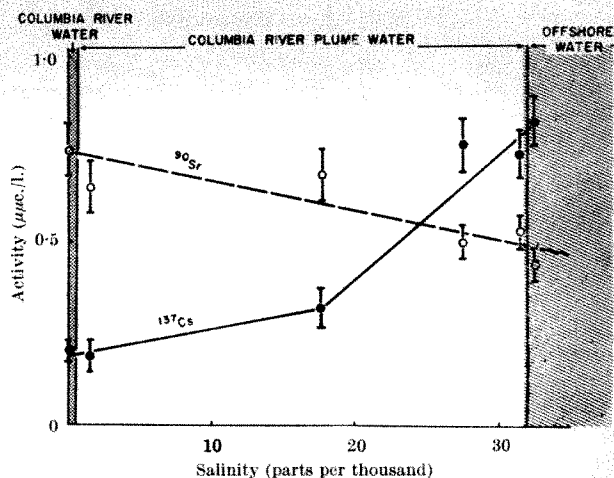


Fig. 2. ⁹⁰Sr and ¹³⁷Cs concentrations in and near the Columbia River plume, July 1964, as a function of salinity

To understand the ocean mixing process of ⁹⁰Sr and ¹³⁷Cs originating in the river, we collected eight 100-l surface-water samples from the river, the river plume, and its vicinity during July 12–24, 1964. The Millipore-filtered water samples were analysed for their ⁹⁰Sr content by the fuming nitric acid method² and for ¹³⁷Cs by the ammonium molybdophosphate method³ at the Meteorological Research Institute, Tokyo. The results and the surface salinity pattern are shown in Fig. 1.

The river water near Portland, Oregon, gave ⁹⁰Sr and ¹³⁷Cs concentrations of 0.75 and 0.20 μCi/l. respectively. The river water contains the radionuclides from two sources: world-wide fall-out and by-product of the operation of the Hanford atomic reactor⁴.

We have no measured concentrations in the river for the isotopes produced at Hanford, but they may be indirectly estimated by comparing the ⁹⁰Sr and ¹³⁷Cs content of Japanese river waters with that of Columbia River water. Japanese waters contain only fall-out. Average Japanese river waters contained 0.19 μCi/l. of ⁹⁰Sr and 0.034 μCi/l. of ¹³⁷Cs in 1961 (ref. 5). At Pasco, downstream from Hanford, the annual average concentration for ⁹⁰Sr in 1962 was 0.7 μCi/l. (ref. 4). If we assume that the ⁹⁰Sr concentrations in Japanese rivers were similar to the fall-out portion of the ⁹⁰Sr content of Columbia River water, then about 0.5 μCi/l. of ⁹⁰Sr would appear to be contributed by the Hanford operation in 1962. Similar ⁹⁰Sr content could have originated from Hanford in 1964.

The sampling station most distant from the shore was more than 300 km off the Oregon coast (Fig. 1). Its water is the least affected by the river plume, and, therefore, should be representative of the oceanic water for this region. We found ⁹⁰Sr and ¹³⁷Cs concentrations of 0.34 and 0.03 μCi/l., respectively, and a salinity of 32.5 parts per thousand. Other waters collected off the coast are in the river plume and can be expected to show effects of a mixture of offshore water and river water. If physical mixing is the only mechanism regulating the concentration of the nuclides, then the plume waters should possess radioisotopic concentrations proportional to the contribution of the two parental water bodies. Fig. 2 confirms this simple mixing theory in first approximation. ⁹⁰Sr appears to have a better linear correlation with salinity than ¹³⁷Cs.

Comparison of the ⁹⁰Sr content in the river water and the offshore water shows that the river water contains about 0.3 μCi/l. more than the offshore water (Fig. 2). Conversely, the ¹³⁷Cs content in the river water is about 0.6 μCi/l. lower than that of the sea water.

A peculiarly high ¹³⁷Cs concentration of 1.2 μCi/l. was observed in the estuarine water of the Nehalem River mouth, about 70 km south of the mouth of the Columbia

River (Fig. 1). No satisfactory explanation is available at present.

The upwelled water at Newport, Oregon, had low ^{87}Sr and ^{137}Cs contents of 0.21 and 0.26 $\mu\text{c./l.}$, respectively. The salinity of this water, 33 parts per thousand, indicates that the water had been upwelled from 100 m depth. The data from the upwelled water appear to confirm the decrease of the nuclide concentration with increasing depth⁶.

Folsom, Mohanrao and Winchell⁷ determined the ^{137}Cs content of sea water along and off the coast of California in 1959 and 1960. Their work is still continuing. According to Folsom (personal communication) ^{137}Cs concentrations off California, between 30° and 35° N., and from the coast to 130° W., were about 0.7 $\mu\text{c./l.}$ in surface waters in 1964. His values are in agreement with ours.

In conclusion we find that both ^{87}Sr and ^{137}Cs concentrations of the Columbia River plume waters are, in first approximation, proportional to the contribution of the river waters as determined from salinity.

We thank Prof. C. L. Osterberg and H. H. Dobson for their assistance. This work was supported by U.S. National Science Foundation grant GP-2232 and by Office of Naval Research contract Nonr 1286(10), project NR 083-102.

KILHO PARK
MARILYN J. GEORGE

Department of Oceanography,
Oregon State University, Corvallis.

YASUO MIYAKE
KATSUKO SARUHASHI
YUKIO KATSURAGI
TERUKO KANAZAWA

Meteorological Research Institute,
Mabashi, Suginami, Tokyo, Japan.

¹ The river-originated ^{51}Cr has been used to investigate the Columbia River plume in 1965 by Osterberg, C., Cutshall, N., and Cronin, J. (in preparation).

² Miyake, Y., Saruhashi, K., and Katsuragi, Y., *Papers Meteorol. Geophys.*, Tokyo, 11, 188 (1960).

³ Miyake, Y., Saruhashi, K., Katsuragi, Y., and Kanazawa, T., *Papers Meteorol. Geophys.*, Tokyo, 12, 85 (1961).

⁴ Wilson, R. H., *Evaluation of Radiological Conditions in the Vicinity of Hanford for 1962* (Hanford Laboratories, AEC Res. and Dev. Rep. HI-76526, 1963).

⁵ Miyake, Y., Teubota, H., *Radioisotopes in Hydrology*, 425 (Intern. Atomic Energy Agency, Vienna, 1963).

⁶ Miyake, Y., and Saruhashi, K., *Disposal of Radioactive Waste*, 169 (Intern. Atomic Energy Agency, Vienna, 1960).

⁷ Folsom, T. R., Mohanrao, J. G., and Winchell, P., *Nature*, 187, 480 (1960).

Metal Grains in Chondritic Meteorites

THE nickel-iron minerals taenite and kamacite in octahedrites (iron meteorites) are inhomogeneous, as shown in Fig. 1: taenite crystals are richest in nickel and kamacite crystals are poorest in nickel at the phase boundaries where they abut one another¹⁻³. This is a natural consequence of the behaviour of nickel-iron alloy systems if they are cooled slowly and steadily, as in the interior of a planet⁴⁻¹⁰. The binary nickel-iron phase diagram predicts that beneath $\sim 800^\circ\text{C}$, as temperature falls, taenite and kamacite should continuously react with one another and change in composition. Nickel concentration should increase steadily in taenite, while in kamacite at $T < \sim 500^\circ\text{C}$ it should decrease.

A point inside a taenite or kamacite crystal must be able to receive nickel or iron atoms via lattice diffusion from the nearest taenite-kamacite interface (where reaction is occurring) in order to maintain itself at the continuously changing equilibrium composition. But lattice diffusion becomes more and more sluggish with falling temperature, and less able to serve crystal interiors. Interior compositions must eventually lag behind the equilibrium values, leaving centres of taenite crystals poor in nickel, centres of kamacite crystals rich in nickel (at $T < \sim 500^\circ\text{C}$). These are just the inhomogeneities observed in octahedrites.

Chondrites, members of the principal class of stony meteorite, also contain nickel-iron, in minute grains. Urey and Mayeda¹¹ examined these metallographically, and showed that the grains are usually pure kamacite or pure taenite, rarely composite. They noted concentric etch patterns in the taenite grains which indicated that the latter are also inhomogeneous in composition, surrounded by high nickel borders just as is taenite in octahedrites.

Reed¹² analysed metal grains in ten chondrites with an electron microprobe, and found them comparable in composition to kamacite and taenite in octahedrites. He noted that chondritic taenite and most chondritic kamacite are inhomogeneous, but did not define the nature of the inhomogeneities.

More can now be said about chondritic metal grains. An electron-microprobe examination of metal grains in 34 chondrites has been carried out; detailed results will appear elsewhere. Microprobe profiles of nickel content were run over a number of grains in ordinary chondrites. Three representative profiles appear in Fig. 2. Taenite grains were found to be richer in nickel at their surfaces than in their centres, confirming Urey and Mayeda's metallographic observation. Kamacite grains were found to be poorer in nickel at their surfaces than in their centres. In short, taenite and kamacite exhibit exactly the same

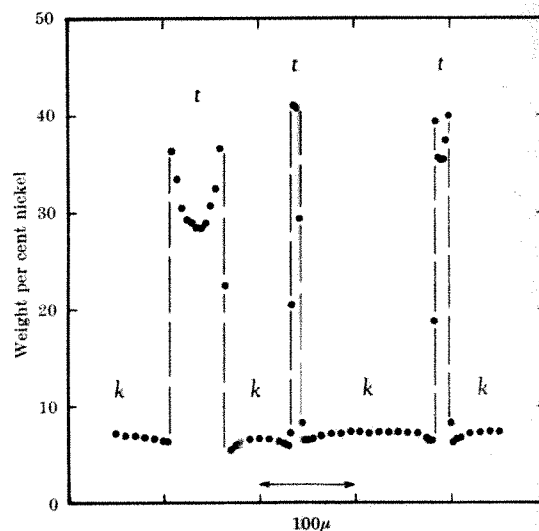


Fig. 1. Electron-microprobe profile across Widmanstätten structure in a typical octahedrite (Anoka), showing compositional inhomogeneity of both taenite (t) and kamacite (k) phases.

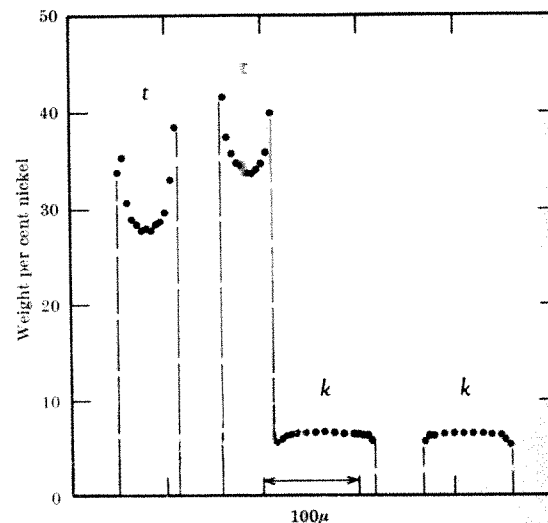


Fig. 2. Typical microprobe profiles across metal grains in chondrites. Left, taenite grain in Moes; centre, composite grain in Bjurböle; right, kamacite grain in Forest City.

inhomogeneities in chondrites as they do in octahedrites. The only important difference is that in octahedrites, taenite and kamacite are in contact with one another, while in chondrites, they usually occur as discrete grains separated by silicate minerals.

Silicate minerals in ordinary chondrites show textural evidence of thermal recrystallization^{13,14}. The parent chondritic material must have been held at high temperature for a long time. The metal grains could not have survived such an event unchanged: the compositional inhomogeneities we now observe must have been impressed on them as they cooled from this heating. For that to have happened, iron and nickel must have been able to move with great facility from one metal grain to another through intervening silicate material, so that taenite-silicate and kamacite-silicate interfaces in chondrites behaved effectively like taenite-kamacite interfaces in octahedrites.

If taenite crystals in octahedrites have nickel-poor interiors because the effective range of nickel diffusion shortens with lowering temperature, it is obvious that nickel concentration will be lowest at the centre of a crystal, and that the smaller a crystal is or the slower it cooled, the longer diffusion will have been able to supply its centre, and the higher its central nickel content will be. In fact, it is possible, given the dimension of a taenite crystal and its central nickel content, to derive the rate at which it cooled through the temperature range $\sim 600^\circ\text{C} - \sim 400^\circ\text{C}$ in which inhomogeneity developed. (The iron-nickel phase diagram and the coefficient of diffusion of nickel in taenite also must be known, of course.) This has been done for taenite in several octahedrites by programming a digital computer to simulate the diffusion of nickel into slowly cooling taenite crystals^{6,8}.

Since metal grains in chondrites appear to have evolved in a manner quite analogous to that of taenite and kamacite in octahedrites, this method of determining the cooling rate of the system can legitimately be applied to them as well. Theoretical curves relating the central nickel content of a spherical taenite grain to its radius, for several cooling rates (assumed constant), are plotted in Fig. 3.

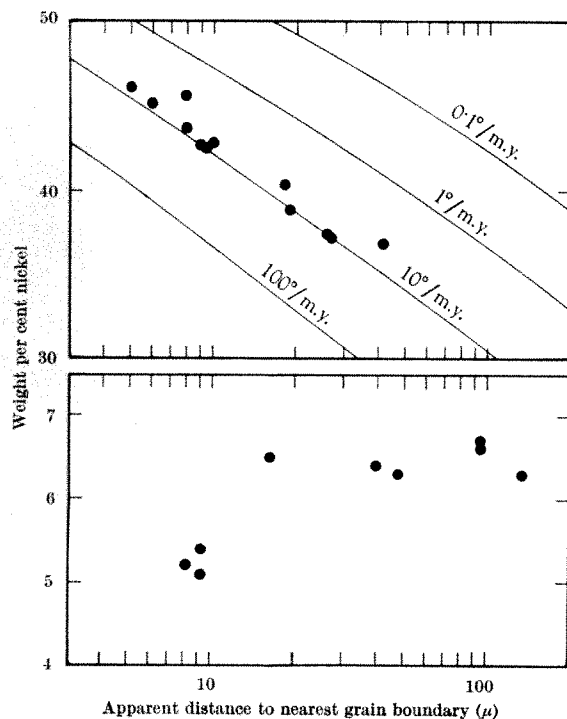


Fig. 3. Relationship between composition at centres of metal grains and their apparent dimensions, in the chondrite Forest City. Above, taenite; below, kamacite. Curves in upper plot show relationships predicted by theory, for taenite grains in systems that cooled at various rates (given in deg. C per million years)

The method of calculating these appears in ref. 6. Analyses at the centres of taenite grains in the Forest City chondrite are plotted over them. Forest City appears to have cooled through $550^\circ - 450^\circ\text{C}$ at a rate of about 10°C per million years. Similar results were obtained for taenite grains analysed in the ordinary chondrites Moes and Ehle, and in the light portions of the light-dark structured chondrites Pantar and Salles.

It is interesting to note that the analogous diffusion-imposed relationship between size and central nickel content holds for Forest City kamacite grains: the smaller the grain (and hence the shorter the diffusion path), the lower the central nickel content.

The cooling rate obtained allows us to say something about the parent planet or planets in which the ordinary chondrites evolved. Ten degrees per million years is the rate at which the centre of an asteroid some 70 km in radius would have cooled⁶. Alternatively, cooling could have occurred $\sim 70\text{ km}$ beneath the surface of a larger body.

This work was supported by the National Aeronautics and Space Administration. Meteorite samples were kindly supplied by the Chicago Natural History Museum, the U.S. National Museum, and Dr. Edward Anders.

J. A. Wood

Enrico Fermi Institute for Nuclear Studies,
University of Chicago, and
Smithsonian Astrophysical Observatory,
Cambridge,
Massachusetts.

¹ Yavnel', A. A., Borovski, I. B., Il'in, N. P., and Marchukova, I. D., *Doklady Akad. Nauk S.S.S.R.*, **123**, 256 (1958).

² Maringer, R. E., Richard, N. A., and Austin, A. E., *Trans. Amer. Inst. Min. (Metall. Eng.)*, **215**, 56 (1959).

³ Agrell, S. O., Long, J. V. P., and Ogilvie, R. E., *Nature*, **198**, 749 (1963).

⁴ Derge, G., and Kommel, A. R., *Amer. J. Sci.* (5), **34**, 203 (1937).

⁵ Perry, S. H., *U.S. Nat. Museum Bull.*, **184** (1944).

⁶ Wood, J. A., *Icarus*, **3**, 429 (1964).

⁷ Reed, S. J. B., *Geochim. Cosmochim. Acta*, **29**, 535 (1965).

⁸ Goldstein, J. I., and Ogilvie, R. E., *Geochim. Cosmochim. Acta*, **29**, 893 (1965).

⁹ Short, J. M., and Andersen, C. A., *J. Geophys. Res.*, **70**, 3745 (1965).

¹⁰ Goldstein, J. I. (in the press).

¹¹ Urey, H. C., and Mayeda, T., *Geochim. Cosmochim. Acta*, **17**, 113 (1959).

¹² Reed, S. J. B., *Nature*, **204**, 374 (1964).

¹³ Wood, J. A., *Geochim. Cosmochim. Acta*, **26**, 739 (1962).

¹⁴ Dodd, R. T., and van Schmus, R., *J. Geophys. Res.*, **70**, 3801 (1965).

RADIOCHEMISTRY

Site of Attack in the Radiolysis of Pyrimidine Compounds

PREVIOUSLY reported experiments with thymine in dilute aqueous solutions have shown that the site of attack in radiolysis with cobalt-60 γ -rays depends on the pH (ref. 1). In acid and neutral solutions, the principal attack is on the double bond at the carbon atom remote from the methyl group, with ultimate formation of saturated products²⁻⁵. In strongly alkaline solution the attack is largely at the methyl group, with formation of the unsaturated compound, 5-hydroxymethyluracil, as the principal product. The shift in the site of attack as the pH is increased correlates well with a model based on two changes in the reaction path. At least one of these changes appears to be related to ionization of thymine¹.

These findings immediately raise the question of the site of attack in other pyrimidine compounds and in their nucleosides and nucleotides. A set of compounds was therefore selected to permit investigation of this problem relative to the presence of amino, methyl, ribose, deoxyribose, ribose phosphate, and deoxyribose phosphate substituent groups. Strongly acid, neutral, and strongly basic aqueous solutions of each of the compounds, approximately $2 \times 10^{-4}\text{ M}$, were exposed to cobalt-60 γ -radiation. Ultra-violet absorption spectra were measured on a Cary recording spectrophotometer and loss of absorption in

the region 260–280 m μ was determined. The 5,6-double bond of the pyrimidine ring is an essential part of the chromophore group, and a decrease in absorption may be related to saturation of the double bond⁴.

In 0.1 N sulphuric acid solutions, the optical density decreases linearly with radiation dose, and the disappearance yields for the chromophore of uracil, cytosine, and 5-methylcytosine are, within experimental error, the same and equal to 2.9–3.0 molecules per 100 eV absorbed. The yield for thymine⁶ is slightly lower, 2.5–2.7. The presence of a pentose unit on any of the compounds reduces the yields to about 2.4–2.6; the presence of a phosphate on the pentose has little additional effect, lowering the yields only slightly, to 2.3–2.5. Thus radiolytic attack in acid solutions occurs largely at the 5,6-double bond. Neither the 5-methyl group of 5-methylcytosine nor the amino-group of this compound or of cytosine is attacked sufficiently to change the double-bond disappearance yield. The pentose and the pentose-pyrimidine link in the nucleosides and nucleotides are attacked at most to the extent of about 20 per cent of the total reaction, a result in agreement with reference 4.

In alkaline solutions (2 N sodium hydroxide), in contrast, the optical density generally changes non-linearly with radiation dose, and the initial chromophore disappearance yields vary greatly among the different compounds (Table 1). In agreement with the findings of Ranadine, Korgaonkar and Sahasrabudhe⁷, the initial disappearance yield for pyrimidines which lack a 5-methyl group, uracil and cytosine, is less than in acid solution, but saturation of the double bond remains a major reaction. However, with the pyrimidines which have a 5-methyl group, thymine and 5-methylcytosine, the initial yield for chromophore loss is zero. Since the amino group takes little part in the reaction (cf. uracil and cytosine) it is probable that with 5-methylcytosine, as has been reported previously for thymine, attack is occurring on the 5-methyl group, leaving the double bond unsaturated and the chromophore unchanged. The expected product, by analogy with thymine, is 5-hydroxy-methylcytosine.

The presence of a pentose group on the pyrimidine ring has a marked effect on radiolysis in alkaline solutions. Irradiation of the ribose compounds, uridine and cytidine (nucleosides), in 2 N sodium hydroxide results in an increase in optical density. Irradiation of ribose (and deoxyribose) alone in 2 N sodium hydroxide causes an increase in optical density approximately equal to that observed in uridine and cytidine with equivalent doses. It may be tentatively concluded, then, that the ribose unit successfully competes with the double bond of the uracil and cytosine units for the radiation-produced active species. Attachment of a phosphate to the pentose, as shown by results obtained from nucleotides (Table 1), has no appreciable effect. Irradiation of alkaline solutions of the nucleosides, thymidine and 5-methyldeoxycytidine, and the nucleotide, thymidylic acid, does not cause a change in optical density. This suggests that the 5-methyl group is more subject to attack than the deoxyribose unit. The relative sensitivity of the ribose and deoxyribose units in comparison with the sensitivity of the 5,6-double bond is shown by experiments with the nucleotides, cytidylic and deoxycytidylic acids. The former gives an increase in optical density, the latter a decrease amounting to an apparent initial chromophore disappearance yield of 0.4. Thus the ribose unit competes with the double bond for the attacking species more successfully than does the

deoxyribose; that is, in strongly alkaline solution ribose attached to a pyrimidine compound appears to be more sensitive to radiation than is deoxyribose, similarly attached.

Irradiation of neutral solutions of the various pyrimidine compounds gives results which are not grossly different from those observed in acid solutions.

From these results, it may be concluded that in acid solution the initial radiolytic attack occurs chiefly at the 5,6-double bond of the pyrimidine ring. Amino and methyl groups do not appear to be attacked, and the presence of a pentose or pentose phosphate unit diverts no more than 20 per cent of the attacking species from the double bond. In alkaline solutions, where the molecules are ionized, both methyl and ribose groups compete successfully with the double bond for the attacking species, that is, are more sensitive. Ribose appears to be somewhat more sensitive than deoxyribose when attached to cytosine. The phosphate group appears to play no important part in the initial radiolytic attack under any of the conditions reported here.

This work was supported by contract AT(04-1) GEN-12 between the Atomic Energy Commission and the University of California.

L. S. MYERS, JUN.
J. F. WARD
W. T. TSUKAMOTO
D. E. HOLMES

Laboratory of Nuclear Medicine and Radiation Biology,
Department of Biophysics and Nuclear Medicine,
and
Department of Radiology,
School of Medicine,
University of California,
Los Angeles.

¹ Myers, jun., L. S., Tsukamoto, W. T., Ward, J. F., Holmes, D. E., and Julca, J. R., *Science*, **148**, 1234 (1965).

² Scholes, G., Weiss, J., and Wheeler, C. M., *Nature*, **178**, 157 (1956).

³ Ekert, B., and Monier, R., *Nature*, **184** (B.A.), 58 (1959).

⁴ Scholes, G., Ward, J. F., and Weiss, J., *J. Mol. Biol.*, **2**, 379 (1960).

⁵ Barszcy, D., and Shugar, D., *Acta Biochim. Polonica*, **8**, 455 (1961).

⁶ Ward, J. F., and Myers, jun., L. S., *Radiat. Res.* (in the press).

⁷ Ranadine, N. S., Korgaonkar, K. S., and Sahasrabudhe, M. B., *Proc. Intern. Conf. Peaceful Uses of Atomic Energy, Geneva, 1955*, **11**, 299 (1956).

CHEMISTRY

Advances in Liquid Chromatography

THE development of liquid chromatographic techniques has been restricted because of the non-existence of sensitive detection systems. Apart from the tedious manual analysis of evaporation and determination by weight or titration the only available continuous method has been ultra-violet absorption. In this process, the sample, for example amino-acid, peptide or protein, is developed by an external reagent like ninhydrin to give strong absorption in the ultra-violet. This procedure is carried out at a single wave-length and is restricted to those samples with the required characteristic.

A detection system based on a moving wire has been developed which continuously monitors the effluent of a liquid chromatographic column¹. The principle of operation is similar to that reported by James, Scott and Ravenhill². The system is applicable to all relatively involatile organic compounds irrespective of structure, for example, fatty acids, amino-acids, peptides, lipids, steroids, etc. A great advantage is that the process is independent of the mobile liquid phase, which could be a mixture of hexane, benzene, ether and water and all the intermediate solvents as quoted in Trappe's elution table. At present this method has detected a concentration of 5 μ g triglyceride in 1 ml. of hexane. It is feasible that this performance can be improved.

The advantages of a continuous detection system possessing this high degree of sensitivity will be immed-

Table 1. INITIAL YIELDS (MOLECULES/100 eV ABSORBED) FOR THE DISAPPEARANCE OF THE PYRIMIDINE CHROMOPHORE GROUP ON EXPOSURE OF AQUEOUS SOLUTIONS, 2 N SODIUM HYDROXIDE, TO COBALT-60 γ -RADIATION

	Uracil	Thymine	Cytosine	5-Methyl- cytosine
Pyrimidine only	1.6	~0	1.9	~0
Nucleoside	++ (r)	~0 (d)	++ (r)	~0 (d)
Nucleotide	++ (r)	~0 (d)	++ (r) 0.4 (d)	—

r, Ribose compound. d, Deoxyribose compound. ++, Optical density increase on irradiation.

ately recognized by workers in liquid chromatography as being of value for the improvement of liquid columns.

Present sample injections are of the order 0.5–10 ml. With the improved sensitivity of the wire detector, injections of the order 10–100 μ l. will be sufficient for quantitative analyses. The reduction in sample size makes practical the use of high-efficiency micro or capillary columns for analytic separations. The high performance of these columns will give a reduction in analysis time by reducing column length and/or increasing the velocity of the moving liquid phase, resulting in an analysis time comparable to a gas-liquid chromatographic separation.

A liquid-chromatographic column requires a stationary phase which can be either solid (for example, alumina, silicic acid) or liquid (for example, water). In using micro or capillary columns it will be necessary to select the operating conditions to minimize the loss of stationary liquid phase via column bleed. When the stationary phase is a solid, column bleed is negligible. The column is, therefore, stable, and can be used for many analyses.

B. MAKIN
F. P. SPEAKMAN

W. G. Pye and Co., Ltd.,
Cambridge.

¹ U.K. Patent No. 998,107.

² *Chem. and Indust.*, 746 (May 1964).

Microstructure of Fibrous Sulphur

PRINS *et al.*^{1–4} have shown, using X-ray diffraction, that fibrous sulphur (S_8) is made up of a long-chained polymeric component (S_μ) together with a crystalline allotrope (S_γ). Prins *et al.*² have also proposed a model for the polymer in which the sulphur atoms are arranged in a helical structure with $3\frac{1}{2}$ atoms per turn; later, Prins and Tuinstra³ suggested the possibility of a spiral superstructure of these helices, with a longitudinal repeat distance of 78 Å.

The similarity between the molecular structure thus described and that of some synthetic carbon-chain polymers⁵ suggested to us that another feature sometimes found in drawn polymers, namely, the presence of a fibrillar structure as demonstrated by electron microscopy⁶, might also be observed in fibrous sulphur.

Sulphur fibres a few μ in diameter were therefore prepared by drawing directly from the melt, using a glass rod; the melt consisted of sulphur of 99.99 per cent purity, maintained at a temperature in the range 250°–300° C. These air-quenched fibres were first examined directly in the electron microscope. As expected, the effects of high vacuum and the heating action of the electron beam led to evaporation and partial destruction; during this process, however, it was possible to observe and record a characteristic macro-fibrillar structure in the specimen (Fig. 1). These macro-fibrils, of diameter about 2000 Å, appeared to resist evaporation longer than the rest of the specimen, which presumably consisted of S_8 . They also repelled one another laterally, probably as a result of electrostatic charging in the electron beam. This effect can be seen in an extreme form at A, in which a macro-fibril has broken away from the fibre along part of its length and bowed outwards.

In one or two cases these macro-fibrils could be seen to consist of bundles of fibrils, about 250 Å in diameter. At the edge of the fibre, single fibrils of about the same size extended perpendicularly to the surface (B, Fig. 1); it seems probable that they were held in this position by their electrostatic charge, which, in combination with the sublimation effect, produced stretching, thinning and eventual disappearance of the fibrils.

The surface of sulphur fibres prepared as already described was investigated by means of single-stage carbon replicas; carbon was evaporated directly on to the fibre surface, followed by removal of the sulphur by slow sublima-

tion *in vacuo*. The surface structure appeared to consist (Fig. 2) of fibrils running parallel to the length of the fibres with a diameter of 370 ± 10 Å. Some of these showed a still finer structure (A, Fig. 2), consisting of dark, approximately parallel lines about 80 Å apart. These may have been micro-fibrils, but the precise interpretation is not yet clear.

During the preparation of air-quenched specimens it was found that variations in melt temperature and strain rate could lead to the production of fibres with a wide range of mechanical properties; some permitted visco-elastic extension to very high ratios at low stress, while in others the stress rose rapidly until a fully stress-hardened relatively inextensible fibre was formed. This behaviour is typical of those synthetic polymers in which stress-induced crystallization occurs during drawing⁷.

In an attempt to obtain greater reproducibility, water-quenched fibres were made by pouring molten 99.999 per cent sulphur into cold water; it was found that, for a melt temperature of 250° C, these could always be fully extended and stress-hardened by hand at moderate strain rates.

In the as-quenched condition little or no surface structure was visible in the electron microscope, but fully stress-hardened fibres showed a well-developed fibrillar surface (Fig. 3), the fibrils having a mean diameter of 300 ± 25 Å. They were often curled, as though broken under tensile stress.

An exactly similar fine structure to that shown at A in Fig. 2 was also found, although the line spacing, at about 50 Å, was somewhat smaller.

The main difficulty in preparing single-stage carbon replicas of sulphur fibres lay in subliming the sulphur

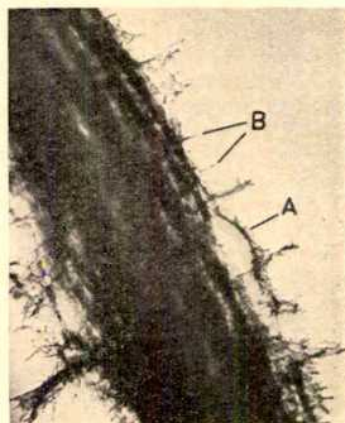


Fig. 1. Transmission micrograph of air-quenched sulphur fibre A, macrofibril; B, fibrils ($\times 8,000$)

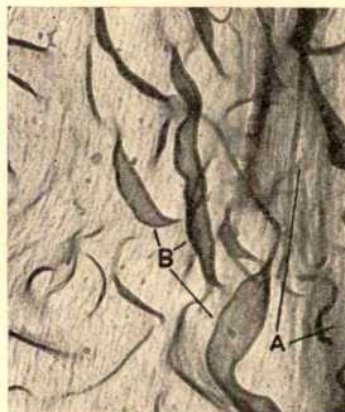


Fig. 2. Carbon replica of air-quenched fibre A, fine structure; B, replica distortion ($\times 20,000$)



Fig. 3. Carbon replica of water-quenched drawn fibre, showing fibrillar surface structure ($\times 12,000$)

sufficiently slowly to prevent distortion of the carbon film. At a pressure of 10^{-4} torr, a temperature of 60° – 80° C gave complete sulphur removal in 1 h, in most cases without replica distortion. Fig. 2 shows some examples (at B) of the type of distortion usually produced; the fibrillar structure is on a much finer scale, and is apparently unaffected.

It therefore appears that three distinct levels of sub-structure in fibrous sulphur can be detected in the electron microscope. The long polymer chains postulated from X-ray evidence are, we suggest, aggregated into fibrils 250–400 Å in diameter, perhaps via micro-fibrils 50–100 Å in diameter. These fibrils then bunch together into macro-fibrils 2000 Å in diameter. The nature of long carbon chain polymer fibres is by no means fully understood, particularly in regard to their crystallinity, and the study of similar fibres composed of a single element, sulphur, may help to throw some light on this problem.

This work was carried out during the tenure of a United Nations Fellowship by one of us (A.J.). We also thank the Sulphur Institute for a gift of high-purity sulphur.

A. JASKOLSKA*
A. E. B. PRESLAND

Department of Chemical Engineering
and Chemical Technology,
Imperial College of Science
and Technology,
London, S.W.7.

* Present address: Institute of Sulphur Technology, Rydygiera 8, Warsaw Poland.

¹ Prins, J. A., and Schenk, P. A. M., *Physica*, **22**, 770 (1956).

² Prins, J. A., Schenk, P. A. M., and Wachters, *Physica*, **23**, 746 (1957).

³ Prins, J. A., and Tuinstra, F., *Physica*, **29**, 328 (1963).

⁴ Prins, J. A., and Tuinstra, F., *Physica*, **29**, 884 (1963).

⁵ Hearle, J. W. S., and Peters, R. H., *Fibre Structure*, chap. 4 (Textile Institute, Manchester, 1963).

⁶ Ref. 5, Chap. 8.

⁷ Ref. 5, Chap. 14 and Fig. 14. 10.

Infra-red Emission Spectra of Minerals

THIS study was undertaken to explore the feasibility of measuring infra-red emission spectra of bulk, opaque minerals. As this involved the examination of the rough, irregular surfaces of bulk specimens at temperatures close to room temperature, emission methods using conventional spectrometers¹ were not applicable. The emittance of specimens near room temperature is too low to operate dispersion spectrometers.

A Block Model I-4T interferometer spectrometer was used, with the detector at about 30° . The theory and

operation of the instrument are described elsewhere^{2,3}. Repetitive interferometric spectra of specimens at about 40° were digitized and coherently added at a rate of 2 scans per second with a 'Coadder' (Block Engineering Co.) in order to enhance the signal. The cumulative spectrum was then processed with a wave analyser, to result in plots of emittance against wave-number. The resolution was 40 cm^{-1} and is shown at R in the figures. The ordinates are arbitrary and are displaced in Figs. 2 and 3 to avoid overlapping of spectra.

Fig. 1 shows the results of the coherent addition of spectra of asbestos, each plot except that marked 1 representing the summation of several individual scans. The spectrum 'grows' with increasing number of scans, so that detailed spectra can be obtained even though the emittance of the specimen is very low. Figs. 2 and 3 show other examples of interferometric infra-red emission spectra of homogeneous bulk mineral specimens. In each case the spectrum shown is the sum of multiple scans. Detailed structure is revealed, indicating the feasibility of developing the method for the direct examination of bulk specimens.

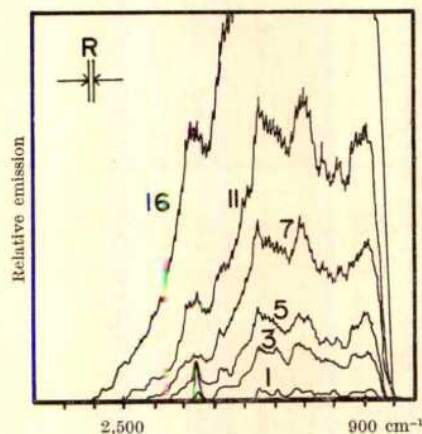


Fig. 1. Infra-red emission spectra of asbestos fibres. The number by each spectrum indicates the number of scans taken

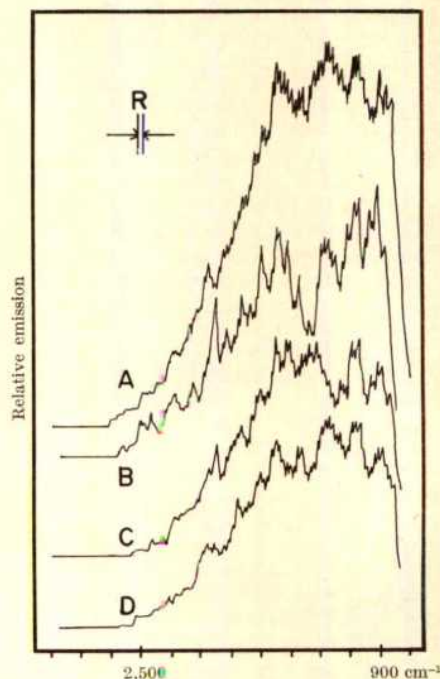


Fig. 2. Infra-red emission spectra of bulk mineral specimens. The specimen and number of scans are: A, nodular turquoise from Arizona, 120; B, interior of turquoise nugget from Nevada, 180; C, jade, 60; D, Psilomane, 32

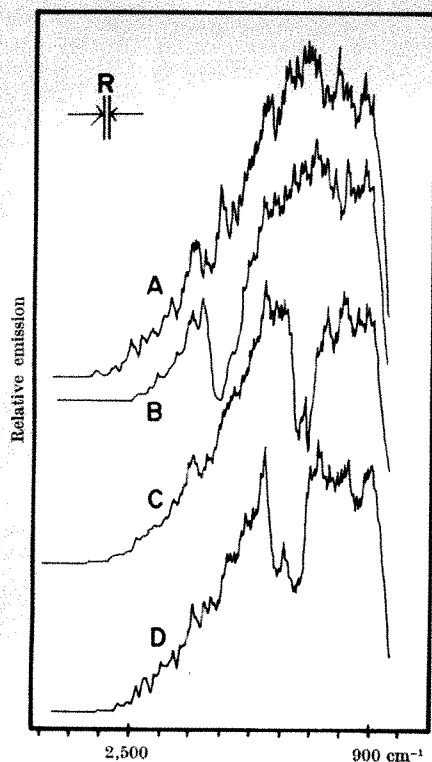


Fig. 3. Infra-red emission spectra of bulk mineral specimens. A, azurite; B, rhodocroite; C, muscovite; D, calcite. 120 scans were taken for each spectrum.

The emission was monitored from areas of about 1 cm diameter on the surface of each sample. The coherent addition of spectra, however, will permit examination of much smaller areas, so that inhomogeneous specimens or small homogeneous specimens could be examined.

This work was supported by U.S. Public Health Service grant AP 00211-01A2. I thank Block Engineering Co. for their help.

M. J. D. Low

School of Chemistry,
Rutgers, The State University,
New Brunswick, N.J.

¹ Low, M. J. D., and Inoue, H., *Anal. Chem.*, **36**, 2397 (1964).

² Persky, M. J., "Atmospheric Infrared Optics - Flux Measurements," Report AFRL 63-439 (1963).

³ Block, L. C., and Zachor, A. S., *Appl. Optics*, **3**, 209 (1964).

Nitroso Formaldehyde

In recent papers by McGrath and McGarvey^{1,2} a new spectrum was reported and the carrier identified as the methoxyl radical. This spectrum, consisting of diffuse bands at 2064 Å, 2036 Å and 1999 Å, was obtained from the flash photolysis of methyl nitrite. Under presumably adiabatic conditions McGrath and McGarvey found that the spectrum appeared at short delay and the intensity increased to a maximum within 2.5 msec.

We find, under isothermal conditions using a 2600 J flash, that the spectrum reaches maximum intensity 70–100 μsec after flashing and that a fourth band at about 1985 Å is also present. The latter band appears to be present in McGrath and McGarvey's photograph but is partially obscured by a group of silicon reversal lines.

We feel that the identification of the spectrum is erroneous since it can be obtained by flashing—in the presence of nitric oxide and excess nitrogen—formaldehyde, formic acid, methyl formate, acetaldehyde or glyoxal. In those cases investigated in detail, namely, glyoxal and formaldehyde, the spectrum reached maximum intensity about 60 μsec after flashing and slowly disappeared over a period of 100 msec. In experiments with glyoxal, an

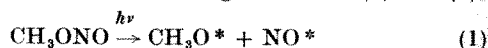
aqueous sodium nitrite filter with a cut-off at 4000 Å was used to ensure that excitation occurred only through the 4000–5000 Å bands of glyoxal.

Further confirmation that these spectra arise from a common carrier was found by flashing fully deuterated formaldehyde with nitric oxide in the presence of nitrogen and also fully deuterated methyl nitrite. In both cases the spectra observed were the same. The effect of deuteration on the 2064 Å band was a 6 Å shift to shorter wavelength and with sharpening and apparent splitting. Little difference was noted in the 2036 Å band, but the 1999 Å band disappeared and the 1985 Å band was sharper and more intense. No new bands were observed when CHDO was flashed with nitric oxide. However, this does not necessarily prove the existence of only one hydrogen atom in the carrier since the small extent of the shifts coupled with the diffuse character of the bands could well obscure new bands.

The compounds giving rise to this spectrum are all known to produce formyl radicals on flashing^{3,4}. This was confirmed in the case of glyoxal but it was found that formyl radicals could not be observed when mixtures of glyoxal and nitric oxide were flashed isothermally. In this case the spectrum of HNO appeared at 14 μsec delay, reached a maximum at about 100 μsec then decayed over a period of 46 msec. Both glyoxal and nitric oxide were depleted to a small extent by the flash.

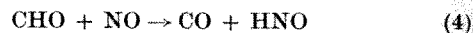
Continuous photolysis of 40 mm glyoxal and 80 mm nitric oxide at 18° C, using a 600-W medium pressure mercury arc, resulted in the decomposition of 33 mm glyoxal and the reaction of 20 mm nitric oxide after 90 min photolysis. The major gaseous products were carbon monoxide and nitrogen with small amounts of nitrous oxide and nitrogen dioxide (tentatively identified) and a trace of carbon dioxide. In the absence of nitric oxide the same amount of glyoxal (33 mm) was decomposed in 15 min. The gaseous products in this case were carbon monoxide and a trace of hydrogen as found by Norrish and Griffiths⁵.

The flash photolysis experiments provide reasonably strong evidence that the 2064–1985 Å spectrum is that of CHONO. Light absorbed by methyl nitrite at its maximum (2150 Å) corresponds to 130 kcal/mole, while only 39 kcal/mole⁶ is required to break the O–NO bond. Some of the excess energy may appear as activation of CH₂O, which could undergo reactions (2) and (3):

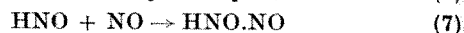
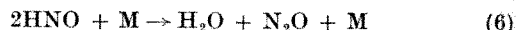


It is presumed that formyl radicals generated by flashing the other compounds giving rise to the 2064–1985 Å spectrum also react as in (3).

The formation of HNO may be ascribed to the following two reactions, the latter being similar to that proposed by Fischer and Buchanan⁶ for the unimolecular decomposition of formyl fluoride:



The formation of nitrogen, nitrous oxide and nitrogen dioxide from the photolysis of glyoxal/nitric oxide mixtures is similar to the results obtained by Strausz and Gunning⁷ for the photolysis of formaldehyde/nitric oxide mixtures. They proposed that reaction (4) was followed by reactions (6), (7) and (8):



If our identification of the spectrum is correct, CHONO may be removed by a similar reaction scheme, with reaction (5) as the primary decomposition reaction.

One of us (I. M. N.) was supported in this work by a scholarship from the Public Service Board of Australia.

I. M. NAPIER
R. G. W. NORRISH

Department of Physical Chemistry,
University of Cambridge.

McGrath, W. D., and McGarvey, J. J., *Nature*, **201**, 991 (1964).
McGrath, W. D., and McGarvey, J. J., *Trans. Faraday Soc.*, **60**, 2196 (1964).
McKellar, J. F., and Norrish, R. G. W., *Proc. Roy. Soc. A*, **254**, 147 (1960).
Hertzberg, G., and Ramsey, D. A., *Proc. Roy. Soc. A*, **233**, 34 (1956).
Fray, P., *Trans. Faraday Soc.*, **51**, 1387 (1955).
Fischer, G., and Buchanan, A. S., *Trans. Faraday Soc.*, **60**, 378 (1964).
Strausz, O. P., and Gunning, H. E., *Trans. Faraday Soc.*, **60**, 347 (1964).
Norrish, R. G. W., and Griffiths, J. G. A., *J. Chem. Soc.*, 2829 (1928).

Use of 'Sephadex' Gel for the Estimation of Molecular Weight of Humic Substances in Natural Water

THOUGH little is actually known about the shape of humus molecules, and only a few experiments have been described^{1,2} on the fractionation of humus on 'Sephadex' gel (Pharmacia, Uppsala, Sweden), there is strong evidence or the existence of a relationship between the elution rate through the 'Sephadex' column and the molecular weight^{3,4}.

In the course of studies on the nature of the soluble humic substances that cause the brown-yellow colour of Norwegian surface-water, 'Sephadex' gel has been used in attempts to fractionate and characterize these substances according to their molecular size.

The molecular exclusion properties of the different gel types are reported by the manufacturers of 'Sephadex' to be as follows: 'Sephadex G-50' excludes solutes with molecular weights exceeding 8,000–10,000; correspondingly 'G-75' excludes solutes with molecular weights greater than 10,000–50,000, whereas with grades 'G-100' and 'G-200' the lower molecular weight limits for complete exclusion are 100,000 and 200,000 respectively. The molecular weights of humus reported in the literature vary from 600–300,000 (ref. 5).

Water from a typical moorland area was filtered through glass fibre pads (Whatman 'F/A') and concentrated by vacuum evaporation (400:1) at 35° C. During concentration, approximately 18 per cent of the total dry matter of the concentrate was precipitated. This was removed by filtration before proceeding further. Results of some analyses of the water sample and its concentrate are shown in Table 1. Portions of the filtered concentrate were passed through columns of 'Sephadex' and were eluted with distilled water containing a trace of thymol to prevent bacterial growth. The fractions obtained were analysed for colour, total iron, hardness (EDTA method) and permanganate oxidation value (COD).

Attempts to isolate small humus molecules (mol. wt. < 8000), by means of lower 'Sephadex' grades 'G-25' and 'G-50', were unsuccessful and resulted in an irreversible desorption or coagulation, visible as brownish-coloured flocs all through the bed material. As far as I am aware no irreversible phenomenon of this kind has previously been reported^{6,7}. Flotation experiments with gel 'G-50', containing the flocculated humus, seem to indicate that the phenome-

Table 1. COMPOSITION OF FILTERED MOORLAND WATER BEFORE AND AFTER CONCENTRATION

Date	Colour mg Pt/l.	Filtered water				Hardness (total) mg CaO/l.	Concentrated filtered water	
		GOD (K ₂ Cr ₂ O ₇) mg O/l.	Fe++ μg/l.	Fe+++ μg/l.	pH		pH	Evaporation residue mg/ml.
May 19 62	13 1	90	12	5 4	5.9	4.6	10.5	

non is the result of coagulation, probably caused by desalting due to the small negative charge on the 'Sephadex'. When the humus concentrate was applied to columns of 'Sephadex G-75' and 'Sephadex G-100' the separation shown in Fig. 1 a and 1 b resulted. In each case the humic substances are separated into two peaks with distribution coefficients (K_D) of 0 and approximately 1, respectively. The fractions representing peak C (Fig. 1 b) were evaporated in vacuum to a suitable volume and filtered through a column of 'Sephadex G-200'. This filtration (Fig. 1 c) resulted in only one peak with distribution coefficient 0.1. These experiments indicate that apart from the precipitate formed during concentration the organic matter in typical moorland water consists of at least two types of humic substances that differ considerably in molecular size. The larger molecular fraction probably has a molecular weight of between 100,000–

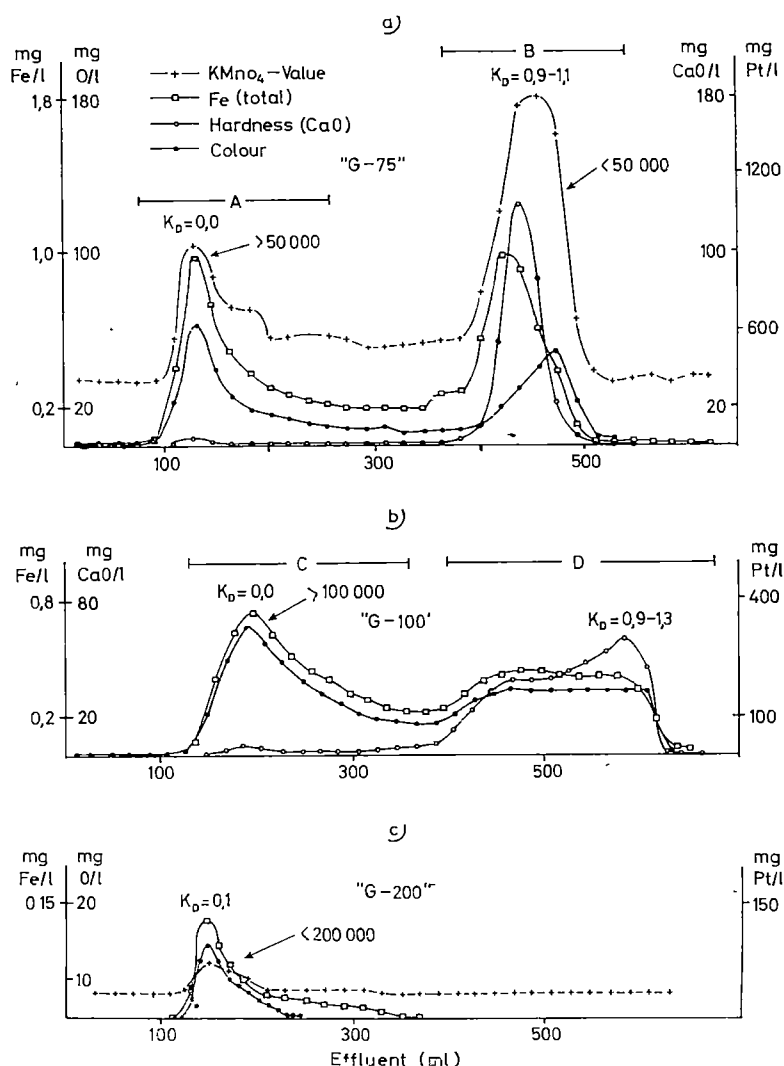


Fig. 1 Fractionation of water-soluble humic substances on 'Sephadex' with distilled water as eluant.
(a) 'Sephadex G-75'. Column dimensions, 49 × 3.3 cm. Sample: 4 ml. water concentrate containing 42.3 mg solute. (b) 'Sephadex G-100'. Column dimensions, 47 × 3.6 cm. Sample: 4 ml. water concentrate containing 2.8 mg solute. (c) 'Sephadex G-200'. Column dimensions, 49 × 3.6 cm. Sample: Peak C from the 'G-100' separation concentrated in vacuo to 2 ml.

200,000 and the smaller possibly below 10,000. Both these types are believed to consist of organic complexes of ferrous iron⁸. It is interesting to note that one of these molecular types contains calcium. This has been confirmed by continuous electrophoresis experiments, which indicate that about 3/4 of the calcium in the fractions of peak *B* (Fig. 1 *a*) is associated with the coloured iron complexes. The manner in which this calcium is bound to the humus is unknown.

E. T. GJESSING

Norwegian Institute for Water Research,
Blindern, Norway.

¹ Ferrari, G., and Dell'Agnola, G., *Soil Sci.*, **96**, 418 (1963).

² Posner, A. M., *Nature*, **198**, 1161 (1963).

³ Flodin, P., *Dextran Gels and their Applications in Gel-filtration* (Pharmacia, Uppsala, Sweden, 1962).

⁴ Whitaker, J. R., *Anal. Chem.*, **35**, 1950 (1963).

⁵ Scheffer, F., and Ulrich, B., *Humus und Humusdüngung*, **1**, 39 (Enke, Stuttgart, 1960).

⁶ Gelotte, B., *J. Chromatog.*, **3**, 330 (1960).

⁷ Glazer, A. N., and Welner, D., *Nature*, **194**, 862 (1962).

⁸ Gjessing, E., *Limnol. Oceanogr.*, **9**, 272 (1964).

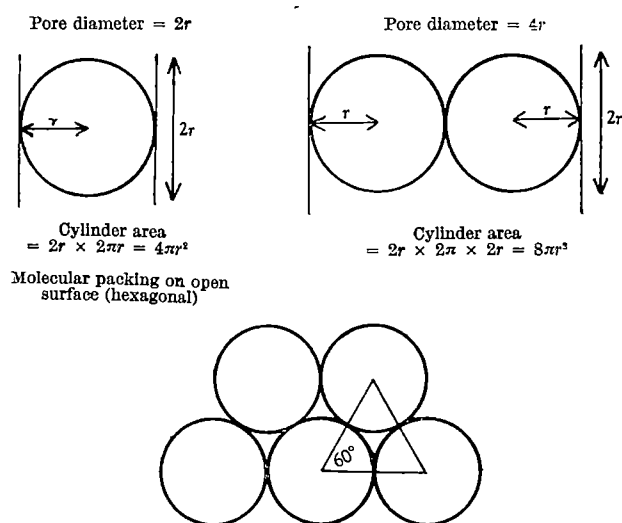
Errors in the Calculation of Surface Area and Average Pore Radius

In the determination of surface areas from adsorption data the area of the adsorbed molecules A_m is usually calculated from the liquid density, assuming hexagonal close packing in the liquid state on the surface, namely:

$$A_m = 1.091 \left(\frac{M}{d_l N} \right)^{\frac{2}{3}} \quad (1)$$

where M is the molecular weight of adsorbate, d_l is liquid density in g/cm³, N is Avogadro's number.

The constant 1.091 is a packing factor to account for hexagonal close packing. This calculation assumes that the molecule only covers an area on one flat, or almost flat, surface. In very small pores, which occur in certain zeolites, charcoals, and silica², this is not true. To take an extreme case, if only one molecule can fit into the pore touching the walls (as shown in Fig. 1), it actually covers an area of $4\pi r^2$, the area of the cylinder into which it fits,



The area of the triangle is the area that three 60° angle segments of the projected area of one molecule cover; that is, half the total projected area. Area of triangle = $\frac{1}{2} \times \text{base} \times \text{height} = \frac{1}{2} \times 2r \times \sqrt{3}r = \sqrt{3}r^2$. Therefore area covered by a molecule = $2\sqrt{3}r^2$. Area covered by two molecules = $4\sqrt{3}r^2$.

$$\frac{\text{True area}}{\text{Apparent area}} = \frac{4\pi r^2}{2\sqrt{3}r^2} = \frac{2\pi}{\sqrt{3}} = 3.63$$

$$\frac{\text{True area}}{\text{Apparent area}} = \frac{8\pi r^2}{4\sqrt{3}r^2} = \frac{2\pi}{\sqrt{3}} = 3.63$$

Fig. 1. Apparent and actual molecular coverage in molecular sized pores

where r is the radius of the pore. The simple adsorption theory assumes it to cover an area of $2\sqrt{3}r^2$. Thus the actual area is $\frac{2\pi}{\sqrt{3}}$ ($= 3.63$) times the apparent area.

If the pore is two molecular diameters wide, the factor is again 3.63. For increasing pore size the factor will decrease quickly to unity, but substances with pore approaching molecular size will show apparent surface areas which are really too low. Silica samples of this type have been shown to have areas of the order c 300 m²/g or less¹ compared with commercial silica gels with areas of 600–700 m²/g. This is the reverse of what might have been expected assuming a similar total pore volume in the two types of silica.

Average pore radius is often calculated from total pore volume V_p cm³/g and surface area S cm²/g. Assuming the pores to be open cylinders of radius \bar{r}_p cm, then

$$\bar{r}_p = \frac{2V_p}{S} \quad (2)$$

$$\text{However } V_p = \frac{X_T}{1,000d_l} \text{ cm}^3/\text{g} \quad (3)$$

where X_T is adsorption at saturation in mg/g and d_l is density of liquid adsorbate in g/cm³

$$\text{Also } S = \frac{X_m N A_m}{1,000 M} \text{ cm}^2/\text{g} \quad (4)$$

where X_m is monolayer adsorption in mg/g and the other terms are as previously defined.

$$\text{Then } \bar{r}_p = \frac{2X_T}{1,000d_l} \frac{1,000 M}{X_m N A_m} = \frac{X_T}{X_m} \frac{2M}{d_l A_m N} \text{ cm} \quad (5)$$

Substances with very fine pores, similar to those already mentioned, give type I adsorption isotherms with very sharp 'knees'. In these cases the monolayer capacity (calculated by the use of the Langmuir equation) is found to be very nearly equal to the total adsorption. Thus X_m and X_T in equation (5) are equal and this leaves \bar{r}_p as a constant. Substituting the values for nitrogen adsorption

$$\bar{r}_p = \frac{2 \times 28}{0.808 \times 6.03 \times 10^{23} \times 16.2 \times 10^{-16}} = 7.08 \times 10^{-8} \text{ cm} \quad (6)$$

Thus whatever the true pore size might be, a value less than about 7 Å radius cannot be obtained from adsorption data. This error in radius is connected with the error previously described in that 16.2 Å is not the area covered by the nitrogen molecule in these pores.

Combining equations (1) and (5), cancelling X_m and X_T and ignoring the packing factor gives

$$\bar{r}_p = 2 \left(\frac{M}{d_l N} \right)^{\frac{1}{3}}$$

where $\left(\frac{M}{d_l N} \right)^{\frac{1}{3}}$ may be taken to be approximately equal to the molecular diameter D_m . Thus in general pore radius never appears less than twice the adsorbate diameter for calculation. This type of error has also been shown for very fine-pored silica² where radii appear always 7–10 Å by calculation based on nitrogen adsorption, whereas molecular sieve effects show the pores to be mainly 3.5 to 4.8 Å in diameter³.

D. DOLLIMORE
G. R. HEAL

Department of Chemistry
and Applied Chemistry,
Royal College of
Advanced Technology,
Salford, Lancashire.

¹ Dollimore, D., and Heal, G. R., *Trans. Farad. Soc.*, **59**, 2386 (1963).

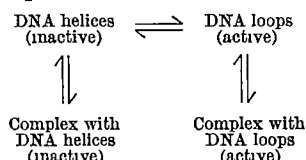
² Heal, G. R., thesis, University of London, 68 (1962).

³ Heal, G. R., thesis, University of London, 95 (1962).

BIOCHEMISTRY

Correlation of the Binding to DNA Loops or to DNA Helices with the Effect on RNA Synthesis

RECENT investigations have revealed that template DNA is found natively in the form of single-stranded loops during the active transcription of selected portions of the genome in higher organisms^{1,2}. Conversely, during repression of such transcription the DNA is found natively in the form of double-stranded helices^{1,2}. A variety of organic molecules which function *in vivo* as inhibitors or stimulators of RNA synthesis within pre-selected portions of the genome³ have been shown to be capable of a reversible physical binding to DNA *in vivo* or *in vitro*. Each of these inhibitors or stimulators binds preferentially to either single-stranded or to double-stranded DNA. In every case for which adequate data are available (Table 1), a strong correlation exists between the form of DNA preferred for binding and the effect of the ligand on DNA synthesis within pre-selected portions of the genome. These strong correlations suggest that such ligands may exert their characteristic effects on RNA synthesis by preferentially stabilizing either the inactive helical form or the active loop form of DNA^{2,10} in the equilibrium:



The mechanisms of such preferential binding to either double-stranded helical DNA or to single-stranded loop DNA are little understood. Preliminary thermodynamic analyses have revealed that the equilibrium between the helical and the loop forms of DNA can be shifted during binding by an effect of the ligand on one or more of the physical forces existing within the DNA-solvent system. These forces include: (a) the hydrophobic solute-solvent interactions between DNA and water¹⁹; (b) the hydrogen bond interactions between the complementary bases of opposing DNA strands²⁰; (c) the electrostatic charge interactions between the phosphate groups of the same or opposing DNA strands²¹; (d) the stacking (van der Waal's) interactions between the successive bases of the same or opposing DNA strand²². In addition, the ability of particular ligands to (e) cross-link opposing DNA strands²³ or to (f) fit sterically into certain regions of the DNA molecule²³ is of importance in the inhibition or stimulation of RNA synthesis. Thus, both histone-type inhibitors and actinomycin-type inhibitors bind preferentially to double-stranded helical DNA by utilizing properties (e) and (f)²³. In addition, histones alter physical force (c)³, while actinomycins may alter forces (a), (c) and (d)⁶. By contrast, both testosterone-type stimulators and oestrogen-type stimulators bind preferentially to single-stranded loop DNA by utilizing property (f)²⁴, and by altering physical forces (a) and (d)²⁴. Before such inhibitors or stimulators can bind to DNA and alter the rates of RNA synthesis they must often be first concentrated within the particular sensitive tissue by specific, non-DNA binding agents^{13,25}.

All the foregoing inhibitory or stimulatory ligands (Table 1) except complementary RNA are molecules which are capable of reacting with all portions of the DNA genome non-selectively. RNA by contrast is capable of a selective interaction with specific portions of the DNA genome¹⁷. It is this selective ability which appears to be the basis for its role as the agent of specific de-repression of RNA synthesis during selective transcription of the genome^{1,2,18}. In a similar fashion, polyoma viral DNA binds preferentially to single-stranded host DNA²⁶. The result of such oncogenic viral DNA interaction with the host DNA genome is a de-repression of host DNA synthesis and of host enzyme synthesis^{27,28}. A concurrent selective de-repression of host RNA synthesis is also likely²⁷.

This work was carried out during the tenure of a research career development award (CA-17857) from the U.S. Public Health Service.

JOHN H. FRENSTER

Laboratory of Cell Biology,
Rockefeller Institute,
New York.

- ¹ Frenster, J. H., *Nature*, **206**, 1269 (1965).
- ² Frenster, J. H., *Nature*, **208**, 394 (1965); Frenster, J. H., in *The Chromosomes: Structural and Functional Aspects*, ed. by Dawe, C. J., and Yerganian, G. (Williams and Wilkins, Inc., Baltimore, 1965).
- ³ Akhtar, E. O., Bonner, J., and Ts'o, P. O. P., *J. Mol. Biol.*, **11**, 128 (1965).
- ⁴ Huang, R. C., and Bonner, J., *Proc. U.S. Nat. Acad. Sci.*, **48**, 1216 (1962); Allfrey, V. G., and Mirsky, A. E., *Proc. U.S. Nat. Acad. Sci.*, **48**, 1590 (1962).
- ⁵ Allfrey, V. G., Littau, V. C., and Mirsky, A. E., *Proc. U.S. Nat. Acad. Sci.*, **49**, 414 (1963).
- ⁶ Haselkorn, R., *Science*, **143**, 682 (1964). Reich, E., *Science*, **143**, 684 (1964). Gellert, M., Smith, C. E., Neville, D., and Felsenfeld, G., *J. Mol. Biol.*, **11**, 445 (1965).
- ⁷ Reich, E., Franklin, R. M., Siatkin, A. J., and Tatum, E. L., *Proc. U.S. Nat. Acad. Sci.*, **48**, 1238 (1962).
- ⁸ Lerman, L. S., *J. Cell. Comp. Physiol.*, **64**, Suppl. 1, 1 (1964).
- ⁹ Cohen, S. N., and Yielding, I. K., *J. Biol. Chem.*, **240**, 3123 (1965); *Proc. U.S. Nat. Acad. Sci.*, **54**, 521 (1965).
- ¹⁰ Ts'o, P. O. P., and Lu, P., *Proc. U.S. Nat. Acad. Sci.*, **51**, 17 (1964).
- ¹¹ Kochakian, C. D., Hill, J., and Harrison, D. G., *Endocrinology*, **74**, 635 (1964). Loeb, P. M., and Wilson, J. D., *Clin. Res.*, **13**, 45 (1965).
- ¹² Talwar, G. P., Segal, S. J., Evans, A., and Davidson, O. W., *Proc. U.S. Nat. Acad. Sci.*, **52**, 1059 (1964).
- ¹³ Robert, F., *J. Chim. Phys.*, **60**, 684 (1963).
- ¹⁴ Loeb, L. A., and Gelboin, H. V., *Proc. U.S. Nat. Acad. Sci.*, **52**, 1219 (1964).
- ¹⁵ Berg, P., Kornberg, R. D., Faneher, H., and Dieckmann, M., *Biochem. Biophys. Res. Comm.*, **18**, 932 (1965).
- ¹⁶ Chamberlin, M., and Berg, P., *Proc. U.S. Nat. Acad. Sci.*, **48**, 81 (1962); *J. Mol. Biol.*, **8**, 297 (1964).
- ¹⁷ Schildkraut, C. L., Marmur, J., Fresco, J. R., and Doty, P., *J. Biol. Chem.*, **236**, PC 2 (1961). McCarthy, B. J., and Hoyer, B. H., *Proc. U.S. Nat. Acad. Sci.*, **52**, 915 (1964).
- ¹⁸ Frenster, J. H., *Nature*, **206**, 680 (1965).
- ¹⁹ Herskovits, T. T., Singer, S. J., and Gelduschek, E. P., *Arch. Biochem. Biophys.*, **94**, 99 (1961). Sinanoglu, O., and Abdunur, S., *Fed. Proc.*, **24**, Suppl. 15, S 12 (1965).
- ²⁰ Watson, J. D., and Crick, F. H. C., *Nature*, **171**, 737, 964 (1953).
- ²¹ Kotin, L., *J. Mol. Biol.*, **7**, 3C3 (1963). Schildkraut, C. L., and Lifson, S., *Biopolymers*, **3**, 195 (1965).
- ²² Luzzati, V., Mathis, A., Mason, F., and Witz, J., *J. Mol. Biol.*, **10**, 28 (1964). Holcombe, D. N., and Tinoco, jun., I., *Biopolymers*, **3**, 121 (1965).
- ²³ Wilkins, M. H. F., Zubay, C., and Wilson, H. R., *J. Mol. Biol.*, **1**, 179 (1959). Luzzati, V., and Nicolai, A., *J. Mol. Biol.*, **7**, 142 (1963). Hamilton, L. D., Fuller, W., and Reich, E., *Nature*, **198**, 538 (1963). Lloyd, P. H., and Peacocke, A. R., *Biochim. Biophys. Acta*, **95**, 522 (1964).
- ²⁴ Munck, A., Scott, J. F., and Engel, L. L., *Biochim. Biophys. Acta*, **26**, 397 (1957).
- ²⁵ Noteboom, W. D., and Gorski, J., *Arch. Biochem. Biophys.*, **111**, 559 (1965).
- ²⁶ Axelrod, D., Habel, K., and Eoltan, E. T., *Science*, **146**, 1466 (1964).
- ²⁷ Dulbecco, R., Hartwell, L. H., and Vogt, M., *Proc. U.S. Nat. Acad. Sci.*, **53**, 403 (1965).
- ²⁸ Klt, S., Dubbs, D. R., Anken, M., and Melnick, J. L., *J. Cell Biol.*, **27**, 52A (1965).

Table 1. CORRELATION OF THE PREFERRED FORM OF DNA FOR BINDING WITH THE EFFECT ON RNA SYNTHESIS

Ligand	Preferred form of DNA for binding	Effect of ligand on RNA synthesis
Histones	Double-stranded (ref. 3)	Inhibition (refs. 4, 5)
Polylysine	Double-stranded (ref. 3)	Inhibition (ref. 6)
Actinomycin D	Double-stranded (ref. 6)	Inhibition (ref. 7)
Acridine orange	Double-stranded (ref. 8)	Inhibition (ref. 8)
Chloroquine	Double-stranded (ref. 9)	Inhibition (ref. 9)
Testosterone	Single-stranded (ref. 10)	Stimulation (ref. 11)
Oestradiol	Single-stranded (ref. 10)	Stimulation (ref. 12)
Methylcholanthrene	Single-stranded (ref. 13)	Stimulation (ref. 14)
RNA polymerase	Single-stranded (ref. 15)	Stimulation (ref. 16)
Complementary RNA	Single-stranded (ref. 17)	Stimulation (ref. 18)

Isolation and Amino-acid Sequence of β -LPH from Sheep Pituitary Glands

SEVERAL adenohipophyseal hormones have been demonstrated to possess *in vitro* lipotropic activity; these include growth, adrenocorticotrophic, thyrotrophic, α -melanocyte-stimulating and β -melanocyte-stimulating hormones. Recently, Rudman, Astwood and their colleagues reported the preparation of Fraction H, peptides I and II, and showed these preparations to be lipotropic agents^{1,2}.

In the course of investigating a simplified procedure for the isolation of ACTH from sheep pituitary glands, we have obtained a lipotropic peptide³ which is chemically distinct from ACTH and other known adenylohypophyseal hormones. In this communication, we wish to describe the isolation of another lipotropic peptide (designated as β -LPH) from sheep pituitaries. The complete amino-acid sequence of β -LPH will also be presented here.

The procedure for the isolation of the sheep β -LPH was similar to that previously described³, except that acid-acetone extraction, sodium chloride precipitation and dialysis were carried out at 0° C. In addition, the solution was adjusted to pH 8 before dialysis and the dialysed fluid was maintained at this pH. The insoluble material encountered during dialysis was centrifuged off and the clear supernatant fluid was chromatographed on the carboxymethyl-cellulose (CMC) column. The final step of purification involved chromatography on a cation exchanger IRC-50 column which has been equilibrated with 0.01 M ammonium acetate buffer of pH 4.6. The final product was shown to be homogeneous by various criteria including exclusion chromatography on 'Sephadex G-75', paper chromatography, ultracentrifugational analyses, and terminal amino-acid studies.

The amino-acid content in molar ratios as determined by the automatic amino-acid analyser⁴ is as follows: Lys₁₀, His₂, Arg₄, Asp₄, Thr₄, Ser₅, Glu₁₆, Pro₅, Gly₈, Ala₁₃, Val₂, Met₂, Ileu₁, Leu₄, Tyr₃, Phe₃, Try₁. The calculated molecular weight based on this composition is in good agreement with that obtained by sedimentation equilibrium investigations. Terminal analysis using the carboxypeptidase procedure indicates the sequence . . . Gly-Glu(NH₂) at the COOH terminus. By means of the phenylisothiocyanate method, the NH₂-terminal sequence Glu-Leu . . . was established.

Five peptide fragments were obtained by the reaction of β -LPH with cyanogen bromide⁵ and purified by CMC column chromatography. Amino-acid and terminal group analyses revealed that the five peptide fragments were derived from the parent molecule with the amino-acid sequences as follows: I, 1-47; II, 1-65; III, 66-90; IV, 48-65; and V, 48-90 (Fig. 1). After these fragments were subjected to enzymatic digestions with pepsin, trypsin and chymotrypsin; the digests were purified by zone electrophoresis on paper followed by paper chromatography. The purified peptides were analysed for amino-acid composition, NH₂- and COOH-terminal residue. Most of the purified peptides were also subjected to the step-wise degradation procedure of Edman⁶. From these results, the complete amino-acid sequence of β -LPH was proposed as shown in Fig. 1.

It is of great interest to note the presence in β -LPH of a core, Met-Glu-His-Phe-Arg-Try-Gly, a sequence identical with that held in common by adrenocorticotrophic and melanotropic hormones from various species. In addition, the amino-acid sequence 37-58 in β -LPH is identical to that of human^{7,8} β -MSH except that amino-acid residues in positions 42 and 46 are serine and lysine instead of glutamic acid and arginine as in the case of the human hormone.

The detailed account of this work will be published in the *Proceedings of the Sixth Pan-American Congress on Endocrinology*. This work was supported in part by grant (G-2907) from the National Institutes of Health U.S. Public Health Service. L. B. and M. C. wish to acknowledge fellowships from the Guggenheim Foundation and the Jane Coffin Childs Memorial Fund for Medical Research respectively.

CHOH HAO LI
LIVIO BARNAFI
MICHEL CHRÉTIEU
DAVID CHUNG

Hormone Research Laboratory,
University of California,
Berkeley, California.

¹ Rudman, D., *J. Lipid Res.*, **4**, 119 (1963).

² Friesen, H., *Metabolism*, **10** (Part 2), 1214 (1964).

³ Birk, Y., and Li, C. H., *J. Biol. Chem.*, **239**, 1048 (1964).

⁴ Spackman, D. H., Stein, W. H., and Moore, S., *Anal. Chem.*, **30**, 1190 (1958).

⁵ Gross, E., and Witkop, B., *J. Biol. Chem.*, **237**, 1856 (1962).

⁶ Edman, P., *Acta Chem. Scand.*, **4**, 277 (1950).

⁷ Harris, J. I., *Nature*, **184**, 187 (1959).

⁸ Pickering, B. T., and Li, C. H., *Biochim. Biophys. Acta*, **74**, 156 (1963).

Reactivity of the ϵ -Amino Group of Collagen

In view of the frequency with which the results of Bowes and Moss¹ are quoted as indicating that only about 60 per cent of the ϵ -amino groups of lysine in collagen are available for reaction with fluorodinitrobenzene (FDNB), we should like to put on record the following observations.

Out of 27 millimoles lysine per 100 g collagen², 13-14 millimoles were recovered as ϵ -DNP-lysine. After correction for hydrolytic losses based on recovery of ϵ -DNP-lysine added to collagen and hydrolysed under the same conditions, this represents reaction with 17 millimoles per 100 g. Analyses by the Moore and Stein³ procedure revealed only 3 to 4 millimoles of lysine in the DNP-protein, indicating much more extensive reaction and leaving 10-11 millimoles of ϵ -DNP-lysine unaccounted for. This, together with the relative constancy of the amounts of ϵ -DNP-lysine recovered regardless of the pre-treatments given to the collagen, led to the suggestion that "failure to account for all the lysine as the ϵ -DNP derivative was not primarily due to non-reactivity of the lysine to FDNB" but to the fact that " ϵ -DNP-lysine is much less stable to acid when combined in the collagen than when present as the free amino-acid".

Since that time various values have been reported for the availability of ϵ -amino groups in skin collagen, for example, Sykes⁴, 70-80 per cent; Solomons and Irving⁵, 64-70 per cent; Hormann⁶, 90 per cent; Hallsworth⁷, up to 70 per cent; and Courts⁸, 90-100 per cent in gelatine derived therefrom. Using a procedure based on that of Courts we ourselves have been able to account for all the lysine in DNP-collagen as ϵ -DNP-lysine⁹.

It is possible that variations in reactivity reported are, at least in part, related to the difficulties involved in establishing a reliable correction factor for losses of

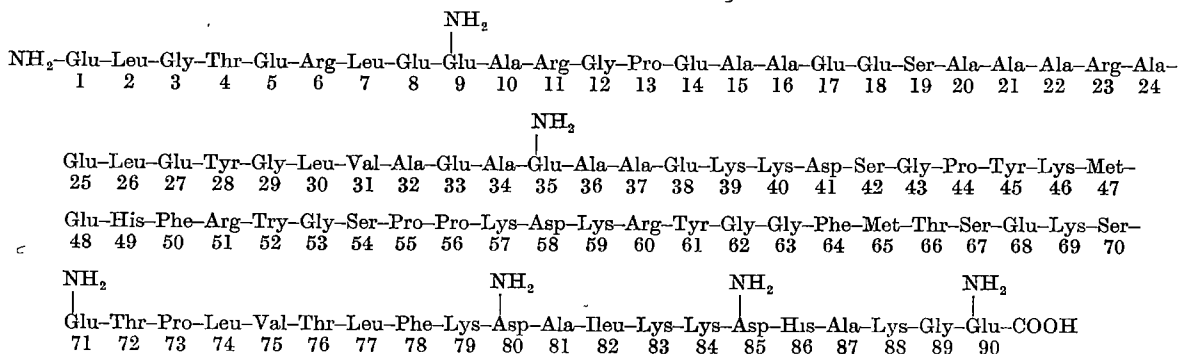


Fig. 1. The amino-acid sequence of the sheep pituitary β -LPH

DNP-lysine during hydrolysis and separation. There seems to be little evidence that under favourable conditions reaction of FDNB with ϵ -amino groups of collagen does not approach completion.

J. H. BOWES
A. S. RAISTRICK

British Leather Manufacturers' Research Association,
Milton Park, Egham, Surrey.

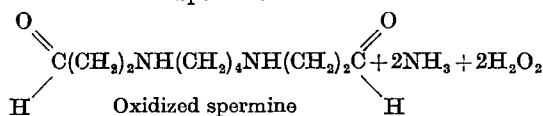
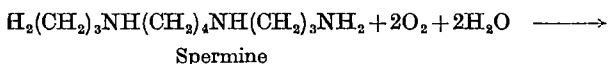
J. A. MOSS

Tunstall Laboratory,
Shell Research Limited,
Broad Oak Road,
Sittingbourne,
Kent.

Bowes, J. H., and Moss, J. A., *Biochem. J.*, **55**, 735 (1953).
Bowes, J. H., Elliott, R. G., and Moss, J. A., *Biochem. J.*, **61**, 143 (1955).
Loore, S., and Stein, W. H., *J. Biol. Chem.*, **192**, 663 (1951).
Lykes, R. L., *J. Soc. Leather Trades' Chem.*, **36**, 267 (1952).
Solomons, C., and Irving, J., *Biochem. J.*, **68**, 499 (1958).
Jermann, H., *Leder*, **13**, 79 (1962).
Gallsworthy, A. S., *Biochem. J.*, **93**, 255 (1964).
Gourts, A., *Biochem. J.*, **53**, 70 (1954).
Bowes, J. H., and Raistrick, A. S., *J. Amer. Leather Chem. Assoc.*, **59**, 201 (1964).

Antiviral Action of Oxidized Spermine— Inactivation of Plant Viruses

SPERMINE, $\text{NH}_2(\text{CH}_2)_3\text{NH}(\text{CH}_2)_4\text{NH}(\text{CH}_2)_3\text{NH}_2$, a naturally occurring polyamine, is oxidized by purified serum amine oxidase as follows (ref. 1):



Oxidized spermine (OS) was shown to inhibit the growth of *Mycobacterium tuberculosis*^{2,3}, *Escherichia coli*, and *Staphylococcus aureus*⁴, and to inactivate various other bacteria⁵ and bacterial viruses⁶. It was therefore of interest to study also the effect of OS on several plant viruses.

The following virus inocula were used: Potato virus Y (PVX) (a yellow strain) was purified from *Nicotiana glutinosa* L. leaves, by rate-zonal, density-gradient centrifugation, after charcoal adsorption⁷. Tobacco mosaic virus (TMV) was purified from *N. tabacum* L. leaves by differential centrifugation⁸, or used in the form of a crude extract. Alfalfa mosaic virus (AMV) was obtained as a crude extract from *N. glutinosa*. The crude extracts, TMV and AMV, were prepared by grinding 0.5 g leaves in ml. 0.06 M phosphate buffer pH 7.0; TMV extract was further diluted 1:25 in the same buffer. OS was prepared by oxidation of spermine hydrochloride with purified def-plasma amine oxidase⁶.

The reaction mixture, containing OS and virus, was incubated for 90 min at 37° C, and diluted to 4 ml. with 0.6 M phosphate buffer at pH 7.0. In the control, OS was replaced by an equal volume of phosphate buffer, or phosphate buffer and amine oxidase.

Test and control mixtures of TMV and AMV were assayed by rubbing with gauze pads on 16 opposite half leaves of *N. glutinosa*, or *Phaseolus vulgaris* var. 'Pinto', respectively, previously dusted with carborundum. PVX was assayed on the opposite leaves of *Gomphrena globosa*. Lesions were counted 3–5 days after appearance and the results were analysed by the 'sign-test'⁹.

With all three viruses tested, either purified or as crude extracts, OS caused a significant reduction in lesion number (Table 1). Residues of amine oxidase in the reaction mixture did not affect infectivity of TMV.

Table 1. INACTIVATION OF TMV, PVX AND AMV BY OXIDIZED SPERMINE (OS)

Virus	Reaction mixture ml. virus	ml. OS (600 µg/ml.)*	Average No. of lesions per half leaf†	Reduction of lesions No. in log
TMV, purified (0.02 mg/ml.)	0.2	0.2	8	95§
TMV, purified†	0.2	0.2	31	211§
TMV, crude extract	0.2	0.8	3	58§
PVX, purified	0.2	0.2	1	13§
AMV, crude extract	0.4	0.4	0.13	5.69§

* Based on the amount of spermine added to the original incubation mixture.

† The number of lesions per half leaf represents the average of 16 half leaves.

‡ In this experiment the control contained amine oxidase at same concentration as OS.

§ Significant at 1 per cent level.

When increasing concentrations of OS were incubated with a constant concentration of TMV (0.02 mg/ml.), maximum activity was observed around 100 µg OS in 0.6 ml. of the reaction mixture (Fig. 1). The rate of viral inactivation by OS increased by prolonging the incubation of the reaction mixture (Fig. 2).

The inactivating effect was not eliminated when free OS was removed from the incubated reaction mixture by 2 cycles of centrifugation at 105,000g for 1 h, washing and resuspending the TMV containing pellets in buffer. The rate of inactivation in these suspensions was highly significant and similar to that of those virus samples which were inoculated together with OS (Table 2). This would indicate that OS is bound to the viruses, which are then inactivated, and does not act on the host. No deleterious effect of OS on the leaves was observed.

OS was found to inactivate 3 different plant viruses *in vitro*; a rigid rod type (TMV), a flexible rod (PVX) and a

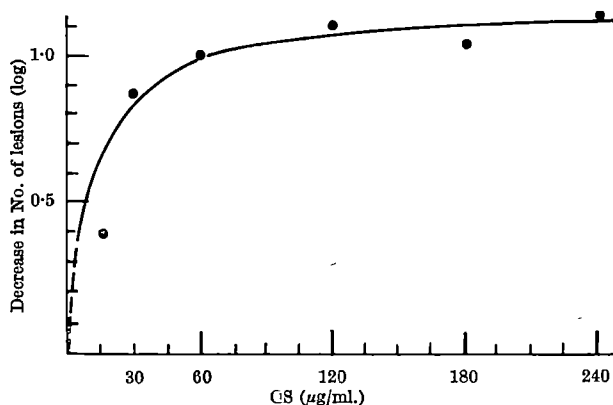


Fig. 1. Effect of concentration of OS on TMV inactivation

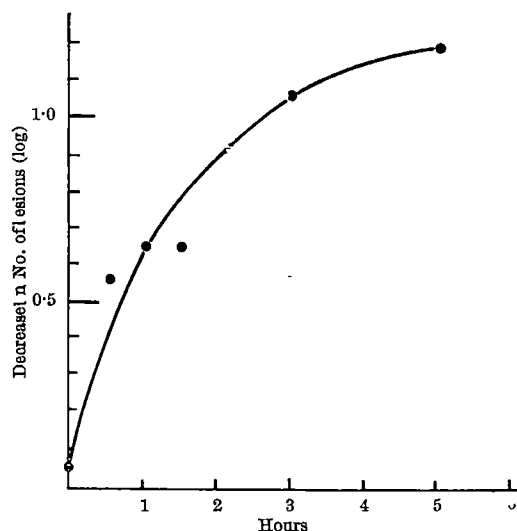


Fig. 2. Effect of incubation time on TMV inactivation

Table 2 ANTIVIRAL EFFECT OF BOUND OXIDIZED SPERMINE (OS)

Incubation time (min)	Inoculum	Average No. of lesions per half leaf		Reduction of lesion No. in log
		OS	Control	
90	OS+TMV	36	73†	0.306
	TMV*	10	25†	0.402
180	OS+TMV	3	62‡	1.408
	TMV*	3	42‡	1.140

* Free OS was removed by ultra-centrifugation and washing.

† Significant at 5 per cent level.

‡ Significant at 1 per cent level.

spherical virus (AMV). This suggests, though further proof is necessary, that OS may inactivate a broader range of plant viruses. The inactivation *in vitro* is a function of the incubation time and concentration of OS. This suggests that the inactivation is due to a chemical interaction. It is not unlikely that this chemical interaction is a reaction between OS and viral nucleic acids. Such a reaction has previously been proposed to explain the inactivation of T_4 coliphages by OS (ref. 10).

This work was aided by grant CA-06867 from the National Cancer Institute, U.S. Public Health Service.

U. BACHRACH
SHLOMIT RABINA
G. LOEBENSTEIN
G. EILON

Department of Clinical Microbiology,
Hebrew University,
Hadassah Medical School,
Jerusalem, Israel,
and
Virus Laboratory,
Volcani Institute of Agricultural Research,
Beit Dagon, Israel,

¹ Tabor, C. W., Tabor, H., and Bachrach, U., *J. Biol. Chem.*, **239**, 2194 (1964).

² Hirsch, J. G., *J. Exp. Med.*, **97**, 327 (1953).

³ Fletcher, F., Epstein, C., and Jewell, P. I., *J. Gen. Microbiol.*, **8**, 323 (1953).

⁴ Tabor, C. W., and Rosenthal, S. M., *J. Pharmacol. Exp. Therap.*, **118**, 139 (1956).

⁵ Bachrach, U., and Persky, S., *J. Gen. Microbiol.*, **37**, 195 (1964).

⁶ Bachrach, U., Tabor, C. W., and Tabor, H., *Biochim. Biophys. Acta*, **78**, 768 (1963).

⁷ Corbett, M. K., *Virology*, **15**, 8 (1961).

⁸ Steere, R. L., *Adv. Virus Res.*, **6**, 1 (1959).

⁹ Siegel, S., *Nonparametric Statistics*, pp. 68-75 (McGraw Hill, New York, 1956).

¹⁰ Bachrach, U., and Leibovici, J., *Proc. Sixth Intern. Congr. Biochem.*, **32**, 42 (1964).

Maintenance of *Escherichia coli* and the Assimilation of Glucose

It has been shown^{1,2} that bacteria require a threshold level of an energy source for maintenance without growth. However, there is a possibility that the thresholds observed might represent some form of concentration requirement for permeability. Thus it is desirable to demonstrate directly the uptake of glucose fed at or below the threshold level. Any glucose assimilated in the absence of growth can then be correlated only with a maintenance requirement for a carbon-energy source.

Cultures of *Escherichia coli* B were grown, collected and washed as previously described¹, providing cells in the log phase of growth, keeping them cold to avoid uncontrolled starvation and maintaining a relatively constant osmotic environment. Suspended cells were then distributed among 15 experimental tubes so that each tube contained 7×10^8 cells per ml. in 10 ml. of the salt medium without glucose. Each tube contained 2.1 mg cells on a dry basis. One set of 5 tubes served as the starvation control receiving no exogenous energy source, another set of 5 tubes received unlabelled glucose, and the third set of 5 tubes received uniformly labelled glucose (glucose- $U^{14}C$). Glucose was fed to the appropriate tubes every 6 h at a level of 0.28 μ moles in 0.10 ml. of salt medium, a rate of 0.022 μ mole/mg cells/h. The radioactive glucose was so diluted with unlabelled glucose as to provide 7,700 counts/min/0.28 μ mole. The control group received an equivalent

volume of salt medium. Every other day one tube from each series was removed for plate count, turbidity measurement and determination of radioactivity. Methods for plate counts and turbidity measurements have been described elsewhere³, representing rather minor modifications of standard methods adopted to improve reproducibility.

To determine the radioactivity of incubated cells, 7-10 ml. aliquots of the cell suspensions were withdrawn from each tube and centrifuged for 10 min in a 'Serval' motor SS-1 at 1,800g and 4° C. The supernatant solution was discarded, and the pellet of cells washed twice by suspending in 5-ml. portions of salt medium and re-centrifuging as above. The cells were washed a third time with 5 ml. distilled water to improve counting efficiency removing salts from the residue. The resulting pellet of cells was re-suspended in 0.1 ml. distilled water and transferred with a micropipette to a rectangular piece of Whatman No. 1 filter paper (3.5 x 2 cm). A continuous current of warm air facilitated drying. Both the tube holding the cell suspension and the micropipette were rinsed three times with 0.5-ml. portions of distilled water and the washings were evaporated on the same paper. Lastly, each paper was placed upright in a 20-ml. vial and dried at 100° C for 1 h. The radioactivity of each sample was counted in a liquid scintillation system⁴.

Fig. 1 reveals that constancy of turbidity resulted from the periodic feeding of the small amount of glucose. Since turbidity records both dead cells and cellular debris, loss of cells or leakage of internal materials must have been limited for growth of other cells. Therefore, the threshold level reported earlier¹ does in fact represent a maintenance rather than a permeability requirement. Because the rate of feeding was too low for growth, the added glucose could have been completely oxidized to carbon dioxide and

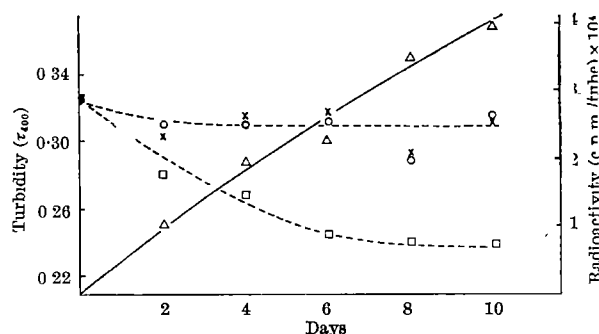


Fig. 1. The incorporation of carbon from glucose- $U^{14}C$ and the maintenance of turbidity in suspensions of *E. coli*. All suspensions initially contained 7×10^8 cells/ml. These data are adjusted for the volume changes resulting from the additions made to the individual suspensions. Evaporation was prevented by use of tightly sealed culture tubes which were opened every 6 h both for the appropriate additions and to replenish the oxygen supply. Δ , Radioactivity of cells fed glucose- $U^{14}C$; \circ , turbidity of cells fed glucose- $U^{14}C$; \times , turbidity of cells fed unlabelled glucose; \square , turbidity of starved cells.

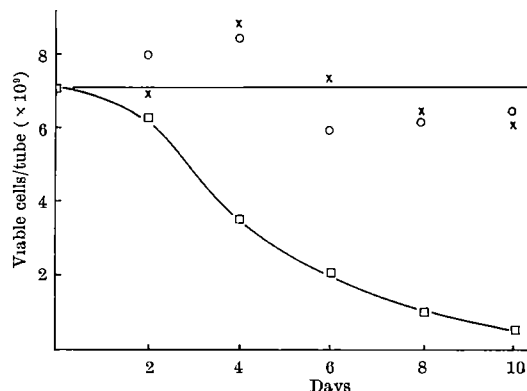


Fig. 2. Maintenance of viability on adding glucose to suspensions of *E. coli*. These data were obtained by plate counts made on aliquots of the suspensions described in the legend of Fig. 1. \circ , Fed glucose- $U^{14}C$; \times , fed unlabelled glucose; \square , starved cells.

ater. However, carbon from the glucose was assimilated to cellular components.

The corresponding Fig. 2 demonstrates the preservation viability in the cell suspensions fed glucose. Obviously a low level of radioactivity had no biological effect within the experimental period. Carbon from glucose was continually accumulated (Fig. 1) with a declining fraction tained as might be expected. After two days, 19 per cent the radioactivity fed was present in the cells while the action had dropped to 13 per cent of the much larger tal fed in ten days. Apparently glucose was utilized ore or less normally. Certainly it entered the cells and as partly assimilated and retained without concomitant owth.

This work was supported in part by a grant from the rmy Research Office, Department of the Army.

SARAH B. MCGREW
M. F. MALLETTE

Department of Biochemistry,
ennsylvania State University,
niversity Park, Pennsylvania.

McGrew, S. B., and Mallette, M. F., *J. Bacteriol.*, **83**, 844 (1962). Mallette, M. F., *Ann. N.Y. Acad. Sci.*, **102**, 521 (1963).

Iarr, A. G., Nilson, E. H., and Clark, D. J., *Ann. N.Y. Acad. Sci.*, **102**, 536 (1963).

Davidson, E. A., *Technical Bulletin*, No. 4, Packard Instrument Company; Wang, C. H., and Jones, D. B., *Biochem. Biophys. Res. Comm.*, **1**, 203 (1959).

PHYSIOLOGY

Hydrolysis of Disaccharides during Absorption by the Perfused Small Intestine of Amphibia

ALTHOUGH it is now generally conceded that disaccharides may be absorbed intact at appreciable rates from the lumen of the small intestine, as found for maltose by Waymouth Reid in 1901, very little is known about the factors which affect the process. It is established that in mammals, including human subjects, disaccharides such as lactose, sucrose and maltose undergo hydrolysis at some site in or on the mucosal cells and not in the intestinal lumen¹⁻⁴. We have now investigated the absorption of some disaccharides by the small intestine of some Amphibia using a system for the perfusion of the mesenteric vascular bed.

In this system the vascular perfusion was used to control the ionic composition of the sub-mucosal faces of the absorbing cells and to carry away for collection the material extruded from these cells. The oxygenation of the mucosal epithelial layer was achieved by the recirculation of bicarbonate-Ringer through the lumen of the intestinal segment by means of a gas lift (equilibration gas, 95 per cent oxygen, 5 per cent carbon dioxide) in a manner similar to that used for mammalian intestine⁵. The vascular perfusion was maintained by pumping bicarbonate-Ringer containing 1 g/100 ml. bovine serum albumin and equilibrated with 95 per cent oxygen, 5 per cent carbon dioxide through a nylon cannula (1 mm outer diam., 0.5 mm inner diam.) inserted through the systemic aortic arch into the coeliac-mesenteric artery. The vascular effluent was usually collected as a fluid comprising exudate from the cut mesenteric veins together with the 'sweat' extruded from the serosal surface of the perfused segment which is immersed in liquid paraffin. In some experiments the portal vein was also cannulated and the venous effluent collected separately. The rate of arterial perfusion at 26° C was of the order of 1 ml./min/g wet weight about 300 μ l./min for each intestine). Glucose was measured by use of *tris*-buffered glucose oxidase as described by Dahlqvist⁶.

With either glucose or maltose (initial concentration 1 mg/ml.) present in the mucosal fluid of intestine from *Rana pipiens*, it was found that the rates of appearance of glucose in the vascular effluent are almost the same (Table 1). In *R. pipiens* we also found similar evidence

for a trehalase activity which results in a vascular appearance rate of glucose similar to that from maltose. Lactase and sucrase appear to be absent. On the same basis, maltase and trehalase are also present in the small intestine of *R. temporaria* and of *Bufo vulgaris*. The small intestine of these two species also appears to be deficient in lactase and sucrase activity.

During these investigations, little disaccharidase activity was found in the mucosal fluids. When Ringer which contained maltose and had been circulated through the intestinal lumen for 1 h. was removed and incubated at 26° C the further rise in glucose concentration could be used to estimate the maltase activity in solution. In a series of eleven experiments on *R. pipiens* it was found that only 7.2 per cent of the total maltase activity of the preparations was in solution in the luminal fluid after luminal circulation for 1 h.

By measuring separately the rate of appearance of glucose in the vascular effluent and in the mucosal fluid it was possible to examine simultaneously the effects of differing maltose concentrations on the total rate of disaccharide hydrolysis and on the rate of glucose appearance in the vascular effluent. At concentrations below 5.56 mM these processes appear to conform to Michaelis-Menten type kinetics and hence it is possible to calculate an affinity constant (K_m) and a maximum velocity constant (V) for each. In Table 2 are given representative values of these parameters for maltose and for glucose transport into the vascular effluent from luminal solutions of maltose for specimens of *R. pipiens* of a different batch from those from which the data in Table 1 were derived. Also included in Table 2 are the values of K_m and V for glucose transport into the vascular effluent from luminal glucose solutions. When these are compared with the corresponding values for maltase it is seen that the values (in molar units) of the constants for the enzyme are greater than those for the glucose transport process. In other words, the rate of hydrolysis of maltose by the enzyme is not rate-limiting to transfer of glucose into the vascular effluent at high concentrations of the disaccharide in the intestinal lumen. On the other hand, the mechanisms underlying the transport of glucose into the vascular effluent are saturated at lower molar concentrations than the disaccharidase. In accord with these findings, the fraction of glucose liberated from the maltose by the maltase, and which appears in the vascular effluent, decreases as the concentration of the disaccharide in the lumen is increased. At low concentrations of the disaccharide the efficiency of capture of the liberated glucose molecules is remarkably high, no hexose liberation to the luminal circulation being detectable in some experiments using *Bufo vulgaris* (Fig. 1).

Our finding that, with disaccharide present in the intestinal lumen, glucose appears in the perfusate at a higher concentration than in the mucosal fluids is consistent with the view that, in the Amphibia we have examined, the disaccharides maltose and trehalose are absorbed as such and hydrolysis takes place in or on the intestinal epithelial cells. Similar results have been

Table 1

Sugar in mucosal fluid	Initial concentration	Glucose concentration after 1 h μ g ml. ⁻¹		Glucose appearance in vascular effluent μ M h ⁻¹ g ⁻¹ FFDW*
		Mucosal fluid	Vascular effluent	
Maltose	2 mg ml. ⁻¹	6 \pm 1	53 \pm 4	94 \pm 7
Glucose	2 mg ml. ⁻¹	\approx 2,300	66 \pm 4	147 \pm 8

Each value is mean \pm S.E. of 43 observations on 11 animals.

* FFDW, fat-free dry weight of whole wall of segment used.

Table 2

K_m	Total maltase 3.5×10^{-3} M	Appearance of glucose in vascular effluent	
		From maltose 0.45×10^{-3} M	From glucose 0.32×10^{-3} M
V	336 μ M maltose h ⁻¹ g ⁻¹ FFDW	71*	83
		μ M glucose h ⁻¹ g ⁻¹ FFDW	

Kinetic parameters of maltase and of glucose appearance in vascular effluent of small intestine of *R. pipiens*. Each value is mean of observations on 2 (or 3*) animals.

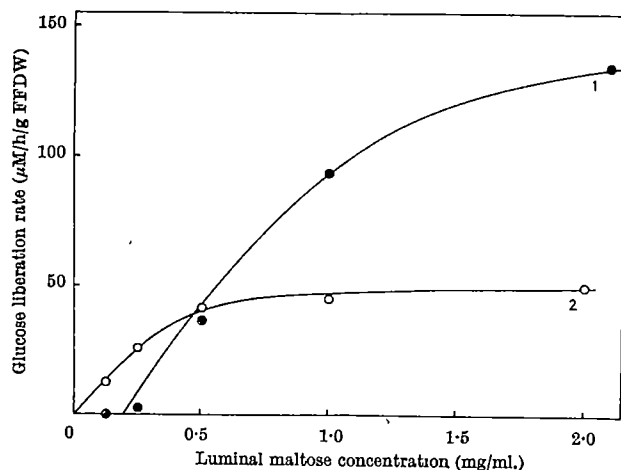


Fig. 1. Relationship between concentration of maltose in intestinal lumen and rate of liberation of glucose into (1) luminal circulation and (2) vascular effluent. Each point is the mean of 2 or 3 observations all on a single specimen of *Bufo vulgaris*.

found for maltose in the mammal³. If, as in the hamster, some of the disaccharidases are localized in the brush border of the absorbing cells⁷, the efficiency of capture of the products of hydrolysis by any hypothetical separate hexose transporting system must be high. One way of describing the phenomenon is to ascribe a vectorial component to the hydrolytic activity of the enzyme. In other words, for a disaccharidase in the brush border, the probability of escape of the products of hydrolysis in one direction, that is, into the cell, is higher than that of the escape in any other direction. If this is so, the vectorial effect is not only most evident on low disaccharide concentrations, but also depends on the presence of Na⁺ ions in the mucosal fluid, for we have found that the substitution of potassium or of lithium for the sodium in the mucosal fluid greatly reduced the transfer of glucose to the vascular fluid without affecting the total rate of disaccharide hydrolysis. Phloridzin (5×10^{-6} M) produced a similar effect. Such a vectorial effect could be due at least in part to the cyto-architectural arrangement of the disaccharidase molecules. For example, with the disaccharidase located in pores in the limiting membranes of the brush border of the absorbing cells the escape of glucose molecules from the inner end of the pore into the cell could be a rate-limiting step to the transfer of glucose at high rates of hydrolysis. A hypothesis of this sort raises the question as to what extent the ability of the mucosal cells to transfer single hexose units from the mucosal fluid into the vascular bed depends on the presence and orientation of oligosaccharidase molecules in the limiting membranes of the absorbing cells.

D. S. PARSONS
J. S. PRICHARD

Department of Biochemistry,
University of Oxford.

¹ Reid, E. W., *J. Physiol.*, **26**, 427 (1901).

² Cajori, F. A., *Amer. J. Physiol.*, **104**, 659 (1933).

³ Miller, D., and Crane, R., *Biochem. Biophys. Acta*, **52**, 281 (1961).

⁴ Chain, E. B., Mansford, K. R. L., and Pocchiarini, F., *J. Physiol.*, **154**, 39 (1960).

⁵ Fisher, R. B., and Parsons, D. S., *J. Physiol.*, **110**, 36 (1949).

⁶ Dahlqvist, A., *Anal. Biochem.*, **7**, 18 (1964).

⁷ Miller, D., and Crane, R., *Biochim. Biophys. Acta*, **52**, 293 (1961).

Protective Effect of Oestrogens against the Toxic Decomposition Products of Tribromoethanol

THE purpose of the present communication is to record that oestrogens have a protective effect against the toxic decomposition products of the anaesthetic tribromoethanol ('Avertin').

Tribromoethanol decomposes slowly on long-standing in the dark; but this process is much accelerated on exposure to the light. Stock solutions of tribromoethanol generally contain amylene hydrate, which acts as a solvent and also retards the breakdown process, but the manufacturers recommend that the stock solution should be tested before use for the presence of toxic decomposition products (dibromoacetaldehyde and hydrobromic acid) by assessing the colour changes when an aqueous solution of congo red indicator is added to the anaesthetic solution. When toxic breakdown products are present the indicator gives a blue coloration instead of the normal red.

In this laboratory we have used tribromoethanol extensively as a general anaesthetic for operative procedures in rabbits, guinea-pigs, rats and mice. We have used stock solutions of tribromoethanol which have been kept in the laboratory away from light for more than two years and they have shown no signs of decomposition or loss of potency, and the overall mortality due to the use of this anaesthetic has been negligible. The present observations were noted in an experiment where it was discovered that a stock solution of tribromoethanol purchased only one month beforehand and not tested by us for toxicity, contained lethal decomposition products.

The experiment was designed to investigate the effect of various doses of 17 β -oestradiol and diethylstilboestrol on the foreign body response. One hundred and twenty male white mice (T.O. Swiss strain) of 20–25 g body weight were used in the experiment. The mice were divided into ten groups each containing ten animals, and twenty animals were used as controls. Each of the test groups received oestrogen in the doses shown in Table 1 and each dose of oestrogen was administered orally by stomach tube in 0.1 ml. sterile distilled water². Immediately following the initial dose of oestrogen, the animals were taken to two separate operating rooms, six groups being taken to each. The mice were then anaesthetized by the intra-peritoneal injection of a 2.5 per cent aqueous solution of tribromoethanol given on the basis 0.01 ml. per g body weight. In one operating room, all the animals received anaesthetic freshly prepared from a stock solution of tribromoethanol which had been in use for the previous eighteen months. In the other operating room, the animals (groups marked * in Table 1) received anaesthetic freshly prepared from a stock solution of tribromoethanol which had been purchased one month beforehand. Bilateral subcutaneous implantation of sterile cotton wool dental pellets was then performed through a single dorsal mid-line incision so that a single pellet was implanted in each flank³. On recovery from the anaesthetic, the animals were returned to a thermostatically controlled heated animal house.

Twenty-four hours after operation it was discovered that a number of deaths had occurred in the groups which had received the three lower doses of 17 β -oestradiol (0.001, 0.01 and 0.1 mg); and the surviving animals in these groups, and also those in the group which had received 1.0 mg 17 β -oestradiol daily, showed signs of toxicity. The animals in all the other groups appeared to be well, and oestrogen treatment was administered to all the surviving mice. Forty-eight hours after operation, it was noted that further deaths had occurred in the groups which had received all except the highest dose of 17 β -oestradiol. It was also noted that the animals which had received the highest doses of 17 β -oestradiol and diethylstilboestrol showed signs of toxicity and two of the diethylstilboestrol-treated mice had died. The other diethylstilboestrol-treated groups and the controls were not affected, and oestrogen treatment of the survivors was carried out. Seventy-two hours after operation, it was noted that further deaths had occurred in all the groups which had received 17 β -oestradiol and also in the group which had received the highest dose of diethylstilboestrol. The other diethylstilboestrol-treated groups and the control animals had remained well.

Table 1. EFFECTS OF VARIOUS DOSES OF OESTROGEN ON SURVIVAL OF MICE AFTER INTRAPERITONEAL INJECTION OF TOXIC DECOMPOSITION PRODUCTS OF TRIBROMOETHANOL ('AVERTIN')

17 β -Oestradiol daily dose for 3 days (mg)	No. of animals in group	No. of survivors after operation			Diethylstilboestrol daily dose for 3 days (mg)	No. of animals in group	No. of survivors after operation		
		24 h	48 h	72 h			24 h	48 h	72 h
0.001	10*	5	1	0	0.001	10	10	10	10
0.01	10*	7	3	0	0.01	10	10	10	10
0.1	10*	9	5	2	0.1	10	10	10	10
1.0	10*	10	7	3	1.0	10	10	10	10
10.0	10*	10	10	5	10.0	10*	10	8	4
Untreated controls	10	10	10	10	Untreated controls	10	10	10	10

* Groups received toxic tribromoethanol.

At the conclusion of the experiment, the stock batches of tribromoethanol were tested for the presence of toxic decomposition products, and it was discovered that the batch used to anaesthetize the mice which had received all the doses of 17 β -oestradiol and the highest dose of diethylstilboestrol had undergone partial decomposition.

The observations recorded in this experiment show clearly that the administration of oestrogens has a marked protective effect against the lethal decomposition products of tribromoethanol (dibromoacetaldehyde and hydrobromic acid), and that the period of survival after administration of these toxins depends on the dose of oestrogen administered and lengthens with increasing doses of the oestrogen (Table 1).

Nicol and Zikry⁸ reported that oestradiol benzoate has a protective effect against the toxicity of trypan blue in guinea-pigs, and they attributed their findings to the stimulating effect of oestrogen on the reticulo-endothelial system which thus becomes more capable of taking up the dye and detoxicating it. It has also been reported that the reticulo-endothelial system plays an important part in protection against a variety of noxious agents⁹, and it seems possible that the protective influence of oestrogen against the toxic effects of dibromoacetaldehyde and hydrobromic acid may be the result of oestrogen-produced stimulation of the reticulo-endothelial system.

T. NICOL
B. VERNON-ROBERTS
D. C. QUANTOCK

Department of Anatomy,
King's College,
London, W.C.2.

The Merck Index of Chemicals and Drugs, sixth ed., 967 (Merck and Co., Rahway, 1952).

Nicol, T., Vernon-Roberts, B., and Quantock, D. C., *J. Endocrinol.* (in the press).

Nicol, T., Vernon-Roberts, B., and Quantock, D. C. (unpublished work, 1965).

Nicol, T., and Zikry, A. A., *Nature*, **170**, 239 (1952).

Berry, L. J., and Spies, T. D., *Medicine*, **28**, 239 (1949).

Aldosterone Secretion: Differences in Direct Effects of Rubidium and Potassium Perfusion into Dog Adrenals

MORE than eighty years ago, Ringer¹ observed that rubidium ions had effects on the frog's ventricles similar to those produced by potassium. Since then these two ions have been found to be very nearly interchangeable in a wide variety of biochemical and physiological processes².

The systems most extensively examined have been rat skeletal muscle³, rat acid-base balance⁴, dog renal excretions⁵ and the human erythrocyte⁶⁻⁸. In fact, Lahn⁸ has suggested that rubidium might be more effective than potassium in treating digitalis overdosage because rubidium is more resistant to the action of ouabain in depressing the entry of erythrocytes.

We now offer evidence that this mutual interchange of these two ions does not operate in one very important system; that is the adrenal cortex of dogs. Isolated adrenal glands of hypophysectomized dogs were prepared by the technique of Hilton *et al.*⁹. Aldosterone, hydro-

cortisone and corticosterone secretion rates were measured in the adrenal venous effluent during control collection periods, following perfusion of either potassium chloride or rubidium chloride, and after an injection of ACTH given at the end of each experiment. Potassium chloride was perfused in each experiment to achieve an arterial blood concentration of approximately 8 m.equiv./l. and RbCl was perfused to achieve a concentration of approximately 4 m.equiv./l. (Rb + K = 8 m.equiv./l.).

The effects of the potassium chloride infusion experiments are shown in Fig. 1. Potassium produced its expected stimulation of aldosterone secretion without effect on hydrocortisone or corticosterone secretion¹⁰. ACTH given at the end of each experiment caused stimulation of all three steroids. In contrast, as shown in Fig. 2, rubidium failed to alter aldosterone secretion. ACTH in these experiments again increased production of all three steroids.

These results were quite unexpected in view of the heretofore demonstrated interchangeability of these two ions in a wide variety of biological systems and the high penetrability of rubidium into cells. In experiments somewhat relevant to ours, Kunin *et al.*¹¹ and Tarail *et al.*¹² observed that electrocardiographic alterations in the dog were similar during infusions of potassium chloride, producing plasma potassium levels of 8 m.equiv./l., to those induced by RbCl producing plasma Rb levels of 4 m.equiv./l. (Rb + K = 8 m.equiv./l.). Also Glasser and Ellis¹³ found that rubidium could prevent the lipid depletion of the zona glomerulosa of rat adrenals otherwise produced by potassium deficiency and on this evidence suggested that rubidium could substitute for potassium in their system. However, aldosterone production was not measured in their experiments.

Lacking specific evidence for or against rubidium penetration into dog adrenal cortical cells, we cannot offer

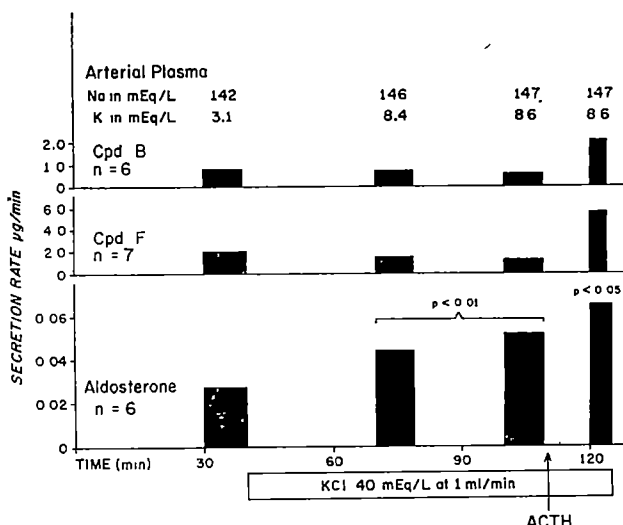


Fig. 1. Stimulatory effect of potassium on aldosterone secretion in adrenals of hypophysectomized dogs. KCl was perfused at a concentration of 40 m.equiv./l. to achieve a final arterial concentration of approximately 8 m.equiv./l.

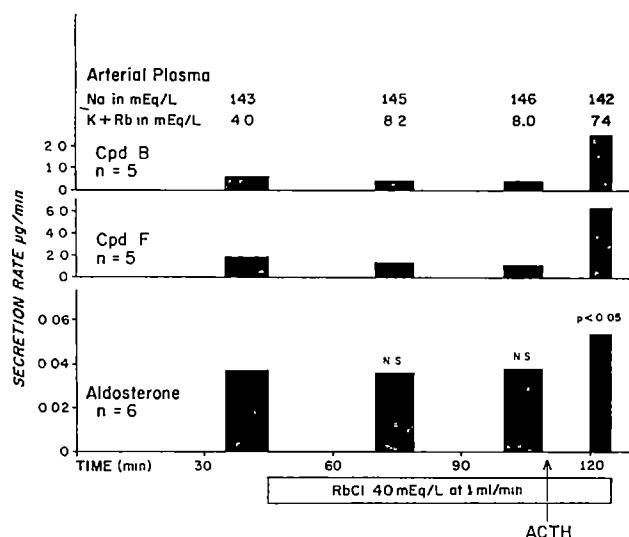


Fig. 2. Lack of effect of rubidium on aldosterone secretion in adrenals of hypophysectomized dogs. RbCl was perfused at a concentration of 40 m.equiv./l. to achieve a final arterial concentration of Rb+K=8 m.equiv./l.

any cogent explanation for our results and must conclude that, so far as aldosterone production in the dog adrenal is concerned, rubidium, at the concentration used, cannot be substituted for potassium. This would appear to be the first example of such a dichotomy between these two ions.

This work was supported by grants from the American Heart Association, the N.Y. Heart Association, Life Ins. Res. Fund, and U.S. National Institutes of Health (grant A2828). One of us (J. G. H.) is investigator of the Health Research Council of the City of New York (contract I-130)

JAMES G. HILTON
SHELDON ALTER
PAUL CUSHMAN

Department of Medicine,
St. Luke's Hospital,
421 West 113th Street,
New York.

- ¹ Ringer, S., *J. Physiol.*, **4**, 370 (1882).
- ² Relman, A., *Yale J. Biol. and Med.*, **29**, 248 (1956).
- ³ Relman, A., Lambie, A., Burrows, B., and Roy, A., *J. Clin. Invest.*, **38**, 1249 (1957).
- ⁴ Lambie, A., Relman, A., and Schwartz, W., *J. Clin. Invest.*, **38**, 1538 (1958).
- ⁵ Kunin, A., Dearborn, E., Burrows, B., and Relman, A., *Amer. J. Physiol.*, **197**, 1297 (1959).
- ⁶ Solomon, A., *J. Gen. Physiol.*, **38**, 57 (1952).
- ⁷ Love, W., and Burch, G., *J. Lab. and Clin. Med.*, **41**, 351 (1953).
- ⁸ Kahn, J., *J. Pharm. and Exp. Therap.*, **136**, 197 (1962).
- ⁹ Hilton, J., Weaver, D., Muelhelms, G., Glaviano, V., and Wegria, R., *Amer. J. Physiol.*, **192**, 525 (1958).
- ¹⁰ Davis, J., Urquhart, J., and Higgins, J., *J. Clin. Invest.*, **42**, 597 (1963).
- ¹¹ Kunin, A., Dearborn, E., and Relman, A., *Amer. J. Physiol.*, **183**, 636 (1955).
- ¹² Tarall, R., Bennett, T., and Noell, W., *J. Clin. Invest.*, **34**, 966 (1955).
- ¹³ Glasser, L., and Ellis, J., *Amer. J. Path.*, **38**, 103 (1961).

A New Property of the Early Receptor Potential of Rat Retina

In the dark-adapted eye, the amplitude of the early receptor potential (e.r.p.)¹ is linearly proportional to the number of molecules of rhodopsin bleached by a flash.² The second of a train of intense flashes evokes a smaller response because less rhodopsin is available.² However, with further stimulation, the e.r.p. reaches a steady voltage, about 10 per cent of the first response. This occurs even though each molecule of rhodopsin should, theoretically, have bleached many times over.³ The relationship established by Corie² breaks down after the second or third maximal flash. The reason for this is that

the flash causes regeneration of photopigment from stable product of bleaching, by a mechanism different from that analysed by Williams³. The regeneration can be demonstrated by exposing the eye to a series of intense flashes (Fig. 1) until the e.r.p. amplitude has reached stable lower level.

The eye is then exposed to continuous illumination from a tungsten source. Following this, the e.r.p. is again evoked with intense flashes. The first response is much smaller than the previous lower limit, but the subsequent e.r.p.s increase in amplitude. The experiment can be repeated several times.

The phenomenon shown in Fig. 1 is less pronounced if the retinal circulation is maintained. It is exaggerated if the tungsten illumination lasts for not longer than 5 min (Fig. 2A). It is greatly reduced if a filter, excluding wavelengths shorter than 460 nm, is placed in the light path. It is therefore likely that the flash photo-isomerizes a *trans*- to *cis*-retinal, thus initiating the synthesis of rhodopsin^{4,5}. The increase of e.r.p. amplitude is associated with an increase in amplitude of the electroretinogram. In separate experiments, it has been found that the change of amplitude corresponds to an increase in sensitivity of about 0.3 log units. The increase in rhodopsin concentration, predicted from the e.r.p. experiments, would account for this.

These experiments therefore support the theory of photo-regeneration. Our measurements of e.r.p. amplitudes show that the rhodopsin formed by this mechanism is partly bleached during the same flash. Since the regeneration time permits little diffusion, the retinal must still be bound to the opsin.

Fig. 2A shows that after adaptation to tungsten light the e.r.p. wave-form changes. At body temperature, a change that can be seen is that the initial, descending negative phase becomes more rapid. The e.r.p. can be slower and the negative phase abolished, by cooling^{6,7}. A positive potential is then revealed. If this experiment is performed with an eye which has been previously exposed to tungsten light, the wave-form is different from the already described^{6,7} (Fig. 2B). The positive e.r.p. is preceded by a negative deflexion. It is the latter which

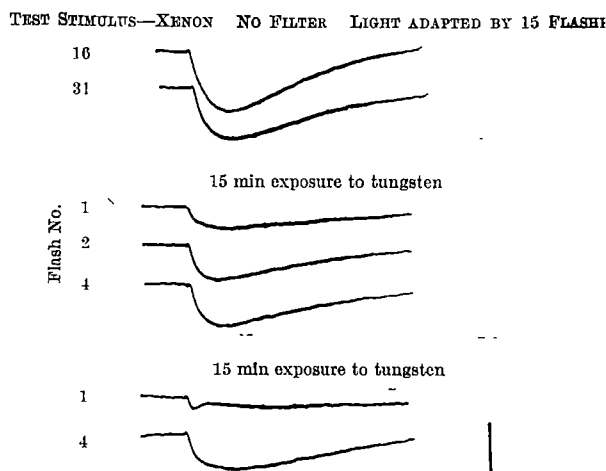


Fig. 1. E.r.p.s of albino rats. Light shielded corneal wick electrode. Reference electrode in scalp. Pupil dilated. Stimulus, 50 J, 800 μ sec, xenon flash delivered by fibre optic bundle 1 cm in diam placed 1 mm from cornea, ensuring total uniform retinal illumination. Effective stimulus intensity, 2.8 quanta absorbed/rhodopsin mol./flash. Animal killed by overdose of urethane immediately prior to experiment. Calibration bars, 100 μ V, 2 msec. Stimulus delayed from start of trace. 30-sec interval between flashes. Ordinal number of flash indicated on left. E.r.p.s produced by sixteenth and thirty-first flash nearly equal in amplitude. Exposure to tungsten light, through same bundle, of intensity calculated to bleach total rhodopsin in about 5 min if no regeneration occurs. Subsequent to this exposure, the first test flash evokes a small e.r.p., but subsequent flashes evoke larger responses. The experiment can be repeated. Note rapid downward excursion of the trace after tungsten exposure. The initial positive potential is not visible, since the experiment was carried out near body temperature.

E.R.P. AFTER EXPOSURE TO TUNGSTEN LIGHT—XENON FLASH, WHITE

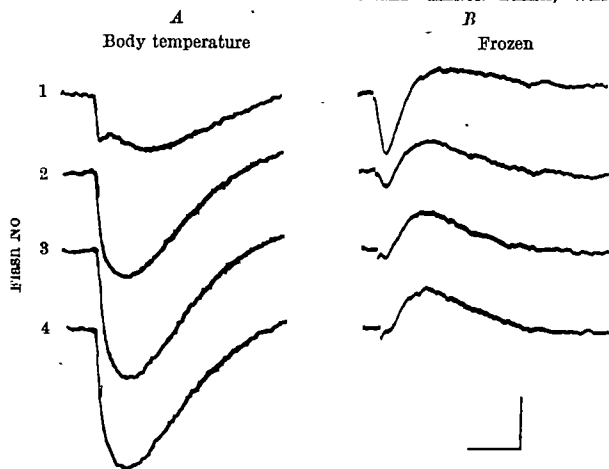


Fig. 2. E.R.P.s obtained after exposure to 5 min tungsten light. A, At body temperature, showing increasing responses to successive flashes. Note rapid negative deflexion. B, Responses obtained when excised eye placed on block of ice cooled by methanol-carbon-dioxide mixture. The eye was placed so that it did not become opaque (frozen). The later negative potential seen in 2A has disappeared. The first flash evokes a negative wave, previously undescribed, which is as rapid as the initial positive phase. In subsequent flashes, the negative potential decreases, while the positive potential grows. Calibration, 2 msec, and A, 100 μ V. B, 50 μ V.

causes the rapid downward swing at body temperature. The negative deflexion can only be seen after intense tungsten illumination. It cannot be seen if wave-lengths shorter than 460 nm are excluded. After the fourth flash it seems to have disappeared from the records. It may still be present, but obscured by the positive potential, which has grown. In some of our experiments, the positive response to the first flash is much smaller than in Fig. 2B, and it can then be seen that the time course of the negative response is much the same as that of the positive. It is distinct from the extra-retinal potential described by Brown⁸.

It therefore appears that the photolysis and synthesis of rhodopsin *in situ* are associated with potentials of opposite polarity. Both resist anoxia and low temperature, and have similar time courses. It is therefore possible that they are generated by the same reversible mechanism. This would imply that the responses have a closer relationship to the primary action of light than hitherto supposed⁷—either photoconduction in oriented molecules, or rotation of molecular dipoles.

G. B. ARDEN
HISAKO IKEDA

Department of Neurophysiology,
Institute of Ophthalmology,
Judd Street, London, W.C.1.

¹ Brown, K. T., and Murakami, M., *Nature*, **201**, 626 (1964).

² Cone, R. A., *Nature*, **204**, 736 (1964).

³ Williams, T. P., *J. Gen. Physiol.*, **47**, 679 (1964).

⁴ Hubbard, R., and Kropf, A., *Proc. U. S. Nat. Acad. Sci.*, **44**, 130 (1958).

⁵ Hubbard, R., and Wald, G., *J. Gen. Physiol.*, **36**, 269 (1952-3).

⁶ Pak, W. L., and Cone, R. A., *Nature*, **204**, 836 (1964).

⁷ Pak, W. L., and Ebrey, T. G., *Nature*, **205**, 484 (1965).

⁸ Brown, K. T., *Nature*, **207**, 1249 (1965).

PHARMACOLOGY

Inhibition of Conjugation by Anti-inflammatory Drugs

IBUFENAC (4-isobutyl-phenylacetic acid) has recently been introduced as an anti-inflammatory drug. In animals it possesses analgesic, anti-inflammatory and antipyretic properties¹. Thompson, Stephenson and Percy² found raised serum transaminase-levels in 12 of 36 patients treated with ibufenac. Five cases of jaundice have now been recorded in about 400 patients receiving ibufenac³.

Phenylbutazone, another anti-inflammatory drug, occasionally causes jaundice; 12 cases have been recorded⁴. The liver shows cholestatic and cytotoxic lesions⁵. Phenylbutazone has been given to patients with cirrhosis of the liver for 1-5 months without alteration of liver function tests⁵.

Using *in vitro* systems, we have examined the effect of ibufenac and phenylbutazone on conjugating mechanisms in the rat and rabbit liver. Bilirubin conjugation was determined in rat liver slices and rabbit liver homogenates by the method of Lafhe and Walker⁶. *o*-Aminophenol conjugation was determined in rat liver slices by the method of Levvy and Storey⁷ and in rabbit liver homogenates by the method of Stevenson and Dutton⁸.

The addition of ibufenac and phenylbutazone lowered the rates of conjugation of bilirubin and *o*-aminophenol by rat liver slices (Table 1). The transferase stage of conjugation was examined in rabbit liver homogenates incubated with ample uridine diphosphate glucuronic acid. Ibufenac and phenylbutazone reduced the rates of conjugation of bilirubin and *o*-aminophenol (Table 2).

Table 1. EFFECT OF ANTI-INFLAMMATORY DRUGS ON CONJUGATION IN RAT LIVER SLICES

Concn. (mM)	Rate of conjugation (μ g/g/h)			
	Ibufenac Bilirubin	<i>o</i> -Aminophenol	Phenylbutazone Bilirubin	<i>o</i> -Aminophenol
0	54	70	31	57
0.01	50	62	26	48
0.1	43	61	21	44
1.0	50	39	15	48
10.0	0	0	6	35

Table 2. EFFECT OF ANTI-INFLAMMATORY DRUGS ON CONJUGATION IN RABBIT LIVER HOMOGENATES

Concn. (mM)	Rate of conjugation (μ g/g/h)			
	Ibufenac Bilirubin	<i>o</i> -Aminophenol	Phenylbutazone Bilirubin	<i>o</i> -Aminophenol
0	248	300	190	243
0.01	248	273	120	252
0.1	216	182	0	245
1.0	220	50	0	226
10.0	0	14	0	194

Clinical trials of ibufenac have indicated that the drug is hepatotoxic; the reported incidence of jaundice is about 1 in 80 patients. The type of jaundice has not been defined, but liver biopsy in one case showed preservation of normal architecture with no evidence of bile stasis. The jaundice was mild and liver function rapidly returned to normal after withdrawal of ibufenac³. Rats given 100-250 mg/kg ibufenac orally for 27 weeks showed no evidence of liver damage, but doses of 625-1,000 mg/kg caused liver cell damage⁹. Ibufenac inhibits *o*-aminophenol and bilirubin conjugation in *in vitro* conjugating systems; the circulating concentration of ibufenac after normal dosage (2.4 g) is about 0.1 mM, so it is possible that glucuronyl transferase can be inhibited at therapeutic concentrations.

Phenylbutazone jaundice may be due to a direct action of the drug which is dose-dependent⁹. Cholestatic jaundice rarely results from phenylbutazone therapy; the jaundice has been associated with generalized allergic manifestations¹⁰. Therapeutic blood levels in man are 5-15 mg/100 ml; this is 0.15-0.5 mM, at which level inhibition of conjugation occurs *in vitro*.

Ibufenac and phenylbutazone uncouple oxidative phosphorylation¹¹; 50 per cent inhibition is produced by about 0.25 mM phenylbutazone and 1.0 mM ibufenac. Phenylbutazone inhibits a number of other enzymes, for example glutamic acid dehydrogenase¹² at similar concentrations.

Ibufenac and phenylbutazone cause jaundice, the former with probably greater frequency than the latter. The drugs inhibit conjugation at therapeutic concentrations in preparations *in vitro*.

I thank Prof. Nicholas H. Martin and Dr. J. Hall of Boots Pure Drug Co., Ltd., for their advice, Miss J. McNichol for technical assistance, and the British

Empire Cancer Campaign for Research and Boots Pure Drug Co., Ltd., for financial assistance.

T. HARGREAVES

Department of Chemical Pathology,
St. George's Hospital Medical School,
London, S.W.1.

- ¹ Adams, S. S., Cliffe, E. E., Lessel, B., and Nicholson, J. S., *Nature*, **200**, 271 (1963).
- ² Thompson, M., Stephenson, P., and Percy, J. S., *Ann. Rheum. Dis.*, **23**, 397 (1964).
- ³ Reichenberg, H. K. von, *Phenylbutazone*, second ed. (E. Arnold, Ltd. London, 1962).
- ⁴ Smetana, H. F., *Ann. N.Y. Acad. Sci.*, **104**, 821 (1963).
- ⁵ Weiner, M., Chenkin, T., and Burns, J. J., *Amer. J. Med. Sci.*, **228**, 36 (1954).
- ⁶ Lathe, G. H., and Walker, M., *Biochem. J.*, **70**, 705 (1958).
- ⁷ Levy, G. A., and Storey, I. D. E., *Biochem. J.*, **44**, 295 (1949).
- ⁸ Stevenson, I. H., and Dutton, G. J., *Biochem. J.*, **82**, 330 (1962).
- ⁹ Markoff, N., *Medizinische*, **46**, 2219 (1959).
- ¹⁰ Schwartz, H. N., Marshall, D., and Robinson, B. D., *N.Y. State J. Med.*, **54**, 265 (1954).
- ¹¹ Whitehouse, M. W., *Nature*, **201**, 629 (1964).
- ¹² Bassler, K. H., and Lang, K., *Naunyn-Schmiedeberg's Arch. Exp. Path. Pharmacol.*, **231**, 254 (1956).

Preferential Release of Adrenaline from the Adrenal Medulla by Muscarine and Pilocarpine

HISTOCHEMICAL examination of the adrenal medulla of several species, including the cat, has revealed two distinct types of chromaffin cell, one rich in adrenaline and the other rich in noradrenaline^{1,2}; and with appropriate physiological stimuli it is possible to release one or the other of these catecholamines more or less selectively³⁻⁵. Pharmacological investigations, on the other hand, have indicated that two types of 'acetylcholine receptor' are present in the cat's adrenal medulla. Attention was directed to this by Feldberg, Minz and Tsudzimura⁶, who found that both nicotine and pilocarpine had pressor effects when injected 'close-arterially' into the adrenal gland and that the effect of the former was blocked by excess nicotine while that of the latter was blocked by small doses of atropine. They were further able to demonstrate both nicotine-sensitive and atropine-sensitive components in the stimulant action of the splanchnic nerve on adrenal medullary secretion. Our purpose here is to present evidence suggesting that the two sets of phenomena are related: that, in the cat at least, pilocarpine and muscarine release adrenaline with little or no noradrenaline; while nicotine releases large amounts of noradrenaline as well as adrenaline.

Our experiments have been carried out on cat's adrenal glands acutely denervated and perfused *in situ* with Locke's solution following the method previously described⁷. Adrenaline and noradrenaline escaping from the adrenal vein were assayed by the fluorometric technique⁸. During the course of perfusion the gland was made to discharge catecholamines either by stimulating the splanchnic nerve for 20-30 sec with supramaximal shocks at 30/sec or by adding to the perfusion fluid various cholinomimetic drugs for the same length of time. These drugs fell into three groups: (a) acetylcholine (10^{-5} g/ml.); (b) muscarine and pilocarpine ($1-2 \times 10^{-4}$ g/ml.); (c) nicotine ($2 \times 10^{-6}-10^{-5}$ g/ml.).

Stimulation of the splanchnic nerve or the addition of acetylcholine or nicotine to the perfusion fluid caused the liberation of large amounts of both adrenaline and noradrenaline in each of many tests on different glands: the mean percentage of total catecholamines appearing as noradrenaline was 61 per cent with splanchnic nerve stimulation (24 tests), 58 per cent with ACh (10 tests) and 55 per cent with nicotine (7 tests). In contrast, pilocarpine and muscarine yielded quite different results: in 8 tests with pilocarpine, noradrenaline accounted for only 4 per cent of the total amines released and in 7 tests with muscarine, only 16 per cent. The contrasting effects of nicotine and pilocarpine in a single gland are shown in responses (N) and (P) of Fig. 1. Hexamethonium, in doses

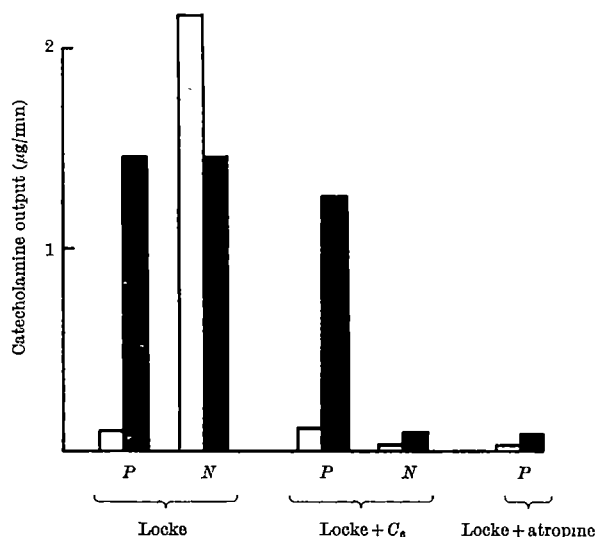


Fig. 1. Secretion of catecholamines in response to pilocarpine (P), 10^{-4} g/ml., and to nicotine (N), 2×10^{-5} g/ml., given during perfusion with Locke's solution or Locke's solution with the addition of hexamethonium (C_6), 10^{-4} g/ml. or atropine 10^{-5} g/ml. Each agonist was introduced for 30 sec. The solid black columns indicate the secretion of adrenaline during perfusion with the agonists and the open columns indicate the corresponding secretion of noradrenaline.

that strongly inhibited the responses to nicotine, had little effect on the responses to pilocarpine or muscarine which, however, were readily blocked by atropine (Fig. 1). Feldberg, Minz and Tsudzimura⁶ noted in their experiments that large doses of nicotine failed to block completely the pressor effects of stimulating the splanchnic nerve, but that the residual response was reduced by atropine. We have obtained essentially similar results with hexamethonium, and have been able to show that the residual secretion persisting in the presence of large doses of hexamethonium (and blocked by atropine) is largely made up of adrenaline.

From the evidence provided by these various agonists and antagonists, it seems clear that there are two sets of acetylcholine receptors in the cat's adrenal medulla and that these are related to the preferential release of the two catecholamine hormones: the receptors activated by pilocarpine and muscarine appear to be associated principally with the release of adrenaline, while those activated by nicotine are involved in the release of both adrenaline and noradrenaline. A simple interpretation of the effect of muscarine and pilocarpine would be that the receptors they activate are mainly on cells containing only adrenaline; while the fact that nicotine releases adrenaline and noradrenaline would suggest either that 'nicotine receptors' are present on both adrenaline and noradrenaline-containing cells or, alternatively, that nicotine is capable of activating the same receptors that respond to muscarine.

It is remarkable that so little attention has been paid to the presence in the adrenal medulla of receptors responding to muscarine and related drugs to which the work of Feldberg *et al.*⁶ so clearly pointed. Time and again cholinomimetic substances have been screened for adrenal medullary stimulating activity in animals given atropine. Obviously adrenal medullary stimulants of the muscarine type are missed in such tests. Confusion has arisen from the common pharmacological practice of equating 'nicotinic sites' of drug action (in the gross anatomical sense) with 'nicotine receptors' at the cellular level. The adrenal medulla, where nicotine is certainly an outstandingly effective agent, numbers among the familiar 'nicotinic sites', and there has been a tendency to assume an absence of muscarine receptors there. This assumption is certainly unfounded in the cat where muscarine receptors appear to be intimately related to the secretion of adrenaline. Feldberg *et al.*⁶ report that they performed only

a few experiments with muscarine, and that, because of its depressant effects on heart and general circulation, it was difficult to be certain of its action on the adrenal medulla. Nevertheless they state that they did record, in several instances, an undoubted, though weak, pressor effect abolished by atropine. They do not mention the doses they employed. In our own experiments where we have used *d*-l-muscarine iodine we have found that muscarine is capable of evoking very strong secretory responses; for example, in each of four tests a brief exposure (20 sec) to muscarine in a concentration of 10^{-4} g/ml. raised catecholamine output during this period from resting values under $0.05 \mu\text{g}$ to more than $9 \mu\text{g}/\text{min}$.

The fact that atropine is a highly effective blocker of the muscarine receptors may prove to be of considerable heuristic value in the physiological analysis of their function in the body's economy. It will, of course, be necessary to examine other species to determine the general significance of the muscarine receptors and their relation to the preferential release of adrenaline.

It is pertinent that receptors activated by muscarine-like drugs and blocked by low doses of atropine also occur in sympathetic ganglion cells⁹ which are developmental homologues of the medullary chromaffin cells. The sympathetic ganglia have also been most commonly considered as 'nicotinic sites'. In the ganglia, however, the functional distinction between muscarine and nicotine receptors is not so evident as it is in the adrenal.

W. W. DOUGLAS
A. M. POISNER

Department of Pharmacology,
Albert Einstein College of Medicine,
New York.

- Hillarp, N. A., and Hokfelt, B., *Acta Physiol. Scand.*, **30**, 55 (1953).
Wood, J. G., *Amer. J. Anat.*, **112**, 285 (1963).
Frankö, O., *Acta Anat.*, **18**, Suppl. 17 (1952).
Redgate, E. S., and Gellhorn, E., *Amer. J. Physiol.*, **174**, 475 (1953).
Folkow, B., and von Euler, U. S., *Circ. Res.*, **2**, 191 (1954).
Feldberg, W., Minz, B., and Tsudzimura, H., *J. Physiol.*, **81**, 286 (1934).
Douglas, W. W., and Rubin, R. P., *J. Physiol.*, **159**, 40 (1961).
Anton, A. H., and Sayre, D. F., *J. Pharmacol.*, **138**, 360 (1962).
Trendelenburg, U., *Ann. Rev. Pharmacol.*, **1**, 219 (1961).

HAEMATOLOGY

Quantitative Determination of Haemoglobin A_2 by Acrylamide-gel Electrophoresis

A PROBLEM of great interest to the clinician is the diagnosis of thalassaemia trait. An objective and specific test for detection of this disease was first offered by Kunkel *et al.*^{1,2} who, using starch block electrophoresis, found that in cases of thalassaemia trait the A_2 haemoglobin was characteristically elevated. This fraction has been extensively investigated by electrophoresis using paper³, starch block^{4,5}, starch gel⁶, cellulose acetate⁷, and acrylamide gel⁸ as the supporting media. Both starch block and starch gel electrophoresis are widely used to-day for haemoglobin analysis but are difficult to standardize and require a great deal of 'laboratory technique'. Acrylamide gel is superior to starch in that it is easier to work with, is more reproducible, is faster and the fractions can be quantitated by direct densitometry^{9,10}.

The work recorded here was undertaken to compare quantitatively starch-block and acrylamide-gel electrophoresis and to establish the normal ranges of haemoglobin A_2 as determined by acrylamide gel electrophoresis.

Blood specimens collected with any standard anticoagulant were prepared as described by Pearson and McFarland⁴. In lieu of ultracentrifugation satisfactory clear haemolysates may be obtained by filtering after routine centrifugation through *S* and *S* No. 289 analytical filter pulp. Prior to electrophoresis the haemolysates were diluted 1:1 with the working buffer solution.

The working buffer solution was the *tris*-borate-EDTA buffer described by Peacock¹¹. Electrophoresis was performed using a vertical cylindrical cell (Arden Instruments, Inc., Rockville, Md., U.S.A.) designed by us¹². After electrophoresis for 1 h at 300 V the gel patterns were scanned unstained by a recording densitometer equipped with a 500-m μ interference filter¹⁰. The area below the curves is integrated automatically and the percentage distribution of the fractions is calculated in the usual manner.

A typical separation of normal and abnormal haemoglobins by acrylamide-gel electrophoresis and a composite densitometer scan of the various components is shown in Fig. 1. Blood specimens from apparently healthy donors were analysed quantitatively by the starch block technique of Pearson and McFarland⁴. Thirty-six specimens with the A_2 haemoglobin in Pearson's normal range (1.5–3.1 per cent) were then separated on acrylamide gel and the fractions quantitated by direct densitometry. The mean value of the A_2 haemoglobin by this method was 2.9 per cent; the standard deviation was 1.5 and the normal range (mean \pm S.D.) was 1.4–4.4 per cent.

Several investigators have reported the normal range of the A_2 haemoglobin content obtained by various methods. Aksoy *et al.*³, using paper electrophoresis, and Pearson and McFarland⁴, using the starch-block technique, reported the normal range to be 2.6–3.5 per cent and 1.5–3.1 per cent respectively. Goldberg and Ross⁶, using starch-gel electrophoresis, found the normal range to be 2.4–4.5 per cent. Rozman *et al.*⁷ obtained a normal range of 2.4–4.1 per cent for A_2 using cellulose acetate electrophoresis. Nakamichi and Raymond⁸, using acrylamide-gel electrophoresis, found the normal range to be 0.5–5.0 per cent. In work recorded here the normal range was 1.4–4.4 per cent. From an analysis of the foregoing it is apparent that the upper range of the A_2 haemoglobin determined

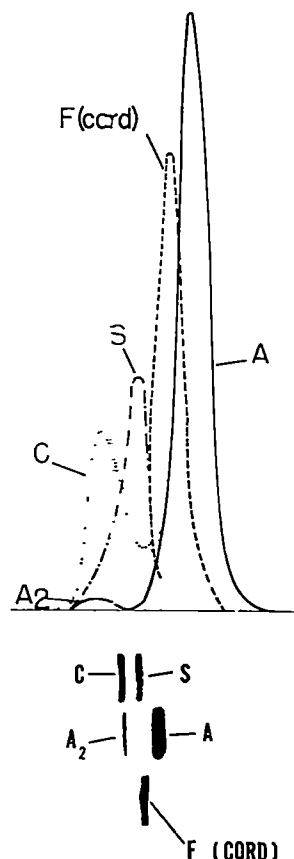


Fig. 1. Normal and abnormal haemoglobins separated by acrylamide gel electrophoresis with a composite densitometric scan

by acrylamide-gel electrophoresis is generally higher than that obtained by other methods.

THOMAS G. FERRIS
ROBERT E. EASTERLING
RICHARD E. BUDD

Rear-Admiral G. W. Calver's
Physical Chemistry Research Laboratory,
National Naval Medical Center,
Bethesda, Maryland.

- ¹ Kunkel, H. G., and Wallenius, G., *Science*, **122**, 288 (1955).
- ² Kunkel, H. G., Ceppellini, R., Muller-Eberhard, U., and Wolf, J., *J. Clin. Invest.*, **36**, 1615 (1957).
- ³ Aksoy, M., Lehmann, H., and Eng, L., *Lancet*, **i**, 762 (1957).
- ⁴ Pearson, H., and McFarland, W., *U.S. Armed Forces Med. J.*, **10**, 693 (1959).
- ⁵ Wolff, J. A., *Blood*, **18**, 807 (1961).
- ⁶ Goldberg, C. A. J., and Ross, A. C., *Clin. Chem.*, **6**, 254 (1960).
- ⁷ Rozman, R. S., Sacks, R. P., and Kates, R., *J. Lab. Clin. Med.*, **62**, 692 (1963).
- ⁸ Nakamichi, M., and Raymond, S., *Clin. Chem.*, **9**, 135 (1963).
- ⁹ Ferris, T. G., Easterling, R. E., and Budd, R. E., *Blood*, **19**, 479 (1962).
- ¹⁰ Ferris, T. G., Easterling, R. E., and Budd, R. E., *Anal. Chem.*, **36**, 955 (1964).
- ¹¹ Peacock, A. C., Bunting, S. L., and Queen, K. G., *Science*, **147**, 1451 (1965).
- ¹² Ferris, T. G., Easterling, R. E., and Budd, R. E. (unpublished work).

IMMUNOLOGY

Time-course Studies on Antibody Response in Thymectomized and Sham-thymectomized Mice

THE observation that neonatal thymectomy is associated with immunological defects¹⁻⁴ has stimulated new interest in the thymus as an organ important in the production of immunity. Antibody response and reaction to homologous skin grafts are the usual criteria of the immune capacity of the animal. Whether or not any particular, completely thymectomized animal shows immunological defects depends on the species and strain of the animal and the age of the animal when thymectomized. More recently⁵⁻⁷ thymectomized animals of the same strain have been shown to have decreased responsiveness to some antigens and not to others. In all these investigations, one or two time-intervals between injection of antigen and sampling for antibody activity were chosen. This communication deals with the haemolysin response to sheep red blood cells at various times after immunization. A delay in antibody formation in the thymectomized mice is demonstrated, and the danger inherent in the use of a single time interval between injection and serum collection is pointed out.

Swiss albino mice, inbred in this laboratory for 25 generations, were thymectomized or sham-thymectomized within 24 h of birth according to the method of Miller^{8,9}. Only the occasional mouse showed signs of wasting and only normal-appearing mice without abscesses were used in this investigation. Sheep red blood cells in Alsevers solution (Baltimore Biological Laboratory—BBL) were washed four times in Krebs-Ringer phosphate (KRP) solution at pH 7.4. At 6 weeks of age, each mouse received by intraperitoneal injection 0.1 ml. of a 10 per cent suspension of red blood cells for immunization. Blood was obtained from cut tails. Serum was inactivated, then serially diluted in 'Microtiter' plates (Cooke Engineering Co., Arlington, Va., U.S.A.)¹⁰ and guinea-pig complement (BBL) added. The plates were incubated at 37° C for 30 min; sheep red blood cells were added and incubated for 2 h at 37° C. The plates were then stored for about 20 h at 30° C. A dilution giving approximately half haemolysis was considered positive. All the titres are expressed as the negative logarithm to the base 2 of the dilution.

Fig. 1 shows the haemolysin response during the first 20 days following immunization with the sheep red blood cells. Sham-thymectomized animals displayed a two-

peaked response with the first peak occurring at 4 days, and the second peak occurring at 12–14 days. Thymectomized animals had a single-peaked response. The peak of this response occurred at 10 days and the titre reached one-sixth to one-eighth that of the sham-operated animal at 4 days. In the thymectomized animal there was no detectable haemolysin activity on or before 6 days.

By ultracentrifugation in a 10–40 per cent linear sucrose gradient in KRP solution the 19S and 7S classes of antibody could be separated¹¹. The first peak in the sham-thymectomized animal's response was found to be of the 19S class, and the second of the 7S class. In the thymectomized animals the antibody was of the 19S class at the peak, the 7S antibody appearing later.

It seems unlikely that the delayed response in thymectomized animals can be related directly to the lack of competent cells, since this lack, without any complicating compensating mechanisms, should lead to a decrease in the strength of response without alteration of the time relationships of the response. To produce a delay, competent cells stimulated by the sheep red blood cells must reach the stage of producing antibody at a later time. On the other hand, the decreased haemolysin titres observed in thymectomized mice can best be explained on the basis of a lack of competent cells or on a more rapid removal of circulating antibody⁶.

Keeping in mind the various responses to different antigens observed using a single time interval after immunization, it is worth looking at some arbitrary times in the present haemolysin response. If 6 days had been chosen, no haemolysin response in the thymectomized animals and a considerable response in the sham-thymectomized animals would have been observed. If 9 days had been chosen, no difference in the total haemolysin response between thymectomized and sham-thymectomized animals would have been detected. If later arbitrary times had been chosen, a decreased haemolysin response would have been observed in the thymectomized mice. Therefore, as a distinct possibility, the varying response in the same strain to different antigens observed by others could be explained at least in part on the time chosen for the antibody testing, or, if the time chosen for investigating the various antigens was kept constant⁵, on the time-course of the antibody response to that particular antigen. Using similar reasoning, the differing effects of thymectomy on primary and secondary response¹² could be due to the fact that the antibody activity of the thymectomized animals may have been sampled near the peak times for one response but not for the other. One of the more prominent sources of variation in the effect of neonatal thymectomy on antibody response is strain differences. While it seems unlikely that the

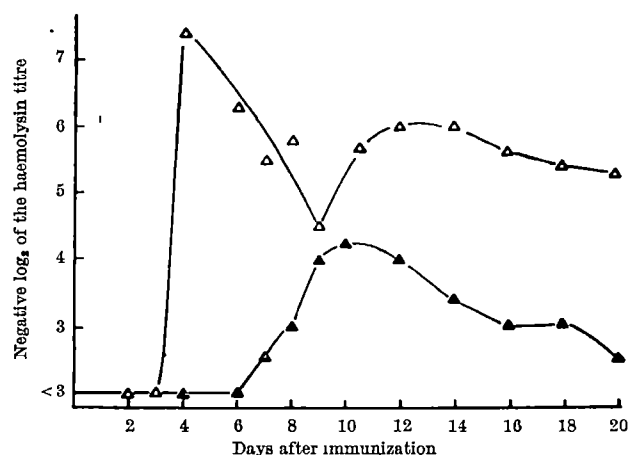


Fig. 1. The negative logarithm to the base 2 of the average haemolysin titre at various times after immunization is shown for both thymectomized (▲) and sham-thymectomized (△) animals. Each point represents at least 6 serum haemolysin determinations

degree of responsiveness observed in various strains would be altered to any great extent by using different time intervals between injection of the antigen and collection of the antibody-containing sera, the possibility should not be eliminated without investigation.

Thymectomized animals of a strain that has not shown an antibody response at a particular time may not respond at any time, or may have an antibody response at some later time. It may be important to distinguish between these possibilities when looking at the mode of action of an agent which protects a thymectomized animal against the usual immunological defects characteristic of that strain. Is the agent allowing the development of an immune response normally absent in the thymectomized animals, or is it merely shifting the response present in the thymectomized animals so that the response can now be detected at the particular time interval chosen? To answer this and related questions, one must determine antibody responses at various times after immunization.

I thank Prof. J. A. McCarter for his advice.

NICHOLAS R. SINCLAIR*

Department of Biochemistry,
Dalhousie University,
Halifax, Nova Scotia,
Canada.

* Medical Research Fellow, Medical Research Council, Canada. Present address: Chester Beatty Research Institute, Pollards Wood Research Station, Chalfont St. Giles, Buckinghamshire.

¹ Miller, J. F. A. P., *Lancet*, ii, 748 (1961).

² Miller, J. F. A. P., *Proc. Roy. Soc. B*, 156, 415 (1962).

³ Martinez, C., Kersey, J., Papermaster, B. W., and Good, R. A., *Proc. Soc. Exp. Biol. and Med.*, 109, 193 (1962).

⁴ Miller, J. F. A. P., Marshall, A. H. E., and White, R. G., *Adv. Immunol.*, 2, 111 (1962).

⁵ Good, R. A., Dalmasso, A. P., Martinez, C., Archer, O. K., Pierce, J. C., and Papermaster, B. W., *J. Exp. Med.*, 116, 773 (1962).

⁶ Humphrey, J. H., Parrott, D. M. V., and East, J., *J. Immunol.*, 7, 419 (1964).

⁷ Parrott, D. M. V., and East, J., in *The Thymus in Immunobiology*, edit by Good, R. A., and Gabrielson, A. E., 523 (Hoeber-Harper Publishers, New York, 1964).

⁸ Miller, J. F. A. P., *Brit. J. Cancer*, 14, 93 (1960).

⁹ Miller, J. F. A. P. (personal communication).

¹⁰ Sever, J. L., *J. Immunol.*, 88, 320 (1962).

¹¹ Kunkel, H. G., *The Plasma Proteins*, edit. by Putnam, F. W., 1, 279 (Academic Press, 1960).

¹² Hess, M. W., Cottler, H., and Stoner, R. D., *J. Immunol.*, 91, 425 (1963).

Inactivation of T-2 Bacteriophage by Sensitized Leucocytes *in vitro*

ALTHOUGH *in vitro* investigations of the primary¹ and secondary^{2,3} phases of the immune response have been reported, few investigators have used blood as a source of immunologically competent cells⁴⁻⁶. The use of blood instead of lymphoid organs as a source of cells enables the investigator to obtain multiple samples of a homogeneous cell population. This communication reports a preliminary approach to an immunological investigative system consisting of sensitized rabbit blood leucocytes and T-2 bacteriophage, a particulate antigen that can be assayed by a procedure which is relatively simple, sensitive and quantitative.

T-2 bacteriophage is assayed by the soft agar method described by M. H. Adams⁷. *E. coli* (strain B 295) is used to prepare bacteriophage stock and serves as the susceptible bacterium in the plaque assay. This organism is maintained on agar slants (stock) and in 'L. C.' broth⁸. The assay is carried out by overlaying 'L. C.' agar with 3.5 ml. of soft agar (0.5 per cent agar in water) in which a 0.1-ml. sample of T-2 phage has been mixed with 0.1 ml. of *E. coli*. The bacteria, prepared daily from an overnight culture, are held in early log phase (O.D. 60-90 Klett-Summerson units, red filter) by maintenance at room temperature. Phage dilutions, producing 20-40 plaques per dish, are plated in triplicate and the results are averaged.

T-2 bacteriophage is prepared according to a modified method described by M. H. Adams⁷. Phage at a total concentration of 5×10^7 P.F.U. (plaque forming units) is added to 20 ml. of *E. coli* in log phase, and the mixture incubated for 6 h. The cells are lysed by adding 0.5 ml. chloroform. Bacterial debris is cleared by centrifugation at 3,000g for 30 min. The virus is pelleted by centrifugation at 27,000g for 2 h. The phage is resuspended in medium 56 (ref. 9). This yields a titre of about 1×10^{11} P.F.U. per ml.

Larger amounts of phage are prepared by proportionately increasing the volume of reagents added. To avoid the time-consuming high-speed centrifugation, phage is precipitated by adjusting the pH of the supernatant of the low-speed centrifugation to pH 4.2 with concentrated HCl. The virus is then pelleted by centrifugation at 9,750g for 15 min. It is resuspended as outlined above.

Two intraperitoneal injections of 5×10^{10} P.F.U. of phage were given to male New Zealand rabbits twice a week for three weeks. Ten days later they were given 1×10^{11} P.F.U. of phage subcutaneously, and this dose was repeated twice at six-day intervals. The first blood sample was drawn two weeks after the last injection; the last sample was drawn a month later.

Blood was obtained by cardiac puncture. Heparin (Mann Research Labs.) was used to prevent coagulation (4×10^{-2} mg/ml. of blood). The blood was mixed with a 3.5 per cent gelatine solution (Fisher USP) in a 3:1 ratio (15 ml. of blood to 5 ml. of gelatin) and allowed to stand for about 20 min at 37° C (ref. 10). The supernate containing the leucocytes was removed and diluted to 70 per cent with CM (culture medium; Eagle's medium with 10 per cent foetal bovine serum)¹¹. The cells were centrifuged into a loose button at 1,000 r.p.m. for 6.5 min in a clinical centrifuge. The contaminating red cells were removed by resuspending the cells in 0.85 per cent NH_4Cl for 5 min¹². The leucocytes were then washed three times in CM.

The leucocytes were counted by a Coulter 'Model B' counter using a 50- μ pore, coupled to a size distribution write-out¹³. A dye exclusion test using Erythrosin B demonstrated a 98 per cent viability¹⁴.

Suspensions of leucocytes and phage in 4 or 5 ml. of CM were incubated at 37° C in 1 oz. stoppered prescription bottles filled with 5 per cent CO_2 in air. The degree of neutralization was determined by assaying the P.F.U. in the medium after centrifuging the cells out of suspension.

The results of several experiments employing varying phage and leucocyte concentrations are shown in Table 1. The rapid and steady decline in the number of P.F.U. is

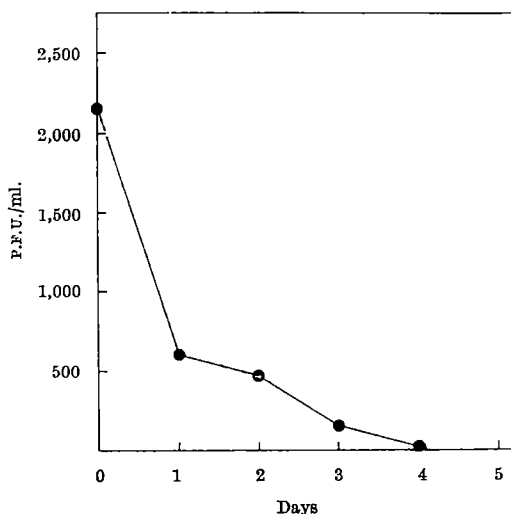


Fig. 1. P.F.U./ml. present in culture after incubation with sensitized leucocytes (experiment 3)

Table 1. NUMBER OF INFECTIVE PHAGE PARTICLES REMAINING AFTER INCUBATION WITH SENSITIZED LEUCOCYTES

Experiment	Animal	Cell concentration/ml.	P.F.U. added/ml.	Days							
				0	1	2	3	4	5	6	7
1	F	3.5×10^6	5,000	6,000	2,000	1,700	700	230	200	220	200
2	H	2.0×10^6	1,500	1,150	400	100	n.a.	n.a.	n.a.	n.a.	n.a.
3	I	2.5×10^6	2,500	2,150	600	480	160	30	n.a.	n.a.	n.a.
4	J	2.0×10^6	3,700	2,800	430	75	n.a.	470*	120	120	n.a.

n.a.—not assayed.

* Additional phage added to increase the antigen titre

Table 2. NUMBER OF INFECTIVE PHAGE PARTICLES REMAINING AFTER INCUBATION WITH NORMAL (UNSENSITIZED) LEUCOCYTES OR WITH NO CELLS PRESENT

Experiment	Animal	Cell concentration/ml.	P.F.U. added/ml.	Days					
				0	1	2	3	4	5
5	O	3.62×10^6	2,500	2,200	2,600	1,700	2,650	1,900	3,000
6		no cells	2,500	2,500	2,100	1,550	2,150	1,200	2,000
7	L	1.96×10^6	2,000	2,250	1,300	1,250	1,430	1,100	1,000
8		no cells	2,000	1,850	1,700	1,700	600	1,200	1,000

graphically demonstrated in Fig. 1, which is a plot of experiment 3. The discrepancy between the number of P.F.U. added and the number present on day 0 is due to the neutralization of T-2 phage by residual immune serum and a slight shift in plating characteristics when the virus is suspended in CM.

When similar cultures of phage and leucocytes from an unsensitized animal were prepared and assayed (expt. 5), the number of P.F.U. did not decline steadily. Comparable results were observed when phage was incubated alone in CM (expt. 6; Table 2). The results of these experiments are plotted in Fig. 2 for comparison with Fig. 1.

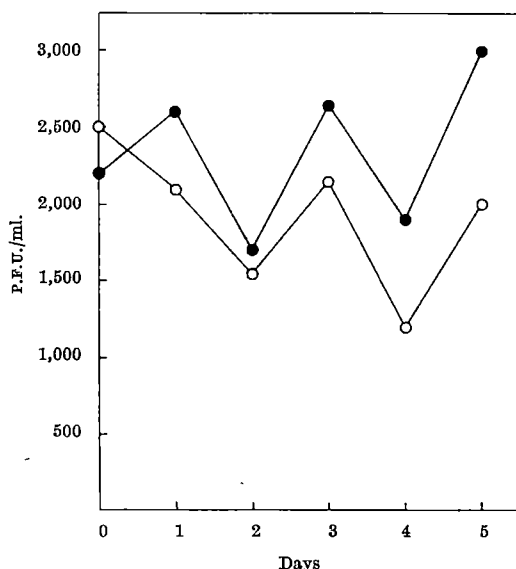


Fig. 2. P.F.U./ml. present in culture after incubation with unsensitized leucocytes or no cells present (experiments 5 and 6). ●, Normal leucocytes; ○, tissue culture media

The titre of the control cultures (phage incubated with unsensitized leucocytes or no leucocytes) did not remain constant. A kinetic study of this problem has been carried out in another laboratory and it appears that the original virus sample contained two populations of T-2 phage, one of which has a tendency to aggregate and dissociate¹⁵. This factor was not of critical importance in the present investigation, in which only the steady decline in P.F.U. was investigated.

Immunofluorescent studies have suggested that antibody production *in vitro* is most active on the third or fourth day of incubation^{4,16}. Plaque assay analysis³ and localized haemolysis techniques⁶ have detected specific

antibody formation after 24-h incubation, the latter method demonstrating peak synthesis on the fourth day. Our work confirms antibody production early in the incubation period, but suggests that somewhat different kinetics are involved. Whether the specific inactivation of phage in our system is due to antibody synthesis *in vitro*, release of preformed antibody, and/or phagocytosis is a subject for continued research.

This work was supported by U.S. Public Health Service grants 1 SOL FR 05358, GM-10789 and GM-9966. The bacteria and virus were obtained from Dr. Ruth Beloff, Department of Microbiology, Yale University. We thank Dr. Beloff for her suggestions and instructions in the use of plaque techniques.

ROBERT YOUNG
FRANK H. RUDDLE

Department of Biology,
Yale University,
New Haven, Connecticut.

- ¹ Fishman, M., *J. Exp. Med.*, **114**, 837 (1961).
- ² Michaelides, M. C., and Coons, A. H., *J. Exp. Med.*, **117**, 1035 (1963).
- ³ Attardi, G., Cohn, M., Horibata, K., and Lennox, E. S., *J. Immunol.*, **92**, 335 (1964).
- ⁴ Elves, M. W., Roath, S., Taylor, G., and Israels, M. C. G., *Lancet*, **i**, 1292 (1963).
- ⁵ Hulliger, L., and Sorkin, E., *Nature*, **198**, 299 (1963).
- ⁶ Landy, M., Sanderson, R. P., Bernstein, M. T., and Jackson, A. L., *Nature*, **204**, 1320 (1965).
- ⁷ Adams, M. H., *Bacteriophages* (Interscience Publishers, New York, 1959).
- ⁸ Luria, S. E., Adams, J. N., and Ting, R. C., *Virology*, **12**, 348 (1960).
- ⁹ Monod, J., Cohen-Bazile, G., and Cohn, M., *Biochim. Biophys. Acta*, **7**, 585 (1951).
- ¹⁰ Coulson, A. S., and Chalmers, D. G., *Lancet*, **i**, 468 (1964).
- ¹¹ Eagle, H., *Science*, **130**, 432 (1959).
- ¹² Dioguardi, N., Agostoni, A., Lomanto, B., and Piccaluga, A., *Haematologica Latina*, **5**, 99 (1962).
- ¹³ Harris, M., *Cancer Res.*, **19**, 1020 (1959).
- ¹⁴ Phillips, H. J., and Cherryberry, W. W., *Exp. Cell Res.*, **13**, 341 (1957).
- ¹⁵ Beloff, R. (personal communication).
- ¹⁶ O'Brien, T. F., Michaelides, M. C., and Coons, A. H., *J. Exp. Med.*, **117**, 1053 (1963).

Electrophoretic Mobility of an Immune Globulin from Rainbow Trout Serum

CONSIDERABLE interest has been focused recently on the comparative aspects of the immune response in vertebrates. A sound knowledge of the development of the immune response at various phylogenetic stages in existing vertebrates may clarify the evolution of the various aspects of vertebrate immunity through its highest development in mammals and birds, and provide a better understanding of the mechanisms involved. This communication reports findings concerning the immunoelectrophoretic mobility of an immunologically active fraction of serum from a teleost fish, rainbow trout (*Salmo gairdneri*).

Rainbow trout were immunized by injecting washed rabbit erythrocyte suspensions intracoelomically into a number of fish maintained at a constant water temperature of 15° C. The antisera of highest titre from two of these fish were selected for preparing anti-trout immune globulin sera. Both of these trout anti-rabbit erythrocyte sera were obtained from bleedings made more than 2 months after initial stimulation. The anti-trout immune globulin sera were prepared by injecting three rabbits intraperitoneally three times a week for 2 weeks with saline suspensions of rabbit erythrocytes coated with trout globulin. The rabbit erythrocytes were sensitized by incubation in 1:10 saline dilutions of the trout anti-rabbit erythrocyte sera followed by three washings in 10 volumes of 1 per cent saline solution. One-ml. aliquots of freshly prepared 25 per cent suspensions of sensitized rabbit erythrocytes in saline were used as inocula.

Two other antisera were used in this study. One, a rabbit anti-human serum reagent furnished by Dr. R. S. Weiser, was used to compare electrophoretic migrations of rainbow trout and human serum components. The other, prepared in rabbits from injections of sockeye salmon (*Oncorhynchus nerka*) red cell haemolysate preparations, was used for comparison of electrophoretic mobilities and immunological specificities with salmon globulins previously studied by immunoelectrophoresis¹.

The anti-trout immune globulin reagents were initially screened by the double-diffusion precipitation technique of Ouchterlony as modified by Ridgway *et al.*² and were found to contain precipitins. That these were immune and not normal precipitins was confirmed by similar tests comparing pre-immunization sera with post-immunization sera. Immunoelectrophoretic tests were made as described by Krauel and Ridgway¹.

Fig. 1 represents an immunoelectrophoretic slide comparing components in normal rainbow trout serum detected by the anti-trout immune globulin and anti-sockeye salmon haemolysate reagents with components of human serum detected with antihuman serum. When antisera from the other two rabbits were tested against normal trout serum, results were obtained comparable with those of the anti-trout immune globulin reagent used in Fig. 1. These arcs are asymmetrical, indicating that trout immune globulin is heterogeneous with respect to electrophoretic mobility and possibly to molecular size. The anti-haemolysate reagent detected two components in rainbow trout serum of distinct electrophoretic mobilities, the faster component having the same mobility as the component detected by the anti-trout immune globulin reagent. It is of interest to observe in the latter case that, while the mobilities are identical, the two components are not immunologically cross-reactive. Using the human serum-anti-human serum lines as a standard, the two faster components of the rainbow trout serum migrate as beta globulins while the slower component detected by the anti-haemolysate reagent has the mobility of a fast gamma or slow beta globulin.

Neither the beta nor the gamma globulin components of the rainbow trout serum which were detected by the anti-haemolysate reagent have been associated with immunological activity. These components have not been found in all sockeye salmon sera¹, while the component detected by the anti-trout immune globulin reagent has been present in sera of all trout or salmon tested.

Fairley and Harris³ incubated rabbit erythrocytes in human sera containing normal agglutinins for rabbit red cells. They subsequently found that these sensitized red cells were agglutinated by antisera against complement as well as by antisera against human antibody. In our studies with anti-trout immune globulin sera, the possibility cannot be excluded that the precipitins may represent trout complement-rabbit antibody precipitation as well as trout antibody-rabbit antibody precipitation.

No immunologically active globulin with true gamma 2 mobility was detected by this procedure. This accords with the finding of little or no gamma 2 globulin reported by Deutsch and McShan⁴ and Engle *et al.*⁵ for several species of teleosts; by Clem and Sigel⁶ for the margate (*Haemulon album*); and by Post⁷, who partially purified rainbow trout anti-bacterial antibody and studied its mobility by paper electrophoresis. However, Uhr *et al.*⁸ have reported significant levels of gamma 2 globulin in anti-phage sera from the goldfish (*Carassius auratus*) 2-4 weeks after immunization, and much increased levels of gamma 2 globulin two months after immunization. Also, Papermaster *et al.*⁹ found 'multiple gamma bands' by starch-gel electrophoresis and immunoelectrophoresis in the teleosts they studied. The variations in globulin mobilities reported suggest that either the various experimental approaches result in different responses, or that different species of teleost fish cannot be considered to be identical with respect to the molecular species of globulins with antibody activity.

This study was supported in part by U.S. Public Health Service grant CRT 5040 from the National Cancer Institute.

HAROLD O. HODGINS
GEORGE J. RIDGWAY
FRED M. UTTER

U.S. Fish and Wildlife Service,
Bureau of Commercial Fisheries Biological Laboratory,
Seattle, Washington.

¹ Krauel, K. K., and Ridgway, G. J., *Intern. Arch. Allergy*, **23**, 246 (1963).

² Ridgway, G. J., Klontz, G. W., and Matsumoto, C., *Bull. S. Intern. North Pac. Fish Comm.*, 1962.

³ Fairley, G. H., and Harris, G., *Vox Sang.*, **7**, 175 (1962).

⁴ Deutsch, H. F., and McShan, W. H., *J. Biol. Chem.*, **180**, 219 (1949).

⁵ Engle, jun., R. L., Woods, K. E., Paulsen, E. E., and Pert, J. H., *Proc. Soc. Exp. Biol. and Med.*, **98**, 905 (1958).

⁶ Clem, L. W., and Sigel, M. M., *Fed. Proc.*, **22**, 1138 (1963).

⁷ Post, G., *Departmental Information Bulletin*, **63-7**, 42 (State of Utah, Department of Fish and Game, 1963).

⁸ Uhr, J. W., Finkelstein, M. S., and Franklin, E., *Proc. Soc. Exp. Biol. and Med.*, **111**, 13 (1962).

⁹ Papermaster, B. W., Condie, E. M., Finstad, J., and Good, R. A., *J. Exp. Med.*, **119**, 105 (1964).

PATHOLOGY

Lactic Dehydrogenase Isoenzymes in Muscle from Patients with Duchenne Muscular Dystrophy

THE enzyme lactic dehydrogenase (LDH) can be separated into five different isoenzymes, each composed of a tetramer of one or both of two distinct polypeptides^{1,2}. Isoenzymes 1 and 5 are composed solely of one of these two polypeptides, and isoenzymes 2, 3 and 4 consist of combinations of the two polypeptides. The proportions of the five isoenzymes vary from tissue to tissue, during development and with certain other environmental factors^{3,4}. In skeletal muscle one such factor is the amount of muscle activity⁴.

Several reports⁵⁻⁹ have indicated that the proportion of LDH 5 in muscle from patients with Duchenne's dystrophy was significantly reduced. Two of these authors^{6,7} were

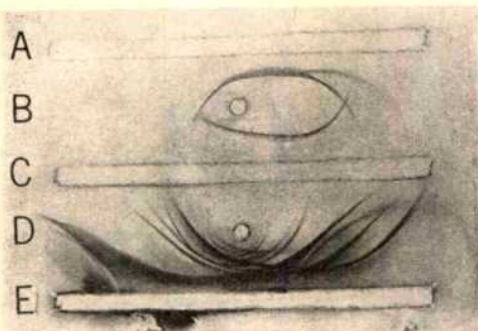


Fig. 1. Immunoelectrophoretic mobilities of globulin components of normal rainbow trout serum compared with components of human serum. A, Rabbit anti-sockeye salmon red cell haemolysate antiserum; B, normal rainbow trout serum; C, rabbit anti-trout immune globulin antiserum; D, human serum; E, rabbit anti-human serum antiserum.

unable to detect any LDH 5 activity. These findings suggested a plausible biochemical cause for Duchenne's dystrophy, namely the incorrect construction of the polypeptide that solely constitutes LDH 5. The other polypeptide of LDH being normal would allow for some activity in the remaining four tetrameres, assuming that the inactive polypeptide could remain structurally competent to bind the functional polypeptide. Furthermore, the fact that LDH 5 functions under anaerobic conditions more effectively than LDH 1 might contribute a physiological explanation for muscle weakness or premature degeneration in a fibre lacking the sub-units of LDH 5. Therefore, it seemed important to investigate in further detail LDH activity from muscle biopsies obtained from dystrophic patients. Besides starch electrophoretic separation of the LDH isoenzymes from biopsy and tissue culture the results of pyruvic inhibition and histochemical determinations of LDH are reported briefly in this communication.

The three subjects of the investigation were boys with the onset of progressive muscular weakness at approximately five years of age. Although none had other affected family members, all had pseudohypertrophy of muscles and the typical biopsy and serum findings associated with Duchenne's dystrophy. Patients 1 and 2 were eight years of age and were only mildly affected. Patient 3 was fourteen years old and confined to a wheel-chair. Investigations were carried out on biopsies from deltoid muscle.

The fresh muscle was homogenized in 0.2 tris buffer (pH 8.3) and the supernatant LDH activity was measured by the method of Bergmeyer *et al.*¹⁰. The isoenzymes were separated by starch-gel electrophoresis (5 V/cm)¹¹ and stained for LDH activity¹². The total activity added to the slots was made equal through appropriate dilution of the supernatants. Cells derived from tissue culture of the biopsy were washed and homogenized before addition to the starch-gel slots. The frozen tissue was sectioned at 5 μ and 8 μ thicknesses and stained, together with appropriate controls, for LDH activity by the method of Thomas and Pearse¹³.

Although LDH 5 was clearly present in all three (Fig. 1), the first two patients showed some decrease in its relative proportion. The third showed no decrease. The isoenzyme patterns obtained from the cells tissue-cultured from the muscles predominantly showed LDH 5, as did the controls. An unexpected observation was that in patient 3 a neurogenic type of atrophy was found in the deltoid region, although the previous muscle biopsy from the calf was compatible with progressive muscular dystrophy. The

Table 1. ENZYME ACTIVITIES IN SERUM AND MUSCLE

	Serum units/ml.	Muscle LDH
	LDH*	Units* per g 2.9 $\times 10^{-4}$ M pyruvate
Patient 1	420	< 18
Patient's mother	125	0.9
Patient 2	—	404
Patient's brother	—	7.6
18-yr.-old male	—	—
Patient 3	320	77
Patient's mother	185	1.7
Heart muscle	—	—
		200,600

* Wroblewski units.

total LDH activity in the muscle from the dystrophic boy was less than the control (Table 1). The percentage of LDH activity measured using 10^{-3} M pyruvate was reduced but not to the extent of heart muscle, which contained practically no LDH 5 (Table 1). The histochemical stains for LDH revealed no differences in amount or intracellular location of precipitate.

The isoenzyme patterns showing LDH 5 in these three particular patients presumably affected by the clinical syndrome of Duchenne's dystrophy differ from those reported by Emery, who found no LDH 5. In view of Zondag's work¹⁴, which shows that LDH 5 is more labile when frozen, it is possible that Emery in storing his samples at -15° C destroyed LDH 5. In confirmation of Zondag's findings, loss of LDH 5 activity was found after freezing homogenates obtained from both normal and dystrophic muscle; however, partial preservation was accomplished by adding nicotinamide adenine dinucleotide. The present observations tend to suggest that the small reduction in LDH 5 isoenzyme is secondary to physiological changes in the muscle rather than a primary biochemical change in progressive muscular dystrophy.

This work was supported by grants HD00449 and HD00836 from the U.S. Public Health Service and in part through the Clinical Research Facility of the University of Washington supported by the National Institutes of Health (FR 37). We thank Drs. Stanley Gartler and Elizabeth Smith for carrying out the tissue cultures and the creatine phosphokinase determinations respectively.

THOMAS H. SHEPARD
LAWRENCE H. GORDON
JOHN E. WOLLENWEBER

Department of Pediatrics and Department of Surgery,
School of Medicine,
University of Washington, Seattle.

¹ Markert, C. L., and Moller, F., *Proc. U.S. Nat. Acad. Sci.*, **45**, 753 (1959).

² Markert, C. L., in *Cytodifferentiation and Macromolecular Synthesis*, edit. by Locke, M., 65 (Academic Press, 1963).

³ Cahn, R. O., Kaplan, N. O., Levine, L., and Zwilling, E., *Science*, **136**, 965 (1962).

⁴ Dawson, D. M., Goodfriend, T. L., and Kaplan, N. O., *Science*, **143**, 929 (1964).

⁵ Drefus, J. C., Demos, J., Schapira, F., and Schapira, G., *C.R. Acad. Sci., Paris*, **254**, 4384 (1962).

⁶ Pearson, C. M., *Amer. J. Med.*, **35**, 632 (1963).

⁷ Emery, A. E. H., *Nature*, **201**, 1044 (1964).

⁸ Lauryssens, M. G., Lauryssens, J. J., and Zondag, H. S., *Clin. Chim. Acta*, **9**, 276 (1964).

⁹ Brody, I. A., *Neurology*, **14**, 1091 (1964).

¹⁰ Bergmeyer, H.-U., Bernt, E., and Hess, Benno, in *Methods of Enzymatic Analysis*, edit. by Bergmeyer, H.-U., 736 (Academic Press, 1963).

¹¹ Smithies, O., *Biochem. J.*, **71**, 585 (1959).

¹² Vesell, E. S., and Bearn, A. G., *J. Gen. Physiol.*, **45**, 553 (1962).

¹³ Thomas, E., and Pearse, A. G. E., *Histochemie*, **2**, 266 (1961).

¹⁴ Zondag, H. S., *Science*, **142**, 965 (1963).

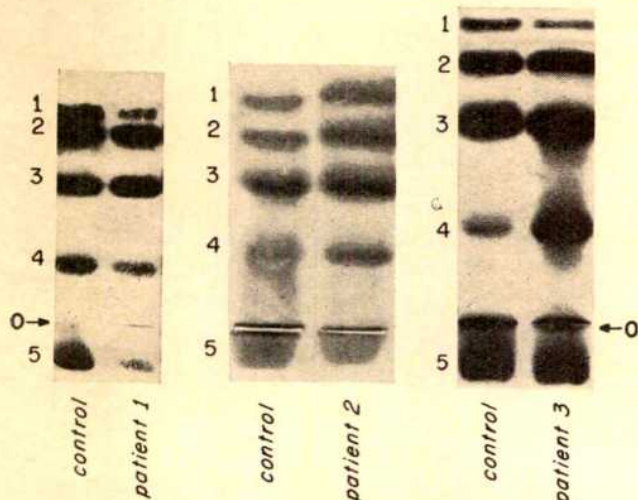


Fig. 1. LDH isoenzyme patterns from homogenates of muscle from three dystrophic patients. The control for patient 1 was from a normal brother, while controls for patients 2 and 3 were obtained from autopsy material. O signifies the origin and the anode is at the top.

RADIOBIOLOGY

A Reduced Oxygen Enhancement Ratio for X-ray Survival of HeLa Cells *in vitro*, after Treatment with 'Methotrexate'

THE importance of anoxic cells within human tumours as a potential cause of failure when these tumours are treated by X- or γ -ray therapy has been realized for more than ten years^{1,2}. Clinical attempts have been made

render all tumour cells well-oxygenated by irradiating patients while they are breathing pure oxygen under increased pressure in a tank, and radiations of higher ionization density such as some fast neutrons, which are relatively independent in their effects of the presence or absence of oxygen, have passed the stage of preliminary trials^{3,4}. A similar clinical result, however, might be obtained by using a chemical modifier of radiation effects which produced greater potentiation of X-ray cell-killing under anoxic conditions than under well-oxygenated conditions⁵.

As part of a continuing investigation of the mechanism of cellular recovery from X-ray damage⁶, the reproductive survival of HeLa S-3_{oxf} cells was determined by clonal growth after single and fractionated X-irradiations delivered under oxygenated or anoxic conditions. Growth media and criteria for reproductive survival were as previously described⁷; all irradiations were carried out 8–24 h after the cells were plated in Falcon TC Petri dishes so that they were well attached to the plastic surface at the time of irradiation. Oxygenated conditions are defined as a humidified mixture of 5 per cent carbon dioxide in air flowing over the medium at the time of irradiation; anoxic conditions were produced by gassing the Petri dishes in a brass pressure tank for 6 h with humidified 5 per cent carbon dioxide in white spot purity nitrogen (British Oxygen Co., Ltd.) at a flow rate of 50 ml./min. The oxygen contamination of the effluent gas was measured by a Hersch cell and was less than 15 parts per million. (These measurements were kindly performed by Dr. E. J. Hall and Mr. J. S. Bedford.) Irradiation with 250 kVp X-rays (half-value layer = 1.3 mm copper) was by a simultaneous, parallel-opposed field technique (focal-skin distance = 50 cm) at a dose-rate of 43 rad/min measured under aerated conditions by a ferrous sulphate dosimeter inside the brass pressure tank. In additional experiments with and without 'Methotrexate', cells were irradiated under oxygenated conditions with 90 kVp X-rays (half-value layer = 1.1 mm aluminium) at a dose-rate of 169 rad/min. This latter technique produced dose-response curves which were indistinguishable from those produced by irradiating the cells under oxygenated conditions inside the brass pressure tank with 250 kVp X-rays.

'Methotrexate' (4-amino-10-methylpteroylglutamic acid, Lederle) was added to the growth medium at the time of plating, removed 24 h later by aspiration, and replaced with identical medium without the drug, after washing the plates once with complete growth medium without serum. This treatment sensitized the cells to X-irradiation even at concentrations of the drug which did not themselves produce any cell-killing, as is shown in Fig. 1. Higher concentrations of 'Methotrexate' present in the medium for only 24 h killed a proportion of the cells outright, although the growth medium contained folic acid in a concentration of 0.01 $\mu\text{g/ml}$. Those cells not synthesizing DNA during the 24-h period while the 'Methotrexate' was present in the medium escaped its effect regardless of the concentration of the drug in the medium (Fig. 2); this is similar to earlier observations of effects of the anti-metabolite 5-fluorouracil on survival of HeLa cells *in vitro*⁷.

At a drug concentration of 0.3 $\mu\text{g/ml}/24\text{ h}$, the X-ray dose-response curve for 'Methotrexate' survivors showed a significantly reduced oxygen enhancement ratio (OER), as shown in Fig. 3. If this observation is confirmed in other cell lines and in tumours *in vivo*, it would predict a therapeutic 'gain factor'⁸ of 1.56 over X-irradiation without the drug present, for killing anoxic tumour cells. Even if a major effect of 'Methotrexate' is simply to partially synchronize the cell population by selecting as survivors only those cells which have not yet begun DNA synthesis⁹, the fact that the subsequent survival after X-irradiation is less sensitive to the presence or absence of oxygen suggests that this drug may be useful in com-

bination with radiotherapy. One would expect, however, that the total X-ray dose administered would have to be suitably reduced both because the effect of single X-ray doses is potentiated by 'Methotrexate', and because HeLa cells show a markedly decreased ability for recovery between fractionated irradiations in the presence of 'Methotrexate'⁶ which would magnify the effects of a fractionated course of irradiation as compared with a similar course without the drug present when normal intracellular recovery could take place. This effect of 'Methotrexate' on recovery is likely to explain the recent finding of Condit *et al.*¹⁰, that the combination of intravenous administration of 'Methotrexate' with radiotherapy produced unacceptable normal tissue response if protracted treatment schedules were used, but no significant toxicity if short, intensive radiation courses were given.

It is hoped that the laboratory finding of a reduced oxygen enhancement ratio in the X-ray dose-response of a strain of human cells treated previously with 'Methotrexate', combined with the favourable early clinical

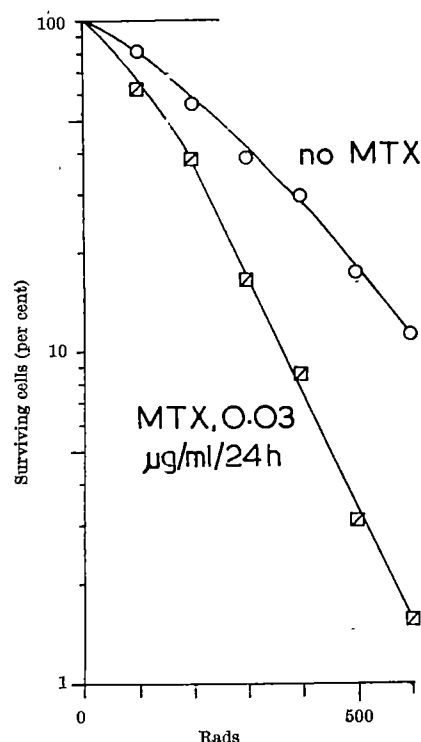


Fig. 1. X-ray dose-response, HeLa S-3_{oxf}

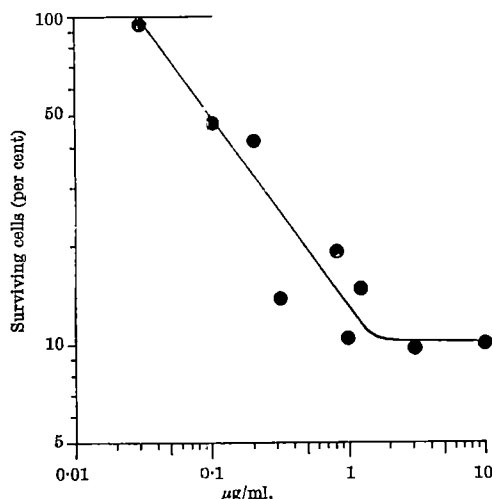


Fig. 2. Reproductive survival of HeLa S-3_{oxf} cells after 24-h exposure to 'Methotrexate'

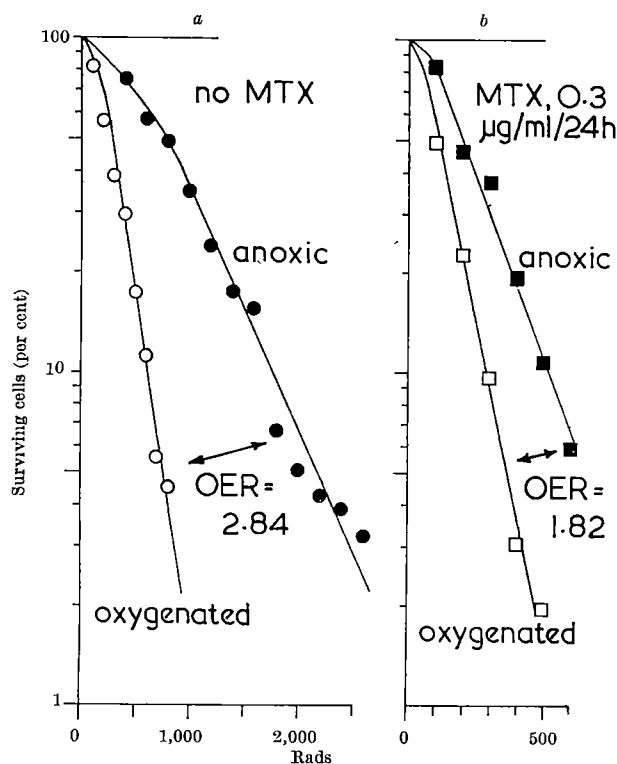


Fig 3. X-ray dose-response, HeLa S-3 cells under anoxic or oxygenated conditions. Survival curve parameters are as follows (a) no 'Methotrexate', $n=2.0$, D_0 anoxic = 595 rads, D_0 oxygenated = 210 rads, (b) 'Methotrexate' $0.3 \mu\text{g/ml/24 h}$, $n=1.25$, D_0 anoxic = 200 rads, D_0 oxygenated = 110 rads

evaluation of this drug in conjunction with radiotherapy^{10,11}, may lead to larger-scale, systematic and controlled clinical evaluation.

I hold a Helen Hay Whitney fellowship in radiobiology in the University of Oxford. This work was begun with support from the Damon Runyon Memorial Fund, and is continuing with the support of the Medical Research Council.

ROGER J. BERRY

Radiobiology Laboratory,
Radiotherapy Department,
Churchill Hospital, Oxford.

- ¹ Gray, L. H., Conger, A. D., Ebert, M., Hornsey, S., and Scott, O. C. A., *Brit. J. Radiol.*, **26**, 638 (1953).
- ² Churchill-Davidson, I., Sanger, G., and Thomlinson, R. H., *Lancet*, **1**, 1091 (1955).
- ³ Churchill-Davidson, I., *Brit. J. Radiol.*, **37**, 721 (1964).
- ⁴ Fowler, J. F., and Morgan, R. L., *Brit. J. Radiol.*, **36**, 115 (1963).
- ⁵ Alper, T., in *Cellular Radiation Biology*, 272 (Baltimore, Williams and Wilkins Co., 1965).
- ⁶ Berry, R. J., *Brit. J. Radiol.*, **38**, 155 (1965).
- ⁷ Berry, R. J., *Nature*, **203**, 1150 (1964).
- ⁸ Bewley, D. K., and Hornsey, S., in *Biological Effects of Neutron and Proton Irradiations*, 2, 173, Vienna, I.A.E.A. (1964).
- ⁹ Dalen, H., Oftebro, R., and Engeset, A., *Cancer*, **18**, 41 (1965).
- ¹⁰ Condit, P. T., Ridings, G. R., Com, J. W., Williams, G. R., Mitchell, D., and Boles, G. W., *Cancer Res.*, **24**, 1524 (1964).
- ¹¹ Friedman, M., and Daly, J. F., *Amer. J. Roent.*, **90**, 246 (1963).

Effects of Dietary Levels of Phosphorus and Calcium on the Comparative Behaviour of Strontium and Calcium

THE body burden of radioactive strontium to be attained in the population depends directly, among other factors, on the comparative metabolic behaviour of ingested strontium and calcium¹. Early work demonstrated a degree of constancy in the relative behaviour of the two elements that made such an approach useful for approximate estimates of radiation dosages to be expected from given intakes of calcium and radioactive strontium. An important practical implication was that if the relative behaviour were constant, then the body burden of radio-

active strontium to be developed could be proportional, reduced by dietary supplementation with uncontaminated calcium². More detailed investigation, however, shows that the relative behaviour of these two alkaline earths did vary under certain conditions³⁻⁵. Therefore the work recorded here was done in growing rats to investigate systematically two important variables which were shown to affect the relative absorption and retention of calcium and strontium; namely, the dietary levels of calcium and phosphorus.

The terminology is that of the 'observed ratio' where $OR_{\text{bone/diet}} = \frac{\text{Sr/Ca of bone}}{\text{Sr/Ca of diet}}$ and values of OR were determined by short-term double tracer investigations. The details of this procedure have been described previously⁶. The rats were of a highly inbred albino strain and were 8-9 weeks of age at the time of the experiment. After weaning they were raised on a stock diet containing 1.2 per cent calcium and 0.8 per cent phosphorus. At the start of the experimental period the animals were placed on the given experimental diet; 2 days later they receive tracer levels of ⁸⁵Sr and ⁴⁵Ca (or ⁴⁷Ca) incorporated in the drinking water for a 2-day period. After the end of the radionuclide application the animals were maintained on the experimental diet for another 2 days and then killed for tissue analysis. Thus, the rats were on the experimental diet for a total of 6 days, receiving the tracer strontium and calcium during the 3rd and 4th days. The skeletons were dissected from the soft tissue and ashed at 800° for 18 h. The ashed skeletons were dissolved in warm hydrochloric acid and the solution made to volume for the radioactive assays.

The effects of calcium and phosphorus levels on the values of $OR_{\text{bone/diet}}$ are presented in Figs. 1A and B. Various combinations of levels were studied with calcium ranging from 0.4 to 2.4 per cent and phosphorus from 0.4 to 2.0 per cent. As shown in Fig. 1A, the OR value generally

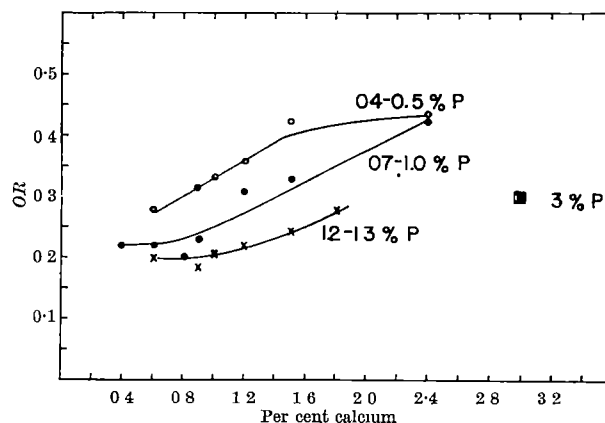


Fig. 1A Effect of dietary calcium on $OR_{\text{bone/diet}}$ values at varying dietary levels of phosphorus

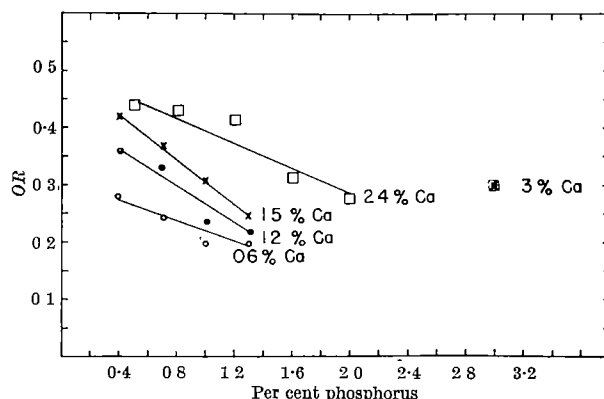


Fig. 1B Effects of dietary phosphorus on $OR_{\text{bone/diet}}$ values at varying dietary levels of calcium

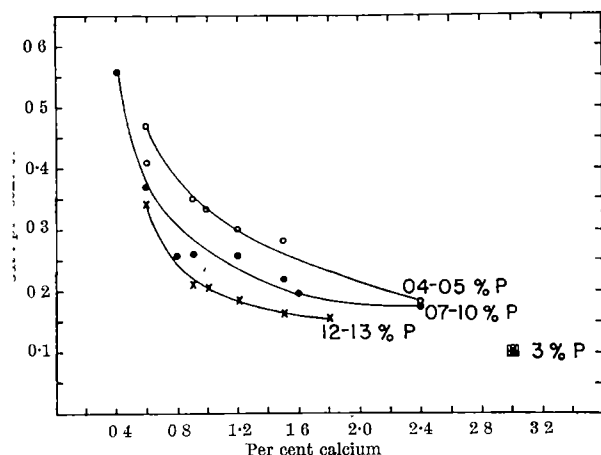


Fig. 2A. Effect of dietary calcium on $OR \div \text{per cent calcium}$ at varying dietary levels of phosphorus

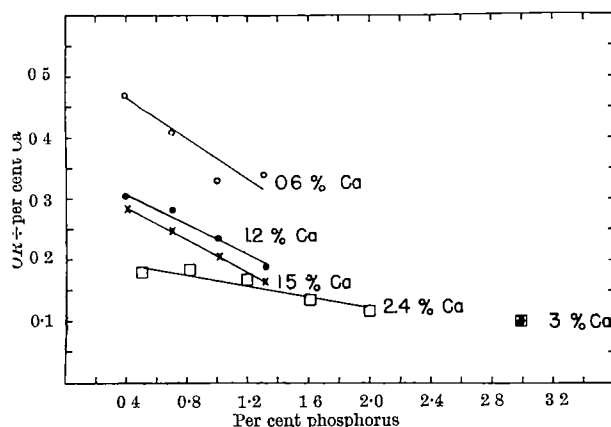


Fig. 2B. Effect of dietary phosphorus on $OR \div \text{per cent calcium}$ at varying dietary levels of calcium

increased with the calcium content of the diet. There seemed to be little change in the OR value at 0.7–1.3 per cent phosphorus between calcium levels of 0.4–1.0 per cent. Roughly, a change from 1 to 2 per cent calcium (2-fold) caused an increase of 1.5-fold in the OR value.

Fig. 1B shows that the OR value decreased as the phosphorus content of the diet was increased at given levels of calcium. In general, when the phosphorus level was increased from 0.4 to 1.2 (3-fold) the OR value was decreased by a factor of about 1.5.

A consideration of some interest is the degree to which the body burden of ingested radiostromium can be decreased by dietary supplementation with uncontaminated calcium³. The prediction of such a reduction is reflected not by the value of OR but rather by the value of $OR \div \text{per cent calcium}$. Therefore, the data have been so expressed in Figs. 2A and B. In Fig. 2A it is noted that as the calcium content increased from 0.6 to 2.4 per cent (4-fold) the $OR \div \text{per cent calcium}$ decreased by a factor of about 2.3. The effect of changing the concentration of phosphorus is shown in Fig. 2B. At calcium levels of 0.6–1.5 per cent the slopes of the curves were similar and as the phosphorus content varied from 0.4 to 1.2 per cent (3-fold), the $OR \div \text{per cent calcium}$ decreased by a factor of 1.5; at the 2.4 per cent level of calcium the effect was smaller giving a comparative factor of decrease of about 1.2.

It was of interest to determine the practical extremes at which these effects were demonstrable. Accordingly an investigation was carried out using an experimental diet containing 3 per cent calcium and 3 per cent phosphorus. The results are shown as single points in Figs. 1A to 2B; the OR value was 0.30 and the $OR \div \text{per cent calcium}$ was 0.10, which represented a reasonable fit with the other data as far as could be determined.

Although previous experience had indicated that short-term investigations of this nature do reflect long-term behaviour, it is satisfying to note the close agreement with data based on a 94-day feeding period recently published⁴; Harrison's experiments compared diets of 0.56 and 1.1 per cent dietary calcium at a phosphorus-level of 0.66 per cent and gave values of $OR \div \text{per cent calcium}$ ratios of 0.41 and 0.28, respectively. These compare with values of 0.40 and 0.25 as taken from Fig. 2A. In general the present findings are not in disagreement with the limited number of values and comparisons that are to be found in the literature^{3-5,8}.

It is not the intention here to discuss the reasons for this behaviour. Many factors are undoubtedly operative; in particular, any effects on gastrointestinal absorption and renal excretion. The work recorded here was done with growing animals since this phase is of most interest as regards potential harm from deposited radionuclides. There is an indication that mature animals respond somewhat differently⁴. Also, since the OR value is markedly higher in the very young than in the older individual, the considerations presented here become less applicable with increasing age.

Perhaps the important feature of the investigation is the demonstration that, in the growing rat, the OR value tends more or less regularly to increase with increasing dietary calcium and to decrease with increasing dietary phosphorus. The minimum body burden of ^{90}Sr would be attained if the calcium level of the diet were increased to a maximum by use of uncontaminated calcium and if, at the same time, the phosphorus level were also increased to a maximum. At levels of calcium between about 0.4 and 1.0 per cent and at phosphorus concentrations of 0.7–1.3 per cent, the decrease in body burden of ^{90}Sr would be expected to be roughly proportional to the increase of dietary calcium. At higher levels of calcium and lower levels of phosphorus an increased calcium would lower the body burden of ^{90}Sr to an extent less than proportional. The extent to which levels of ^{90}Sr in the human infant are affected by dietary calcium and phosphorus is not clearly known.

This work was supported by the Federal and Republic Councils for Scientific Research, Belgrade and Zagreb.

K. KOSTIAL
S. VOJVODIC
C. L. COMAR

Institute for Medical Research,
Zagreb, Yugoslavia, and
Department of Physical Biology,
Cornell University, Ithaca, New York.

¹ Comar, C. L., and Wasserman, R. H., in *Mineral Metabolism*, 2, Part A, ed. by Comar, C. L., and Bronner, F., 2, Part A, 523 (Academic Press, New York, 1964).

² Wasserman, R. H., Comar, C. L., and Papadopolou, D., *Science*, **126**, 1180 (1957).

³ Wasserman, R. H., and Comar, C. L., *Proc. Soc. Exp. Biol. and Med.*, **103**, 124 (1960).

⁴ Thompson, R. C., and Palmer, R. F., *Amer. J. Physiol.*, **199**, 94 (1960).

⁵ Kostial, K., Luttkie, A., Gruen, N., Vojvodic, S., and Harrison, G. E., *Intern. J. Rad. Biol.*, **6**, 421 (1963).

⁶ Harrison, G. E., *Intern. J. Rad. Biol.*, **8**, 177 (1964).

⁷ Comar, C. L., Wasserman, R. H., and Nold, M. M., *Proc. Soc. Exp. Biol. and Med.*, **92**, 859 (1956).

⁸ Palmer, R. F., and Thompson, R. C., *Amer. J. Physiol.*, **207**, 561 (1964).

BIOLOGY

Occurrence of *Salvinia auriculata* Aublet on the Congo River

THE floating water fern *Salvinia auriculata* Aublet has caused considerable anxiety by its rapid spread on the newly formed Kariba lake in Rhodesia¹⁻³.

The possibility therefore of the plant spreading to other man-made lakes is a matter of great concern.

This danger is of particular importance to West Africa, where the Volta lake is already filling, and a large lake is due to be formed on the River Niger. Both these river

basins at present appear to be free from both *S. auriculata* and the equally important water hyacinth, *Eichhornia crassipes* Solms.

Therefore the recent discovery (in October 1964) that *S. auriculata* is abundantly present on the Congo river in association with the well-established *E. crassipes* indicates a significant hazard to other countries. Floating masses of hyacinth enclosing *Salvinia* plants are discharged into the Atlantic where some individuals may survive to be accidentally or even deliberately carried elsewhere.

That the presence of *S. auriculata* on the Congo is relatively recent is indicated by botanical collections made on the Congo in 1957 by Lebrun⁴ which included specimens of *S. rotundifolia*, a less economically important species, while *S. auriculata* was not observed.

This collection and two others by Wagemans in 1958, also from the Congo, were all identified by Compère⁵ as *S. rotundifolia*. This identification has since been confirmed by Jarrett⁶ at Kew.

The collection of *S. auriculata* was made by me at, and a few miles above, Leopoldville. Large amounts of the plant were observed as small clumps apparently broken off from larger mats upstream. The material was in the 'tertiary' or folded leaf form and carrying abundant sporocarps. The *Salvinia* was to be found among the numerous clumps of hyacinth stranded on islands in the river and along the bank.

Vigilance by countries within range of the Congo would appear to be desirable so that any isolated introductions of either *S. auriculata* or *E. crassipes* can be readily recognized and rapidly exterminated.

That this hazard is real is shown by the recent (possibly deliberate) introduction, it is believed from the Congo, of *E. crassipes* to Senegal, where action is being taken by the Department of Agriculture at Dakar⁷ to eradicate the weed.

E. C. S. LITTLE

Agricultural Research Council
Weed Research Organization,
Begbroke, Kidlington, Oxford.

¹ Schelpe, E. A. C. L. E., *J. South African Bot.*, 27 (3), 181 (1961).

² Hattlingh, E. R., *Weed. Res.*, 1, 803 (1961).

³ Boughey, A. S., *Adansonia* III, 1, 49 (1963).

⁴ Lebrun, J., *Herb. Hort. bot. Brux.*, 11, 190 (1957).

⁵ Compère, C., *Bull. Jard. bot. Etat. Brux.*, 32, 200 (1962).

⁶ Jarrett, F. M. (personal communication).

⁷ Pradat, A. (personal communication).

Air Ion Effects on the Oxygen Consumption of Barley Seedlings

IN previous work we demonstrated that exposure of higher plants such as *Avena sativa* and *Hordeum vulgare* to (+) or (-) air ions generated in a pollutant-free atmosphere produced a statistically significant increase in growth under laboratory conditions as well as under greenhouse conditions^{1,2}. Since then, our interests have been focused on the biochemical mechanisms involved in air-ion-enhanced growth. It was found that (+) or (-) air ions materially increase the biosynthesis of cytochrome c and other iron-containing enzymes^{3,4}. We have postulated that the increased concentration of these enzymes may be an important metabolic factor in making possible the accelerated growth-rate regularly observed in ionized air⁴. If this is so, one would expect to find evidence of increased respiratory activity in ion-treated plants. The experiments described here were performed to detect such an increase.

Barley seeds (*Hordeum vulgare* var. 'Musashino') were soaked in distilled water and aerated for 3-5 days. Germinated seeds with several roots 1-2 cm long, but without an emerging coleoptile, were placed on 'Cashmiron' wool moistened with distilled water in a plastic Petri dish. Each of three dishes containing 30 seeds was placed in a plastic ion chamber⁵. The seeds were exposed in the dark

to air-ion densities of 4.5×10^7 (-) ions/cm²/sec; 4.0×10^7 (+) ions/cm²/sec or to normal non-ionized air. At stated intervals 5 seedlings were collected from each chamber and their oxygen consumption was measured by conventional manometric methods at 30° C (ref. 6) repeating each determination three times. Next, the seedlings were dried, first in an oven at 60° C, then over calcium chloride in a desiccator, and they were weighed.

Even as early as 24 h after the start of exposure to air ions, there was a clear-cut increase in oxygen consumption of the treated seedlings as compared with the control (Fig. 1) and the percentage increase at each time-interval remained relatively constant throughout the 96-h period of observation. Evidently an increased oxygen consumption is a significant element in the response of higher plants to treatment with air ions. This finding is compatible with data cited later obtained during the course of experiments on iron chlorosis.

First, it was observed that the development of iron chlorosis in seedlings cultivated in iron-free nutrient solution is markedly accelerated by exposing the seedling to air ions of either charge³. As the concentration of chlorophyll drops at the onset of chlorosis there is a marked rise in the concentration of cytochrome c (as determined by spectrophotometric absorbance and the weight of protein and iron in the extracted cytochrome c fraction)⁴. When seedlings are grown in an iron-containing medium and are exposed to positively or negatively ionized atmosphere during growth, the typical ion-induced acceleration in growth rate is accompanied by stimulation of cytochrome c synthesis, but chlorosis fails to develop and there is no essential difference in the concentration of chlorophyll between exposed and control plants.

Next, studies were made on the effects of gaseous ion on the active and residual iron content of barley seedling during the course of iron chlorosis⁷. Active iron is the fraction concerned with the production of chlorophyll; it is contained in the chloroplasts and is soluble in hydrochloric acid^{8,9}. Residual iron occurs outside the chloroplasts; it is not concerned with chlorophyll production nor is it soluble in 1 N hydrochloric acid. Air ions of either charge induce a significant decrease in active iron content of the seedlings as the chlorophyll content declines. At the same time there occurs an increase in residual iron and the cytochrome c fractions of the seedlings. There is evidence that the rise in residual iron content involves not only cytochrome c but other cytochromes and iron-containing enzymes as well.

Our present working hypothesis, based on earlier experimental results and compatible with the work reported here, suggests that one part of iron within the seed exists in a non-specific form and can be used as required for any purpose (free-state iron). When seeds are soaked in water some of the iron is diverted to the enzyme system concerned in germination. The remainder of the free

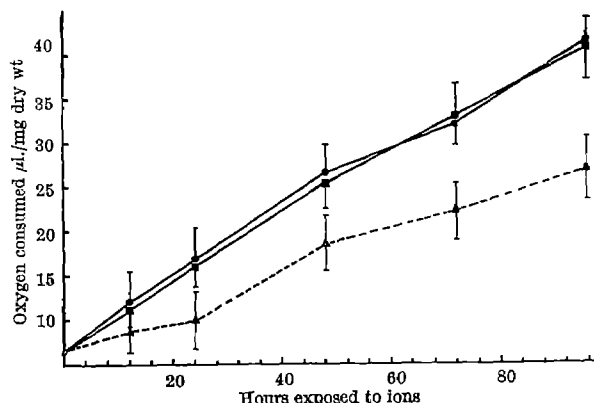


Fig. 1. The effect of exposure to (+) or (-) air ions on the oxygen consumption of barley seedlings. Each point represents 3 determinations and the vertical bars are 95 per cent confidence intervals. ●, Negative; ■, positive; ▲, control.

ate iron is used in a balanced fashion for cytochrome synthesis and for synthetic processes leading to chlorophyll formation. If the seeds are grown in an iron-free medium and are exposed to dense atmospheres of $(-)$ $(+)$ air ions, the balance normally prevailing between the two processes is upset; iron is used preferentially for the production of cytochromes and other iron-containing enzymes with the result that insufficient iron is available for the synthesis of chlorophyll and in consequence chlorosis develops.

Under the conditions usually obtaining in Nature, external stores of iron are available, the normal pattern of chlorophyll synthesis prevails and chlorosis is avoided. Here, too, the synthesis of cytochrome and other iron-containing enzymes is stimulated by air ions and the plant provided with metabolic equipment essential for the support of an increased rate of growth. That the enzymes are actually utilized for this purpose is evidenced by the increased rate of oxygen consumption reported in this communication. We are inclined to the belief that the effect of air-ion action in higher plants may be the regulatory systems which control iron metabolism in the seed and young seedlings⁷.

This work was supported by: (1) a research grant from the U.S. Public Health Service (2 R01-AP00002-06), Division of Air Pollution, Bureau of State Services; (2) contract Nonr 3656(06) (NR 102-587) between the Office of Naval Research, Department of the Navy, and the University of California; (3) a grant from the Committee on Research of the University of California.

SADAO KOTAKA

ALBERT P. KRUEGER

PAUL C. ANDRIESE

Air Ion Laboratory,
School of Public Health and Naval Biological Laboratory,
School of Public Health,
University of California, Berkeley.

KAZUTOSHI NISHIZAWA
Department of Plant Biochemistry,
Faculty of Science,

Tokyo University of Education, Ohtsuka, Tokyo.

TAKESHI OHUCHI

MITSUKO TAKENOBU

YASUO KOZURE

Department of Biological Chemistry,
College of Agriculture,
Ibaraki National University,
Ami-machi, Ibaraki-ken, Japan.

Krueger, A. P., Kotaka, S., and Andriese, P. C., *J. Gen. Physiol.*, **45**, 579 (1962).

Krueger, A. P., Kotaka, S., and Andriese, P. C., *Intern. J. Biometeor.*, **6**, 33 (1962).

Krueger, A. P., Kotaka, S., and Andriese, P. C., *Intern. J. Biometeor.*, **7**, 17 (1963).

Krueger, A. P., Kotaka, S., and Andriese, P. C., *Nature*, **200**, 707 (1963).

Kotaka, S., Krueger, A. P., and Andriese, P. C., *Plant Cell Physiol.*, **6** (in the press).

Umbreit, W. W., Burris, R. H., and Stauffer, J. F., *Manometric Techniques*, 305 (Burgess Publishing Co., Minnesota, 1964).

Krueger, A. P., Kotaka, S., and Andriese, P. C., *Intern. J. Biometeorol.*, **8**, 5 (1964).

Oserkowsky, J., *Plant Physiol.*, **8**, 449 (1933).

Jacobson, L., *Plant Physiol.*, **20**, 233 (1945).

Presence of Plant Growth Substances in Earthworms demonstrated by Paper Chromatography and the Went Pea Test

REPORTS have been published describing increases in growth and vigour of pasture in New Zealand following the experimental transplantation of lumbricid earthworms of the species *Allolobophora caliginosa* into soils where they were not normally found¹⁻³. Fourteen species of Lumbricidae are known in New Zealand, although the family is not native to the region⁴. Since they have been introduced accidentally during the past hundred years or so there has not been sufficient time for them to spread by natural means to all areas under grass. The increased

pasture yields may be due to the presence of plant growth-promoting compounds elaborated by earthworms and secreted by them into their casts and thus into the soil. A similar suggestion was, in fact, advanced by Hopp and Slater⁵, who found in pot experiments that the addition of both living and dead earthworms was associated with increased growth of the test plants.

The application of paper chromatography to earthworm extracts has shown the presence in them of indole compounds, some of which when examined by the Went pea test⁶ have displayed biological activity. The method was as follows. Earthworms were killed by brief immersion in water at 45° C and then homogenized in distilled water (100 g/l.). The homogenized preparation was centrifuged and the supernatant fluid was removed for solvent treatment without pH adjustment. For each species examined the pH was about 6.3. The fluid was extracted twice into peroxide-free diethyl ether and the combined extracts were evaporated in a current of air, leaving residues, which were taken up in water and filtered. The filtrate was then extracted with ethyl acetate which was in turn evaporated, leaving a tarry deposit. The tar-like material was largely eliminated by further extraction with water and filtration. The filtrate was finally extracted with ethyl acetate which was evaporated to 0.1 ml. Of this volume one-tenth was used for spot detection, and the remainder was applied as a streak a few centimetres away on the starting line, to provide material for the biological test. Ascending chromatograms were developed for 6 h at 22° C in 8 : 1 : 1 isopropyl alcohol, aqueous ammonia and water⁷ and indole substances detected by the modified Ehrlich reagent of Jepsen⁸. After drying, the paper was cut 0.5 cm above and below the corresponding spot position, while spot-free areas were cut into strips of 2 cm width. Strips were eluted by shaking with 20 ml. distilled water and the extracts assayed by the pea test.

For each of the species examined, there were several indole compounds present, but only one was biologically active. The results are summarized in Table 1; the R_F figures shown are mean values derived from several tests.

It is apparent that the biologically active compound is different in each of these species. With four of them the separate identity of the compounds was confirmed by the pea test. Aqueous extracts of earthworms were tested in serial dilution against pea shoots. The curvatures obtained were plotted against the log of the concentration of extract and the straight lines so derived differed in slope from each other and from indolyl-3-acetic acid (IAA). The mean angular differences from IAA were as follows: *A. caliginosa*, + 15°; *L. rubellus*, + 30°; *E. foetida*, + 43°.

As well as earthworms, two species of common garden slugs have been found to contain plant growth substance. Centrifuged homogenates of *Agriolimax reticulatus* and *Milax gracilis* both yielded pea curvatures similar to those of earthworms at the same concentration. Solvent extracts were prepared in the same way as for earthworms; chromatograms of these produced, in the case of *M. gracilis*, an intense indigo spot which was not biologically active, whereas the pea responses were obtained from the region of R_F 0.62-0.76, where no indole compounds were detected. With *A. reticulatus*, on the other hand, no indole colours developed, while pea curvatures were obtained at R_F 0.27-0.40.

Table 1. INDOLE COMPOUNDS PRESENT IN EARTHWORM EXTRACTS

Species	Positive in the pea test R_F value	Ehrlich colour	Ninhydrin reaction	Not responsive in the pea test R_F values
<i>Allolobophora caliginosa</i>	0.59	Indigo-brown	+	0.47, 0.78
<i>Allolobophora longa</i>	0.09	Red-brown	—	0.69, 0.71
<i>Lumbricus rubellus</i>	0.54	Violet	—	0.68, 0.88
<i>Lumbricus terrestris</i>	0.25	Violet-brown	—	0.65
<i>Eisenia foetida</i>	0.73	Violet	+	0.35
<i>Dendrobaena rubida</i>	0.45	Indigo	—	0.08, 0.14, 0.65, 0.82
Indolyl-3-acetic acid	0.50	Purple	—	

Apart from the possible effects on plant growth of these substances secreted by earthworms, they might well subserve a useful function in solving problems of taxonomy in the Oligochaeta. For example, among the Lumbricidae it may be possible by further investigation of the growth substances to establish the validity of species such as *Dendrobaena subrubicunda*, the identity of which is now in doubt⁶.

R. L. NIELSON

Ruakura Agricultural Research Centre,
Department of Agriculture,
Hamilton, New Zealand.

¹ Hamblin, C. J., and Dingwall, A. R., *N.Z. J. Agric.*, **71**, 55 (1945).

² Richards, J. G., *N.Z. J. Agric.*, **91**, 559 (1955).

³ Stockdill, S. M. J., *N.Z. J. Agric.*, **98**, 227 (1959).

⁴ Lee, K. E., *N.Z. Dept. Sci. Ind. Res. Bull.*, **130** (1959).

⁵ Hopp, H., and Slater, C. S., *J. Agric. Res.*, **78**, 347 (1949).

⁶ Went, F. W., and Thimann, K. V., *Phytohormones* (The Macmillan Company, New York, 1937).

⁷ Stowe, B. B., and Thimann, K. V., *Arch. Biochem. Biophys.*, **51**, 499 (1954).

⁸ Jepson, J. B., in *Chromatographic Techniques, Clinical and Biochemical Application*, edit by Smith, I. (Heinemann, London, 1958).

⁹ Gates, G. E., *Ann. Mag. Nat. Hist.*, Ser. 13, **1**, 33 (1958).

Promotion of Germination in Light-requiring Seed by Chloramphenicol

In this laboratory we are investigating germination processes in light-requiring seeds of *Lactuca sativa* L. (var 'Grand Rapids') including protein and nucleic acid synthesis in relation to the photocontrol of germination. During this investigation the effects of some inhibitors of protein synthesis have been examined. This communication reports certain results which have been obtained with chloramphenicol.

Our sample of seeds (batch No. 18639, of the 1964 harvest, from Ferry-Morse Seed Co.) gives approximately 10 per cent germination in darkness at 24°C, the maximum being reached after 20 h. 95–98 per cent germination is achieved following irradiation with red light (1 min at 120 $\mu\text{W}/\text{cm}^2$) given 1.5 h after the start of imbibition.

In preliminary experiments, when seeds were incubated on filter paper moistened with chloramphenicol solutions (750–3,500 $\mu\text{g}/\text{ml}$) at 24°C, a marked stimulation of germination in darkness was noted. This stimulation was much more pronounced if the seeds were infiltrated with the chloramphenicol solutions under reduced pressure. In this treatment, the seeds were allowed to imbibe on filter paper moistened with the test solutions for 1.5 h. They were then placed in approximately 10 ml. of the solution (or water, in the control) in a tube which was evacuated three or four times until the seeds failed to rise to the top of the liquid. They were then returned to filter paper wetted with the appropriate solution and the germination percentages were measured after 24 h in darkness at 24°C. All manipulations were carried out under a green safelight. Fig. 1 shows that the amount of germination in darkness increases linearly with chloramphenicol concentration over the range 500–3,000 $\mu\text{g}/\text{ml}$. These results have been confirmed, and we have also been informed that other workers found slight stimulation of dark germination by low concentrations of chloramphenicol¹.

Far-red light markedly reduces the promotive effect of chloramphenicol. To show this, seeds were irradiated, after one hour's imbibition and again after infiltration, with far-red light for 5 min at 230 $\mu\text{W}/\text{cm}^2$, and then returned to darkness. The lowest curve of Fig. 1 represents the results of this treatment. In further experiments it was found that chloramphenicol was ineffective in causing germination in darkness if the seeds were allowed to imbibe on water for 24 h prior to application of this chemical. In these experiments also, the seeds were infiltrated after the period on water and were incubated on the chloramphenicol solutions for 24 h before counting.

It is well known that the response of 'Grand Rapids' seeds to a given energy-level of red light decreases if the

imbibed seeds are held in darkness for some time before irradiation². This is described as skotodormancy. The curve in Fig. 2 illustrates this for our sample of seed. The other curve in Fig. 2 shows the effect of chloramphenicol on the development of skotodormancy. In these experiments seeds were kept on filter paper wetted with 2,000 $\mu\text{g}/\text{ml}$. chloramphenicol, without infiltration, for varying lengths of time in darkness. The ungerminated seeds were removed, infiltrated several times with water and spread on papers moistened with water. They were then illuminated with red light for 1 min at 120 $\mu\text{W}/\text{cm}^2$ and returned to darkness. Control seeds were manipulated

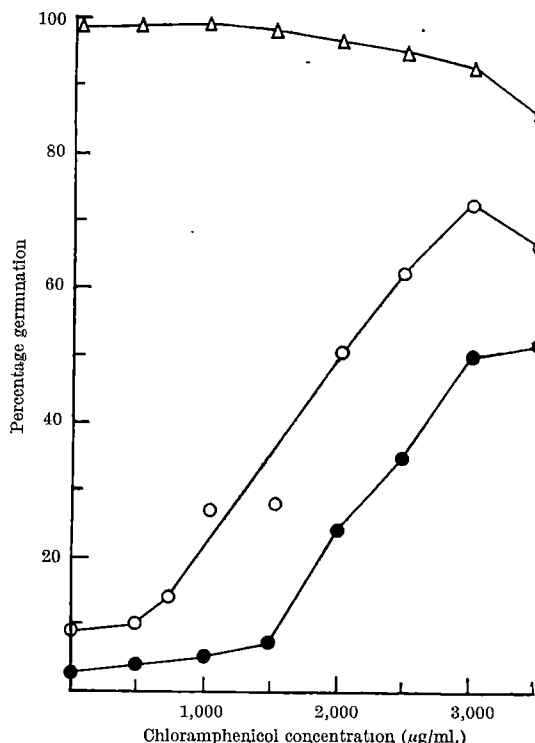


Fig. 1. Effect of chloramphenicol on germination of 'Grand Rapids' lettuce seeds at 24°C. Figures represent the means of 4 replications. Δ, Seeds exposed to red light (1 min at 120 $\mu\text{W}/\text{cm}^2$) 1.5 h after start of imbibition; ○, seeds maintained in darkness; ●, far-red treated seeds

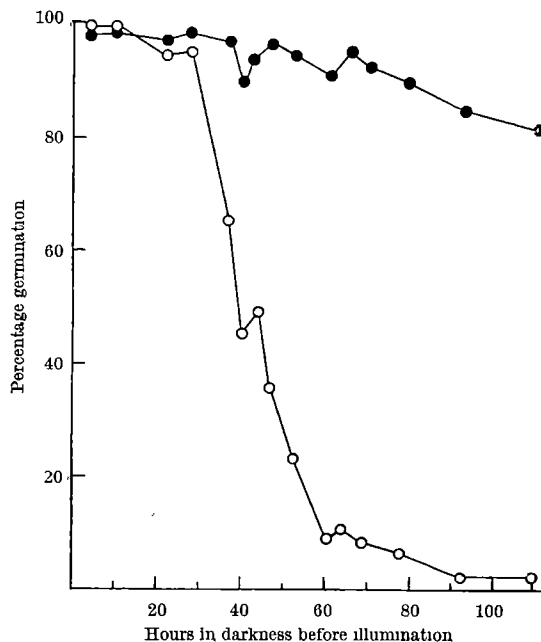


Fig. 2. Effect of chloramphenicol on skotodormancy. ●, Chloramphenicol-treated seeds (2,000 $\mu\text{g}/\text{ml}$); ○, water-imbibed seeds

narily except for the exposure to chloramphenicol. Germination percentages were determined 24 h after irradiation. Fig. 2 shows that seeds treated with chloramphenicol retain their sensitivity to light even after 24 h in darkness. It is unlikely that residual chloramphenicol in the seeds is acting additively with red light, for in further experiments the washing period after treatment with the chemical was prolonged to 24 h without any accompanying loss of response to red light.

In our experiments we have found no appreciable stimulation of germination in darkness by chloramphenicol one at concentrations lower than 500 $\mu\text{g/ml}$. However, these levels do increase the ability of very low concentrations of gibberellic acid (GA_3) to promote dark germination. For example, 20 $\mu\text{g/ml}$ GA_3 induced approximately 50 per cent germination in darkness, but this value was increased to the order of 70 per cent when the gibberellin was combined with 150–500 $\mu\text{g/ml}$ chloramphenicol. The effect of much lower concentrations of GA_3 (5 $\mu\text{g/ml}$) was similarly enhanced.

Two hypotheses concerning the promotion of germination in darkness by chloramphenicol can be suggested. First, this substance might have a positive promotive effect such that normal control by way of the phytochrome system is by-passed. On the other hand, although its action may be dependent on phytochrome it might primarily arrest an inhibitory process which commences in darkness when the seeds imbibe water. The results presented here lead us to favour the second suggestion. Since short irradiation with far-red light reduces the effect of chloramphenicol, it is not likely that this substance behaves entirely in a positive promotive fashion unless it acts directly on the phytochrome system to convert P_R to P_{FR} , which does not seem plausible. That chloramphenicol is almost completely ineffective if applied to the seeds after a 24-h delay on water, also supports this conclusion. It seems probable, from the experiments with far-red light, that germination even in chloramphenicol-treated seeds is still to some extent under phytochrome control. Now, dark-imbibed seeds are thought to contain a small proportion of phytochrome in the active (P_{FR}) form. This is inferred from the observation³, which we have confirmed, that germination, in darkness, of control seeds on water is substantially reduced by a short exposure to far-red radiation. We would suggest, therefore, that the effect of the low level of P_{FR} present in dark-imbibed seeds is fortified by chloramphenicol.

The question now arises as to the mechanism of action of chloramphenicol. This substance has been shown to inhibit protein synthesis in a number of plant systems including seeds. Young and Varner⁴, for example, found that chloramphenicol strongly reduces amylase and phosphatase synthesis in pea seeds although germination still proceeds. It is possible, then, that chloramphenicol acts in a similar manner in lettuce seeds and here prevents the development of some inhibitory system in which protein synthesis is involved. It is assumed that this inhibitory system normally impedes the action of the low level of P_{FR} . Although this has not yet been shown directly we have found, using ^{14}C -labelled leucine and ^{14}C -orotic acid, that there is indeed protein and RNA synthesis occurring in dark-imbibed seeds even though they do not germinate. Moreover, there are indications that this protein synthesis is almost completely confined to the endosperm and cotyledons. Further evidence of protein synthesis in darkness comes from our finding of a different protein pattern (using disc electrophoretic separation on polyacrylamide gel^{5,6}) in the soluble proteins of dark-imbibed seeds from that in dry or germinating seeds.

The hypothesis developed above, that the low level of P_{FR} in dark imbibed seeds is antagonized by an inhibitory system preventing germination, might explain why chloramphenicol is ineffective if applied to seeds which have previously been kept on water for 24 h. Under these

conditions, not only does the inhibitory system assume dominance but there is also a loss of P_{FR} by dark reversion or destruction. The possibility also arises that skotodormancy is caused by an active inhibition during darkness.

We have implied in the foregoing discussion that chloramphenicol does not directly affect the phytochrome system but that it acts to relieve some sort of inhibitory process. One further possibility should, however, be considered—that chloramphenicol prevents the loss of P_{FR} , occurring by conversion to P_R or by dark destruction⁷. In either case the result would be the maintenance of the low level of P_{FR} . Some evidence has been presented³ that P_{FR} , if maintained even in small proportions for an extended duration, can induce germination over several days. Since the effect of chloramphenicol in our experiments is apparent after 20 h it does not seem likely that it can act only to preserve the low level of P_{FR} .

In conclusion, we believe that the foregoing results indicate the possibility that germination in light-requiring lettuce seeds is under the control of two systems, a promotive one governed by phytochrome and an inhibitory one which commences after imbibition and for which protein synthesis is required. It is interesting to note here that birch seeds, for which there is good evidence against an active inhibitory process in darkness⁸, show no response to chloramphenicol in darkness.

M. BLACK

M. RICHARDSON

Department of Biology,
Queen Elizabeth College (University of London),
Campden Hill, London, W.8.

¹ Smith, H. (personal communication).

² Borthwick, H. A., Hendricks, S. B., Toole, E. H., and Toole, V. K., *Bot. Gaz.*, 115, 295 (1954).

³ Scheibe, J., and Lang, A., *Plant Physiol.*, 40, 485 (1965).

⁴ Young, J. L., and Varner, J. E., *Arch. Biochem. Biophys.*, 84, 71 (1959).

⁵ Ornstein, L., and Davis, B. J., *Disc Electrophoresis*, Parts I and II (Distillation Products Industries, New York).

⁶ Steward, F. C., Lyndon, R. F., and Barber, J. T., *Amer. J. Bot.*, 52, 155 (1965).

⁷ Slegelman, H. W., and Butler, W. L., *Ann. Rev. Plant Physiol.*, 16, 383 (1965).

⁸ Black, M., and Wareing, P. F., *Physiol. Plantarum*, 8, 300 (1955).

Oxidative and Biosynthetic Utilization by Human Spermatozoa of a Metabolite of the Female Reproductive Tract

In vitro investigations of the metabolism of mammalian spermatozoa have amply demonstrated their ability to utilize a variety of glycolysable and oxidizable substrates¹. No information is, however, available on the metabolism of spermatozoa within the female reproductive tract. If, as is probable, the epithelial cells use up not only the glucose supplied by the circulatory system, but also oxidizable intermediates such as pyruvate and lactate, the spermatozoa in contact with those cells would have to oxidize their endogenous reserves of lipid material, provided that the oxygen tension in the various regions of the tract is adequate to support the aerobic metabolism of the spermatozoa. In the absence of external substrates the conditions in the female genital tract would not be favourable for the survival of spermatozoa for prolonged periods of time. Whereas this may not affect fertility in animal species in which mating occurs at the time of oestrus, with rapid ascent of an adequate number of spermatozoa, a limited survival capacity due to the lack of substrates may affect the prospects of fertilization in man.

During human fertility investigations, motile spermatozoa have been found in cervical mucus 12 h and even several days after coitus, although in 'hostile mucus' motility is lost rapidly². Among a number of factors which may be involved, the possibility suggests itself that the secretions of certain sites of the female reproductive tract may contain metabolites which are not utilized by the

epithelial cells of the tract but can be taken up by spermatozoa. This hypothesis is suggested by the report of Kurzrok and Birnberg³ of the occurrence of glucosamine in human cervical mucus at the time of ovulation. Since hexosamines are not known to serve as substrates of spermatozoa, experiments were designed to test the ability of spermatozoa to metabolize glucosamine.

Suspensions of washed human spermatozoa in Krebs saline were incubated under aerobic conditions with D-glucosamine-1-¹⁴C and the 'respiratory CO₂' was collected and counted. This technique had been found sufficiently sensitive to measure the rates of oxidation of such substrates as glucose, pyruvate and acetate in human sperm suspensions using normal cell counts which are too low to permit measurements of gaseous exchanges⁴. As shown in Table 1, human spermatozoa produced ¹⁴CO₂ from D-glucosamine-1-¹⁴C at somewhat lower rates than from glucose-¹⁴C (randomly labelled). The production of ¹⁴CO₂ from glucosamine was suppressed in the presence of non-radioactive glucose, whereas glucosamine did not inhibit the oxidation of glucose-¹⁴C. When bull spermatozoa were tested under similar experimental conditions and with the same batch of radioactive substrates, different results were obtained; whereas glucose stimulated the respiration of bull spermatozoa and its carbon appeared in the respiratory carbon dioxide, the respiratory rate in the presence of glucosamine remained at the endogenous level and only negligible amounts of ¹⁴CO₂ were produced. Since brain extracts are known to phosphorylate glucosamine^{5,6}, a respiring hamster brain homogenate was incubated with glucose-¹⁴C and with D-glucosamine-1-¹⁴C. Like bull spermatozoa, hamster brain actively oxidized glucose, but failed to liberate ¹⁴CO₂ from glucosamine.

In recent investigations in this laboratory it was found that human spermatozoa utilize glucose and acetate as precursors in the biosynthesis of glycerolipids⁷, extending previous reports on the biosynthesis of lipids by bull⁸ and fish spermatozoa⁹. The possibility of a biosynthetic pathway of glucosamine was therefore examined. Human spermatozoa, freed from seminal plasma by centrifugation, were suspended in Krebs saline and incubated with D-glucosamine-1-¹⁴C for 4 h at 37°. After re-isolation and washing of the cells in Krebs saline containing unlabelled glucosamine, the lipids were extracted and purified by the procedure of Folch¹⁰ and fractionated on silicic acid columns. After both aerobic and anaerobic incubation the glyceride and phosphatide fractions were found to be carbon-14-labelled in a pattern resembling the labelling of animal spermatozoa by glucose-¹⁴C. The rate of incorporation of glucosamine into the total lipids of human spermatozoa was of the same order of magnitude as the rate of incorporation of glucose (0.3–0.5 μmoles/10⁸ spermatozoa/h).

Although the metabolism of glucosamine by spermatozoa of other species remains to be tested, the preliminary finding of a difference in enzymatic properties between the spermatozoa of man and the bull is intriguing. The intermediary steps of glucosamine breakdown in human spermatozoa are not known at present, but from the observed suppression of the production of ¹⁴CO₂ from glucosamine-1-¹⁴C when glucose was added, it may be presumed that the initial step is a phosphorylation catalysed by a hexokinase of greater affinity for glucose than for glucosamine. This step may be followed by the deamination of

glucosamine-6-phosphate to yield fructose-6-phosphate, reaction known to occur in animal and bacterial systems^{11,12}. The ability of human spermatozoa to channel glucosamine carbon into the glycolytic pathway would account for the oxidation of glucosamine and its incorporation into the glycerol portion of the glycerolipids.

The finding that human spermatozoa can utilize as substrate a metabolite of the cervical secretion is of significance because it shows that the energy metabolism of spermatozoa can be supported within the female reproductive tract. Furthermore, the demonstration of the incorporation of this metabolite into the lipids of spermatozoa suggests that these cells may replenish the reserves while passing through certain sites of the female tract. These observations also open up an approach to the study of the phenomenon of capacitation. As a working hypothesis it is suggested that capacitation is the result of the uptake by spermatozoa of a metabolite of the female reproductive tract, enabling the biosynthesis of a component necessary for the functional integrity of spermatozoa.

This work was supported by U.S. Public Health Service research grant GM-07078.

CHARLES TERNER

Biological Science Center,
Boston University, Boston, Massachusetts.

- ¹ Mann, T., *The Biochemistry of Semen and of the Male Reproductive Tract* (Methuen and Co. Ltd., London, 1964).
- ² Sturgis, S. H., *Fertil. and Steril.*, **7**, 463 (1956).
- ³ Kurzrok, R., and Birnberg, C., *Intern. J. Fertil.*, **3**, 134 (1958).
- ⁴ Turner, C., *Amer. J. Physiol.*, **198**, 48 (1960).
- ⁵ Harpur, R. P., and Quastel, J. H., *Nature*, **164**, 693 (1949).
- ⁶ Brown, D. H., *Biochim. Biophys. Acta*, **7**, 487 (1951).
- ⁷ Turner, C., and Minassian, E. S., *Fed. Proc.*, **24**, 476 (1965).
- ⁸ Turner, C., and Korsh, G., *Biochemistry*, **1**, 387 (1962).
- ⁹ Turner, C., and Korsh, G., *J. Cell and Comp. Physiol.*, **62**, 251 (1963).
- ¹⁰ Folch, J., Lees, M., and Sloane Stanley, G. H., *J. Biol. Chem.*, **226**, 4 (1957).
- ¹¹ Leloir, L. F., and Cardini, C. E., in *Methods of Enzymology*, edit. by Colwick, S. P., and Kaplan, N. O., **5**, 418 (Academic Press, New York, 1962).
- ¹² Comb, D. G., and Roseman, S., *ibid.*, **442**.

Electromagnetic Recording Diver Balance

THE use of the Cartesian principle in a very sensitive device for measuring manometric reactions was suggested by Linderström-Lang in 1937 (ref. 1). In the hands of Holter² the diver method was developed into a reliable and versatile technique; further increase in the sensitivity was accomplished by Zeuthen³.

The concept of reduced weight, RW (= submerged weight) was introduced by Linderström-Lang and Holt in connexion with density gradient work⁴. Subsequently Zeuthen⁵ invented a Cartesian diver balance on which determinations of RW may be made.

The diver balance has so far only been used in biologic work, but in this field it has turned out to be a very useful tool.

Its applications include the determination of water permeation, using D₂O as a tracer^{6–10}. Exchange studies with the Cartesian diver balance are rather tedious, as an automatically recording diver balance would therefore be a great advantage in this work. To meet this requirement we have constructed an electromagnetic diver balance (Fig. 1). The diver is made either of plastic (polyethylene) or of glass; in either case a small piece of magnetic material ('Koerzit T') is contained in the lower end of the diver. The RW of the finished diver must be adjusted so that it rises to the surface in the appropriate medium; the force required to pull it down must not, on the other hand, exceed the maximum force of the electromagnetic magnet (see below).

An ordinary photocell cuvette, containing medium as diver, is placed in the light path between a lamp and photocell. The current in a magnetic coil below the cuvette is passed through an amplifier controlled by the lig

Table 1. OXIDATION OF GLUCOSE AND GLUCOSAMINE BY SPERMATOZOA

Substrate	Labelled substrate (μmoles/10 ⁸ /h) appearing in respiratory CO ₂	
	Man	Bull
Glucose- ¹⁴ C	3.62	88.0
Glucosamine-1- ¹⁴ C	2.87	1.0
Glucose- ¹⁴ C; glucosamine	3.94	83.0
Glucosamine-1- ¹⁴ C; glucose	0.90	0.1

Washed spermatozoa were incubated in Krebs saline at 37°; gas, air. Glucose-¹⁴C (randomly labelled) and D-glucosamine-1-¹⁴C, 0.002 M, 1 mCi/mole. Human spermatozoa: sperm count, 2.18 × 10⁸/vessel, incubation period 4 h; bull spermatozoa: sperm count, 3.08 × 10⁸/vessel, incubation period 2 h.

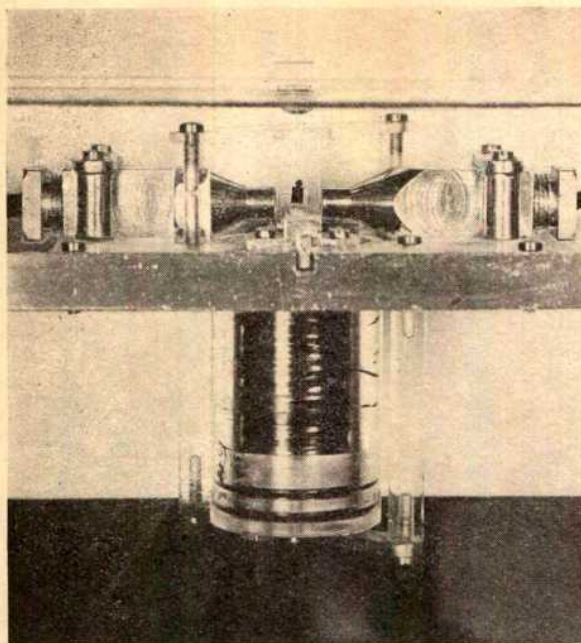


Fig. 1. Photograph showing part of the diver balance set-up. In the cuvette a diver is floating, loaded with a frog's egg

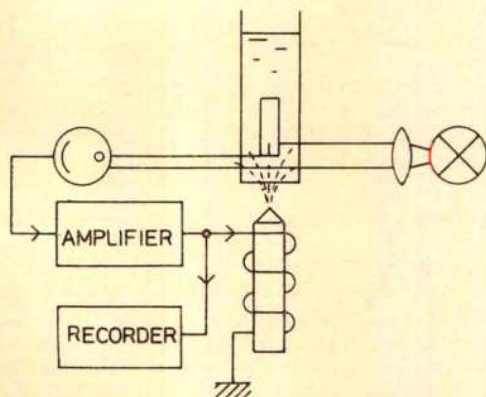


Fig. 2. Diagram of the operating principle of the electromagnetic diver balance

reaching the photocell (Fig. 2). The current is proportional to the amount of light, and the diver will sink in the cuvette until the current in the coil just balances the buoyancy. When loaded, less current is required. The decrease in current, proportional to the load, is registered on a recorder. The unit, comprising cuvette, photocell, lamp, and electromagnet, is submerged in a water bath. Besides weighings and permeability (diffusion) determinations the balance may also be used to follow gasometric reactions. If a small capillary, sealed with a fluid stopper (like the Cartesian diver), is placed on the balance, the change in RW associated with consumption or production of gas may be registered.

The range of the balance shown in Fig. 1 is about 10–300 μg . More sensitive balances may be constructed, but great demands must in that case be made on the temperature regulation of the water bath. No upper limit seems to obtain, and there seems to be no reason why a diver balance for RW determination on whales cannot be made, should the need arise.

Note added in proof. After the present communication was submitted for publication, a paper has appeared (Brzin, M., Kovic, M., and Oman, S., *Compt. Rend. Lab. Carlsberg*, 34, 407; 1965) describing an electromagnetic

recording diver balance. This device is, however, manually controlled and not automatic like that described here.

SØREN LØVTRUP
SUNE LARSSON

Department of Histology,
University of Göteborg,
Sweden.

¹ Linderström-Lang, K., *Nature*, **140**, 108 (1937).

² Holter, H., *Compt. Rend. Lab. Carlsberg, Sér. Chim.*, **24**, 399 (1943).

³ Zeuthen, E., *Compt. Rend. Lab. Carlsberg, Sér. Chim.*, **24**, 479 (1943).

⁴ Linderström-Lang, K., and Holter, H., in *Die Methoden der Fermentforschung*, edit. by Bamann and Myrbäck (1940).

⁵ Zeuthen, E., *Compt. Rend. Lab. Carlsberg, Sér. Chim.*, **26**, 243 (1948).

⁶ Pigon, A., and Zeuthen, E., *Experientia*, **7**, 455 (1951).

⁷ Løvtrup, S., and Pigon, A., *Compt. Rend. Lab. Carlsberg, Sér. Chim.*, **28**, 1 (1951).

⁸ Løvtrup, S., *J. Exp. Zool.*, **155**, 139 (1960).

⁹ Løvtrup, S., *J. Theoret. Biol.*, **5**, 341 (1963).

¹⁰ Bernström, K.-E., Haglund, B., and Løvtrup, S., *J. Exp. Zool.*, **155**, 317 (1964).

Photo-induced Hydroxylation of Cinnamic Acid in Gherkin Hypocotyls

THE influence of light of different spectral regions on the synthesis of hydroxycinnamic acids in the hypocotyls of gherkin seedlings has been described previously¹. This phenol synthesis can be stimulated considerably by supplying segments from the hypocotyls with *trans*-cinnamic acid. In the latter case about 95 per cent of the end-product consists of a glucose ester of *p*-coumaric acid, as was determined with paper chromatography. Light stimulates the conversion of cinnamic acid to *p*-coumaric acid. The occurrence in plant material of a hydroxylase which catalyses this reaction has been demonstrated by Nair and Vining². They showed that tetrahydrofolic acid and a reduced pyridine nucleotide were necessary to obtain maximum enzyme activity. The feeding experiments with cinnamic acid enable us to investigate how far the accumulation of hydroxycinnamic acids in the hypocotyls of intact seedlings in response to light is determined by changes in hydroxylase activity.

To examine whether enzyme synthesis is involved we have used cycloheximide (actidione), a drug that inhibits the transfer of amino-acid from sRNA to polypeptide^{3,4}. The following experiment was performed. Hypocotyl segments, 5 mm in length, were cut immediately below the plumular hook of 3-day-old gherkin seedlings (*Cucumis sativus* 'Venlose niet plekkers', strain 'Tercken VI'), grown in the dark. Lots, of 30 segments each, were floated on 10 ml. distilled water in Petri dishes, kept in darkness for 2 h, and then transferred to a cabinet with blue light (700 $\mu\text{W}/\text{cm}^2$). To different duplicate samples 50 μg cycloheximide was added at the following times: 2 h before transfer to the light, immediately before transfer to the light, and after 2, 4 and 6 h in the light, respectively. All segments remained in the light during 24 h. Afterwards the cycloheximide-treated lots were washed with distilled water, and all the samples were transferred to Petri dishes with 10 ml. of 10^{-3} M *trans*-cinnamic acid and 1 per cent glucose in water (pH 5.0). Fig. 1 gives the amounts of *p*-coumaric acid glucose ester that accumulated in the segments during the next 24 h, in which they were kept on the precursor solution in darkness. To two of the four lots that had not previously been treated with the drug, 50 μg cycloheximide was added in the precursor solution. The average value obtained for these samples is given by the closed symbol in Fig. 1. Hydroxycinnamic acid esters in this and in the following experiments were determined as described previously¹. Each point in the graphs represents the mean of three experiments, each run in duplicate.

The foregoing experiment excludes the possibility of a direct photochemical reaction involving cinnamic acid. The inhibition by cycloheximide indicates that *de novo*

synthesis of one or more enzymes is involved. Addition of the inhibitor has an effect similar to that found when transferring the seedlings to darkness, as can be seen by comparing Figs. 1 and 2. That the inhibition is not an unspecific effect is demonstrated by the fact that *cycloheximide* has very little effect once light has been given for more than 6 h. Whether this light-induced enzyme synthesis concerns cinnamic acid hydroxylase itself, or one or more enzymes involved in the synthesis of a co-factor, has not yet been established. Evidence that *de novo* synthesis of enzymes plays a part in the increase in phenol synthesis in response to light has also been obtained for chlorogenic acid in potato tuber tissue⁵ and of anthocyanin in mustard seedlings⁶.

The influence of light quality, light intensity and duration of irradiation on the conversion of cinnamic acid into *p*-coumaric acid in hypocotyl segments is shown in Fig. 2. For these experiments the same light and filter combinations were used as for those with intact seedlings¹. Duplicate lots of 30 segments each were floated on an aqueous solution of 10^{-3} M *trans*-cinnamic acid and 1 per cent glucose (pH 5.0), irradiated for the times indicated in the graphs, and afterwards kept in the dark until 24 h from the beginning of the irradiation.

In a number of aspects the results are remarkably parallel with those relating to the effects of light on the accumulation of hydroxycinnamic acids in the hypocotyls of intact seedlings¹.

(1) In all three wave-length regions the induction is an increasing function of the duration of irradiation up to a certain limit. This limit of about 8 h is fairly independent of light quality and light intensity. It may be that this has some bearing on the problem of photoperiodism.

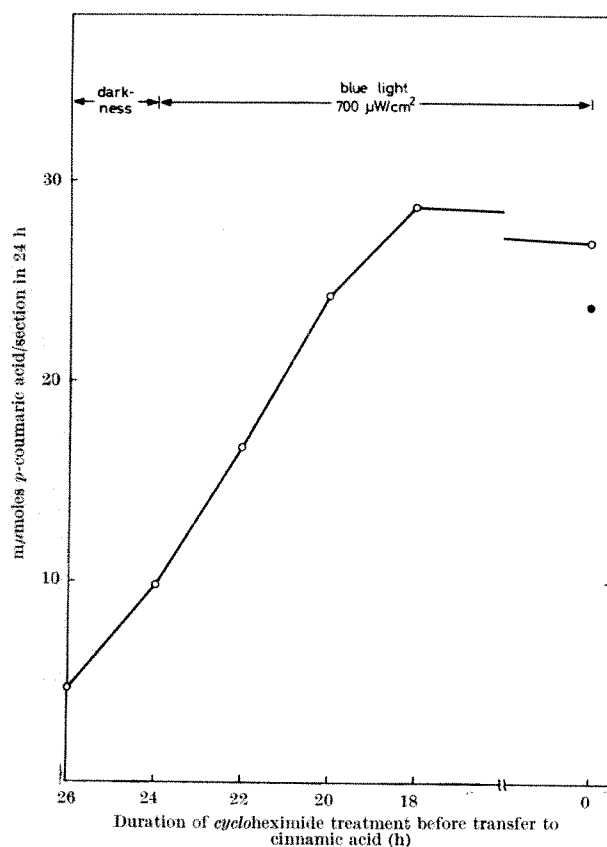


Fig. 1. Effect of *cycloheximide* on cinnamic acid hydroxylation in gherkin hypocotyl segments induced by blue light ($700 \mu\text{W}/\text{cm}^2$). Segments were floated on distilled water and irradiated for 24 h. *Cycloheximide* ($5 \mu\text{g}/\text{ml}$) was added to different lots at the times indicated in the graph. The points give the amounts of *p*-coumaric acid accumulated in the segments during the next 24 h in darkness from aqueous solutions of 10^{-3} M *trans*-cinnamic acid and 1 per cent glucose (open symbols) and 10^{-3} M *trans*-cinnamic acid, 1 per cent glucose and *cycloheximide* ($5 \mu\text{g}/\text{ml}$) (closed symbol).

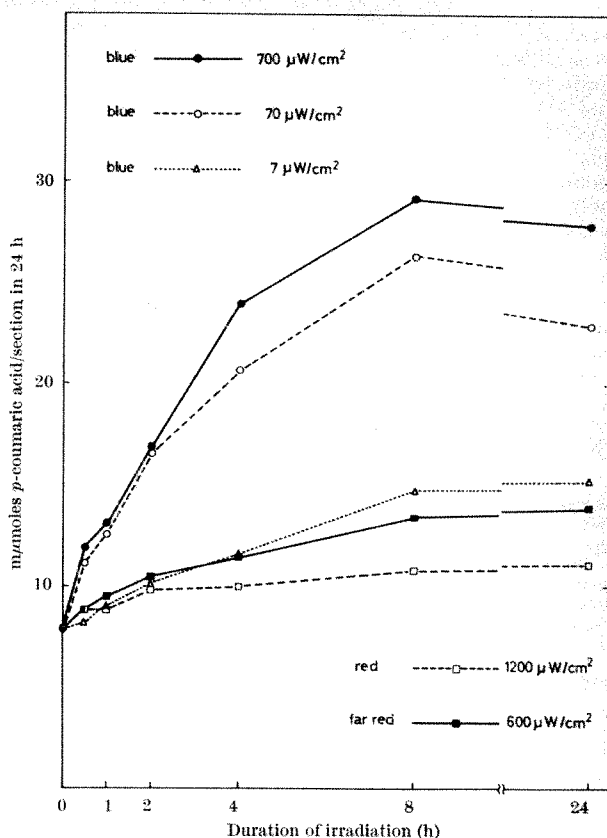


Fig. 2. Accumulation of *p*-coumaric acid during 24 h in hypocotyl segments floated on an aqueous solution of 10^{-3} M *trans*-cinnamic acid and 1 per cent glucose as a function of duration of irradiation with blue, red and far-red light.

(2) Blue light is much more effective than light of the other wave-length regions, the induction being an increasing function of the light intensity.

(3) The induced hydroxylase activity is always smaller in the basal part of the hypocotyl than in the apical part, as was revealed in experiments with segments excised from different parts of the hypocotyl⁷. The spectral distribution of the light sensitivity did not alter significantly over the length of the hypocotyl. Therefore, if more than one pigment is involved we may expect a rather similar distribution.

An important difference between light-induced phenol synthesis in hypocotyls of intact seedlings and induced hydroxylase activity in hypocotyl segments is that short irradiations only on the former process have a relatively pronounced effect¹. Moreover, the relative effectiveness of blue, red and far-red light in inducing hydroxylase activity in segments is more in agreement with the induced phenol synthesis in hypocotyls of decotyledonized seedlings than in those of intact plants. A recent finding that a much higher stimulation of hydroxylase activity in segments can be obtained when the intact plant receives the short light treatment instead (10 min), and the segment to be excised remains part of the plant for at least 1 h after the light treatment, may have some bearing on this⁷. Excision before or covering during the irradiation of the cotyledons has a lowering effect. Therefore, besides hydroxycinnamic acid precursors, other transportable factors are probably involved in the light-induced phenol synthesis. They may play a role in the induction of enzymes or in the modification of their activity.

Feeding with phenylalanine also results in increased synthesis of *p*-coumaric acid in hypocotyl segments. In this plant no stimulation has so far been obtained with a number of other hydroxycinnamic acid precursors⁸; glucose, quinic acid, shikimic acid, phenyllactic acid and

tyrosine. It may be that at this stage of development the seedling depends for its phenol metabolism on the supply of phenylalanine originally present in the cotyledons. We do not yet know whether in the gherkin hypocotyl the phenylalanine deaminase activity is already high enough or is also increased by light to meet the demands for cinnamic acid under all circumstances. Photo-induced hydroxylation of this compound seems to be determined by local factors and by influences from other parts of the plant. There is a striking correlation between enzyme activity and accumulation of phenols in a particular part of the hypocotyl⁷. This points to an important controlling function of the hydroxylase step in hydroxycinnamic acid metabolism and in cell elongation, so far as the latter is regulated by phenols⁸.

G. ENGELSMA

Philips Research Laboratories,
N.V. Philips' Gloeilampenfabrieken,
Eindhoven, The Netherlands.

- Engelsma, G., and Meijer, G., *Acta Bot. Neerl.*, **14**, 54 (1965).
Nair, P. M., and Vining, L. C., *Phytochem.*, **4**, 161 (1965).
Ennis, H. L., and Lubin, M., *Science*, **146**, 1474 (1964).
De Kloet, S. R., *Proc. Ned. Akad. Wetensch.*, Ser. B (in the press).
Zucker, M., *Plant Physiol.*, **38**, 575 (1963).
Mohr, H., and Lange, H., *Naturwissenschaften*, **52**, 261 (1965).
Engelsma, G., *Acta Bot. Neerl.* (in the press).
Neish, A. C., *Biochemistry of Phenolic Compounds*, 295 (Academic Press, London and New York, 1964).
Engelsma, G., and Meijer, G., *Acta Bot. Neerl.*, **14**, 73 (1965).

Histological Investigations of the Mechanism of Sterility Induced by Deuterium Oxide in Mice

DURING investigations in which normal females were mated to male mice treated with deuterium oxide, it has been shown that D₂O produces sterility in the males^{1,2}; however, the site of the damage has not been demonstrated. Since embryonic death after implantation did not increase in such females, cleavage stage embryos were examined 18–72 h after mating³. Most of the embryos present in the oviducts were either one-celled or, though 'multicellular', were abnormal and degenerating; few normal embryos were found. These results did not indicate whether developmental failure was due to failure of the sperm to fertilize the egg or to a failure of the fertilized egg to develop. The experiment reported here utilized standard histological techniques to demonstrate clearly that the previously observed pre-implantation death is due to the failure of the sperm to fertilize the egg, not to subsequent abnormal cleavage of the fertilized egg.

C₅₇ male mice from the University of California Cancer Research Laboratory, 10–14 weeks old at the beginning of the experiment, were used. This inbred strain has been shown to be more sensitive to the sterility-producing effects of D₂O than Swiss strain mice, which are random bred⁴. Because C₅₇ males are known to have a high innate sterility, they were tested for fertility before the start of the experiment, and only those males which sired two viable litters were used. D₂O was given at the concentration of 30 per cent by volume in the drinking water *ad lib*. At the end of treatment for 4 weeks, water was given instead of deuterium oxide as drinking water. Control males, mated weekly, were maintained under conditions identical to those of the experimental animals except that they drank water throughout the experiment. All females were virgin Swiss mice, from Simonsen Laboratories, Gilroy, California.

Each male was caged with 8 (later 3) females nightly from 10 p.m. to 9 a.m. At 4-h intervals the females were examined for vaginal plugs. Thus the time of copulation could be estimated within 4 h. As females were mated, they were removed and other females were placed in the cage. Males were killed 12–16 h after mating. Some mated females were allowed to go to term; their litters were examined at birth.

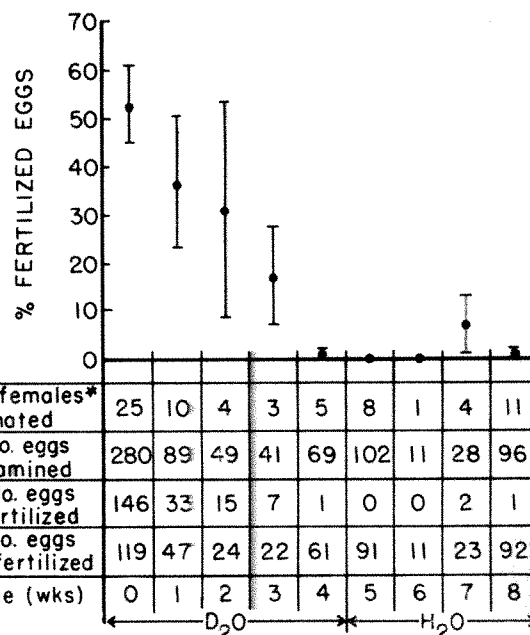


Fig. 1. Effect of D₂O on the number of fertilized eggs obtained from females mated to treated males. The number of fertilized eggs as percentage of the total eggs examined, with the standard error of the mean, is presented; also a tabulation of total eggs, fertilized and unfertilized eggs. Those eggs scored as degenerating or undiagnosable are not included in the tabulation.

* The data from females mated to control males were used in the analysis of variance calculations. However, since the sample size for each week was small, the data have been pooled with those from the treated males at zero time.

Oviducts were removed *in toto* and fixed in Bouin's solution. They were embedded in paraffin, serially sectioned at 10 μ , and stained with haematoxylin-eosin. All sections of each oviduct were examined with a light microscope; magnifications up to times 640 were used. All slides were code-numbered, so that the experimenter did not know whether the eggs came from a control female or one mated to a treated male. Eggs were scored as 'fertilized' if two pronuclei were observed or if a sperm tail and enlarging sperm head were visible in the ooplasm and/or if the orientation, condensation and chromosome position in the oocyte meiotic spindle indicated that emission of the second polar body was in progress or complete. In eggs scored 'unfertilized', an unchanged second meiotic spindle was present in the ooplasm and there was no evidence of sperm head or tail. Fragmented eggs, and those in which the ooplasm showed unusual condensation, were scored as 'degenerating'.

The results are shown in Fig. 1. By two weeks on D₂O, the average number of fertilized eggs had dropped from 5.8 per female (pooled data for females mated with control males and treated males at week zero) to 3.8 per female; after males had been on D₂O for four weeks, no fertilized eggs were obtained from the mated females. A two-way analysis of variance was carried out on the data⁵. The group treated with D₂O was significantly different from the control group ($F_{1,44}=8.5$; $P\leq 0.01$). There was also a systematic change over the eight-week period ($F_{8,44}=4.5$; $P\leq 0.01$), and this change was a function of whether the animals had been treated with D₂O, that is, the interaction between treatment and time was significant ($F_{8,44}=2.6$; $P\leq 0.05$). Table 1 gives the results of the number and size of litters obtained from those females which were

Table 1. EFFECT OF D₂O ON THE NUMBER AND SIZE OF LITTERS OBTAINED FROM FEMALES MATED TO TREATED MALES

No. of females mated	28*	8	5	6	3	8	2	7	8
No. of females littered	25	8	5	1	0	0	0	0	2
No. of viable offspring	206	52	18	2	0	0	0	0	7
Time (weeks)	0	1	2	3	4	5	6	7	8

* The data from females mated to control males were pooled with those from females mated to treated males at time zero.

allowed to go to term. From a control average of 7.3 viable offspring per female, there was a decrease to 0.3 offspring per female after two weeks' treatment and to no offspring after three weeks of D_2O administration to the males.

Under the conditions of this experiment, sperm penetration usually occurred within 6-8 h after the females were removed from the males. Thus, by collecting eggs 12 h after copulation, one could be sure that sperm entry would have been effected, yet most eggs should not be old enough to start degenerating. The results presented here clearly indicate that failure of the D_2O -treated males to produce viable offspring, as previously reported, was due to the failure of the sperm to fertilize the egg and not to a failure of the subsequent development of the fertilized egg. These conclusions are supported by subsequent examination of 630 living eggs by phase contrast microscopy.

Histological examination of the testes of D_2O -treated male mice is in progress; initial observations suggest that acrosome formation may be abnormal. However, at present, the mechanism of action of D_2O in producing sterility is not known. Autoradiographic studies of eggs from females mated to D_2O -treated males (in which the sperm are labelled isotopically) and electron microscopy of sperm from D_2O -treated males are also in progress in further attempts to pinpoint the site of action of D_2O .

We thank Dr. Gordon Pryor of the Stanford Research Institute, Menlo Park, California, for the statistical analysis of the data. We also thank Prof. Melvin Calvin and Dr. Edward L. Bennett for their advice.

This work was sponsored in part by the U.S. Atomic Energy Commission.

ANN M. HUGHES

Lawrence Radiation Laboratory,
University of California,
Berkeley.

LAUREL E. GLASS

Department of Anatomy,
University of California Medical School,
San Francisco.

¹ Hughes, A. M., and Calvin, M., *Science*, **127**, 1445 (1958).

² Hughes, A. M., Bennett, E. L., and Calvin, M., *Proc. U.S. Nat. Acad. Sci.*, **45**, 581 (1959).

³ Hughes, A. M., Bennett, E. L., and Calvin, M., *Ann. N.Y. Acad. Sci.*, **84**, 763 (1960).

MICROBIOLOGY

Simplified Technique for Preparative Disc Electrophoresis

THE disc electrophoretic method¹ is becoming an important analytical tool in the study of components of complex mixtures. While engaged in work on the separation of antigenic constituents of connective tissue, we did some experiments to adapt this method to a preparative scale. Our aim was to construct as simple and inexpensive an apparatus as possible. In the course of our experiments we became aware of the publications of Lewis and Clark² and Racusen and Calvanico³ on preparative disc electrophoresis. One of the instruments described by the former investigators seemed, after minor modifications, to be suitable for our purpose. The procedure finally devised differed from that of Lewis and Clark mainly in the use of a 'Sephadex G-200'-sucrose mixture instead of the sample gel⁴ and in the arrangements for eluting the material from the polyacrylamide gel.

The apparatus (Figs. 1, 2 and 3) consists of (A) a 'Perspex' box used as the upper buffer reservoir, (B) a

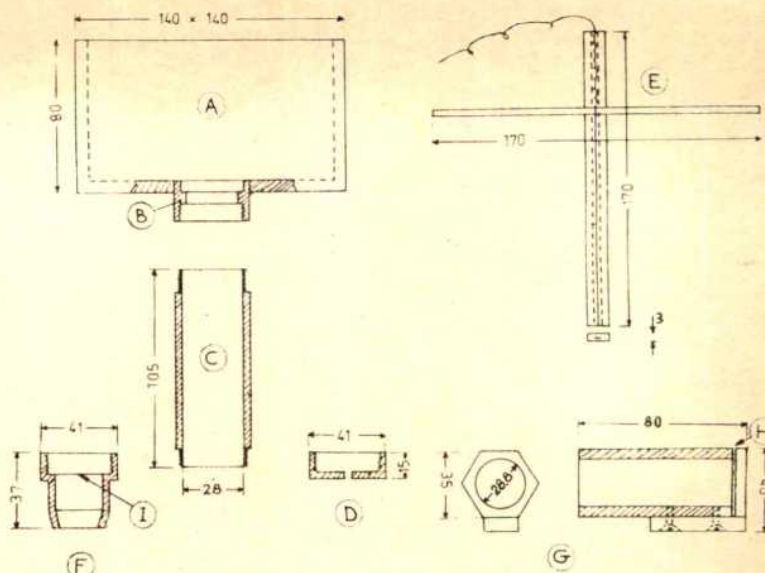


Fig. 1. Drawing of preparative disc electrophoresis apparatus. E, Upper electrode; A, upper reservoir; C, tube containing the gel system; D, cap covering lower end of C; F, 'Perspex' attachment used for elution; G, brass jig with slot H.

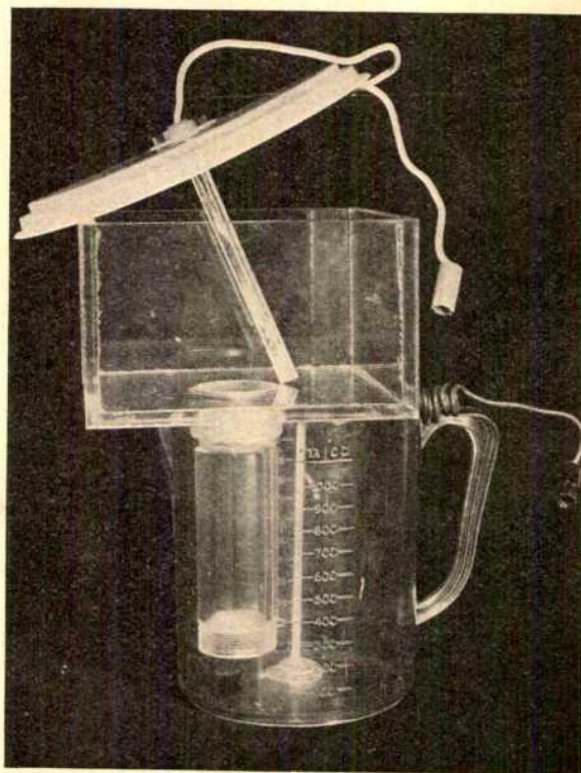


Fig. 2. Apparatus assembled for electrophoresis. In practice the upper reservoir was supported on a stand a few cm above the lower reservoir.

short 'Perspex' tube attached to a hole cut in the centre of A, (C) a 'Perspex' tube with threaded ends, which can be screwed into B, (D) a 'Perspex' cap with a 1-mm hole drilled in the centre, which can be screwed on the lower end of C. The connexions of C to B and D are made water-tight by means of rubber washers. A plastic insert serving as the reservoir for the lower buffer has a flat platinum wire electrode affixed to the bottom centre. A similar electrode, E, attached to a round polyethylene plate, is used for the upper reservoir. F is a short 'Perspex' tubing, which can be screwed on to the top of C. H is a brass jig with a slot, H, 3 mm from one end.

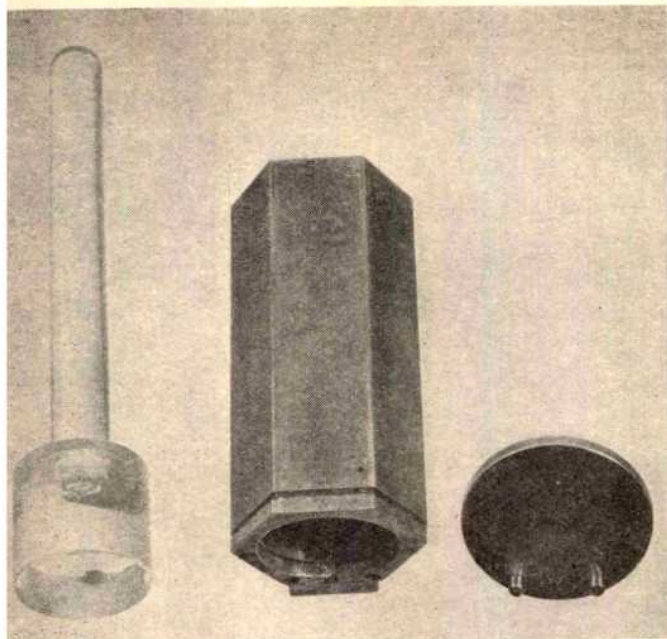


Fig. 3. Jig for cutting gel slices (centre) with cover (right) and 'Perspex' piston (left) used in holding the gel in position during cutting

ise with two projecting pins, which fit into holes in the wall of *G*, is used to close one end of the jig.

Lower and spacer gels were prepared as described by Ornstein and Davies¹. The sample gel was replaced by a suspension of 'Sephadex G-200' in 20 per cent sucrose⁴. The 'Sephadex' suspension was centrifuged for 15 min at 3,000 r.p.m. in an MSE 'Multex' centrifuge and the supernate was discarded. To 5 ml. of the packed 'Sephadex' was added 2 ml. of *tris*-glycine buffer pH 6.7 (ref. 1) diluted 1/10 (TGB) and 1 ml. of the sample previously dialysed against TGB. The use of 'Sephadex' obviated the difficulties sometimes encountered in the polymerization of the sample gel and shortened the time required to set up the experiment.

Electrophoresis was carried out as follows: the centre hole in cap *D* was plugged with a piece of 'Plasticine'. The cap was screwed on the lower end of tube *C* and the tube was clamped in a vertical position. 50 ml. of the lower gel were introduced into *C* and the gel was covered with a 2-mm layer of distilled water. Polymerization was completed in 50–60 min. The water was removed and the lower gel was rinsed with spacer gel in the usual way. 15 ml. of the spacer gel were poured on the lower gel and covered with water as before. Photopolymerization was carried out with a 15 W fluorescent daylight lamp, placed at a distance of 2 cm from *C*. The lamp was moved to the opposite side of *C* after 15 min, and then placed above and close to *C* after another 10 min. The polymerization required approximately 50 min.

The water layer was removed and *D* was unscrewed, after first unplugging the hole. The apparatus was assembled as shown in Fig. 2. The lower reservoir was filled with TGB, the level of the buffer solution being adjusted so that *C* was immersed to a depth of 2 cm. The sample (incorporated in 'Sephadex-TGB') was placed on the spacer gel, followed by 2 ml. of packed 'Sephadex' in sucrose. The latter served to protect the sample from mixing with the buffer solution. TGB, containing 1.5 ml./l. of 0.01 per cent bromphenol blue, was carefully placed over the 'Sephadex'. The total amount of buffer solution in the upper reservoir was 500 ml. Electrophoresis was carried out in a cold room at a constant current of 45 mA and 320 V. The current was adjusted by changing the position of the upper electrode in box *A*. The run was completed in about 4 h. During this time

the bromphenol blue band travelled down tube *C* for 8–9 cm.

Tube *C* was now unscrewed, the gel was rimmed under water and allowed to slide into the cutting jig *G*, the latter being closed with its cover. 3-mm slices were cut with a small hand saw, through slot *H*. A small piece was cut from each slice and placed along the side of a microscope slide coated with 2 per cent Noble agar in veronal buffer pH 8.2, μ : 0.05. Antiserum containing antibodies to the mixture of antigens being separated was placed in the centre trough. Antigens present in sufficient concentration produced lines within 24 h, but final results were read after 4–5 days.

Direct elution of the antigens by suspending the broken-up gel slices in buffer solutions did not give satisfactory results. Elution was therefore carried out by electrophoresis. Slices giving precipitation lines were broken up in a 'Teflon' homogenizer. A piece of dialysis tubing knotted at one end was drawn over *F* and fastened with rubber bands. *F* was filled with TGB and several layers of filter paper were placed at *I*. *F* was screwed on to *C* and the broken-up gel to be eluted was placed on the filter paper and covered with a disc of filter paper on which was placed a small amount of 'Sephadex' (1 mm high). The apparatus was assembled as before and electrophoresis was carried out at 45 mA, 300 V, for 3 h. A test with antigen labelled with ¹²⁵I showed that about 95 per cent of the radioactivity was eluted from the gel during this time. The antigen solution was concentrated by pervaporation and the antigen was identified by diffusion in agar.

We have successfully used this technique to prepare a purified antigen from a complex mixture of rat liver connective tissue antigens. The antigen was separated by preparative disc electrophoresis after preliminary fractionation on a column of 'Sephadex G-100' and treatment with acetate buffer, pH 5.5. A description of this procedure is being prepared. The antigen was concentrated in two gel slices.

The technique described here was also found useful for preparing antibodies to single components of the mixture, without having first to isolate the antigen. The slice containing the desired component was homogenized in borate buffer, pH 8.0, incorporated in Freund's adjuvant and injected into rabbits. Antibodies were detected by diffusion in agar 2 weeks after injection. The difficulty with which proteins are eluted from polyacrylamide⁵ suggests that the gel itself might have adjuvant properties, through a slow release of antigenic material. Weintraub and Raymond⁶ have indeed demonstrated the ability of the gel to enhance antibody production and they have pointed out the advantage of this technique in preparing antibodies to single components of complex mixtures. We were not aware of their work when these experiments were carried out.

This investigation was supported by Public Health Research Grant AM 07629 from the National Institute of Arthritis and Metabolic Diseases, U.S. Public Health Service.

We thank Mr. Gideon Einav for constructing the apparatus.

D. SULITZEANU
W. F. GOLDMAN

Department of Bacteriology,
Hadassah Medical School,
Hebrew University,
Jerusalem.

¹ Ornstein, L., and Davies, B. J., *Disc Electrophoresis* (Distillation Products Industries, Rochester, New York, 1962).

² Lewis, U. J., and Clark, M. O., *Anal. Biochem.*, **6**, 303 (1963).

³ Racusen, D., and Calvanico, N., *Anal. Biochem.*, **7**, 62 (1964).

⁴ Broome, J., *Nature*, **199**, 179 (1963).

⁵ Reusser, F., *Arch. Biochem. Biophys.*, **106**, 410 (1964).

⁶ Weintraub, M., and Raymond, S., *Science*, **142**, 1677 (1963).

ENTOMOLOGY

An Inhibitory Effect of Allatectomized Males and Females on the Sexual Maturation of Young Male Adults of *Schistocerca gregaria* (Forsk.) (Orthoptera: Acrididae)

As shown by Loher¹, removal of the corpora allata from immature adult males of the desert locust (*Schistocerca gregaria* Forsk.) prevents the onset of sexual maturity and also the production of the pheromone which has an accelerating effect on the maturation of young males.

An investigation has recently been made into the relative effects of allatectomy and of severing the nervous connexions of the corpora allata on sexual behaviour, oocyte development and pheromone production in adult desert locusts². In the course of these experiments it became apparent that allatectomized males not only fail to accelerate maturation but actually retard it.

In one experiment, 19 young males were placed in separate jars within 24 h after fledging. Seven of them were kept each with one allatectomized male. The other twelve were kept each with a normal female of its own age. The allatectomized males were about four weeks old at the beginning of the experiment and had been operated on a few days after fledging—well before sexual maturation. The age at which the young males began to develop the yellow coloration on the abdomen (which is indicative of the onset of maturity³) was recorded. As soon as this point was reached, or earlier in the case of the last ones to become mature, the young males were put each with one fully mature female for about 5 h daily during which time they were kept under observation and their ages at first copulation or attempted copulation were noted. Those young males which were being kept with females were left with their own female during this period provided that she was fully mature; otherwise a fully mature female was substituted. At the end of each observation period, the young males were replaced with their usual partners. The males kept with allatectomized males started to become yellow in an average of 24.3 ± 9.4 days and first copulated, or tried to copulate, in an average of 29.5 ± 11.4 days. The males kept with females started to become yellow in an average of 17.0 ± 3.7 days and copulated, or tried to copulate, in an average of 19.2 ± 4.7 days. The difference in ages at copulation was significant ($P < 0.02$) and the difference in yellowing-times was also significant ($P < 0.05$). Of the males kept with allatectomized males the last one to copulate failed to become yellow and could not therefore be included in the calculation for yellowing-time. This accounts for the greater time elapsing between the average yellowing-time and the average copulation-time in this treatment.

The number of males kept with allatectomized males in the above experiment was small, so a larger experiment was next carried out in which young males were kept in four different ways as follows:

- | | |
|--|--|
| (A) Each with one allatectomized male | } allatectomized locusts operated on as in the previous experiment |
| (B) Each with one allatectomized female | |
| (C) Each with one normal female of the same age as the young males | |
| (D) Each in isolation | |

The results of this experiment are summarized in Table 1.

It is apparent that allatectomized males and allatectomized females have a similar retarding effect on male maturation and that the normal females are neutral in effect.

In a subsequent experiment the effect of the presence of allatectomized females on oocyte development in young adult females was investigated. Sixteen young females were kept in pairs with each other, another sixteen were

Table 1. EFFECT OF THE PRESENCE OF ALLATECTOMIZED MALES, ALLATECTOMIZED FEMALES AND NORMAL FEMALES ON THE MATURATION-RATE OF YOUNG MALE ADULTS OF *Schistocerca gregaria*

Figures represent average \pm S.D. in days after fledging. (In parentheses number of young males investigated)

Each young male kept:	First appearance of yellow coloration	First copulation
(A) With one allatectomized male	24.1 ± 6.9 (13)	27.7 ± 7.7 (13)
(B) With one allatectomized female	25.1 ± 6.7 (12)	26.5 ± 6.3 (12)
(C) With one normal female	18.6 ± 4.0 (12)	20.9 ± 4.2 (12)
(D) In isolation	17.5 ± 3.8 (13)	18.7 ± 3.5 (13)
Significance tests (P)	A/B not sig. A/C < 0.05 A/D < 0.01 B/C < 0.01 B/D < 0.01 C/D not sig. A + B/C + D < 0.001	A/B not sig. A/C < 0.02 A/D < 0.001 B/C < 0.02 B/D < 0.001 C/D not sig. A + B/C + D < 0.001

kept each with one allatectomized female, and another sixteen were kept each in isolation. When dissected at the age of 3 weeks the average lengths of the first or largest oocyte were respectively 4.4, 4.2 and 4.2 mm. The result was therefore negative, no effect of the allatectomized females being demonstrated.

It has previously been shown^{3,4} that, although the presence of mature males of *Schistocerca* has an accelerating effect on maturation, very young adult locusts within a week of fledging have the reverse effect and retard maturation of young males. The retarding influence of the allatectomized locusts is similar to that of very young locusts and it seems that allatectomy, as well as preventing maturation, prolongs the period during which an inhibitory influence on the maturation of others is exerted.

M. J. NORRIS
M. P. PENER*

Anti-Locust Research Centre,
Wrights Lane, London, W.8.

* Present address: Laboratory of Entomology, Department of Zoology, Hebrew University, Jerusalem, Israel.

¹ Loher, W., *Proc. Roy. Soc., B*, **153**, 380 (1960).

² Pener, M. P., *J. Zool.*, **147**(2), 119 (1965).

³ Norris, M. J., *Anti-Locust Bull.*, No. 18 (1954).

⁴ Norris, M. J., *Nature*, **203**, 784 (1964).

VIROLOGY

Nuclear Surface N-Acetyl Neuraminic Acid Terminating Receptors for Myxovirus Attachment

INSIGHT into the molecular organization of cellular membranes may be gained by defining their molecular components in terms of functional groups which serve as receptors for virus attachment. Thus, the specific attachment of myxovirus to neuraminic acid-terminating glycoprotein groups on the cell surface and the loss of virus binding capacity following exposure of cells to neuraminidase serve to define the neuraminic acid molecule in its role as a functional receptor on the plasma membrane¹. This report describes the first use of this approach to map the surface of nuclei by comparing the characteristics of myxovirus attachment and elution of whole cells with isolated nuclei. Here we demonstrate that the surface of cytoplasm-free nuclei prepared by treating cells with an anionic detergent contains specific receptors for the attachment of myxovirus, and that in terms of virus adsorption and elution the surface of nuclei isolated from HeLa cells reacts much like that of an erythrocyte, or of a host cell the viral engulfment capacity of which has been destroyed by heat.

Isolated nuclei are prepared by suspending monolayer dispersed HeLa S3 cells to a density of 2×10^6 cells/ml. in a cold 0.01 per cent aqueous solution of purified sodium dodecyl sulphate (SDS)². The cold SDS-cell suspension is pipetted 3–6 times through a 10-ml. pipette or mixed vigorously for about 30 sec on a 'Vortex-Junior' mixer. This brief treatment produces complete dissolution of the

asma membrane and most intracytoplasmic structures, and results in a quantitative yield of nuclei. This technique is similar in principle to several methods described recently³⁻⁶, but simpler in practice. The nuclei used in these experiments were washed with a K^+ -free phosphate-buffered-saline by centrifugation at 1,600 r.p.m. for 1 min in an International Model PR-2, and resuspended in attachment solution⁷ or K^+ -free saline. Nuclei were removed for adhering cytoplasm by means of oil immersion phase-contrast and dark-field optics, and with fluorescent microscopy of acridine orange-stained unfixed material under conditions known to reveal cytoplasm as brilliant red-orange fluorescence⁸. By these tests, more than 99 per cent of the nuclei were judged completely free of adhering cytoplasm and presented an extremely uniform and smooth appearance at the membrane-fluid interface. Electron microscopic investigations are underway to determine whether the surface of nuclei isolated in this manner consists of a single or double unit membrane⁹.

Virus attachment studies on isolated nuclei exposed to Newcastle disease virus (NDV), a representative of the myxovirus group the members of which characteristically bind to receptors terminating in *N*-acetyl neuraminic acid^{9,10}, are carried out as follows at 2° C: nuclei and cells are suspended in cold attachment solution to equal densities, usually 2.0×10^7 /ml., and an equal number of plaque-forming particles (PFU) of NDV are added to each of the two suspensions. Aliquots of the virus-cell and virus-nucleus mixtures are removed at various intervals, immediately diluted 1:20 in cold attachment solution, and centrifuged to remove cells, nuclei and bound virus. The supernatant fluid is assayed for unattached plaque-forming particles as previously described¹¹. Fig. 1 represents the characteristic attachment of NDV to HeLa cells and the loss of virus binding following the treatment of the cells with neuraminidase. Fig. 2 demonstrates that NDV binds equally efficiently to isolated nuclei, and that, as with whole cells, binding capacity is lost following incubation with neuraminidase. Values of the velocity constants for attachment¹², k , of NDV to whole cells and nuclei were experimentally equi-

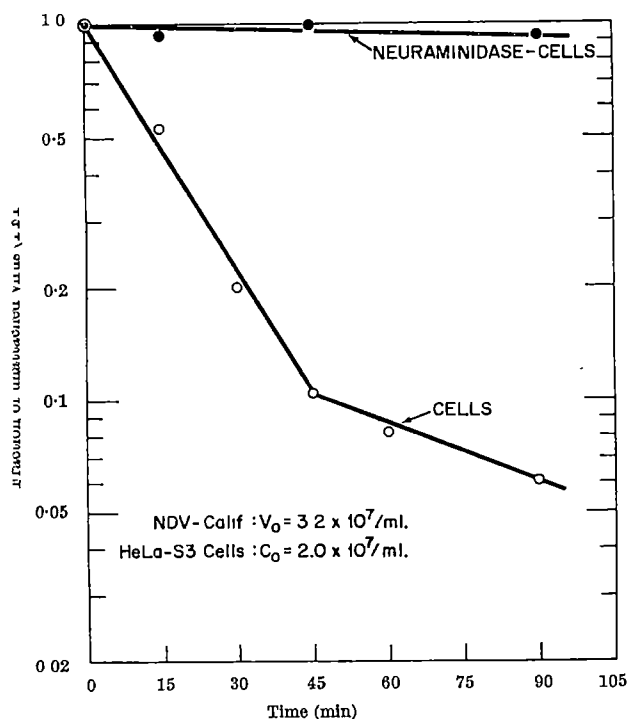


Fig. 1. Newcastle disease virus attachment to neuraminidase-treated and non-treated HeLa cells. Temperature of attachment = 2° C

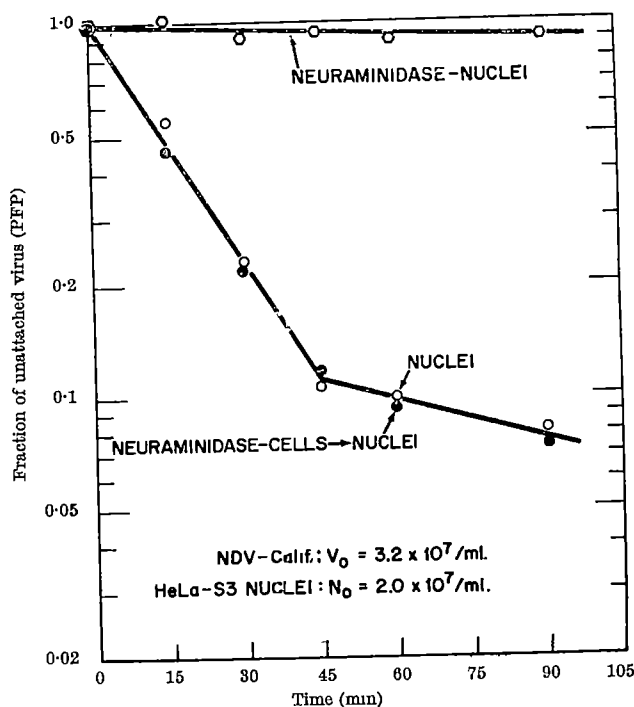


Fig. 2. Newcastle disease virus attachment to neuraminidase-treated and non-treated nuclei derived from neuraminidase-treated and non-treated HeLa cells. Temperature of attachment = 2° C

valent, with $k = 2 \times 10^{-9}$ cm³ min⁻¹. The patterns of NDV attachment to nuclei and cells were similar, that is, attachment was rapid and uniform for about 85 per cent of the virus population, and for the remainder slowed markedly to about one-tenth of the original rate.

When NDV at the low multiplicities (virus/cell ratio ≈ 1) used here is attached to HeLa cells at 2° C less than 0.2 per cent of the virus elutes when the temperature of the virus-cell complex is raised to 37° C (ref. 13), and within 10 min essentially all of the particles are engulfed by the cell¹⁴. In contrast, when virus-nuclei complexes formed at 2° C are incubated at 37° C virus elution commences, and within 30 min all initially bound virus is recoverable in the supernatant fluid as free plaque-forming particles (Fig. 3). This elution is accompanied by receptor destruction, and a consequent inability of the nuclei to bind virus as evidenced by a ten-fold reduction in uptake of a second virus input. Similarly, viral elution from the host cell surface may be demonstrated if engulfment of virus is prevented by heating the cells for 15 min at 50° C prior to virus attachment. Then, viral elution occurs at essentially the same rate as from the surface of isolated nuclei or bovine red blood cells, the latter used to verify the well-documented elution characteristics of myxovirus from erythrocytes (Fig. 3).

To test whether receptors for attachment of myxovirus to the surface of nuclei are artefacts, derived from the cell membrane during the isolation procedure, the following experiment was performed: HeLa cells were treated with neuraminidase (Grand Island Biological Co., New York) for 15 min at 37° C to remove all cell surface-situated receptors as tested by: (a) release of *N*-acetyl neuraminic acid (NANA) determined chemically¹⁵; (b) loss of virus binding capacity; or (c) loss of cell susceptibility to infection¹⁶; then cooled to 2° C, washed, and treated with SDS to obtain nuclei. The curve labelled 'neuraminidase-cells → nuclei' in Fig. 2 demonstrates that under these conditions complete removal of receptor *N*-acetyl neuraminic acid molecules located in the cell membrane has no effect on the receptor complement of subsequently isolated nuclei as judged by the rate of NDV attachment.

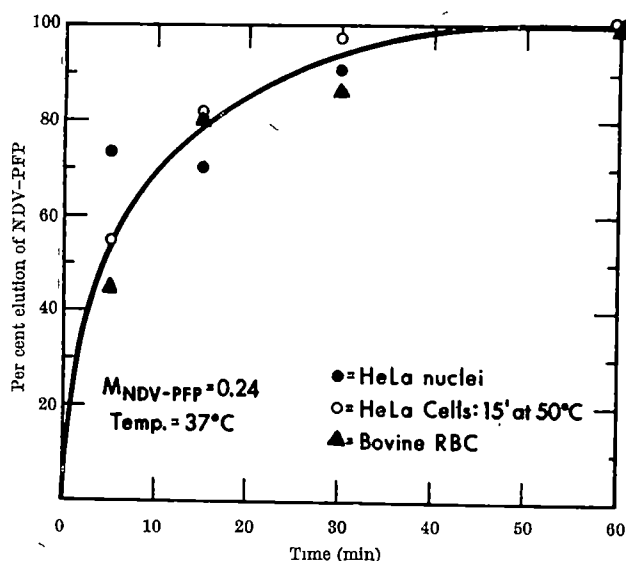


Fig. 3. Newcastle disease virus elution at 37° C from HeLa cells heated for 15 min at 50° C, nuclei derived from HeLa cells, and bovine erythrocytes. Virus attachment initially at 2° C for 30 min

We were concerned with the relative amounts of sialic acid on the surface of whole cells and nuclei isolated from them, and hence determined the amount of neuraminic acid freed from these two surfaces following treatment with neuraminidase (15–25 units enzyme/ 1×10^7 cells or nuclei for 20 min at 37° C in phosphate-buffered-saline containing 10^{-3} M Ca^{++} ; conditions adequate to release maximal amounts of sialic acid as assayed by the Warren procedure¹⁵). Table 1 lists the sialic acid (NANA) content of HeLa S3 cell and nuclear surfaces as the average and standard deviation of from 5 to 10 determinations made on cells, or nuclei from cells, grown in spinner culture. To determine whether the low NANA content of the nuclear surface was due to removal of membrane bound-NANA moieties by SDS, we tested the sensitivity of plasma membrane-bound NANA to removal by the detergent, since the normal content of NANA on the cell surface was known (Table 1). Sialic acid freed from the cell surface by neuraminidase was measured after exposing the cells to SDS under conditions that do not produce dissolution of the plasma membrane, namely, 0.01 per cent SDS in cold isotonic K^+ -free saline. Cells exposed in this manner, first to SDS, release the usual amount of NANA ($0.16 \mu\text{M}/1 \times 10^8$ cells) after enzyme treatment, indicating that our standard treatment with detergent apparently does not free NANA-containing molecules from membrane.

In light of the equivalent velocity constants for NDV attachment to nuclear and cell surfaces, and hence the presumed equivalent collision efficiency of the two systems, the 24-fold excess of neuraminic acid groups on the cell surface revealed in Table 1 presents somewhat of an anomaly, and suggests that many of the NANA prosthetic groups are not functional in a receptor capacity (perhaps inaccessible as Burnet¹⁶ has thought in accounting for the receptor gradient), a concept consistent with the fact that only a small fraction of plasma membrane-located carboxyl groups of NANA may effectively contribute to the net negative charge on the cell surface¹⁷. On the other hand, when the contribution of microvilli to the cell surface is considered, the surface area per cell

approximates $10^4 \mu^2$, or about 10^5 molecules of NANA per μ^2 . With the surface area of the nucleus estimated at about $500 \mu^2$, there are also about 10^5 molecules of NANA per μ^2 . The errors inherent in evaluating the total cell and nuclear surface areas are of such magnitude that the density of NANA molecules on each surface might, as a first approximation, be considered equal. The equivalent k values obtained for the attachment constants are thought to support this view.

During the sialic acid determination we noted that nuclei treated with neuraminidase to release surface NANA did not leak significant amounts of 2-deoxyribose as measured spectrophotometrically¹⁸, attesting to the integrity of the membrane of isolated nuclei, since rupture of the nuclear membrane by sonication or acid hydrolysis was shown to release large amounts of this sugar. In the connexion, the relative stability of the nuclear membrane of mammalian cells in hypotonic solutions of detergent of general interest. For the preparation of cytoplasm-free nuclei, the concentration of cells suspended in aqueous SDS is critical. As higher densities of cells are used, correspondingly larger fraction of nuclei is found with cytoplasmic debris adhering to their periphery; noticeably, the Golgi-region of the cytoplasm is most tenaciously bound, and hence invariably the last portion of the cytoplasm to fall free.

Throughout these investigations we have been impressed with the specificity and sensitivity of the biological test system, viral attachment, over that of the chemical reactions used to detect NANA-terminal groups. For example, it was necessary to amass more than 10^6 nuclei for a single chemical determination, whereas 10^4 nuclei suffice to detect receptors, or the loss of same, by viral attachment techniques.

These investigations demonstrate that viral attachment to particular molecular groups on the surface of cell or nuclei can be used as a biological approach of exquisite specificity and sensitivity to define the complex mosaic of chemical entities that constitute the functional group of cytomembranes. To pursue this approach further we have initiated a complementary investigation designed to determine the rate and pattern of reappearance, and conditions governing the replacement of NANA terminating groups on the surface of normal individual cells¹⁹.

This work was aided by grant AI-03619-05 from the U.S. National Institutes of Health. One of us (P. I. M.) is a research career development awardee of the National Institute of Allergy and Infectious Disease, U.S. Public Health Service (2-K3-GM-15, 461-05).

PHILIP I. MARCUS
JESSE M. SALT
VERA GALINOVIC SCHWARTZ

Albert Einstein College of Medicine,
Bronx, New York.

Table 1 NEURAMINIC ACID CONTENT OF HELa CELL AND NUCLEAR SURFACES

Surface	NANA ($\mu\text{M}/1 \times 10^8$ cells or nuclei)	Molecules NANA/surface
Cell	$0.168 \pm 0.041^*$	$1,050 \times 10^6$
Nucleus	0.007 ± 0.002	44×10^6

* The direct Ehrlich procedure gave exactly one-half this value for cell surface NANA.

- ¹ Gottschalk, A., in *The Viruses*, IV, edit. by Burnet, F. M., and Stanley W. M., 3 (Academic Press, 1959).
- ² Mandel, B., *Virology*, **17**, 288 (1962).
- ³ Hodes, M. R., Palmer, C. G., and Williams, D. S., *Nature*, **182**, 529 (1958).
- ⁴ Fisher, H. W., and Harris, H., *Proc. Roy. Soc., B*, **156**, 521 (1962).
- ⁵ Hubert, M. T., Favard, P., Carasso, N., Rosencwajg, R., and Zalta, J., *J. de Microscopie*, **1**, 435 (1962).
- ⁶ Hymner, W. C., and Kuff, E. L., *J. Histochem. Cytochem.*, **12**, 359 (1964).
- ⁷ Marcus, P. I., and Carver, D. H., *Science*, **149**, 938 (1965).
- ⁸ Robbins, E., and Marcus, P. I., *J. Cell. Biol.*, **18**, 237 (1963).
- ⁹ Klenk, E., and Lempfrid, H., *Hoppe-Seyl. Z.*, **307**, 278 (1957), quoted by Cohen, A., in *Mechanisms of Virus Infection*, chap. 4, edit. by Smith, W. (Academic Press, 1963).
- ¹⁰ Howe, C., Rose, H. M., and Schneider, L., *Proc. Soc. Exp. Biol. and Med.*, **96**, 88 (1957).
- ¹¹ Marcus, P. I., *Virology*, **9**, 546 (1959).
- ¹² Tolmach, L. J., in *Advances in Virus Research*, **4**, 67 (1957).
- ¹³ Marcus, P. I., *Bact. Rev.*, **23**, 232 (1959).
- ¹⁴ Silverstein, S. C., and Marcus, P. I., *Virology*, **23**, 370 (1964).
- ¹⁵ Warren, L., *J. Biol. Chem.*, **234**, 1971 (1959).
- ¹⁶ Burnet, F. M., in *Principles of Animal Virology*, second ed., 114 (Academic Press, 1960).
- ¹⁷ Wallach, D. F. H., and Eylar, E. H., *Biochim. Biophys. Acta*, **52**, 549 (1961).
- ¹⁸ Marcus, P. I., and Hirsh, D. (abst., *J. Cell. Biol.*, **18**, 47A (1963)).

Effect of β -Propiolactone and Thiomersal on Growth of B.H.K. Cells in the Hamster Cheek Pouch

THE use of tissue cultures of continuous cell-lines for preparing vaccines poses a number of problems which are assumed not to exist when primary cell-cultures are used. Undoubtedly the most interesting aspect requiring clarification is the potential of these cells to multiply when inoculated into an animal. There are several factors which could have a bearing on this, in particular the physical treatment that the cells undergo during the preparation of a vaccine. This treatment is probably most severe in the preparation of inactivated vaccines, and we have examined the various stages of this process to determine their effect.

Newcastle disease vaccine can be prepared by growing the virus in tissue culture, inactivating with 1:1,000 β -propiolactone and afterwards adding 0.013 per cent thiomersal as a preservative. Virus, inactivating agent and preservative are each capable of causing cell damage and were therefore investigated. The test involved virus growth in cells derived from the B.H.K. 21 cell line which had been previously modified until they were capable of growing in suspended culture. Inoculating the hamster cheek pouch was used for detecting the presence of viable cells. This system could also detect oncogenic substances that might be released from the treated cells.

A suspension of B.H.K. cells in the log phase of growth, with a viable count of 1.5×10^6 cells/ml., was concentrated by centrifuging at 166*g* for 15 min. The cells were immediately resuspended in maintenance medium at concentrations of 10×10^6 , 20×10^6 and 30×10^6 cells/ml. Each of these suspensions was divided into three portions, one being treated with β -propiolactone, one with thiomersal and one left untreated.

Treatment with β -propiolactone was carried out by adding a freshly prepared 10 per cent solution of β -propiolactone in distilled water to the cell suspension to give a final concentration of 1:1,000; the mixture was then kept at 37°C for 2 h, and after adjustment of the pH to 7.2 it was inoculated into hamsters. A further sample was treated with thiomersal at a final concentration of 0.013 per cent by adding it to cells maintained at +4°C. This mixture was inoculated into hamsters after treatment for 2 h. The control cells received no treatment apart from storage at +4°C for 2 h before inoculation.

Syrian hamsters weighing 50–60 g were used. The technique adopted was that described by Handler and Foley¹, except that the animals were anaesthetized with ether before inoculation and when they were examined for signs of tumour development. 0.1 ml. of a suspension of cells was implanted under the epithelium of the cheek pouch with a 26-gauge needle. Only one cheek pouch was inoculated. Each cell suspension was given to a group of four hamsters; two of them received 0.1 ml. cortisone solution ('Cortelan' 25 mg/ml.) subcutaneously at the time of inoculation. This dose was repeated three times a week for 3 weeks. The hamsters were observed daily during the first week and three times a week thereafter.

Small nodules, approximately 1 mm in size, were evident in most hamsters during the first few days after inoculation. These nodules either regressed or became visibly larger, depending on the type of cell suspension administered. In certain instances the nodules were associated with a prominent vascularity. When a nodule developed, the hamster was retained until the nodule became large or showed signs of breaking down. The nodule was then excised for histological examination. Transplantations of nodules to other cheek pouches were not made. In those hamsters in which nodules failed to develop, the animals were retained for 42 days before being killed, and the area of the cheek pouch that had been inoculated was removed for histological study.

When untreated B.H.K. cells were inoculated at the three different cell concentrations, the initial nodules enlarged considerably from the seventh day onward and became markedly vascular by the tenth day. No apparent difference was noticeable between the pairs of hamsters receiving cortisone and the others. The hamsters were retained for 14–24 days before the nodules were excised. Histological examination of the nodules revealed extensive cellular proliferation. The pattern was that of an undifferentiated cell sarcoma; numerous mitotic figures were present and there were signs of tissue necrosis in the central area. The interpretation was that cells in the inoculum could proliferate in the hamster cheek pouch and produce tumours similar to those we have seen in earlier studies with HeLa cells.

B.H.K. cells treated with thiomersal at the two higher cell concentrations produced nodules with an associated vascularity in the cheek pouches. Hamsters inoculated with the smallest concentrations of thiomersal-treated B.H.K. cells, 10×10^6 cells/ml., developed no nodules during 42 days' observation. The histological sections on the cheek pouches of these animals revealed no abnormalities except occasional local inflammatory responses.

B.H.K. cells treated with β -propiolactone produced no nodules in hamsters at any of the three concentrations of cells. Histological sections of cheek pouches were normal, apart from minimal inflammatory responses in some sections.

An attempt was made to assess the effect of Newcastle disease virus on the viability of B.H.K. cells. Unfortunately this had to be combined with inactivation by β -propiolactone in order to avoid the presence of live virus in the animal house. A suspension of cells with a viable count of 2×10^6 per ml. was infected with a tissue-culture adapted strain of Newcastle disease virus. Four days later the cells were removed and concentrated by centrifuging; they were then resuspended in supernatant to give a final cell concentration of 30×10^6 per ml. Inactivation with 1:1,000 β -propiolactone was carried out as previously described. The sample was divided into halves; one received no further treatment, but thiomersal was added to the other at a final concentration of 0.013 per cent. None of the inoculated hamsters developed tumours, and there was no histological sign of cell proliferation.

It would seem that β -propiolactone at a concentration of 1:1,000 is highly efficient for preventing subsequent multiplication of the cells. Thiomersal at a concentration of 0.013 per cent appears to have a marginal effect during the short period of treatment used. The fact that it was able to inhibit cell growth when low concentrations of cells were used may be related to the lower protein content of this particular cell suspension; with higher cell concentrations non-specific absorption of the substance may occur, reducing its availability. Alternatively, the initial low concentrations of the preservative or the short treatment may be responsible for lack of inhibition at higher cell concentrations. In practice the cell concentration of a vaccine is likely to be less than one-fifth of the lowest concentrations we used, and at these levels 0.013 per cent thiomersal totally inhibits cell growth.

Table 1

Cell suspension	Cells per 10 ml.	Regression (R) or progression (P)	Vascularization	Host inflam. response	Mitosis. Few or none — Many +
B.H.K. alone	30×10^6	P	Marked	—	+
	20×10^6	P	Marked	—	+
	10×10^6	P	Marked	—	+
B.H.K. + thiomersal	30×10^6	P	Marked	—	+
	20×10^6	P	Marked	—	+
	10×10^6	R	Poor	+	—
B.H.K. + β -propiolactone	30×10^6	R	Poor	+	—
	20×10^6	R	Poor	+	—
	10×10^6	R	Poor	+	—
B.H.K. + NDV virus + β -propiolactone	No thiomersal	R	Poor	+	—
	Thiomersal	R	Poor	+	—

Under the conditions of these experiments, the physical treatment that a virus vaccine undergoes during the various production stages of virus growth, inactivation and addition of preservative, was shown to be deleterious to the tissue culture cells from which the virus was produced; using a highly sensitive test system we were unable to demonstrate surviving cells. We were unable to demonstrate any breakdown product capable of producing tumours.

From these experiments it seems that the preparation of an inactivated vaccine from virus grown in these cells would carry no risk of inducing malignant tumour growth in hamsters and hence would also carry no risk for a heterologous host, in which there would, moreover, be an immunological barrier.

S. A. KEEBLE
C. S. HEYMANN

Virus Research Unit,
Glaxo Laboratories, Ltd., Greenford, Middlesex.

¹ Handler, A. H., and Foley, G. E., *Proc. Soc. Exp. Biol. Med.*, **81**, 237 (1958).

GENETICS

Linkage in Man: the Inv and the Lp Serum Type Systems

THE new serum type system Lp¹ is a useful genetic marker system, the frequency of the Lp^a gene being approximately 0.18 in Caucasians^{2,3} and test reagents unrestrictedly available. Concerning the serum type system Inv (refs. 4 and 5), test reagents have been relatively difficult to obtain, but are now increasingly available³⁻⁵. The established Inv^a or Inv^{1a} gene frequencies, 0.06-0.10 in Caucasians and considerably higher in other populations, would indicate common use of this marker system in the future. This pre-supposes the elucidation of the genetics of this system, including analysis of linkage relationships. This communication provides data on the Inv-Lp linkage relation.

The material comprised 87 unrelated parents and 20 children from Norwegian families tested for Lp(a) and Inv(a). Of the unrelated individuals 67 were derived from a family material considered earlier as regards Lp(a) by Mohr and Berg², and 20 derived from a material concerning *Epidermolysis bullosa* (Gedde-Dahl, in preparation).

Anti-Inv(a) Math. serum and anti-D Roehm serum were kindly provided by Dr. A. G. Steinberg, while anti-Lp(a) serum was of our own production (K. B.). The Inv typing was performed as outlined by Steinberg⁵ with minor modifications, and the Lp typing as described by Berg².

Among the 87 unrelated individuals 13.79 per cent (12 individuals) were found to be Inv(a⁺) and 33.33 per cent (29 individuals) Lp(a⁺) (Table 1). Four matings of the type Inv(a⁺)Lp(a⁺) × Inv(a⁻)Lp(a⁻) were observed among the 40 pairs of parents where both parents were tested. As appears from Table 2, recombination between the Inv and Lp loci must have taken place in each of these families. The Fisher-Finney method¹⁰ gave values of 73.225 units, a linkage score of -7.233 and a test value of -0.845. The most likely recombination frequency is 50 per cent, that is free recombination. By the Morton-Smith method¹¹, linkage between the Lp and Inv loci may be excluded with a probability of 0.985, and linkage below 30 per cent with a probability of 0.996. It may be noted that practically all the information, 66 units by the

Fisher-Finney method, was derived from a single 12 s family. In all four families the double heterozygote parent was the mother.

Berg and Mohr¹² found the phenotype Lp(a⁺) in 34.6 per cent of 314 unrelated Norwegians. 67 of these, namely 23 Lp(a⁺) and 44 Lp(a⁻) individuals, are included in the present material. The frequency of the phenotype Inv(a⁻) has not previously been estimated for the Norwegian population. The distribution in the present material however, does not differ significantly from our own finding of 14 Inv(a⁺) individuals among 143 Norwegians not included here ($\chi^2: 0.86, 0.5 > P > 0.3$). Both materials combined gave a phenotype frequency of Inv(a⁻) of 11.2 per cent. These results of Inv(a) will be included in later publication.

From the gene frequencies of Lp^a 0.1877² and Inv 0.059 an expected number of 0.8 double backcross matings among 40 random Norwegian matings may be calculated. Actually, 2 such matings were observed among 33 from a unselected family material, and 2 out of 7 in families selected by *Epidermolysis bullosa* and by the number of children.

It appears that the linkage relations of the Inv loci have not previously been analysed beyond an exclusion of linkage closer than 30 per cent recombination between the Inv and Gm loci¹³.

Work on the relations between the Lp locus and the loci of the ABO, MNS, Rh, Le, Lu, P, Fy, Jk, K, Hp, G, Gm, and Ag systems has not revealed any linkage, but information on some of the relations was scarce^{2,9}.

The calculations were made by Mr. Dan Wøien, of the University Institute of Mathematics, Oslo, by means of an IBM electronic computer 1620 II.

This work was in part supported by U.S. Public Health Service research grant GM 6842 from the Division of Research Grants.

T. GEDDE-DAHL, jun.

University Institute of Medical Genetics,
Oslo, Norway.

K. BERG

University Institute of Forensic Medicine,
Rikshospitalet, Oslo, Norway.

¹ Berg, K., *Acta Path. Microbiol. Scand.*, **59**, 369 (1963).

² Mohr, J., and Berg, K., *Acta Genet. (Basel)*, **13**, 343 (1963).

³ Berg, K., and Wendt, G. G., *Humangenetik*, **1**, 24 (1964).

⁴ Ropartz, C., *Nature*, **189**, 586 (1961).

⁵ Steinberg, A. G., in *Progress in Medical Genetics*, 2 (Grune and Stratton, London and New York, 1962).

⁶ Herzog, P., and Drdova, A., *Vox Sang.*, **6**, 636 (1961).

⁷ Ropartz, C., Rivat, L., and Rousseau, P.-Y., Ninth Congr. Int. Soc. Blood Transf., Mexico (1962).

⁸ Rittler, H., and Schmidtmann, E., *Humangenetik*, **1**, 144 (1964).

⁹ Berg, K., *Acta Path. Microbiol. Scand.*, **62**, 278 (1964).

¹⁰ Finney, D. J., *Ann. Eugen.* (Lond.), **10**, 171 (1940).

¹¹ Smith, C. A. B., *Amer. J. Hum. Genet.*, **11**, 289 (1959).

¹² Berg, K., and Mohr, J., *Acta Genet. (Basel)*, **13**, 349 (1963).

¹³ Steinberg, A. G., and Matsumoto, H., *Hum. Biol.*, **36/2**, 77 (1964).

Artificial Insemination of Dystrophic Mice with Mixtures of Spermatozoa

MUSCULAR dystrophy in mice (*Dystrophia muscularis* symbol *dy*) is transmitted as an autosomal recessive and is linked to Steel (*Sl*) and Ames waltzer (*av*) in linkage group IV³⁻⁵. Mice of genotype *dy/dy* have pronounced weakness of the hind limbs and die as young adult. Dystrophic male mice do not breed, and dystrophic females may have one or sometimes two litters early in their reproductive life, when mated to normal male. The disease can be recognized in mice 2-3 weeks old and has a characteristic manifestation, described in detail by Michelson *et al.*¹ and Loosli *et al.*².

In a previous report⁷, litters consisting entirely of dystrophic (*dy/dy*) mice were obtained by inseminating dystrophic *F*₁ hybrid females (*F*₁ hybrid from 12 *Re-dy/+* × *C57BL/6-dy/+*) (ref. 8) with spermatozoa from *F*₁ hybrid males of the same genotype. The number of dystrophic females producing litters following artificial insemination approaches that observed with natural

Table 1. PHENOTYPE DISTRIBUTION OF 87 UNRELATED PARENTS

	Inv(a ⁺)	Inv(a ⁻)
Lp(a ⁺)	4	25
Lp(a ⁻)	8	50

Table 2. PHENOTYPE DISTRIBUTION OF CHILDREN IN FOUR FAMILIES OF MATING TYPE Inv(a⁺)Lp(a⁺) × Inv(a⁻)Lp(a⁻)

Family	No. of children	Inv(a ⁺) Lp(a ⁺)	Inv(a ⁻) Lp(a ⁻)	Inv(a ⁻) Lp(a ⁺)	Inv(a ⁺) Lp(a ⁻)
Eb XXV	12	2	3	3	4
678	3	1	1	1	
688	3	1	1		1
Eb LXIII	2		1		

Table 1. RESULTS OF ARTIFICIAL INSEMINATIONS OF HORMONALLY PRIMED DYSTROPHIC FEMALES VERSUS NATURAL MATINGS OF HORMONALLY PRIMED NORMAL FEMALES

	Litters/ No. inseminated	Mean litter size	Total offspring
dystrophic females (A) inseminated with spermatozoa from dystrophic males (A)	73/235 (31%)	7.5	549
natural matings of wild type females (B) and wild type males (B)	127/317 (43%)	8.9	1,222
A = 129(Re)B6-+/p dy/dy derived from 129/Re-p/p +/dy × C57BL/+/+ +/dy			
B = 129(Re)B6-+/p +/+ derived from 129/Re-p/p +/+ × C57BL/+/+ +/+			

Table 2. RESULTS OF INSEMINATIONS OF DYSTROPHIC FEMALES WITH MIXED SPERMATOZOA

pink-eyed dystrophic females (C) inseminated with spermatozoa from a pink-eyed dystrophic male (C) mixed with those from a dark-eyed non-dystrophic male (D).

Experiment	Litters/No. inseminated	Mean litter size	Offspring		Total
			Pink-eyed	Dark-eyed	
1	9/20	6.1	14	39	53
2	2/18	9.0	1	4	5
3	5/20	4.2	8	13	21
4	5/20	5.0	13	12	25
5	1/22	8.0	4	4	8
6	3/19	7.0	12	9	21
7	5/17	11.0	26	29	55
8	14/24	7.9	67	43	110
9	8/20	5.5	23	21	44
10	15/20	7.2	62	46	108
Totals	67/200 (34%)	6.7	230	220	450

C = 129(Re)B6-p/p^m dy/dy derived from 129/Re-p/p +/dy × C57BL/p^m/p^m +/dy
D = 129(Sv)B6-+/+ +/+ derived from 129/Sv-+/+ +/+ × C57BL/+/+ +/+

matings of hormonally primed 129B6F₁ normal females (Table 1). The offspring are homozygous for dystrophy (dy/dy) but are otherwise a genetically segregating population. Variation in genetic background does not, however, cause any appreciable differences in the expression of dy/dy genotype^{6,10}.

This communication reports reproductive performance of dystrophic females following insemination with mixtures of genetically-tagged spermatozoa, along with information on genotypes of offspring. The eye-colour markers, pink-eyed dilution (p) and its wild-type allele (+), were used to identify normal and potentially dystrophic individuals from 12 days of gestation forward. This method permits within-litter biochemical and histopathological comparisons of dy/dy and dy/+ muscle in pre-clinical stages.

All mice used in these experiments were 129B6F₁ hybrids, but special mutant-carrying sub-lines of strains 129 and C57BL/6 were required to provide the desired colour markers. A mutation at the pink-eyed dilution locus (p^m) in the C57BL/6 strain permitted the development of a C57BL/6-p^m dy strain from the existing C57BL/6-dy strain, the latter described by Russell *et al.*⁸. A 129/Sv-CP sub-strain homozygous for the wild-type alleles of pink-eyed dilution and dystrophy (N10) was available through the courtesy of Dr. Leroy Stevens.

Ovulation was hormonally induced in all females by intraperitoneal injections of 2 i.u. of pregnant mare serum (PMS) (Ayerst Laboratories, Inc., New York) followed in 36–40 h by 2 i.u. of human chorionic gonadotropin (HCG) (Ayerst Laboratories, Inc., New York). In production of all dystrophic litters, spermatozoa from the ductus deferens and epididymis of one 129B6F₁-dy/dy male, suspended in 0.4 ml. 0.85 per cent sodium chloride at room temperature, were sufficient to inseminate approximately five 129B6F₁-dy/dy females. In production of mixed dystrophic-normal litters, spermatozoa from a mature pink-eyed dy/dy male and from a dark-eyed +/+ male of comparable age were suspended together in 0.8 ml. saline. Each female received 0.04–0.05 ml. of the spermatozoa through the cervix (method of Dziuk and Runner, 1960) (ref. 9), 10–12 h after injection of HCG. Immediately following insemination each female was put with a vasectomized male until a vaginal plug was observed. The mean age of all females was 55 days, range 32–89 days. Dystrophic females frequently do not withstand the strains of pregnancy and lactation, hence it was often necessary to take their litters by Caesarean section at the 19th or 20th day of gestation. Pregnant

normal females, which had been primed and mated 2 days prior to the artificial insemination of the dystrophic females, were used as foster-mothers for these litters.

When spermatozoa were mixed from two males, one of genotype p^m/p dy/dy and the other of genotype +/+ +/+, and then inseminated into females of genotype p^m/p dy/dy, approximately equal numbers of pink-eyed offspring (230) and dark-eyed offspring (220) resulted (Table 2). However, in any single experimental group the proportion of pink-eyed and dark-eyed offspring often deviated markedly from the expected 1:1 ratio. The pink-eyed offspring were presumed to be dystrophic whereas dark-eyed offspring were presumed to be normal heterozygous carriers of dystrophy. The majority of the potentially dystrophic young were used in various pre-clinical studies and thus could not be checked for expression of dystrophy. These results suggest equal transmission and fertilizing ability of p dy and p^m dy spermatozoa compared with +/+ spermatozoa. Extensive counts (unpublished) from dystrophic and normal F₁ hybrid males have shown no significant differences in numbers of spermatozoa.

We thank Mrs. Ada Grindle for her technical assistance.

This work was supported by a grant from the Muscular Dystrophy Associations of America.

JANICE L. SOUTHARD
H. GLENN WOLFE
ELIZABETH S. RUSSELL

The Jackson Laboratory,
Bar Harbor, Maine.

¹ Michelson, A. M., Russell, E. S., and Harman, P. J., *Proc. U.S. Nat. Acad. Sci.*, **41**, 1079 (1955).

² Stevens, L. C., Russell, E. S., and Southard, J. L., *Proc. Soc. Exp. Biol. and Med.*, **95**, 161 (1957).

³ Wolfe, H. G., *Mouse News Letter*, **29** (1963).

⁴ Schaible, R. H., *Mouse News Letter*, **29** (1963).

⁵ Russell, E. S. (unpublished results).

⁶ Loosli, R., Russell, E. S., Silvers, W. K., and Southard, J. L., *Genetics*, **46**, 347 (1961).

⁷ Wolfe, H. G., and Southard, J. L., *Proc. Soc. Exp. Biol. and Med.*, **109**, 630 (1962).

⁸ Russell, E. S., Silvers, W. K., Loosli, R., Wolfe, H. G., and Southard, J. L., *Science*, **135**, 1061 (1962).

⁹ Dziuk, P. J., and Runner, M. W., *J. Reprod. Fertil.*, **1**, 321 (1960).

¹⁰ Meier, H., West, W. T., and Hag, W. G., *Arch. Pathol.*, **80**, 165 (1955).

PSYCHOLOGY

Shift Working: the Arrangement of Hours on Night Work

PROBLEMS of adaptation to shift-working routines may be increased by physiological disturbance when changing to night work is involved. It has been shown that performance on some kinds of work can be affected by inversion of normal time routines so that output at night is lowest in the early hours of the morning^{1,2}. This is believed to be associated with diurnal rhythms which persist with old periodicities into the new routines. Experiments have shown, however, that complete inversion of the environment is accompanied after a period by synchronization of diurnal rhythms with the new routines³. For this reason physiologists have sometimes suggested that, to facilitate adaptation and improve efficiency, night work should be permanent or for a long period^{4,5}.

While this recommendation may be suitable for special occupational groups it fails to take into account many variables in the industrial situation. Social, domestic and recreational interests also have their place in the shift-worker's life, and these make it impossible to live in a completely inverted environment. In these circumstances adaptation of the diurnal rhythms becomes less probable and there is some evidence that it does not occur. Van Loon⁶ has shown that the temperature periodicity of three men, although modified, failed to invert after 13 weeks on night shift when continuity was inter-

Table 1. THE TWO SHIFT CYCLES

Week	Average weekly hours 42							
	1	2	3	4	1	2	3	4
Mon.	6-2	Off	10-6	2*-10	6-2	Off	10-6	2-10
Tues.	6-2	Off	10-6	2-10	6-2	Off	10-6	2-10
Wed.	6-2	10-6	Off	2-10	2-10	6-2	Off	10-6
Thurs.	6-2	10-6	Off	2-10	2-10	6-2	Off	10-6
Fri.	6-2	10-6	2-10	Off	10-6	2-10	6-2	Off
Sat.	6-12 30	10-6	12 30-10	Off	10-6	12 30-10	6-12 30	Off
Sun.	Off	10-6	2-10	6-2	Off	10-6	2-10	6-2

* 6 a.m., 2 p.m., 10 p.m.

rupted by a weekly rest-day. Studies by Wyatt and Marriott⁷ of absence from work and work performance of shift-workers, both on a fortnightly and on a monthly cycle, showed an increase in absence during successive weeks of the night shift and a decrease in absence and an increase in output in the following weeks of the day shift. Although not based on physiological measures, this suggests a failure of adaptation and a consequent accumulation of fatigue over the period of the night shift which was dissipated on the subsequent weeks of the day shift. Finally, night workers appear to retain some day-time eating habits, and if times of food intake are a secondary synchronizing factor⁸ this will interfere with adaptation.

Two studies have been carried out of the behaviour and preferences of men engaged on shift work. In the first, chemical workers in a large plant had been operating a continuous shift system, in which they worked seven shifts followed by a rest period, before changing to a different shift. On the insistence of the men and after balloting, the system was changed to a 3 × 2 × 2 cycle (Table 1). This type of cycle results in short but frequent bursts on the night shift. Interviews with a sample of fifty men, after three years experience of the new cycle, showed that all but seven men preferred the shorter cycle. The main reasons given for this preference were that they felt less tired and that they had more opportunity for participating in normal social and domestic life each week. Of those preferring the longer cycle, only four gave a reason which indicated some degree of physical adaptation during the seven-shift cycle.

The second study⁹ examined the behaviour of men on shift work who were allowed to exchange shifts among themselves. They could also ask to be placed on a permanent night list. The men worked a variety of shifts arranged in a complex 32-week rota in which night duties were listed for two in every seven or eight weeks. The study showed that one in five of all listed duties were exchanged and that 87 per cent of these changes were between day and night duties. The original rota was planned so that each man worked 25-35 per cent of his time on night duty. As a result of the exchanges and of permanent night-duty listings the numbers of men working different proportions of night duty were distributed as shown in Fig. 1. The bimodal distribution indicates preference for either permanent day- or permanent night-working. That this preference is associated with adapta-

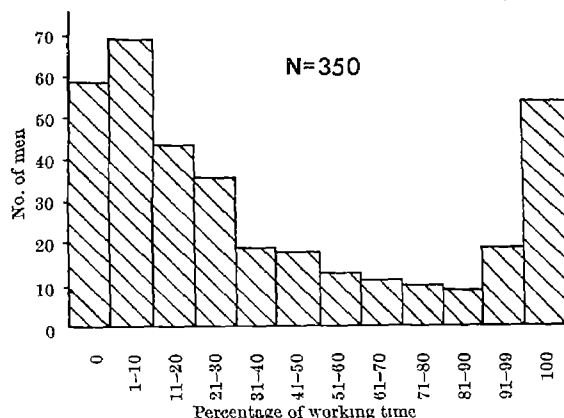


Fig. 1 Distribution of population according to percentage of working time spent on night shift

tion of diurnal rhythms, however, is doubtful. The night duty worked was one of alternate nights—one night on and one night off—and adaptation to a 48-h cycle has not, up to now, been demonstrated⁴. In addition, the 48-h cycle was broken by the fairly frequent interpolation of overtime nights. A more detailed study of the population is planned.

The variety of behaviour observed in these investigations suggests that adaptation to shift working is a complex process involving the balancing of many factors in both the internal and external environment. For this reason no single solution to the problem of the best arrangement of shift cycles is likely to be generally applicable. An extension of shift work and night work is anticipated and with shortened weekly hours there will be greater flexibility in their arrangement. Before theories of the optimum arrangement of shifts are formulated more must be known of the nature and interaction of psychological and social, as well as physiological, factors in the working situation.

GWYNETH DE LA MARE

J. WALKER

Medical Research Council,
Industrial Psychology Research Unit,
Psychology Department, University College,
London, W.C.1.

¹ Bjerner, B., Holm, A., and Swenasson, A. *Brit. J. Indust. Med.*, **12**, 105 (1955).² Browne, R. C., *Occup. Psychol.*, **23**, 121 (1949).³ Lobban, M. C., *J. Physiol.*, **165**, 75 (1962).⁴ Kleitman, N., *Sleep and Wakefulness* (University of Chicago Press, 1968).⁵ Kleitman, N., *Sci. Amer.*, **187**, 34 (1952).⁶ Van Loon, J. A., *Ergonomics*, **6**, 267 (1963).⁷ Wyatt, S., and Marriott, R., *Brit. J. Indust. Med.*, **10**, 164 (1953).⁸ Halberg, F. J., *Perspect. Biol. Med.*, **3**, 491 (1960).⁹ de la Mare, G., and Shummin, S., *Occup. Psychol.*, **38**, 203 (1964).

Effect of Infantile Treadmill Experience on Body-weight and Resistance to Exhaustion in the Rat

AN earlier experiment reported an inverse relationship between the age of rats and their exhaustion time on a slowly and continuously revolving wheel¹. The experiment reported here show the effect of early treadmill experience on later resistance to exhaustion and a hitherto-unnoticed weight-gain effect.

The apparatus used in these experiments was similar to that used previously¹⁻³. The rats were placed in individual 5.5 m. by 9.5 in. cubicles, on wheels two-third submerged in water and constantly rotating at 2 r.p.m. Each animal was in a separate compartment and food was available at all times.

The Ss in these investigations were male Long-Evan rats from the University of Florida colony.

In Exp. 1, eight Ss were placed on the wheel for 2 day at 40 days of age and 4 days at 60 days of age (the split period was inadvertently brought about by a wheel breakdown). A control group matched by litter and mean group body-weight was given no wheel experience. At 120 days of age both groups were placed on the wheel until exhaustion. The criterion for exhaustion was inability of the S to maintain himself on the wheel. The data from this and the second experiment are summarized in Table 1. The mean hours to exhaustion for the experimental and control animals were 82 and 67 respectively. In addition it was noted that at 120 days the experimental group weighed considerably more than the control group.

Table 1 BODY-WEIGHT AND EXHAUSTION TIME AS A PERCENTAGE CHANGE FROM CONTROL GROUP

	Body-weight before infantile experience	Body-weight after infantile experience	Body-weight before final exhaustion test	Exhaustion time
Exp. I	100%	86%	107%	122%
	40 days old	64 days old	120 days old	
Exp. II	101%	69%	114%	143%
	60 days old	68 days old	160 days old	

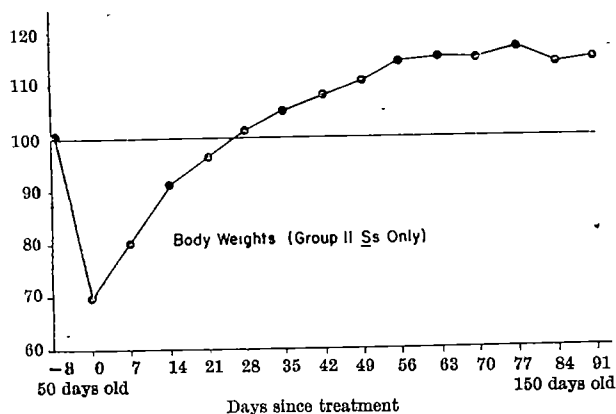


Fig. 1. Mean experimental group weight as a percentage of control group

Encouraged by these results—and intrigued by the gain in weight figures, six *Ss* were placed on the wheel at 50 days of age for 8 days. Six litter- and weight-matched controls were not given wheel experience. Weights were obtained prior to and immediately after the wheel experience and weekly thereafter.

At 160 days of age both groups of *Ss* were placed on the treadmill until exhausted. The mean hours to exhaustion were 70 and 49 for the experimental and control groups respectively. An analysis of variance of the exhaustion times for the combined data from Exps. I and II showed a significant difference between the experimental and control groups ($P < 0.001$); however, no significant experiment effect (Exp. I versus Exp. II) or interaction (between experiment and infantile experience) was found.

Fig. 1 presents the weight of the treated group in Exp. II as a percentage of the control group. It can be seen both in Fig. 1 and Table 1 that the infantile treadmill experience caused the experimental groups to fall behind the control *Ss* in weight and that, following the treatment, the experimental *Ss* caught up with and by-passed the control group.

Two possible interpretations of these results have been eliminated by further experiments. Animals starved to 72 per cent of a matched group weight at 50 days of age (taking about 48 h) caught up with the control group weight by 66 days of age and remained there with no increase above the control group weight (total $N = 14$). When both groups were placed on the wheel at 150 days of age no significant difference in resistance to exhaustion was present. Thus neither the weight gain nor the increased resistance to exhaustion can be attributed to infantile weight loss *per se*.

Another possibility was that the increased resistance to exhaustion of the treated *Ss* was mediated by their heavier weight. When normal *Ss* from another study³ were grouped so as to match same-aged litter-mates and compare the heavier with the lighter *Ss* as to resistance to exhaustion, it was found that at a given age lighter animals walked longer on the wheel ($t = 3.2$, $n = 16$, $P < 0.01$).

It appears that the treadmill acted as a stressor and that this 'acute stress' during infancy was responsible for the increased growth of muscle and other tissue. It is not known whether the improved treadmill performance is indicative of a general increased resistance to stress or is a specific effect of the early treadmill experience perhaps reflecting a 'learning' component.

This work was supported in part by a National Institutes of Health grant (NIH-MH-03881-03) to W. B. Webb and a National Aeronautics and Space Administration predoctoral fellowship (NASA-BSG (T)-13) to R. A. Levitt.

ROBERT A. LEVITT
WILSE B. WEBB

University of Florida, Gainesville.

¹ Webb, W. B., and Agnew, jun., H. W., *Science*, **136**, 1122 (1962).

² Webb, W. B., *J. Exp. Psychol.*, **53**, 162 (1957).

³ Levitt, R. A., and Webb, W. B., *J. Pharmacol. Sci.*, **53**, 1125 (1964).

Form-similarity between Phosphenes of Adults and pre-School Children's Scribbles

We reported earlier^{1,2} a method which permits reproducible electrical stimulation of phosphenes—that is subjective light patterns, visible with closed eyes—without surgery. It was shown that these patterns resembled line drawings of 'geometrical' forms, and that a great number of them could be stimulated within a rather small bandwidth (± 10 per cent of the mean excitation frequency) within the electro-encephalographic frequency range. In the meantime, the dependence of this effect on the shape of the stimulating current³, the identification and lifetime of single phosphenes⁴ and the influence of hallucinogenic drugs on their shape⁵ have also been investigated. In addition, copies of 520 phosphenes observed by 313 subjects have been collected⁶: it was possible to classify these into 15 form groups ('phosphene-Linnaeus', 1-15, first two columns of Table 1).

As reported elsewhere⁷, a particular similarity was found between scribbles of young children and such phosphenes induced electrically in adults. According to about 300,000 drawings and paintings from pre-school children of American, Chinese, French, English and Negro origin, collected by one of us (E. K.) in the Golden Gate Nursery School, San Francisco⁷, it would seem that, at this age,

Table 1. OCCURRENCE OF 15 TYPICAL PHOSPHENE FORM GROUPS (COLUMN A, LIT. 3) IN 329 SCRIBBLES OF ONE SINGLE CHILD (CYNTHIA LIS, GOLDEN GATE NURSERY SCHOOL)

Form groups	A	B	C	D	E	F	G
1. Arcs	95	0	0	0	0	2	3
2. Radials	75	12	3	0	3	3	3
3. Waves	68	28	22	24	19	26	28
4. Lines	59	0	5	7	3	10	10
5. Combined fig.	51	4	6	0	6	0	1
6. Circles	44	24	12	36	12	45	47
7. Multiple fig.	34	16	52	23	19	23	23
8. Odd figures	28	24	1	51	1	13	12
9. Quadrangles	18	6	2	5	2	2	2
10. Spirals	14	3	3	0	3	28	29
11. 'Poles'	13	0	0	0	0	0	0
12. Lattices	8	7	6	6	5	10	10
13. Triangles	6	0	0	0	0	1	1
14. Fingers	5	0	1	1	1	1	0
15. 'Cherries'	2	0	1	1	1	3	3
Sum of numbers of recognizable phosphene patterns (col. A) and of recognizable scribbles (cols. B to G)	520	124	114	154	75	167	172
Sum of scribbling form groups corresponding	15 (Lit. 3)	9 (R.K.)	12 (M.K.)	9 (J.K.)	12 (E.L.)	13 (H.R.)	13 (W.G.)
Percentage of scribbling form groups corresponding	100	60	80	60	80	86	86

Column A: Number of phosphenes belonging to each phosphene form group.

Columns B-G: Numbers of recognizable scribbles (out of 329) similar to each phosphene form group, according to 6 different experimenters.

such 'outlinings in geometrical style' play an important part in the development of a child's expression in drawing or painting.

The scribbling starts early (from an age of about two years); about twenty typical 'scribbles' and six typical 'diagrams' (Greek cross, square, circle, triangle, odd-shaped area and diagonal cross) have been observed (Fig. 1, below) and classified (ref. 7, pp. 131-132). From these basic geometrical forms ('scribbles' and 'diagrams'), the child later develops 'combines' and 'aggregates' (Fig. 1, middle) which each include two or more diagrams and enable him, from about an age of four years onwards, to compose more complicated representations of objects of his environment, such as Sun, people, houses, animals and flowers, from the basic geometrical outlinings (Fig. 1, above). If we compare the basic form groups of the Munich 'phosphene-Linnaeus' (first two columns on the left of Table 1), with those of the San Francisco 'scribbles-Linnaeus' (ref. 7, pp. 131-132) we see that nearly 90 per cent of the phosphene forms can be found in the 'scribbles-Linnaeus', although they were collected independently from each other. We note, for example, that they have the following figures in common: arcs, radials (crosses), waves, lines, combined patterns, circles, dots, odd figures, quadrangles, spirals, poles, triangles and 'cherries'. Lattices and fingers do not appear in the 'scribbles-Linnaeus' (ref. 7), but are scribbled occasionally.

An interesting question arises as to how many different basic form groups one particular child can produce by

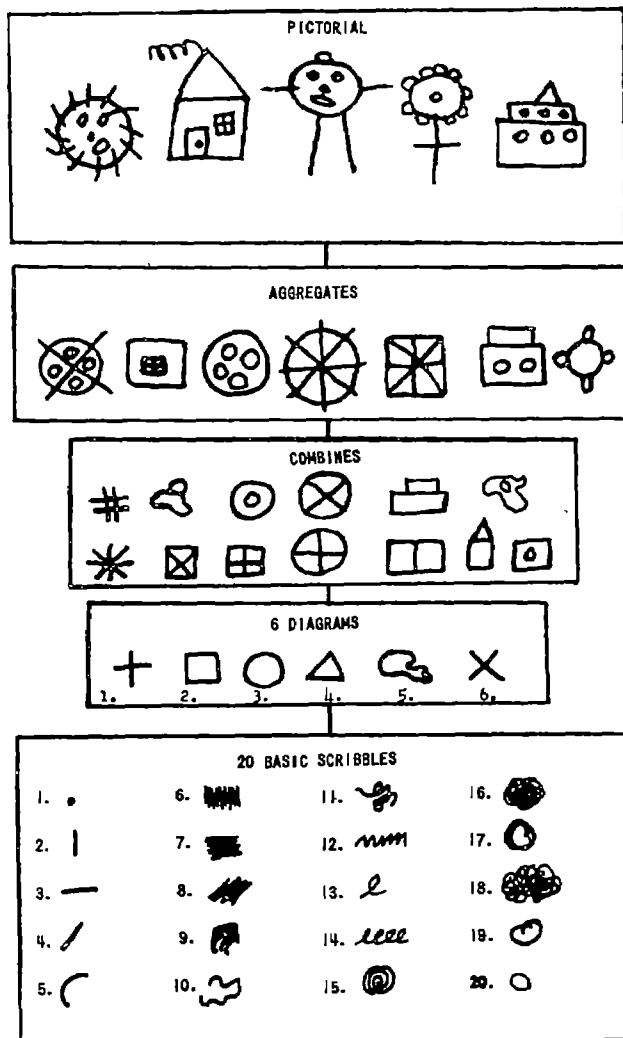


Fig. 1 Development of outlinings of 3-6-year-old children (ref. 7)

scribbling, as compared with the number of basic form groups of phosphenes in adults. Table 1 shows in its columns B-G (besides the classification of 520 electrically induced phosphenes of adults already discussed in A) the number of basic scribbles selected from 329 spontaneous outlinings of one single child (Cynthia Lis, 36-43 months old). The scribbles have been collected and checked by the Golden Gate Nursery School.

The selection of recognizable scribbles and the comparison with the phosphene form groups was made by six different adult experimenters: one of us (R. K.) found 9 such groups (Nos. 2, 3, 5-10, 12) in 124 basic patterns recognizable for her; another (M. K.), 12 groups in 114; the other (J. K.), 9 groups in 154. Our colleagues found E. L., 12 groups in 75; H. R., 13 groups in 167; W. G. 13 groups in 172. Each column B-G shows the number of basic scribbles corresponding to each phosphene form group in column A.

Although we are comparing here the phosphene patterns of 313 adults with the scribbles of only one child, it can be seen that a high proportion (60-86 per cent) of the child's 'eligible' patterns fits into one of the 13 form groups of the 'Phosphene Linnaeus'. To obtain significant statistical results it is, of course, desirable to analyse scribbles of a greater number of children as well as to increase the number of phosphenes in the Linnaeus further. It can be concluded, however, that the forms of phosphenes and children's scribbling have in common quite a number of elementary geometrical forms. (A similar relationship seems to exist between the form of phosphenes and the forms of certain Neolithic rock drawings (see ref. 6). The furnishing of comparative data for both relationships by the Werner Reimers Foundation for Anthropogenetic Research, Frankfurt, is gratefully acknowledged.)

This result may help to clarify the neurophysiological mechanism for the production of both phenomena, about which, so far, very little is known. As the behaviour of young children shows, the ability to scribble 'geometric patterns' does not exist in a child under the age of three. It seems to develop, however, starting from elementary motoric movements, rather rapidly (within months) as result of the very process of scribbling. At the end of this period, however, each child does not scribble an innumerable number of different patterns but only a limited number of 'basic' ones. From this one may conclude that we are dealing here with the activation of pre-formed neuronal networks in the visual system.

Certain networks in the visual system (neuronal oscillators or coincidence filters with small bandwidth within the electro-encephalographic frequency range) have been considered as a model for the phosphene phenomena^{5,8}. In case this or similar approaches confirm the experimental results, one can perhaps hope for a satisfactory general neurological clarification of the mechanism of pattern- or Gestalt-recognition.

For the comparison of phosphene patterns and scribbles we thank Dr. W. Grozinger, Mrs. E. Ledig and Prof. H. Richter.

RHODA KELLOGG

Golden Gate Nursery School, San Francisco, California.

M. KNOLL

Institut für Technische Elektronik,
Technische Hochschule, München.

J. KUGLER

Universitäts-Nervenklinik, München.

¹ Knoll, M., *Schweiz. Z. Psychol.*, 17, 110 (1958).

² Knoll, M., and Kugler, J., *Nature*, 184, 1823 (1959).

³ Höfer, O., *Elektromedizin*, 8, 72 (1963).

⁴ Knoll, M., Höfer, O., Lawder, S. D., and Lawder, U. M., *Elektromedizin*, 235 (1962).

⁵ Knoll, M., Kugler, J., Höfer, O., and Lawder, S. D., *Confinia Neurol.*, 201 (1963).

⁶ Knoll, M., and Knoll-Grelling, U., in *Buddhism and Culture*, edit. Yamaguchi, P., 201 and 213 (Kyoto, 1960).

⁷ Kellogg, R., *What Children Scribble and Why* (San Francisco, 1955).

⁸ Knoll, M., and Kugler, J., I.C.M.C.I.—Lecture, Tokyo, Sept 10 (1964).

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, December 13

OPERATIONAL RESEARCH SOCIETY (at the Royal Aeronautical Society, 4 Hamilton Place, London, W.1), at 4.30 p.m.—Mr. D. W. Trigg: "Logic of Stock Replenishment Subject to Production Constraints".

SOCIETY OF CHEMICAL INDUSTRY, COLLOID AND SURFACE CHEMISTRY GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Mr. S. A. Mitchell: "The Surface Properties of Amorphous Silicas".

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS (at 9 Bedford Square, London, W.C.1), at 6 p.m.—Annual General Meeting.

INSTITUTION OF MECHANICAL ENGINEERS, HYDRAULIC PLANT AND MACHINERY GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Application of Control Theory to Control of Surges".

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, W.C.2), at 6.30 p.m.—Mr. L. Truquer—Presidential Address.

ROYAL SOCIETY OF MEDICINE (at 1 Wimpole Street, London, W.1), at 8 p.m.—Prof. P. E. Ireland (University of Toronto): "Preparation of Man for Travel into Space".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Dr. R. B. Sarjeant: "Background to the Yemen War".

Tuesday, December 14

INSTITUTION OF THE RUBBER INDUSTRY, LONDON SECTION (at the Royal Society of Tropical Medicine and Hygiene, 26 Portland Place, London, W.1), at 2.30 p.m.—Mr. D. A. Mackintosh: "Automation"; 4.15 p.m.—Mr. I. C. Heatham: "Rolling Ball Loss Spectrometer".

ZOOLOGICAL SOCIETY OF LONDON (at the Zoological Gardens, Regent's Park, London, N.W.1), at 5 p.m.—Scientific Papers.

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, Westminster, London, S.W.1), at 5.30 p.m.—Meeting on "Forth Road Bridge".

INSTITUTION OF ELECTRICAL ENGINEERS, CONTROL AND AUTOMATIVE DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. D. J. Ray: "Hot-Strip-Mill Adaptive Computer Control".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. E. H. Cooke-Yarborough: "Recent Developments in Nuclear Instrumentation".

PARLIAMENTARY AND SCIENTIFIC COMMITTEE, GENERAL COMMITTEE (in Committee Room 12, House of Commons, Westminster, London, S.W.1), at 3.30 p.m.—Discussion on "Water Resources" initiated by Mr. N. A. F. Rowntree and Prof. Jansen (University of Delft).

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. J. P. Shillingford: "Cardio-pulmonary Function Following Myocardial Infarction". (Fourteenth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation)*

INSTITUTION OF MECHANICAL ENGINEERS, AUTOMOBILE DIVISION (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Mr. B. J. Albert and Mr. J. C. Walker: "Tyre to Wet Road Friction and High Speeds".

Tuesday, December 14—Wednesday, December 15

INSTITUTE OF METALS (at the Royal Commonwealth Society, Craven Street, London, W.C.2)—Inaugural Meeting of the Joint Committee on Heat Treatment, including discussions on: (a) Scientific Aspects, (b) Heat-Transfer Practice, and (c) Equipment.

Wednesday, December 15

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 2 p.m.—Dr. F. V. Flynn: "Data Processing and Computers in Clinical Pathology".*

ROYAL METEOROLOGICAL SOCIETY (at 49 Cromwell Road, London, S.W.7), at 5 p.m.—Mr. D. R. Grant: "Some Aspects of Convection as Measured from Aircraft"; Mr. S. D. R. Wilson: "Instability of a Geostrophic Wind with a Transverse Wind-speed Gradient"; Mr. G. O'Mahoney: "Rainfall and Moon Phase".

ROYAL STATISTICAL SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.15 p.m.—Mr. B. P. Emmett: "The Design of Investigations into the Effects of Radio and Television Programmes and other Mass Communications".

INSTITUTE OF FUEL (at the Royal Institute of British Architects, 66 Portland Place, London, W.1), at 5.30 p.m.—Dr. P. J. Foster: "Carbon in Flames".

INSTITUTE OF MATHEMATICS AND ITS APPLICATIONS (in the Large Physics Lecture Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. M. F. Atiyah, F.R.S.: "Global Aspects of Elliptic Differential Equations".*

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. K. K. Schwarz: "Further Developments in the Design and Performance of High-Voltage Terminal Boxes".

INSTITUTION OF MECHANICAL ENGINEERS (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Mr. E. J. Richards, O.B.E.: "Considerations of Noise in the Design of Industrial Machinery and Factories".

INSTITUTE OF SCIENCE TECHNOLOGY, LONDON BRANCH (in the Chemistry Department, University College, Gower Street, London, W.C.1), at 6.30 p.m.—Mr. A. G. Jones: "Is It Safe in Your Laboratory?" (followed by a film).

PLASTICS INSTITUTE, LONDON SECTION ENGINEERING SUB-GROUP (at the Coachmakers Arms, 88 Marylebone Lane, London, W.1), at 6.30 p.m.—Mr. P. T. Hughes: "Thin Wall Injection Mouldings—Mould Design and Technique".

Thursday, December 16

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, JOINT I.E.E./I.E.R.E. COMPUTER GROUPS (at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 2.30 p.m.—Colloquium on "Storage—Future Possibilities".

SOCIETY OF CHEMICAL INDUSTRY, ROAD AND BUILDING MATERIALS GROUP (at 14 Belgrave Square, London, S.W.1), at 2.30 p.m.—Meeting on "New and Revised Test Methods".

INSTITUTION OF MINING AND METALLURGY (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Mr. H. Squair: "A Reflectometric Method of Determining the Silver Content of Natural Gold Alloys"; Dr. E. F. Stumpff and Mr. A. M. Clark: "Electron-Probe Micro-analysis of Gold-Platinoide Concentrates from South-east Borneo"; Mr. A. A. North and Mr. R. A. Wells: "Solvent Extraction of Uranium from Slurries by Means of a Rotary-Film Contactor".

LINNEAN SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Meeting on "Factors Influencing the Distribution of Amphioxus". Prof. J. E. Webb: "The Influence of Ocean Currents" and "The Nature of Deposits Colonized by Amphioxus"; Dr. W. A. M. Courtney: "Temperature as a Limiting Factor".

LONDON MATHEMATICAL SOCIETY (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. J. A. Todd, F.R.S.: "A Representation of the Mathieu Group M_{21} as a Collineation Group".

INSTITUTE OF PETROLEUM, EXPLORATION AND PRODUCTION GROUP (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. L. S. Ramsay: "Geophysical Work in the North Sea".

INSTITUTION OF ELECTRICAL ENGINEERS, CONTROL AND AUTOMATION DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Recording Instruments" opened by Mr. F. R. Axworthy, Mr. R. L. Hales and Mr. T. Whiteley.

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. R. A. Gregory: "Gastrin". (Last of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation)*

CHEMICAL SOCIETY AND THE SOCIETY FOR ANALYTICAL CHEMISTRY (in the Lecture Theatre, School of Pharmacy, Brunswick Square, London, W.C.1), at 6 p.m.—Prof. W. Kemula (University of Warsaw): "The Partition of Mixtures of Homologues and Isomers by Chromato-Polarography".

INSTITUTION OF MECHANICAL ENGINEERS, APPLIED MECHANICS GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "The Influence of Hold Time at Elevated Temperatures on High Strain Fatigue".

Friday, December 17

INSTITUTE OF NAVIGATION (at the Royal Institution of Naval Architects, 10 Upper Belgrave Street, London, S.W.1), at 5.30 p.m.—Mr. R. Bennett: "Recent Navigational Aids to Fishing".

Monday, December 20

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Mr. D. C. Drummond: "Recent Developments in the Control of Commensal Rodents".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

EXPERIMENTAL OFFICERS (2) with a degree or H.N.D. or H.N.C. in physics or electrical engineering, and preferably experience in a research laboratory or in development laboratory) in the DEPARTMENT OF ELECTRICAL AND CONTROL ENGINEERING, to undertake the design and construction of electrical apparatus and to assist in research—Head of the Department of Electrical and Control Engineering, Battersea College of Technology, Battersea Park Road, London, S.W.11 (December 17).

LECTURER (preferably with experience in electrochemistry) in PHYSICAL CHEMISTRY (Post F/70S); and a LECTURER (preferably with experience in physiology or invertebrate ecology) in BIOLOGY (Post F/71S) in the DEPARTMENT OF CHEMISTRY AND BIOLOGY, Liverpool Regional College of Technology—The Director of Education, 13 Sir Thomas Street, Liverpool, 1 (December 20).

KEEPER (with an appropriate degree and/or the Museums Association Diploma and museum or field experience) of ARCHAEOLOGY—The Director, City of Liverpool Museums, William Brown Street, Liverpool, 3 (December 22).

ASSISTANT LECTURER (with a good honours degree in botany and/or zoology, and a special interest in cell biology and microbiology) in BIOLOGY—The Secretary, University of Exeter, Northcote House, The Queen's Drive, Exeter, Devonshire (December 31).

FACULTY LECTURER IN THEORETICAL PHYSICS—The Secretary of Faculties, University Registry, Oxford (December 31).

LECTURER or ASSISTANT LECTURER in MATHEMATICS (applications will be considered in either pure mathematics or mathematical physics and it is hoped to make appointments in both fields)—The Secretary to the Council, Westfield College (University of London), Hampstead, London, N.W.3 (December 31).

LECTURER or ASSISTANT LECTURER in SOCIOLOGY in the DEPARTMENT OF SOCIAL STUDIES—The Registrar, The University, Leeds, 2 (December 31).

READER, SENIOR LECTURER and a LECTURER in the SCHOOL OF PHYSICS—The Registrar, University of Warwick, Coventry (December 31).

LECTURER or ASSISTANT LECTURER (graduate in medicine, veterinary science, dentistry, or biology) in the DEPARTMENT OF HISTOLOGY—The Registrar, University of Liverpool, Liverpool, quoting Ref. CV/345/N (January 3).

LECTURER/SENIOR LECTURER (with experience in the application of statistics to biological science) in STATISTICS/BIOMETRY at the University of New England, Armidale, New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 7).

LECTURER (preferably with interests in one or more of the following: applied geography, social geography, the countries of the European

Economic Community) IN GEOGRAPHY—The Registrar, The University, Newcastle upon Tyne, 2 (January 8).

LECTURERS (2) IN CHEMISTRY (Organic, Physical or Inorganic) at the University College, Dar es Salaam (The University of East Africa)—The Inter-University Council, 33 Bedford Place, London, W.C.1 (January 8).

ASSOCIATE PROFESSOR (with high academic qualifications and practical training and experience in medical microbiology as well as experience in teaching and research) IN THE DEPARTMENT OF MEDICAL MICROBIOLOGY, University of New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 10).

LECTURER (Grade II) IN METALLURGY WITHIN THE DEPARTMENT OF MECHANICAL ENGINEERING—The Registrar and Secretary, University of Bristol, Bristol (January 12).

ASSISTANT LECTURERS OF LECTURERS (3) IN THE DEPARTMENT OF PHYSICS—The Registrar, University of Essex, Wivenhoe Park, Colchester, Essex (January 15).

LECTURER OR ASSISTANT LECTURER IN BOTANY at the University of Singapore—The Inter-University Council, 33 Bedford Place, London, W.C.1 (January 15).

LECTURER OR SENIOR LECTURER (suitably qualified person with special interests in organic chemistry) IN CHEMISTRY at the University of Canterbury, Christchurch, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, January 15).

LECTURER (with a good honours degree in geography and if possible post-graduate research experience and specialization in surveying, cartography and mathematical geography, and preferably a knowledge of statistics) IN GEOGRAPHY at the University of Hong Kong—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Hong Kong and London, January 15).

CHAIR OF GEOGRAPHY in the School of Earth Sciences, Macquarie University—The Registrar, Macquarie University, P.O. Box 458, North Sydney, Australia, or The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (January 17).

SENIOR LECTURER, LECTURER, ASSISTANT LECTURER and a RESEARCH ASSISTANT IN THE DEPARTMENT OF PROBABILITY AND STATISTICS (applicants may have research interests in any branch of probability and statistics)—The Registrar, The University, Sheffield (January 17).

LECTURER (specialized in theoretical and practical aspects of physical methods of analysis, particularly in relation to drugs) IN PHARMACEUTICAL CHEMISTRY IN THE DEPARTMENT OF PHARMACY, University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 31).

LECTURERS (2) IN THE DEPARTMENT OF BACTERIOLOGY AND IMMUNOLOGY—Prof. D. G. Evans, F.R.S., London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C.1 (January 31).

ASSISTANT (graduate or non-graduate) FOR A MEDICAL PROFESSIONAL UNIT for biochemical research involving automated amino acid analysis—The Clerk to the Governors, St. Bartholomew's Hospital, London, E.C.1, quoting Project No. 611, Ref. No. ASC/522.

ASSISTANT LECTURER IN ANALYTICAL CHEMISTRY IN THE DEPARTMENT OF PURE AND APPLIED CHEMISTRY—The Registrar, University of Strathclyde, George Street, Glasgow, C.1.

ASSISTANT OR ASSOCIATE PROFESSORS IN THE DEPARTMENT OF PHYSICS (special consideration for one position will be given to candidates with research interests in theoretical physics)—Department of Physics, University of Victoria, Victoria, B.C., Canada.

ASSISTANT PROFESSOR (national of the United Kingdom or the Republic of Ireland, with a good honours degree and relevant experience in teaching first degree students, and preferably with a higher degree) OF PHYSICS at the Middle East Technical University, Ankara—The Appointments Office, Ministry of Overseas Development, Room 404, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 203/181/011.

EXPERIMENTAL OFFICER (with a degree in some branch of biology) IN THE DEPARTMENT OF PATHOLOGY to work as a member of a team investigating the behaviour of children's tumours in tissue culture—Dr. J. K. Steward, Tissue Culture Laboratory, Department of Pathology, University of Manchester, Manchester, 13.

EXPERIMENTAL PHYSICIST (Low Energy Nuclear Physics) (preferably with experience in the use and operation of nuclear accelerators)—Prof. L. Krause, Head, Department of Physics, University of Windsor, Windsor, Ontario, Canada.

GRADUATE (preferably with previous experience in cytogenetics) to work in the CHROMOSOME LABORATORY OF THE DEPARTMENT OF CLINICAL RESEARCH at the Royal Marsden Hospital, Fulham Road, S.W.3—The Secretary, Institute of Cancer Research, 34 Sumner Place, London, S.W.7, quoting Ref. 300/G/24.

POSTDOCTORAL BIOCHEMIST to work on the metabolism of the lens in relation to eye disease—Dr. R. van Heyningen, Nuffield Laboratory of Ophthalmology, University of Oxford, Walton Street, Oxford.

PROFESSOR (preferably candidate in the field of solid state, structure of liquids, spectroscopy or thermodynamics) IN PHYSICAL CHEMISTRY—Prof. Harry E. Gunning, Head, Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada.

RESEARCH ASSISTANT (graduate in psychology) on a three-year project concerned with the psycho-physiological investigation of chronic schizophrenia—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1.

SENIOR LABORATORY TECHNICIANS AND LABORATORY TECHNICIANS IN THE DEPARTMENT OF CHEMISTRY (for some of the posts technical experience in radiochemistry, spectroscopy, mass spectrometry and gas chromatography is required)—Head of the Department of Chemistry, Northern Polytechnic, Holloway, London, N.7.

SENIOR LECTURER (honours graduate) IN ZOOLOGY; and a LECTURER (honours graduate) IN ZOOLOGY—The Secretary, Sir John Cass College, Jewry Street, London, E.C.3.

TUTOR OR LECTURER (specialist in any branch of physics) IN PHYSICS—The Principal, Somerville College, Oxford.

Kingscourt, Ireland. By John S. Jackson. Pp. 131–152 + plate 11. 7s. 6d. Series A, Vol. 2, No. 9. Lower Carboniferous Volcanic Rocks Near Tulla, Co. Clare, Ireland. By R. W. Schultz and G. D. Sevastopulo. Pp. 153–162. plates 12 and 13. 5s. Series B, Vol. 1, No. 15. Varietal and Clonal Differences in White Clover. By P. L. Curran and M. L. Reilly. Pp. 163–172. 4s. Serie B, Vol. 1, No. 16. Molassed Beet Pulp in the Rations of Pigs. By F. X. Ahern and Dr D. M. McAleese. Pp. 173–183. 4s. (Dublin: Royal Dublin Society, 1965.) [31]

The Night Sky: The Stars Month by Month, 1966. Pp. 29. (London: The Times Publishing Company, Ltd., 1965.) 5s. [32]

University of Oxford. Annual Reports 1963–1964. Pp. 14. (Supplement No 10 to the *University Gazette*, August 1965.) (Oxford: The University, 1965.) 2s. 6d. [31]

The Countryside in 1970, Second Conference, London, 10–12 November 1965—a Review of the Preparatory Studies. Pp. 48. (London: The Royal Society of Arts, and The Nature Conservancy, 1965.) [31]

The Association of Commonwealth Universities. List of Universit Institutions in the Commonwealth. Ninth edition. Pp. 12. (London: The Association of Commonwealth Universities, 1965.) Gratis. [31]

A Critical Review of Assessment Procedures in Secondary School Science (with special reference to C.S.E.). By J. F. Eggleston. (Research Unit for Assessment and Curriculum Studies.) Pp. 56. (Leicester: The Secretary (Research Unit), School of Education, Leicester University, 1965.) 5s. [31]

Imperial College of Science and Technology (Royal College of Science Royal School of Mines; City and Guilds College). Prospectus 1966–67. Pp xviii + 52 + 7 photographs. Calendar 1965–66. Pp. xi + 519. (London Imperial College of Science and Technology, 1965.) [31]

Other Countries

Museum of Comparative Zoology, Harvard University. Breviora No 224 (July 15, 1965). New Species of Land Molluscs, with Notes on Other Species from the Solomon Islands. By William J. Clench. Pp. 8 (2 plates). No. 225 (July 15, 1965). The Asian Species of *Galeritula* Strand (Coleoptera Carabidae). By Hans Reichardt. Pp. 16. No. 226 (July 15, 1965). The Larval Form of the Heteromil (Pisces). By Giles W. Mead. Pp. 5. No. 227 (September 10, 1965). The Species of Hispaniolan Green Anoles (*Sauria Iguanidae*). By Ernest E. Williams. Pp. 16. No. 228 (September 15, 1965). Relationships Among Indo-Australian Zosteropidae (Aves). By Ernst Mayr. Pp. 6. No. 229 (September 15, 1965). The Genus *Darlingtonia* (Serpentes) in Hispaniola, including a New Subspecies from the Dominican Republic. By Albert Schwartz and Richard Thomas. Pp. 10. No. 230 (September 15, 1965). Notes on Some Non-Passerine Birds from Eastern Ecuador. By David W. Norton. Pp. 11. (Cambridge, Mass.: Museum of Comparative Zoology, Harvard University, 1965.) [41]

Bulletin of the Museum of Comparative Zoology, Harvard University Vol. 133, No. 4. The Genera of the Chilocorini (Coleoptera, Coccinellidae). By Edward A. Chapin. Pp. 227–271. (Cambridge, Mass. Museum of Comparative Zoology, Harvard University, 1965.) [41]

Desert Locust Control Organization for Eastern Africa. Second Annual Report of the Director, 1st July 1963 to 30th June 1964. Pp. ii + 31. (Nairobi Government Printer, 1965.) 2s. [41]

United States Department of Interior: Fish and Wildlife Service. Bureau of Sport Fisheries and Wildlife. Research Report No. 68. Bibliography or Reservoir Fishery Biology in North America. By Robert M. Jenkins. Pp. iv + 57. 40 cents. Statistical Digest No. 55. Propagation and Distribution of Food Fishes for the Calendar Years 1961 and 1962. Prepared by the Division of Fish Hatcheries. Pp. ii + 49. 25 cents. (Washington, D.C.: Government Printing Office, 1965.) [41]

World Health Organization. Public Health Papers, No. 29. Mass Campaign and General Health Services. By C. L. Gonzalez. Pp. 87. 4 Sw. francs 6s. 8d.; 1.25 dollars. Technical Report Series No. 310. Diabetes Mellitus—Report of a WHO Expert Committee. Pp. 44. 3 Sw. francs, 5s.; 1 dollar. No. 311: Special Courses for National Staff with Higher Administrative Responsibilities in the Health Services—Report of a WHO Study Group. Pp. 31. 2 Sw. francs; 3s. 6d.; 0.60 dollars. No. 312: WHO Expert Committee on Dependence-Producing Drugs—Fourteenth Report. Pp. 16. 2 Sw. francs; 3s. 6d.; 0.60 dollars. No. 314: Nutrition and Infection—Report of a WHO Expert Committee. Pp. 30. 2 Sw. francs; 3s. 6d.; 0.60 dollars. (Geneva: World Health Organization; London: H.M. Stationery Office, 1965.) [41]

F. and M. Scientific Corporation. Gas Chromatography Methods for Biochemistry and Medicine: a Rapid Method for Serum Cholesterol Analysis by Gas Chromatography. Pp. 8. (Avondale, Pennsylvania: F. and M. Scientific Corporation; London: F. and M. Scientific Europa, N.V., 1965.) [41]

Metropolitan Life Insurance Company. Statistical Bulletin, Vol. 40 (August, 1965): Recent Mortality Trends in the Western World. Accidental Falls: Fatal and Nonfatal. Reported Frequency of Chronic Respiratory Diseases as Causes of Death—Age Variations. Incidence of Disability in the First Half of 1965. Pp. 12. (New York: Metropolitan Life Insurance Company, 1965.) [41]

New York State Museum and Science Service. Bulletin No. 397: Pollen Studies in the Cruseo Lake Area of Prehistoric Indian Occupation. By Donald D. Cox and Donald M. Lewis. Pp. iii + 29. (Albany, New York: The University of the State of New York—The State Education Department, 1965.) 50 cents. [41]

United Nations: Food and Agriculture Organization of the United Nations World Food Program Studies. No. 2. The Impact of Food Aid on Donor and Other Food-Exporting Countries. By G. R. Allen in association with R. G. Smethurst. Pp. vii + 52. 5s.; 1 dollar. No. 4: Operational and Administrative Problems of Food Aid. By D. A. Fitzgerald. Pp. vii + 63. 5s.; 1 dollar. No. 6. Food Aid and Education. Pp. v + 22. 5s.; 1 dollar. (Rome: Food and Agriculture Organization of the United Nations; London: H.M. Stationery Office, 1965.) [41]

Editorial and Publishing Offices of "NATURE"

MACMILLAN (JOURNALS) LIMITED

4 LITTLE ESSEX STREET, LONDON, W.C.2

Telephone Number: Temple Bar 6633. Telegrams: Phusis London W C 2

Annual subscription: Inland £14, Overseas £13 15s., payable in advance, postage paid to any part of the world.

Advertisements only should be addressed to

T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2.

Telephone Number: Holborn 4743

Registered as a newspaper at the General Post Office

Copyright © Macmillan (Journals) Limited, December 11, 1965

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

The Scientific Proceedings of the Royal Dublin Society. Series A, Vol. 2, No. 6. The Carboniferous Stratigraphy of the Area North-West of Newmarket, Co. Cork, Ireland. By W. H. Morton. Pp. 47–65 + plate 4. 4s. 6d. Series A, Vol. 2, No. 8. The Upper Carboniferous (Namurian and Westphalian) of

NATURE

No. 5016

SATURDAY, DECEMBER 18, 1965

Vol. 208

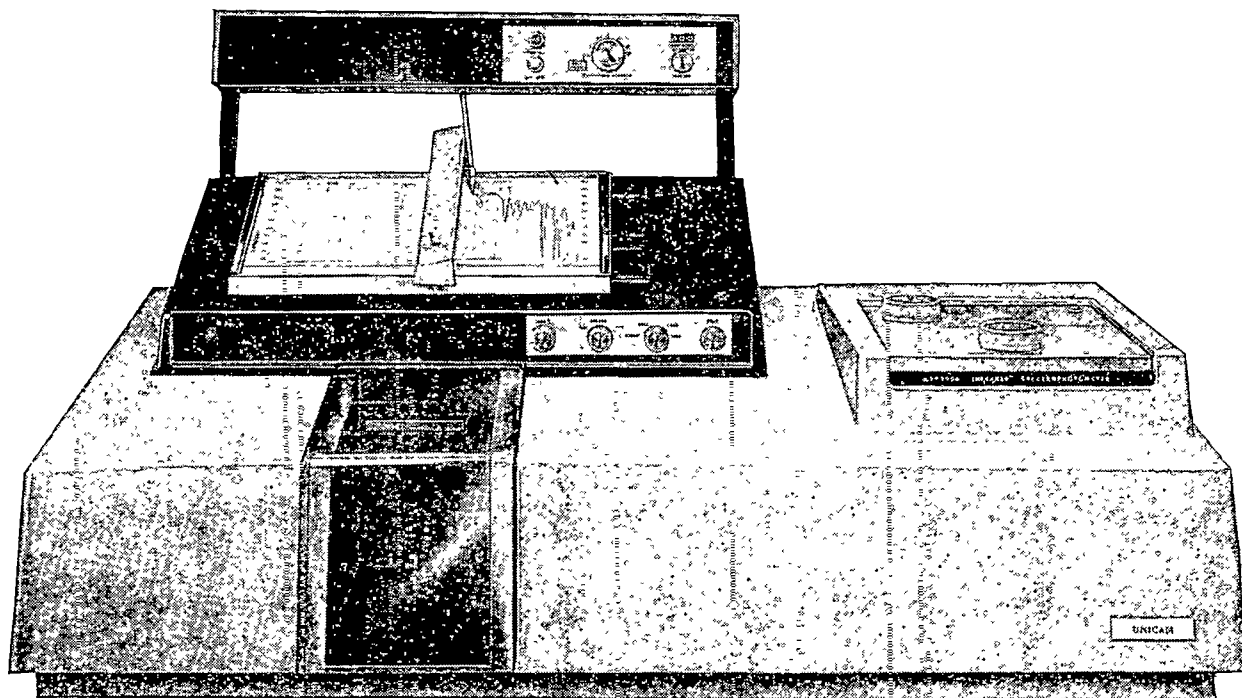
Published by
MACMILLAN (JOURNALS) LTD.

CONTENTS

	<i>Page</i>
ORGANIZATION OF THE SCIENTIFIC CIVIL SERVICE	1133
THE FOUNDER OF ABDOMINAL SURGERY. By Sir Arthur MacNalty, E.C.B.	1135
PHYSICS OF THE EAR. By Prof. I. G. Taylor	1136
MAMMALIAN POPULATIONS. By H. N. Southern	1136
PLATES AND SHELLS. By Prof. B. N. Cole	1137
THE DUTCH STUDY CIRCLE FOR AGRICULTURAL HISTORY. By G. E. Fussell	1138
NOMAD PEOPLES OF THE STEPPES. By M. C. Burkitt	1138
INFORMATION SERVICES IN BRITAIN. By R. Brightman	1139
IRISH BIBLIOGRAPHY. By H. J. Heaney	1139
THE ROYAL SOCIETY. By Lord Florey, O M., P.R.S.	1141
CONSERVATION AND MAN'S ENVIRONMENT. By Dr. Ian McTaggart Cowan	1145
OBITUARY :	
ACADEMICIAN EUGENE N. PAVLOVSKY. By Dr. Cecil A. Hoare, F.R.S.	1151
NEWS AND VIEWS	1152
VISUAL OBSERVATION OF SATELLITES. By D. G. King-Hele	1156
SCIENTIFIC AND INDUSTRIAL RESEARCH IN NEW ZEALAND	1156
FOREST HYDROLOGY. By John C. Rodda	1157
RECONSTRUCTION OF PHASE OBJECTS BY HOLOGRAPHY. By Prof. D. Gabor, F.R.S., Prof. G. W. Stroke, D. Brumm, A. Funkhouser and A. Labeyrie	1159
MODERN LOW-TEMPERATURE CALORIMETRY. By Dr. I. M. Firth	1162
PROGRESS IN THE PLANKTONIC FORAMINIFERAL BIOSTRATIGRAPHY OF THE NEOGENE. By Dr. F. T. Banner and Dr. W. H. Blow	1164
2-TRIFLUOROMETHYLBENZIMIDAZOLES : A NEW CLASS OF HERBICIDAL COMPOUNDS. By Dr. D. E. Burton, Dr. A. J. Lambie, J. C. L. Ludgate, Dr. G. T. Newbold, Dr. A. Percival and D. T. Sagers	1166
ACTIVITY OF 2-TRIFLUOROMETHYLBENZIMIDAZOLES AS UNCOUPLERS OF OXIDATIVE PHOSPHORYLATION. By Dr. O. T. G. Jones and W. A. Watson	1169
REPLICATION AND THE ACTIVATION OF MUSCLE DIFFERENTIATION. By Dr. Paul Pietsch and Susan B. McCollister	1170
NOREPINEPHRINE AND SEROTONIN IN THE TISSUES AND VENOMS OF TWO PIT VIPERS. By Prof. Aaron H. Anton and Prof. Joseph F. Gennaro, jun.	1174
FRACTIONATION OF MOUSE DEOXYRIBONUCLEIC ACID ON HYDROXY-PATITE. By Dr. P. M. B. Walker and Dr. Anne McLaren	1175
STIMULATION OF SYNTHESIS OF RIBONUCLEIC ACID IN SUB-APICAL SECTIONS OF <i>Avena</i> COLEOPTILE BY INDOLYL-3-ACETIC ACID. By Prof. T. H. Hamilton, R. J. Moore, A. F. Rumsey, A. R. Means and Prof. A. R. Schrank	1180
PRINCIPLES OF CELL MOTILITY : THE DIRECTION OF CELL MOVEMENT AND CANCER INVASION. By Dr. S. B. Carter	1183
A SEX-DEPENDENT FACTOR IN APLASTIC ANAEMIA ? By Dr. D. E. Peeg and J. E. Burns	1187

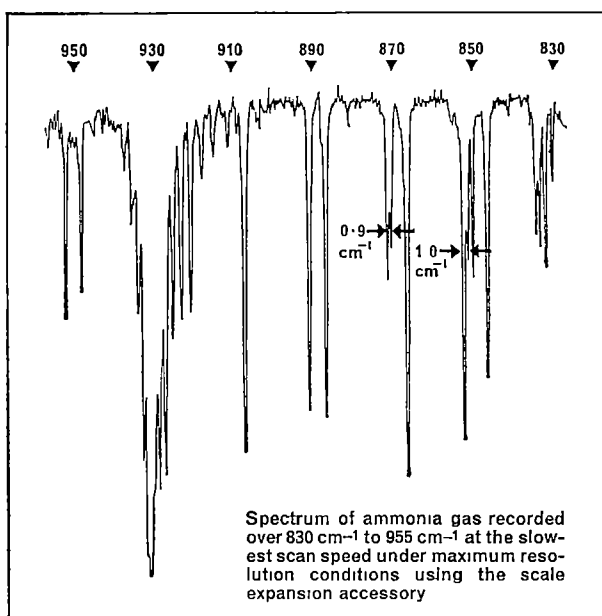
(Continued overleaf)

NEW **grating** SP200G infrared spectrophotometer



high resolution

Unicam now offers all the advantages of a grating filter monochromator combined with the proved features of the SP200 prism spectrophotometer. The first order of the NPL grating is isolated by four interference filters, brought into play by an automatic mechanism exclusive to Unicam. The resolution is as high as 3 cm^{-1} at 3000 cm^{-1} and stray light is less than 1% throughout most of the range and never exceeds 2%. The wavenumber accuracy is better than 2 cm^{-1} at 1000 cm^{-1} and 4 cm^{-1} at 3000 cm^{-1} . To this performance are added the advantages of the flat bed recorder, the accessible sample compartment and the convenient operation of the SP200. The new SP200G is constructed and finished to the high degree of precision for which Unicam instruments are famous. Descriptive literature is available on request.



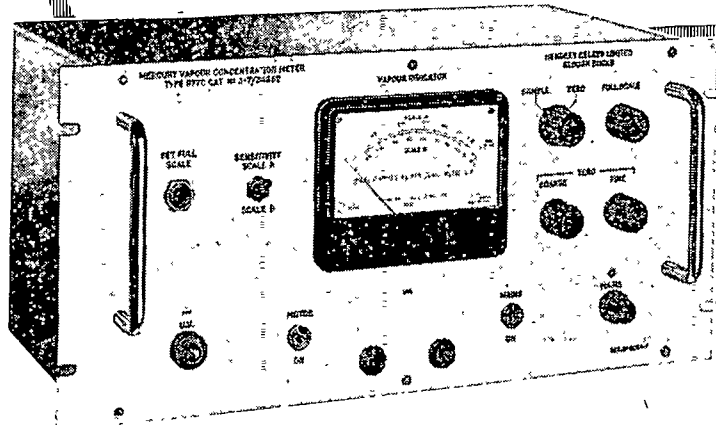
Precision Spectrophotometers

UNICAM

LETTERS TO THE EDITOR

	Page		Page
RADIO ASTRONOMY		PHYSIOLOGY	
Radio Structure of the Galactic Centre Region.—Dr. D. Downes, Prof. A. Maxwell and Prof. M. L. Meeks . . .	1189	Further Purification and Characterization of a Placental Protein with Immunological Similarity to Human Growth Hormone.—Dr. Henry Friesen . . .	1214
ASTROPHYSICS		Influence of the Cerebral Cortex on the Cuneate Nucleus of the Monkey.—Dr. F. Harris, Dr. S. J. Jabbur, Dr. R. W. Morse and Dr. A. L. Towse . . .	1215
Common Features of the Optical Continua associated with Violent Cosmic Events.—Prof. L. Searle, Dr. A. W. Rodgers, W. L. W. Sargent and J. B. Oke . . .	1190	Differences in Variability of Discharge Frequency between Primary and Secondary Muscle Spindle Afferent Endings of the Cat.—R. B. Steir and Dr. P. B. C. Matthews . . .	1217
Growth of Interstellar Grains.—Dr. M. J. Baines and I. P. Williams . . .	1191	Squid Giant Axon: Repetitive Responses to Alternating Current Stimulation.—Prof. H. R. Hirsch . . .	1218
RADIOPHYSICS		PHARMACOLOGY	
Anomalous Intensity Ratios of the Interstellar Lines of OH in Absorption and Emission.—R. X. McGee, Dr. B. J. Robinson, F. F. Gardner and J. G. Bolton . . .	1193	Teratogenic Effects of Synthetic Compounds related to Trypan Blue: the Effect of 1,7-Diamino-8-naphthol-3,6-disulphonic Acid on Pregnancy in the Rat.—Dr. George A. Christie . . .	1219
Formation of Hydroxyl Molecules in Interstellar Space.—Dr. J. L. Symonds . . .	1195	Biphasic Dose-response Relationship with Acetylcholine on the Heart of the Mollusc <i>Tapes turgida</i> .—P. R. Carroll and G. B. Chesher . . .	1220
PHYSICS		HAEMATOLOGY	
Laminar and Turbulent Flow in Open Channels.—Dr. John Allen . . .	1196	Synthesis of the α_1 -Glycoprotein (Darcy) of Rat Serum by the Liver.—Prof. H. E. Weimer, D. C. Benjamin and D. A. Darcy . . .	1221
ENGINEERING		A New <i>Bisalbuminaemia</i> Family.—Dr. Georges Sandor, Dr. Louis Martin, Dr. Michel Porsin, Dr. Alain Rousseau and Dr. René Martin . . .	1222
Detonability of Nitroglycerin contained in Porous Rock.—J. Edmund Hay and Fred H. Scott . . .	1197	IMMUNOLOGY	
CRYSTALLOGRAPHY		Bone Marrow as the Major Source of Potential Immunologically Competent Cells in the Adult Mouse.—Dr. M. L. Tyan and Dr. L. J. Cole . . .	1223
Structure of Calcium Oxalate Monohydrate.—Prof. H. J. Arnott, Dr. F. G. E. Pautard and Prof. H. Steinfink . . .	1197	Immunogenic Activity of Lipids of <i>M. tuberculosis</i> .—Dr. B. Stöss and Dr. R. Herrmann . . .	1224
CHEMISTRY		PATHOLOGY	
Thermoluminescence and Ultra-violet Phosphorescence of the Nucleic Acid Bases.—Dr. R. J. Fleming and R. M. Kerr . . .	1198	Cutaneous Elastin in Ehlers-Danlos Syndrome.—Dr. David P. Varadi and Dr. David A. Hall . . .	1224
Dissociation Energy of the N—H Bond in Hydrazine.—Dr. I. P. Fisher and Dr. G. A. Heath . . .	1199	Prevention of Cholesterol-induced Cholelithiasis by Neomycin.—Dr. E. H. Mosbach, V. Bokkenheuser, D. P. Chattopadhyay, M. Schmidt, R. L. Hirsch and Dr. A. F. Hofmann . . .	1226
Effect of pH and Addition of Organic Materials on Denitrification Losses from Soil.—D. M. Ekpette and Dr. A. H. Cornfield . . .	1200	EADIOBIOLOGY	
Absence of Diffusion Control of Reaction Rate in the Thermal Decomposition of Amorphous Polymers.—Derek A. Smith . . .	1200	<i>In vitro</i> Effect of Gamma-radiation on Different Cholinesterase Preparations.—Dr. R. E. Catalan and Dr. A. M. Municio . . .	1227
Kinetics of Formation of the Monoacetylacetonato-Iron (III) Complex.—W. K. Ong and Dr. R. H. Prince . . .	1201	BIOLOGY	
Heterogeneity of Titan Yellow demonstrated by Thin-layer Chromatography.—R. J. Hall and L. R. Flynn . . .	1202	Evolutionary Tendency of Relative Growth in Arthropods and Man.—Dr. Ryuichi Matsuda . . .	1228
Charge Transfer Properties of <i>dl</i> -Thioctic Acid and Other Disulphides.—Wayne M. Moreau and Prof. Karl Weiss . . .	1203	Phytotoxin Production by <i>Verticillium albo-atrum</i> Reinke et Berthold.—Dr. G. F. Pegg . . .	1228
Homogeneous Hydrogenation and Hydroformylation using Ruthenium Complexes.—D. Evans, J. A. Osborn, F. H. Jardine and Prof. G. Wilkinson, F.R.S. . . .	1203	Establishment of a Pure Infection of the Nematode <i>Ostertagia circumcincta</i> in the Sheep.—J. W. Copland . . .	1229
BIOCHEMISTRY		Gametogenesis of <i>Plasmodium vinckei</i> .—Dr. J. Bafort, I. H. Vincke and G. Timperman . . .	1230
Lysozymes characterized in Duck Egg-white: Isolation of a Histidine-less Lysozyme.—Dr. J. Jollès, G. Spotorno and P. Jollès . . .	1204	Phosphorus-deficiency Symptoms in Tobacco and Transpirational Water Loss.—Prof. A. Wallace and E. Frolich . . .	1231
Evidence discriminating between the Chemical and the Chemiosmotic Mechanisms of Electron Transport Phosphorylation.—Dr. Peter Mitchell and Dr. Jennifer Moyle . . .	1205	Undernutrition and Early Embryonic Mortality in Merino Ewes.—T. N. Edey . . .	1232
Enzymatic Formation of β -Cyanoalanine from Cyanide by <i>Escherichia coli</i> Extracts.—Dr. Patricia M. Dunnill and Prof. L. Fowden, F.R.S. . . .	1206	Human Lymphocytes cultured in Microplates.—Dr. Jacob A. Brody and Bob Huntley . . .	1232
Enzymatic Formation of β -Cyanoalanine from Cyanide.—Dr. H. G. Floss, Dr. L. Hadwiger and Prof. E. E. Conn . . .	1207	A Well-preserved Grizzly Bear Skull recovered from a Late Glacial Deposit near Lake Simcoe, Ontario.—R. L. Peterson . . .	1233
Thalidomide as a Possible Biological Acylating Agent.—S. Fabro, R. L. Smith and Prof. R. T. Williams . . .	1208	Direct Action of Thyroxine on the Trout Pituitary <i>in vitro</i> .—Dr. Bridget I. Baker . . .	1234
'Burned' Tyrosyl Residues and the Activity of Trypsin.—James F. Riordan, Dr. Warren E. C. Wacker and Prof. Bert L. Vallee . . .	1209	MICROBIOLOGY	
Comparison of Salivary Amylase-levels in the Human Male and Female.—Prof. Robert L. McGeachin, Patricia A. Hay and Patricia A. Prell . . .	1211	Differences in the Antigenic Pattern of <i>Azotobacter</i> grown on Different Nitrogen Sources.—Prof. Tord Holme and Bengt Zacharias . . .	1235
Acid Mucopolysaccharide and Hexosamine Metabolism in Dermal Connective Tissue of Adrenalectomized Rats.—Prof. John Fabianek, Dr. Anthony Herp and Dr. Leonor Martinez . . .	1212	Human Indigenous Microflora in Gnotobiotic Rats.—Dr. H. A. Dymaza, G. S. Stoewsand, Dr. J. J. Enright, Prof. P. C. Trexler and L. C. Gall . . .	1236
Occurrence of Odd-numbered Fatty Acids in the Mullet <i>Mugil cephalus</i> .—Dr. R. G. Ackman . . .	1213	PSYCHOLOGY	
		Neural Volleying: Upper Frequency Limits detectable in the Auditory System.—Capt. James C. Boudreau . . .	1237

Essential *for* sampling and monitoring...



HENDREY

Mercury Vapour Concentration Meter

Type E 3472

Measures concentration of mercury vapour present in air. Essential where quantities of mercury are regularly used or where danger exists of leakage of mercury vapour from apparatus.

Two sensitivities are available at the touch of a switch, giving either full-scale meter reading at a concentration of 770 microgrammes per cubic metre of air or full-scale meter reading at a concentration of 200 microgrammes. Terminations provided at rear for operation of a recorder to give continuous readings of concentration present.

Hendrey Relays

HENDREY RELAYS LTD · 390-394 BATH ROAD · SLOUGH · BUCKS. Telephone : Burnham 609/612
MANUFACTURING ELECTRICAL ENGINEERS. CONTROL & LABORATORY APPARATUS. Telegrams : Hendrelay, Slough
On Admiralty, Principal Ministries and Post Office Lists. A.R.B. and M.O.A. Approved Design and Inspection

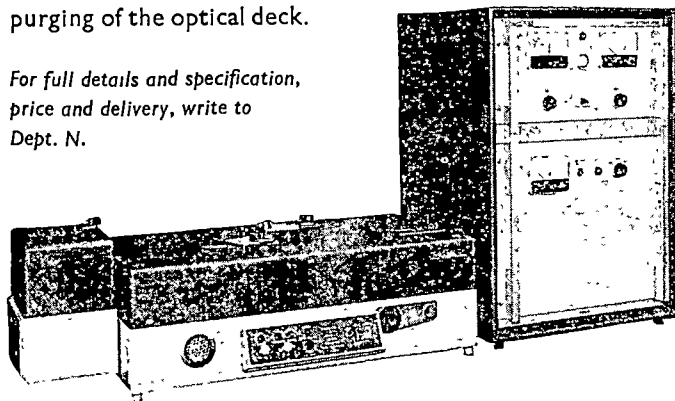


PRECISION INSTRUMENTS

NEW FEATURES—

This equipment now incorporates automatic Verdet Dispersion Correction and range change switching, water jacketed sample chamber and nitrogen purging of the optical deck.

For full details and specification,
price and delivery, write to
Dept. N.



Manufactured under licence. Patent No.918078

The electronic unit for this instrument is manufactured by Messrs. Bandix Electronics Ltd., of Nottingham

***The
POLARMATIC 62
Automatic
Spectropolarimeter***

RANGE—181 to 600 millimicrons.

ROTATION—Up to 1.4 degrees at 200 millimicrons positive or negative.

SAMPLE TUBES—5, 10 and 20 mm
standard. Water jacketed types available.

RECORDER—Flat bed plotter.

RECORDING TIME—200 to 600 millimicrons in 20 minutes.

Bellingham & Stanley Limited

71 HORNSEY RISE, LONDON, N.19. PHONE: ARCHWAY 2270

ORGANIZATION OF THE SCIENTIFIC CIVIL SERVICE

THE sixth report of the Estimates Committee, which included some comments on the organization of the Scientific Civil Service in general, has been swiftly followed by a report from the Treasury on the organization of the Scientific Civil Service*. Much of this report confirms the views expressed in the Estimates Committee's report and, broadly, the Scientific Civil Service emerges well from both enquiries. The report from the Treasury, however, as might be expected, goes in further detail into the organization and recruitment of the Scientific Civil Service. It is accordingly of greater interest to the scientist and technologist, quite apart from the authoritative character of the Committee conducting the enquiry.

This Committee was appointed in March 1964 with Sir Mark Tennant as chairman, and included among its eight members, Sir Frederick Brundrett, Sir Charles Cawley, Sir Walter Cawood, Dr. F. M. Lea, Prof. D. G. Tucker and Sir Owen Wansbrough-Jones, with Mr. P. L. Towers as secretary. The remaining members were Mr. F. J. Doggett and Mr. D. S. Watson. The Committee was to review the organization of the Scientific Civil Service, particularly in relation to the scope for appointments made for finite periods in substitution for appointments on standard established terms, and to examine whether, in practice, the duties assigned to the three component classes were fully appropriate to their qualifications and experience. The Committee was accordingly to consider whether the 'scientific officer' class might devolve some of its responsibilities to the 'experimental officer' class, and the latter some of its responsibilities to the 'assistant officer' class and, if so, whether devolution would require changes in the structure of the classes. Any form of organization, to be effective, must be related to the purposes it is designed to serve; it should be such as to attract, and retain, sufficient people possessing the requisite qualifications, and their ability should be developed and deployed to the best possible advantage. These interacting considerations lie at the root of the matter and are accordingly covered by the Committee in its review. The report relates to the situation as it existed at the time, but the Committee believes that the substance of its comments still holds good, in spite of the substantial re-organization of civil science in the public sector.

The present structure of the Scientific Civil Service dates from the reconstruction period immediately following the Second World War, and the basis of the reorganization is set out in the White Paper on the Scientific Civil Service issued in September 1945, with the annexed report of the Barlow Committee on Scientific Staff. The new scientific officer and experimental officer classes came into existence on January 1, 1946, and a new class of assistants (scientific) was introduced a year later to relieve the experimental officer class of the more routine work. The senior class in the Scientific Civil Service is thus the scientific officer class, but a few research fellowships are also available in Government scientific establishments

at the three different levels of principal, senior and junior research fellowships. Moreover, in each of the three classes of the Scientific Civil Service, some officers serve in a temporary or unestablished capacity, constituting some 15 per cent of the scientific officer class and 12 per cent of the experimental officer class.

In both classes 80 per cent of the officers have been employed in the Civil Service for 5 years or less, while in the scientific officer class 60 per cent, and in the experimental officer class 50 per cent, have been employed for 2½ years or less. At present, including unestablished or temporary officers, some 4,100 officers are employed in the scientific officer class, 7,200 in the experimental officer class and 5,500 in the scientific assistant class. No class-to-class promotions to the three scientific classes are made under the age of 31; individuals over that age are selected at departmental discretion. Promotion within the three classes is strictly on merit.

For class-to-class promotion to the scientific officer class especially, it is clearly desirable that the standard should be both high and reasonably uniform throughout the service, and for this reason the Civil Service Commission maintains a central panel of scientists to assist departments in making selections for such promotions, as well as for promotions within the class to the principal scientific officer grade. Above this grade a substantial measure of managerial responsibility is normally involved in the work; but there are always some officers in the class whose particular interest remains centred on research and whose work clearly merits recognition. For this reason, some appointments are made each year on grounds of special merit, and the officers so promoted are enabled to continue with the work best suited to their particular bent. There are no set limits to the number of such appointments, but during 1962-64 such appointments have numbered some twenty to thirty in each year.

Commenting next on the functions and requirements of the Scientific Civil Service, the Tennant Committee points out that, while a striking feature of the Scientific Civil Service is the great variety of its work, basic research is an essential element. This wide variety of work requires a considerable diversity of talents and abilities and, while the Service needs men who can make an individual contribution to the frontiers of knowledge, there is only limited scope for those who are interested in research for its own sake and who are reluctant to accept any restriction on this field of activity. Men are needed who can visualize an end-product or a specific application and direct their research to that end. Those who are to fill the higher posts in the scientific officer class must possess, or be capable of developing, considerable managerial ability, in addition to their high scientific qualifications. The general tone and standard of Government scientific work is necessarily set by those who occupy, or may later rise to, the more senior posts, although they need the support of the experimental and other skills provided by the experimental officer class and also in turn by the scientific assistant class.

* H.M. Treasury. Report of a Committee appointed to review the Organization of the Scientific Civil Service. Pp. 28 (London: H.M.S.O., 1965.) 2s. 3d. net.

As to the merging of the scientific officer and experimental officer classes, the Tennant Committee has reservations. A unified Service might facilitate the full and proper use of the graduate talent and reduce the effect of the apparent tendency on the part of some universities to discourage graduate entry to the experimental officer class. If the classes were merged, however, there would no longer be a class with a special prestige of its own, and the Committee believes that the prestige which is attached to the scientific officer class does, in fact, appeal to the right type of recruit, while from the practical point of view, fusion of the two classes would complicate the question of probation. It is not easy to assess the relative weight of these advantages and disadvantages, but even if the Committee had believed that the balance was in favour of a unified class, it would have done no more than recommend that the idea should be further investigated. It is, however, satisfied that, in the present circumstances, the balance of advantage lies in favour of retaining the present arrangements.

Discussing next the recruitment situation, the Committee concludes that, although in 1964 the number of vacancies in established posts considerably exceeded the number of those recruited as established officers, in general the Service is attracting to the scientific officer class a fair share of the graduates it seeks to recruit, but it is rather less successful than its competitors in attracting really outstanding young scientists. This agrees with the evidence given to the Estimates Committee. Nevertheless the Committee does not believe that a system of recruitment based wholly or substantially on contract appointment would be satisfactory. If, however, ways could be found to make such employment attractive to some of those who would not otherwise enter the Service, its use might have some value as a supplementary method of recruitment, and it recommends that the possibility should be further explored.

The Committee emphasizes that the Service must be equipped to take full advantage of the increasing flow of science graduates from the universities. A proper leavening of outstanding scientific ability is essential if the standards of the Service are to be maintained. In recruitment, the nature of the work and the opportunities it offers, the idea of a permanent pensionable career, salary and career prospects all weigh differently with different people. So far as immediate salary prospects may affect the balance of individual judgment, the Civil Service may be at a disadvantage in competing with employers who have greater freedom and flexibility in making initial offers, particularly with the more talented individuals. The Committee suggests that a limited degree of flexibility in relation to starting salary might improve the ability of the Government to attract the most outstanding candidates; this possibility should be examined further. Everything possible, however, should be done to dispel present-day misconceptions about work in the Service and to build up an accurate picture of this work. This can be done, to some extent, by informal contact between members of the Scientific Civil Service and other scientists working in related fields, and every opportunity should be taken to develop such links both with universities and with industry. In particular, the Committee recommends that interchange of staff with the universities or industry, whether on a short-term loan or on permanent transfer, should be encouraged.

Quoting at length from the Barlow Report of 20 years ago, the Tennant Committee endorses the view, which the

Barlow Committee then expressed, that prospects for scientists of average ability in the Civil Service do not compare unfavourably with those offered in most universities and in industry for men of like ability. It is in regard to the man of more than usual ability that improvement was necessary then and is now. The Barlow Committee held that it was a first essential that the scales of pay and system of promotion of scientific classes should ensure that the best scientific men should have equal prospects of pay and promotion with the best men in the administrative class at least up to the top of the principal grade. This view is reiterated by the Tennant Committee, and although the Government, in the White Paper of 1945, agreed that staff complements should be so arranged as to ensure that the outstanding scientist had a reasonable expectation of reaching the principal scientific officer grade in his early thirties, its general conclusion is that the structure of the class up to and including principal scientific officer is well adapted to the needs of the work and, having regard to the terms of the White Paper quoted, provides the basis for a reasonable career. It does emphasize, however, that at a comparatively early stage in their careers some formal training in management should be available to members of the scientific officer class, although the Committee did not regard it as falling within the scope of its own review of the organization of the Scientific Civil Service to consider the question of permanent transfer to the administrative class as general purpose administrators, although recognizing the importance of the suggestion. It is, however, important that departments themselves should do their utmost to promote mobility of staff within and between their establishments. The Committee recognizes that this may involve establishments accepting some temporary inconvenience in the interests of the Service as a whole, and that the scientist himself must appreciate the advantages to be gained by widening his horizons. Nevertheless, it recommends that the effort should be made.

The Tennant Committee considered very carefully the position of the older scientists who may no longer be able to contribute materially to original research. It does not believe that the difficulty here is as extensive or as serious as is sometimes suggested, and it considers that the recommendations made as to career management and managerial training should help to minimize the problem. There may be occasional difficulties in the small establishments and in those where the main emphasis of the work is on research rather than on development. Here every effort should be made to arrange for officers to be transferred to some centre of work offering greater scope for their particular capacities.

As might be expected from a body of people of the experience and standing of the Tennant Committee, it is regarded as fundamental that highly qualified staff should not be employed on work which does not make a full call on their special qualifications, skill and experience. To do so is to waste talent and skills which neither the Service nor the country can afford, and the Committee believes that the importance of devolution of suitable tasks cannot be over-emphasized. While it is considered impracticable to define specific areas or types of work which could be devolved in this way, the Committee believes that there is still scope for further devolution but the extent can only be determined within the Department or Establishment itself. This is above all a matter for management, and is one reason for desiring to see scientific training associated with management and administration.

Commenting on the experimental officer class itself the Committee observes that many of the entrants now possess either degrees or equivalent qualifications or Higher National Certificates. This trend can be expected to become more pronounced as the flow from the universities, colleges of advanced technology and technical colleges increases, and it recommends that the arrangements for recruitment to the experimental officer class should be reconsidered. It envisages a system under which the normal age of direct entry would be the early twenties—the academic qualification being a degree or its equivalent or Higher National Certificate. Candidates without such qualifications would be recruited into the scientific assistant class in the first instance. Transfer to the experimental officer class could come when some practical skill and experience had been acquired and possibly subject also to obtaining some qualification at least equivalent to Higher National Certificate.

The Committee considers that the present structure of this class may not adequately reflect the balance and variety of its work. There is a wide gap between the accepted standard for the grade of experimental officer and that for the senior experimental officer, and the Committee thinks that there is work to be done, and being done, which is of higher quality than is normally expected of an experimental officer but nevertheless does not merit senior experimental officer grading. This situation could be met by creating a new intermediate grade, and the Committee recommends that this should be considered as part of a thorough revision of the grading and structure of the class. The future frontiers and structure of the scientific assistant class will obviously depend on the nature of any new arrangements made for the experimental officer class and could be considered when the problems associated with the latter class have been resolved.

The main recommendations of the report are in line with the comments of the Estimates Committee and also with the evidence tendered to that Committee. The report of the Tennant Committee clearly goes far deeper into the organization and use of the Scientific Civil Service than the enquiry conducted by the Estimates Committee into the Civil Service as a whole could be expected to do. While the Tennant Committee is satisfied that the scientific officer class, up to and including principal scientific officer, is well adapted to the needs of the work and provides the basis for reasonable careers, it is apparent that the recommendations of the Barlow Committee have, even after 20 years, still to be fully implemented and the way made clear to the highest posts in the Civil Service for those with the necessary administrative ability. Nor is it possible to feel entirely reassured that highly qualified staff is not being employed on work which does not make full demands on its special qualifications, skill and experience. This is never an easy task and demands much of the administrator, who may well be ill-equipped to discharge his responsibilities unless he himself has at least some modicum of scientific training or experience. There remains also the question of ensuring that the Scientific Civil Service attracts a sufficient number of really outstanding scientists. It is to be hoped that, following so swiftly after the Estimates Committee's own survey of the situation, the recommendations of this report of a Treasury Committee will not be neglected, and that steps will be taken to ensure that the necessary measures are taken as a matter of urgency.

Finally, as a tailpiece to the recommendation that the Service should take full advantage of the increasing flow of science graduates from the universities, it is worth noting that the optimism of the Robbins Report in this respect has been firmly challenged, perhaps most recently by Prof. R. V. Jones in his Joseph Payne Memorial Lecture, "The Advancement of Learning: 1605–2005 A.D." (*Nature*, 206, 129; 1965). Prof. Jones doubts whether the number of students of high ability going to universities, judged by the Ph.D. status, has appreciably increased. While university places offered in physics are not all being taken up, an increasing proportion of students do not appear to make the grade. This view is supported by the evidence of further enquiries at Aberdeen specifically in relation to physicists. If, therefore, the number of graduates of outstanding ability in physics and in other subjects is unlikely to be increased in proportion, it is the more imperative to ensure that the maximum use is made of such graduates wherever they may be employed. Prof. Jones points out further that in order to cope with the expansion planned in physics departments in the universities in the next few years some 50 per cent of all those graduating as Ph.D.s in physics would be needed to staff the universities. This is not a practical proposition; only 50 per cent would then be left for Government service, for loss by emigration, and for industry. It is in this context, moreover, that the Civil Service requirements have also to be considered. Obviously much more thought has to be given to the use made of our first-class scientific talent, including its distribution, and the most strenuous efforts made to see that such talent is not employed on duties for which those with lesser qualifications could be used.

THE FOUNDER OF ABDOMINAL SURGERY

Spencer Wells

The Life and Work of a Victorian Surgeon. By John A. Shepherd. Pp. xi+152+14 plates. (Edinburgh and London: E. and S. Livingstone, Ltd., 1965.) 30s. net.

THIS is a book with special appeal to surgeons who are interested in the history of their profession. Mr. Shepherd, a Liverpool surgeon, has carefully investigated the career and numerous writings of Spencer Wells, and is well able to appraise the results of his subject's work.

Thomas Spencer Wells was the eldest son of a builder near St. Albans. After apprenticeship to a Barnsley doctor he completed his medical education at Leeds, Dublin and St. Thomas's Hospital, London. He qualified as M.R.C.S. in 1841 and entered the Royal Naval Medical Service in the same year. Already, he had gained some distinction by a Dublin prize essay on "Bronchotomy" and was known to Sir William Burnett, the director-general of the Service.

For about seven years Wells was assistant surgeon to the Naval Hospital at Malta, where he enhanced his surgical reputation, being elected F.R.C.S. in 1844 and promoted surgeon in 1848. Between 1848 and 1850 he studied in Paris and Vienna, and travelled in Egypt and Italy in attendance on a nobleman suffering from tuberculosis. From 1851 until 1853 he was surgeon to H.M.S. *Modeste*.

A pulmonary illness caused him to be invalided home and put on half-pay. He soon recovered and was appointed surgeon to the Samaritan Free Hospital for Women and Children and lecturer in surgery to the Grosvenor Free School of Medicine. In 1855 he was appointed by the War Office as a civil surgeon to serve in the Crimean War. In 1856 he was recalled by the Admiralty, resigned

from the Navy, and resumed his duties at the Samaritan Hospital.

In the first half of the nineteenth century the mortality from surgical operations was high, and only the most daring of surgeons opened the abdominal cavity. In the majority of cases, death occurred from sepsis, and in the large metropolitan hospitals morbidity and mortality were highest from this cause. One well-recognized condition was the ovarian cyst, a slow-growing tumour.

Mr. Shepherd describes at some length the work of the early operators, the ovariologists, both British and American, their limited successes and their failures through sepsis. By 1857 the operation was in disuse and was considered an unjustifiable one by many surgeons. Nevertheless, Wells developed this operation, his first success being on a woman of thirty-eight in 1858. He faithfully recorded his cases, and soon became famous for his masterly technique. Later, when Lister had introduced antiseptics into surgery, the mortality from ovariectomy was greatly diminished.

Wells had to face much hostility and opposition. Even when the success of his operation was established, attempts were made by Clay, Lawson Tait and others to challenge his priority. Wells met all these attacks with dignity and calmness. "Only an individual possessed of the strongest will and determination could have stood up to the opposition which he had to encounter." The account of these professional jealousies makes sad reading in regard to men who must have adopted the healing art in order to benefit mankind.

In the end the claims of Wells were fully vindicated. He was elected president of the Royal College of Surgeons for 1882-83, created a baronet and accorded international fame. He died of apoplexy on January 31, 1897.

Sir Frederick Treves wrote of Spencer Wells that he "by his determination succeeded in founding abdominal surgery as we know it", and also said that his work contributed to make surgery a precise science. In this biography Mr. Shepherd gives chapter and verse for these outstanding achievements.

A. MACNALLY

PHYSICS OF THE EAR

The Physics of the Ear

(International Series of Monographs on Physics, Vol. 3.) By T. S. Littler. Pp. ix + 378. (Oxford, London and New York: Pergamon Press, 1965.) 80s.

DR. T. S. LITTLER is well known to the medical and educational profession as the director of the Wernher Research Unit on Deafness. His book, *The Physics of the Ear*, contains fifteen chapters with supplementary sections giving mathematical treatment of physical data. As the title would suggest, the subjects of anatomy, physiology and measurements of hearing are dealt with from the point of view of the physicist rather than the clinician, who would wish a greater discussion on the application to various clinical states. The approach given in the book lays an excellent theoretical and practical basis for the clinician.

The subjects of hearing and deafness have captured the attention of physiologists especially during the past twenty years when experimental approaches have been possible due to the advances in techniques for investigating nerve physiology. Dr. Littler makes a very comprehensive and critical survey of the literature to date and is to be specially commended for referring to the very early work both in Great Britain and the United States.

In the section on anatomy the most up-to-date data are described and discussed, with the possible exception that insufficient space has been given to the efferent pathways described by Rasmussen and to such work as that by Fex and Hernandez P6on. The work on the

physiology of the ear during the past 20 years has been most exciting. Dr. Littler manages to convey the wealth of knowledge we have gained in this period in a careful analytical approach to the literature, bringing out the work of Von Békésy, Davis and his collaborators but paying respect to the early pioneers.

The book gives comprehensive coverage on both the physical and physiological aspects of thresholds of hearing with reference to auditory fatigue and adaptation—a subject with which the author is himself more particularly familiar in his research. The chapter on audiometry is covered rather from the aspects of the theories of testing hearing than from a detailed clinical application to varying pathologies. This is not a criticism of a book which is not intended to be primarily a clinical text-book.

The final chapter, on "Theories of Hearing", is a most valuable conclusion to the main body of the book. It is of interest to see that Dr. Littler in planning his own book has placed this subject last. Perhaps it is a reflexion of his own thinking, which has always placed greater emphasis on experimental data as his basis of thought and action. As throughout the book, this final chapter is dealt with most thoroughly and critically.

Dr. Littler's contribution to the standardization of audiometric threshold is universally recognized. In the chapter on audiometry he reports fully on his own work in this field. The chapter dealing with the alleviation of deafness bears the stamp of his own experience, as does the section describing the development of hearing aids. The detailed description of the development of the Medresco hearing aid will be of interest to the reader. The criticism that must be offered here is that there does not appear to have been sufficient differentiation between the congenitally and acquired deaf among children and adults. The possibility that congenitally deaf children will need a different type of hearing aid from the deafened adult does not appear to have been given sufficient critical analysis.

Throughout, the book is written in a clear, readable style. Dr. Littler manages in his lucid way to bring together the many facets from the old and the new and weld together a coherent story, giving each part a perspective which will help the reader to gain an insight into the development of audiology. The book is to be highly recommended to those entering this field as well as to the experienced anatomist, physiologist and clinician. It is a book which offers a fund of information, including a valuable reference section. The publishers ought also to be congratulated on this publication. I. G. TAYLOR

MAMMALIAN POPULATIONS

Physiological Mammalogy

Edited by William V. Mayer and Richard G. Van Gelder. Vol. 1: Mammalian Populations. Pp. xii + 381. 86s. Vol. 2: Mammalian Reactions to Stressful Environments. Pp. xii + 326. 92s. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1963 and 1965.)

POPULATIONS of small mammals (that is, rodents and insectivores approximately up to the size of the brown rat, *Rattus norvegicus*) have attracted the attention of ecologists for many years and, especially since the end of the First World War, much labour has been spent on measuring their population dynamics and putting forward theories concerning the natural regulation of their numbers. Their importance lies first in their notably wide distribution and abundance as primary and, to a lesser extent, secondary consumers in most animal communities; secondly, in the wide ranges within which their numbers fluctuate and in the cyclic nature, especially in Arctic and sub-Arctic habitats, of these fluctuations. The pervasive nature of this behaviour and the frequent economic

significance of peak populations ("plagues of mice") have been broadly covered by Charles Elton in his book, *Voles, Mice and Lemmings*.

In spite of the energy which has been devoted to this problem, no clear answer has yet emerged to the question, "How are these small-mammal numbers regulated in Nature?" Nevertheless, there is no lack of theories and, indeed, of controversy about the matter. At various times the observed fluctuations have been attributed to changes in the physical environment, especially climatic cycles, to interaction between the small mammals and their natural resources, competitors and 'antagonists' (that is, predators, parasites and disease organisms) and to interaction between the small mammals themselves. The extended work of D. H. Chitty on population cycles in the short-tailed vole (*Microtus agrestis*) showed that the cycles were not necessarily synchronous and so directed attention away from the environment to endogenous factors within the populations themselves. The puzzling fact that the decline or 'crash' of a population continued down to exceedingly low densities in spite of little or no apparent damage to resources, swung attention to the possibility that physiological and/or genetic changes coincided with different phases of the population cycles. Although much work has continued on measuring the effect of food and predators, a tremendous attack has been made in the past twenty years from the physiological and social angles.

The planned series of publications entitled *Physiological Mammalogy*, of which the first two volumes are reviewed here, attempts to synthesize the vast amount of information from what might be called classical animal physiology, drawn from restricted laboratory types, with more recent work on field populations of a wide variety of species. Volume 1 contains two long articles, "The Social Use of Space", by John B. Calhoun, and "Endocrine Adaptive Mechanisms and the Physiologic Regulation of Population Growth", by T. T. Christian, both of which present evidence for the self-regulating nature of population control in (mainly) small mammals. Volume 2, with the sub-title of *Mammalian Reactions to Stressful Environments*, contains three articles, "Reproduction and Development", by S. A. Asdell, "Water Metabolism of Mammals", by Robert M. Chew, and "Hibernation", by Ch. Kayser, which can be characterized as review articles, covering an immense amount of literature, which it is valuable to have brought together here.

Calhoun has a very clear thesis to develop and argue, which is that mammalian evolution has been based on the efficiency of a social group of about twelve individuals and that most of the processes of population control, except where herding habits have been evolved, are concerned to redress aberrations from this norm. His argument concentrates on the way in which sensory contact with neighbours and exploratory behaviour conduce to home ranges organized in terms of such a social unit and in the way in which aberrations reduce viability of individuals in various physiological characteristics. Many of the data used to substantiate the theoretical steps in the argument have not been published previously, and in every way Calhoun's contribution goes far beyond the review level which predominates in Volume 2. In detail, the theoretical steps are not easy to follow for readers unused to thinking in terms of mathematical notation, but the presentation and discussion of relevant data at each step bring the argument into focus for the non-mathematical biologist. On the whole, it may be said that the data presented at each step do not invalidate the hypothesis, but whether they are susceptible of other interpretations or will be invalidated by fresh data remains to be seen.

The contribution of Christian is mainly concerned to show the mechanisms through which the various physiological disabilities subsumed under the term 'stressed' can arise in response to increasing population densities. There is more of a review quality about his article than about

that of Calhoun, and his careful and detailed account of the structure, functioning and integration of the endocrine system in mammals is an extremely valuable background to any thinking about population problems in these terms. The rest of the article examines what is known about endocrine upsets in laboratory and wild populations in relation to density, with the general theme that here is a biological system with (in the modern phraseology) a built-in homeostatic mechanism.

Both these articles deal with ways in which automatic adjustment to an 'optimum' equilibrium of numbers may be maintained, but are obscure, or reticent, about how this 'optimum' equilibrium is determined. It seems probable that this problem will remain obscure until equal or greater energy is devoted to measuring the resources of the environment.

In Volume 2 the first article, by S. A. Asdell, consists of a fairly condensed summary of the facts of mammalian reproduction and of the patterns observed in different groups. Robert M. Chew gives a very thorough account of the physiology of water metabolism in mammals (with an extensive bibliography) with special attention to their capacity to resist dehydration in arid environments. Ch. Kayser deals somewhat diffusely with hibernation.

The editors are to be congratulated on a worth-while accomplishment, involving different levels of presentation, and further volumes will be eagerly awaited.

H. N. SOUTHERN

PLATES AND SHELLS

Introduction to Plate and Shell Theory

By C. E. Turner. Pp. xii + 208. (London: Longmans, Green and Co., Ltd., 1965.) 35s.

STRUCTURES which qualify as 'plates' or 'shells' have tended for years past to receive rather limited treatment in standard texts on applied elasticity and strength of materials. This is no doubt due to the extremely varied conditions of geometry and loading that can arise, and consequently compendious and advanced specialist texts have emerged separately, of which Timoshenko and Woinowsky-Krieger's *Theory of Plates and Shells* and Flügge's *Stresses in Shells* might be quoted as classical examples. *Introduction to Plate and Shell Theory* is thus to be welcomed as a soundly written text of moderate length and moderate price which provides a thorough introduction to the field and neatly bridges the gap in existing literature.

One is glad to see a student text in which the essential and ever-repeating pattern of the actual work of theoretical stress analysis is so consciously and deliberately displayed: namely, construction of equations of equilibrium, of geometric deformation, of the assumed (normally linear) stress-strain behaviour, leading to the final and purely mathematical task of producing working equations for whatever parameters are deemed of special interest.

A short introductory chapter gives a clear account of the basic concepts of a loaded shell element. This is followed by a wide-ranging discussion of the membrane theory of shells, which, within the limits imposed by its simplifying assumptions, can so often yield remarkably useful design information in exchange for time spent on comparatively short and simple methods of calculation. Flat circular plates are then discussed for situations which involve symmetrical stretching (as with rotation or with inplane edge loading) and symmetrical bending. The conclusion to the introductory function of the book then follows in a presentation of a general theory of symmetrically loaded shells of revolution, in which cylindrical, spherical, conical and toroidal forms—together with combinations of these—are examined in some detail.

The remaining parts of the book chiefly present variations and elaborations of the foregoing themes; but

among the selected problems some useful discussion is offered of unsymmetrical bending, the influence of elastic foundations and—more briefly—instabilities, thermal stresses and melastic behaviour.

The text is supported by about sixty very clear diagrams, some short tables of mathematical functions, and a quite adequate bibliography. It should find a substantial readership among students of stress analysis and design, and has much to offer to established workers.

B. N. COLE

THE DUTCH STUDY CIRCLE FOR AGRICULTURAL HISTORY

De Studiekring voor de Geschiedenis van de Landbouw, 1939–1964

(Study Circle for Agricultural History, 1939–64.) Pp. 37. (Wageningen, Netherlands: Royal Association for Agricultural Science, 1964.)

ON the initiative of Dr. W. A. J. Oosting of Benneken an effort was made in 1937 to establish a Dutch Study Circle for Agricultural History. The plan came to fruition in 1939, but the Studiekring was set up as a part of the Nederlandsch Genootschap voor Landbouwwetenschap. Dr. Oosting was elected chairman, and J. A. van der Loeff was elected secretary with an influential committee. The intention was to study agricultural history in the broadest sense of the word; but the activities of the nascent Studiekring were brought to a standstill by the German invasion in 1940. The death of Dr. Oosting in 1942 was a great loss.

In 1944 Prof. Z. W. Sneller wrote to van der Loeff saying he had been approached by several people to form a society for the study of agricultural history, and enquiring whether the Studiekring was still operative. It was in fact in being, but in a state of war could do little, and it was decided to wait for more peaceful times. The members of the Studiekring are historians and scientists working on agricultural and kindred subjects.

Activities were resumed in April 1946. An open meeting was held and a programme of work planned. It was (1) to organize a 'history day' during the Nederlandse Landbouwweken in conjunction with the Nederlandsch Genootschap voor Landbouwwetenschap; (2) to encourage research in the field of agricultural history, and to publish the results in a series of *Agronomisch-Historische Bijdragen*; (3) to promote the publication of brief contributions to the subject in the *Landbouwkundig Tijdschrift* and to collect reprints of these in an *Agronomisch-Historisch Jaarboek*; (4) to foster the compilation of a bibliography of important articles on Dutch agricultural history from early Dutch journals; (5) to encourage the reprinting of scarce old publications of value for the purpose.

Until 1950 it was possible for the Studiekring to participate in the Agricultural Week, and the annual lectures presented were printed in the *Agronomisch-Historisch Jaarboek*. After that date this arrangement ceased. Lectures, however, continued to be read at the annual meeting of the Circle. Lectures, combined with excursions to the part of the Netherlands discussed, were also arranged each year after 1951.

Five volumes of the *Agronomisch-Historisch Jaarboek* have been published containing reprints that had been contributed to the *Landbouwkundig Tijdschrift* and other material. These appeared in 1940, 1942, 1947, 1949 and 1951. Unfortunately the *Jaarboek* had to be discontinued for lack of material. Six volumes of *Agronomisch-Historische Bijdragen* have been published, 1948, 1949, 1950, 1958, 1959, 1964. The last-mentioned bears the title *Ceres en Olio: Seven Essays on Agricultural History written to celebrate the 25th Year of the Studiekring*.

One of the Circle's desiderata was the compilation of a bibliography as a fundamental basis for the study of

farming history. A committee was set up in 1946, but after collecting some material it laid this programme aside in anticipation of the foundation of a readership in farming history at the Agricultural University. Instead a chair of agricultural, economic and social history was established in 1949. At the same time the Dutch Agricultural History Institute was formed at Groningen in connexion with the State University, one of the purposes of which was to solve the problem of publication of sources for Dutch farm history. Prof. B. H. Slicher van Bath became the first appointed to the new chair at Groningen. Thus ten years after the formation of the Studiekring the study of farming history was recognized as a subject for higher education. The bibliography that was one of the first aims of the Studiekring was finally compiled by Dr. J. M. G. van der Poel in 1953 while he was director of the Agronomisch-Historisch Instituut, and published under the title *Wegwijzer in de Landbouwwgeschiedenis*; besides its primary purpose this was intended as a guide for teachers in secondary and primary agricultural and market garden schools, who wished to teach simple farming history. A general international background is provided, but naturally the contents are mainly Netherlands sources.

Another object of the Studiekring was research into old land measures, and in 1956 a committee of three members was appointed to go into this subject. This small committee came to the conclusion that, in addition to land measures, other ancient measures and weights ought to be collected and related to those of modern times. Prof. Slicher van Bath, a member of this committee, published an *Alphabetical List of Books and Articles of Importance in Relation to Ancient Dutch Weights and Measures in A.A.G. Bijdragen* No. 11 issued by the Department of Agricultural History of the Agricultural University at Wageningen in 1964.

The work of the Studiekring has been very effective during its 25 years of life. Not only has it encouraged research and publication of results, but also it has been largely responsible for the development of farming history at all levels of education throughout the Netherlands.

G. E. FUSSELL

NOMAD PEOPLES OF THE STEPPES

The Royal Hordes

Nomad Peoples of the Steppes. By E. D. Phillips. Pp. 144 (141 illustrations). (London: Thames and Hudson, Ltd., 1965.) 30s. clothback; 15s. paperback.

THIS is a first-class publication, useful both for the specialist and for the interested layman. It is profusely illustrated, many of the illustrations being in colour. Most people have heard of the Huns, and of the danger to western civilization due to their incursions. But not everyone realizes that the Huns were only one of many hordes who roamed about the vast areas of Central Asia, stretching from Modern Russia to China, and who, starting as early as 4000 B.C., continued to flourish until only a few hundred years ago when they were finally suppressed by the growing urban civilizations of Russia and China. Nor must one think of these nomad hordes as mere crowds of savages. Nomadism was a real substitute for urban civilization, which, given the suitable environment, produced fine works of art and a not uncivilized way of life. Anyone studying the illustrations in *The Royal Hordes: Nomad Peoples of the Steppes* will have to admit that in the realm of art these nomads were by no means behind their urban opponents. The ability to produce a fine art is one of the criteria which separate man from the animals.

It is suggested that nomadism grew out of an early neolithic way of life by emphasizing the domestication of animals rather than agriculture; and with the development of the horse, a true pastoral as against a settled agricultural

mode of life became possible. It is suggested that such a development from the neolithic Tripolye culture took place as long ago as 4000–2000 B.C. in southern Russia and similarly in Caucasia, western Siberia, Turkestan, the Tarim basin and in the Far East during the same period. From 2000–1500 B.C. the movement spread, and the new way of life developed.

Naturally, the nomads absorbed useful knowledge from their neighbours. Thus the use of bronze, so important for horse harness, became general. Pottery was frequently painted and real works of art in metal, including gold, were produced. The next 500 years see the rise of the true mounted nomadism, which reached its full development about the start of our era. This mounted nomadism, of course, was especially dangerous to the urban civilizations to the West and to the Far East, and it was only when each of these latter really united that they were strong enough to destroy the nomad hordes.

There is no doubt that this is a book to possess. There is a tremendous amount of information packed into a small compass and the illustrations give a vivid picture of the splendid art of these folk.

M. C. BURKITT

INFORMATION SERVICES IN BRITAIN

The Government Explains

A Study of the Information Services. By Marjorie Ogilvy-Webb. (A Report of the Royal Institute of Public Administration.) Pp. 229. (London: Allen and Unwin, Ltd., 1965.) 28s. net.

THIS examination of the information services of the central government made by a study group of the Royal Institute of Public Administration, of which Sir Kenneth Grubb was chairman, and Mrs. Marjorie Ogilvy-Webb, writer of the report, was research officer, does not cover local government services, and the overseas information services are also excluded. It gives a thoroughly competent and readable account of the British information services and their development, which not only demonstrates the necessity of such services to-day and their essential part in the functioning of a democracy but is also to some extent an antidote to the undertones of such a book as Jacques Elliot's *The Technological Society* which visualizes the citizen being conditioned by the mass media of communication. That danger is fully recognized in this report, but sound reasons are given for believing that it is not serious in Britain.

For the most part the report is factual. After an introductory chapter describing the scope of the communications between government and governed, there are two chapters reviewing the historical development of information services from before 1914 to the present day. The next three chapters describe in succession first, the organization developed for the co-ordination of information; next, the way the departmental divisions work; and then the functioning of the Central Office of Information and of the Stationery Office. Two chapters deal with staffing the information services and with training civil servants for their relations with the public, and the final chapter contains the comments and conclusions of the examination.

It is this calm appraisal of the information services that provides the reassurance, not least because the difficulties are frankly faced. No charge of political bias has ever been properly sustained against the information services nor are individual members of the information services known to have engaged in political controversy. Nevertheless, the whole question of impartiality is very difficult, and there is much in this final chapter for the scientific or technical adviser to ponder. The creation of an informed public opinion where scientific and technical issues are involved is not always simple and, although

such issues are not discussed directly in this book, its implications for such issues are easily discovered, though the only factual slip I have noted is an inaccurate reference to the Advisory Council on Scientific Policy. Bibliographical references appear to be fairly complete although the manner of citation is not always the most convenient and there are places where more biographical references might have been included: apart from Lord Reith's *Into the Wind*, these are largely lacking. One thing at least this report should do: it should prevent any raising of the old controversies and ensure acceptance of the information services by the major political parties, while stimulating the continuous thinking about their functioning and the constant vigilance that are the safeguards against abuse. Not least, it should contribute to establishing the information services on a level of esteem and status that will help to attract to such a career men and women of the character and ability demanded if the service is to be of the highest standard.

R. BRIGHTMAN

IRISH BIBLIOGRAPHY

A Guide to Irish Bibliographical Material

By Alan R. Eager. Pp. xiii+392. (London: The Library Association, 1964.) 96s.

THE National Library of Ireland is at present preparing what will be the first attempt at an Irish national bibliography. Eleven volumes, dealing with manuscript sources, should appear later this year. As an interim measure, Mr. Eager has compiled a bibliography of bibliographies listing more than 3,800 items arranged broadly according to Dewey.

Much of the material consists of catalogues and printed bibliographies. Many of these, however, are not specifically, or even predominantly, Irish in coverage. General works such as the *British Union Catalogue of Periodicals* and *World List of Scientific Periodicals* are included so that Eager duplicates much of Walford and Winchell, without their annotations.

The comparative paucity of Irish bibliographies has tempted Mr. Eager to stray beyond the limits one would expect. In his anxiety to record at least one reference for every subject he has "included works which are not bibliographies *per se* but are standard or representative, also articles which appeared in periodicals and newspapers that are of value but are not generally catalogued separately and are thus overlooked". The dangers of such a policy are obvious, and Mr. Eager has not avoided them. Under "Domestic Science", for example, the only sub-heading is "Hotels, Restaurants, etc.", and of the five items listed, four are accommodation guides; the fifth is the history of a Dublin hotel. In such circumstances would it not have been better to ignore the subject completely?

Articles from periodicals, the other type of material, are very often from journals such as the *Proceedings of the Royal Irish Academy*, to which reliable indexes already exist and which any competent research worker would consult as a matter of course.

Criticism of this book revolves around Mr. Eager's too-good intentions. Sins of omission are rare, and if the compiler had set out to produce a vade-mecum to Irish studies the book would have been a praiseworthy beginning. Even so, one might have wished for a standard approach where the expansion of initials into forenames was concerned. An unfortunately haphazard presentation has been adopted.

Nevertheless, Mr. Eager's book has already become a standard reference tool, and we should be grateful for the accuracy and care in its compilation which are obvious throughout, especially in recording unpublished manuscripts and work in progress.

H. J. HEANEY

The Fossil Evidence for Human Evolution

An Introduction to the Study of Palaeoanthropology. By Sir Wilfrid E. Le Gros Clark. Second edition, revised and enlarged. (The Scientist's Library.) Pp. xii + 201. (Chicago and London: University of Chicago Press, 1964.) 6 dollars; 45s.

THIS revised edition of *The Fossil Evidence for Human Evolution* comes at an appropriate time and 10 years after the original publication. In 1954, Piltdown had been relegated to the history of fakes and forgeries and the human evolutionary picture was all the better for the removal.

The 10 years that have passed have seen fewer crises and the emergence of a much more satisfactory realization of man's ancestry. Much of this has depended on the labours of a few men: the prescience and persistence of Kenneth Oakley, who has added a new system of time to human studies; the labours of John T. Robinson on the splendid collections of Australopithecines that he has had to leave in South Africa on his translation to Wisconsin; and always the genius of L. S. B. Leakey in charming fossils from the ground.

All this has clarified the general view, if some aspects of it are a little clouded. Most systematists will heartily agree with Sir Wilfrid that there are too many names along the route of man's emergence. While it is true that in these pages *Pithecanthropus* and *Sinanthropus* are now less frequently mentioned and *Homo erectus* stands more neatly in their place, yet the Australopithecines bristle with genera from A to Z. There is much to be said for the author's plea that, until they become systematically established, specimens might bear such names as "the Steinheim skull", the "Swanscombe skull", etc., rather than a too hasty attribution to a new genus and species. On the other hand, human palaeontology is of general interest and a new genus may more easily and more widely be recorded.

The increase in the size of the new text is largely due to valuable descriptions of the methods of dating remains. Fluorine, carbon-14, potassium-argon and uranium-lead are dealt with. It is interesting to note that dates in the Pleistocene are being lengthened and put further back. *Homo sapiens* had a history of 50 thousand years in the 1954 edition. Now it is 200 thousand.

This well-produced volume is a necessity for all who work in human palaeontology. Others will also appreciate the quiet wisdom and the well-turned phrases of its distinguished author.

W. E. SWINTON

Mathematical Analysis

Functions, Limits, Series and Continued Fractions (International Series of Monographs in Pure and Applied Mathematics, Vol. 69). Edited by L. A. Lyusternik and A. R. Yanpol'skii. Translated by D. E. Brown. Pp. xiv + 404. (Oxford, London and New York: Pergamon Press, 1965.) 70s.

THE content of *Mathematical Analysis* is somewhat wider than the sub-title suggests. There is a chapter on n -dimensional spaces, functions defined there, linear operators, convex bodies; the work on series includes a considerable amount of material on orthogonal series and systems of orthogonal polynomials; there is a short section on special functions, such as the Bernoulli and Euler numbers, the elliptic functions, the gamma function, Bessel functions. The material is logically arranged and clearly set out, but no theorems are proved. It is thus difficult to know how the book is to be used, and it is a pity that the editors do not tell us how this and succeeding volumes fit into the Russian techniques of instruction. The arrangement is scarcely suitable for a book of reference, but the absence of proofs would prevent it being an adequate text for private study. If a teacher were to select the more difficult key theorems and go through them in detail with his pupils, they might then, granted time

and patience, derive very substantial benefit by working carefully through the rest of the book, proving the subsidiary theorems and verifying the numerous formulae. There is, for example, an immense amount of information stored away in the chapter on orthogonal polynomials; and to verify the couple of dozen integral forms, not all entirely trivial, for Catalan's constant would make an admirable revision exercise in the technique of definite integrals. Perhaps if later volumes are translated, a prefatory word on how best to use them could be added.

T. A. A. BROADBENT

Power Travelling-wave Tubes

(Applied Physics Guides.) By J. F. Gittins. Pp. xii + 276. (London: The English Universities Press, Ltd., 1964.) 50s.

TRAVELLING-WAVE tubes have played such a unique part in the development of microwave communication links that they well merit close examination. Not only in their conception are they the result of an impressive flight of imagination, but also in their construction they incorporate some of the most recent technological developments.

Power Travelling-wave Tubes gives a very successful presentation of most problems involved in the design of travelling-wave tubes. In view of the emphasis put these days on the importance of advanced engineering education, it is encouraging to find a book which is prepared to tackle not only the fundamental but also the technological side of a problem.

The author discusses both forward and backward wave amplification processes, including crossed-field interaction. The operating of travelling-wave tubes is explained using Pierce's approach, but a commendable use is made of vector diagrams to illustrate many points. Separate chapters discuss slow-wave structures, electron guns and collectors and transitions, severs and radiofrequency windows. The two final chapters deal specifically with vacuum and measurement techniques.

The book can be recommended as an up-to-date description of an important field of vacuum tube technology and it is a welcome addition to technical literature.

P. A. LINDSAY

Structure Antigénique de Tumeurs Expérimentales

Par C. Deckers. Pp. 180. (Bruxelles: Editions Arscia S.A.; Paris: Librairie Maloine, S.A., 1964.) n.p.

THIS is a monograph covering several years' work by Dr. Deckers, dealing with the analysis of antigens in rat tumours by the methods of Ouchterlony double diffusion and immunoelectrophoresis. Several methods for increasing the sensitivity of the gel diffusion technique were described. The most sensitive of these used a combination of immunoelectrophoresis and double diffusion in gel. Hyperimmune rabbit sera were used in all the investigations involving rat tissues. Five organ-specific liver antigens were demonstrated in normal rat liver. During the course of chemical carcinogenesis of the liver, the concentration of organ specific antigens progressively diminished. In transplantable hepatomata, there was only one such antigen remaining. However, a new antigen was detected in a transplantable (HA) hepatoma. This new antigen could not be detected in other tumours. Attempts to provoke circulating antibodies to the HA antigen in rats were unsuccessful, as far as the means of detection would allow. Inoculation of rats with HA tumour extracts either slightly decreased or increased the rate of growth of tumours transplanted later.

The efforts of some of the other workers in this field were mentioned. The author deserves credit for persisting in a problem which poses many experimental difficulties, and it will be interesting to follow the progress of the work.

ROBERT R. DOURMASHKIN

THE ROYAL SOCIETY

ANNIVERSARY ADDRESS*

By Lord FLOREY, O.M., P.R.S.

AWARD OF MEDALS, 1965

Copley Medal : Prof. A. L. Hodgkin, F.R.S.

THERE are few areas of modern knowledge the development of which can be identified with the leadership of one person. Yet, in the field of neurophysiology, the important and indeed decisive advances which have been made during the past 30 years are almost wholly due to the original work of A. L. Hodgkin and to the influence which the theoretical concepts and experimental techniques developed by him have had on research workers all over the world.

In his earliest paper, published in 1936, Hodgkin showed that the cable-like structure of a nerve fibre provides an essential link in the conduction of the nerve impulse, and that the electric current generated during excitation of a small part of a nerve fibre can stimulate a region of a few millimetres length ahead and so propagate with a large safety margin. This was Hodgkin's first experimental exercise, in which he still used established techniques. To advance the study of the nerve impulse further, he decided to adopt a more direct approach, by working on isolated cells and measuring their membrane properties with the help of micro-electrodes introduced into the cell interior. This led to a new kind of experimental analysis, far in advance of what had been attempted before. Hodgkin's decision to embark on this project and his refusal to be deterred by what must have seemed appalling technical difficulties have led, during the past 20 years, to a gradual revolution of the whole neurophysiological field.

Hodgkin's experiments resulted in the discovery of the ionic mechanism of the nerve impulse. He showed that the action potential wave arises from a rapid entry into the cells of a small quantity of sodium ions followed by an equivalent loss of potassium ions from the cell interior. The initial influx of sodium is a regenerative process which propagates along the whole length of the fibre. The impulse derives its immediate energy from the ionic concentration differences between the cell and the surrounding fluid.

In a series of investigations extending over 20 years, Hodgkin and his pupils have worked out the quantities of the ion movements across the nerve membrane and the rates of the underlying permeability changes, and more recently have been bringing to light the chemical reactions involved in the restoration and maintenance of the ionic store of the cell.

Hodgkin has had the enthusiastic collaboration of many distinguished pupils and has built up a school of physiologists of high international reputation. Moreover, his techniques of intracellular recording and the directness of his single cell analysis have served as a model which has raised the standards of experimentation in a wide area of physiological research. The new concepts explaining bioelectric phenomena in terms of specific ionic conductance changes have not only had a far-reaching influence on the study of peripheral nerve and muscle, but have also led to important advances in the investigation of the synaptic mechanisms in the central nervous system.

A Royal Medal : Dr. R. A. Lyttleton, F.R.S.

Dr. Lyttleton is distinguished for his theoretical studies in many fields of astronomy and geophysics. He has shown outstanding ability in the application of dynamics to astronomical problems, and his book, *Rotating Liquid Masses*, is a unique description of work on obscure classical problems that few scientists have hitherto been able to understand. In his work he has finally clarified problems which puzzled many distinguished theorists such as Jacobi and Jeans. In particular, he elucidated and clarified the important result that the disruption of a fluid mass by rotation would not lead to a pair of bound bodies, as Jeans believed, but to two independent bodies. His work on this subject has had major repercussions on several contemporary problems in astronomy, especially on the formation and stability of galaxies, on the origin of the Solar System and the capture of satellites by planets. In earlier work (in collaboration with Hoyle), Lyttleton evolved from Cowling's pioneer effort the first satisfactory model of a main sequence star and gave the first correct specification of the boundary conditions in stellar interiors. He has made many contributions to the problems of the interstellar medium, particularly in the examination of the interaction between stars and interstellar matter and the problem of accretion. His work on comets, on which he has evolved almost the only existing truly physical theory, is widely known and he has made many contributions to the problems of the constitution of the Earth and the planets. Lyttleton is also well known for his studies in cosmology; indeed there are few subjects in contemporary astronomy which have not been stimulated and enriched by his work.

A Royal Medal : Dr. J. C. Kendrew, F.R.S.

Dr. Kendrew has for many years conducted an intensive investigation of the molecular structure of sperm-whale myoglobin and has the distinction of having revealed for the first time the full three-dimensional arrangement of the atoms in a protein molecule. This successful culmination of more than 20 years' work is a landmark in the history of biology. It is now possible to interpret much of the biological function of this molecule in chemical terms and to begin to see how the many activities in living organisms which are controlled by proteins can be explained at the molecular level.

Kendrew and the group working in the Medical Research Council Unit at Cambridge have given a powerful impulse to the establishment of molecular biology as a new branch of science. This group has brought together in the elucidation of macromolecular structure a large number of different techniques and, in particular, have shown how X-ray diffraction can extend the range of structural analysis down to the atomic level, even for the most complex molecules, by the use of electronic computers. Kendrew's success is an indication of his insight in the choice of a particular molecule on which to concentrate his efforts, but he has constantly directed attention to the general principles of macromolecular architecture which are exemplified in the structures he describes. • It is largely

* Delivered at the Royal Society on November 30.

due to him that the ultimate solution of the problem of form and function in biology is no longer a matter for the philosophers but is a goal which can be reached by patient experimentation.

A Royal Medal : Dr. H. C. Husband, C.B.E.

Dr. Husband is distinguished for his work in many aspects of engineering, particularly for his design studies of large structures, such as those exemplified in the radio telescopes at Jodrell Bank and Goonhilly Downs. He has achieved great success in his co-operative work with the scientists in the evolution and construction of these instruments which have been so important to British science and technology. Husband was presented with the concept of the large radio telescope in 1949 and agreed to undertake the design and construction of the fully steerable 250-ft. aperture paraboloid, at a time when several other engineers had rejected the problem as practically insoluble. The scientific specifications presented him with a complex of interesting structural, mechanical and electrical problems which had never before been faced. His work on these problems and on those later arising in the smaller precision telescopes is typical of his skill in co-ordinating the efforts of engineers and scientists in the solution of unusual structural difficulties, which has led to the successful completion of many public works in Britain and abroad. He brings to this work some of the width of interest shown by the great engineers of the nineteenth century, together with the persistence so necessary for success in such fields.

Davy Medal : Prof. H. W. Thompson, F.R.S.

Prof. Thompson's major contribution to chemistry has been the application of spectroscopy, especially infra-red and Raman, to a wide range of chemical problems, although he has also been responsible for important pioneering work in gas kinetics and in photochemistry.

The early spectroscopic work involved the determination and assignment of the vibrational frequencies of small molecules, the calculation from these of the force constants of chemical bonds and the correlation of both with structural features of molecules. He can justly be described as one of the pioneers in the application of infra-red absorption spectroscopy to the problems of structure and analysis of larger molecules, for which he had to devise experimental methods which at the time were novel. These techniques involved improvement of methods for measuring the spectra and also the devising of methods of handling samples in all phases and over wide temperature ranges. During this period Thompson made substantial contributions to the establishment of characteristic group frequencies covering many structural classes of chemical and biological interest. He was the first to study polymers in this way and to use polarized infra-red radiation for the study of oriented polymers and crystals. A novel and fascinating extension of this work was his combination of the reflecting microscope with an infra-red spectrometer to investigate minute crystals and fibres.

He has also made extensive measurements and theoretical analysis of the rotational fine structure of vibration bands of small molecules in the vapour phase, in order to obtain the molecular constants and geometry, and to extend the theory of molecular energy-levels. Some 20 years ago, he was a pioneer in the use of the newer photoconductive cells so as to obtain much higher resolution in the infra-red. Some of the resulting data on small molecules are now accepted for secondary wave-length standards, by the international commission concerned. The command of these techniques permitted him to execute a lengthy programme on the intensities of vibrational absorption bands of key groups, and their interpretation in terms of bond polar properties and molecular-

structural features. He was one of the first to construct a photoelectrically recording Raman spectrometer, and has measured Raman spectra in studies of molecular structure, for the determination of molecular vibration frequencies, and in studies of intermolecular interactions.

More recently, he has used spectroscopic methods to investigate intermolecular forces and the formation of complexes, using solvent effects on vibrational spectra, pressure-broadening studies on rotational lines, the measurement of hydrogen bonding equilibria, and other systems.

Hughes Medal : Prof. D. H. Wilkinson, F.R.S.

Prof. Wilkinson is outstanding in the group of experimental nuclear physicists who started research in Britain in the closing years of the Second World War. At that time he was enrolled as a member of the British atomic energy team working in Cambridge, and he very quickly established himself as an investigator of originality and drive. Wilkinson has made a very detailed study of the validity of charge independence in nuclear physics, and in a long series of papers (written in collaboration with his students) he has analysed isobaric spin selection rules applicable in many nuclear transitions. Recently he has developed a mass formula applicable to the components of iso-spin multiplets.

In recent years his interests in nuclear structure problems has led to a series of important experimental studies of short-lived excited states of light nuclei using pulsed beam techniques. Wilkinson has also made contributions in the field of high-energy physics. Perhaps more than any other physicist he has been quick to see the relation between the concepts of high-energy physics and those of nuclear physics. For example, in a series of papers he has analysed the validity of parity conservation in strong nuclear interactions.

He has been particularly fascinated by the possibility of using elementary particles as probes for the study of nuclear structure. He pointed out that certain observations on the capture of *K*-mesons by complex nuclei received their natural interpretation on the assumption that nucleons in the surface of nuclei tend to form closely knit clusters, and he has proposed methods of studying the detailed structure of the nuclear surface by measurement of the intensity of *K*-mesic X-rays. These methods appear likely to be of considerable importance when stronger beams of *K*-mesons become available.

Wilkinson has also made important contributions to the discussion and evolution of national policy in relation to nuclear energy and high-energy physics, and recently acted as chairman of a representative committee to report particularly on future British policy with respect to high-energy accelerators.

PRESIDENTIAL ADDRESS

No doubt more than one view can be taken of the purpose of the President's address. Some might consider that a specific scientific field should be singled out for pointed comment and a large, if figurative, sign erected to indicate clearly the road ahead. Others might expect some philosophical discussion of difficult contemporary problems while still others, possessed of the necessary cynicism characteristic of modern sophisticated society, will know that to-day the address is largely to fill in some 30 minutes or so before the very important manoeuvre of inducting my successor.

I propose to devote this time to considering some of the affairs of the Society, but I wish to avoid giving any impression that even now I know everything about how the Society works, still less that I can instruct my successor and his colleagues in how to deal with the many problems which confront it.

While preparing for to-day I re-read Sir Cyril Hinshelwood's masterly Tercentenary address (*Nature*, 187, 274; 1960). He gave a summary of the past activities of the Fellows of the Society, he touched on the functions performed by an Academy, and he mentioned the uncertain and unknown future of science and its relationship to creative thought and even to the evolution of society. He said, after discussing specialized organs of Government and the universities, "an Academy, therefore, is a natural body to provide for several vital things; non-commercial periodicals for the publication of discoveries, a measure of financial support for ideas still too embryonic to be of immediately obvious practical application, the mutual stimulus of association and discussion and the immaterial award of honour for intellectual achievement".

Perhaps I can begin by discussing 'financial support for embryonic ideas'. The Society serves a useful purpose by dispensing sums of money which are in fact almost negligible compared with those now handled by a number of Government agencies. In spite of the small amount of money available, the grants we are able to make are greatly valued by the recipients, and Fellows are glad to devote their time to the careful scrutiny of applications. From 1947 until 1955 Council was able to grant about 66 per cent of the amount applied for. In the year ending March 1965 the figure was 36 per cent. Expressed another way, to have granted last year as big a proportion of requests as 10 years ago would have needed £170,000 instead of the £100,000 which we had at our disposal. It should be added that the drop in the number of people we were able to support was not due to any falling off in the excellence of the proposals made to us. Perhaps our recent experience merely underlines what is becoming only too painfully obvious, and that is that the resources the country is prepared to devote to scientific activities are insufficient to meet the demonstrable current needs, and present indications are that the amount of money available is not likely to increase substantially in the foreseeable future.

Perhaps the deployment of Government resources is the modern equivalent of events in the early days of the Society when Fellows contributed—or sometimes did not contribute—a shilling a week towards demonstrating experiments at meetings. There never was enough money.

We are now all familiar with the large financial backing that has to be put at the disposal of those who wish to pursue what is called 'big science'. Even the general public seems prepared to back these expenditures, or at least no loud outcry against them has arisen.

The idea that very expensive experimental equipment must be shared at some central institute or laboratory is fully accepted on both a national and an international basis, and in the most advanced form of expensive physics we are approaching the time when co-operation on a world scale will be achieved for the construction and working of gigantic apparatus. But perhaps we should be taking a sharper look at whether more could not be accomplished by greater national and international integration in smaller, though still expensive, scientific endeavours. So far as scientific discoveries are concerned it should not matter whether they are made in Oshkosh or Vladivostok. The same, of course, cannot be said of technological innovations or developments, simply because the world is divided into fiercely competitive units each one trying to outdo the others for economic reasons. Nevertheless, within the framework of contemporary political systems, much more could, I believe, be done to forward discovery by making use of selected environments throughout the world in which concerted efforts could be deployed. By this I mean that instead of spreading our national resources too thinly between too many institutions we should concentrate on 'centres of excellence', and from firm national bases international 'centres of excellence' could be

formed. We might then hear less of 'brain drains', and if some of our best people did go to work in other countries, their going would be balanced at least partially by a reverse flow.

This might not be entirely unpractical in dealing with, for example, institutions in the United States with which scientists of Britain have such good relations and with which it is easy to communicate. It should not be insuperable in dealing with Western Europe where, although something has been attempted, we are still making far too few conscious efforts to organize co-operative work. At the present time we are even deficient in the amount of scientific discussion we have with our near neighbours. Is it that we are unwilling to become good Europeans? If so we should consider whether this is wise.

When we look at the Iron Curtain countries we have to be content with relatively little. Agreements reached with the U.S.S.R. and other communist countries spell out in detail exchange arrangements with which we are all pleased, but real international working has scarcely been touched.

I am sure that most, if not all, hard-headed administrators of science would look on the accomplishment of what I have dimly outlined as far too Utopian to contemplate at all. And yet the Royal Society prides itself on its extensive international connexions, its contributions to the International Council of Scientific Unions and its fostering of the exchange of scientific ideas. I wonder if the time might not come in the next 15 or 20 years when the Society might find it to be for the benefit of scientific endeavour to lead in the direction of greater international co-operation, not only at the conference-level but at that of the bench. Perhaps we could play a real part here.

But of course Britain needs something not only in which scientists and technologists can take a pride but in which the general populace, who now have to provide the resources, can also be interested and about which they can be informed. What an inestimable boost to national morale the great programmes for the exploration of space must give in the United States and the U.S.S.R. Is it now quite beyond our capacity as a nation to become really mentally and physically stretched to the limit in cultivating some field of activity? Can we canalize any great scientific adventure of which the whole country could be proud? Could we make a really great mark in exploring the under-water world, for example, rather than making what at present seem to be somewhat pathetically inadequate, though scientifically and technically competent, efforts to enter the same fields as the great rocket powers? Could not a country that mounted the great *Challenger* expedition foster oceanography and the exploration of the possibilities of the sea, its water, and what is beneath it, so that it would be our national pride and joy? But perhaps you are thinking that I am asking too many silly questions.

I will instead make a statement. It is that there is now overwhelming evidence that rapid population growth is bringing with it dire consequences, not only in the great Asiatic countries but probably even here. Evidence is slowly accumulating that the question is not simply whether food can be supplied for an ever-increasing population, but whether overcrowding *per se* does not lead to obscure and so far ill-defined difficulties of mental and social adjustment to a crowded and rapidly changing environment. Observations on animals are certainly pointing to some such possibility. Perhaps we should be paying more attention to the generally unpleasing form that life is assuming in great cities.

It may be that to relate population to environment optimally is the greatest technological task of the end of this century. Its solution will not depend on biological science or medicine alone. It will involve every discipline from economics to psychology. Would the Royal Society serve the nation in a wider sense and science in a narrower sense by taking a greater interest in some of the questions

I have mentioned? To become significantly involved in such matters as these will require more resources than the Society can at present command, and indeed perhaps more than the country conceives that it can afford, though this would depend on the great modern game of 'allotting priorities'.

At the moment it is considered to be desirable to give free medicine to all. The application of free calamine lotion to the irritated skins of the populace may be more important than administering to the needs of irritated scientists; but this sort of judgement is in the realm of politics and, as you know, we do not mix ourselves in these matters here. Still, one cannot help wondering, only of course when we are outside Burlington House, whether the priorities now being chosen are right for the ultimate well-being of Britain.

In connexion with resources for research I need scarcely mention that we have been witnessing during the past 18 months or so a reorganization of the ways in which Government assistance is given for scientific and technological research, development and education. Fellows of the Society might well be advised to take an increasing interest in the way in which these organizational matters are handled, for we may be approaching a state which was popularly advocated some 20 years ago, when it was said that scientists should be on tap and not on top. Perhaps the Royal Society as a collection of active scientists might have something to say on these tendencies.

Coming now to the "immaterial award of honour for intellectual achievement", there is one point that particularly deserves comment this year. This is the first time that the new Royal Medal for merit in the technological field has been awarded. The gracious act of Her Majesty in instituting this new honour is very greatly valued by the Society, for we are particularly delighted to have been enabled by this Medal further to proclaim that the Society wishes to continue to foster the applied sciences.

Perhaps I could now turn to the theme of "the mutual stimulus of association and discussion". During about nine months of the year the Society meets frequently on Thursdays, as is its ancient custom. I suppose that anyone who has sat in this chair, and has been in the privileged position of not understanding the paper being given and so of being able to survey the audience, will have wondered whether these exercises still serve a useful purpose. These doubts seem to be shared by Fellows, for it must be confessed that at times I look around to see how many of them are present and I have long learnt on such occasions not to ask if there is a quorum for conducting the business of the Society. The great growth of specialized societies may have diminished the importance of the delivery of papers at our meetings. Perhaps in view of the multiplicity of calls on the time of Fellows and the numerous activities of the specialized societies we now meet formally too often. Be that as it may, it has long been the policy of the Society to have symposia and lectures, and we are greatly indebted to Prof. W. T. J. Morgan and the Hooke Committee for setting in motion in the recent past the organization of many admirable meetings of this kind. They have grown to such an extent that they have almost succeeded in displacing the delivery of original papers. Some of these meetings have been very popular not only with Fellows but also with many other scientists working in the broad fields covered by the meetings and, thanks to the Tercentenary Appeal and other sources, we have been able to welcome among our speakers prominent scientists from abroad. The popularity of such gatherings has brought difficulties. Thus, on one occasion, we had to migrate to the lecture theatre of the Shell Building on the South Bank. On another occasion we went to the Zoological Society in Regent's Park, and on the occasion of a joint meeting with the Genetical Society we went to University College, London. To all our hosts on these occasions we are much indebted. But one consequence of this peripatetic existence has been that we have had to procure a coffin-

like box for the transport of the mace, and I am sure that our original Fellows, and even Charles II himself, might have been somewhat astonished at the adventures of their royal emblem.

The increased volume of publication associated with these activities has thrown a crushing burden on the editorial staff of the Society, which brings me to another of Sir Cyril Hinshelwood's headings, the support of 'publication'. A number of the symposia and papers at discussion meetings are published as separate issues of the *Philosophical Transactions* and the *Proceedings*, and this puts great demands on Mr. J. C. Graddon and his staff. I should like to take this opportunity to express to them on behalf of the Fellows our gratitude for all their splendid efforts to keep our publications up to their excellent standard. But of course it has not escaped the attention of the Officers that a number of Fellows are dissatisfied with the time taken for publication in our *Proceedings*, especially *Proceedings B*. I would ask Fellows to be patient, and to be sympathetic with those who are trying to produce these journals under considerable physical difficulties. I should like to say, however, that so far as *Proceedings B* are concerned, we lack that vigorous support from Fellows which we could legitimately expect. May I take this opportunity to appeal to those on the biological side to support the journal more than they do at present, either by contributing papers themselves or by seeing that those whom they can influence submit work for publication.

I have now the very great pleasure of announcing the biggest single benefaction that has been received in the history of the Society. The trustees of the estate of the late Mr. M. S. Napier have arranged to transfer to us the sum of £275,000 to be used in the broad field of cancer research. For the first 5 years the income will be used to assist a project at the Institute of Cancer Research at the Royal Marsden Hospital, and thereafter the Society will be free to apply the income in the ways it deems best under the terms of the Trust. We are greatly indebted to the trustees for so munificently increasing our resources for research in biological fields.

There is one matter which affects the future welfare of the Society and of all its Fellows. I refer to accommodation. Before, however, I mention what is happening I should like to make another quotation from Sir Cyril Hinshelwood's address. He said: "There has seldom, if ever, in the world's history been a time when existence was not in some degree precarious, yet the right response to danger lies in action. Faith in the future has indeed a very great survival value. The better equipped are certainly more likely to survive than the worse equipped and not only to save themselves but to save others".

These noble sentiments, if such I may call them, were of great assistance to me and I think to the other Officers and members of Council as we clasped our heads while making some crucial decisions in the so-called 'dog days' of last summer.

You will remember that we had been confidently assured that we should be able to transform the inside of our proposed premises in Carlton House Terrace to meet our purposes for about £500,000. In the summer, after a very careful look at the detailed plans and estimates, it became clear that we could not get into Carlton House Terrace without having raised £850,000. Fortified, at least in my case, by the words I have quoted, the lease for Nos. 6-9 Carlton House Terrace has now been signed and work has begun on transforming the interior. Unless unforeseen circumstances arise, the building should be ready for occupation about July 1967.

In order to proceed with our plans, which those intimately concerned with the Society regard as essential to our future welfare, we shall have to run into a very serious debt unless we receive further generous donations. Steps are being taken to extract us from this extremely difficult position. We have sufficient faith in our friends in the

community in general to feel confident that the oldest scientific Society in the world, and one which in the past has inspired so much scientific work not only in Britain but in other countries, will not be allowed to fail or even falter from the lack of the material assistance which we now so urgently require. Preparations are well advanced for a general appeal not only for the new accommodation and facilities but also for funds to make the best use of them.

I must confess that it is for me a cause of great regret that I shall leave office without having been able to see completed the task of raising the money to equip the Society with the accommodation which it so desperately needs, if it is to continue to play its part and to expand its activities as it should in the modern, rapidly changing scientific world.

The somewhat gloomy thought that possibly through some misjudgement of mine something will now go seriously wrong with the finances of the Society gives emphasis to the fact that I have never ceased to be astonished that the Society should have done me the supreme honour of electing me to this office. I came very ill-prepared for the tasks it involves, and I should not have lasted until to-day but for the very sympathetic understanding and help of all the officers, the support of Council, and the forbearance of the Fellows.

To-day there is what might be called a considerable turn-over in officers. I am most indebted to the retiring 'A' Secretary, Sir William Hodge, and the Foreign Secre-

tary, Sir Patrick Linstead. Both have had a great deal to do with the handling of the ever-increasing activities of the Society. During his period of 8 years as Physical Secretary Sir William Hodge has played a significant part in the Society's contribution to the nation's scientific research programme in space, and during Sir Patrick Linstead's term of office there has been a very substantial increase in the Society's international relations not only within the International Council of Scientific Unions but also in inter-academy relations and in exchange arrangements. I am sure you would like me to express to them the gratitude of the Society for what they have done for it during their term of office. We welcome the new officers and wish them well in what they will find to be an onerous job.

My thanks and those of the Society are, as always, due to Dr. D. C. Martin, Dr. R. W. J. Keay and their colleagues for all the loyal and informed work they have done for us during the past year, often in ways which are quite unperceived by Fellows in general.

It now remains only for me to wish my successor in office as happy a time as I have had. I am sure the Society will face the future with greater confidence than it has had before, for I believe that, by suitable adaptations to the changing environment in which scientific and technological work will be carried out, the Society will have an even more honourable future than it has had a glorious past.

CONSERVATION AND MAN'S ENVIRONMENT*

By DR. IAN McTAGGART COWAN

Dean of the Faculty of Graduate Studies, University of British Columbia

THROUGH the millennia of his birth, man was a poorly equipped, struggling omnivore inhabiting environments which offered special favour. Populations were small, and the product of a limited area was the sole support of its humans.

As man the inventor, however, he added to his inadequate physical attributes a long series of devices which extended his ability as a food gatherer and expanded his environmental tolerance.

With increasing competence, the itinerant hunter-food gatherer in his family group became the neolithic agriculturist. This was certainly subsistence agriculture, but it permitted the first permanent settlements and therewith the first truly man-made environments. The discovery of the river basins with their rich soils led to the production of food surpluses and with these the specialists, the villages and later the cities.

Through these years when every man was an intimate daily participant in the struggle to wrest survival from an unpredictable environment, a rich store of folk images grew from the day-to-day experiences. These guided his biological routines and provided acceptable explanations for the commonplace physical and biological phenomena. He was an observant and rational creature, and here and there across the world developed some effective practices to prolong the food-producing ability of his habitat. These folk techniques, however, were family or tribal in scope and died with the group. In general, early man lacked a concern for the environment, for the creatures in it and for the consequences of man's activity. Great cities were born in the Mediterranean basin and elsewhere, many to be abandoned in a few hundred years as desolate monuments to man's ineptitude. Climatic change has been proposed as explanation for these early failures of

urban man. The overwhelming weight of evidence, however, points clearly to man, not climate, as the agency that let in the desert, or destroyed the capability of the soil.

Then scarcely a century ago, man turned his talents to vital invention. Aseptic surgery, vaccination, public-health measures, antibiotics and chemotherapy introduced a new era in which man emerged as the first creature to influence directly the answers to the ageless question: Who dies, when, and of what? The outcome of these discoveries is clearly revealed in the burgeoning human populations.

It was the thought processes of science that consolidated the era of vital invention and started man on the harried course to large-scale environmental manipulation. The scientific image emerged, frequently in sharp conflict with the folk image of the living world and its relationships. As Boulding has pointed out, even a relatively imperfect shift from the folk image of man and society to a scientific image involves man in at least two large, irreversible, and related changes. The first of these is the increase in self-consciousness, not only of the individual himself, but also of the society in which he has been placed. The second change is the development of the integrated systems point of view, where the world is seen as functioning in an orderly and predictable way, where imposed changes have predictable consequences.

Man is no longer the frail primate, surrounded on every hand by baleful and mysterious forces, wild beasts and pestilence. He glories in his new capacity to go where he wills when he wishes, to conquer all natural obstacles, to guide his own star. We have man the despoiler, the casual pursuer of short-term goals, the arbiter of survival for so much of the world's biota. At the same time, this is man the creator of majestic works, self-conscious man, the only moral creature, man the conservator ready to answer for his errors and to extend the umbrella of his competence

* Substance of an address delivered at the Bicentennial Celebration held in Washington during September 16-18 to commemorate the birth of James Smithson (see *Nature* of October 23, p. 321).

over many lesser forms of life with which he shares his environment.

This, to me, is one of the great revolutions of attitude of all time; man the fearful becomes man the master.

Roots of Conservation

Conservation as we know it to-day is a complicated and interesting area of activity. In very large part, it is the expression of the enlightened self-interest of a population, arising from the understanding, scientifically gained, of the laws of growth, the known facts of population regulation, and the discovery that, for wild crops, as for time, the environment has a capacity which it cannot exceed, but can sustain.

This is conservation as it is properly applied to the living, self-replacing resources on which man can draw for his sustenance, his energy needs and his economic enrichment. The doctrine of wise use is the operating principle. Properly stated, it is that a living resource may not be used at a rate faster than its capacity to replace itself. Sustained yield is the objective of the management programmes in forestry, in fisheries and in wild-life management. In these areas of conservation, self-consciousness is happily bolstered by the profit motive. It profits man in the long run to conserve the renewable resources.

The evolution of the principle of sustained yield has its roots in the folk learning of antiquity, given form, substance and conceptual veracity by science. It first received public acknowledgement as the operating framework of a national policy when, in 1910, President Theodore Roosevelt promulgated what has come to be known as the Roosevelt Doctrine. This recognized all outdoor resources as an inseparable whole, established a public responsibility for the wise use of these resources and declared science as the working instrument to guide public policy.

But though the Roosevelt Doctrine marked the inauguration of the era of scientific conservation, it was itself the outcome of half a century of struggle acted out in the political arena of the United States, as the old concepts of the private right to all public resources were defeated in the devastated forest lands of America, and the role of water on the arid lands of the central continent became established in law. The names of Carl Schurz, U.S. Secretary of the Interior under President Hayes, and of John Wesley Powell are prominent among those who saw the message of conservation boldly written in a troubled landscape nearly a century ago. But, as usual, reason was slow in acceptance, as its adversary was the easy short-term profit where wealth and political influence were bedfellows.

In almost every instance it was born of human tragedy; ghost towns in a chaos of ravished forest land, towering clouds of top-soil which carried with them the hopes of thousands in Oklahoma and the other dust-bowl areas of the world, surging flood waters on the delta lands, stinking rivers carrying sickness to all who used them. Always the task faced by the ecologist in conservation has been to rescue man from the consequences of ignorance, avarice or folly.

The continent is still repaying the debt which was ruthlessly extracted from the landscape in the nineteenth century. "In the forests, as on the ranges, and in the mines, it was every man for himself, and it would take a generation of protest, and a Rough Rider President, to slow down the onslaught and put the get-rich-quick capitalists on the defensive. The nineteenth century lumber tycoons, to give them full credit, housed a growing nation, cleared land, and hastened the pace of westward expansion. However, they set world records for waste, and their prodigal prosperity consumed the stored capital of Nature—which, by right, belonged to other generations" (Udall, 98, 1963).

The other parent of to-day's conservation takes its origin from more complicated sources: moral conscience offended by killing beyond need, religious concepts of cruelty and a genuine concern to retain for our enjoyment creatures whose beauty of colour, form, movement and sound appeal to the senses and give us pleasure. The protectionist movement has its roots as deep in human antiquity as art, music and religion. In its earliest manifestations, it is a folk movement strongly espoused by an ever-enlarging segment of our society. However, along the way, it is gaining the strength of true understanding derived from scientific inquiry and the unarguable power of the market place. People will pay for it.

This aspect of conservation was at first concerned with the protection of birds by the establishment of refuges, but rapidly broadened to encompass the preservation of entire areas of special beauty or unique biota. The concept of the national park emerged as one of the most powerful popular movements of our time.

Perhaps the dominant trend in conservation to-day arises out of our growing realization of the influence human populations have already had on their environment. If indeed we seek mastery of our fate, it is of fundamental importance to control ourselves and regulate our actions as degraders of the potential contribution that environments may make to future generations. The frontier philosophy of do what most profits without thought for to-morrow is no longer tolerated as a working principle by any advanced society.

It is near 30 years since Aldo Leopold gave expression to the "ecological conscience", recognizing each generation not as owners outright of the land and its resources, but as the holders of life rent with the responsibility of wise custodianship without reduction in potential. Ecologists have been slow to involve themselves with a study of man as the dominant influence in the world's terrestrial ecosystems. But, even so, our knowledge of human ecology is growing rapidly, paced by such exhaustive summaries as the Wenner-Gren Foundation report on *Man's Role in Changing the Face of the Earth* (1956); the searching studies of the British scene by the Nature Conservancy; and the recent conference on the "Future Environments of North America" (1964). As the understanding of the ecologists increases, so also does the appreciation of our potential for actions detrimental to human environment.

The complex web of man's impact on his environment defies neat compartmentalization; but there are five areas that, by virtue of differing group interest and research, need justify separate comment: (1) Soil conservation; (2) The role of man in the survival of the biota or of its productive capacity; (3) The ecology of man-made pollution; (4) The maintenance of natural beauty and the opportunity to relate to Nature; (5) The maintenance of genetic variety and the preservation of opportunity to learn.

Soil Conservation

Here and there in time and location, man has gleaned bits of information on the nature of the soil as he extracted his crops from the arid lands or sought his livelihood on the steepening hillsides of an overcrowded habitat. The great drought period of the 1930's in central North America, however, for the first time found man ready for massive, effective, science-based attack on the soil problems of a continent. The Soil Conservation Service of the United States established under Franklin Roosevelt's administration can fairly claim to have changed the face of a continent in its 30 years of existence. In so doing, it has mustered an understanding and a technical force that is carrying its influence to many lands.

Soil conservation in North America has made possible the tremendous food-producing potential of the continent;

but beyond this it has had immeasurable influence on all other aspects of the conservation of natural resources.

Conservation of Species

The expansion of natural history into ecology during the scientific revolution saw the principles on which the idea of conservation rests added to abundantly, both at the operating level and in concept. The community as a vital entity operating in accord with discernible laws that could yield prediction; the idea of the limiting factor, and of density-dependent feed-back between organism and environment, were among those hypotheses that provided new conceptual equipment.

The idea of altering the natural forces that were regulating the lives of creatures other than ourselves is a major landmark in the flowering of human ideas—its emergence marked the transition between simple protection and management; the purposeful attempt to alter the environmental impact on a species or community to produce a preconceived result. Management includes the regulation of the direct or indirect impact of man on the species or community, as well as all attempts to alter such other features of the environment as water, food, shelter, parasites, disease, predation, special facilities, competition or distribution. The objective of management in conservation to-day is much broader than the mere maximizing of profit. Each living organism is seen as the repository of a unique assortment of biological information gained through the eras via the process of evolution. Each offers a potential enrichment of human knowledge, and enjoyment that is limited only by our capacity to appreciate. The loss of any single element in the world's store of varied life is viewed as an erosion of the quality of the human environment.

In general terms, management is directed to the encouragement of those species we desire to assist, to reduce populations of creatures we regard as damaging our interests or to maintain the integrity of an entire assembly of plants and animals; that is, to maintain a community for its riches of species and associations. Species-oriented conservation falls into three main categories: management for survival; management of distribution; and management for harvest.

Management for Survival

In nineteen hundred years the world has lost 107 kinds of mammals, and close on 100 kinds of birds. The extinction of plants and the lesser animals is not known but probably vastly exceeds that of birds and mammals. Nearly 70 per cent of these losses have occurred in the past century and mostly through the activity of man. Here and there throughout the world, on every continent and on many of the remotest islands a host of other species, more than 1,000 strong, faces the imminence of complete and final passage from the world's fauna.

Extinction has been an essential companion of evolution since the beginning of time, and there is no reason to believe that the process is complete. Nevertheless, it is an ideal of conservation that no creature should pass from the face of the Earth through the instrumentality of man. If we would pose as the masters of creation, to prevent extermination of a large and obvious form of life stands as a challenge to our ingenuity and our competence.

There is an element of drama also in the plight of a vanishing creature which captures the imagination. The challenge to aid the troubled species thus has consequences far beyond the retention of its genotype. It becomes an instrument of enlightenment; thousands of people develop an increased awareness of the principles of conservation through identification with the endeavour.

Several special agencies make their particular concern the assembly of all available data on endangered species. The International Union for the Protection of Birds, the

Survival Service Commission of the International Union for the Conservation of Nature as well as many agencies of Western Governments, Japan and several other nations contribute in important degree to the identification of species in trouble and in co-ordinating assistance programmes. The International Union and the World Wildlife Fund muster the skills and organize the support for emergency attempts to redress the havoc man has wrought on wildlife in the farthest corners of the world. In the United States the recent Land and Water Conservation Fund Act provides for the protection of endangered species there. There are substantial successes, but the tasks are huge and without precedent. Species that are in trouble as an outcome of man's alterations of habitat have proved most difficult to assist, as have insular endemics. Long periods of evolution out of contact with the specialized competitors, predators and diseases of the continents have rendered island species most vulnerable to the impact of man-induced changes in environment. Islands are unique and desperately fragile. They require special care. Introductions of exotics, domestic species, or of diseases are almost certain to be catastrophic.

A recent approach to the restoration of endangered species is through the instrument of captive rearing for later release into the wild. It cannot be regarded as the perfect answer as many species are not susceptible to confined rearing, and others have been shown to possess a heritable factor for wildness which may be selected against in captive breeding.

There are two other potential hazards to releasing hand-reared or even wild-caught stock from elsewhere in an effort to increase the numbers of an insecure population. One is the danger of introducing a different and less well-adapted genotype. The second is the risk of introducing disease organisms foreign to the creature.

An ever-present danger of such programmes of introduction or re-introduction is the loss of the genetic distinction of a local endemic form. The widespread transplanting of such native North American species as bobwhite quail and cottontail is suspected of having changed the genotype of some local forms. On occasion, the hybrid has locked the time-earned adaptive features of the local race, and what started as a logical attempt to aid a struggling population became the kiss of death.

We have slowly learned to appreciate the tolerance developed between disease organism and host during eons of mutual evolution. But experience has been a hard teacher and many an organism has suffered the devastating consequence of either our ignorance or our stupidity as we refused to apply what knowledge we had gained. A case in point can be seen in the plight of the largest wild-living stock of American bison in the world. The transplantation of a disease-ridden herd of plains bison into the last remaining stronghold of the wood bison in Wood Buffalo Park, Canada, led to the predicted but ignored consequences. These may not only finish the race but may see the transmission of the disease into clean stocks of moose and barren ground caribou.

It is impossible to foresee the direction that our interests in the biota will take as human tenure of the Earth lengthens, as our population increases, our demands on the resources expand and our understanding of the environment becomes ever more detailed. To-day our concern is for the forest trees, certain more obvious plants, and for the mammals, birds, some fishes and some reptiles. Our knowledge of the ecological facts pertinent to the management of most of these is inadequate and we are totally innocent of the data which would permit us to manage the populations of most of the living creatures of the world. The only tenable approach to the maintenance of the largest part of the biota is through the management of ecosystems rather than individual species.

Where climax situations are concerned, the task, in theory, is relatively simple. On the other hand, the restoration and maintenance of any of the transitory seral stages

in a living community of plants and animals is a task of great complexity. So much so that we are at present almost powerless to plan for the successful ecological management of even the smaller national parks of America.

The usual approach to the conservation of vanishing plants or animals has been to create a refuge or park to contain it and to exclude fire. Special reserved areas have been established to maintain stands of climax redwood forest, Douglas firs, Monterey cypresses, organ pipe cactuses, Joshua trees and the entire flora of some of the Hawaiian craters. These measures are seldom adequate, and the truly ecological view of the objectives is only beginning to enter into planning and administration.

Management of Distribution

In general, a species becomes less vulnerable as its distribution widens. Of special importance is discontinuity of distribution as this protects against the inadvertent catastrophe that can overwhelm a single small population.

We can sometimes foster discontinuity of distribution by carefully selected transplants of a species into unoccupied but apparently suitable habitat.

This, at the same time, provides a unique opportunity for the establishment of a disease-free nucleus population. Natural extinction has been an active process through all existence. Although we have little knowledge of the causes of extinction, epizootic disease is a possibility. Thus, the establishment of disease-free discontinuous populations should give added survival value to the species and will give additional surety to our objective of management for variety.

Management of Numbers

In general, the utilization of a new element in the biological resource still follows the primitive pattern. Thus, for each new species for which we find a use, there occurs first a period of uninhibited exploitation, as if the resource was unlimited. Sooner or later, declining availability arouses concern that the stock will be commercially eliminated. Too frequently, the rising cash value emerging from progressive scarcity obscures the biological situation and renders politically difficult the establishment of measures adequate to restore the productivity of the resource and to place it on a basis of sustained yield management.

Conservation practices designed to manage for sustained yield consist of fact-finding, restrictive regulation and positive management. The important difference between the last two is that, while restrictive regulation is designed to regulate human exploitation of a wild species to a level at or below the mean replacement rate, positive management is oriented toward increasing the production or survival of young, and to lengthening the life of adults of the managed species. In terms of the classical sigmoid of population growth the objective of conservation of a harvested population is to maintain the population at the level of greatest rate of increase while at the same time moving this upward by raising the ceiling imposed by the environment.

Most wild populations exist within a delicately balanced complex of species that make mutual use of the food potential of the environment. Some competition between species is frequent. The consequence of human depletion of certain species is often to promote a new balance within which the preferred species plays a lesser part. A biological vacuum often does not arise, and for this reason it may be impossible to restore the population to its early productivity even under the best of management.

Where the demand is greatest, the concept of maximum sustainable yield has come into being. This may be expressed in terms of number, weight, or cash return.

The simpler task of sustained yield management is that confronting the forester whose product is wood. His

populations are immobile and easily measured; the regulation of size of harvest presents few biological problems of decision. The unique factors are the long period of growth between harvests and, in the north, the vast areas of almost single species forests. These render protection from fire and pestilence the major hazards to success, and the technology of these tasks becomes limiting.

Increasingly, the value of many forests arises not from their primary product but from their contribution to maintaining the integrity of watersheds, as an environment for wildlife and as a place for human recreation. Here the designation of goals is more difficult, and the knowledge demanded for successful management more precise. In only a few areas is adequate research information available.

Few among the world's fishes provide commercially important harvests and for only a handful of species can it be claimed that effective, sustained yield management is in force. In many instances our biological ignorance is inhibiting the development of management routines. For more of the marine fishes the political complications of the multi-national competition for the crop frustrate the application of even existing information to the task of conservation.

The principle of abstention which is being pioneered with respect to certain of the North Pacific fisheries is a useful experiment in international conservation. Under this Canada, Japan and the United States have agreed to abstain from fishing stocks of fish under full use and scientific management by any one of them. To be effective, however, agreements of this sort require the participation of all those nations that are competing for the fish resource of the management area. An important effect of such an agreement is the incentive for additional studies and better management.

The worst example of the failure of conservation, not for want of biological information but from bad faith, commercial avarice and political iniquity, is to be seen in our treatment of the marine mammals of the world. Completely adequate demonstration has been available for at least a decade that species after species among the larger whales is being reduced to the point of extinction, and the industrial potential of the industry thus destroyed. Despite this, the responsible international organization of whaling nations has been repeatedly prevented from establishing the essential conservation measures through the political influence wielded by certain commercial interests bent only on recouping an investment as quickly as possible.

The harp and hood seals of the North American east coast are among the remaining commercially useful seal populations, and they are suffering a similar fate.

Ecology of Pollution

The most insidious influences of man in the environment arises from the disposal of wastes and from the purposeful distribution of biocide chemicals to destroy plants, insects and related organisms regarded as inimical to certain human activities. These two forms of activity have the common denominator of so altering the environment chemically or physically that it is no longer a suitable habitat for many native forms of life and is often damaged as a habitat for man himself.

Egler (1964) has stated that "the problem of pesticides in the human environment is 95 per cent a problem—not in the scientific knowledge of pesticides, not in the scientific knowledge of the environment, but in the scientific knowledge of human behaviour". It is a combination of apathy and organized stupidity frequently motivated by the market-place. The same can be said for pollution in the more usual sense. The devastating consequences of the ecological ignorance that fosters and permits such action was realistically presented to millions of people the world over by Rachel Carson in *Silent Spring* (1962).

Again to quote Egler: "In general, we have acted with remarkable arrogance to the whole of Nature of which we are a part. Any part we do not want, we seek to destroy, completely and utterly. . . . With the destruction of each such 'pest' by the use of the handiest, cheapest, most quickly acting pesticide, goes the destruction of anything else about which we do not care at the moment, or the eventual destruction of other things about which we may care but by such remote side-effects that the actual connection can be disputed".

The problem is of world-wide scope and increases with the rise of human populations, but is most intense in the sophisticated societies with the most advanced chemical industries.

Despite the growing public awareness that there are grave consequences from our present introduction of destructive chemicals into the ecosystem, the manufacture and distribution of these are increasing annually. There is no limit to our ingenuity in designing new forms in which we can introduce chemicals into the complicated web of our ecosystems, while we are powerless to influence where they will travel and impotent to remove them.

The biological destruction of rivers and lakes through the introduction into them of sewage and the chemical effluent of industry has aroused widespread public concern. The problem has become a national emergency in many countries and has generated powerful corrective efforts. Despite local success, the pollution of fresh waters remains one of the most devastating consequences of civilization.

Nowhere else in our relationship with the biological world in which we live are the lines of our understanding, our communication, our sources of political action, our economic ambitions, our biases, and our fears more hopelessly interwoven. It is difficult not to despair that an economic society is impotent to prevent the pollution of land, air and water which we now support or condone. Viewed the world over, mankind to-day is indeed managing his environment. This management, however, is not the outcome of a studied attempt to proceed toward a desired objective; it is rather the cumulative result of varied extemporizing, unplanned and unco-ordinated, directed toward the satisfaction of the immediate need. Individuals, societies and governments frequently compete and promote conflicting attitudes and acts of strong environmental consequence. We are completely without any well-defined and generally accepted philosophy to direct our specific behaviour toward our surroundings.

Even our governments are not organized to react effectively to a comprehensive management of our actions as they influence the human biophysical environment. We are geared for local crises; the epidemic, the crop failure, the forest fire, the devastation of riparian lowlands by flood, all trigger prompt action by some appropriate authority. These are crises easily seen and understood, dramatic in their impact on our immediate desires. The environmental changes of the greater ultimate importance take place so gradually, insidiously, unobtrusively that they escape our attention until irreparable harm is done. Cumulative contamination of the environment by the waste products of our factories, kitchens and bathrooms; gradual destruction of wild-life habitats with all they contain; the sprawling blight that flows from our cities farther and farther into the countryside; the indestructible wastes of our technology that beer can after 'auto-carcass', plastic bottle after 'pliofilm' bag spread filth over our beauty spots, these have not yet reached that point in public understanding where consensus can lead to effective corrective action.

Man and the Enjoyment Resources

The world of to-day falls, perhaps loosely, into two categories of human societies. There are those which, despite improvements in scattered technologies, are concerned, at the level of the average individual, with the

day-to-day task of staying alive. For them, it can be truly said that the immediate objectives and concepts have changed little since the days of human origin. The concern is living, not the quality of life. At the other extreme are those fortunate societies that have evolved through science and social ingenuity a competence that has to a very large extent banished the folk fears of starvation and pestilence and introduced new horizons to the image of life. Concern has shifted to the richness of experience that any individual can expect from his environment. It has become a proper objective of all mankind to equalize, as far as possible, the opportunity available to all individuals in all societies. There is no gain for man, however, if equalization is downwards.

The logical concomitant of this principle is that those societies that have progressed farthest in the search for quality of living should exercise a concern extending far beyond their borders. Mankind's to-morrow will be found on the world scene, not within the parochial confines of a contemporary political unit. The contribution to the food stocks and to the economic potential of a country that is to be found in its renewable consumptive resources makes these obvious first candidates for attention. But, Sir Julian Huxley has so well said: "Human ecology involves finding out what resources are available in our environments and how to make best use of them. We have to think first of all of the material resources—minerals, water power, soil, forests, agricultural production—but we must also think of the non-material or enjoyment resources of the habitat, such as natural beauty and solitude, interest and adventure, wild scenery and wildlife.

"We should set about planning a Fulfilment Society rather than a Welfare Society, an Efficiency Society, or a Power Society."

It can safely be said that one of the important criteria of an advanced society is its devotion to the maintenance of the ecological well-being of the human environment in all its attributes. Prominent among these will be the non-consumptive uses for recreational enjoyment and scientific enrichment.

The recognition of the deep need of man for opportunity to associate himself with Nature first occurred as a revulsion from the stark surroundings of the factory environment which became the lot of the majority during the Industrial Revolution. The easily accessible commons, though not then recognized as such—for psychiatrists were as unknown as jet propulsion—became psychiatric safety valves for the toiling thousands of Britons.

A century ago, the land-grabbing aristocracy of Britain, who had already taken to themselves one acre of every seven in the nation, attempted to enclose Wimbledon and Epsom Commons. This was the touchstone to a legal battle of classical import. Henry Fawcett, M.P., and professor of political economy in the University of Cambridge, championed the cause and saw in it the great principle that was at issue—the public right to open space reserved in its name. The legal battle was fought between the Corporation of London and fourteen lords of manors who sought to divide Epping Forest. The Corporation won the suit and established the all-important legal principle on which so much of our more recent conservation legislation has rested. The Act of 1876 permanently declared in Britain the public interest in open spaces as taking precedence over private desires. Since 1925, British law has given to the public a statutory right of access for air and exercise on every common or place of manorial waste and to any rural common (Gibson, 1964).

An ocean away, the practical dreamers of the New World were forming ideas of similar philosophy. With the expansiveness of thinking that accompanied the great spaces, American concepts spread from such fine civic beauty spots as New York's Central Park to California's Yosemite and the magnificent two million acres of the first National Park—Yellowstone: all this before 1870.

The first voices also were decrying two and a half centuries devoted to plundering the natural resources of North America. The buffalo herds were gone, as were the vast flocks of passenger pigeons; but worse, none had successfully challenged the view that the natural wealth of the biological resources was free for the taking; the continent's devastated forests were prime testimony.

It took the combination of a brilliant, visionary forester, Gifford Pinchot, and Theodore Roosevelt, a president of the United States with deep roots in the wilderness, to turn the tide and to introduce the concepts of conservation. Among the first large-scale tangible results was the establishment of the great National Forest system of the United States. Designed to produce timber for the long-time good of the nation, these forests now contribute richly to the recreational lands of the continent. None in the new world could then foresee the crowded cities, the airports, super-highways, the clatter, speed and tensions to come, and the desperate need of people to find themselves again in the impersonality of unspoiled landscapes, in the surging vitality of many small lives.

"Modern man is turning almost instinctively to the last remnants of the primeval scene, to know again the mystery of the unknown and the beauties of unchanged terrain. While it is doubtful if his ancestors appreciated the intangible qualities of wild country, he is developing that capacity. Now that wilderness is no longer a threat to security or survival, he is beginning to look at it for the first time with some measure of appreciation and understanding, realizing that within it may be the answer to confusion and a source of inspiration closely allied to beauty. . . . National Parks, as reservations of beauty, are sanctuaries where people may recapture, at least in part, some glimmer of the visions that may have stirred their forebears" (Olson, 1962).

With pathetic frequency our groping hands have left irreparable scars on the beauty we sought to serve. Super-highways, garbage dumps, hydroelectric impoundments, mining and deforesting (cattle grazing), are only a few of the incongruous and destructive activities we have condoned in our parks, but we have hammered out some principles along the way.

(1) On the world scene the national park concept has usefully served many objectives. The most frequent has been the preservation of endangered species where this depends on intact segments of entire ecosystems. A new and exciting concept has emerged on the American continent. National parks, as we now view them, may have great value as museums of ecology, as wild-life reserves, archaeological sites or for the protection of natural wonders, but their first function is to provide the setting, the beauty, timelessness and natural order in which man can regain the perspective he needs.

(2) The national parks belong to all people and no part of the policy that guides their operation should be oriented to provide private profit to local residents.

(3) An attested ecological objective to guide park policy is essential. The Leopold Committee urges that this be to retain or restore the ecological conditions obtaining when the region was discovered.

(4) Protection of park values from increasing hordes of users is among the most challenging problems to-day. Zoning for quality of use and the limitation of access are growing necessities.

(5) The social organization requisite to the protection of State or Provincial parks whose policy and survival can be altered on executive whim has so far eluded our political ingenuity. On the American continent, where the political voice of the *entrepreneur* is loudest, the integrity of all parks required the constant vigilance of militant citizen groups.

(6) For the economically oriented, it has been shown beyond doubt that well-mannered national parks pay dividends beyond their operating costs and may, as in parts of Africa, form the basis of a major industry.

At this point, the conservation road forks again into the scientific and the aesthetic. With or without our consent, the evolutionary process will continue, new forms will arise and others vanish, most of them without our ken. The advent of man introduced a new and dominant force into the biosphere. By his selection as by his modification of the environment he has greatly altered the *tempo* and nature of evolutionary change. It is certain that the practical consequences of the revolution in biology will further increase our capacity for positive influence in the evolutionary process. But this does not mean that we should ignore the challenge to interfere with the consequences of our actions or even to deny to Nature the right to extirpate. The world's store of genetic material is seen as an inexhaustible source of novel combinations which can be used for the future benefit of man. Each genotype lost before evolution has replaced it is another step in the degradation of our environment. This is an expression of our pragmatic concern with conservation.

The ecologist sees yet another reason for attempting to preserve intact samples of the various biotic communities.

The task of extracting the ecological truths is far from complete and new techniques offer constant new opportunities to search more deeply. Lost segments of the ecosystem take with them their unexposed truths. Our opportunity to learn and to understand is permanently impoverished. Strong reasons, therefore, can be advanced for conserving segments of all major communities for the sole purpose of research.

In the other direction, it is being ever more emphatically asked why man should have to seek beauty only in far places. There is creative capacity in man that, if given full rein, could replace much that is sordid and ugly in our urban environment with beauty, clean air and green space.

As the history of this age is written, conservation as a concept will be regarded as perhaps the greatest contribution of the New World to human ideas. For the idea had its birth and saw most of its evolution in the United States of America. In a century Powell's vision of sustained-yield forestry spread and adapted to encompass human contact with the entire living world. It gained depth of perception and an almost religious fervour from the Marshs, Muirs and Thoreaus, and scientific rationale from the host of naturalists and ecologists that have emerged from the universities of the northern hemisphere. It provided the banner around which rallied all those whose vision of the man at his finest involved a sensitive integration into the biophysical world. The Sierra Clubs, Audubon Societies, Wilderness Societies and Unions for the Conservation of Nature have given power to the cause without which the concept could not have found political and physical expression.

But the cause is far from won, and at an increasing rate the twin forces of a burgeoning technology and a surging human population are posing ever more difficult problems for the conservators to solve. At an increasing rate we pollute the land, the air and the sea, convert our rivers into sewers and spread our indestructible wastes along the remotest shores. An urgent challenge to our ingenuity is the disposal of our wastes.

Over vast areas of the world even the most elementary conservation concepts have still to penetrate. Here fire and destructive agriculture rapidly narrow the gap between man's numbers and his food supply; balanced ecosystems are degraded to uselessness, biotas vanish for ever.

We have not even approached the fascinating, but vital, problem of man in an enclosed ecosystem. In a very real sense we are denizens of a space capsule to which nothing enters but solar energy. What population of men will the renewable resources of the world support? At what level does the addition of another million reduce rather than increase the quality of human life? These

are questions as close to the core of morality as to conservation. To attack them at all demands the attention of the finest ecological, sociological and political minds we can muster. The answer is urgent, as each passing decade brings us either further on the down grade or nearer the asymptote. We know not which.

It has been relatively easy to find support for conservation on the American continent, where our man-to-space ratio has been low, hunger has not been an alternative where an acre was allocated to quality of living rather than to food for survival, and where the economic advantages were obvious. The pressures will change as the alternatives gain more immediacy.

Central to conservation on this continent is the gradual change in the legal view of the rights of the individual in relation to the long-term benefits to society. In this context certain recent interpretations of the Supreme Court of the United States of America have the greatest significance. That the individual as a member of a society retains only those liberties specifically allocated to him by the society is an interpretation with broad impact in the natural sense. There still remains in many quarters, however, the narrow interpretation that only consumptive use is really use and should take priority in competitions. There is the demand also that even the aesthetic qualities of our lives

should be justified in dollar values when alternative uses of land are an issue. Conservation lives in both our worlds, the economic and the aesthetic; the contribution of the ideology is equally to both, but the standards of comparison are probably invalid.

It is the unique revelation of man that he is not only consciously sensitive to his own environment, but also relates himself to much larger and more complex processes in which he plays a part. His image of the world then becomes an important element in the processes of the world itself.

A central element in our vision of the kind of world we would inhabit is the ideology of conservation. Within it we find values that we will defend and ideas that we seek to propagate. Man has come full circle from the unwilling participant in the processes of survival to become the only creature whose vision influences those processes. Conservation and other ideology have played so important a part in human affairs and hold so much for man's future.

Even in those unfortunate areas of our own land and others, where avarice and ignorance still triumph, the achievements and ideals of conservation stand as a constant reminder of what could be. Self-consciousness once awakened can no longer be escaped.

OBITUARY

Academician Eugene N. Pavlovsky

THE death of Eugene Nikanorovitch Pavlovsky on May 27, 1965, in his eighty-second year, deprived Soviet science of one of its most eminent representatives, who dominated the scene of medical zoology in his country for more than half a century and whose outstanding contributions in the field of biology and parasitology found recognition throughout the world.

E. N. Pavlovsky was born on March 5, 1884, in the town of Birjutch, Voronezh Province. After receiving his secondary education at the Borisoglebsk Gymnasium, where he gained a gold medal on matriculation in 1903, he was admitted to the Military Medical Academy of St. Petersburg. Though primarily intended for the training of army doctors, the Academy was—and still is—one of the greatest medical schools in Russia, where young Pavlovsky studied under some of the most renowned medical scientists, including I. P. Pavlov, the physiologist, A. A. Maximov, the histologist, and N. A. Cholodkovsky, the zoologist.

Pavlovsky showed an early interest in zoology, and while still a student he started the research work in the laboratory of Prof. Cholodkovsky on the anatomy of lice and on the poison glands of arthropods and fishes, for which the Academy awarded him a gold medal. On graduating with distinction in 1909, Pavlovsky was appointed assistant to his teacher, N. A. Cholodkovsky, and in 1913 he took the M.D. degree with a thesis on the structure of poison glands in arthropods, and was elected Privat-docent. The first period of Pavlovsky's scientific career was devoted mainly to investigations on venomous animals, for the study of which he devised refined methods of manual dissection, described in a series of handbooks and recently in *Methoden der Sektion von Insekten* (Berlin, 1960). The results of some of this work were incorporated in his M.Sc. thesis of the University of Petrograd, and eventually in an important monograph on venomous animals published in Germany (1927).

In the meantime, on the death of Cholodkovsky in 1921, Pavlovsky succeeded him as professor of biology and parasitology (as the chair was renamed). During this period, the upheaval caused by the First World War

and then by the civil war brought about an aftermath of pandemic outbreaks of malaria, typhus and other infectious diseases which were ravaging the young Soviet Republic. Pavlovsky responded to the urgent necessity of dealing with the disastrous situation by directing all his energy and knowledge to problems of public health, thereby initiating the second, and most fruitful, period of his scientific activities, to which he dedicated the rest of his life. From 1928 onwards Pavlovsky and his teams carried out more than 160 parasitological and epidemiological surveys throughout the U.S.S.R. These expeditions revealed a close connexion between diverse arthropod-borne infections of man and lower mammals, which were shown to be zoonoses (for example, seasonal encephalitides, relapsing fevers, leishmaniasis), and laid the foundation, in 1939, of the concept of natural foci (or focality) of transmissible diseases, which was Pavlovsky's most outstanding achievement.

He demonstrated that some of these diseases are naturally localized in foci within well-defined geographical areas (biotopes), where the pathogenic organisms, their vectors and hosts form an ecological association (biocenose) within which the infection circulates for indefinite periods of time from donor-hosts via vectors to recipient-hosts—represented by wild animals—quite independently of man. It is only when human beings intrude on such an enzootic locality or settle down to live there that they expose themselves to the attacks of the vectors, which then transmit to them the parasites acquired from the wild reservoir hosts. On the basis of this discovery, Pavlovsky formulated his idea of "landscape epidemiology": since natural enzootic foci are characterized by definite geographical peculiarities of the terrain, these can serve as indicators of the presence in a given area of certain diseases presenting a potential danger to man. The examination of the geographical background of zoonoses enabled Pavlovsky and his associates to detect and control numerous foci of human infections within the Soviet Union. It has also inspired a great volume of epidemiological investigations throughout the rest of the world. The recognition of its importance is reflected in the creation of centres for the study of zoonoses in South America, the United States and East Africa, as well as at the World Health Organization.

To Pavlovsky also belongs the credit of developing an ecological approach to the phenomenon of parasitism, based on the recognition of the organism of the host as the environment of its parasites. He introduced the original concept of parasitocenoses, concerning the mutual relations between the components of the parasitic fauna of a host. One of the great merits of his scientific activities was the application of scientific principles to the solution of problems of practical importance in public health, which resulted in the local eradication of certain diseases. Pavlovsky was the author of more than 1,000 publications, devoted to parasitology, entomology, microbiology, epidemiology, toxicology and evolution, as well as to the history of biology and medicine, and including several monographs and text-books. Some of his most important works have recently been brought together in three volumes (in Russian): *General Principles of Parasitology and Zoology* (1961); *Collected Papers on Experimental Zoology and Toxic Animals* (1963); and *Collected Papers on Experimental Parasitology* (1963), as well as in the following English versions: *Human Diseases with Natural*

Foci (Moscow, 1963) and *Natural Nidality of Transmissible Diseases, with special reference to the Landscape Epidemiology of Zoonanthroposes* (in the press: U.S.A.). Pavlovsky was also an outstanding teacher, who created an imposing school of Russian parasitologists.

In addition to the professorship at the Military Medical Academy, which he relinquished in 1958 with the rank of Lieutenant-General in the Soviet Army Medical Service, Pavlovsky was for many years director of the Zoological Institute of the Academy of Sciences and president of the Geographical and Entomological Societies of the U.S.S.R. He was also an honorary member of several academies of science and of numerous learned societies outside his own country. For his outstanding scientific achievements he was elected a member of the Academy of Sciences in 1939 and of the Academy of Medical Sciences of the U.S.S.R. in 1944; he was awarded the honorary degree of D.-ès-Sc. of the Sorbonne, and was the recipient of many prizes and medals, including the Darwin-Wallace Medal of the Linnean Society in 1959.

CECIL A. HOARE

NEWS and VIEWS

Civil Engineering in the Welsh College of Advanced Technology, Cardiff: Prof. J. D. Geddes

DR. J. D. GEDDES, senior lecturer in civil engineering materials in the University of Newcastle upon Tyne, has been appointed professor of civil engineering in the Welsh College of Advanced Technology, Cardiff, from January 1. Dr. Geddes gained a first-class honours degree at the University of Newcastle upon Tyne in 1949. Later, after work with consulting civil engineers, he returned to his *alma mater* to work on the subject of piled foundations; he was awarded a Ph.D. for this work at the age of twenty-five. His research was carried out under the Radley postgraduate studentship of the Institute of Civil Engineers, of which body Dr. Geddes already held the distinction of a Miller Prize. In later years he was to be awarded prizes and a Bronze Medal by the Institution of Structural Engineers for his work on mining subsidence. After a year or two as head of the Federal Laboratory of the Public Works Department of Nigeria, controlling all testing and site investigation, he again returned to the University of Newcastle upon Tyne and, for the past 10 years, has been head of the Materials and Building Science Division of the Department of Civil Engineering in the University. His researches have covered mining subsidence and its effects, heat flow through concrete, stresses in buried pipelines, the part played by grout in pre-stressed concrete construction, and studies in site investigation. Dr. Geddes has controlled an effective testing laboratory run by the Department as a regional service, and has forged a bond between the profession and the University. He has found time to become an authority on the history of the British regiments and will return in the spring to give a University lecture on the subject in Newcastle. His new students will also welcome his contributions to *Problems in Engineering Soils*—a text-book soon to be published under joint authorship.

Fast Reactor Agreement

THE United Kingdom Atomic Energy Authority and Aktiebolaget Atomenergi, Sweden, have concluded an agreement for exchange of information and collaboration on fast reactor research and development. Information to be exchanged concerns design and operating experience from research and experimental reactors, basic physical and chemical properties of materials for use in fast reactors

and reactor physics, as well as operating experience with certain fuels. The agreement will be effected by means of reports and visits. There may be exchanges of staff from time to time. The progress of the agreement will be reviewed periodically at meetings between representatives of the Authority and Aktiebolaget Atomenergi.

Ministry of Technology

IN written answers in the House of Commons on November 30, the Minister of Technology, Mr. F. Cousins, listed sixteen projects on which he had required the Atomic Energy Authority to carry out research at estimated costs varying from £500 to £73,000 during the financial year 1964-65 and totalling more than £377,800. These projects involved 108 people full-time or part-time, and accounted for about 1 per cent of the Authority's effort on civil research and development in 1965. In addition, work on some minor projects had been authorized, and certain preliminary investigations were being made, while Aldermaston was undertaking non-atomic work on repayment for Government departments, mainly in the defence field, to the amount of about £600,000 in the present year. Desalination research, to the extent of £278,000, was proceeding at Winfrith, Culcheth, Harwell and Risley; work is also under way on hydrostatic extrusion (£54,000) at Springfields, on a medical centrifuge (£15,000) at Capenhurst, and on a design study for scientific payload for the European Space Research Organization satellite (£41,000) at Culham and Aldermaston. The Authority's expenditure on civil nuclear research and development is given as £47.9 million. Of the £35 million shown in the Authority's civil trading account as the proceeds of sales for the year ended March 31, 1965, 60 per cent is attributed to fuel elements for civil reactors, 20 per cent to electricity and 6 per cent to radioactive isotopes. Of the £6.5 million expenditure on the Ministry of Technology in the past 12 months, £4.5 million was for research stations, just under £2 million on grants for research to research associations and other bodies, and £650,000 on the Ministry's headquarters. Appropriations in aid amounted to £850,000.

Mechanical and Electrical Engineering

IN a written answer in the House of Commons on December 1, the Prime Minister, Mr. H. Wilson, stated that the Ministry of Technology would now assume responsibility for sponsoring the mechanical and electrical

engineering industries as a whole, as well as for engineering standards. On these, it would deal direct with the British Standards Institution, but it would not deal with the Institution's work on consumer protection. It would also take over from the Board of Trade the responsibility for determining standard weights and measures. The central co-ordination of measures to promote the growth of industrial productivity, through the National Economic Development Committees and by other means, was a primary concern of the First Secretary of State and Secretary of State for Economic Affairs, but departmental Ministers carried direct responsibility for encouraging productivity in those industries which their departments sponsored. The Minister of Technology would, accordingly, have this responsibility for the mechanical and electrical engineering industries. The Board of Trade would continue to deal direct with these industries on exports, commercial relations and on its other functions and responsibilities which affect industry as a whole.

Scottish Hydroelectric Schemes

IN a statement to the House of Commons on November 29, the Secretary of State for Scotland, Mr. W. Ross, said that he had completed his study of the report into the Enquiry into the Fado-Fionn and Laidon hydroelectric schemes. The report concluded that neither scheme was needed, at least up to 1975, because adequate capacity would be provided by large new stations which must be commissioned by that time elsewhere in Scotland. These would provide power at a lower cost than either of the projected schemes. The assumptions on which these schemes were assessed are subject to change with economic circumstances. Other factors affecting the pattern of electricity generation might also arise out of the continuing development of methods of generation and the fuller exploitation of pumped storage. While, in the light of the report, Mr. Ross was not prepared to confirm the schemes now, he did not regard this as precluding their reconsideration in the future, and when more information was available about future generation plans the North of Scotland Hydro-Electric Board would be free to submit them again.

Colour Television

IN a written reply in the House of Commons on December 1, the Postmaster General, Mr. W. Benn, stated that the Television Advisory Committee had recommended to him that colour television be introduced on 625 lines only, using the PAL system of transmission, and that he was now considering this advice.

British Government Aid Overseas

IN reply to questions in the House of Commons on November 30, the Minister of Overseas Development, Mrs. B. Castle, said that we had now reached the second year of the arrangement under which the British Government contributed £250,000 a year for three years to the recurrent costs of University College, Salisbury. Since the College provides higher education for students of all races in Rhodesia, and operates under Royal Charter, the Government had decided to continue its assistance to the College so long as it is satisfied that conditions continue to exist under which the College is able to discharge this function. If it became clear to the Government that the multi-racial function of the University was being undermined, the Government would reconsider the allocation of aid. British Government contributions in 1964-65 of £6.5 million to the International Bank for Reconstruction and Development, £5.1 million to the International Finance Corporation, £45 million to the International Development Association, £22.7 million to the United Nations Expanded Programme of Technical Assistance and Special Fund, £31.3 million to the United Nations Relief and Works Agency, £3.1 million to the United

Nations Children's Fund, £10.1 million to the United Nations Korean Reconstruction Agency, £1.5 million to United Nations Assistance to the Congo, £1.2 million to the World Food Programme and £11 million to the Indus Basin Development Scheme, brought the total contributions to multilateral agencies since 1946-47 to £137.5 million. Official aid and private investment in developing countries amounted to 1.1 per cent of Britain's gross national product in 1954.

The Bodleian Library

THE annual report of the Curators of the Bodleian Library for the year ended July 31, 1964, notes an overall increase of 10,000 on the previous year's figures for issues of books and manuscripts to readers and staff, while an increase in the number of newly admitted readers was almost wholly in those who were not members of the University. The ratio between members and non-members, for long steady at 3 : 1, is now almost 5 : 2 (University of Oxford. Pp. 28. Supplement No. 9 to the *University Gazette*, August 1965. Oxford: The University, 1965. 2s. 6d.). Besides lists of publications by members of the staff during the year, the report refers to the work of the Department of Western Manuscripts and to that of the Department of Printed Books, where accessions totalled 56,244 volumes and 110,330 parts, compared with 44,826 and 121,276, respectively, in 1962-63; 293,909-microfilm exposures, 16,460 photostats and 712 bromide prints were made from library material and from manuscripts deposited for the purpose.

Lord Nuffield's Benefactions for the Advance of Medicine

THE *Report for 1963-64* of Lord Nuffield's Benefactions for the Advance of Medicine (University of Oxford. Pp. 28. Oxford: The University, 1965) includes lists of staff and publications by the Department of Clinical Medicine, the research interests of which are in gastroenterology, haematology and population pathology. Other Departments, the publications of which are listed, include Surgery, Obstetrics and Gynaecology; Anaesthetics; Orthopaedic Surgery, Pathology, Clinical Biochemistry, and the Nuffield Institute for Medical Research. Research projects in these departments are also briefly listed.

Soils and Foundations

AN important application of soil research concerns the behaviour of soil particles under the influence of loading, and a recent publication, entitled *Soils and Foundations: 1 (Building Research Station Digest (Second Series), No. 63. Pp. 6. London: H.M.S.O., 1965. 4d.)*, deals with the numerous factors that interact with each other and must be kept in mind in building-design. The movement of water in the soil is determined by its texture and by the pressure exerted by a foundation load. Shrinkage and swelling may operate simultaneously, as when structural load is equal to weight removed in excavation. Settlement of the load is rapid on sands but may continue for many years on clays. The differences in settlement between strips, rafts and piles are discussed. Attention is also directed to shrinkage and swelling brought about by rainfall and vegetation and removal of trees on a building site, and the tendency for outside walls to move and produce cracks. Other matters dealt with include peat soils, made-up ground, frost heave and 'quick-sand' conditions.

Timber as Building Material in Hot Climates

THE behaviour of timber used as a building material, under what may be called 'aggressive' environmental conditions, has long been a matter of discussion and practical importance; it is a subject of almost endless research. In Africa the effects of climatic extremes on

timber have engaged the attention of Dr. A. J. Du Toit, research officer, Timber Unit, South African Council for Scientific and Industrial Research, Pretoria, who has published a paper, *The Use of Timber as a Building Material in Hot Climates* (National Building Research Institute. Reprinted from *RILEM Bulletin* No. 25, December 1964. Council for Scientific and Industrial Research. Ref. No. RU I.13). Dr. Du Toit points out that, in Africa, two types of hot conditions can be distinguished, the arid hot type, such as is encountered in the region between Timbuktu and Agadès, and the humid hot type in the region between Douala and Abidjan. The average temperature in both these regions is approximately 80° F, while the average humidity is approximately 35 per cent in the dry region and 83 per cent in the humid region. Obviously wood, being a hygroscopic material, will react quite differently in these contrasting climates. The theme of this paper is that wood can only give satisfactory service as a building material if its properties and behaviour in varying circumstances are known and well understood. There exists a certain amount of prejudice against the use of timber as building material in tropical countries, but this may be partly due to lack of knowledge of its reactions in such environments on the part of builders. It is considered that correct application of present-day knowledge of the properties and behaviour of various woods under tropical conditions would do much to overcome this prejudice. Such information includes primarily climatic agents: the effects of temperature, humidity, rain, exposure to sun, evaporation, dust storms, cyclical influences, maritime influences, direct effects of sea-water (continuous or alternating immersion), saline atmosphere, spray; and biological agents: cryptogams (rot, mould, algae, lichens, fungi), insects (termites, borers, etc.), and vegetation. Dr. Du Toit discusses all these agents, and his paper contains much practical advice on ways and means of protection of timber exposed to these potentially destructive forces, including notes on various West African woods considered to be resistant at least to some of them.

Progress in Protozoology

THE second international conference on "Protozoology", held in London during July–August 1965, discussed many aspects of this subject. Protozoologists the world over will welcome the publication of abstracts of the many papers read at the Conference, entitled *Progress in Protozoology (Abstracts of Papers read at the Second International Conference on Protozoology, London, July 29–August 5, 1965)* (International Conference Series, No. 91. Pp. 278. Amsterdam, New York, London, Milan, Tokyo and Buenos Aires: Excerpta Medica Foundation, 1965. 65s.). Prefaced by a message of welcome and goodwill from Prince Philip, the volume discusses so many aspects of the biology, morphology and ecology of a wide variety of Protozoa that it is impossible to summarize its contents in a brief note. An idea of the number and variety of the papers read may be gained from the fact that the index of authors belonging to many nationalities covers two pages, each with four columns of names.

Antimalarial Prophylaxis in Eastern Nigeria

EVERYONE concerned with the world-wide campaign against malaria, a disease that was once aptly described as 'public enemy No. 1', will be interested in Dr. Hay Arthur's account of antimalarial prophylaxis in primary schools in Eastern Nigeria (*Antimalarial Prophylaxis in Primary Schools in Eastern Nigeria*. February–March, 1965. Pp. 41. London: Wellcome Museum of Medical Research, 1965). Dr. Arthur shows that in the suburbs of a town in Eastern Nigeria, where one out of every two children is infected with malaria, the number of children suffering from the disease can drastically be reduced by a regular weekly dose of 'Daraprim'.

Human Intelligence

SOME reasons why the evolution of human intelligence should be a continuing process have been described by Prof. Sheldon C. Reed, director of the Dight Institute of Human Genetics at the University of Minnesota. When parents' income, education, social status, or I.Q. is correlated with the number of children they produce, correlation coefficients are obtained which are negative in sign. These well-established negative correlations are statistically biased and seriously misleading, because they ignore the important segment of the parental generation which had no children. The negative correlation (-0.30 ± 0.02) between family size and intelligence of the children in it has no meaning for the next generation because the mentally retarded children will have little income, education or social status; they will seldom marry or have offspring. The mentally retarded who do produce may have large families, but the average number of children produced by the retarded is lower than that of normal persons. It is impossible to determine what is happening to the intelligence of mankind as a whole, but it is clear that it is not decreasing at an appreciable rate. Prof. Reed's main conclusion is the perhaps euphoric concept that genetic orthoselection for higher intelligence is continuing at the present time. The challenge of a complex world, with an ever greater premium placed on higher intelligence, should result in an acceleration of the rate of evolution toward that end (*American Scientist*, September 1965).

International Commission on Zoological Nomenclature

NOTICE is hereby given of the possible use of its plenary powers by the International Commission on Zoological Nomenclature in connexion with the following cases, full details of which will be found in the *Bulletin of Zoological Nomenclature* (22, Part 3; November 2, 1965): (1) Suppression of *Drassus atropos* Walckenaer, 1830 (Araneae). (2) Validation of *Pan* and *Panthera* from Oken, 1816 (Mammalia). (3) Neotype for *Ceratophyllus soricis* Dale, 1878 (Insecta, Siphonaptera). (4) Suppression of *Euclidaris* Pomel, 1883, *Papula* Bayle, 1878, *Cidaritis papillataconoidea* Parkinson, 1811, and *Cidaritis savignyi* Audouin, 1826 (Echinoidea). (5) Type-species for *Phasia* Latreille, 1804 (Insecta, Diptera). (6) Suppression of *Papilio lintingensis* Osbeck, 1765 (Insecta, Lepidoptera). (7) Type-species for *Monopsyllus* Kolenati, 1875; suppression of *Geratopsyllus sciuri* Kolenati, 1856, *Monopsyllus sciuri* Kolenati, 1857, and *Ceratopsyllus monotenus* Kolenati, 1856 (Insecta, Siphonaptera). (8) Type-species for *Stizus* Latreille, [1802–1803] (Insecta, Hymenoptera). (9) Type-species for *Diodontus* Curtis, 1834 (Insecta, Hymenoptera). (10) Type-species for *Trychosis* Foerster, 1868 (Insecta, Hymenoptera). (11) Type-species for *Prospaltella* Ashmead, 1904 (Insecta, Hymenoptera). (12) Suppression of *Mullus auriflamma* Forskal, 1775 (Pisces). (13) Type-species for *Chamaemyia* Meigen, 1803 (Insecta, Diptera). (14) Suppression of *Xyleborus* Bowdich, 1825 (Insecta, Coleoptera). Any zoologist who wishes to comment on any of the foregoing cases should do so, in writing, within five months, to the Secretary, International Commission on Zoological Nomenclature, c/o British Museum (Natural History), Cromwell Road, London, S.W.7, England. Those received early enough will be published in the *Bulletin of Zoological Nomenclature*.

Selective Control of Weeds in Rice Fields

IN vast areas of the world, rice is the basic food crop. There are as many different varieties of rice as there are different ways of growing it; but wherever it is cultivated, weed control has always been, and still is, a primary concern of the rice grower. The most universally prevalent and troublesome weed to contend with in rice fields is what is known as barnyard grass; this is so much like rice that even experienced farmers find some difficulty in

distinguishing one from the other in early stages of the growth of the plant. Until comparatively recent times, traditional methods of weed control in rice fields were hand-weeding and flooding; herbicides were tried, but it was found that many of these, which were effective with barnyard grass, had the unfortunate side-effect of also killing the rice. A few years ago a remarkable, highly selective post-emergence herbicide for use on rice was developed by Rohm and Haas known as 'Stam F-34' (3',4'-dichloropropionanilide); this compound is unique in its selective activity in rice; it kills grasses and broad-leaf weeds, but not the rice. An illustrated article in *Rohm and Haas Reporter*, entitled "How Stam Works", explains the facts (23. No. 3; May-June 1965. Rohm and Haas Company, Philadelphia, Pennsylvania; Lennig Chemicals, Ltd., London, W.C.1). 'Stam' is supplied as an emulsifiable concentrate; after dilution with water, this herbicide is applied as a spray, generally by aerial means. The mechanism by which this product operates is briefly and technically described: simply stated, 'Stam' is deactivated by an amazing enzymatic system in rice; barnyard grass and other weeds infesting rice fields are not able to protect themselves, so they wilt and die, and the rice flourishes. The contrast between a field treated with this product and an untreated plot is strikingly shown in one of the coloured photographs illustrating this article; in sprayed fields, the mature rice crop is seen as a clean stand of golden, rice-laden heads; untreated fields are described as "splochy with green and brown—with weeds often choking out the rice plants". It is claimed that when 'Stam' is properly applied to a grassy rice field a substantial increase in yield and a finer quality of rice may be expected.

University of Oxford: Vice-Chancellor's Oration

THE Vice-Chancellor's Oration, which prefaces the annual report for 1964-65 in Supplement No. 3249, of October 1, 1965, to the *Oxford University Gazette* (University of Oxford. Oration by the Vice-Chancellor and Annual Report, 1964-1965. Pp. 37-56. Supplement to *Oxford University Gazette*, No. 3249. Oxford: The University, 1965. 2s.) emphasizes the value of the work of the Franks Commission and refers also to the appointment of a Committee on University Libraries under the chairmanship of Mr. R. Shackleton. The annual report, besides noting the appointment of this Committee, refers also to the creation of new graduate societies and to entitlement to fellowships as recommended by the Norrington Committee and to grants from the Common University Fund to the poorer colleges. Matriculated students in residence increased by 487 on the corresponding figures for 1963-64. Candidates entered for the Final Honours School in engineering numbered only 50 compared with 72 in 1963 and 62 in 1964; for physics, the corresponding figures are 140, 135 and 159, respectively; for chemistry, Part I, 180, 141 and 165; for chemistry, Part II, 147, 130 and 115; for biochemistry, 14, 17 and 18; and for mathematics, 130, 91 and 117. An additional £70,000 was made available to departments for 1964-65 over and above normal growth in expenditure on salaries, including about £28,000 for new proposals. Most of this went to the creation of 16 new posts of lecturer status, 5 in physical sciences and 2 in biological sciences, and 5 faculty lectureships. A new Diploma in the Science of Materials has also been introduced.

Royal Society of London: Montague Napier Trust Fund

THE Council of the Royal Society has accepted the offer by the Trustees of the late Mr. Montague Napier, one of the pioneers of the motor-car and aeroplane engine industry, of a capital sum of the order of £275,000. A new trust fund will be formed with this sum, the income from which will be used for research, with the view of ascertaining the cause of cancer, including any corresponding or allied disease and the means of its preven-

tion, cure and alleviation. In accordance with the wishes of the Trustees, the income from this Montague Napier Trust Fund for the first five years will be transferred to the Institute of Cancer Research for the purpose of developing the bridge of collaboration, which already exists between the Foyal Marsden Hospital and the Institute, for the support and development of those fields of cancer research and cancer treatment generally referred to as 'clinical research'.

Christmas Holiday Films and Talks on Geology

A SPECIAL Christmas holiday programme of films and talks will be held at the Geological Survey and Museum, South Kensington. The programme will include: "The Underwater Search" (December 28, film); "North Slope, Alaska", and "Fire Fight at Ahwaz" (December 29, films); "Volcanoes in Britain" (December 29, illustrated lecture); "Landforms from the Air in Canada and Iceland and New Zealand" (December 30, films); "Continents on the Move" (December 30, illustrated lecture); "The Forth Road Bridge" (December 31, film); "Geology with a Camera" (December 31, illustrated lecture); "The Story of Camp Century" and "The Glacier Climbers" (January 1, films); "The Search for Oil in Nigeria" and "Rig Move" (January 3, films); "The Forth Road Bridge" (January 4, film); "Channel Tunnel" (January 4, illustrated talk); "First Catch Your Fossil" (January 5, demonstration). Further information and tickets can be obtained from the Museum Lecturer, Geological Survey and Museum, Exhibition Road, South Kensington, London, S.W.7.

Announcements

SIR WILLIAM HILDRED, director-general of the International Air Transport Association, has been awarded the Edward Warner Award for 1965 of the Council of the International Civil Aviation Organization, for outstanding contributions to the development of international civil aviation.

ENTRIES are invited for the 1966 "Interaction of Technologies" award, made jointly by the Shell Chemical Company and the British Association for the Advancement of Science. The prize of 250 guineas, and a discretionary second prize of 100 guineas, will be awarded for the best paper submitted, before February 28, 1966, on the subject "The Problem of Technological Barriers". Further information and forms of application can be obtained from the Secretary, Shell/B.A.A.S. Award, Shell Chemical Co., Ltd., Shell Centre, Downstream Building, London, S.E.1.

A MEETING of the Burton-on-Trent Section of the Institution of the Rubber Industry will be held at Burton-on-Trent on January 31. Further information can be obtained from the Institution of the Rubber Industry, 4 Kensington Palace Gardens, London, W.8.

A COURSE of eight evening lectures on "Modern Physical Aids for the Chemist" will be held at the John Dalton College of Technology, Manchester, commencing on February 8. Further information can be obtained from Mr. P. R. Falkner, John Dalton College of Technology, Chester Street, Manchester 1.

A SEMINAR on "Relations Between the Administration and the Scientific Establishment", arranged by the Science of Science Foundation, will be held at the Ciba Foundation on January 17. Further information can be obtained from the Science of Science Foundation, c/o the Ciba Foundation, 41 Portland Place, London, W.1.

A SYMPOSIUM on "Scientific Aspects of Pest Control", arranged by the National Academy of Sciences-National Research Council and other United States Federal agencies, will be held in Washington during January 31-February 3. Further information can be obtained from the Agricultural Board, National Academy of Sciences-National Research Council, 2101 Constitution Avenue, Washington, D.C.

VISUAL OBSERVATION OF SATELLITES

ON October 20 there was an all-day discussion meeting at the Royal Society on the subject of visual observation of satellites. The meeting was organized jointly by the Royal Society and the British Astronomical Association, under the chairmanship of Prof. C. W. Allen (University of London Observatory), and was the first gathering of visual observers of satellites to be arranged by the Tracking Working Group of the British National Committee on Space Research.

The first subject discussed was the purposes of making observations. This topic was introduced by D. G. King-Hele (Royal Aircraft Establishment, Farnborough), who explained how visual observations of satellites fitted into the general pattern of satellite observations, and how they could best be used in geophysical studies. Investigations of the changes in satellite orbits have brought about great advances in two quite different branches of geophysics: the study of the Earth's gravitational field and the upper atmosphere. Visual observations will probably not be accurate enough for future investigations of the gravitational field; but they are quite accurate enough for most examinations of upper-atmosphere density and temperature, and, for the vast majority of satellites, visual observations are the only ones available. Visual observations, therefore, continue to be of great scientific importance, and since a large number of observations, ideally about 100 per week, are needed to determine accurately the orbit of a satellite, it is probably best for the limited number of visual observers to concentrate their attention on a relatively small number of satellites, particularly those with lifetimes of less than about twenty years.

Although the determination of orbits is the most important use for visual observations, nine other uses were briefly mentioned, including the improvement of predictions, observations of re-entry, the identification of satellites, observing satellites made of materials which are almost transparent to radar, observing satellites from the same launch which pass over very closely together, timing fluctuations in brightness, and various other uses.

The second subject on the programme was the techniques of observing. First there is the problem of searching for the satellite, especially when the predictions are in error, and the various stratagems for overcoming these difficulties were discussed. Next there is the question of accuracy. Since visual observations are not so accurate as those made by cameras, visual observers always have to strive for the best possible accuracy. Normally, observations are made relative to a pair of reference stars, and it was generally agreed that the best accuracy can be achieved by noting the time and position of the satellite as it passes two stars very close together, such that

the line between them lies perpendicular to the satellite's track. The accuracy to be aimed at by a visual observer, even though it will not often be achieved, is about 2 min of arc in direction and 0.1 sec in time. There was also discussion of the number of observations which an observer should make. Two observations are better than one if they are equally accurate, but there is less to be gained by making a large number of observations because of the bias error in timing. Discussion on the types of instruments used by observers showed very clearly that this is a matter of personal preference and depends more on the temperament of the observer than on objective scientific criteria. Many observers use binoculars and say that they can see twice as well with two eyes as with one, while other observers prefer to use telescopes, though their optical design and mountings are very varied.

The next topic discussed was the timing of observations, introduced by H. G. Miles (director of the Artificial Satellite Section, British Astronomical Association). Visual observers normally time their observations by starting (or stopping) a stop-watch at the time of the observation and synchronizing it with standard time signals, such as the *MST* radio signals or the Post Office speaking clock. The various possible errors arising from both stop-watches and time signals were reviewed, and the general conclusion was that the various time-standards at present used by observers are just accurate enough to introduce no significant error in visual observations, which can rarely expect to have an accuracy better than 0.1 sec. The stop-watches could introduce errors unless care was taken in rating them frequently, and running them for as short a time as possible before or after the observations.

The final subject for discussion was predictions, introduced by Mr. D. E. Smith (Radio and Space Research Station, Slough). Mr. Smith described some of the difficulties encountered by the Satellite Prediction Service at the Radio and Space Research Station and explained improvements which it was hoped to make in the near future. In the discussion which followed, many suggestions were made for particular improvements in the prediction service, and these suggestions will be taken into consideration in deciding future policy.

The meeting was attended by twenty leading British visual observers, four observers from other countries in Europe who report their observations to the British Prediction Centre, members of the kinetheodolite teams at the Royal Greenwich Observatory, Herstmonceux, and the Royal Observatory, Edinburgh, and representatives of various other organizations concerned with satellite tracking.

D. G. KING-HELE

SCIENTIFIC AND INDUSTRIAL RESEARCH IN NEW ZEALAND

IN the report of the Department of Scientific and Industrial Research, New Zealand, for 1965*, the Director-General records a staff on March 31, 1965, of 1,334. Of these, 511 were scientific officers, and the total represented an increase of 32 in the year. In all there were 562 technical officers, and of the 1,334 staff, 175 were employed in the Physics and Engineering Laboratory, 172 in the Chemistry Division, 91 in the Grassland Division and 90 in the Geological Survey. The total expenditure on these four Divisions was £343,611,

£310,086, £141,697, and £163,887, respectively, out of a total expenditure by the Department of £3,024,792. Expenditure on the Geophysics Division, with a staff of 82, was £195,950; on the Plant Diseases Division (68), £127,327; on the Soil Bureau (79), £152,516; on the Antarctic Division (23), £105,380; on the Crop Research Division (58), £107,332; and on the Information Service (47), £104,867. Expenditure on the incorporated Research Associations amounted to £230,840—the major share going to the Dairy Research Institute (£74,205) and the Wool Research Organization (£75,000).

Steady progress has been made in implementing the recommendations of the Mineral Resources Committee,

* New Zealand. Report of the Department of Scientific and Industrial Research for the year ended 31 March, 1965. Pp. 88. (H. 34.) (Wellington: Government Printer, 1965.) 3s.

and equipment is being built up and the staff strengthened. Sand deposits have been surveyed both for their economic value and also to collect information for shoreline research. The detailed survey so far has covered much of the east coast of Northland, where the modern shore and off-shore flats contain between 50 per cent and 75 per cent of lime soda feldspar. Clay deposits near Coalgate and Hororata in Canterbury, examined by the Geological Survey and Chemistry Division, contained an excellent grade of bentonite. A new series of geological maps of the main cities is being prepared and the Seismological Observatory is investigating the mechanisms of earthquakes, including theoretical research on alternative mechanisms which could cause a sudden change in the conditions of elastic equilibrium within the Earth. Earthquake occurrence has been subjected to statistical analysis, with respect both to position and to time, and members of the Applied Mathematics Division co-operating with the Geophysics Division are making a statistical analysis of the patterns of occurrence and location of earthquakes.

The Physics and Engineering Laboratory is developing a method of presenting information on the radiation output from various types of heaters for guidance in calculating the effect of the radiation on the comfort of people, by engineers and architects. The Chemistry Division has also developed automatic apparatus for separating the two types of silver and cupro-nickel coins in circulation in view of the change-over to decimal currency.

A detailed analysis of results obtained from the Bluff radar during the International Geophysical Year revealed a number of new and unsuspected features in the pattern of behaviour of the radar aurora and gave the first clues as to the over-all pattern. Increases in ground radioactivity from nuclear weapons tests continue to be studied, while the principle of the electromagnetic flowmeter has been applied to the measurement of blood flow in the heart lung machines of the cardio-thoracic surgical unit at Green Lane Hospital and also for measuring fluid flow in the artificial kidney.

Soil studies by the Grassland Division have shown that organic phosphorus may progressively accumulate under permanent pastures forming about 60 per cent of the total phosphorus in such soils. A survey of the vegetation and soils of the Tongariro National Park, initiated in 1960, is now nearing completion. A new variety of oats named 'Mapua', bred at the Cross Research Division, was selected from a 1953 cross of 'Milford', a lodging-resistant Welsh variety, and 'Onward', a plump-grained English oat. In full-scale trials since 1957-58 it was superior to all present varieties, highly resistant to lodging and of reasonably good milling quality. Since 1962 the Division has also produced three promising hybrid varieties of onion for field trials, and commercial release of New Zealand Hybrid No. 1 has already been requested. From crosses of turnips with aphid- and mosaic-resistant swedes, turnips have been selected with high field tolerance to these diseases. The work of the Plant Diseases Division on heat therapy for the control of post-harvest fruit rot has concentrated on hot-water treatment for pip fruits and peaches. With apples and pears *Botrytis* rots were not controlled at temperatures tolerable to the fruits, but the method would appear to have applications in the control of stone fruit brown rot in peaches, especially canning peaches. The new compound zeatin, extracted from the sweet corn kernels, has now been shown to be 6-(4-hydroxy-3-methylbut-2-enyl) amino-purine.

As a preliminary to detailed study of the movement of behaviour of red deer and other animals, the Animal Ecology Division is developing techniques for capture and marking. The Tobacco Research Station has undertaken a programme of breeding for resistance to *Verticillium* wilt black root rot, *Phytophthora* and mosaic virus.

Included with the Director-General's report are reports from the branches and from the incorporated research associations. The seventh annual report of the Ross Dependency Research Committee is appended. Lists of publications are included in the reports from the various branches.

FOREST HYDROLOGY

AN Advanced Science Seminar supported by the U.S. National Science Foundation was held at the Pennsylvania State University, State College, during August 29-September 10. The symposium was conducted by the School of Forestry with three other bodies: the North-eastern Forest Experimental Station, United States Forest Service; the International Association of Scientific Hydrology; and the International Union of Forest Research Organizations. Twenty-one countries were represented at the symposium by eighty-five participants, the largest single group being from the United States. Eighty-five papers and reports were delivered and the proceedings will be published during the next nine months. Sound organization and critical discussion stimulated by several invited scientists made this a successful meeting.

Many countries are concerned about the adequacy of existing and future water resources, and in most, research is being conducted into these and other problems in hydrology. Particular attention has been given to investigating the hydrological significance of different types of land use and, during the past 30-40 years, the hydrological role of forests has been studied in various parts of the world. Some well-known experiments have been conducted, particularly in the United States, Switzerland and South Africa, but investigations commenced more recently have had the benefit of modern instrumentation and better techniques from the start. The aim of these experiments is to compare the water balance of forest

catchments with adjoining catchments of a different vegetational character by showing differences in the volume and distribution of run-off. Now, energy budgets for catchments are being drawn up in addition to water balances and, with the use of neutron scatter devices for soil moisture measurement, considerable advances are being made in the study of forest hydrology. The importance of research of this type and also the timely nature of the symposium were emphasized by the severe water shortage in New York City and surrounding areas during August-September.

The symposium commenced with a review of activities in each country represented. In Israel, a country of few trees, difficulties arise from lack of water, in contrast to Taiwan with too much water and many trees. This distinction between countries where water conservation is important and those with the problem of soil conservation was most apparent. In many countries there seemed to be an unnecessary division of responsibility for hydrological research among a number of different organizations.

The second session considered forests and precipitation—the forest being regarded as a redistributor rather than a stimulator if occult precipitation was not taken into account. The problems involved in assessing precipitation over a catchment were described by Corbett (U.S.A.) using material from San Dimas Watershed. Leonard (U.S.A.) was concerned with the mathematics of inter-

ception, while Leyton, Reynolds and Thompson (Great Britain) showed that the reduction of transpiration from wetted leaves was exceeded by the accompanying increase in evaporation. Through-fall, stem-flow and interception in different stands as related to gross precipitation were given attention with the distribution of moisture in forest soils. Such matters as surface detention and drip studies, canopy cover measures and through-fall drop sizes were touched on by Zinke in a thorough review of interception investigations carried out in the United States. Snow in forests was considered in relation to transport processes and the mechanics of interception, and Martinelli (U.S.A.) gave an account of methods of increasing high altitude snow packs.

In the session on forests and soil water, the difficulties involved in defining and separating components of the hydrograph were evident in papers dealing with overland flow and sub-surface flow. Hewlett (U.S.A.) devised a response factor for classifying the hydrological performance of drainage basins in Appalachian forest areas, while Zahner (U.S.A.) dealt with estimation of moisture régimes for three forest soils, by using a balance between rainfall and potential evapotranspiration with the appropriate soil moisture release curve. Field determinations of moisture régimes beneath different vegetation types were described by Shachori (Israel), who showed that a greater reduction in moisture content was caused by maquis than by pine or pasture. There were five papers on various aspects of forest management and yield as related to soil moisture and ground water. In two of these, rise in the water table after thinning of pine and birch stands to various densities was reported, first by Holstener-Jørgensen (Denmark) for a clay area and then by Heikurainen (Finland) for peat.

Penman (Great Britain) started the session on forests and evapotranspiration by comparing theoretical evaporation from forests with observed results. Records from the Castricum lysimeters and two East African forest catchments were used to indicate how calculated evaporation values approached the difference between rainfall and run-off. Baumgartner (Germany) showed energy reasons for differing evapotranspiration rates over various types of surface, and he demonstrated that the energy available for evaporation at a grass surface was only three quarters of that for a forest. A six-year investigation of the evaporation from a Scots pine stand in south-east England was described by Rutter, and the result ($E_T = 1.20 E_0$) was accounted for in terms of the increase in evaporation of intercepted water over the reduction in transpiration. Knoerr (U.S.A.) solved the energy balance equation for a single leaf for different temperatures, humidities and stomatal resistances, and Swanson (U.S.A.) gave an example of the use of the heat-pulse velocity method of sap flow measurement in his investigation of transpiration from pine and spruce. The value of the dual approach on water balance and energy budget lines was apparent from Pereira's account of land use hydrology experiments conducted in East Africa. Various aspects of the relation between evapotranspiration and forest management (including the use of anti-transpirants) were dealt with in several papers. In one, Waggoner (U.S.A.) reported that chemical sprays used to close stomata caused a 20 per cent reduction in transpiration from single trees, but that a method of treating whole forests had to be found before a significant increase in run-off and water yield could be achieved.

In the 'Forests and Run-off' session, Lull and Sopper (U.S.A.) used records of discharge from a large number of small catchments in the north-east United States, with topographic and other characteristics of those catchments, in a series of multiple regression analyses to evolve a method of predicting various parameters of run-off. Ogiwara (Japan) was concerned with predicting flood discharges from forest catchments, and, by using a non-linear relationship which included a measure of catch-

ment wetness and rainfall, he was able to show the effect of forest cutting on floods. Differences in the volume and distribution of run-off from cleared north and south facing catchments at Coweeta were mentioned by Hibbert (U.S.A.), and later it was suggested that these differences might be linked to the small size of the cleared areas, making them unrepresentative. A second Japanese paper (Nakano) dealt with clearing forest catchments and showed that the effect of cutting in increasing flow is statistically significant at the 5 per cent level only for the first few years.

Several papers in the session on forests and soil stabilization dealt with the factors controlling erosion, such as rainfall characteristics, topography and litter cover. The quantitative measurement of erosion was discussed and the use of Middleton's dispersion ratio and Anderson's surface aggregate ratio mentioned. Margaropoulos showed how small dams and woven barriers with contour planting had been used to control erosion in Greece and the potential of high altitude sites in Austria for re-establishment of forest was reported by Aulitzky. In this paper it was reported that measurements of solar radiation had been made in excess of the solar constant by 18 per cent for periods of 10–20 min. Vegetational control of erosion in loess areas and strip mined areas of the U.S.A. was described along with New Zealand investigations of erosion.

Errors in run-off measurement, calibration methods for catchments and data processing of stream-flow were subjects discussed in the first papers in the instrumentation and techniques session. Anderson (U.S.A.) reported on new techniques in multivariate analysis applied to determining sediment yield. Wicht discussed the validity of the South African catchment experiments, but doubted that a method of analysis had been evolved which could readily show the effects of burning, cutting or the development of forest cover. Papers on digital hydrological synthesis and the use of isotopes concluded the session.

A panel discussed a variety of new instruments including several neutron scatter devices, a pressure pillow for snow measurement, snow depth markers that can be read from the air, a photo-electric device for measuring snow in the air, a snow resistograph, and an economical radiometer fitted with a mercury current integrator. A display of these and other instruments took place at the end of the symposium.

Films of a number of experimental areas in North America were shown, including one giving vivid portrayal of the fire at the San Dimas Watershed. Several experimental sites were visited, including a flood-prevention project and a waste-water renovation project. In this latter experiment, treated sewage effluent was being sprayed over croplands and over a forest area and there had been considerable increases in yield and growth, as well as appreciable ground water recharge.

From the papers and discussions it was apparent that a considerable effort had already gone into forest hydrology. In this context it is perhaps surprising that for a country with a rapidly accelerating demand for water, Britain has had such a limited research programme for evaluating relationships between the hydrology and land use of catchment areas. Although some worth-while investigations of forest hydrology have been carried out, not one has been on a catchment scale and has involved a land use change. Several experiments of this type are proposed in the programme of the Hydrological Research Unit, Natural Environment Research Council, but owing to administrative difficulties they have not yet been started. These experiments should considerably assist the understanding of the hydrological cycle in a forest environment, as they will incorporate recently developed methods of automatic data collection and analysis. It is hoped that these new methods will help in overcoming the handicap of Britain's late start in forest hydrology.

JOHN C. RODDA

RECONSTRUCTION OF PHASE OBJECTS BY HOLOGRAPHY

By PROF. D. GABOR, F.R.S.

Imperial College of Science and Technology, London

AND

PROF. G. W. STROKE, D. BRUMM, A. FUNKHOUSER and A. LABEYRIE

University of Michigan, Ann Arbor, Michigan

THE principle of wave-front-reconstruction imaging, first described by one of us in 1948 (refs. 1-3), has recently resulted in spectacular advances, notably in the form of three-dimensional 'lensless' photography and imaging, with both macroscopic and microscopic objects³⁻⁸. Excellent images have been obtained in a number of variations of the basic method, notably when the objects used were 'half-tone' intensity objects, or transparencies, rather than 'phase' objects.

'Phase' objects may be of a primary interest in a number of holographic applications, notably in microscopy, and in several other applications, where phase rather than amplitude variations in the light field may be predominantly characteristic of the physical phenomena under investigation, for example, in work with wind tunnels and in acoustical applications.

As holograms are recorded on photographic emulsions which register only intensities, not phases, one might easily believe that a hologram is not a full substitute for a real object. Indeed, the total wave-front which issues from a hologram in the reconstruction cannot be the same as the original wave-front emitted by the object, because one half of the information is missing. In order to obtain the total information one requires two holograms, which are in sine-cosine relation to one another. Two such 'complementary' or 'quadrature' holograms have been used in the 'total reconstruction microscope' by one of us⁹. Adding up the wave-fronts issuing from two such holograms, one obtains in the reconstruction the original wave-front, and nothing else, except a uniform background.

However, somewhat paradoxically, the original wave-front is also contained in the modified wave-front diffracted in the reconstruction by a single hologram. The incompleteness shows itself in the fact that this wave-front is mixed up with an additional wave, which appears to issue from a 'conjugate object'. But the two

partial wave-fronts can be separated by various methods, the simplest of which is using a skew reference beam at an angle to the plate, in the taking of the hologram. The information-theoretical paradox that an incomplete record contains the full information in a retrievable form is explained by the fact that there is a loss of one half of the definition. This, however, is as good as unnoticeable in almost all present-day applications of holography.

Consequently, since one of the two (or more) waves diffracted by the hologram in the reconstruction process contains information on both the amplitude and the phase distribution in the object, both the phase and the amplitude information may be extracted from the reconstructed wave-front, for example, by suitable 'filtering' of the aerial reconstructed images, or diffraction patterns, before the final image is recorded on a photographic film. In essence, the image-forming wave-fronts reconstructed from holograms are indistinguishable from the wave-front which would be obtained from an ideal lens or mirror looking directly at the object, when it is possible to form an image in the ordinary 'one-step' imaging, for example, in microscopy. It has therefore been clear to us for some time that we may display the phase in the holographically reconstructed images of 'phase' objects, when necessary, with the aid of any one of the several well-known methods^{10,11} (for example, phase contrast, interferometry, Foucault or Schlieren methods) used to display the phase variations in the form of amplitude variations, as used in microscopy and other phase measuring applications in optics.

Because of the great present interest in holography, and because some of our recent advances seem to indicate a good likelihood that high resolutions may indeed be attainable in microscopy at very short wave-lengths (for example, X-rays), it may be of interest to demonstrate that phase-preserving imaging and 'phase-contrast' image

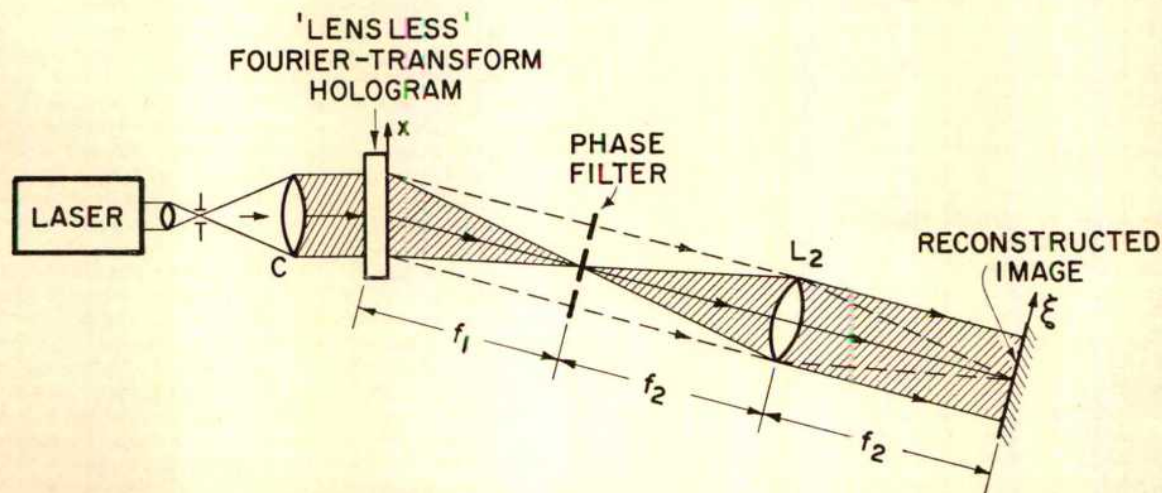


Fig. 1. Modified Fourier-transform holographic image-reconstruction arrangement, permitting 'phase-contrast' detection and imaging of phase objects. The distance f_1 is equal to the distance of the object, respectively point-reference, from the hologram in the 'lensless' recording of the Fourier-transform hologram^{3,12}. 'Conventional' Fourier-transform reconstruction of the images from the Fourier-transform holograms is obtained in the absence of the 'phase filter'. (The geometrical magnification obtained in Fourier-transform holographic imaging is equal to the ratio f_2/f_1 . An additional magnification factor equal to λ_2/λ_1 is obtained, when the reconstructing wave-length λ_2 exceeds the recording wave-length λ_1 . In this work, $f_1 = 415$ mm, $f_2 = 600$ mm, $\lambda_2 = \lambda_1 = 6328$ Å.

reconstruction may indeed be readily achieved in holography, using single holograms.

As one example of the 'phase-preserving' reconstruction of the image of a phase object, we have used the arrangement shown in Fig. 1. The phase object used is shown (barely visible) in Fig. 2a, and an enlarged transmission two-beam interferogram of the object is shown in Fig. 2b.

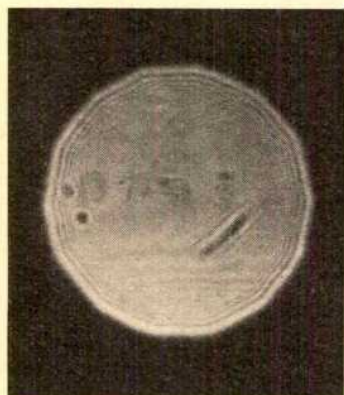


Fig. 2a. Direct (not holographic) image of phase object used in this work, showing degree to which a 'pure' phase object was obtained by suitable bleaching of a Kodak 649F emulsion (see text, and Fig. 2b). The object is the word 'phase' and the letter 'Φ' (the word phase being 20 mm long). The slight contrast detectable is due to some slight defocusing, and some residual absorption in the plate

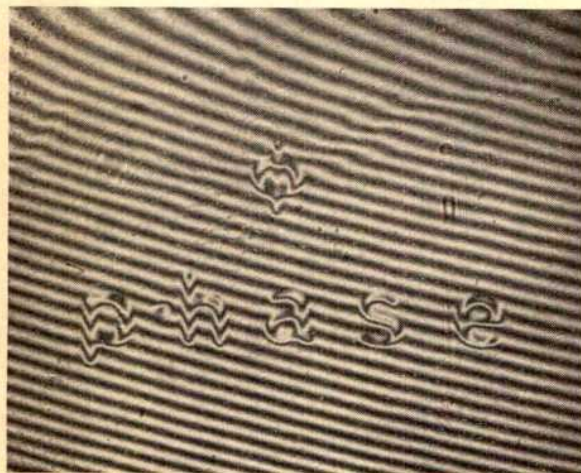


Fig. 2b. Two-beam single-pass interferogram (6328 Å) of the phase object used in this work. The hologram of this phase object was recorded in the 'lensless' Fourier-transform hologram recording arrangement according to ref. 12

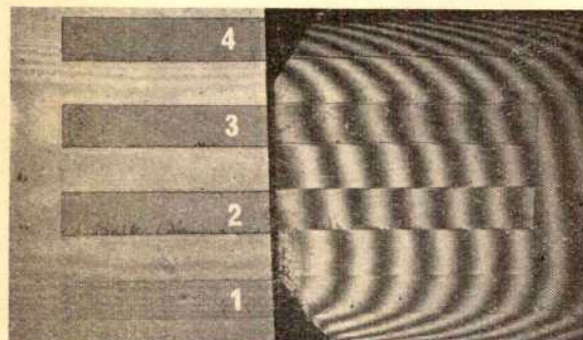


Fig. 2c. Two-beam single-pass interferogram (6328 Å), illustrating the amount of emulsion shrinkage and corresponding phase variation achievable in a Kodak 649F emulsion with four different exposures (in ratios $\times 1$, $\times 2$, $\times 3$, $\times 4$), and suitable bleaching with Kodak chromium image intensifier, used to obtain almost pure 'phase objects', with minimum residual absorption

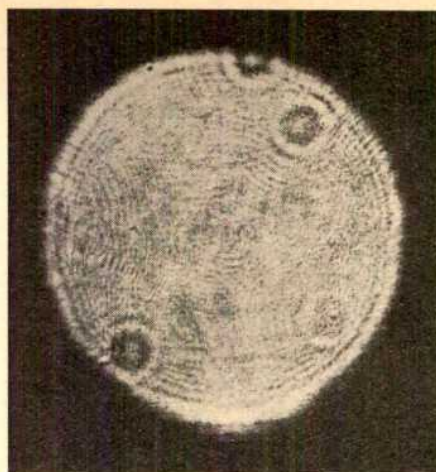


Fig. 3. Reconstructed image, obtained by Fourier-transform reconstruction in the arrangement of Fig. 1, without the use of any phase-contrast enhancement. (The 'lensless' Fourier-transform recording of the hologram of the object was obtained according to ref. 12.) The image shown here is characterized by an almost complete absence of any amplitude contrast in the phase-portion of the image (the various interference effects are spurious, and are caused mainly by imperfectly clean reconstructing optics). It may be noted that excellent imaging is obtained under similar conditions when the original objects are amplitude or intensity objects^{12,13}, rather than pure phase objects

The phase object was formed by photographing the word 'phase' and the letter 'Φ' on a Kodak 649F plate, and by bleaching the emulsion (using Kodak chromium intensifier as the bleacher). It is well known that photographic emulsions will shrink with the density of the exposure. (Typical emulsion shrinkage with exposure factors, 1, 2, 3, 4, is shown in Fig. 2c.) The phase object was recorded by projecting an image on to the plate deliberately slightly out of focus, in order to avoid steep gradients at the edges of the letters. (The amount of shrinkage shown in Fig. 2b was achieved in a 20-sec exposure, with a 75 W bulb, at $f/11$, in 1:1 imaging in the enlarger, and suitable bleaching.)

The hologram of the phase object was recorded in the 'lensless Fourier-transform' hologram recording arrangement, first described by one of us^{12,13}, in which the spherical waves originating from the various object points are made to interfere with a 'single' spherical reference wave, originating from a source 'point' in the mean plane next to the object. A reference wave of a radius $f_1 = 415$ mm was used in the recording. (We may note that the 'lensless' recording of the hologram permits storage of the information about the phase distribution in the object without introducing any other optical elements between the object and the emulsion, thus avoiding any extraneous scattering, which might reduce the fidelity and sensitivity of the method.)

The images reconstructed from the 'lensless Fourier-transform' hologram are shown in Figs. 3, 4 and 5. Fig. 3 shows a Fourier transform reconstruction, without filtering, obtained by simply projecting a plane monochromatic (6328 Å) wave through the hologram, and by recording one of the side-band images in the focal plane of a $f_2 = 600$ -mm lens. Unlike the reconstruction-imaging of intensity or amplitude objects, the reconstructed image of the phase object in Fig. 3 shows no amplitude contrast, because of the 'pure' phase nature of the object.

Figs. 4 and 5 show well-contrasted images of the phase object, obtained by a number of the well-known phase filtering or phase-contrast methods, in which the phase variations are made visible in the form of amplitude (that is, intensity) variations in the image.

Figs. 4a and b show the reconstructed images, obtained by a 'defocusing' phase-contrast enhancement, in a Fourier-transform reconstruction arrangement, as in the sharply focused Fig. 3, but now by recording the images together slightly ($\pm 1/4 f_2$ at $f/24$) out of focus, with respect to the in-focus image of Fig. 3.

Fig. 4c shows an in-focus image, in which the phase-contrast enhancement was obtained in the filtering arrangement shown in Fig. 1, with the help of the corner of a phase-contrast filter (also recorded photographically, and bleached, similarly to the object recording already described here).

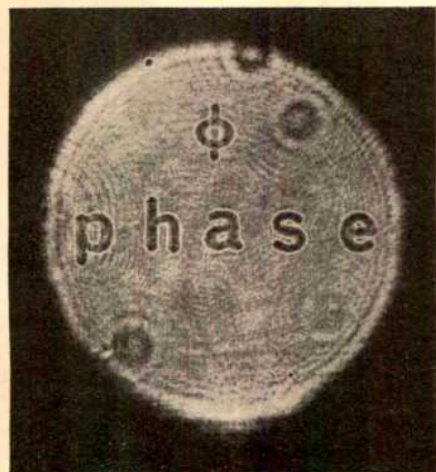


Fig. 4a. Reconstructed image of phase object, obtained with 'phase-contrast' enhancement by defocusing (here towards the L_1 lens of Fig. 1 by $-f_2/4$, with $f_2 = 600$ mm). The length of the word in the object was 20 mm (in the image, it is 30 mm, because of the f_2/f_1 magnification (see Fig. 1))

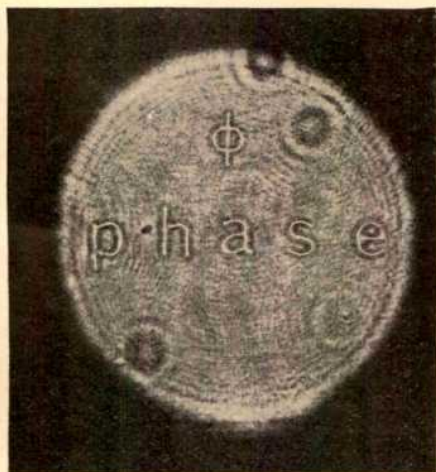


Fig. 4b. Reconstructed image of phase object, with 'phase-contrast' enhancement, obtained by defocusing (here, out of focus, away from the lens L_1 by $+f_2/4$)

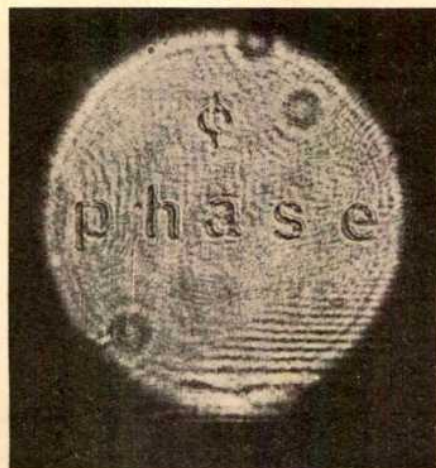


Fig. 4c. Reconstructed image of phase object, with phase-contrast enhancement obtained by using a phase-contrast filter (the corner of one of the rectangular phase strips, shown in Fig. 2c) in the arrangement of Fig. 1. (Here, the interference effects are spurious, and due to some imperfect cleanliness in the reconstruction optics)

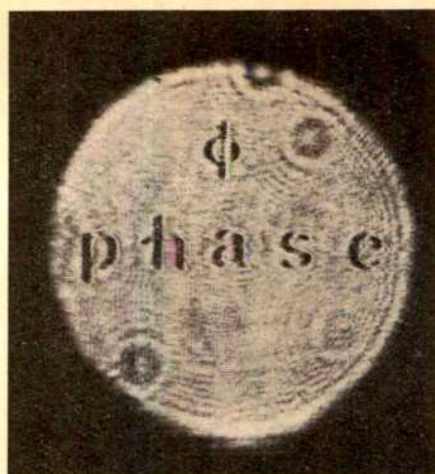


Fig. 4d. Reconstructed image of phase object, with phase-contrast enhancement, obtained with a Foucault knife-edge used in the phase-filter plane of Fig. 1

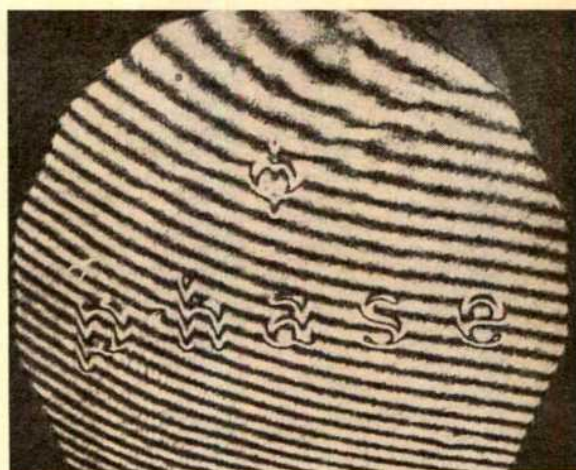


Fig. 5. "Hologram of the hologram." Two-beam single-pass interferogram (6328 Å laser light) of the interference pattern between the reconstructed aerial image and a plane reference beam. Comparison of the image-interferogram shown here with the similarly obtained object-interferogram of Fig. 2b demonstrates that phase-distribution in the object is indeed preserved and completely reconstructed in Fourier-transform wavefront-reconstruction imaging, using a modified 'phase-contrast' enhancing arrangement, such as that illustrated in Fig. 1. (We may note that phase preservation and phase-enhancing reconstruction apply to holograms recorded at one wave-length, and reconstructed in a second wave-length, for example, when λ_1 is in the X-ray domain and λ_2 in the visible-light laser domain)

Fig. 4d shows an in-focus image, with 'phase-contrast' enhancement obtained in the filtering arrangement of Fig. 1, with the help of a Foucault knife-edge filter (at right angles to the word 'phase').

Fig. 5 shows a two-beam interferogram of the reconstructed aerial image, formed by interference of the aerial image with a plane wave (in a suitable beam-splitting arrangement): by comparing the interferogram of the phase object (Fig. 2b), the degree of phase preservation and of fidelity of 'phase-preserving' reconstruction may be readily assessed.

It is clear from a comparison of the images and interferogram of the image, of Fig. 4 and of Fig. 5, with the phase object and object-interferogram of Fig. 2, that the phase-distribution in the phase object was not only retrievably recorded in the hologram, but also that the phase in the image of the phase object can be readily displayed as an amplitude (respectively intensity) in the reconstructed image, with the aid of phase-contrast or other image-filtering methods, including interferometry of various types. We may note that there is some indication that holograms of phase objects, recorded with a $1/1$

ratio of reference/diffracted field intensity (rather than the about 5/1 used here), appear to display some noticeable 'phase-contrast' enhancement simply in the focus of the 'conventional' Fourier-transform reconstruction arrangement.

We thank R. C. Restrick for his advice. This work was supported in part by the U.S. National Science Foundation and the U.S. Office of Naval Research.

¹ Gabor, D., *Nature*, **161**, 777 (1948).

² Gabor, D., *Proc. Roy. Soc., A*, **197**, 475 (1949).

³ Gabor, D., *Proc. Phys. Soc., B*, **244** (1951).

⁴ Leith, E. N., and Upatnieks, J., *J. Opt. Soc. Amer.*, **53**, 1377 (1963).

⁵ Leith, E. N., and Upatnieks, J., *J. Opt. Soc. Amer.*, **54**, 1295 (1964).

⁶ Leith, E. N., Upatnieks, J., and Haines, K. A., *J. Opt. Soc. Amer.*, **55**, 981 (1965).

⁷ Stroke, G. W., *Optics of Coherent and Non-Coherent Electromagnetic Radiations* (Univ. of Michigan, first ed., May 1964; second ed., March 1965).

⁸ Stroke, G. W., and Falconer, D. G., *Phys. Letters*, **13**, 306 (1964); **15**, 283 (1965); with Funkhouser, A., *Phys. Letters*, **16**, 272 (1965); with Restrick, R., Funkhouser, A., and Brumm, D., *Phys. Letters*, **18**, 274 (1965); *App. Phys. Letters*, **7**, 178 (1965).

⁹ Gabor, D., with Goss, W. P., British Patent No. 727,893/1955, application date July 6, 1951.

¹⁰ Zernike, F., *Physica, Haag*, **1**, 43 (1934); *Physik. Z.*, **36**, 848 (1935); *Z. Techn. Physik*, **16**, 454 (1935).

¹¹ Francon, M., *Le Contraste de Phase en Optique et en Microscopie* (Revue d'Optique, Paris, 1950).

¹² Stroke, G. W., *App. Phys. Letters*, **6**, 201 (1965).

¹³ Stroke, G. W., Brumm, D., and Funkhouser, A., *J. Opt. Soc. Amer.*, **55**, 1327 (1965).

MODERN LOW-TEMPERATURE CALORIMETRY

By DR. I. M. FIRTH

Department of Physics, University of St. Andrews

PRECISION calorimetry at liquid helium temperatures began in 1929 with the development of a very sensitive phosphor bronze thermometer by Keesom and Van Den Ende¹. Since that time our knowledge of the heat capacity of solids has increased greatly; some of the discoveries in this field of measurement have been very important, such as the electronic heat capacity in metals² and the heat capacity associated with the superconductive phase transition³, both measurements agreeing remarkably well with theoretical predictions. Before 1929 the main stimulus for specific heat measurements was the known decrease in the specific heat of solids at low temperatures from the famous room temperature value of 6 cal mole⁻¹ degree⁻¹ of Dulong and Petit. In this period both theory and experiment were advanced, producing the theory of specific heats of solids by Einstein⁴, the refinement to this by Debye⁵, and the vacuum calorimeter of Nernst⁶.

Since those early days of calorimetry, experimental methods have been continually improved and now a new era of precision has been entered. This has come opportunistically, for theoreticians interested in phenomena in the neighbourhood of critical points⁷ are calling for more accurate data close to such singularities. Although modern refinements now allow an accuracy of 0.05 per cent and a temperature resolution of 10⁻⁶ °K in some cases, a great many of the older troubles of calorimetry still persist, and this remains a research topic where many experimental data are taken. Modern ways can make such recordings automatic, but calorimetry at low temperatures is still a research problem requiring all the ability of a trained physicist and the facilities of a good workshop.

Basic Measurements

The heat capacity of a specimen is defined by:

$$C(T) = \lim_{\Delta T \rightarrow 0} \frac{\Delta Q}{\Delta T}$$

and is inherently a measurement averaged over some temperature interval when made by direct methods, that is, measuring the temperature rise on adding a known quantity of heat. Only when $C(T)$ is determined indirectly, from theoretical connexions with some other parameter, is it defined at a unique temperature. At room temperature, where $C(T)$ is varying slowly, standard two-phase methods may give average values that are adequate, but at low temperatures, where the variation is rapid, it is highly important to reduce as much as possible the temperature interval over which the average is taken. The experimental problem reduces, therefore, to isolating the specimen from its surroundings to a high degree and measuring the heat input ΔQ and the temperature rise ΔT as accurately as possible.

At low temperatures the most convenient source of ΔQ is Joule heating so that ΔQ is evaluated from measurements of electrical power and time. Where heat is applied intermittently in pulses the total heat input is correlated with the temperature difference between the initial and final states of equilibrium. The other method of applying heat in practical calorimetry is to do so continuously and requires electrical power to be compared with the rate of change of temperature. There are important differences between these two techniques which dictate their suitability to modern methods and methods of automatic recording.

As well as controlled Joule heating there will be an extraneous heat input over which the experimenter has limited control. This unwanted heat input can be said to occur through heat conduction and heat transfer by radiation. By the first, heat leaks to the calorimeter through its suspension threads and through the residual gas in the vacuum chamber surrounding the calorimeter, but both can be kept very small. Radiation of heat to the calorimeter occurs through emission and absorption of light and other electromagnetic radiation, and acoustical energy transfer. Often the latter two processes are the more important and can be minimized by screening apparatus from high-frequency radio sources—television transmissions often contribute to this factor through pick-up—and by isolating the whole calorimeter apparatus from vibrations.

In sensitive specific heat measurements the heat leak must be measured and corrected for, or kept low and sensibly constant so that it does not affect measurements. In the heat-pulse method a correction for the extraneous heat input is found for each pulse. Individual corrections of this type would be indispensable where the heat leak is large and varying with time over the temperature range of interest, but at low temperatures the heat leak can be kept small and constant for fairly long times. The method is therefore more powerful than is required here and has, in addition, certain shortcomings. First, as the heat pulse is generally of short duration, the electrical measurement of ΔQ may be difficult to make accurately, for currents of a few μ amp and voltages of a few μ V have to be measured. Secondly, as there is inevitably some thermal resistance between the calorimeter and the sample, and as the thermal conductivity of the sample may not be high, the recorded temperature, that is, of the calorimeter, is always somewhat higher than the mean temperature of the specimen. This temperature difference can become quite large during a heating pulse and gives rise to the so-called 'over-heating' effect⁸ (see Fig. 1), and the time to re-establish equilibrium may be long.

Corrections for over-heating can be made accurately by extrapolating the linear portions of the temperature

variation curve to the middle of the heating pulse and equating areas x , y to z . This method, therefore, requires a continuous recording to be made of temperature against time on both sides of the pulse. Corrections and extrapolations are then made from geometrical manipulations of this record, and the number of points so treated is limited by the time it takes the calorimeter temperature to reach equilibrium and by the patience of the observer. A maximum of data per degree Kelvin may be twenty.

There is also experimental evidence for not using this technique: Shiffman⁹ has observed that in the same temperature range the calculated specific heat may vary from pulse to pulse indicating that electrical transients lead to discrepancies between the real heat input and that measured.

Automatic schemes¹⁰ have been reported which use the heat-pulse technique. Temperature readings are taken at specified time intervals before and after the pulse. In manipulating the data so recorded, a computer can be programmed to make the appropriate extrapolations if the linear portions only of the drift curves are used. Important data may have to be neglected intentionally, and from the discussion of over-heating by Parkinson and Quarrington, it is apparent that results of great accuracy may not be obtained.

The continuous heating method, on the other hand, requires accurate instantaneous measurement of temperature and of input of power at specified times. Because heat is applied continuously, a state of dynamic thermal equilibrium is attained even in samples of low conductivity. Where the highest temperature resolution is required the heater power can be reduced accordingly, but the reduction can only be made to the level of the extraneous heat input so that again, to obtain high resolution, the experimenter must emphasize high thermal isolation in his design.

This method has been discussed in detail by Cochran¹¹. During continuous heating the electronic bridge used to measure temperature is repeatedly unbalanced by very small steps in a sense that the bridge returns to balance as the heating progresses. The time taken between successive balances is measured by electronic counters and, as the temperature jump between balances is small, the heat capacity is related linearly to the power input and the time interval. There is little doubt about the meaning of recordings here, as there may be in the pulse method, and automatic recording schemes can be used with ease, for data can be presented in digital form. As many readings can be taken as the warning rate will allow, and about a hundred readings can be made per degree Kelvin. Although the accuracy of each reading is high in itself, the large amount of data that can be recorded in this fashion makes it possible to achieve higher accuracy by statistical methods of analysis.

The method can be used to evaluate the extraneous heat input to the calorimeter, heating is stopped and the temperature drift caused by the various heat leaks is observed. A check on the heat leak is obtained also by

repeating heat capacity measurements with varying electrical heat inputs knowing that the heat capacity remains constant, so obtaining the heat leak from a difference equation. Careful attention to technique and design has enabled heat leaks of 1–10 ergs per sec, a temperature resolution of 10^{-6} °K and an accuracy in specific heat of 0.05 per cent to be obtained.

Thermometry

Temperature in the range of 0.2–5 °K is generally measured by means of a secondary thermometer. Commercial carbon radio resistors, especially made to be temperature insensitive at room temperature, were found some years ago to be most usefully sensitive at low temperatures¹². For calorimetry they have the desirable properties of high sensitivity, low heat capacity, low sensitivity to measuring current and to magnetic fields, and low cost. Carbon resistors are affected, however, by cycling to room temperature and their resistance-temperature response is reproducible to about a millidegree. More undesirable than this is, in principle, the fact that full equilibrium of the response is not attained for some 8 days when the resistors are held at low temperature, implying, in general, some change in characteristic during any experiment. It is most likely that, now they have been made available commercially, doped germanium thermometers will supplant carbon resistors as the most used secondary thermometer, since they exhibit little change of response in cycling to room temperature and much more rapidly attain equilibrium.

The most important aspect of calorimetry is the calibration of the thermometer because the accuracy of the whole experiment depends largely on this. As much experimental time is spent in comparing the resistance of the carbon resistance thermometer to the temperature of the liquid helium bath, it is important that an exact analytical description is obtained. There have been many suggestions how improvements can be made to the approximate formula suggested by Clement and Quinell¹²:

$$\left(\frac{\log R}{T}\right)^{\frac{1}{2}} = a + b \log R$$

where R is the measured resistance, T is the absolute temperature, and a and b are constant parameters. (Once the constant parameters have been evaluated for a resistor, this formula is used with a correction graph showing the deviation of the actual response in later experiments from the response defined by the particular a and b .) The most notable of these improvements is that by Moody and Rhodes¹³, who introduced an expression involving a large number of adjustable parameters in the form:

$$\frac{1}{T} = \sum_n a_n (\log R)^n$$

where $n = 0, 1, 2, \dots$. This formula permits easy recalculation of all the parameters to high accuracy after each calibration and removes the necessity for using inconvenient correction graphs. An exact analytical description of the temperature response facilitates subsequent conversion of resistance to temperature in calculations to determine the specific heat and indicates a great advance in the manipulation of the calibration procedure.

An important part of the calibration procedure is that a computer is programmed to perform the calculations. The root-mean-square deviation is calculated and calibration points that are above a pre-set value, for a given value of n , are rejected: the parameters are then recalculated until the allowed deviation is not exceeded. This method is compatible and can be used to advantage with the scheme already outlined here where the resistance of the thermometer is recorded automatically against the bath temperature.

For highest precision, carbon resistance thermometers must be used in a suitable bridge circuit where the power dissipation is kept low, that is, less than 10^{-8} W. Prior

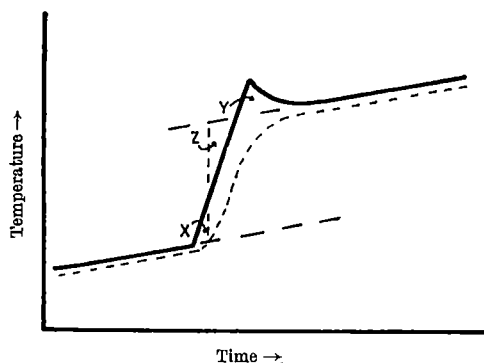


Fig. 1. Over-heating of calorimeter during an input pulse of heat.
—, Calorimeter temperature, ----, sample temperature

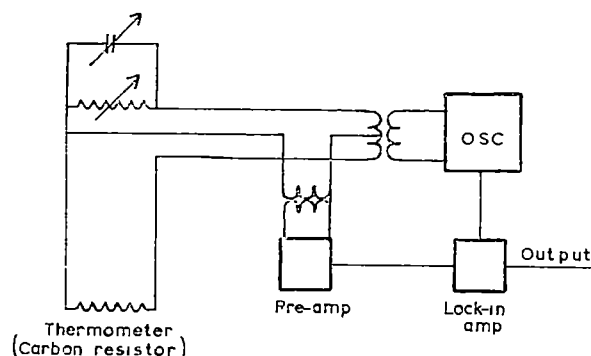


Fig. 2. A.C. bridge to determine the resistance of the temperature sensitive resistor using a lock-in amplifier

to the carbon-resistance thermometer, resistance thermometry had utilized a variety of resistance-measuring techniques among which were the potentiometer method, the Thomson double-bridge method and the Wheatstone bridge method. All are capable of high resolution but require to be adjusted by hand, involving up to four operations for the measurement of resistance between branch points and are not suited to any experiment that requires an instantaneous indication of temperature.

With the carbon resistance thermometer came the necessary advance in accurate instantaneous resistance measurement¹⁴. A simple Wheatstone bridge is most generally used with a high-gain, lock-in amplifier as detector. Such a bridge is shown in Fig. 2 and is similar to that used by Buckingham and Fairbank¹⁵ in their famous experiment on the specific heat of liquid helium at the superfluid transition temperature. Bridges of this type can detect changes of 10^{-7} °K, that is 0.02 ohm in 50 kohms where the power dissipated in the carbon resistor is about 10^{-10} W. It is this type of temperature-measuring system that was referred to earlier.

Automatic Recording and Calculation

There can be now few workers who manually calculate specific heats from experimental data, for the repetitive calculations involved can be better done, in less time, by computer. With a well-designed method of undertaking the experiment, data can be recorded automatically on to any of the many media used for feeding data to digital computers, paper tape, magnetic tape or punched cards, so reducing the overall time required to perform the calculations and, at the same time, reducing the errors in recording large amounts of data—the human operator with a desk calculator is only about 95 per cent accurate.

From what has been said already, it seems more meaningful to use the continuous heating technique with auto-

matic recording equipment. In this technique the power input can be monitored by repeatedly recording current and voltage to the calorimeter heater; the time between known small temperature jumps is easily available from an electronic timer as is the temperature jump which is in the form of a digital setting of a calibrated resistance box. The extraneous heat input, manifested by a temperature drift, can be recorded in a similar fashion as can the thermometer calibration information, except here the bath temperature would be recorded against a definite balance point of the Wheatstone bridge.

Data recorded in this way would be processed by a computer when programmed appropriately. The programme would be in two parts: first, the thermometer calibration data would be converted into analytical form; secondly, from measurements of ΔQ and ΔT and of the extraneous heat input and from information about the heat capacity of the empty calorimeter and mass of the sample, the specific heat of the sample would be calculated.

It should be emphasized that to take full advantage of this modern, logical modification to calorimetric practice both technique and data recording procedures have to be carefully considered. Precautions must be taken to ensure that necessary information is not discarded in the process of recording and that sufficient checks are included in the whole process, from apparatus to final results, so that meaningful results are obtained with the required overall accuracy.

Much has been accomplished since the time of Nernst in this field of research to ease the burden of the experimentalist. Automatic methods will not reduce the time necessary to take experimental data, however, but final results, which before might have taken between 30 and 40 h by manual calculation, can be obtained in 30 min of computer time. The research worker must not be entranced, however, by these advances and must be continuously vigilant of his techniques in order to maintain the older standards.

¹ Keesom, W. H., and Van Den Ende, J. N., *Leiden. Comm.*, 203C (1929).

² Kok, J. A., and Keesom, W. H., *Physica*, 1, 175 (1934).

³ Keesom, W. H., and Van Laer, P. H., *Physica*, 5, 193 (1938).

⁴ Einstein, A., *Ann. Phys.*, 22, 180 (1907).

⁵ Debye, P., *Ann. Phys.*, 39, 789 (1912).

⁶ Nernst, W., *Z. Elektrochem.*, 20, 357 (1914).

⁷ Green, M. S., Conf. "Phenomena in the Neighbourhood of Critical Points", Washington, D.C. (1965) (to be published).

⁸ Parkinson, D. H., and Quarrington, J. E., *Proc. Phys. Soc.*, A67, 569 (1954).

⁹ Shiffman, C. A. (personal communication).

¹⁰ Blythe, H. J., Harvey, T. J., Hoare, F. E., and Moody, D. E., *Cryogenics*, 4, 28 (1964).

¹¹ Cochran, J. F., *Ann. Phys.*, 19, 186 (1962).

¹² Clement, J. R., and Quinell, E. H., *Rev. Sci. Instr.*, 23, 213 (1952).

¹³ Moody, D. E., and Rhodes, P., *Cryogenics*, 3, 77 (1963).

¹⁴ Blake, C., Chase, C. E., and Maxwell, E., *Rev. Sci. Instr.*, 29, 715 (1958).

¹⁵ Buckingham, M. J., and Fairbank, W. M., *Prog. Low Temp. Phys.*, 3, 80 (1961).

PROGRESS IN THE PLANKTONIC FORAMINIFERAL BIOSTRATIGRAPHY OF THE NEOGENE

By DR. F. T. BANNER and DR. W. H. BLOW

Exploration and Production Research Division, British Petroleum Co., Ltd., Chertsey Road, Sunbury-on-Thames

CURRENT research into the value of planktonic foraminifera (particularly the Globigerinacea) as biostratigraphical indexes for the Neogene has now reached the stage of refinement wherein 23 zones may be recognized within the interval from the Miocene to the Recent. These zones may be identified in sediments of the circum-equatorial areas of the world, and many have also been directly recognized by us in temperate areas. The zones have been established by the examination of the succession of fossil faunas in Venezuela, Colombia,

Ecuador, Trinidad, Barbados, Carriacou, Jamaica, U.S. Gulf Coast, Fiji, Saipan, Guam, Christmas Island, Papua, New Guinea, West Irian, Java, Sumatra, New Zealand, Australia (Victoria), Japan (Shikoku), Tanganyika, Iran, Greece, Sicily, Italy, Libya, south-west France, Austria, the Atlantic and Pacific oceans (deep-sea cores), and other areas. The sequence of zones distinguished is as follows, from top to base stratigraphically:

Zone N. 23: *Globigerina calida*—*Sphaeroidinella dehiscens excavata* n. sub-sp. assemblage-zone; characterized by

the assemblage of *G. calida forma typica* Parker, *S. dehiscens excavata* (n. sub-sp. diagnosed by its deeply excavated sutures exposing the inner whorls¹), *Globigerina megastoma*, *Hastigerinella digitata*.

Zone N. 22: *Globorotalia* (*G.*) *truncatulinoides* partial-range-zone; defined by that part of the range of the nominate taxon prior to the first appearance of the Zone N. 23 assemblage.

Zone N. 21: *Globorotalia* (*G.*) *tosaensis* consecutive-range-zone; defined by that part of the range of the nominate taxon prior to the first appearance of its immediate descendant, *G. (G.) truncatulinoides*, the two taxa being phylogenetically consecutive.

Zone N. 20: *Globorotalia* (*G.*) *multicamerata*—*Pulleniatina obliquiloculata* (s.s.) partial-range-zone; defined by that part of the concurrence of the ranges of the two nominate taxa subsequent to the extinction of *Globoquadrina altispira* (which occurs within the range of *P. obliquiloculata* s.s.) and prior to the advent of *Globorotalia* (*G.*) *tosaensis* (which, in turn, occurs at a horizon virtually the same as that of the extinction of *G. (G.) multicamerata*²).

Zone N. 19: *Sphaeroidinella dehiscens* (s.s.)/*Globoquadrina altispira* (s.s.) concurrent-range-zone; defined by the interval of concurrence of the ranges of the two nominate taxa.

Zone N. 18: *Globorotalia* (*G.*) *tumida tumida*—*Sphaeroidinellopsis subdehiscens* partial-range-zone; defined by that part of the concurrence of the ranges of the nominate taxa which occurs before the first evolutionary appearance of *Sphaeroidinella dehiscens* (s.s.) from its immediate ancestor, *Sphaeroidinellopsis subdehiscens* (s.l.).

Zone N. 17: *Globorotalia* (*G.*) *tumida plesiotumida* consecutive-range-zone; defined by that part of the range of the nominate taxon which occurs prior to the first appearance of its immediate descendant, *G. (G.) tumida* (s.s.), the two taxa being consecutive in appearance, phylogenetically³.

Zone N. 16: *Globorotalia* (*Turborotalia*) *acostaensis* (s.s.)—*G. (G.) merotumida* partial-range-zone; defined by that part of the range of *G. (T.) acostaensis* (s.s.) which occurs before *G. (G.) merotumida* gives rise to its immediate descendant, *G. (G.) tumida plesiotumida*³.

Zone N. 15: *Globorotalia* (*Turborotalia*) *continuosa* partial-range-zone; defined as that part of the range of the nominate taxon which occurs after the extinction of *G. (T.) siakensis* and before the first evolutionary appearance of *G. (G.) acostaensis* from its immediate ancestor, *G. (T.) continuosa*.

Zone N. 14: *Globigerina nepenthes*/*Globorotalia* (*Turborotalia*) *siakensis* concurrent-range-zone; defined by the concurrence of the two nominate taxa.

Zone N. 13: *Sphaeroidinellopsis subdehiscens*—*Globigerina* n.sp.aff. *nepenthes* partial-range-zone; defined by that part of the range of *S. subdehiscens* (s.s.) which occurs before the evolutionary first appearance of *Globigerina nepenthes* from its immediate ancestor, *G.n.sp.aff. nepenthes*.

Zone N. 12: *Globorotalia* (*G.*) *fohsi* partial-range-zone; defined by that part of the range of the nominate taxon as redefined and restricted⁴, which occurs before the first evolutionary appearance of *Sphaeroidinellopsis subdehiscens* from its immediate ancestor, *S. seminulina* (s.s.).

Zone N. 11: *Globorotalia* (*G.*) *praefohsi* (n.sp., MS⁴), consecutive-range-zone; defined by that part of the range of the nominate taxon which occurs before the first evolutionary appearance of its immediate descendant, *G. (G.) fohsi*, the two taxa being phylogenetically consecutive.

Zone N. 10: *Globorotalia* (*Turborotalia*) *peripheroacuta* (n.sp., MS⁴) consecutive-range-zone; defined by that part of the range of the nominate taxon which occurs before the first evolutionary appearance of its immediate descendant, *G. (G.) praefohsi*, the two taxa being phylogenetically consecutive.

Zone N. 9: *Orbulina suturalis*—*Globorotalia* (*Turborotalia*) *peripheroronda* (n.sp., MS⁴) partial-range-zone; defined by that part of the range of *O. suturalis* which occurs before *G. (T.) peripheroronda* gives rise to its immediate evolutionary descendant, *G. (T.) peripheroacuta*, the two latter taxa being phylogenetically consecutive.

Zone N. 8: *Globigerinoides sicanus*⁵—*Globigerinatella insueta* partial-range-zone; defined by that part of the range of *Globigerinatella insueta* which is concurrent with the earliest part of the range of *Globigerinoides sicanus*, but which occurs before the first evolutionary appearance of *Orbulina suturalis*⁶.

Zone N. 7: *Globigerinatella insueta*—*Globigerinoides quadrilobatus trilobus* partial-range-zone; defined by that part of the range of *Globigerinatella insueta* which occurs after the extinction of *Globigerinita dissimilis* (s.l.) but which also occurs before the first evolutionary appearance of *Globigerinoides sicanus* from its immediate ancestor, *G. quadrilobatus trilobus*, the latter two taxa being phylogenetically consecutive.

Zone N. 6: *Globigerinatella insueta*/*Globigerinita dissimilis* (s.l.) concurrent-range-zone; defined by the concurrence of the two nominate taxa.

Zone N. 5: *Globoquadrina dehiscens praedeheiscens*—*G. dehiscens dehiscens* partial-range-zone; defined by that part of the interval during which the evolution from *G. dehiscens praedeheiscens* to *G. dehiscens* (s.s.) occurs and during which morphologically intermediate specimens are frequent, which is above the horizon of extinction of *Globorotalia* (*Turborotalia*) *kugleri* and yet which is below the horizon of first appearance of *Globigerinatella insueta*.

Zone N. 4: *Globorotalia* (*Turborotalia*) *kugleri* total-range-zone; defined by the total range of the nominate taxon.

Zone N. 3: *Globigerina angulisuturalis* partial-range-zone; defined by that part of the range of the nominate taxon which occurs above the horizon of extinction of *Globorotalia* (*T.*) *opima* (s.s.)⁷ and below the horizon of first appearance of *G. (T.) kugleri*.

Zone N. 2: *Globigerina angulisuturalis*/*Globorotalia* (*Turborotalia*) *opima* (s.s.) concurrent-range-zone; defined by the concurrence of the nominate taxa.

Zone N. 1 (= P.20): *Globigerina ampliapertura* partial-range-zone; defined by that part of the range of *G. ampliapertura* (s.s.) which occurs after the horizon of extinction of the last-surviving species of *Pseudohastigerina* (e.g., *P. naguawichiensis*) yet which occurs before the first appearance of *G. angulisuturalis*.

Careful re-assessment of the zones proposed for parts of the Neogene by Bolli⁸, Blow⁹, Blow and Banner¹⁰ and others¹¹⁻¹³ has shown that, while much of the zonation deduced from Caribbean (Trinidad, Venezuela) and East African (Tanzania) successions retains considerable validity when applied on a world-wide basis, modifications have proved necessary, as already indicated. For example, the '*Globigerina ampliapertura* zone' and the '*Globorotalia opima opima* zone', as previously understood⁸, had to be redefined following observation of the stratigraphic overlap of these two nominate taxa. The zones already proposed not only can be recognized directly in tropical and sub-tropical assemblages, but many sequences from the complete succession have been observed to occur in temperate latitudes. The complete documentation for the zonal sequence will be published elsewhere, when the succession of planktonic foraminiferal faunas will be described in relation to these zones, to the local stratigraphic succession in each area examined, to the biostratigraphical evidence of the associated fossils, and to geographic provincialization of the fossil faunas. The principal geostatigraphic results may be briefly summarized here.

The Oligocene has been found to contain two planktonic foraminiferal zones (termed P.18 and P.19)¹⁴; Zone N. 1 appears to straddle the boundary between the Late Oligocene and the Early Miocene, and has thus been

equated, in the above scheme, to Zone P. 20, the last zone of the Palaeogene. Zone N. 4 occurs at or near the base of the holostratotype Aquitanian stage¹⁵, older Neogene zones being present in parastratotypes of this stage (which may have to be accommodated by the Bormidian¹⁶ stage). The holostratotype Langhian¹⁷ contains Zones N.8, N.9 and N.10 (at least), and the holostratotype Tortonian contains the uppermost part of Zone N.15, all of Zone N.16 and the lower part of Zone N.17. Stratigraphically intermediate zones may be represented in the Serravallian¹⁸; but this has not yet proved directly determinable, as the stage lacks a precisely designated stratotype. The higher levels of the Vindobonian¹⁹, as exemplified by the 'Bulimina-zone' (or *Bolivina dilatata* zone) of the Badener series²⁰ at Nussdorf²¹, include Zone N.15 and/or Zone N.16. Zone N.17 is present in the Messinian²², as stratotypified²³. Zone N.19 occurs in the lower Pliocene (Zanclean²⁴ and Piacenzian²⁵), whilst Zone N.18 appears to straddle the Pliocene/Miocene boundary. The 'Sphaeroidinella-datum' of Bandy¹³ does not correspond precisely to the base of our Zone N.19, for that 'datum' was based upon an incomplete succession in the Philippines^{26,27}, where *Sphaeroidinella dehiscentis* appeared abruptly immediately above a hiatus marking the Pleistocene/Miocene disconformity. *Globorotalia* (*G.*) *truncatulinoides* (s.s.), which also first appeared at the so-called 'Sphaeroidinella-datum' in the Philippines, marks the base of Zone N.22, the zone which we have recognized to occur in the lower part of the stratotype Calabrian of Santa Maria di Catanzaro²⁸, the agreed earliest Quaternary²⁹.

In the Far East, it appears that the top of the 'Te-stage'³⁰ occurs within Zone N.9, and that the top of the 'Tf-stage' occurs either within Zone N.14 or near the base of Zone N.15. The base of the 'Tf₃' division³¹ has been estimated to occur within Zone N.12–N.13.

It must be noted that a re-investigation of the so-called 'Globorotalia fohsi lineage'⁸ has been completed, resulting in a complete taxonomic, nomenclatural and biostratigraphical revision⁴ of the indexes and of the zones as previously understood⁹ and described from Trinidad³ and Venezuela⁹ in particular. Comparison with other areas has shown the presence of a diastem between the Lengua and Cipero formations in Trinidad³, Zone N.13 being eliminated there by the hiatus; this has necessitated revision of the so-called 'Globorotalia mayeri zone'⁸. Very rich sequences of planktonic foraminiferal faunas from Jamaica have shown that the Bowden Shell Bed, long famed for its rich mollusc assemblages³², lies within Zone N.19. The planktonic foraminiferal assemblage of Zone N.19 has independently been recognized to occur in at least three deep-sea cores from c. 100 cm below sea-floor in the Pacific Ocean³³.

We thank Dr. F. E. Eames, chief palaeontologist, British Petroleum Co., Ltd., for his advice, and all our

colleagues for their advice, criticisms, sample donations and geological information.

- ¹ Paratype, BM(NH) No. ZF. 2369, illustrated in Brady, H. B., *Rep. Voy. Challenger*, 9 (Zool.), Pl. 84, Fig. 8 (1884); cf. *S. dehiscentis dehiscentis*, lectotypified by Banner and Blow, *Contr. Cushman Found.*, 11, 35, Pl. 7, Fig. 3 (1960).
- ² Forms referred (*auctt.*) to *G. multicaerata* from Recent seas are referable to *G. cultrata* (s.l.) *et al.*
- ³ Banner, F. T., and Blow, W. H., *Nature*, 207, 1351 (1965).
- ⁴ Blow, W. H., and Banner, F. T., *Micropaleontology* (in the press).
- ⁵ *G. sacanus* de Stefani, *Plinia*, 3, 9 (1952) is regarded as a synonym of *G. bisphericus* Todd, *Amer. J. Sci.*, 252, 681 (1954).
- ⁶ Blow, W. H., *Micropaleontology*, 2, 57 (1956).
- ⁷ 'Globigerina wilsoni bolivariana Petters', *Contr. Cushman Found.*, 5, 39 (1954) is represented by a single specimen (holotype) in the U.S. National Museum; our examination of this specimen has shown it to be so similar to *Globorotalia optima* Bolli, 1957, that we suspect that its supposed provenance from the Middle Eocene is incorrect; if the two taxa prove to be synonymous, *bolivariana* is the senior species-name, but more specimens would be necessary to establish this, because the holotype may be an atypical homocormorph. In our work, the species *G. optima optima* is maintained for nomenclatorial stability.
- ⁸ Bolli, H. M., *U.S. Nat. Mus. Bull.*, 215, 97 (1957).
- ⁹ Blow, W. H., *Bull. Amer. Pal.*, 39, 67 (1959).
- ¹⁰ Blow, W. H., and Banner, F. T., in Eames, F. E., *et al.*, *Fundamentals of mid-Tertiary Stratigraphical Correlation* (Camb. Univ. Press, 1962).
- ¹¹ Cita, M. B., *Inst. "Lucas Mallada", C.S.I.C. (Esp.)*, *Curs. y Conf.*, 9, 203 (1964).
- ¹² Jenkins, D. G., *Micropaleontol.*, 6, 345 (1960).
- ¹³ Bandy, O. L., *Micropaleontol.*, 10, 1 (1964).
- ¹⁴ *Globigerina selhi* does not occur below Zone P.19, but it is known to persist to within Zone N.3.
- ¹⁵ Valley of St.-Jean-d'Etampes, as described by Mayer-Eymar, K., *Schweiz. Ges. Naturwiss., Verh. Allg. Trogen*, Aug. 17–19, 1857, 192 (1858).
- ¹⁶ Of Pareto, L., *Bull. Soc. Géol. Fr.* (2), 22, 220 (1885), as re-described by Lorenz, C., *Bull. Soc. Géol. Fr.* (7), 6, 192 (1965).
- ¹⁷ Of Pareto, L., *Bull. Soc. Géol. Fr.* (2), 22, 229 (1885), as re-described by Cita, M. B., and Silva, L. P., *Rept. Twenty-first Sess., Intern. Geol. Congr. Norden*, 22, 89 (1960).
- ¹⁸ Of Pareto, L., *Bull. Soc. Géol. Fr.* (2), 22, 232 (1885); suggested by Drooger, C. W., *Inst. "Lucas Mallada", C.S.I.C. (Esp.)*, *Curs. y Conf.*, 9, 210 (1964).
- ¹⁹ Of Depéret, Ch., *C. R. Soc. Géol. Fr.* (2), 23, xxxiv (1895), to include the succession described by himself, *Bull. Soc. Géol. Fr.* (3), 21, 221 (1893).
- ²⁰ Series named by Papp, A., *Mitt. Geol. Ges. Wien*, 56, 240 (1963); zonation after Kapounek, J., *et al.*, *Sond. Erdöl-Erdgas-Zeitschr.*, 8, 109 (1965).
- ²¹ Stratigraphic position of the Nussdorf material confirmed by Dr. K. Turnovsky (personal communication).
- ²² Of Mayer-Eymar, K., *Catalogue . . . des fossiles des terrains tertiaires . . . au Musée fédéral de Zurich*, 2, 13 (1867), and *Tableau des terrains tertiaires supérieurs*, fourth ed. (1868).
- ²³ Sell, R., *Geol.* (2), 28, 1 (1960).
- ²⁴ Of Seguenza, Q., *Bull. Soc. Géol. Fr.* (2), 25, 465; v. et: Trevisan, *Lezione Stratigr. Internat.* (1), 11 (1956).
- ²⁵ Of Mayer-Eymar, K., *Versuch einer synchronistischen tabelle der Tertiärgebilde Europas* (in *Schweiz. Ges. Naturwiss., Verh. Allg., Trogen*, Aug. 17–19, 1857) (1858); v. et: di Napoli-Allata, E., *Intern. Geol. Congr., Sess. 19, Alger* (13), 8 (16), 229 (1954).
- ²⁶ Bandy, O. L., *Science*, 142, 1290 (1963).
- ²⁷ Bandy, O. L., *Bull. Amer. Assoc. Petr. Geol.*, 47, 1733 (1963).
- ²⁸ Described by Gignoux, M., *Ann. Univ. Lyon*, 36, 35 (1913), as typical of his Calabrian stage as proposed in *C.R. Acad. Sci., Paris*, 150, 841 (1910).
- ²⁹ *Intern. Geol. Congr., Rep. Eighteenth Sess.*, 1948, *Proc.* (H), 6 (1950).
- ³⁰ For 'letter-stages' see Leupold, W., and van der Vlerk, I. M., *Leidsche Geol. Meded.* (V) K. Martin Feestbundel, 1, 611 (1931).
- ³¹ The Tf₃/Tf₄ boundary was correlated with the Middle Miocene/Early Miocene boundary by Glaessner, M. F., *Roy. Soc. Victoria*, 55, 41 (1943).
- ³² Woodring, W. P., *Carnegie Inst., Washington, Publ. No. 366* (1925); *Science*, 148, 961 (1965).
- ³³ Parker, F. L., *Contr. Cushman Found.*, 16 (4), 151 (1965).

2-TRIFLUOROMETHYLBENZIMIDAZOLES: A NEW CLASS OF HERBICIDAL COMPOUNDS

By DR. D. E. BURTON, DR. A. J. LAMBIE, J. C. L. LUDGATE, DR. G. T. NEWBOLD, DR. A. PERCIVAL and D. T. SAGGERS

Chesterford Park Research Station, Fisons Pest Control, Ltd., Saffron Walden, Essex

THE synthesis of 2-trifluoromethylbenzimidazole was published in 1953¹ and since then a score of benzene-ring-substituted derivatives have been described in the literature, mainly emanating from the Birmingham School of Organic Chemistry^{2–8}. Antibacterial⁸ and antiviral⁹ properties of some of these compounds have been described.

During the past five years, workers in our laboratory have been interested in the preparation of various heterocycles derived from *o*-phenylenediamines¹⁰, for example, benzotriazoles¹¹, quinoxalines¹² and benzimidazoles¹³, and particularly, but not exclusively, in those bearing halogen substituents in the benzenoid ring. This led us, in early 1962, to embark on the systematic study of the synthesis

and biological behaviour of 2-trifluoromethylbenzimidazoles, of which a large number have been made having a free hydrogen on the 1-position, most being hitherto undescribed in the literature. Early in this investigation it became evident that the group possessed interesting herbicidal activities, though these appeared to be associated with high mammalian toxicity. Further synthesis and biological examination, however, showed that these two properties were not inseparably associated and that an acceptably safe and yet active herbicide could be obtained.

Some members of the 2-trifluoromethylbenzimidazole series show interesting and possibly important insecticidal activity principally by ingestion, but their foliar-protectant interest is limited by phytotoxicity. A high level of molluscicidal activity is also shown by certain members, for example, 4-bromo-5,6,7-trichloro-2-trifluoromethylbenzimidazole with an LC_{50} against *Australorbis glabratus* of 0.1 p.p.m.

Aspects of the chemistry of 2-trifluoromethylbenzimidazoles will be published elsewhere; their preparation was effected by reaction of *o*-phenylenediamine with trifluoroacetic acid or by direct substitution of 2-trifluoromethylbenzimidazoles. The resulting compounds are acidic with the pK_a decreased by about 4–4.5 units from that of the corresponding benzimidazoles with hydrogen in the 2-position. pK_a 's of 2-trifluoromethylbenzimidazoles vary from 8.79 in the case of the unsubstituted compound to the highest acidity in our series, 2.96, for 4,7-dichloro-5,6-dinitro-2-trifluoromethylbenzimidazole.

Evaluation of herbicidal activity was made on two stages of plant growth. In the first test (post-emergent) the toxicity to the aerial portions was assessed by applying a solution of the compounds in aqueous acetone to the foliage of a selection of young crop plants.

In the post-emergent test the typical symptoms induced by the active members of the series were rapid loss of turgidity, necrosis and death of plant within about four days. In the most resistant species the necrotic areas were confined to the areas of spray drop retention.

There was no evidence of significant translocation and the rate of necrosis appeared to be independent of light.

Acute oral toxicity values in the female rat were obtained by standard techniques by our colleague, Mr. D. M. Sanderson. The principal toxic effects were dyspnoea, weakness, occasionally salivation and death in an extended position with immediate rigor mortis. The latter observation is similar to that made on other compounds causing uncoupling of oxidative phosphorylation¹⁴. This possibility has been investigated by our colleagues, Dr. O. T. G. Jones and Mr. W. A. Watson, and it has been confirmed that the most biologically active 2-trifluoromethylbenzimidazoles are powerful uncouplers of oxidative phosphorylation¹⁵.

Examples of this class of compounds are the 5-substituted-2-trifluoromethylbenzimidazoles (Table 1). These derivatives show the typical herbicidal spectra associated with this series. The pK_a of the benzimidazole seems to be an important factor influencing good herbicidal activity. Compounds with electron attracting groups in the 5-position would appear to be more active than those with electron-donating groups at this position. The activity of the parent 2-trifluoromethylbenzimidazole is significantly enhanced by 5-halo-substitution in the sequence 5-I = 5-Br > 5-Cl > 5-F (Table 1). The other derivatives containing an electron-withdrawing group, for example, 5-cyano-, 5-nitro- and 5-trifluoromethyl-, have the same overall activity as the 5-iodo-2-trifluoromethylbenzimidazole. An electron-donating group at the 5-position, for example, 5-methyl-, 5-methoxy- or 5-amino-, has little effect on the herbicidal activity of the parent compound. A marked feature of this series is the variation in species selectivity with slight modification to the structure. This can be seen in the halo- series where selectivity in favour of peas is increased four-fold in proceeding from the 5-fluoro to the 5-iodo derivatives, while the reverse occurs in the case of barley. In general the mammalian toxicity tends to follow the herbicidal trend with the exception that the 5-iodo and 5-bromo-compounds, while having greater herbicidal activity than

Table 1. POST-EMERGENT HERBICIDAL ACTIVITY OF A SERIES OF 5-SUBSTITUTED 2-TRIFLUOROMETHYL BENZIMIDAZOLES

<div> </div>	Average post-emergent herbicide toxicity—6 species Dosage in oz./acre	LD_{50} -oz./acre—to individual test species*		Mammalian toxicity—oral LD_{50} to rats—mg/kg												
Table No.	X	pK_a †	160	80	40	20	10	5	2.5	Peas	Mustard	Linseed	Buckwheat	Sugar-beet	Barley	
1	—	8.79	57	26	18	—	—	—	—	>160	46	70	110	95	>160	800
2	5-CH ₃	8.90	63	40	25	—	—	—	—	>160	60	112	<40	80	>160	>1,600
3	5-C(CH ₃) ₃	9.12	70	79	67	—	—	—	—	>160	<40	<40	<40	<40	80	600
4	5-OCH ₃	—	55	31	—	—	—	—	—	>160	<80	130	<80	125	>160	—
5	5-F	8.00	70	79	69	—	—	—	—	<40	<40	<40	<40	<40	>160	400
6	5-Cl	7.97	86	78	76	65	58	—	—	37	<10	<10	<10	<10	>160	140
7	5-Br	7.99	94	—	—	74	73	62	33	32	2.5	3	<2.5	4	50	670–800
8	5-I	7.71	86	—	—	70	74	57	36	160	3	4	<2.5	3	40	>800
9	5-NH ₂ ‡	4.54†	63	9	—	—	—	—	—	>160	85	140	140	96	>160	1,200
10	5-NO ₂ ‡	6.68	95	69	59	67	60	57	43	110	7	4	<2.5	<2.5	110	70
11	5-CF ₃	7.52	95	88	79	79	74	49	34	10	6	5	<2.5	3	70	140
12	5-CN	7.16	76	—	—	67	55	60	47	>160	<2.5	2.5	<2.5	2.5	>160	100

* The species used were peas, *Pisum sativum*; mustard, *Sinapis alba*; linseed, *Linum usitatissimum*; beet, *Beta vulgaris* (var. Sharpe's Klein E); buckwheat, *Fagopyrum esculentum*; and barley, *Hordeum vulgare* (var. Proctor). The chemical was applied by means of twin air-atomizing gravity-fed spray guns (Bullows type L 500) mounted over a turntable, the speed of rotation of which was adjusted so that the plants received a volume equivalent to 80 gallons/acre. The initial application rate was 10 lb./acre which was halved on successive retests until no significant response was observed. The measurement of activity took the form of a visual assessment seven days after chemical application. The species were scored individually on a 0–100 rating where 0 = no effect and 100 = complete kill. In certain of the tables presented the arithmetic mean of the individual scores is given as being the most representative value for overall herbicidal activity. The LD_{50} levels were obtained by plotting the dosage/score curve on log-probit analysis paper and obtained by eye from a line of best fit.

All tests described here were carried out in controlled environment rooms at a temperature of 22° C with 14 h of artificial illumination (at an intensity of 0.000 ft.-candles), using daylight fluorescent lighting. The soil used was John Innes No. 1 potting compost and the plants were grown in small anodized aluminium pans ('Ovenex' were 7.5 × 3.75 × 2 in.) drilled in the base for drainage. Plants were tap watered throughout the period of the experiment.

† Determined spectrophotometrically in water at 20° by Messrs. D. Hughes, W. Mason and Miss P. M. Palmer.

‡ Zwitterion.

§ Dihydrochloride.

the 5-chloro-2-trifluoromethylbenzimidazole, have lower mammalian toxicity.

The effect of the different substitution patterns on the herbicidal activity is shown by the chloro-substituted derivatives (Table 2). Maximum overall activity is shown by the 4,5,6-trichloro-2-trifluoromethylbenzimidazole (pK_a 6.18). Of particular interest is the considerable increase in mammalian toxicity and decrease in herbicidal activity of the 4,5,6,7-tetrachloro- derivative as compared with the trichloro- derivatives. One of the more promising members of the series so far is 4,5-dichloro-2-trifluoromethylbenzimidazole, *NC* 3363, for which the common name chlorflurazole is proposed, which shows a high contact toxicity to many broad-leaf species at a rate which is safe to cereals, combined with a fairly low mammalian toxicity. Further tests with this compound have confirmed that it is active against a range of cereal weeds, such as chickweed, spurry, mayweed and fat-hen, and that it can be markedly synergized by addition of certain herbicides of the phenoxyacetic range. The field performance of such mixtures will be reported elsewhere by our colleague Dr. R. K. Pfeiffer¹⁶.

In comparison the 4,6-dichloro-2-trifluoromethylbenzimidazole shows a relatively high toxicity to barley.

In the mono- and di-substituted halogen series the bromo- derivatives were superior in herbicidal activity and less toxic to mammals than the chloro- compounds; this advantage is lost in higher substitution.

Introduction of a nitro-group into the substitution pattern produces the characteristic selectivity shown by the other members of the series (Table 3). In the nitro-halogen series the 4,6-substitution pattern gives compounds of good herbicidal activity. The 6-nitro-4-halo derivatives produce compounds with an acceptable safety factor to peas and barley without adversely influencing activity on the other broad-leaved weed species or mammalian toxicity. However, 4-nitro-6-halo derivatives have higher mammalian toxicities without improved selectivity. The dinitro- derivatives were not as active as their dichloro- analogues. The nitro-dichloro-substituted compounds had reduced activity compared with the corresponding disubstituted chloro-compounds. The nitro-trichloro- and dinitro-dichloro- derivatives were of only slight interest herbicidally, although they were of

Table 2. POST-EMERGENT ACTIVITY OF HALO-SUBSTITUTED 2-TRIFLUOROMETHYL BENZIMIDAZOLES

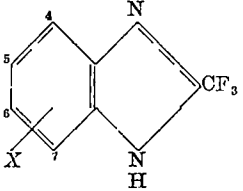
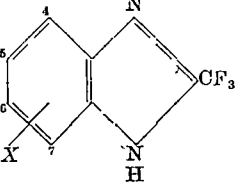
Structure:																
										Average post-emergent herbicide toxicity rating—6 species Dosage in oz./acre						
Table No.	X	pK_a	160	80	40	20	10	5	2.5	LD_{50} levels—oz./acre to individual test species						
			Peas	Mustard	Linseed	Buck-wheat	Sugar-beet	Barley	Mammalian toxicity—oral LD_{50} to rats—mg/kg							
1	—	8.79	57	20	18	—	—	—	—	≥160	46	70	110	95	≥160	800
2	4-Cl	7.57	93	87	82	69	66	28	10	30	6	4	6	10	100	400
3	4-Br	7.37	91	—	84	64	55	37	22	38	5	<2.5	4	10	70	400
4	5-Cl	7.97	86	78	76	65	58	—	—	37	<10	<10	<10	<10	≥160	140
5	5-Br	7.89	94	—	74	73	62	33	—	32	2.5	3	<2.5	4	50	670-800
6	4,5-Cl	6.96	84	91	79	72	57	53	45	11	<2.5	12	<2.5	<2.5	≥160	300-400
7	4,6-Cl	6.78	93	97	96	98	88	74	57	6	<2.5	<2.5	<2.5	<2.5	10	50
8	4,6-Br	6.77	99	—	91	83	88	83	—	20	<2.5	<2.5	<2.5	<2.5	5	50
9	5,6-Cl	7.4	74	72	59	49	56	33	—	>80	13	<2.5	<2.5	6	≥80	120
10	5,6-Br	6.77	92	—	79	69	45	25	—	24	3	5	3	3	55	—
11	4,7-Cl	6.24	—	67	53	—	—	—	—	≥80	<40	<40	<40	40	≥80	40
12	4,7-Br	5.99	60	—	31	30	—	—	—	750	0.7	0.6	26	56	750	150
13	4,5,6-Cl	6.18	100	92	98	94	89	84	71	3	<2.5	<2.5	<2.5	<2.5	10	18
14	4,6,7-Cl	5.64	89	84	73	75	71	67	60	5	3	<2.5	<2.5	<2.5	>160	70-80
15	4,6,7-Br	5.58	95	—	72	66	—	—	—	54	4	1	1	2.5	67	70
16	4,5,6,7-Cl	5.04	72	71	66	62	53	—	—	600	2	3	1	10	300	16

Table 3. POST-EMERGENT ACTIVITY OF HALO-NITRO-SUBSTITUTED 2-TRIFLUOROMETHYL BENZIMIDAZOLES

Structure:																
										Average post-emergent herbicide toxicity rating—6 species Dosage in oz./acre						
Table No.	X	pK_a	160	80	40	20	10	5	2.5	LD_{50} levels—oz./acre to individual test species						
			Peas	Mustard	Linseed	Buck-wheat	Sugar-beet	Barley	Mammalian toxicity—oral LD_{50} to rats—mg/kg							
1	4,6-Cl	6.78	93	97	96	93	88	74	57	6	<2.5	<2.5	<2.5	<2.5	10	50
2	4-NO ₂ -6-Cl	6.33	96	96	96	95	89	80	75	7	<2.5	<2.5	<2.5	<2.5	10	35-50
3	4-Cl-6-NO ₂	5.48	94	96	96	86	84	87	52	3	<2.5	<2.5	<2.5	3	23	140
4	4,6-Br	6.77	99	—	91	83	88	83	—	20	<2.5	<2.5	<2.5	<2.5	5	50
5	4-NO ₂ -6-Br	6.04	97	—	91	88	—	—	—	10	<2.5	<2.5	<2.5	<2.5	<2.5	13
6	4-Br-6-NO ₂	5.45	85	—	70	68	64	51	—	>160	3	<2.5	<2.5	<2.5	80	150
7	4,6-NO ₂	4.96	82	—	79	75	58	38	—	>160	2.5	2.5	<2.5	3	16	150
8	5,6-Cl	7.4	—	74	72	59	49	56	33	>80	13	<2.5	<2.5	6	≥80	120
9	4-NO ₂ -5-Cl	5.98	73	—	49	29	—	—	—	<10	18	>160	12	<2.5	69	80
10	5-Cl-6-NO ₂	6.20	—	—	65	64	67	62	—	>80	<2.5	<2.5	<2.5	<2.5	≥80	200
11	5,6-NO ₂	4.96	—	38	23	—	—	—	—	≥80	50	>80	40	80	≥80	18
12	4,5,6-Cl	6.18	100	92	98	94	89	84	71	3	<2.5	<2.5	<2.5	<2.5	10	>800
13	4-NO ₂ -5,6-Cl	5.25	—	—	28	22	—	—	—	>20	10	>20	>20	>20	160	600
14	4,5-Cl-6-NO ₂	5.24	53	—	—	—	—	—	—	650	65	40	140	55	≥160	400
15	4,6-Cl-5-NO ₂	4.86	83	—	—	75	61	34	20	16	2.5	3	7	6	≥160	—

considerably lower mammalian toxicity than the 4,5,6,7-tetrachloro-compound.

The tetra-halogen substituted 2-trifluoromethylbenzimidazoles are compounds of high mammalian toxicity. Replacement of a chloro group by a methyl, sulphonamido, piperidino or morpholino group did in fact lower mammalian toxicity but the herbicidal activity was correspondingly decreased.

The pK_a values of these compounds are obviously a measure of one of the significant factors effecting herbicidal activity and we found that most of the compounds having good herbicidal activity have a pK_a in the range 5–8. The 5-*t*-butyl compound is an exception; the correlation between pK_a and biological activity will be discussed in a separate paper.

Substitution at the 1-position with alkyl or aralkyl groups gives compounds of considerably less herbicidal activity than the derivatives having a free hydrogen on the nitrogen atom in the 1-position.

By comparison with the post-emergent response, the pre-emergent activity of the 2-trifluoromethylbenzimidazoles, although not insignificant, is of less interest.

However, certain members of the series have shown selective properties, particularly in cotton, which is at present under further investigation. The symptoms of toxicity in the sensitive species are marked germination reduction with chlorosis followed by necrosis and death.

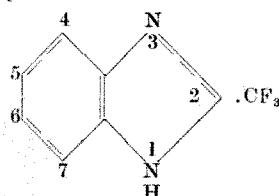
- ¹ Smith, W. T., and Steinle, E. C., *J. Amer. Chem. Soc.*, **75**, 1292 (1953).
- ² Bishop, B. C., Jones, A. S., and Tatlow, J. C., *J. Chem. Soc.*, 3076 (1964).
- ³ Belcher, R., Sykes, A., and Tatlow, J. C., *J. Chem. Soc.*, 4159 (1954).
- ⁴ Brooke, G. M., Burdon, J., and Tatlow, J. C., *J. Chem. Soc.*, 802 (1961).
- ⁵ Fernandez Bolanos, J., Overend, W. G., Sykes, A., Tatlow, J. C., and Wiseman, W. H., *J. Chem. Soc.*, 4003 (1960).
- ⁶ Lane, E. S., *J. Chem. Soc.*, 234 (1955).
- ⁷ Morgan, K. J., *J. Chem. Soc.*, 2343 (1961).
- ⁸ Bishop, B. C., Chelton, E. T. J., and Jones, A. S., *Biochem. Pharmacol.*, **13**, 751 (1964).
- ⁹ Fara, G. M., and Cochran, K. W., *Boll. dell' Ist. Sieroterapico Milan*, **42**, 630 (1964).
- ¹⁰ Fisons Pest Control Ltd., Belgian Patent 621,454; U.S. Patents 3,166,593 and 3,166,594.
- ¹¹ Fisons Pest Control Ltd., British Patent, 990,111.
- ¹² Fisons Pest Control Ltd., Belgian Patent, 631,044.
- ¹³ Fisons Pest Control Ltd., British Application No. 4431/62.
- ¹⁴ Unpublished work. Medical Dept., Fisons Pest Control Ltd.
- ¹⁵ Jones, O. T. G., and Watson, W. A. (following communication).
- ¹⁶ Pfeiffer, R. K., European Weed Control Conference, Paris (December 1965).

ACTIVITY OF 2-TRIFLUOROMETHYLBENZIMIDAZOLES AS UNCOUPLERS OF OXIDATIVE PHOSPHORYLATION

By DR. O. T. G. JONES and W. A. WATSON

Chesterford Park Research Station, Fisons Pest Control, Ltd., Saffron Walden, Essex

THE herbicidal and insecticidal properties of a series of substituted benzimidazoles have been described by Burton *et al.*¹ These compounds are derivatives of 2-trifluoromethylbenzimidazole:



and are related in structure to 4,5,6,7-tetrachlorobenzimidazole, a compound investigated at these laboratories and known to possess activity as an uncoupler of oxidative phosphorylation². Preliminary observations suggested that the 2-trifluoromethylbenzimidazoles also acted as uncouplers and the experiments described in this communication confirm this activity.

Rat liver mitochondria were prepared in 0.25 M sucrose by standard methods³ so that 2 ml. of mitochondrial suspension was obtained from each gram of liver. For oxidative-phosphorylation measurements, incubations were carried out for 20–30 min at 30°; the incubation mixture contained, in a final volume of 3 ml., glycylglycine (50 μ moles), KCl (375 μ moles), $MgCl_2$ (42 μ moles), glucose (180 μ moles), NaF (45 μ moles), AMP (6 μ moles), ATP (6 μ moles), cytochrome *C* (0.036 μ mole), yeast hexokinase (200 *KM* units), potassium phosphate, pH 7.4 (46.2 μ moles), substrate 30 μ moles and about 1 mg mitochondrial nitrogen. Uptake of oxygen was measured using the Warburg apparatus, uptake of phosphate by the method of Fiske and Subbarow⁴ after the addition of perchloric acid (0.5 ml. of 50 per cent v/v) to end the incubation.

ATPase activity was determined by the method of Lardy, Connelly and Johnson⁵.

In Table 1 the uncoupling activity of some of the 2-trifluoromethylbenzimidazoles is compared with that of tetrachlorobenzimidazole and 2,4-dinitrophenol. It can

be seen that the tetrachloro-2-trifluoromethylbenzimidazole is active at much lower concentrations than dinitrophenol; 50 per cent uncoupling being achieved at 8.2×10^{-8} M. Similar results were obtained with other 2-trifluoromethylbenzimidazoles with succinate, glutamate, pyruvate and β -OH butyrate as substrates.

The activity of 2-trifluoromethylbenzimidazoles in stimulating the ATPase of rat liver mitochondria is shown in Table 2. Tetrachloro-2-trifluoromethylbenzimidazole increased ATPase activity at concentrations much lower than those required for 2,4-dinitrophenol to be effective. As would be expected from such uncoupling agents, the 2-trifluoromethylbenzimidazoles stimulated respiration of mitochondria in the absence of a phosphate acceptor. Similar results have been obtained for many 2-trifluoromethylbenzimidazoles.

The 2-trifluoromethylbenzimidazoles behave as weak acids¹ and thus resemble the well-known uncouplers, the

Table 1. ACTIVITY OF SOME 2-TRIFLUOROMETHYLBENZIMIDAZOLES IN UNCOUPLING OXIDATIVE PHOSPHORYLATION COMPARED WITH COMPOUNDS OF KNOWN ACTIVITY

Compound	Conc. required for 50% uncoupling of oxidative phosphorylation (M)
2-Trifluoromethylbenzimidazole	Inactive up to 4.2×10^{-4}
5-Monochloro-2-trifluoromethylbenzimidazole	5×10^{-4}
4,5-Dichloro-2-trifluoromethylbenzimidazole	6×10^{-7}
4,5,6-Trichloro-2-trifluoromethylbenzimidazole	2.5×10^{-7}
4,5,6,7-Tetrachloro-2-trifluoromethylbenzimidazole	8×10^{-8}
2,4-Dinitrophenol	8×10^{-4}
4,5,6,7-Tetrachlorobenzimidazole	1×10^{-4}

Table 2. EFFECT OF 2-TRIFLUOROMETHYLBENZIMIDAZOLES AND 2,4-DINITROPHENOL ON THE ATPASE ACTIVITY OF RAT LIVER MITOCHONDRIA

Exp. No.	Addition	Concentration (M)	iP liberated (μ moles/30 min/0.2 mg N)
1	None	—	0.7
1	4,5,6,7-Tetrachloro-2-trifluoromethylbenzimidazole	2×10^{-7}	3.5
1	"	7.5×10^{-7}	4.3
1	2,4-Dinitrophenol	9.2×10^{-6}	2.6
1	"	9.2×10^{-6}	4.2
2	None	—	0.3
2	4-Nitro-6-chloro-2-trifluoromethylbenzimidazole	5×10^{-7}	1.3
2	"	3×10^{-6}	2.3

substituted phenols. When the ionizable hydrogen of the —NH group at position 1 was replaced by ethyl, there was a loss of uncoupling activity. Thus, although 4,5,6-trichloro-2-trifluoromethyl benzimidazole gave 80 per cent uncoupling at 5×10^{-7} M, its *N*-ethyl derivative was inactive at a concentration of 1×10^{-6} M, which was near the limit of its solubility. The uncoupling activity of the phenols was shown by Parker⁶ to depend on their *pK*_a. Similarly, the activity of the 2-trifluoromethylbenzimidazoles increased as the *pK*_a decreased; thus for halogen substituted benzimidazoles (Table 1) increasing chlorine substitution resulted in a series in which the *pK*_a decreased progressively and the uncoupling activity increased. However, substitution of nitro groups in place of halogens sometimes reduced the uncoupling activity and caused some departures from this relationship.

Replacement of a 5-chloro substituent on the benzene ring by substituents such as amino, methyl and hydrogen which have been found to reduce herbicidal activity¹ also reduced the activity in uncoupling oxidative phosphorylation.

The 2-trifluoromethyl benzimidazoles which are the most effective herbicides¹ have been found to be extremely active as uncouplers of oxidative phosphorylation. Tetrachloro-2-trifluoromethyl benzimidazole appears to be one of the most active uncouplers yet described. It is therefore probable that this activity in uncoupling oxidative phosphorylation contributes to the herbicidal action. They are thus similar in many respects to the nitrophenols and halophenols.

Since this communication was submitted a similar report of the uncoupling activity of NH-acidic benzimidazoles has appeared (K. H. Buchel, F. Korte and R. B. Beechey, *Angew. Chem., Internat. Edit.*, **4**, 788; 1965). Their findings are in substantial agreement with our own.

¹ Burton, D. E., Lambie, A. J., Ludgate, J. C. L., Newbold, G. T., Percival, A., and Sagers, D. T. (preceding communication).

² Parker, V. H. (in the press).

³ Hogeboom, G. H., *Methods in Enzymology*, **1**, 16 (Academic Press, New York, 1955).

⁴ Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, **66**, 375 (1925).

⁵ Lardy, H. A., Connelly, J. L., and Johnson, D., *Biochemistry*, **3**, 1961 (1964).

⁶ Parker, V. H., *Biochem. J.*, **69**, 306 (1958).

REPLICATION AND THE ACTIVATION OF MUSCLE DIFFERENTIATION

By DR. PAUL PIETSCH and SUSAN B. MCCOLLISTER

Biochemical Research Laboratory, The Dow Chemical Co., Midland, Michigan

THE investigation recorded here was conducted to test the possibility that myogenesis can be detonated in events associated with cell replication. The work was facilitated by the antibiotic phleomycin¹, which has the unique property of being able to inhibit DNA synthesis without interfering directly with the production of proteins or RNA^{1,2,18}. Phleomycin, like actinomycin D, appears to owe its specificity of action to selectivity in its binding with DNA. It appears to have an affinity for polynucleotides rich in A-T base pairs and it cancels DNA polymerase activity at concentrations that do not interfere with RNA polymerase². Regeneration in mouse skeletal muscle³ was selected as the principal test system because reasonably close approximations could be made of the time of appearance of the first wave of new muscle fibres or myotubes (96–120 h⁴), and of the interval during which replication takes place (38–72 h⁴). Also, because regeneration is not spontaneous in healthy skeletal muscle, but ensues as an almost inevitable consequence of non-fatal injury, it was possible to gauge the timing of events from a fairly precise starting point—much more so than with other specific myogenic systems (for example, the embryo, regenerating amphibian limbs, rhabdomyosarcoma, etc.).

DNA synthesis during the second–third day of regeneration. When the investigation was begun it was known that increased cell division takes place between the second and third day after injury⁴, but more detailed knowledge of this interval was required in order to undertake the present study. Therefore, the bellies of left tibialis anteriors in C57BL/6J (ref. 5) male mice sharing the same birth-date were inflicted aseptically with three puncture wounds using an 18-gauge hypodermic needle. Animals, weighing $20.1 \text{ g} \pm 0.2 \text{ S.D.}$, received 29 μCi tritiated thymidine (6,700 mc./mmole) intraperitoneally at specific times between 38.5 and 72 h. Injections were accomplished with an automated device, the dose being regulated by duration of infusion. The intervals for wounding and for injection among individuals in a given group of ten or so mice were kept within 3–5 min. At death, 40 min after pulse labelling⁶, the tibialis anterior was dissected out and DNA extracted differentially in 1.1 N perchlorate⁷. DNA was assessed quantitatively and qualitatively by ultra-violet absorption and radioactivity was measured by liquid scintillation

counting. The size of each muscle was accounted for on the basis of its total protein determined by the biuret reaction. Independent experiments were conducted to ascertain errors which might arise from differences in wound sizes. This was done by inflicting a single puncture in one tibialis anterior and three punctures in its contralogue. A correlation of 0.99 was obtained in DNA and actomyosin synthesis for right and left muscles in these cases, suggesting that the reaction would be all or none within the range of traumatization used in these investigations.

There are three waves of thymidine incorporation between 38.5 and 72 h post-wounding (Fig. 1, I, II and III). Peaks and profiles for I and III were confirmed in two independently executed studies. Wave II also was obtained in two separate series, but its peak and period varied by some 2 h. Wounds for all groups had been inflicted at approximately the same time of day, and, of

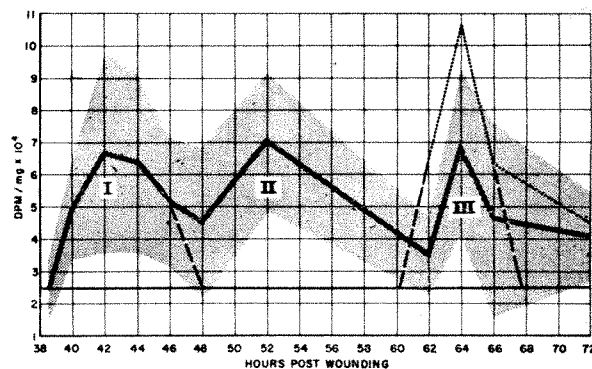


Fig. 1. Incorporation of tritiated thymidine at various times after wounding in tibialis anterior muscle. Unadjusted mean values are represented by the heavy line, and their standard deviations are shown by stippling. Dotted lines at wave III represent values corrected for circadian rhythm. Dashes indicate extrapolations through regions of overlap to the threshold line, that is, the level below which activity is not related to the waves *per se*. Areas under waves I and III were integrated after fitting their respective curves by least squares. The fitted curve for wave I was expressed by the equation: $y = 8.05069149X - 0.00143509987X^2 - 225.365393$; and for III: $y = 31.2881719X - 0.00254415843X^2 - 1,326.22411$. The computer rejected values when they were rounded off. There were from 6 to 10 individual cases per point in this experiment. In an earlier series with three cases per point the same profiles were obtained for waves I and III, but II varied

Table 1. EFFECTS OF CIRCADIAN RHYTHM ON INCORPORATION OF TRITIATED THYMIDINE DURING MOUSE MUSCLE REGENERATION

Wave (cf. Fig. 1)	n	*AZT 1 (activity $\times 10^4$)	n	AZT 7* (activity $\times 10^4$)	n	AZT 19* (activity $\times 10^4$)
I (38.5 h)			8	2.35 \pm 0.86 S.D.	7	2.67 \pm 0.99 S.D.
I (42 h)	8	1.77 \pm 0.38 S.D.	7	2.31 \pm 0.48 S.D.	4	2.26 \pm 0.34 S.D.
III (62 h)			5	7.00 \pm 2.72 S.D.	8	4.76 \pm 1.84 S.D.

While wave I shows no influence on thymidine incorporation that may be ascribed to circadian rhythm, it has been observed that constant exposure to light for prolonged periods does produce effects during this post-wounding interval. It is also noted that while wave III cells incorporate more thymidine at the high point in light, splenic cells show greatest uptake at maximal darkness (AZT 19).

* AZT, arbitrary Zeitgeber time, AZT 1 representing the onset of 12-h period of light.

necessity, the time of day at which tritiated thymidine was introduced varied for each point. This opened the possibility that circadian rhythm would influence wave amplitudes. Therefore, investigations were conducted with wounds (38.5, 42.0 or 62.0 h) receiving tritiated thymidine at different hours of the day. Routinely, all mice are maintained in a windowless, air-conditioned room and are exposed to consecutive, alternating 12-h periods of light and darkness, and this, of course, was maintained in the experiments under immediate consideration. It was learned (Table 1) that wave I (38.5- and 42-h wounds) is not influenced by the light rhythm employed in this laboratory. On the other hand, tritiated thymidine uptake at wave III was influenced by the time of day at which pulse labelling was carried out, for 62-h wounds that had been pulsed at the high point in light showed significantly more activity than did wounds of the same age pulsed at the maximal point in darkness.

These results indicated that, in order to approximate the true characteristics of wave III, it would be necessary to correct for the time of day of injection. In the experiments dealing with circadian rhythm, the arbitrary Zeitgeber time scale (AZT 1-24) suggested by others⁸ was used. In this scheme AZT 1 represents the time when lights go on and AZT 7 (1400 E.S.T. in this laboratory) and AZT 19 the high point in light and darkness, respectively. Such a scale has the obvious advantage over clock time of being readily reproducible anywhere in the world and at any season of the year. It was postulated that AZT 7 would represent the time of maximal operation of circadian factors; in other words, this time was taken as infinitely ideal with respect to increments of enhancement from light, and accordingly this time was assigned a value of zero along the x -axis (because zero steps of progression would be required to achieve it). AZTs were paired reciprocally between 7 and 19 and each set was assigned a consecutively ascending value from zero to twelve (thus $7/7 = 0$, $9/5 = 2$, $19/19 = 12$, etc.). Each set-value corresponded to the numbers of steps along the x -axis from the ideal time and each would represent $x/12$ universe along this axis. Unity was assigned to the y -axis at the ideal point ($x = 0$) and zero to the infinitely non-ideal time of $x = 12$ (AZT 19); that is, infinitely non-ideal time would have zero increments of help from circadian rhythm. Furthermore, it was postulated that circadian factors would decay from the infinitely ideal towards the infinitely non-ideal in an exponential manner. Thus, experimentally obtained values for wave III could be idealized or corrected for circadian rhythm by $R/e^{-x/12}$, R being the observed specific activity and x the set-value for the AZT at the time of pulse labelling. This correction was applied to values for wave III (with x greater than 0 in each case) in Fig. 1 (dotted lines). The applicability of the correction factor was determined by computing $\Delta 40-42$ h/ $\Delta 62-64$ h values from corrected curves and comparing this ratio with that obtained from cases with contralateral tibialis anterioris presenting I and III wave wounds at pulse labelling. The latter ratio was 0.382 and the corrected one 0.372. The corrected wave III curve proved extremely useful in determining the length and period of wave III and also in establishing a base-line for activity of the waves *per se*; that is, it was possible to extrapolate through regions of overlap and to obtain a

truer estimate of background values. On the basis of information to be described in the next section (cf. Tables 2 and 3) of this article it was learned that wave III terminates a little short of 68 h, and extrapolating to this point from the descending shoulder of the corrected curve for this wave, it was possible to traverse regions of activity that could not be handled empirically. The base-line generated horizontally to the left from 68 h intersected the ascending segment of wave I at about 38.5 h, providing an approximation for the onset of its period. After having dropped the point at 48 h to the base-line (once more on the strength of evidence presented in Tables 2 and 3), it was possible to idealize the curve for wave I. This was done by the least-squares method with a Burroughs 5,000 digital computer for waves I and III which produced the equations represented in the legend to Fig. 1. Areas under corrected curves turned out to be 27,000 units for wave I and 34,000 for III. Thus, wave I appears to begin at approximately 38 h and to end just short of 48 h. Wave III spans the interval between 60 and 68 h. The length of wave III, based on corrected expressions, turned out to be almost identical to the time established rather masterfully by others for the s -phase (DNA synthesis interval) in replicating mouse cells⁹. The approximately 10-h length of wave I, 3 h in excess of published s -phase values, no doubt represents asynchrony in the population plus inaccuracies of extrapolation. Interestingly, the similar areas under the curves for waves I and III indicate that the total amounts of DNA being synthesized are of roughly the same magnitude during these two periods, despite amplitude differences. Owing to these uncertainties, wave II was not evaluated.

Effects of phleomycin. With knowledge of the waves of DNA synthesis, their lengths, periods, peaks and characteristics during the important 2-3-day interval, it was possible to investigate the effects of phleomycin with respect both to actomyosin synthesis and DNA replication. Actomyosin synthesis was judged by the incorporation into this protein complex of L-lysine-¹⁴C (u.l. 223 mc./mmole) given intraperitoneally in two doses of 10.0 μ c. each at 94 and 117 h after wounding. At death, seven days after wounding, the entire tibialis anterior muscle was dissected out and actomyosin extracted in buffered 0.6 M potassium chloride^{10,11}. Concentration of actomyosin was determined from its ultra-violet absorption maximum (275 m μ), after the Folin reaction. Size of starting material was judged by determining non-fibrillar protein content of the sample. Radioactivity was measured by liquid scintillation counting. Uninjured muscle was processed similarly in order to develop a base-line for radioactivity not attributable to newly synthesized actomyosin. Phleomycin (kindly supplied by Bristol Laboratories, Syracuse, New York) was dissolved in physiological saline just prior to use, and its biological activity was confirmed on agar-plates seeded with *A. aerogenes*. Phleomycin (0.25 mg) applied before wounding (minus 13 h) or at the very end of waves I and III (48 and 68 h, respectively) failed to suppress actomyosin synthesis (Table 2). When the antibiotic was applied around the peak of wave III activity was moderately suppressed. However, phleomycin virtually eliminated actomyosin synthesis when it was administered to animals between 44 and 45 h; that is, on the descending shoulder of wave I.

The effects of phleomycin on DNA synthesis (Table 3) were judged after a single intraperitoneal dose of the antibiotic (0.5 mg) followed 1 h later by 29 μ c. tritiated thymidine. Animals were killed 40 min later, and tissues were processed in the manner described in the last section. As might be expected from the foregoing account, thymidine incorporation was severely depressed when phleomycin was given during wave I. Incorporation was also markedly suppressed when phleomycin was given during wave III, suggesting that, although these cells are influenced by the antibiotic so far as DNA synthesis is concerned, their future capacity to manufacture muscle proteins is un-

Table 2. TIME-DEPENDENT EFFECTS OF PHELOMYCIN ON ACTOMYOSIN SYNTHESIS IN REGENERATING MOUSE MUSCLE

Administration of phleomycin (hour post-wounding)	n	Control (activity $^{14}\text{C} \times 10^3$)	Phleomycin* (activity $^{14}\text{C} \times 10^3$)	F (%)	Inhibition %
-13	7	9.6 \pm 0.8 S.D.	4 15.20 \pm 0.50 S.D.	1.1	0
44.5 (wave I)	7	9.6 \pm 0.8 S.D.	4 0.04 \pm 0.02 S.D.	61†	97.5
45 (wave I)	6	3.6 \pm 1.3 S.D.	6 0.23 \pm 0.18 S.D.	26†	93.8
48	4	5.3 \pm 2.3 S.D.	4 5.20 \pm 1.50 S.D.	0.006	0
64 (wave III)	5	14.0 \pm 4.7 S.D.	3 11.10 \pm 4.90 S.D.	8.13†	21.5
68	2	12.5 \pm 4.6 S.D.	3 12.60 \pm 1.10 S.D.		0

F refers to analysis of variation (ref. 16).

* Bristol lot 49331-1347 phleomycin used for -13, 44.5 and 64 h experiments. Bristol lot 49331-909 used for 45 h. Bristol lot 49331-616 used for 64 h. Dowco lot 189 for 48 h.

† Low order significance (>10 per cent).

‡ Very highly significant (<0.1 per cent).

CBA/J (ref. 5) male mice used for -13, 44.5 and 64-h experiments, C57BL/6J's (ref. 5) for the rest.

All lots completely inhibited growth on agar of *A. aerogenes* at concentrations employed.

Activity (DPM/mg actomyosin/non-fibrillar protein) minus specific activity of contralateral uninjured tibialis anterior.

Table 3. TIME-DEPENDENT EFFECTS OF PHELOMYCIN ON TRITIATED THYMIDINE INCORPORATION s_2 IN WOUNDED MOUSE MUSCLE

Administration of phleomycin (hour post-wounding)	n	Control (activity $\times 10^3$)	Phleomycin (activity $\times 10^3$)	Uptake inhibited (?)
-17*	4	10.29 \pm 2.38 S.D.	2 13.15 \pm 3.20 S.D.	No
41 (wave I)	7	10.29 \pm 2.38 S.D.	3 2.39 \pm 0.94 S.D.	Yes
41 (wave I)	7	6.70 \pm 1.70 S.D.	8 2.81 \pm 1.10 S.D.	Yes
43 (wave I)	4	16.95 \pm 6.30 S.D.	4 6.23 \pm 0.88 S.D.	Yes
51 (wave II?)	3	11.80 \pm 0.70 S.D.	4 10.17 \pm 3.10 S.D.	No
59†	3	15.40 \pm 8.40 S.D.	4 11.34 \pm 2.20 S.D.	No
63 (wave III)	7	18.18 \pm 2.94 S.D.	8 4.39 \pm 1.22 S.D.	Yes

* Tritiated thymidine given 42 h after wounding to catch wave I (Fig. 1).

† Pulse labelling at 64 h to catch wave III.

‡ Activity, DPM/mg DNA/total protein. Values below about 2.5×10^3 are not attributable to DNA synthesis during the waves (Fig. 1).

impaired. When the antibiotic was given at minus-17 h there was no evidence of curtailed thymidine incorporation at wave I. Similarly, phleomycin at 59 h failed to suppress DNA synthesis at wave III. Phleomycin did not suppress activity when given during wave II; but it is emphasized that there is uncertainty concerning the exact period of this wave; consequently the significance of wave II remains obscure, and for reasons of economy and simplicity it has not been considered further.

Different responses to phleomycin. The pattern of suppression of DNA synthesis in regenerating muscle by phleomycin was not, *a priori*, completely predictable. It was expected, for example, that the antibiotic would influence DNA synthesis at almost any time up to the end of the process. Its failure to do this may have three interesting and not necessarily incompatible explanations: (a) the antibiotic might have restricted access to DNA except in rather circumscribed conditions; (b) the nucleotide sequences where the antibiotic makes its union may have a different operational significance in replication of various cell types; (c) the nucleotide sequences where phleomycin attaches to DNA may be dissimilar among unlike cells; that is, different 'genes' may be involved in binding in varying circumstances. Each of these explanations might underlie the relationship of replication to the read-out of muscle information; each suggests *de facto* differences during replication in unlike cells, and each leads to a single readily verifiable proposition, namely, that specific cell types shall respond differently to the antibiotic. With this in mind, animals were treated with phleomycin and 1 h later they, and a group of untreated siblings, were pulsed with tritiated thymidine. Comparison of specific activities of testicular DNA from treated and untreated mice showed no essential differences (correlation of 1.000). Similar studies were conducted with spleens (Fig. 2, legend) which, like testes, showed no short-term responses to the antibiotic. In another series with spleens (Fig. 2) pulse labelling was delayed until some 18 h after injection of phleomycin, and under these conditions DNA synthesis was suppressed but not altogether inhibited. Evidently some splenic

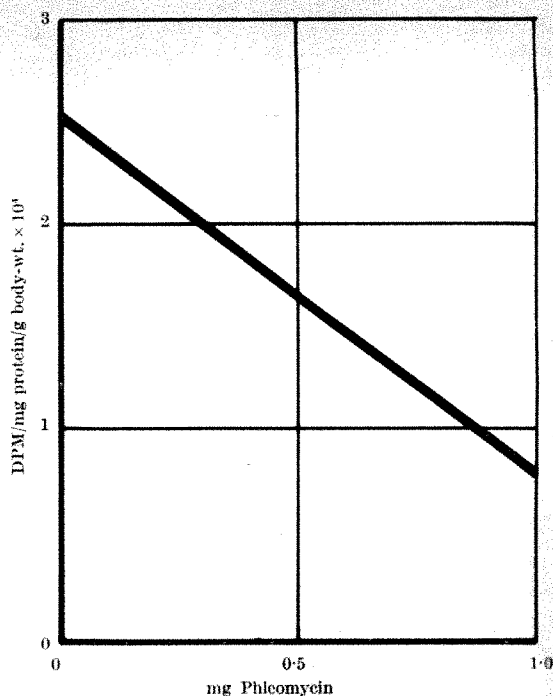


Fig. 2. Effects of phleomycin on incorporation of tritiated thymidine into splenic DNA when pulse labelling was delayed until approximately 24 h after administration of the antibiotic. In a separate series with spleens, tritiated thymidine was given 1 h after various doses of phleomycin. A dose of 0.5 mg/g body-weight produced specimens with specific activities of 10^4 . Spleens of animals that received 1.0 and 1.5 mg/g showed activities of 7×10^3 and untreated control values were 8×10^3 . Thus, the initial effects of phleomycin are not manifested immediately in splenic DNA synthesis. However, as seen in the experiments represented in this graph, a concentration-dependent response to the antibiotic is detectable when tritiated thymidine injection is delayed. In the graph, concentration of antibiotic is represented per gm body-weight along the x-axis.

cells are responsive to phleomycin but at any given moment they represent such a small minority of the total population that it is only after a significant time lapse that their absence from DNA synthesis is amplified to the point where it is detectable. (It seems possible that the responding cells are lymphocytic stem cells.) In a limited number of additional experiments, phleomycin was introduced into animals bearing one of the following transplantable mouse tumours: rhabdomyosarcoma (BW10139), myeloid leukaemia (C1498), adenocarcinoma (BW10232), melanoma (B16) (ref. 5). Tritiated thymidine was injected into treated and untreated tumour hosts 1 h after introduction of the antibiotic. Animals were killed 40 min later and for each the ratio of specific activity between tumour and splenic DNA was computed and compared. Only the phleomycin-treated rhabdomyosarcoma (muscle tumour) showed inhibition; that is, a significant increase in splenic thymidine incorporation as compared with untreated tumour/spleen ratios (Table 4).

In the other cases, tumour/spleen ratios, treated versus untreated, showed insignificant differences, suggesting that, like spleens and testes but unlike regenerating muscle and muscle tumour, there was no immediate, frank reaction to

Table 4. SHORT-TERM EFFECTS OF PHELOMYCIN ON TUMOUR/SPLEEN* RATIOS OF TRITIATED THYMIDINE INCORPORATION

Tumour	n	Untreated ratio	n	Phleomycin ratio
Adenocarcinoma	2	0.117 \pm 0.013 S.D.	3	0.115 \pm 0.052 S.D.
Melanoma	1	0.269	3	0.335 \pm 0.131 S.D.
Myeloid leukaemia	3	0.160 \pm 0.063 S.D.	3	0.159 \pm 0.092 S.D.
Rhabdomyosarcoma	1	0.329	1	0.159

* Spleen does not show an immediate response to phleomycin (see legend, Fig. 2).

Treated and control spleens showed radioactivity within the same range. It is noted that phleomycin produces suppression in splenic DNA synthesis after about 24 h.

phleomycin by leukaemia, carcinoma or melanoma cells. Despite the prediction that this would happen, it must be recognized that testes and spleens are subject to individual physiological variations among animals and the same might very well have been true of tumours; thus, because of this the frame of reference might have been variant. Therefore, more exacting conditions were sought to provide a final test of the proposition. While cells of both waves I and III are highly susceptible to phleomycin, their different causal relationships to actomyosin synthesis suggested, intuitively, that they would vary mutually in response to this antibiotic.

Owing to large standard deviations in data from different individual mice (Fig. 1) it was impossible to design a statistically efficient experiment based on anticipated small comparative differences. It was decided, therefore, to use both tibialis anterior muscles in the same animal, to vary the times of wounding and thereby to make available to wounds of different ages an otherwise uniform set of conditions. Specimens were compared on an individual basis for each animal, the information sought being the comparative value between mean ratios of treated versus those for untreated individuals. The premise was that if phleomycin influenced wounds on both sides of the animal in the same way, then, while absolute values might differ, the ratios would fall within the same statistical range. Conversely, if cells respond in an unlike manner to the antibiotic the ratios between contralateral wounds would differ between treated and untreated. Two series were conducted, 41/63 and 41/43, numerators and denominators representing the ages of the wounds when phleomycin was introduced. Untreated 41/43 ratios showed a mean (four cases) of 0.827 ± 0.27 S.D.; means (five cases) for 41/43 phleomycin-treated animals were 0.873 ± 0.27 S.D., indicating no difference in response to the antibiotic at the two points along wave I. In 41/63 h experiments untreated ratios (seven cases) were 0.382 ± 0.20 , but phleomycin-treated specimens revealed ratios (eight cases) of 0.638 ± 0.18 S.D.; that is, differences between the two groups of very high significance (F distribution beyond 1 per cent).

Thus, phleomycin can virtually eliminate myogenesis but only when it is made available during a critical time-interval. In mouse skeletal muscle regeneration in particular this interval coincides with the period of wave I. As is true with other agents which inhibit regeneration, there is a close correlation between the events going on at the time of application and the mode of inhibitory action (see review of these in Fig. 3). A phase comparable to wave I seems required to set myogenesis into motion, and this appears to entail at least one round of DNA synthesis. Further division may occur but it is not mandatory. It has been reported of tissue-cultured myoblasts that they need not divide in order to accomplish differentiation^{12,13}. Judging from the advanced age of embryonic donors in the last-cited experiments, the cultivated cells, doubtless, had passed the point equivalent to wave I prior to explantation.

Obviously the channels to muscle information are not opened merely by genomic duplication (otherwise all dividing cells would produce muscle) and obviously DNA synthesis cannot be regarded as the proximate cause of myogenesis. Indeed, the nature of wave I's contribution remains to be established. During regeneration also, there is a significant time lapse between wave I and mRNA synthesis (at about 72 h (ref. 14)) and it is fairly well established that when DNA synthesis is going on actomyosin synthesis is not¹⁵. While it is much simpler to regard wave I as the immediate source of myogenic cells the possibility should not be ignored that the wave supplies information that is utilized by other cells.

Differential susceptibility of wave I and III cells (as well as cells of other types) to phleomycin suggests that

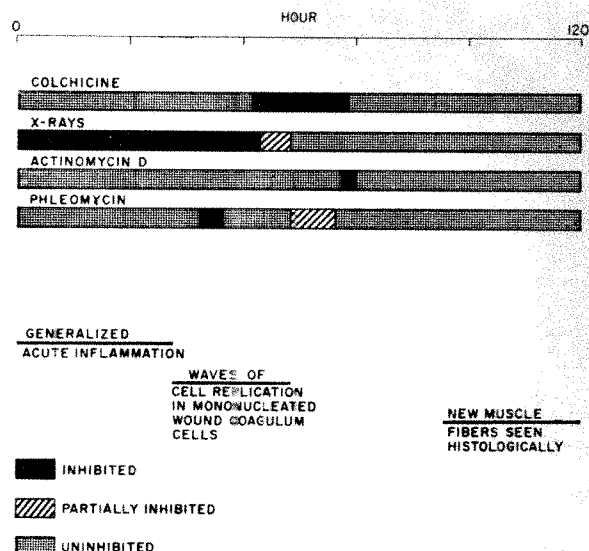


Fig. 3. Summary of time-dependent inhibition of various agents on muscle regeneration and the major events that occur during the sensitive interval. References: colchicine⁴, X-rays¹¹, actinomycin D¹⁴.

specificity in the operational features of thymidine incorporation during wave I, at the molecular or chromosomal level or both—the specific information that may become readable as a result of the uniqueness of factors bestowing differences—may determine the kind of future the daughter cells shall have, whether they shall divide again or enter into relative dormancy or begin to synthesize, anew, polymeric classes previously not among the cell's complement. It seems quite possible, carrying this line of speculation a little further, that the release of specific information could in principle be a function of detailed firing orders among chromosomes, polynucleotide sequences, or both, with read-out being a consequence of the order in which elements were reproduced, and not the gross fact of duplication. If such a method of release is true of myogenesis, then it seems likely that there are other examples of differentiation in which details of replication play an important part. This possibility suggests that it would be quite worth-while to gain comprehensive knowledge concerning the physical chemistry of phleomycin-DNA complexes in different cell types. Indeed, the decision to include these few speculations at all was reached only because technology exists for determining the kinds of interactions possible between this very valuable antibiotic and deoxypolynucleotides.

¹ Maeda, K., et al., *J. Antibiotics* (Tokyo), **9**, A, 82 (1956).

² Falaschi, A., and Kornberg, A., *Fed. Proc.*, **23**, 940 (1964).

³ Lash, J., et al., *Anat. Rec.*, **128**, 679 (1957).

⁴ Pietsch, P., *Anat. Rec.*, **130**, 167 (1961).

⁵ *Handbook on Genetically Standardized JAX Mice* (Bar Harbor Times, Bar Harbor, Maine, 1962).

⁶ Within a few minutes of intraperitoneal injection of tritiated thymidine into mice, their urines became highly radioactive. Within 1 h activity falls to a fraction of this value, approaching background levels. Splenic biopsies 15 min after injection show a level of activity that is maintained in biopsies taken 30 and 90 min later. Thus, it would appear that label is available for a brief interval after administration and that a single injection closely approximates a pulse. These observations were made by Strossberg and Pietsch, 1962 (unpublished), and incidental to the present investigation.

⁷ Ogur, M., and Rosen, G., *Arch. Biochem.*, **25**, 262 (1950).

⁸ Pizzarello, D., et al., *Science*, **145**, 286 (1964).

⁹ Pilgrim, Ch., and Maurer, W., *Exp. Cell Res.*, **37**, 183 (1965).

¹⁰ Mommaerts, W., and Parrish, R., *J. Biol. Chem.*, **188**, 545 (1951).

¹¹ Tassoni, J. P., et al., *Exp. Cell Res.*, **34**, 219 (1964).

¹² Konigsberg, I. R., *Science*, **149**, 1273 (1963).

¹³ Stockdale, F., and Holtzer, H., *Science*, **146**, 866 (1964).

¹⁴ Pietsch, P., *Nature*, **203**, 117 (1964).

¹⁵ Stockdale, F., and Holtzer, H., *Exp. Cell Res.*, **24**, 508 (1961).

¹⁶ Snedecor, G., *Statistical Methods* (Iowa State Univ. Press, Ames, Iowa, 1950).

¹⁷ Williams, R., and Pietsch, P., *Anat. Rec.*, **151**, 434 (1965).

¹⁸ Umezawa, H., et al., *J. Antibiotics* (Tokyo), **15**, A, 275 (1962).

NOREPINEPHRINE AND SEROTONIN IN THE TISSUES AND VENOMS OF TWO PIT VIPERS

By PROF. AARON H. ANTON and PROF. JOSEPH F. GENNARO, jun.*

Departments of Anesthesiology, Pharmacology and Anatomy, College of Medicine, University of Florida, Gainesville

THE serotonin and catecholamine content of snake venom is of interest because of their possible contribution to the profound vascular changes and pain produced by this toxic substance. Venoms which have a defensive use, such as those of insect origin, have been found to contain serotonin, the quantity of which has been said to be directly related to the pain-producing ability¹⁻³. Zarafonitis and Kalas⁴ have implicated these materials in the skin slough and the necrotic lesions produced by reptile venoms because such damage has also occurred after the injection of serotonin and epinephrine. In their work, the authors mentioned that these amines were detectable only from specimens of reptile venom frozen in a dry-ice bath immediately after collection and thawed just prior to testing. They were unable to detect serotonin from the lyophilized venom which is used for the production of antivenin. Because of the implication that lyophilized venom, the preparation commonly used in most researches into the biochemical, physiological and immunological properties of reptile venom, does not contain materials present in the natural state, these observations required confirmation. Hence, several tissues of two species of poisonous snakes as well as their venom collected under various conditions were analysed for norepinephrine and serotonin.

The venom glands were excised and pooled from two specimens each of the Eastern diamondback rattlesnake (*Crotalus adamanteus*) and the cottonmouth moccasin (*Agkistrodon piscivorus*). Large snakes were anaesthetized with intraperitoneal injections of sodium pentobarbital (1-5 ml. of an isotonic saline solution containing 60 mg of barbiturate per ml.) and the venom gland was carefully dissected on each side of the head. Clamps were fixed to the venom ducts to prevent the expression of venom, and the glands of each species were pooled separately in glass dishes kept on ice before homogenizing for analysis. While still anaesthetized various tissues were removed and frozen until analysed for norepinephrine and serotonin.

Fresh venom was collected for analysis from other snakes of the same species into beakers containing 5 mg of β -phenylisopropylhydrazine ('Catron'), a monoamine oxidase inhibitor, and packed in dry ice. For collection of venom the fangs were hooked over the edge of a vessel and the snakes were allowed to bite and eject venom naturally. Little or no pressure was placed on the venom gland during this process as is sometimes done in the 'milking' of venom commercially. Venom was also collected from these two species into Dewar flasks filled almost to the top with liquid nitrogen. Venom ejected into the nitrogen bath was instantly frozen into small droplets which sank to the bottom of the nitrogen pool. The entire container was then transferred to a vacuum chamber and the venom was lyophilized. At no time before dryness did the temperature of the frozen droplets approach ambient temperature.

Catecholamines⁵ and serotonin⁶ were measured fluorometrically after extraction from tissues.

The tissue distribution of norepinephrine (NE) and serotonin (5-hydroxytryptamine, 5-HT) in these snakes is shown in Table 1. NE was detected in all tissues examined, including the venom gland, but not in any of the venom preparations. 5-HT, on the other hand, was found only in the brain of both species, and in the spleen of the rattlesnake. As with NE, no significant amounts

Table 1. TISSUE NOREPINEPHRINE (NE) AND SEROTONIN (5-HT) IN SNAKES
Results are from pooled samples from at least two specimens of each species. Figures in parentheses mean that the fluorescence spectra of these samples were not the same as the serotonin control. Kidney epinephrine of both species was about 50 per cent of the norepinephrine content, the spleen epinephrine of the cottonmouth was about 25 per cent of that of the norepinephrine whereas the other tissues contained insignificant amounts of epinephrine

Tissue	Cottonmouth, $\mu\text{g/g}$		Rattlesnake, $\mu\text{g/g}$	
	NE	5-HT	NE	5-HT
Brain	0.93	1.77	0.44	2.70
Heart	0.64	(0.24)	0.82	(0.06)
Kidney	0.98		0.82	
Liver	0.10	(0.29)	0.24	(0.06)
Lung	0.10		0.20	
Spleen	0.43	(0.18)		1.65
Poison gland	0.72	(0.29)	0.23	(0.63)
Venom:				
Lyophilized	<0.01	(0.03)	<0.01	(0.04)
Fresh milked	<0.01	(0.03)	<0.01	(0.04)
Stored milked	<0.01	(0.03)	<0.01	(0.04)

of 5-HT were found in any of the venom extracts, not even in the specimen collected in the presence of the monoamine oxidase (MAO) inhibitor. The inhibitor was used to prevent the possible destruction of the amines by the MAO that reportedly is present in the venom of these snakes⁴. It should be mentioned that we did not detect MAO, catechol-*o*-methyltransferase or 5-HT/DOPA decarboxylase in the venom or poison gland of the rattlesnake⁷; these enzymes are concerned with the metabolism of NE and 5-HT. However, a contaminating fluorescent substance was extracted from the tissue and venom of these snakes by the procedure that was used for 5-HT, and this is shown in Fig. 1. It can be seen that the fluorescence characteristics of authentic 5-HT and the brain extract are similar, whereas they are dissimilar in the case of the extracts from the poison gland and venom. Although the activation spectrum of the venom extract is shifted only slightly to the left of that of the 5-HT standard, the fluorescence spectrum lies completely out of the range of that exhibited by 5-HT. No differences were noted in the fluorescence produced by venoms which were prepared by

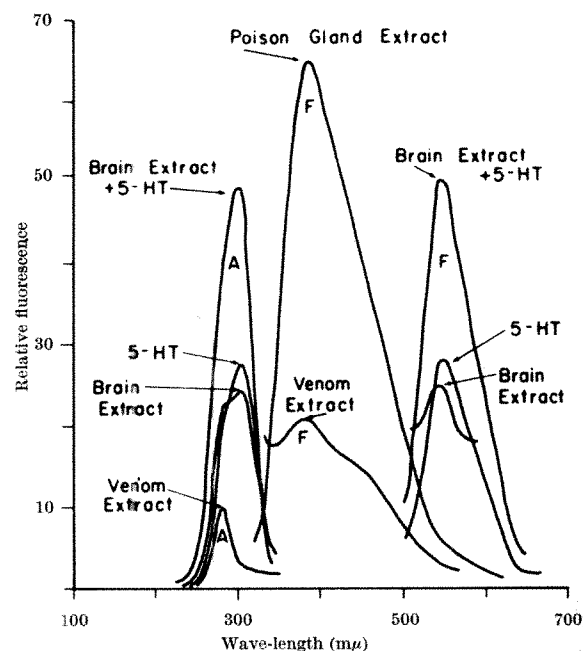


Fig. 1. Fluorescence characteristics of extracts from brain, poison gland and venom of the rattlesnake compared with authentic serotonin (5-HT)

* Present address: Department of Anatomy, University of Louisville, School of Medicine, Louisville, Kentucky.

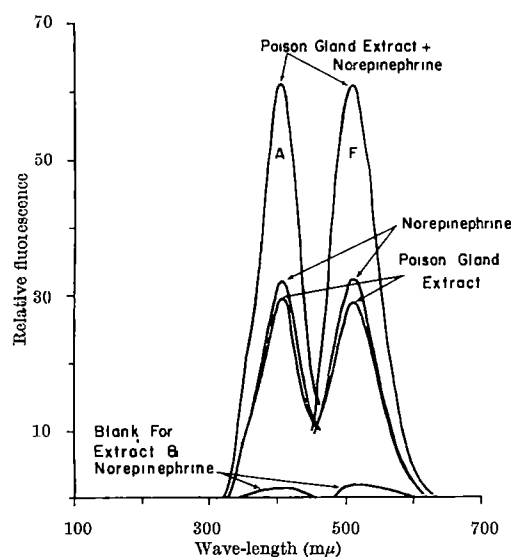


Fig. 2. Fluorescence characteristics of rattlesnake poison gland extract compared to authentic norepinephrine (NE)

lyophilization, or assayed immediately after removal from the snakes or were immediately frozen and assayed at a later time. In neither species studied here was this fluorescence the same as that of authentic 5-HT.

As shown in Fig. 2, the fluorescence characteristics of authentic NE and the substance extracted from the venom gland also were compared. In this case the spectra are identical, indicating that norepinephrine is present in the gland as well as that the analytical procedure for NE is more specific than that for 5-HT.

Norepinephrine was found to be distributed in the tissues of these snakes in a manner similar to that in other animals. Relatively large amounts of NE as well as serotonin were found in brain. This suggests a possible neural function for these amines in the peripheral and central nervous systems of these species analogous to that suggested for other animals. Finding NE in the poison gland may reflect some role in the function of this organ. On the other hand, 5-HT was not detected in the poison gland, and neither amine was found in the venom of these snakes as was reported by Zarafonitis and Kalas⁴. The reason for this discrepancy is probably one of methodology. We have found the Al_2O_3 -trihydroxyindole procedure for catecholamines to be much more specific⁸ than the organic solvent extraction method⁹ used by Zarafonitis

and Kalas. An organic solvent extraction procedure for 5-HT was also used by these workers and ourselves; but our results indicate the presence of a contaminating fluorescing material in venom and the venom gland which could be mistaken for 5-HT. Ordinary 'milking' procedures used to obtain reptile venom traumatize the glandular tissues, and thus could release cells and cellular constituents rich in this unidentified, highly fluorescing substance into the specimen being collected.

Other investigators either regard the minute amounts of these amines, which may be present in snake venom, as being insignificant, or have actually failed to detect their presence^{10,11}. In this respect, workers who have investigated the role of 5-HT in arthropod venom, where it appears in quantity, have found that the removal of this material does not alter the toxicity of the remainder¹². This has led to their questioning the biological significance of 5-HT in such venom¹³. Welsh and Batty have concluded that 5-HT, which can exist in very large quantities in the venom of arthropods and insects, may play a part in defence (as pain-producers), though they cite examples of scorpions, stinging ants, and solitary wasps which produce painful stings and do not contain 5-HT (ref. 3).

Therefore, it seems unwarranted at the present time to conclude that the possible presence of minute amounts of serotonin and norepinephrine contributes significantly to the profound tissue damage caused by the necrotizing and haemorrhagic principles which have been found in the venom of these snakes¹⁴.

This work was supported in part by funds from the National Heart Institute (HE-05251), the Institution of General Medical Studies of the U.S. Public Health Service (GM-07024) and a contract with the U.S. Army Medical Research Laboratory at Fort Knox, Kentucky (DA 15-014-AII-8189).

¹ Welsh, J. H., and Moorhead, M., *J. Neurochem.*, **6**, 146 (1960).

² Keele, C. A., *New Sci.*, **17**, 396 (1963).

³ Welsh, J. H., and Batty, C. S., *Toxicon*, **1**, 165 (1963).

⁴ Zarafonitis, C. J. D., and Kalas, J. P., *Amer. J. Med. Sci.*, **240**, 764 (1960).

⁵ Anton, A. H., and Sayre, D. F., *J. Pharm. and Exp. Ther.*, **138**, 360 (1962).

⁶ Bogdanski, D. F., Pletscher, A., Brodie, B. B., and Udenfriend, S., *J. Pharm. and Exp. Ther.*, **127**, 82 (1959).

⁷ Anton, A. H., Sayre, D. F., and Gennaro, J. F., jun. (in preparation).

⁸ Anton, A. H., and Sayre, D. F., *The Pharmacologist*, **5**, 268 (1963); (in preparation).

⁹ Shore, P. A., and Olin, J. S., *J. Pharm. and Exp. Ther.*, **122**, 295 (1958).

¹⁰ Ersparmer, V., *Prog. in Drug Res.*, **3**, 154 (1961).

¹¹ Welsh, J. H., cited by ref. 10.

¹² Adam, K. R., and Weiss, C., *Nature*, **178**, 421 (1956).

¹³ Adam, K. R., and Weiss, C., *Nature*, **183**, 1398 (1959).

¹⁴ Buckley, E. A., and Porges, N. (edit.), *Venoms* (Amer. Assoc. Adv. Sci., Washington, D.C., 1956).

FRACTIONATION OF MOUSE DEOXYRIBONUCLEIC ACID ON HYDROXYAPATITE

By DR P. M. B. WALKER

Chemical Biology Unit, Department of Zoology, University of Edinburgh

AND

DR. ANNE McLAREN

Agricultural Research Council Unit of Animal Genetics,
Institute of Animal Genetics, Edinburgh

WE have been examining the ability of various fractions of mouse DNA to form specific duplexes with homologous or heterologous DNA in agar^{1,2}. One particularly convenient method of obtaining fractions which differ either in their degree of secondary structure or in their base composition is by the use of hydroxyapatite columns^{3,4}. The results which we have obtained with such fractions may provide evidence concerning both

the hydroxyapatite fractionation procedure and the organization of mouse DNA.

DNA extracted from mouse livers or from mouse L-strain cell cultures¹ is heated for 10 min at 100° C in either 1.5×10^{-3} M sodium chloride and 1.5×10^{-4} M sodium citrate or 3×10^{-3} M phosphate buffer, pH 6.5, and is then cooled in ice water. Chromatography of this material on hydroxyapatite at a constant temperature

with increasing phosphate concentration yields two fractions (Fig. 1): a minor fraction, which at any given temperature of fractionation elutes at a salt concentration similar to native DNA, and a major fraction, which elutes at a lower salt concentration. We shall refer to these as the 'stable' and 'labile' fractions respectively. If the stable material is refractionated under the same conditions, it elutes at the same salt concentration (0.2 M) as before; if it is denatured again before refractionation, it still elutes at the same 0.2 M phosphate position, with only a minor component at the 0.1 M position (Fig. 2b). None of the labile fraction elutes at the 0.2 M phosphate position after a second fractionation (Fig. 2a). In subsequent experiments, the salt concentrations were changed

stepwise, so as to facilitate the recovery and characterization of the fractions. Fig. 3a shows a stepwise elution pattern for a sample of DNA disrupted by ultrasound and fractionated on hydroxyapatite at 70° C. In the stepwise procedure, DNA which elutes at 0.2 M on a gradient will appear between 0.15 and 0.3 M, that is in the 0.3 M washes.

Nature of the stable fraction. The fractionation of denatured DNA on hydroxyapatite depends on the presence of secondary structure, which is stable at the particular temperature at which the columns are run. It is therefore complementary to the thermal elution chromatography procedure also described by Miyazawa and Thomas³, in which native DNA is eluted as a function of temperature at a constant salt concentration. In the latter procedure, the fragments elute as soon as they melt, and it has been shown³ that the lower temperature fractions are rich in adenine and thymine (A + T), and the higher fractions in guanine and cytosine (G + C).

We have compared the two procedures directly by first fractionating native DNA by the thermal elution method, and then re-running these fractions at a constant temperature with stepwise elution on hydroxyapatite (Fig. 4). Those fractions melting at the highest temperatures contained the largest proportion of DNA eluting between 0.16 and 0.3 M phosphate.

Although the stable fraction elutes in the native position, it is not identical with native DNA. The most striking demonstration of this is seen in its ability to form stable duplexes with high molecular weight mouse DNA trapped in agar gels⁵. The high binding ability of the stable fraction is the more surprising since melting curve experiments confirm that it is at least partially duplex in structure. Fig. 3b shows thermal melting curves for stable and labile fractions from a 70° C hydroxyapatite fractionation. The two fractions have a very different melting behaviour: the labile DNA shows a typical 'single-stranded' melting profile, similar to that of RNA, while the stable material more nearly resembles the native material (curve ●, Fig. 5), though its mid-point of melting (T_m) is 5.5° C lower. Its lower T_m , together with the wider range of temperature over which it melts, indicates presumably that the reformed duplexes are less perfect than the native.

The proportion of sonically disrupted DNA eluting as a stable fraction increases as the temperature of fractionation is lowered from 70° C to 20° C. Fig. 5 compares the

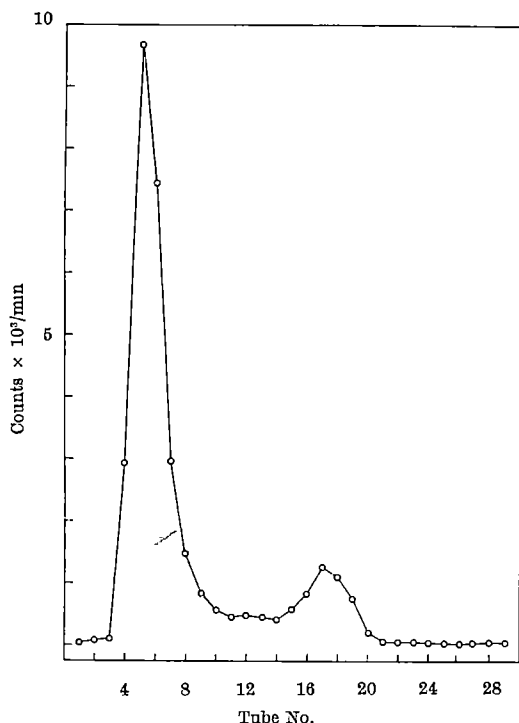


Fig. 1. Fractionation of sonically disrupted and denatured DNA on hydroxyapatite at 70° C. Phosphate gradient increases continuously from 0.03 to 0.3 M

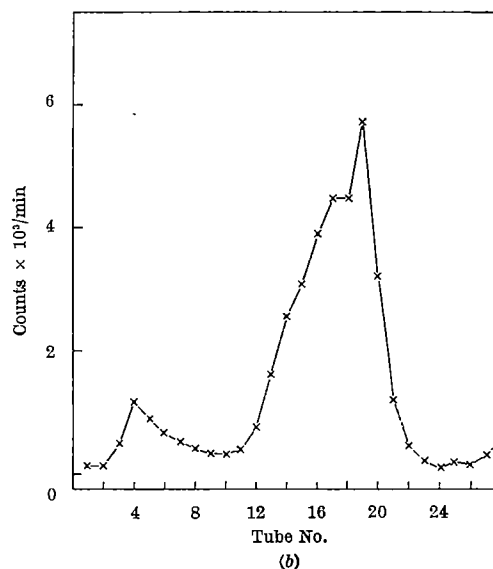
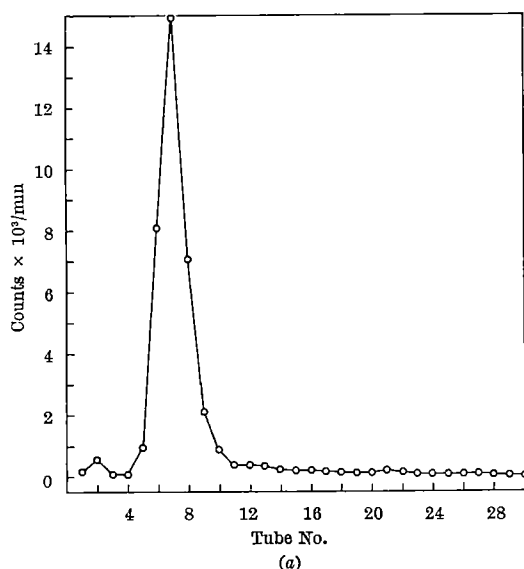


Fig. 2. a, Major fraction from fractionation shown in Fig. 1 re-run; b, minor fraction re-run after a second thermal denaturation. All other conditions as Fig. 1

melting curves of stable fractions obtained at five different temperatures with native DNA of a similar molecular weight distribution. From the 20° C and 40° C fractionations, about three-quarters of the stable material melts gradually below 60° C. The increase in size of the stable fraction as the temperature of fractionation is lowered must, therefore, be due to the inclusion of single-chain molecules, eluting at higher salt because of the secondary structure which forms in single-stranded material on cooling. A corollary of this is that any DNA fraction

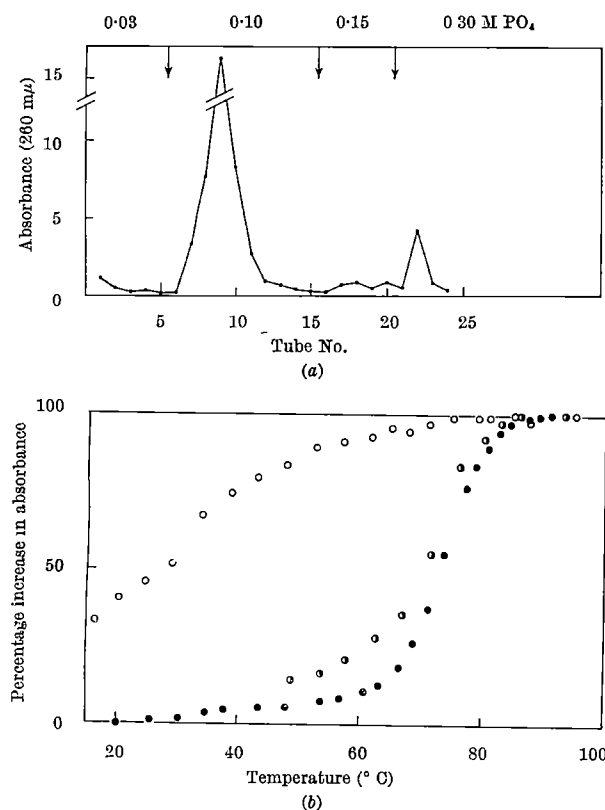


Fig. 3. *a*, 70° C stepwise elution chromatogram of sonically disrupted and denatured DNA, *b*, melting curves of fractionated DNA illustrated in *a*. ○, 0.1 M fraction; ●, 0.3 M fraction, first cycle; ◐, 0.3 M fraction, second cycle. All curves normalized to 100 per cent. Percentage increase in absorbance between 20° and 95° was 19.4 and 32.1 for the 0.1 and 0.3 M fractions respectively. All absorbances recorded in this article are at 260 mμ, and all melting curves are measured in 0.1 M NaCl and 10⁻⁴ cacodylate, pH 6.4

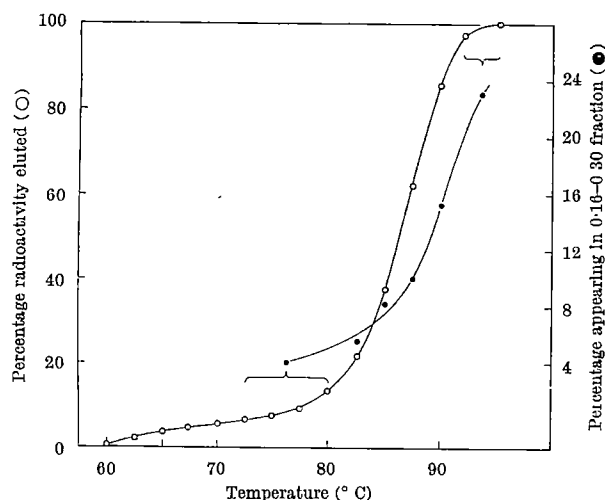


Fig. 4 Thermal elution chromatogram of mouse DNA in 0.08 M phosphate (○), and the percentage (●) of the indicated fractions (brackets show pooling) which eluted between 0.16 and 0.30 M phosphate on a subsequent salt gradient chromatogram at 70° C

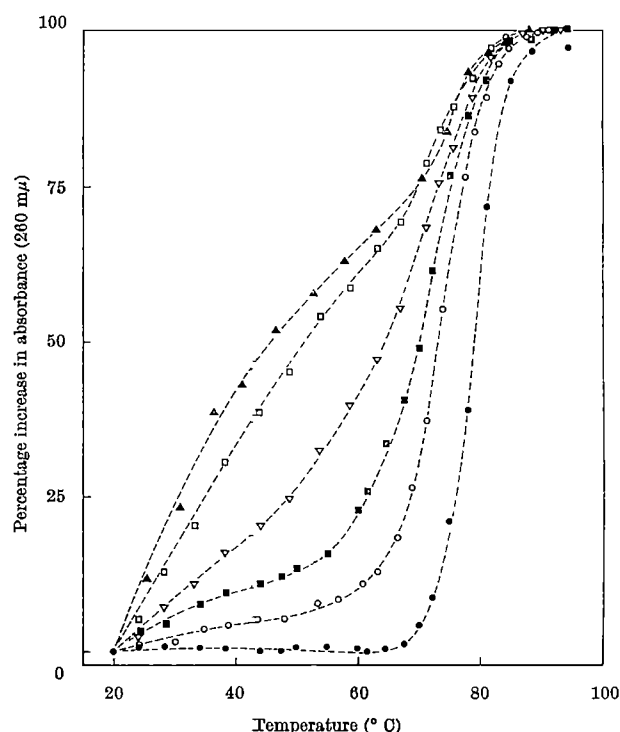


Fig. 5. Comparison of the melting curves of denatured DNA which elutes between 0.16 and 0.3 M phosphate at different temperatures on hydroxyapatite. The temperature of fractionation and percentage increase in absorbance between 20° and 95° are as follows: ▲, 20° C, 21.8 per cent; □, 40° C, 28.8 per cent; ▽, 50° C, 30.2 per cent; ■, 60° C, 29.0 per cent; ○, 70° C, 32.1 per cent; ●, unfractionated, native, 35.7 per cent

with a base sequence or composition which prevents it from forming secondary structure should elute in the 0.1 M phosphate position at room temperature. Our mouse DNA contains no such fraction.

Rate of reformation of duplex structure. The degree of reversibility of a thermal denaturation curve gives some indication of the speed and efficiency with which ordered duplex structure can be reformed. In the experiment illustrated in Fig. 3*b*, after heating to 95° C the cuvettes were rapidly cooled with iced water. On again raising the temperature, the absorbance was found to be higher than its original 20° C value, by only 20 per cent at 20° C, and by 32 per cent at 49° C. After 4 h at 49° C, the percentage increase at this temperature had fallen to 16 per cent and fell little more during a subsequent 12-h incubation at 49° C.

When the solution was then reheated to 90° C, it gave a melting curve very similar to that obtained during the first cycle of heating (●, Fig. 3*b*). These results indicate that a considerable amount of the original ordered structure was almost immediately reformed on cooling, with some further reassortment of strands occurring during incubation at 50° C. This has been confirmed by other experiments in which the second-cycle melting curve followed the first almost immediately.

Evidence of cross-linkage. It appears, then, that the stable fraction from a 70° C hydroxyapatite chromatogram consists of material which elutes at a high salt concentration owing to the rapid reformation of duplex structure after denaturation. This might be expected: (a) if the DNA fraction were made up of relatively short sequences repeated many times, so that association of strands at any one of a number of points gave complementary sequences over an extended region; (b) if the DNA strands either failed to separate completely on heating or if they folded back on themselves from the single-stranded state on account of some special sequence of bases.

On the first hypothesis, rate of duplex reformation should be related to the DNA concentration; while on the

other hypothesis, it should be independent of concentration. We therefore determined whether the concentration at the time of denaturation affected the relative size of the stable fraction. In all cases, DNA solutions in 0.03 M phosphate buffer were heated in a boiling-water bath for 10 min and rapidly chilled in ice; two volumes of distilled water were then added and the DNA immediately adsorbed on to the hydroxyapatite. We found (Table 1) that the size of the stable fraction was affected neither by the concentration at which the DNA sample was denatured nor by the amount of DNA that was put on the hydroxyapatite column.

This evidence might suggest that the strands are held in register by heat-resistant bonds. Our stable fraction may contain a high proportion of CG-rich nuclei (Fig. 4), which are known to resist melting at high temperatures⁶, but which are sensitive to alkaline denaturation⁷. When a sample of DNA was denatured by raising the pH to 12.4 for 10 min at 18° C, the size of the stable fraction was unaffected (Table 1). It therefore seems unlikely that the thermal stability of the CG-rich regions is responsible for holding the strands in register.

Table 1. THE EFFECT OF CONDITIONS DURING DENATURATION ON THE PROPORTION OF DNA ELUTING BETWEEN 1.5 AND 0.3 M PHOSPHATE

Denaturation treatment	DNA concentration during denaturation ($\mu\text{g/ml.}$)	Amount of DNA on hydroxyapatite column (μg)	% of radioactivity eluting between 1.5 and 0.3 M ('stable fraction')
Thermal	30	5	12.7
Thermal	70	12	11.0
Thermal	0.3	3	13.8
Thermal	0.7	0.12	9.2
Alkaline	30	4	8.3
Alkaline	30	4	9.3

Another possibility is that the strands are held together by covalent linkages. These, if present in nature, could be composed of protein. We therefore subjected our DNA before denaturation to digestion by the proteolytic enzyme, 'Pronase B' (Calbiochem Inc.), for 2 and 15 h at 37° C; but this had no effect on the size of the stable fraction.

Cross-linkages may be induced in DNA, for example by nitrous acid. Geiduschek⁸ reported that such cross-linked vertebrate DNA will renature almost completely, and

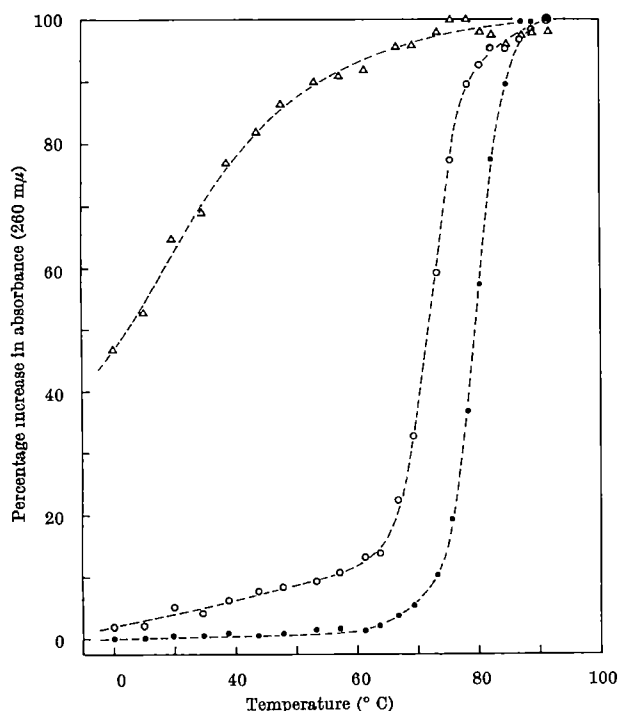


Fig. 6. Melting curves of nitrous acid-DNA. Δ , 0.03-0.12 M phosphate fraction; \circ , 0.12-0.16 M phosphate fraction; \bullet , native DNA. All were from the same batch of sonically disrupted DNA.

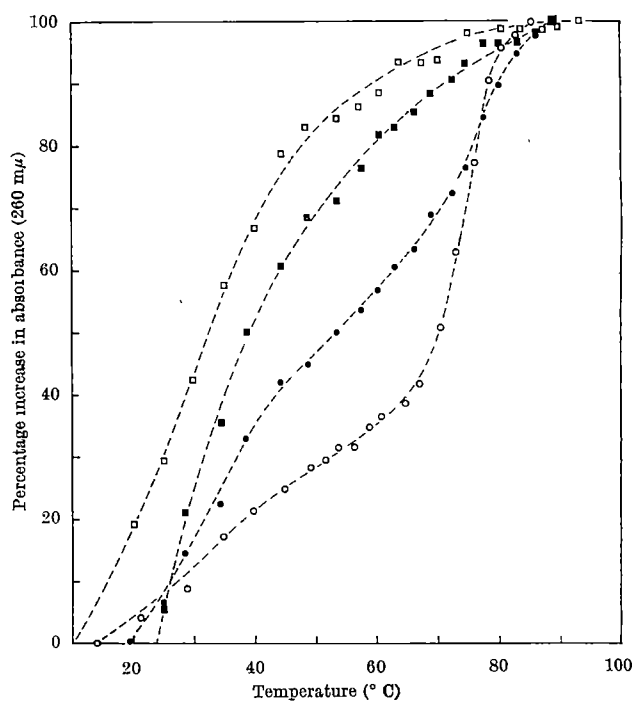


Fig. 7. Melting curves of sheared and high molecular weight DNA. \circ , 0.03-0.10 M, sheared DNA, 20.3 per cent; \square , 0.15-0.30 M, sheared DNA, 26.6 per cent; \bullet , 0.03-0.10 M, high molecular weight DNA, 20.2 per cent; \blacksquare , 0.15-0.30 M, high molecular weight DNA, 21.6 per cent. Numbers refer to molarity of phosphate. The percentages refer to the overall increase in absorbance.

that only a small fraction of the amino groups need be cross-linked to allow this renaturation to occur. Since our stable fraction not only has a T_m 5.5° C lower than that of native DNA, but is also able, unlike native DNA, to form stable duplex molecules with denatured DNA in agar, it cannot be the completely helical molecule which Geiduschek's results might lead one to expect. To investigate the properties of deliberately cross-linked DNA, we treated a sonically disrupted sample with nitrous acid, following Geiduschek's procedure exactly, then denatured it and subjected it to fractionation on hydroxyapatite. This treatment caused 60 per cent of the DNA to elute between 0.12 and 0.16 M phosphate, the remainder appearing between 0.03 and 0.12 M phosphate. The melting curves of these two fractions were strikingly different (Fig. 6), the high salt fraction showing a sharp melting transition 7.0° C lower than that of an untreated native sample of the same molecular weight distribution.

We also fractionated high molecular weight DNA, which had been similarly treated with nitrous acid, and found that all the DNA now eluted between 0.16 and 0.30 M phosphate. This DNA had a melting curve identical with the high salt fraction from the low molecular weight sample. The induced cross-linkages can therefore allow considerable lengths of DNA to renature which are not themselves cross-linked. As will be shown below, the stable fraction of DNA is unable to promote the complete renaturation of higher molecular weight samples.

To determine whether ultrasonic treatment of our DNA might have caused cross-linkages to form, we subjected to hydroxyapatite fractionation our high molecular weight DNA, and also DNA sheared by a single passage through a French pressure cell. The sheared material is trapped in a plain agar gel more readily than is the sonically broken DNA, and must therefore have a higher average molecular weight. On denaturation and subsequent hydroxyapatite fractionation at 70° C, the sheared DNA yielded stable and labile fractions with 0.3 M and 0.1 M phosphate respectively. The melting curve of the stable fraction gave a fairly sharp transition

between 65° C and 80° C (Fig. 7), but unlike the corresponding fraction from ultrasonically broken DNA, it also showed a gradual increase in absorbance between 20° and 60° C. The high molecular weight DNA gave a small stable peak eluting with 0.3 M phosphate, and also an intermediate peak with 0.15 M phosphate. The melting curves of the 0.1 M and 0.3 M phosphate fractions are much more like one another than are those of lower molecular weight DNA, but some evidence for a fairly sharp transition can still be seen at high temperatures.

We interpret these results to mean that at higher molecular weights the molecules which elute at the high salt concentration are partially single-stranded, a relatively short duplex section sufficing to stabilize the whole molecule on the hydroxyapatite. The small size of the 0.3 M fraction obtained from the high molecular weight preparation is probably due to the presence of long single-stranded regions in some of the molecules which also contain duplex regions. A high ratio of the single-stranded to duplex parts might cause them to elute at a lower salt concentration. As the molecular weight is reduced, a progressively larger proportion of each 'stable' molecule is in the duplex state, resulting in a much cleaner fractionation. These results do not support the view that the stable fraction consists of material in which cross-linkages have been induced by ultrasonic treatment.

Discussion. While it is clear that the fractionation of denatured DNA on hydroxyapatite depends on the presence of secondary structure, the method described in this article does not easily distinguish between native DNA, and duplex molecules of stabilities ranging from those melting gradually below 60° C to those showing a sharp thermal transition like that of the native structure. All these can elute from the columns at a phosphate concentration near 0.2 M. The quality of the 0.2 M fractions of DNA, as judged by the shape of their melting curves, depends both on the molecular weight distribution of the initial preparation and on the temperature of fractionation. When these two factors are independently varied, the melting curves produced show a similar pattern, though for rather different reasons.

As we lower the temperature of fractionation, using sonically disrupted DNA of a constant molecular weight distribution, progressively more of the DNA fragments are able to form secondary structure and hence to elute at 0.2 M phosphate. Thus, each melting curve illustrated in Fig. 5 represents a heterogeneous fraction of molecules in which the proportion of the more perfect duplexes decreases with the temperature of fractionation. When DNA of differing molecular weight is fractionated at a constant temperature (70° C), the proportion of the more perfect duplexes again decreases, this time as molecular weight increases, but now the heterogeneity must be along the length of the molecules rather than between molecules. The additional regions must be capable of forming secondary structure at lower temperatures, which melts gradually as the temperature is increased to 60° C. Presumably in their single-stranded condition at 70°, they can be stabilized on hydroxyapatite by the more perfect duplexes to which they are attached.

Our comparison of the chromatographic behaviour of high molecular weight and ultrasonically treated DNA cross-linked with nitrous acid (Fig. 6), as well as earlier evidence⁸, suggests that the bridges which hold the two strands of denatured DNA in register will allow single strands to reform duplexes even if they are at a considerable distance from the bridge. Our stable DNA fraction appears to lack this property, since increasing the molecular weight causes less of the DNA to elute at high salt, and the molecules which do appear at high salt contain a considerable proportion of single strands.

Since the linkages which we believe to be responsible for the properties of the stable fraction are resistant to treatment with heat, alkali and 'Pronase', we are forced to consider models in which the covalent linkage is not

between the two strands of the double helical molecule. For example, the stable covalent bond might consist of a phosphodiester linkage between bases in a single DNA strand, such that the base sequences on either side would allow the strand to fold back on itself and form a duplex region like a hairpin. Discrepancies in the complementary sequences, and the random nature of the original double-strand scissions induced by sonic disruption, could then account for the lower stability of the duplex compared with the native molecule, and for the availability of sites for binding in the agar experiments. The effect of increasing the molecular weight would imply that the sequence restrictions only extend for a relatively short distance from the point of inflexion before becoming non-complementary.

The second model requires further assumptions. If the genome in higher organisms has more than one double helix of DNA per chromosome, then the semi-conservative nature of chromatid reproduction⁹ demands some mechanism for assorting the old and new strands. If covalent linkages joined corresponding halves of the helical molecule, denaturation would give one linked set of old and one of new strands. If the base sequences on either side of the linkages were restricted as in our first model, so as to allow duplex regions to reform, then this model also would explain the properties of stable DNA.

There is accumulating evidence that there may be what might be termed macro-heterogeneity in DNA from higher organisms, that is that there are sections or species of DNA which have properties sufficiently different from the rest to allow them to be isolated or identified. Such sections, which may have special genetic functions, may be sufficiently small to facilitate the eventual determination of base sequences.

The large size of the DNA fraction with the special properties we have been considering, together with the evidence that, in sheared and high molecular weight DNA, the fractions may be joined to other single-chain parts of the denatured molecule, would seem to preclude either cytoplasmic or viral DNA as the source of this fraction. It is also too large to be accounted for by the DNA sequences coding for ribosomal RNA¹⁰, although there is evidence from the structure of such RNA¹¹ that it might be coded by a DNA with similar sequence restrictions to those we have postulated for our DNA fraction. On the other hand, the 'satellite' bands of DNA separated by equilibrium density centrifugation from several mammals¹², including the mouse, are of the same relative size. The satellite band in the mouse is, however, lighter than the main band and probably has a higher AT content.

This work was supported by the Nuffield Foundation and the Medical Research Council. We thank Dr. R. Sinclair for providing tissue-culture cells, and Miss M. McCallum, Mr. A. Mitchell and Mr. T. M. Wadsworth for their assistance. We also thank Dr. C. A. Thomas of the Johns Hopkins University for sending us a copy of his paper with Dr. Y. Miyazawa in advance of publication.

¹ Walker, P. M. B., and McLaren, A., *J. Mol. Biol.*, **12**, 394 (1965).

² McLaren, A., and Walker, P. M. B., *Genet. Res.*, **6**, 230 (1965).

³ Miyazawa, Y., and Thomas, C. A., *J. Mol. Biol.*, **11**, 223 (1965).

⁴ Bernardi, G., *Nature*, **206**, 775 (1965).

⁵ McLaren, A., and Walker, P. M. B. (in preparation).

⁶ Marmur, J., Rownd, R., and Schildkraut, C. L., *Progress in Nucleic Acid Research*, **1**, 231 (1963).

⁷ Murray, K., and Peacocke, A. R., *Biochim. Biophys. Acta*, **55**, 935 (1962); Imman, R. B., and Baldwin, R. L., *J. Mol. Biol.*, **8**, 452 (1964).

⁸ Gelduschek, E. P., *Proc. U.S. Nat. Acad. Sci.*, **47**, 950 (1961).

⁹ Taylor, J. H., Woods, P., and Hughes, W., *Proc. U.S. Nat. Acad. Sci.*, **43**, 122 (1957).

¹⁰ Ritossa, F. M., and Spiegelman, S., *Proc. U.S. Nat. Acad. Sci.*, **53**, 4, 737 (1965). Wallace, H., and Burnstiel, M. L., *Biochim. Biophys. Acta* (in the press).

¹¹ Spencer, M., and Poole, F., *J. Mol. Biol.*, **11**, 314 (1965).

¹² Kit, S., *J. Mol. Biol.*, **3**, 711 (1961). Chun, E. H. L., and Littlefield, J. W., *J. Mol. Biol.*, **7**, 245 (1963).

STIMULATION OF SYNTHESIS OF RIBONUCLEIC ACID IN SUB-APICAL SECTIONS OF *Avena* COLEOPTILE BY INDOLYL-3-ACETIC ACID

By PROF. T. H. HAMILTON, R. J. MOORE*, A. F. RUMSEY, A. R. MEANS and PROF. A. R. SCHRANK

Department of Zoology, University of Texas, Austin

FIG. 1 and its insert illustrate the well-known biological response of *Avena* coleoptile sections to the presence in culture medium of auxin or the plant hormone indolyl-3-acetic acid (IAA). This response represents one of increased growth (= cell elongation) rate over that which occurs in the absence of IAA. Time-sequence investigations (insert, Fig. 1) indicate that the differences in morphological responses between controls and IAA-stimulated coleoptile sections are particularly pronounced after the initial (8–10 h) period of hormone action. By 24 h the IAA-stimulated sections exhibit increases in length which are about 300 per cent greater than those of controls.

In recent years the involvement of RNA and protein synthesis in early hormone action has been demonstrated for a variety of animal¹ and plant² tissues. Furthermore, the inhibition of various biochemical and morphological responses to hormone by actinomycin D (ref. 3) has been interpreted as indicating the necessity for DNA-dependent RNA synthesis for complete manifestation of the biological response to hormone. These investigations^{1,2,4} have emphasized—implicitly if not explicitly—the problem of elucidating biochemical events which occur prior to the morphological ones during sequential responses of specific tissue to hormones. We have thus undertaken for *Avena* coleoptile an investigation of the effect of IAA on RNA synthesis during the initial 8-h period prior to full achievement of morphological response to the hormone.

We now report data which indicate a marked stimulation by IAA of the rate of synthesis of the major classes of RNA in sub-apical sections of the *Avena* coleoptile. We find this stimulation by IAA to be inhibited by actinomycin D. We interpret these observations to indicate that IAA in its early action indirectly or directly exerts an influence on some rate-limiting step in DNA-dependent RNA synthesis. Along with base compositions of total RNA and ribosomal RNA (rRNA), the data presented also provide a partial characterization of the ribosomes (80 S) and classes of rRNA (30 S and 20 S) of *Avena* coleoptile.

Seeds of *Avena sativa* L. (Victory strain: U.S. Department of Agriculture, C.I. 2020) were germinated and grown according to the method of Wiegand and Schrank⁵. After removal of the primary leaf, 5-mm sections of coleoptiles were cut, starting 5 mm below the tip. Absolute growth-rates at times indicated (Fig. 1) were determined by the taking of linear measurements to the nearest 0.1 mm by use of a dissecting scope equipped with an ocular micrometer. For the morphological responses and labelling experiments described as follows, all experimental conditions were identical.

In the course of this work, bacterial contamination of both culture media and cavities of coleoptile sections became evident. Thorough washing of coleoptile sections by the method of Glišin and Glišin⁶ proved partially successful, removing approximately 95 per cent of bacteria in cultures prior to the washings. For an antibiotic, penicillin, streptomycin, tetracycline, mycostatin, erythromycin, and kanamycin were tested. Bacterial levels of samples of culture medium and coleoptile homogenates were assayed by inoculation tests in brain-heart-infusion broth. Briefly stated, with exception of penicillin the antibiotics listed (when used in concentrations necessary for suppression of bacteria) had inhibitory effects either on morphological responses or on RNA synthesis (or

both) of coleoptile sections. Exposures to ultra-violet light—varying length of exposure, distance from source, and intensity of irradiation—were similarly harmful to control responses. Neither by antibiotics nor irradiation techniques have we been able to obtain a bacteria-free coleoptile system without also inhibiting the control responses to culture medium. For the purposes of the work recorded here, penicillin alone provided some protection, but even that resulted in only a slight reduction of bacteria number. We note here that there are no published reports of an effect of IAA on bacterial growth.

In all experiments, 200 coleoptile sections were incubated in 10 ml. of a standard medium (0.01 M tris-maleate, 2 per cent sucrose, 60 units/ml. penicillin GK) having a pH of 5.5 at 5° C. ³H-uridine (1.84 c./mmole) in amount sufficient to maintain linear incorporation for 24 h was added to the medium for a final concentration of 0.002 mM. IAA and actinomycin D, when used, were at final concentrations of 1×10^{-5} M (1.75 µg/ml.) and 8×10^{-6} M (10 µg/ml.). Incubations were carried out under dim red light in a water-bath shaker at 25° C. 8 h later, incorporations were stopped by rapid washing at 0°–2° C of the sections with 500 ml. of an acetate buffer (0.01 M NaAc, 0.1 M NaCl, 0.001 M MgCl₂; pH 5.0 at 5° C).

Coleoptile sections were homogenized at 0°–2° C by 20 strokes in 2 ml. of the acetate buffer in a glass-glass homogenizer run at 600 r.p.m. The homogenate was then adjusted to 1 per cent sodium dodecyl sulphate (SDS) and extracted with an equal volume of buffer-saturated phenol by vigorous shaking at 0° C for 3 min. The layers were separated by centrifugation, and the aqueous layer was extracted twice more with one-half and one-fourth volumes of phenol. Following addition of 2 vol. of cold ethanol to the final aqueous layer, RNA was precipitated

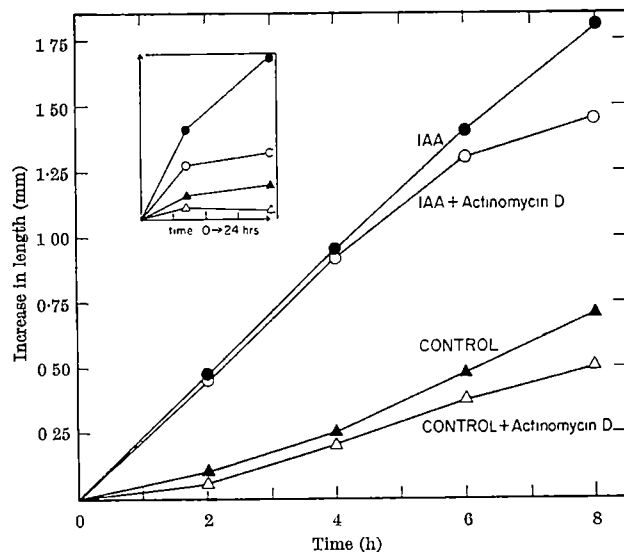


Fig. 1. Growth of *Avena* coleoptile sections as a function of time. Measurements are expressed as increases in length (over a base-length of 5 mm) from 0 to 8 h. Closed (▲) and open (△) triangles denote values for sections incubated in control medium and control medium with actinomycin D added. Closed (●) and open (○) circles denote values for sections incubated in the presence of IAA with actinomycin D absent or present. Concentrations of IAA and actinomycin D and conditions of incubation are described in the text. Insert shows schematically the trends we find for the four experiments run under the same conditions for 24 h. Increases here for IAA-stimulated sections are typically of an order of 3.5–4.0 mm over the 5-mm length at time zero.

* Present address: Department of Biochemistry and Biophysics, Iowa State University, Ames, Iowa.

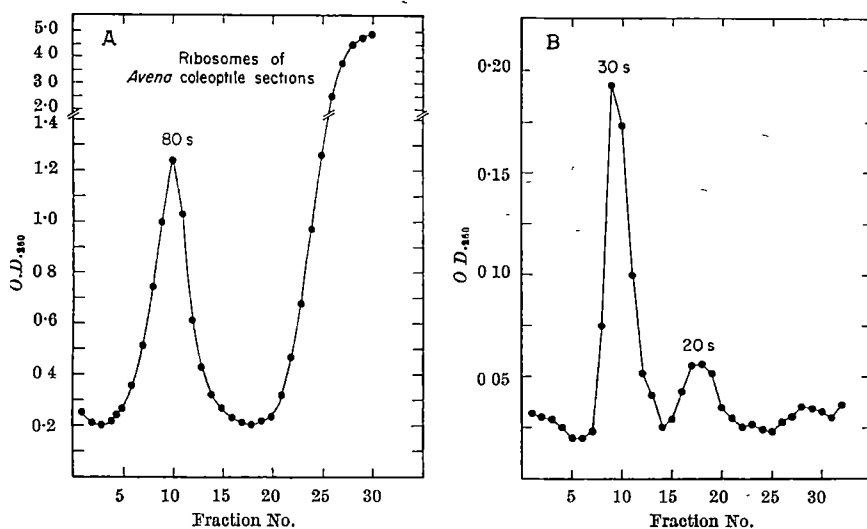


Fig. 2. 80 S ribosomes and their rRNA for *Avena* coleoptile. A, A cytoplasmic extract of coleoptile homogenate (total volume: 2 ml. in 0.02 M *tris*, 0.02 M KCl, 10⁻² M MgCl₂, pH 7.8 at 5° C) was centrifuged at 15,000g for 10 min and made 0.5 per cent sodium deoxycholate. The clarified extract was then run on a 5–20 per cent w/v sucrose gradient (in the *tris* buffer) at 4° C for 4 h at 24,000 r.p.m. 1.0-ml. fractions were collected and their O.D.₂₆₀ determined. B, To show the presence of two sedimentation classes of rRNA in ribosomes thus isolated, fraction 10 (corresponding to 80 S ribosomes) was dialysed against 0.1 M NaCl, 0.005 M MgCl₂, 0.5 per cent SDS, pH 7.8 at 5° C. This was run at 24,000 r.p.m. on a 15–40 per cent w/v sucrose gradient containing NaCl, MgCl₂ and SDS in the concentrations listed. 1.0-ml. fractions were collected, and their O.D.₂₆₀ values determined.

for 2 h at –20° C, collected by centrifugation, and reprecipitated in cold ethanol overnight.

Coleoptile RNA thus obtained was dissolved in 1 ml. of the acetate buffer and layered on to a 20-ml. linear sucrose gradient (5–20 per cent w/v in the acetate buffer already described here). Following centrifugation at 4° C in the SW 25 rotor for 18 h at 24,000 r.p.m., 0.8 ml. fractions were collected in 1.0 ml. water. Aliquots of 0.5 ml. from each fraction were added to 15 ml. of Bray's solution⁷ and assayed for tritium in an Ansitron liquid scintillation counter (counting efficiency: 20 per cent with a background of 24 c.p.m.). The O.D.₂₆₀ values for the fraction volumes remaining were determined by use of a Beckman DU spectrophotometer.

Morphological response to IAA; effects of actinomycin D. In our experimental system, *Avena* coleoptile sections show a linear rate of increase in length during the first 3 or 10 h, and a substantially reduced rate thereafter to 24 h (insert, Fig. 1). This observation is in agreement with that of Shibaoka and Hurusawa⁸. Examining in detail the initial 8-h response for effects of IAA and actinomycin D (Fig. 1), we find the following: (1) a marked acceleration of rate of increase in length for IAA-stimulated sections over that of controls (ratio of rates: 2.6 as reported by Cleland⁹); (2) only light inhibition of either control or IAA responses in the presence of actinomycin D.

This initial lack of sensitivity to actinomycin D (cf. Fig. 1 and insert) may represent either a delay in time for coleoptile uptake of the inhibitor from the medium or that part of the coleoptile's morphological response is truly insensitive to actinomycin D. Supporting the latter possibility is our finding that increased concentrations of actinomycin D (100 µg/ml.) and/or pretreatment periods (30 min; 1 h) also result in the trends noted in Fig. 1 (see following). By 24 h the inhibitory effects of actinomycin

D on IAA and control responses of coleoptiles are clearly discernible.

Characterization of ribosomes and RNA. From 80 S ribosomes¹⁰ isolated from cytoplasmic extracts of coleoptile sections (Fig. 2), and from whole sections, RNA was phenol extracted and characterized on sucrose gradients as already described here (cf. Fig. 2 and Fig. 3A). By comparison with sedimentation profiles of RNA with known *s* values and by analyses in the Model E analytical ultracentrifuge, we determined the presence of 30 S and 20 S classes of rRNA in *Avena* coleoptile sections.

Base composition for total RNA of sections is as follows for percentages of the nucleoside monophosphates UMP, GMP, AMP and CMP; 13.6, 28.2, 23.4 and 24.8 (GC/AU = 1.70)¹¹. For rRNA extracted from 80 S cytoplasmic ribosomes, the values are: for 30 S fractions, 19.2, 32.9, 23.8 and 24.1 (1.33); for 20 S fractions, 18.9, 33.8, 23.1 and 24.2 (1.38)¹¹.

The higher GC/AU ratio of 1.70 for total RNA hints of a higher GC content for soluble RNA of coleoptiles. The base composition for total RNA of *Avena* coleoptile is close to that for tobacco leaf RNA, but its GC/AU is higher than that reported for four other plant species¹².

Stimulation of RNA synthesis by IAA; effects of actinomycin D. As shown in Figs. 3 and 4, the presence of IAA in culture medium results in a marked increase (about 50 per cent) in the incorporation of ³H-uridine into all classes of coleoptile RNA. Under the conditions here used, the extent of this stimulation is nearly the same (48, 60, 47 per cent) for three major classes (30 S, 20 S, 4 S) of RNA. The ranges of variation for triplicate experiments are shown in Fig. 4. Evidence for an effect of IAA on net synthesis of coleoptile RNA is presented in Table 1. In each case, the amount of O.D.₂₆₀-absorbing material extracted as RNA is greater from IAA-stimulated sections than from control sections.

Actinomycin D suppresses the incorporation of ³H-uridine into coleoptile rRNA (control) as well as inhibits the stimulation of such incorporation by IAA (cf. Fig. 5 with Fig. 3). The residual radioactivity remaining on sucrose gradients (Fig. 5) of RNA extracted from actino-

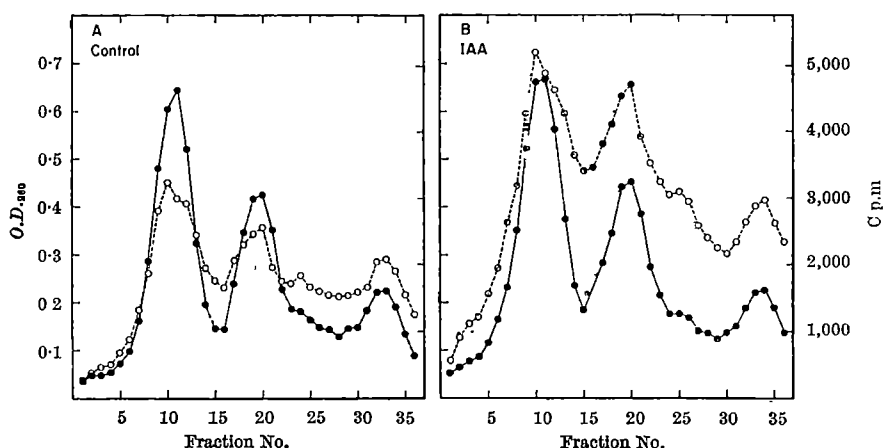


Fig. 3. Sucrose density gradient profiles showing effects of IAA on incorporation of ³H-uridine into RNA in *Avena* coleoptile sections. Closed (●) and open (○) circles denote values for O.D.₂₆₀ and radioactivity (c.p.m.).

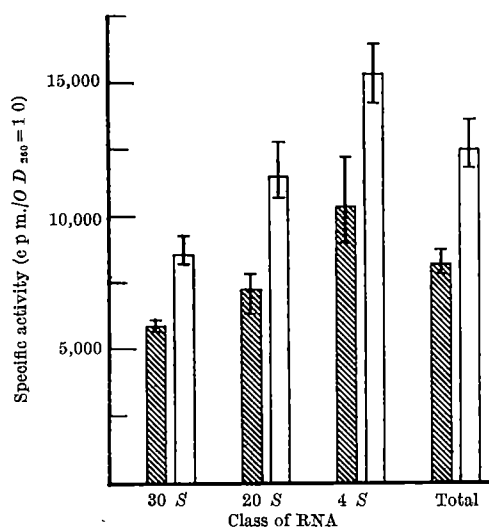


Fig. 4. Specific activities of the labelled RNA extracted from control (shaded bars) and IAA-stimulated (open bars) *Avena* coleoptile sections. Values are derived from triplicate experiments (one of which is shown in Fig. 3), and are expressed for each class of RNA as well as for total RNA. The specific activity of each class is determined from the five fractions comprising each UV peak (the peak fraction plus the two on either side). The specific activity of total RNA is based on the total radioactivity and $O.D._{260}$ values for each gradient.

mycin *D*-treated sections demonstrates, however, two peaks. That the bulk of this residual radioactivity stems from RNA synthesis by actinomycin *D*-resistant bacteria or chloroplasts¹⁰ is evident from the slower rate of sedimentation; on the basis of comparison with $O.D._{260}$ -absorbing boundaries of 30 *S* and 20 *S* coleoptile *r*RNA, these peaks show smaller *S* values corresponding in the main to bacterial¹¹ or chloroplast *r*RNA^{10,12}.

Although the close coincidence of $O.D._{260}$ -absorbing and radioactivity profiles from control and IAA-stimulated systems (Fig. 3) indicated otherwise, we tested the possibility that enhanced incorporation of 3H -uridine into RNA extracted from coleoptiles is a result of influence of IAA on bacterial RNA synthesis. The results noted in Table 2 show this not to be the case; acid-insoluble radioactivity for equal volumes of culture medium containing coleoptile sections incubated for 8 h exhibits no difference for the presence or absence of IAA.

Our findings for *Avena* suggest that the role of indolyl-3-acetic acid as an inducer of plant growth is, in part at least, biochemically manifest by an increase in rate of synthesis of ribosomal and soluble RNA. The incorporation of 3H -uridine into each class of RNA is markedly stimulated in the presence of this naturally occurring hormone. Actinomycin *D* effectively suppresses RNA synthesis in both control and IAA-stimulated systems. This points strongly to the involvement of DNA-dependent RNA synthesis in the early action of the hormone, and we infer that IAA exerts its influence by indirectly or directly activating some unknown rate-limiting step in such RNA synthesis. We note, nevertheless, that inhibition by actinomycin *D* of hormone-stimulated RNA synthesis indicates only that the DNA-RNA complex of the genome must be functional at the time of hormone action. Suppression of hormone action by the inhibitor does not mean that the hormone acts at the site of the gene.

It should be noted that *Avena* coleoptile sections undergo: (1)

Table 1. PHENOL-EXTRACTED RNA FROM SUB-APICAL SECTIONS OF *Avena* COLEOPTILE

Exp.	Control	IAA
1	8.282	9.958
2	8.179	9.078
3	8.806	9.396
Average	8.422	9.477

The $O.D._{260}$ units extracted as RNA are expressed for each experiment as the mathematical summation of the thirty-six $O.D._{260}$ values for the fractions collected from sucrose gradients run under standard conditions (Fig. 3). Each experiment is for 200 control or 200 IAA-stimulated coleoptiles incubated for 8 h as described in the text.

Table 2. ACID-INSOLUBLE RADIOACTIVITY OF COLEOPTILE CULTURE MEDIUM

Exp.	Control	IAA
1	1,176,660 c.p.m.	1,032,900 c.p.m.
2	983,520	1,082,760
3	970,980	984,600
Average	1,043,720	1,033,420

From standard incubation medium (containing 200 coleoptiles, no penicillin, 0.002 mM 3H -uridine, and either no IAA or 1×10^{-4} M IAA) 1.0 ml. media samples were taken after 8 h incubation and assayed for acid-insoluble tritium as follows: 0.5 mg of carrier albumin was added, followed by 10 per cent trichloroacetic acid (TCA) to a final concentration of 5 per cent. The precipitates were collected on 'Millipore' filters, thoroughly washed with cold 5 per cent TCA, dried, and counted by liquid scintillation. Values are expressed for the total 10-ml. culture.

increases in length to some extent in the presence of actinomycin *D*; (2) increases to a greater extent in the presence of actinomycin *D* and IAA (Fig. 1). That this occurs concomitantly with inhibition of RNA synthesis (cf. Fig. 1 with Fig. 5) is further evidence for the possibility that part of the morphological response to control medium and to IAA is insensitive to actinomycin *D*.

A goal of many workers concerned with the roles of nucleic acid formation and function in early hormone action is to determine the degree of similarity or dissimilarity in the pattern of sequential biochemical responses of specific vertebrate, invertebrate, and plant tissues to their characteristic hormones¹³. For two vertebrate hormonal mechanisms (thyroxine \rightarrow rat liver¹⁴; oestrogen \rightarrow rat uterus¹⁵), it is now clear that stimulation of nuclear RNA synthesis precedes stimulation of two DNA-dependent RNA polymerase activities in early hormone action. Whether such sequences will hold true for invertebrate and plant hormonal mechanisms, as well as other vertebrate ones, remains to be determined. For hormone-sensitive plant tissues, however, an interesting set of questions arises concerning nuclear and/or (?) cytoplasmic sites of action and synthesis. There is now evidence¹⁴ for the presence of DNA and DNA-dependent RNA polymerase in chloroplasts—units functioning as 'cells within cells', thus providing extranuclear sites of RNA synthesis. Detailed time-sequence investigations of RNA synthesis and RNA polymerase activities for nuclear, chloroplast and microsomal fractions isolated from *Avena* coleoptile sections cultured in the presence or absence of IAA may provide the answers.

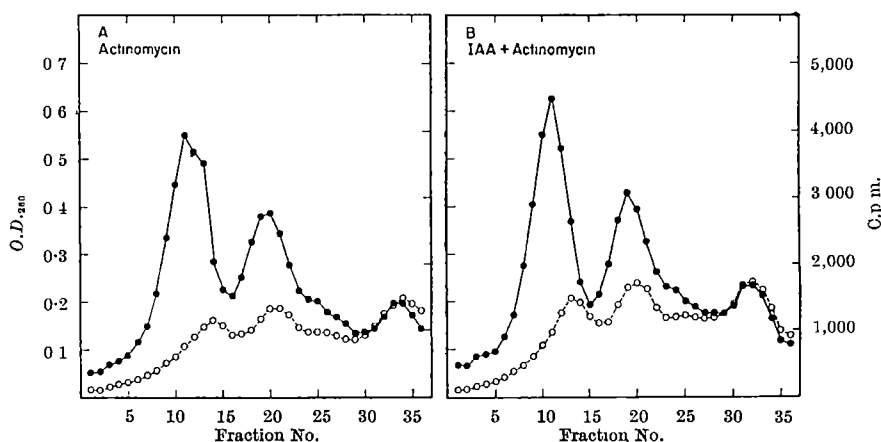


Fig. 5. Sucrose density gradient profiles showing effects of actinomycin *D* in the absence (A) or presence (B) of IAA on incorporation of 3H -uridine into RNA in *Avena* coleoptile sections. Closed (●) and open (○) circles denote $O.D._{260}$ and radioactivity (c.p.m.) values for fractions.

This work was supported by the U.S. Public Health Service (HD-00726-04). We thank Dr. E. Alpert, of Merck, Sharpe and Dohme, for gifts of actinomycin D. We also thank H. S. Forrest, R. Lockart, J. R. Tata, C. C. Widnell, J. Mandelstam, E. M. Martin, O. F. Wiegand, S. E. Harris and W. Hubbard for their advice and assistance.

- ¹ Clever, K., and Karlson, P., *Exp. Cell Res.*, **20**, 623 (1960). Greengard, O., Smith, M., and Acs, G., *J. Biol. Chem.*, **238**, 1548 (1963). Hamilton, T. H., *Proc. U.S. Nat. Acad. Sci.*, **49**, 373 (1963). Korner, A., *Mem. Soc. Endocrin.*, **11**, 60 (1961). Manchester, K. L., and Young, F. G., *Vitam. and Horm.*, **19**, 95 (1961). Mueller, G. C., Gorski, J., and Alzawa, Y., *Proc. U.S. Nat. Acad. Sci.*, **47**, 164 (1961). Tata, J. R., *Nature*, **197**, 1167 (1963). Wool, I. G., *Biochem. Biophys. Acta*, **68**, 28 (1963). Wool, I. G., and Munro, A. J., *Proc. U.S. Nat. Acad. Sci.*, **50**, 918 (1963).
- ² Audus, L. J., *Plant Growth Substances*, 88 (Leonard Hill, Ltd., London, 1959). Bendaña, F. E., and Galston, A. W., *Science*, **150**, 69 (1965). Bendaña, F. E., Galston, A. W., Kaur-Sawhney, R., and Penny, P., *Fed. Proc.*, **24**, 2596 (1965). Bendaña, F. E., Galston, A. W., Kaur-Sawhney, R., and Penny, P., *Plant Physiol.* (in the press). Bonner, J., *Amer. J. Bot.*, **36**, 323 (1949). Click, R. E., and Hackett, D., *Fed. Proc.*, **23**, 525 (1964). Galston, A. W., and Purves, W. K., *Ann. Rev. Plant Physiol.*, **11**, 239 (1960). Gifford, E. M., jun., and Tepper, H. B., *Amer. J. Bot.*, **49**, 706 (1962). Kay, J., *Plant Physiol.*, **38**, Proc. 45 (1963). Key, J. L., and Shannon, J. C., *Plant Physiol.*, **39**, 36 (1964). Key, J. L., and Shannon, J. C., *Plant Physiol.*, **39**, 365 (1964). Knypl, J. S., *Nature*, **206**, 844 (1965). Mitra, P., and Sen, S. P., *Nature*, **207**, 861 (1965). Noodén, L. C., and Thimann, K. V., *Proc. U.S. Nat. Acad. Sci.*, **50**, 194 (1963). Schrank, A. R., *Arch. Biochem. Biophys.*, **61**, 348 (1956). Varner, J. E., and Chandra, G. R., *Proc. U.S. Nat. Acad. Sci.*, **52**, 100 (1964). Zeevart, J. A. D., in *The Nucleo-histones*, edit. by Bonner, J., and T'so, P., 343 (Holden-Day, Inc., San Francisco, 1964).
- ³ For mode of action of actinomycin D, see Goldberg, I. M., and Rabinowitz, M., *Science*, **136**, 315 (1962). Hurwitz, J., Furth, J. J., Malamy, M., and Alexander, M., *Proc. U.S. Nat. Acad. Sci.*, **48**, 1222 (1962). Reich, E., Goldberg, I. H., and Rabinowitz, M., *Nature*, **196**, 743 (1962).
- ⁴ Eboué-Bonis, D., Chambaut, A. M., Volfin, P., and Clausner, H., *Nature*, **199**, 1183 (1963). Greenman, D. C., and Kenny, F. T., *Arch. Biochem. Biophys.*, **107**, 1 (1964). Ferguson, J. J., jun., *Biochim. Biophys. Acta*, **57**, 616 (1962). Hamilton, T. H., *Proc. U.S. Nat. Acad. Sci.*, **51**, 83 (1964). Kenny, F. T., and Kull, F. J., *Proc. U.S. Nat. Acad. Sci.*, **50**, 493 (1963). Moore, R. J., and Hamilton, T. H., *Proc. U.S. Nat. Acad. Sci.*, **52**, 439 (1964). Noteboom, W. D., and Gorski, J., *Proc. U.S. Nat. Acad. Sci.*, **50**, 250 (1963). Rasmussen, H., Arnaud, C., and Hawker, C., *Science*, **144**, 1019 (1964). Sovik, O., and Walaas, O., *Nature*, **202**, 396 (1964). Talwar, G. P., and Segal, S. J., *Proc. U.S. Nat. Acad. Sci.*, **50**, 226 (1963). Talwar, G. P., Segal, S. J., Evans, A., and Davidson, O. W., *Proc. U.S. Nat. Acad. Sci.*, **52**, 1059 (1964). Tashjian, A. H., Ontjes, D. A., and Goodfriend, T. L., *Biochem. Biophys. Res. Comm.*, **16**, 209 (1964). Weber, G., Singhal, R. L., and Stamm, N. B., *Science*, **142**, 390 (1963). Wicks, W. D., and Kenny, F. T., *Science*, **144**, 1346 (1964).
- ⁵ Wiegand, O. F., and Schrank, A. R., *Bot. Gaz.*, **121**, 106 (1959).
- ⁶ Glišin, V. R., and Glišin, M. V., *Proc. U.S. Nat. Acad. Sci.*, **52**, 1548 (1964).
- ⁷ Bray, G. A., *Anal. Biochem.*, **1**, 279 (1960).
- ⁸ Shibaoka, H., and Hurusawa, I., *Plant and Cell Physiol.*, **5**, 273 (1964).
- ⁹ Cleland, R., *Nature*, **185**, 44 (1960).
- ¹⁰ Eisenstadt, B., and Brawerman, G., report respective sedimentation values of 60 S (rRNA: 19 S, 14 S) and 70 S (rRNA: 19 S only) for chloroplast and cytoplasmic ribosomes of *Euglena gracilis*. Our determination of 80 S for sedimentation value of ribosomes of *Avena coleoptile* sections is based on preparations containing both chloroplast and cytoplasmic ribosomes. As such the 80 S value is general and tentative, pending clarification for our system of the two populations of ribosomes. It is interesting that Biswas and Biswas¹⁶ report an actinomycin D-resistant synthesis of RNA by chloroplasts isolated from spinach leaves. Whether this has a bearing on our finding of *Avena* RNA synthesis in the presence of this inhibitor (see Fig. 5A and text) remains to be investigated.
- ¹¹ For base compositions of 30 S and 20 S rRNA, peak fractions from sucrose gradients were collected and analysed separately. Values cited represent averages for triplicate determinations, with less than 1.5 per cent variation. Base compositions were determined by the method of Magasanik (Magasanik, B., in *The Nucleic Acids: Chemistry and Biochemistry*, 1, edit. by Chargaff, E., and Davidson, J. N., 373 (Academic Press, New York, 1955)).
- ¹² Leslie, I., in *Biochemists' Handbook*, edit. by Long, C., 198 (D. Van Nostrand Company, Inc., New York, 1961). Petermann, M. L., *The Physical and Chemical Properties of Ribosomes*, 89 (Elsevier Publishing Co., Amsterdam, 1964).
- ¹³ See Barth, R. H., jun., Buryard, P. P., and Hamilton, T. H., *Proc. U.S. Nat. Acad. Sci.*, **52**, 1572 (1964). Hamilton, T. H., and Teng, C., in *Organogenesis*, edit. by LeHaan, R. L., and Ursprung, H., 681 (Holt, Reinhardt, and Winston, New York, 1965). Karlson, P., *Perspectives Biol. Med.*, **6**, 203 (1963). Samuels, L. D., *New Engl. J. Med.*, **271**, 1252 (1964). Tata, J. R., in *Symp. Fundamental Cancer Research* (M. D. Anderson Hospital, Houston, 1965, in the press). Williams-Ashman, H. G., *Cancer Res.*, **25**, 1096 (1965). Wyatt, G. R., and Linzen, B., *Biochim. Biophys. Acta*, **103**, 588 (1965).
- ¹⁴ Tata, J. R., *Biochim. Biophys. Acta*, **87**, 528 (1964). Widnell, C. C., and Tata, J. R., *Biochim. Biophys. Acta*, **87**, 531 (1964). Tata, J. R., and Widnell, C. C., *J. Biochem.* (in the press).
- ¹⁵ Hamilton, T. H., Widnell, C. C., and Tata, J. R., *Biochim. Biophys. Acta*, **108**, 168 (1965).
- ¹⁶ Biswas, S., and Biswas, B. L., *Experientia*, **21**, 251 (1965). Brawerman, G., and Eisenstadt, J. M., *J. Mol. Biol.*, **10**, 403 (1964). Eisenstadt, J. M., and Brawerman, G., *J. Mol. Biol.*, **10**, 392 (1964). Gibor, A., *Amer. Nat.*, **99**, 229 (1965). Kirk, J. T. O., *Biochem. Biophys. Res. Comm.*, **10**, 233 (1964).

PRINCIPLES OF CELL MOTILITY: THE DIRECTION OF CELL MOVEMENT AND CANCER INVASION

By DR. S. B. CARTER

Imperial Chemical Industries Limited, Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire

INVESTIGATIONS of cell motility are generally carried out with cells attached to glass surfaces, and, in many cases, the influence which the glass itself may have on patterns of cell movement and behaviour is not fully taken into account. The use of unconventional transparent materials serves to emphasize the importance of the part played by the substrate in cell motility. Cellulose acetate has been selected from a wide range of available materials as being of particular value in experiments of this type.

Mammalian cells in culture do not readily adhere to a surface of cellulose acetate, but if this surface is metallized *in vacuo*, cultured cells will adhere to it and spread. By varying the amount of metal deposited it is possible to produce a range of surfaces allowing different degrees of cell adhesion.

The investigation of cell behaviour on various surfaces prepared in this way has led me to draw a number of general conclusions about the principles which govern the direction of cell movement, and the mechanism of cell motility itself.

In all the experiments to be described the cells used were mouse fibroblasts (Earle's L strain), and these were cultured in Eagle's medium¹, with 8 per cent calf serum. A culture chamber of new design was used and this will be described elsewhere. Cover glasses may be coated with an acetate film by immersing them in a 0.5 per cent solution of cellulose acetate (acetyl value 54-56 per cent) in 1,4-dioxan. These are drained in an atmosphere

saturated with dioxan, and dried under vacuum to avoid clouding by atmospheric humidity. The dried films are then heated to 280°C for 10 sec, a procedure which melts the acetate layer, fixing it firmly to the glass and sterilizing it at the same time. The surface of the film must be protected from physical and chemical contamination. Purity of materials is essential.

Metallic palladium is deposited on the acetate surface in a vacuum coating unit of the type used for shadow casting in electron microscopy. A measured length of fine palladium wire is placed on a cleaned molybdenum strip and heated to white heat at 10⁻⁴ mm mercury. The vaporized palladium is deposited on the acetate film by placing the cover glass above the molybdenum strip at a height which can be varied to control the amount of metal deposited. The minimum quantity of palladium necessary to allow full cell adherence to the film gives a very fine deposit which cannot be detected by eye. Several other metals have been found to be equally effective in this respect, but palladium has proved to be the most convenient and there have been no indications of it being responsible for any toxicity.

In order to make a gradient of metal deposition and therefore a surface offering progressively increasing cell adhesion, a number of techniques have been used. The simplest of these is to place a short length of stainless steel rod, 0.5 mm in diameter, in contact with the acetate film. The film is then shadowed by vaporizing 2 mg of fine palladium wire, rolled into a small pellet, at a distance

of 10 cm. This makes a relatively heavy deposition of metal which clearly outlines the intervening rod. Owing to the scattering of metal particles, however, a much finer deposit of palladium extends beyond the visibly shadowed area, and tapers off in the narrowing angle under the curved surface of the rod. A gradient of metal deposition is therefore produced. The advantage of this method is that the upper limit of the gradient is clearly defined by the visible edge of the densely shadowed area.

Cell Movement on a Gradient of Adhesion

Fig. 1 shows a number of cells photographed shortly after attachment, lying on an adhesion gradient prepared in this way. The full metal deposit is visible as a darker area in the upper part of the photograph, and the gradient extends from the bottom of the photograph to the edge of this darker area. Fig. 2 shows the same field after an interval of 8 h, and Fig. 3 shows the tracks of all cells during this period, plotted from a series of intermediate photographs.

It will be seen that the cells on the gradient move in the direction of increasing adhesion to the substrate, and their uniformity of movement contrasts with the apparently random movement of cells on the evenly metallized cellulose acetate, or on glass. As the cells crowd together at the upper end of the gradient, progressively increasing cell-to-cell contacts interfere with the movement of individual cells. Nevertheless the cell migration continues in the same direction, and Fig. 4 shows the same field again after 48 h. At this stage the free edge of the cell sheet is retracting, whereas under more usual conditions a free edge of this type would advance.

Not only is cell movement on the gradient directional, but also the cell tracks are longer and indicate more efficient motility. This is particularly evident in less-crowded cultures where there is more room for individual cell movement, and in a number of experiments of this

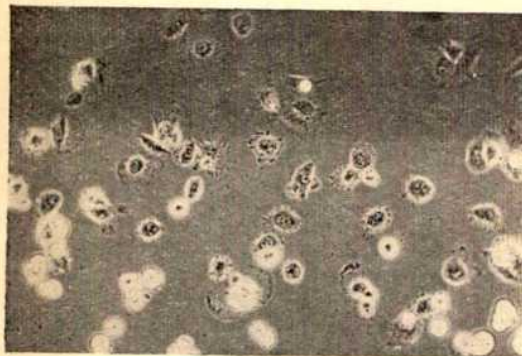


Fig. 1. Newly attached cells on an adhesion gradient. Substrate adhesion increases from the bottom of the photograph to the edge of the darker area. ($\times 125$). (All photomicrographs in this article were taken by phase contrast illumination.)

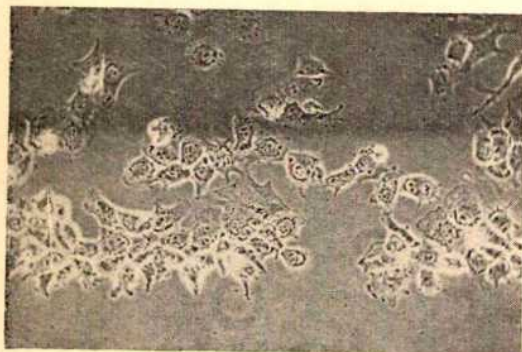


Fig. 2. The same field as Fig. 1 after an interval of 8 h. The cells have moved up the adhesion gradient towards the darker area at the top. ($\times 125$)

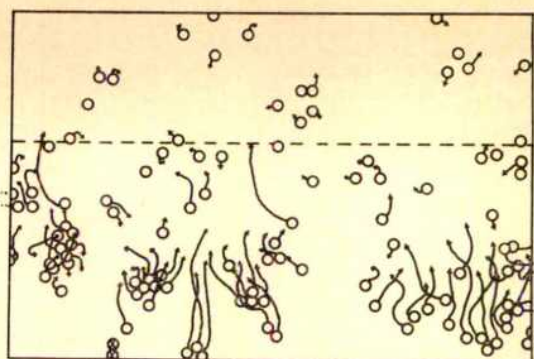


Fig. 3. The movement of individual cells on an adhesion gradient. The circles represent the original positions of the cell nuclei in Fig. 1, and the lines trace the paths followed by these nuclei to their positions in Fig. 2 8 h later. The two nuclei marked with crosses represent cells which became detached. ($\times 125$)

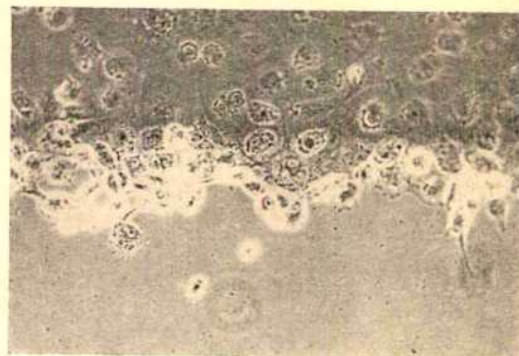


Fig. 4. The same field as in Fig. 1 after 48 h. The cells have continued to move up the adhesion gradient in spite of the crowding which results. The two isolated cells are late-comers which were outside the original field shown in Fig. 1. ($\times 125$)

type the average length of cell track on the gradient was four to five times as long as that on the evenly metallized surface. The speed of cell movement on the evenly metallized surface is of the same order as it is on glass under similar conditions.

It seems probable that movement towards surfaces offering greater adhesion is a general phenomenon applicable to all metazoan cells which are dependent on contact with a surface for their motility, and I believe that this is the essential principle which directs and controls all cell movements of this type. It is generally appreciated that cell motility and cell adhesion are interrelated, but the directness and simplicity of this relationship have not previously been demonstrated by experiment.

Gustafson and Wolpert² suggested on theoretical grounds that cells would tend to move up a gradient of increasing substrate adhesion, but the mechanism they proposed, involving competition between randomly extended pseudopodia, would not predict the immediately directional movement of individual cells which in fact occurs. I suggest that this phenomenon should be called 'haptotaxis' (Greek: haptin, to fasten; taxis, arrangement), conveying the idea that the movement of a cell is controlled by the relative strengths of its peripheral adhesions, and that movements directed in this way, together with the influence of patterns of adhesion on cell shape, are responsible for the arrangement of cells into complex and ordered tissues.

On this basis, the cell migrations involved in morphogenesis, inflammation, wound healing, tumour invasion and indeed all tissue cell movements, are considered to be the result of haptotactic responses by the cells involved, and correspondingly the relative stability of cells in differentiated adult tissues is due essentially to the lack of adhesion differentials on which effective cell movement depends. In discussing the possible mechanisms which

might control cell movement, Weiss³ pointed out that any such mechanism must involve selective conduction or selective fixation. His third principle of selective elimination is not strictly concerned with cell motility. It is interesting to note that the idea of haptotaxis effectively combines both these principles of cell movement.

Contact Inhibition

The concept of contact inhibition⁴ is valuable in providing a primary explanation for the differences in behaviour between normal and malignant cells. Cells which inhibit movement in each other on contact, and therefore tend to form monolayers in culture, are said to show contact inhibition; whereas those which move over each other and pile up into multiple layers are said to show loss of contact inhibition. To a limited extent this distinction divides normal from malignant cells, and a change in the pattern of cell growth towards loss of contact inhibition serves to indicate the transformation of cells, for example by oncogenic viruses.

However, by varying the amount of palladium deposited on a cellulose acetate film and producing surfaces which allow different degrees of cell adhesion, it is possible to change completely the pattern of growth of the same type of cell, without otherwise altering the cultural conditions.

The cells in Fig. 5 are arranged in a monolayer and are demonstrating contact inhibition. These cells are attached to a cover glass which has been coated with acetate and exposed to vaporized palladium. Fig. 6 shows cells from the same culture as they appear on a different part of the cover glass where it has been masked to reduce the amount of palladium deposited. Here the cells are clumped together and would be said to demonstrate loss of contact inhibition. An intermediate stage in which cells show a tendency to monolayer, but with many cells overlapping, can be found in a narrow region between the two areas showing these strikingly different patterns of growth. This finding indicates that contact inhibition, or lack of it, cannot be regarded as an attribute of the cell itself, but describes its relationship to other cells in the presence of a particular alternative substrate.

The phenomenon of contact inhibition can be explained on the basis of haptotactic movement. Cells showing contact inhibition on glass are demonstrating that they can adhere more strongly to this material than they can to each other. Cell movement will therefore be towards any free glass surface so that the monolayer is preserved. Cells showing loss of contact inhibition on glass can adhere more strongly to each other, and will tend to leave the glass surface and pile up in multiple layers. This type of explanation is similar to one of several possibilities considered by Abercrombie and Ambrose⁵, but Abercrombie⁶ has since suggested that the explanation least open to

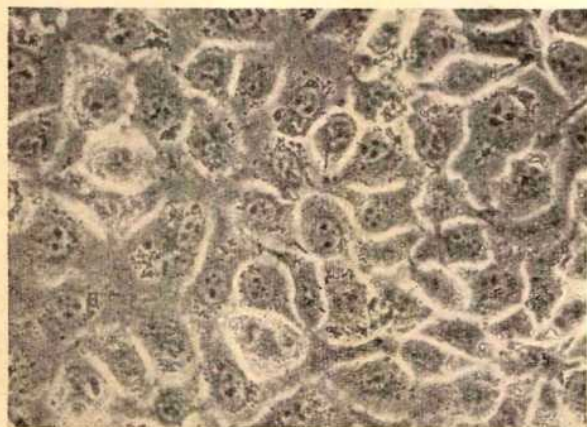


Fig. 5. 'L' cells showing contact inhibition on metallized cellulose acetate. ($\times 325$)

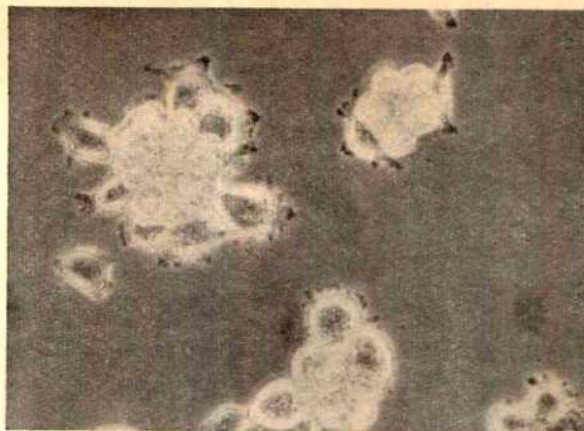


Fig. 6. 'L' cells showing loss of contact inhibition on very lightly metallized cellulose acetate. These cells were growing on a different area of the same cover glass as those shown in Fig. 5. ($\times 325$)

objection is that adhesion between cells inhibits the ruffled membrane, as argued by Curtis^{7,8}. In putting the opposite case it should be made clear that it is a relative lack of adhesion to the new substrate which is suggested as the operative mechanism, and this need not mean a low degree of adhesion in absolute terms. It is also important to emphasize that in discussing the adhesion of cells as it affects their motile behaviour, I am referring to the adhesion which occurs immediately on contact. The adhesion between cells which may develop on more prolonged contact is not at present being considered. The argument used by Abercrombie⁹ against this type of explanation for contact inhibition is that it fails to account for the paralysis of the ruffling movements of the cell. In a later paper concerned with the mechanism of cell motility, I hope to show that this objection can be overcome.

The arbitrary choice of glass as the surface on which most cells in culture are investigated makes the description of a cell in terms of contact inhibition equally arbitrary, though under standardized conditions not without practical value. Unfortunately glass exists in many forms, and its surface properties are readily changed by washing procedures and by substances in the culture medium itself.

Contact Guidance

The principle of contact guidance, elaborated by Weiss¹⁰, can also be explained on the basis of cell movement along a path of preferential adhesion. Although this mechanism is to a large extent implied by Weiss, he argues against the acceptance of any simple explanation in these terms. Orientated structures are likely to present a correspondingly orientated pattern of differing adhesion. This may be so even if the components in the orientated structure are all of the same material, since curvatures of the surface will affect the relative densities of the surface elements which may be concerned with adhesion.

Very precise contact guidance can be achieved by cutting narrow channels in a thin film of cellulose acetate so that the underlying glass is exposed, thereby providing a path offering far greater adherence for cells than the surrounding acetate (Fig. 7). Weiss has suggested that the mechanism of contact guidance may involve micro-orientation of the guiding surface, and has considered how this orientation could be transferred to the molecules of the cell surface in contact with it, and hence to the cell as a whole. It seems unlikely that there should be an appropriately orientated microstructure on a glass surface which has been exposed by removing a narrow strip of cellulose acetate, although such a microstructure could conceivably be imposed by the cutting instrument used to remove it.

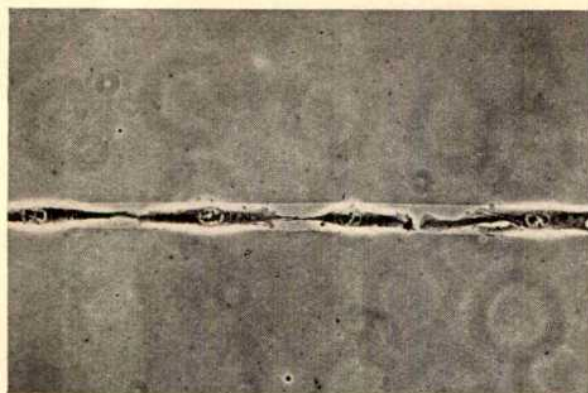


Fig. 7. Contact guidance along a channel cut through a film of cellulose acetate on glass. ($\times 225$)

Contact guidance can also be demonstrated on cellulose acetate when the path of preferential adhesion is made by overlaying with vaporized palladium rather than cutting down to underlying glass.

Such a path can be made by masking the acetate surface and restricting the deposition of metal to a strip of any desired width. Fig. 8 shows cells demonstrating contact guidance along a path made in this way. There is no reason why a metallized strip should have an orientated microstructure, and the simple interpretation involving differential adhesion alone seems adequate as a primary explanation for contact guidance phenomena.

Chemotaxis

There are probably many thousands of substances which can evoke chemotactic responses *in vivo*, but the number which demonstrate this effect under strictly *in vitro* conditions is remarkably small¹¹. The explanation for this may be that the chemotactic substance is acting indirectly by damaging or otherwise altering the surfaces of cells and non-cellular structures in its neighbourhood. This surface change enables the normally non-adherent leucocyte, for example, to adhere. Since the surface change will be greatest in closest proximity to the chemotactic agent, a gradient of surface adhesiveness will be formed to guide the leucocyte to the appropriate area. Such a gradient may be reinforced by the action of substances released by the damaged cells, and it is probable that these substances rather than the chemotactic agent itself are mainly responsible for the adhesion gradient produced. Chemotaxis is difficult to demonstrate *in vitro* because artificial substrates are less susceptible to these surface changes. A direct effect on the leucocyte is also possible since the leucocyte itself could suffer surface changes which would make it more adhesive on the side nearest to the chemotactic stimulus. Failure to demonstrate *in vitro* responses to the majority of agents which are chemotactic *in vivo* suggests that this direct effect may be relatively unimportant. The suggestion that chemotaxis is a special case of haptotaxis applies equally well to negative chemotaxis, but in applying this principle there is no need to consider it in negative terms.

Cancer Invasion

If malignant invasion is due to the operation of haptotaxis, the implication would be that cancer cells can form stronger adhesions to surrounding normal cells than they can to each other.

It was originally shown by Coman¹² that cancer cells have a reduced mutual adhesiveness, and it has also been found that the net negative charge on the surface of a virus-transformed cell is higher than the surface charge on a homologous normal cell¹³. It seems likely that these two findings are closely related, and on theoretical grounds

it might be expected that a cancer cell would adhere more firmly to a normal cell than to another cancer cell because the repulsion due to the combined surface charges would be less.

To obtain absolute measurements of the adhesion between two cells of individually known type would be difficult, but if haptotaxis is accepted as a principle of cell movement, then the direction of cell movement itself can be used to give an indication of the relative adhesiveness of two surfaces for a particular cell.

Abercrombie, Heaysman and Karthaus¹⁴ made the important observation that confronted outgrowths from mouse sarcoma and chick embryo tissue showed more cell overlaps than occurred in a corresponding experiment with two cultures of chick tissue. This involves a comparison of normal and malignant cells from unrelated species, and the experiment is further complicated by the presence of a third surface in the form of a plasma coagulum. Although it points in the right direction, an experiment of this complexity would be difficult to interpret with confidence in terms of relative surface adhesion.

The value of virus transformation in culture is that it makes possible the comparison of normal cells with cells which are as nearly as possible their malignant counterparts. Vogt and Rubin¹⁵ observed that chick fibroblasts transformed by Rous sarcoma virus form multi-layered clumps on glass, but migrate readily over the sheet of untransformed cells. This suggests that the transformed cells are more adherent to themselves than to glass and more adherent to corresponding normal cells than to each other. Even so, it would simplify the interpretation of this experiment to eliminate any possible interference by the glass so that the comparison can be restricted to the cell surfaces only. For this reason some interesting experiments by Stoker¹⁶ are particularly relevant. He showed that whereas hamster cells transformed by polyoma virus formed distinct heaped-up colonies on glass, these same cells did not produce colonies when seeded onto pre-existing monolayers of normal hamster cells. Although he found evidence that the rate of division of the transformed cells was reduced under these conditions, their failure to form multi-layered colonies could also indicate that they were spreading over the pre-existing monolayer and, therefore, demonstrating a stronger attachment to normal cells than to each other. Stoker's experiments were not, of course, designed to test this hypothesis, and his own interpretation involves an entirely different theory in relation to the mechanism of contact inhibition. Nevertheless, this type of experiment closely parallels the experiment previously described in which cells were shown to clump or spread out according to the adhesiveness of the underlying surface.

Although these experiments with transformed cells point to the conclusion that malignant cells can adhere more strongly to their normal counterparts than to each

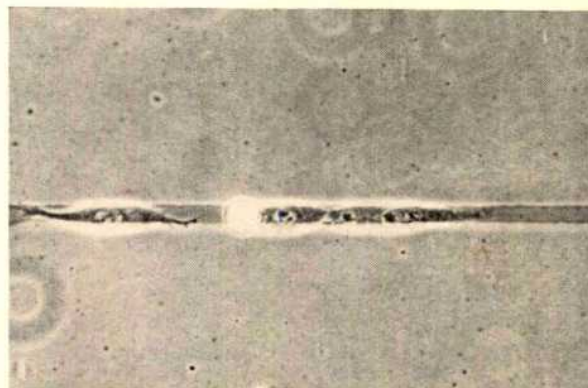


Fig. 8. Contact guidance on a cellulose acetate film coated in a narrow strip with evaporated palladium. ($\times 225$)

other, it is clear that further experiments are required, and these need to be specifically designed to make interpretation in these terms as direct as possible. In addition, it is necessary to establish haptotaxis more firmly as the overriding principle of cell movement. In order to do this it is essential to relate it to the underlying mechanism of cell motility, and this will be attempted in a subsequent paper.

On the basis of haptotactic cell movements, many aspects of the natural history of malignant disease can be interpreted. In local invasion, the peripheral tumour cells move away from the tumour mass into the surrounding tissue in response to the adhesion differential, and are followed immediately by other cells moving under the same influence. In this way, a column of cells tends to be formed and the process of malignant invasion becomes more consistent in direction and consequently more efficient than if individual cells were cut off from the tumour mass so that they were no longer subject to the directional cue provided by the presence of normal cells in front and malignant cells behind.

The pattern of invasion is affected by tissue architecture, not simply on the basis of the physical structures encountered, but also in accordance with the degree of adherence that the cellular and non-cellular components of the structure offer to the invading cells. Similarly the pattern of metastasis for a particular type of tumour is partly determined by anatomical considerations, and partly by the adhesiveness of the endothelial cells lining blood vessels and lymphatics in different tissues for the particular type of malignant cell. The surface properties of these endothelial cells would be expected to vary according to the differing physiological environments to which they are subjected in different types of tissue.

The invasiveness of a particular tumour cannot be regarded as a characteristic solely of the tumour itself, since the nature of the surrounding normal cells plays a part in determining the degree to which tumour to normal cell adhesion exceeds mutual tumour cell adhesion. For this reason, a tumour metastasis in a different tissue may show a greater or a lesser degree of invasiveness in its new

site when compared with the primary tumour, and it would be predicted that some tumours which are non-invasive and regarded as 'benign' in their primary sites could become 'malignant' if artificially transferred elsewhere.

It is important to bear in mind that the cells involved in malignant invasion are not moving in a stationary field. All tissue cells are potentially motile, and normal cells may move in relation to malignant cells for precisely the same reasons as malignant cells move in relation to normal cells. The movement of fibrocytes, for example, in contact with some types of neoplastic cells can result in the effective walling off and containment of a tumour within a fibrous capsule, and the penetration of fibrocytes into the substance of a carcinoma can convert it into a scirrhous type of tumour. The infiltration of some tumours by lymphocytes is also a clear case of the 'invasion' of cancerous tissue by normal cells. It may be more rewarding to consider such phenomena with reference to a defined concept such as haptotaxis than to describe them in non-committal terms of 'reaction' to the tumour. Only a brief indication of the application of such a concept has been attempted here.

I thank Miss Janet Way for technical assistance.

¹ Eagle, H., *Science*, **130**, 432 (1959).

² Gustafson, T., and Wolpert, L., *Intern. Rev. Cytol.*, **15**, 139 (1963).

³ Weiss, P., *Yale J. Biol. and Med.*, **19**, 235 (1947).

⁴ Abercrombie, M., and Heaysman, J. E. M., *Exp. Cell Res.*, **5**, 111 (1953).

⁵ Abercrombie, M., and Ambrose, E. J., *Cancer Res.*, **22**, 525 (1962).

⁶ Abercrombie, M., in *Cells and Tissues in Culture*, edit. by Willmer, E. N., **1**, 189 (Academic Press, London, 1965).

⁷ Curtis, A. S. G., *Amer. Nat.*, **94**, 37 (1960).

⁸ Curtis, A. S. G., *Biol. Rev.*, **37**, 82 (1962).

⁹ Abercrombie, M., *Exp. Cell Res. (Suppl.)*, **8**, 188 (1961).

¹⁰ Weiss, P., *Exp. Cell Res. (Suppl.)*, **8**, 260 (1961).

¹¹ Harris, H., *Exp. Cell Res. (Suppl.)*, **8**, 199 (1961).

¹² Coman, D. R., *Cancer Res.*, **4**, 625 (1944).

¹³ Forrester, J. A., Ambrose, E. J., and MacPherson, J. A., *Nature*, **196**, 1068 (1962).

¹⁴ Abercrombie, M., Heaysman, J. E. M., and Karthausser, H. M., *Exp. Cell Res.*, **13**, 276 (1957).

¹⁵ Vogt, P. K., and Rubin, H., *Virology*, **13**, 528 (1961).

¹⁶ Stoker, M., *Virology*, **24**, 165 (1964).

A SEX-DEPENDENT FACTOR IN APLASTIC ANAEMIA?

By DR D. E. PEGG and J. E. BURNS

Departments of Radiotherapy and Physics, Westminster Hospital, London, S.W.1

APLASTIC anaemia is characterized by the deficient production of peripheral blood cells and is nearly always attended, and presumably caused, by severe bone marrow hypoplasia. The fundamental cause of the condition is usually unknown, and although spontaneous remissions do occur, they are not common, and treatment has usually been conspicuously unsuccessful. Animal experiments in bone marrow transplantation indicated a possible new approach to the management of this disease by the grafting of healthy bone marrow cells: allogeneic marrow infusions are attended by difficulties and hazards which are avoided by the use of syngeneic donors, and on the rare occasions when a patient with aplastic anaemia has a normal identical twin it is possible to duplicate this favourable circumstance clinically. Seven such cases have been reported (Table 1).

In three of the four successful cases recovery was rapid and complete (Cases 2, 4 and 5), while in the fourth (Case 6) the response was slow and incomplete following the first marrow infusion, but a second infusion was more satisfactory although some thrombocytopenia persists*. Of the unsuccessful cases, the first (Case 1) has since undergone a slow and partial recovery while under treatment with steroids and testosterone (severe thrombocytopenia persists)*, Case 3 was not significantly benefited, and Case 7 died one month later. In each of the

four successful cases the author of the report was convinced that the treatment had been responsible. It is of course necessary to consider the possibility that the recoveries were spontaneous and coincided with the treatment purely by chance. This seems highly improbable: the spontaneous recovery rate is low, particularly when the disease has been present for a considerable time, as it had in Cases 4, 5 and 6 particularly. If it is accepted that the treatment was responsible for the recovery of the four successful cases, some attempt must be made to account for its failure in the other three. There appears to be no correlation between the result of the treatment and the cause of the aplasia (in so far as it is known), the age of the subject, or the amount of marrow injected: it is striking, however, that all the female cases recovered whereas all the male cases did not. To calculate the probability of this result occurring purely by chance, where the true reason is the operation of a factor not dependent on sex, it is necessary to make some assumption concerning the number of cases expected to recover. Table 2 shows the calculated probability for a selection of overall success rates. The highest probability is given by assuming that four recoveries will occur in any seven cases treated (the observed success rate) and on this assumption the probability of the observed distribution of successes and failures between males and females

Table 1

Case No.	Age and sex	Aetiology	Duration of aplasia	Amount of marrow	Result	Ref.
1	3 M	Chloramphenicol and/or sulphonamides probably	4 months to first treatment	3.6×10^9 cells	Unsuccessful	1
2	7 F	Paramethadione	6 months to second treatment	2.8×10^9 cells	Complete recovery	2
3	54 M	Unknown	3 weeks	5.6×10^9 cells	Unsuccessful	3
			Unknown, but longer than 2 months	4.8×10^9 cells		
4	9 F	Unknown	2.5 years	7.3×10^9 cells	Complete recovery	4
5	9 F	Unknown	7 months	6.1×10^9 cells	Complete recovery	5
6	55 F	Chloramphenicol	10 months to first treatment	6.0×10^9 cells	Recovery with some persistent thrombocytopenia	6
			3 years to second treatment			
7	15 M	Unknown	1 month	2.0×10^9 cells	Unsuccessful	7

Table 2. PROBABILITY THAT FOUR FEMALE CASES SHOULD ALL SUCCEED AND THREE MALE CASES SHOULD ALL FAIL FOR VARIOUS POSTULATED SUCCESS RATES

Success rate (%)	Probability
90	0.000656 or 1 in 1,520
80	0.00328 or 1 in 305
70	0.00848 or 1 in 154
60	0.00829 or 1 in 121
50	0.00781 or 1 in 128
40	0.00553 or 1 in 181
30	0.00278 or 1 in 366
20	0.000819 or 1 in 1,220
10	0.0000729 or 1 in 13,700

occurring purely by chance can be shown to be 0.0084 or 1 in 119, a rather unlikely (but not impossible) chance. It is, at any rate, sufficient to suggest quite strongly that a sex-determined factor is involved in the treatment of aplastic anaemia by syngeneic marrow infusion.

There appear to be two possible explanations for this quite unexpected observation: either a sex-determined factor is involved in the disease itself and the female disease is actually different from the male disease, or a sex-linked factor determines the success of the treatment. These possibilities will be considered in turn.

(A) *The disease.* Aplastic anaemia appears to affect both sexes equally¹⁰ although cases have been described in which there has been good evidence that sex or hormonal factors have played a crucial part. Havard and Scott themselves described two patients in whom the onset of aplasia was associated with pregnancy and one of these patients relapsed with each of her three pregnancies and remained in remission following her last pregnancy. Similar occurrences have been noted by Lachmann *et al.*¹¹ Shahidi and Diamond¹² reported two cases of aplastic anaemia in male children in which spontaneous remissions occurred precisely at the time of the onset of puberty. It is, in fact, well known that the sex hormones play an important part in the control of haemopoiesis; Gordon and Charipper¹³ showed that the difference in mean haemoglobin levels of male and female rats is due to the action of the sex hormones. The administration of large doses of testosterone in carcinoma of the breast often produces erythroid hyperplasia and even mild polycythaemia¹⁴ and similar therapy has since been advocated for the treatment of juvenile aplastic anaemia¹⁵ and myeloid metaplasia¹⁶. In addition, there is clear evidence that sex-linked genetic factors are involved in haemopoiesis. Rundles and Falls¹⁷ have described a form of sideroblastic anaemia which is hereditary and almost certainly sex-linked, since it affected only males and was recessive in females: the congenital pancytopenia of Fanconi is more common in males than females.

It is quite clear that this evidence falls a long way short of establishing the existence of two, sex-linked forms of aplastic anaemia, and in the present state of knowledge it seems unlikely that this could explain the results of syngeneic marrow infusion.

(B) *The treatment.* It has of course been widely assumed that syngeneic marrow infusion would work, if at all, by cellular transplantation, but it has not perhaps been so readily appreciated that this also involves the assumption that the cause of aplastic anaemia is a primary failure of stem cell activity, presumably brought about by the action of a transient agent which leaves the stem cells

irreparably damaged but the marrow environment intact to accept a syngeneic graft. In fact there is overwhelming evidence against this. In the first place it is very difficult to conceive of an agent producing a specific totally lethal effect on one group of cells in a complex biological system (if it were not totally lethal, then spontaneous recovery would occur). In any event, since the leucocyte, platelet and reticulocyte counts never reach zero, there must be some intact stem cells remaining, and if this is so, and the marrow environment is intact, they would be expected to repopulate the marrow without further intervention their total number would certainly exceed 0.1 per cent of the normal total marrow cellularity, and this amount is adequate to repopulate the marrow spaces of lethally irradiated mice. There is therefore every reason to suppose that aplastic anaemia is not due to stem failure *per se*, but is due to an abnormality of the *milieu intérieur* either the absence of an essential metabolite or hormone or the presence of a specific depressor substance. If a depressor substance was responsible one would expect it also to affect the infused marrow, and prevent grafting. One is led to the conclusion that when syngeneic bone marrow is infused in aplastic anaemia, it does not act by haemopoietic cell transplantation as this is usually understood, but by a quite unknown stimulatory mechanism.

This conclusion is certainly surprising, and in view of the nature of the evidence on which it is based, it must be regarded as highly tentative. But in a disease where so little is understood it is perhaps justifiable to consider any reasonable hypothesis in the hope that it may lead by further observation and experiment to a greater understanding of the disease, and to more effective treatment. In particular it will be most interesting to see further attempts to treat aplastic anaemia with syngeneic bone marrow conform to the same pattern. Limited enquiries have brought to light only one additional case¹ This concerns a female aged twenty-three, who died 3 days after receiving 1.17×10^9 marrow cells from her twin; this is certainly too short a survival to permit an conclusion concerning the effectiveness of the marrow infusion.

¹ Fernbach, D. J., and Trentin, J. J., *Proc. Eighth Intern Congr. Haematol.* 150 (1960).

² Robins, M. M., and Noyes, W. D., *New Engl. J. Med.*, 265, 974 (1961).

³ Pegg, D. E., Fleming, W. J. D., and Compston, N., *Postgrad. Med. J.*, 4, 213 (1964).

⁴ Mills, S. D., Kyle, R. A., Hallenbeck, G. A., Pease, G. L., and Cree, I. (J. *Amer. Med. Assoc.*, 188, 1037 (1964).

⁵ Thomas, E. D., Phillips, J. H., and Finch, C. A., *J. Amer. Med. Assoc.*, 154, 1041 (1964).

⁶ Melvin, K. E., and Davidson, J. N. G., *New Zeal. Med. J.*, 63, 93 (1964).

⁷ McKenna, P. J., and Tocantins, L. M. (personal communication to Thon *et al.*, and quoted by them in ref. 5).

⁸ Trentin, J. J. (personal communication).

⁹ North, J. D. K. (personal communication).

¹⁰ Havard, C. W. H., and Scott, R. B., *Lancet*, i, 461 (1963).

¹¹ Lachmann, A., Lund, E., and Vinther-Paulsen, N., *Acta Obst. et Gyn. Scand.*, 33, 395 (1954).

¹² Shahidi, N. T., and Diamond, L. K., *J. Dis. Child.*, 93, 293 (1959).

¹³ Gordon, A. S., and Charipper, H. A., *Ann. N.Y. Acad. Sci.*, 48, 615 (1944).

¹⁴ Kennedy, B. J., and Nathanson, I. T., *J. Amer. Med. Assoc.*, 152, 11 (1953).

¹⁵ Shahidi, N. T., and Diamond, L. K., *New Engl. J. Med.*, 264, 953 (1961).

¹⁶ Pringle, T. C., and Gardner, F. H., *Clin. Res.*, 7, 210 (1959).

¹⁷ Rundles, R. W., and Falls, H. F., *Amer. J. Med. Sci.*, 211, 641 (1946).

¹⁸ Israels, M. C. G. (personal communication).

LETTERS TO THE EDITOR

RADIO ASTRONOMY

Radio Structure of the Galactic Centre Region

RADIO observations have shown that the galactic centre region consists of a number of discrete sources. The brightest of these, Sagittarius A, is believed to represent the galactic nucleus. This communication describes a new series of observations of the region, made at frequencies of 8.25 and 15.50 Gc/s with a pencil-beam antenna. The angular resolutions were respectively 4.2 and 2.2 arc min, the latter being the highest pencil-beam resolution so far applied to the galactic centre region. The observations confirm that the microwave spectrum of Sagittarius A is non-thermal¹, show that the angular diameter of the source is approximately 3.5 arc min, and demonstrate that, adjacent to Sagittarius A, there is an irregular emission region, which is apparently thermal in nature. The relation between radio data concerning the galactic centre and optical information about the centres of nearby normal galaxies is also examined.

The observations were made with the 120-ft. paraboloid antenna at the Haystack field station of Massachusetts Institute of Technology, Lincoln Laboratory. The antenna has a Cassegrain feed system². The receiver at 8.25 Gc/s had a band-width of 0.5 Gc/s, and with an output time constant of 1 sec the minimum detectable signal was of the order of 0.1° K. The receiver at 15.50 Gc/s used two channels centred at 15.25 and 15.75 Gc/s, each having a band-width of 0.5 Gc/s. With an output time constant of 1 sec the minimum detectable signal for this receiver was 0.2° K. The outputs from the receivers were recorded by both analogue and digital equipment. The observations were made by taking drift-scans, at spacings of 2 arc

min in declination at 8.25 Gc/s, and at 1 arc min at 15.50 Gc/s. This procedure minimizes the effects of ground radiation scattered or diffracted into the antenna. The scans were taken when the galactic centre region was at meridian transit $\pm 2^\circ$, over which period its elevation ranged from 18 to 13 arc deg above the horizon.

Fig. 1a shows the radio brightness contours of the galactic centre region at 8.25 Gc/s and Fig. 1b shows the contours at 15.50 Gc/s. The bright source is Sagittarius A. To the north of Sagittarius A, and partially overlapping it, there is a complex emission region, which consists of an irregular curved ridge. This region may also be distinguished on contour maps at other frequencies^{3,4}. Beneath these sources, there is a well-known emission region extending over several arc degrees, which is not indicated in the diagrams.

Table 1. FLUX DENSITIES, ANGULAR DIAMETERS, AND POSITIONS OF RADIO SOURCES IN GALACTIC CENTRE REGION

	Sgr. A	Sgr. B1	Sgr. B2
Flux density ($\times 10^{-26}$ M.K.S.)			
at 8.25 Gc/s	150	35	125
at 15.50 Gc/s	100	60	190
Angular diameter (arc min)	4.0×2.5	7×5	17×5
Position (1950.0)			
right ascension	17h 42m 27s	17h 42m 34s	17h 42m 59s
declination	$-28^\circ 58' 5''$	$-28^\circ 51' 0''$	$-28^\circ 47' 0''$

Estimates of the flux densities of Sagittarius A at 8.25 and 15.50 Gc/s are given in Table 1. These values were derived on the assumption that the flux density of M87 was 47×10^{-26} M.K.S. units at 8.25 Gc/s and 28×10^{-26} units at 15.50 Gc/s. Beam-width corrections were made for both Sagittarius A and M87. The flux values for Sagittarius A fit closely on the intensity spectral curve of index $\alpha = -0.7$ given earlier by Maxwell and Downes¹, confirming the non-thermal character of the source. The position co-ordinates of Sagittarius A given in Table 1 are mean

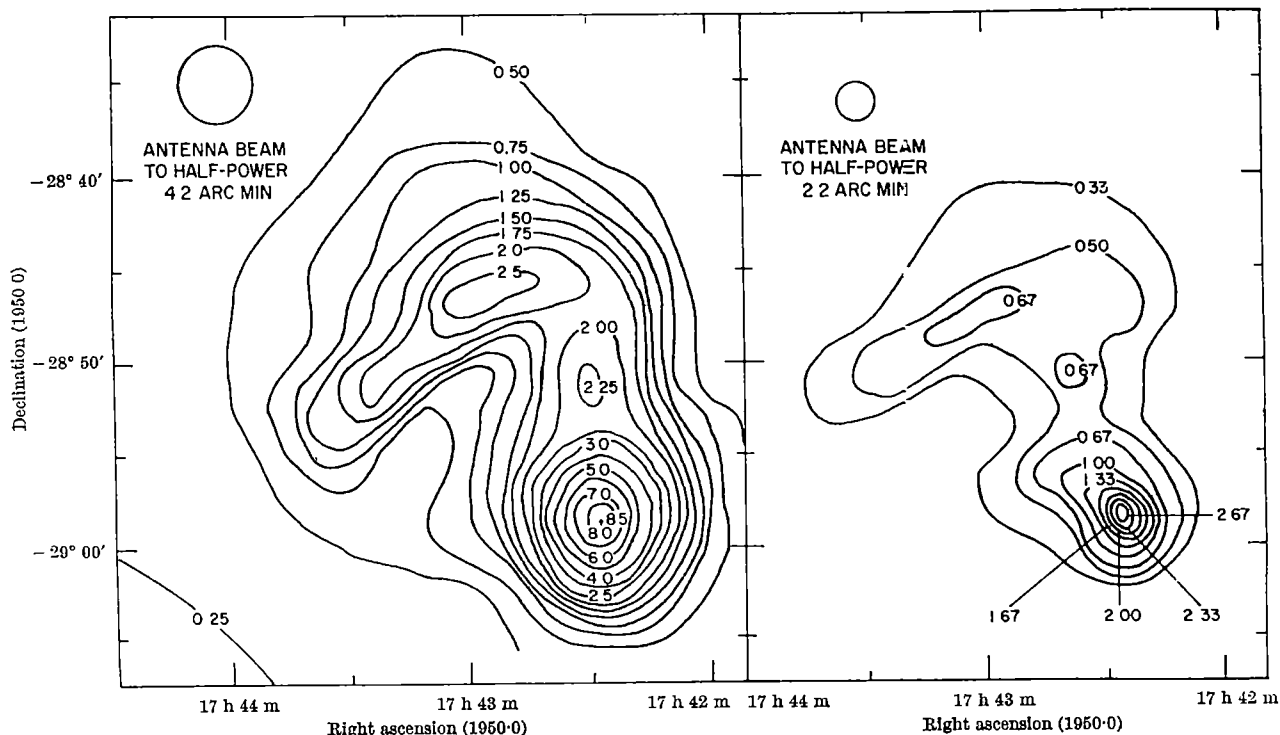


Fig. 1. a (Left), galactic centre region at 8.25 Gc/s. Contours represent antenna temperature in ° K, corrected by 4 per cent for extinction. b (Right), galactic centre region at 15.50 Gc/s. Contours represent antenna temperature in ° K, corrected by 8 per cent for extinction.

values taken from earlier position measurements of Maxwell and Downes, Hollinger³ and Broten *et al.*⁴.

To estimate flux densities from the irregular emission region north of Sagittarius A, we have divided it into two main areas, which we shall refer to as Sagittarius B1 and B2. The centres and angular diameters of these areas are listed in Table 1, the centres having been measured relative to the position of Sagittarius A. On Fig. 1b the centres are represented by the closed contours of antenna temperature 0.67° K. Integrated flux densities for Sagittarius B1 and B2 are given in Table 1. These values, however, should be regarded with caution, particularly at 15.50 Gc/s, since the present survey provides only a few brightness contours for the areas. Comparison of the integrated fluxes from these areas with data taken by Broten *et al.* at 5 Gc/s suggests that the sources are both thermal in nature and that they may therefore be H II regions. There is, however, no evidence to indicate that the sources are physically associated with Sagittarius A.

In considering the structure of the centre of our own galaxy, it is instructive to compare the existing radio data with optical data concerning the centres of two nearby normal galaxies, M31 and M51. (There is, of course, little optical evidence concerning the structure or nature of the centre of our own galaxy, since the centre is heavily obscured by intervening dust clouds.) Both M31 and M51 are observed optically to have a central nucleus of linear dimensions about 20 parsec^{5,6}. In each case this nucleus is embedded in a larger nuclear bulge of dimensions about 1,000 parsec. In the case of our own galaxy, if we believe Sagittarius A to be at the galactic centre and at a distance of 10 kiloparsec⁷, then its angular width of 3.5 arc min to half power would correspond to linear dimensions of the order of 10 parsec, which is comparable with the optical dimensions of the nuclei of the nearby normal galaxies. Similarly, if the extended source underlying Sagittarius A is regarded as radio evidence for a nuclear bulge at the centre of our own galaxy, the radio diameter of about 120 arc min¹ would correspond to linear dimensions of the order of 350 parsec.

Observations of the galactic centre by radio astronomers have now extended over some fifteen years, during which time angular discrimination has increased by a factor of about 30. With each improvement in resolution the central region has generally shown increasing structural complexity. Commencing in 1967, the region will be subject to a series of lunar occultations, and at that time we may look forward to a dramatic increase in the available angular resolution, perhaps by a factor of 100 or more. The detailed structure of this region should then be revealed much more fully.

We thank Mr. A. B. Hull and Dr. S. Weinreb of Lincoln Laboratory, and Mr. R. Rinehart and Mr. J. H. Taylor of Harvard University, for their assistance in making the observations. Lincoln Laboratory is a centre for research and development operated by the Massachusetts Institute of Technology with the support of the U.S. Air Force. The Harvard University part of the programme was supported by the U.S. National Science Foundation.

D. DOWNES
A. MAXWELL

Harvard Radio Astronomy Station,
Fort Davis, Texas.

M. L. MEEKS

Massachusetts Institute of Technology,
Lincoln Laboratory, Lexington,
Massachusetts.

¹ Maxwell, A., and Downes, D., *Nature*, **204**, 865 (1964).

² Weiss, H. G., *IEEE Spectrum*, **2**, No. 2, 50 (1965).

³ Hollinger, J. P., *Astrophys. J.*, **142**, 609 (1965).

⁴ Broten, N. W., Cooper, B. F. C., Gardner, F. F., Minnett, H. C., Price, E. M., Tonking, F. G., and Yabsley, D. E., *Austral. J. Phys.*, **18**, 85 (1965).

⁵ Lallemand, A., Duchesne, M., and Walker, M. F., *Pub. Astron. Soc. Pacific*, **72**, 76 (1960).

⁶ Burbidge, E. M., and Burbidge, G. R., *Astrophys. J.*, **140**, 1445 (1964).

⁷ *Intern. Astro. Union Inform. Bull.*, No. 11, 11 (1963).

ASTROPHYSICS

Common Features of the Optical Continua associated with Violent Cosmic Events

THE purpose of this communication is to point out a similarity in the optical continua of several objects, all associated with violent events, but which otherwise have a diverse character. The objects are the nucleus of the Seyfert Galaxy NGC 4151, the galactic, nova-like object η Carinae, the Crab Nebula and the quasi-stellar radio source, 3C48. (The term nova-like is to some extent descriptive of the spectrum. In using it, we do not mean to imply a mechanism for the outburst in η Carinae. This outburst has involved energies characteristic of supernovae and, unlike either supernovae or novae, has a time scale of the order of a century.) The optical spectrum of NGC 4151 has been discussed by Seyfert¹ and Woltjer², that of η Carinae by Thackeray³, that of the Crab Nebula by Woltjer⁴ and Mayall⁵, and that of 3C48 by Greenstein and Schmidt⁶. The emission lines in all of these spectra show, as a common feature, evidence of internal motions as large as a few thousand km/sec. We indicate in Table 1 some of the principal observed features of these objects.

In independent investigations, we have recently found that NGC 4151 and η Carinae radiate continua that are remarkably similar to one another and to that observed in the Crab Nebula. Further, there are considerable grounds for believing that the optical continua of some of the quasi-stellar sources, such as 3C48, are of similar form. The continua are illustrated in Fig. 1, where we plot the logarithm of the flux per unit frequency interval, $\log F(\nu)$ (with an arbitrary vertical shift for each object), against the reciprocal wave-length (μ)⁻¹.

The observations of η Carinae were obtained with the photoelectric scanner at the Mount Stromlo Observatory. They will be discussed in conjunction with the results of coudé spectroscopy in a forthcoming paper by Rodgers and Searle. For our purpose it is important to note that there is no observable stellar component in the spectrum of η Carinae. Although the spectrum of η Carinae is crowded with emission lines, notably of [Fe II], a smooth, featureless continuum is clearly present and the coudé spectrograms have been used to correct the scan data in order to obtain the curve shown in Fig. 1. η Carinae lies in the galactic plane and suffers considerable interstellar extinction. To obtain the results given in Fig. 1, a large but well-determined correction for this has been applied to the observed fluxes.

The scans of NGC 4151 were obtained by Oke using the 200-in. telescope at Palomar Mountain and the 100-in. telescope at Mount Wilson. They will be discussed, together with photographic spectra obtained by Sargent at Mount Wilson, in a forthcoming paper. The nucleus of NGC 4151 has a continuous spectrum on which are superimposed emission lines; there is no sign in the spectrum of this object of a stellar absorption component such as is found in some of the other Seyfert nuclei, for example, NGC 1068. The continuum shown in Fig. 1 for NGC 4151 has been corrected for the effect of emission lines, but no correction for interstellar reddening has been necessary in this case.

The optical continuous energy distribution of the Crab Nebula has been measured by O'Dell⁷ using narrow band observations over the wave-length range 3210–8440 Å. Estimates of interstellar reddening were made through

Table 1. RADIO AND OPTICAL PARAMETERS

Object	Distance (pc)	Radius (cm)	Luminosity at 45560 (ergs/sec/Å)	Ratio of radio to optical luminosity $F(\nu = 10^9 \text{ epc})$ $F(\nu = 10^{15} \text{ epc})$	Equivalent width of $H\beta(\text{\AA})$
<i>NGC 4151</i>	10^7	1.5×10^{20}	7.8×10^{36}	< 0.1	140
η Car	1.5×10^3	6×10^{16}	2.3×10^{35}	< 0.3	114
Crab Nebula	1.1×10^3	9×10^{17}	1.8×10^{32}	10^3	8
<i>3C48</i>	10^9 *	$< 10^{22}$	$\sim 10^{42}$	10^4	28
<i>3C273B</i>	5×10^8 *	$< 2 \times 10^{21}$	$\sim 4 \times 10^{43}$	10^3	98

* Assuming redshift is cosmological.

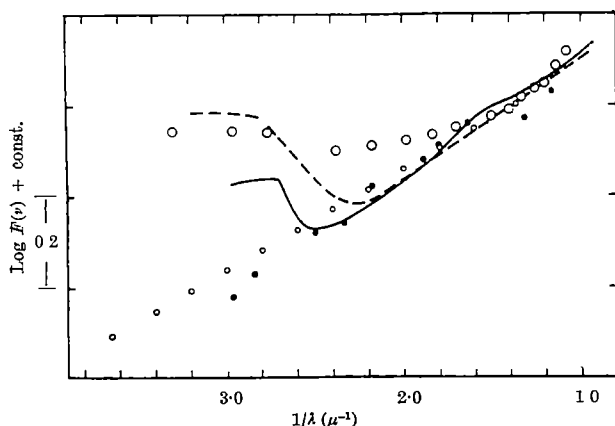


Fig. 1. Optical continua of NGC 4151 (full line), η Carinae (dashed line), Crab Nebula (filled circles), 3C48 (small open circles) and 3C273B (large open circles).

observations of surrounding field stars, the total absorption amounting to 1.10 magnitudes. The photometric observations were corrected for the presence of emission lines due to the filamentary component of the Crab, but the corrections do not exclude noticeable amounts of Balmer recombination continuum at wave-lengths below 3647 Å. Recent unpublished scanner measurements of the Crab by Oke agree in general with O'Dell's observations; but show some evidence for an increase in flux in the Balmer continuum.

We have also plotted, in Fig. 1, Baum's⁸ measurements of the monochromatic fluxes at several wave-lengths for 3C48. These relatively broad band observations have not been corrected for the presence of emission lines but have been reduced to the laboratory frequency system by applying the appropriate redshift corrections for a Doppler displacement factor of 1.367. Again, unpublished scanner observations by Oke show good agreement with those of Baum.

Among the quite diverse objects represented in Fig. 1, there appears to be a remarkable similarity in the radiated optical continua longward of 4000 Å. There are obviously varying contributions of Balmer recombination continua at shorter wave-lengths which correlate well with the observed equivalent widths of H β as listed in Table 1. The resemblance of the overall continua of NGC 4151 and η Carinae is particularly striking in view of their dissimilar nature.

Although the objects so far discussed do have a common slope, it is also clear that there are other objects where this is not strictly the case. As an example, the continuous spectrum of 3C273B as measured by Oke⁹ is much flatter in the blue spectral region, although, as shown in Fig. 1, there is some suggestion that, in the near infrared, the continuum slope of 3C273B approaches that of the other objects represented in Fig. 1. Furthermore, unpublished observations by Oke have shown that other Seyfert nuclei, NGC 1068 and NGC 1275, have considerably redder continuum slopes than NGC 4151; this may be caused by the presence of cool stars in the former.

The point we wish to emphasize is that for all the objects in Fig. 1, there appears to be a common contribution to the spectrum which dominates the Paschen continuum and for which $F(\nu) \propto e^{-\alpha\nu}$ where $\alpha \approx 2.4 \times 10^{-15}$ c.g.s. units. It implies the existence of a common physical process occurring in gas masses which experience violent internal motions. The cases of η Carinae and the Crab Nebula show that this process is not essentially connected with the behaviour of gas masses on a galactic scale.

Shklovsky¹⁰ has proposed that the optical continuum of the Crab Nebula is synchrotron radiation; Mathews and Sandage¹¹ have ascribed the whole of the optical continuum of 3C48 to this process; and Oke⁹ also discusses the red continuum of 3C273B in terms of optical synchrotron

radiation. In all cases the optical spectral index is larger than that found from the radio observations and the difference has been attributed to the effect on the optical fluxes of a high-frequency cut-off in the electron energy spectrum. It is remarkable that in such dissimilar objects so great a uniformity should obtain in the processes which determine the energy spectrum of the relativistic electrons.

If the continua are produced by synchrotron radiation we could hope to detect polarization, given a favourable magnetic field geometry in the objects. The co-existence of a recombination spectrum and a synchrotron spectrum in η Carinae and NGC 4151 would lead one to expect more polarization in the red than shortward of the Balmer jump. In the case of η Carinae, Visvanathan¹² has examined the polarization first observed by Thackeray¹³ and has shown it to be independent of wave-length. This is in contrast to the effect of interstellar polarization, but neither is it to be expected on the synchrotron hypothesis. Investigations of polarization and particularly its wave-length dependence in these violent objects are obviously of great importance in the further understanding of this problem.

L. SEARLE

A. W. RODGERS

W. L. W. SARGENT*

Mount Stromlo Observatory,
Australian National University,
Canberra.

J. B. OKE

Mount Wilson and Palomar Observatories,
Carnegie Institution of Washington,
California Institute of Technology.

* On leave from the University of California at San Diego. Work supported by the U.S. National Science Foundation.

¹ Seyfert, C. K., *Astrophys. J.*, **97**, 28 (1943).

² Woltjer, L., *Astrophys. J.*, **130**, 38 (1959).

³ Thackeray, A. D., *Mon. Not. Roy. Astro. Soc.*, **113**, 211 (1952).

⁴ Woltjer, L., *B. A. N.*, **13**, 301 (1957).

⁵ Mayall, N. U., *Science*, **137**, 91 (1962).

⁶ Greenstein, J. L., and Schmidt, M., *Astrophys. J.*, **140**, 1 (1964).

⁷ O'Dell, C. R., *Astrophys. J.*, **136**, 809 (1962).

⁸ Baum, W., see Sandage, A. R., *Astrophys. J.*, **141**, 1560 (1965).

⁹ Oke, J. B., *Astrophys. J.*, **141**, 6 (1964).

¹⁰ Shklovsky, I. S., *Dokl. Akad. Nauk S.S.S.R.*, **90**, 983 (1953).

¹¹ Mathews, T. A., and Sandage, A. R., *Astrophys. J.*, **138**, 30 (1963).

¹² Visvanathan, N., Thesis, Austral. Nat. Univ. (unpublished results).

¹³ Thackeray, A. D., *The Observatory*, **76**, 154 (1956).

Growth of Interstellar Grains

IN a previous communication¹ we obtained an expression for the rate of growth of interstellar grains moving relative to a gas or to a cloud of grains. The main assumptions were that the grain remained spherical during growth and its size remained small in comparison with the mean free path of the gas molecules, thus enabling the velocity distribution of the molecules to be taken as an equilibrium, in this case Maxwellian, distribution. We also assumed that a fixed proportion, λ , of the colliding gas molecules adhered to the grain.

In order to obtain the actual size of the grain at any instant, we made the further assumption that the speed of the grain remained constant throughout the motion, and was not affected by collisions with the gas molecules. In this communication we dispense with this assumption and take into account the effects of retardation by making use of the formula for the resistance to the motion of small spheres given by Baines, Williams and Asebiomo².

In ref. 1 the rate of increase of the mass M of the grain moving at any speed U through the gas was found to be:

$$\frac{dM}{dt} = 2\pi m n a^2 \lambda \left(\frac{\beta}{\pi}\right)^{\frac{1}{2}} \left\{ \frac{e^{-\beta U^2}}{2\beta} + \left(\frac{U}{2} + \frac{1}{4\beta U}\right) \left(\frac{\pi}{\beta}\right)^{\frac{1}{2}} \operatorname{erf}(\sqrt{\beta} U) \right\} \quad (1)$$

where erf denotes the error function (see, for example, ref. 3). The gas, which consists of n molecules, each of mass m , per unit volume, is assumed to have a kinetic temperature T and as usual we write $\beta = m/2kT$, where k is Boltzmann's gas constant. We found that equation (1) could be written most simply in terms of a non-dimensional molecular speed ratio:

$$S = \sqrt{\beta}U = \frac{2U}{\sqrt{\pi W}}$$

where W denotes the mean thermal velocity of the gas molecules, in the form:

$$\frac{dM}{dt} = mna^2\lambda\left(\frac{\pi}{\beta}\right)^{\frac{1}{2}}\left\{e^{-S^2} + \left(S + \frac{1}{2S}\right)\sqrt{\pi}\text{erf}(S)\right\} \quad (2)$$

Now the resistance to the motion of a small sphere moving through a gas has been calculated in ref. 2 to be:

$$R_S = mna^2\frac{\sqrt{\pi}}{\beta}\left\{\left(S + \frac{1}{2S}\right)e^{-S^2} + \left(S^2 + 1 - \frac{1}{4S^2}\right)\sqrt{\pi}\text{erf}(S)\right\} \quad (3)$$

on the assumption that all the molecules are reflected ($\lambda = 0$), and that the reflexion is specular. (In specular reflexion the component of velocity perpendicular to the surface of collision is reversed while the other component remains unchanged.) Although in the growth problem a proportional λ of the molecules adhere to the sphere, it can be shown using the appendix to ref. 2 that molecules reflected specularly from the sphere carry off no momentum on the average. No modification is therefore required to equation (3) when $\lambda \neq 0$.

The equation of motion for the grain, assuming specular reflexion for the molecules not adhering, is:

$$\frac{d}{dt}(MU) = -R_S$$

and since $S = \sqrt{\beta}U$, we obtain:

$$\frac{d}{dt}(MS) = -\sqrt{\beta}R_S \quad (4)$$

Now:

$$\frac{d}{dM}(MS) = M\frac{dS}{dM} + S$$

or

$$d(MS)/dM = S + 1/\{d(\log M)/dS\} \quad (5)$$

From equations (5), (4), (3) and (2) we can evaluate

$$\frac{d}{dS}(\log M)$$

in terms of S , and integration then gives:

$$\log\left(\frac{M_2}{M_1}\right) = -\int_{S_1}^{S_2}\left[S + \frac{1}{\lambda}\left\{\frac{\left(S + \frac{1}{2S}\right)e^{-S^2} + \left(S^2 + 1 - \frac{1}{4S^2}\right)\sqrt{\pi}\text{erf}(S)}{e^{-S^2} + \left(S + \frac{1}{2S}\right)\sqrt{\pi}\text{erf}(S)}\right\}\right]^{-1}dS \quad (6)$$

where M_1 and M_2 are the masses of the grain for speed ratios $S = S_1$ and $S = S_2$, respectively.

The integration in equation (6) can be carried out numerically. Alternatively, we may approximate to the exponential and error functions in the integrand as in refs. 1 and 2 by using the first few terms of the expansions for large and small S as appropriate (see, for example, ref. 3). The integral can then be evaluated and a simple analytic expression obtained.

For $S \geq 1.4$ we may replace the numerator of the fractional expression in equation (6) by $\sqrt{\pi}(1+S^2)$ and the denominator by:

$$\sqrt{\pi}\left(S + \frac{1}{2S}\right)^*$$

* The first equation in the limiting case (ii) in ref. 1 should contain an additional factor $(\sqrt{\pi}/2)$.

while for $S \leq 1.4$ the numerator can be replaced by:

$$\frac{8}{3}\left(S + \frac{S^3}{5}\right)$$

and the denominator by:

$$2\left(1 + \frac{S^2}{3}\right)$$

Equation (6) then becomes:

$$\log\frac{M_2}{M_1} = -\int_{S \geq 1.4}^{\infty}\left\{1 + \frac{2}{\lambda}\frac{(1+S^2)}{(1+2S^2)}\right\}^{-1}\frac{dS}{S} - \int_{S \leq 1.4}^{\infty}\left\{1 + \frac{4}{5\lambda}\frac{5+S^2}{3+S^2}\right\}^{-1}\frac{dS}{S} \quad (7)$$

and integration gives:

$$\left.\begin{aligned} \frac{M_2}{M_1} &= \left(\frac{S_2^2 + a/b}{S_1^2 + a/b}\right)^{-\lambda/ab} \left(\frac{S_2^2}{S_1^2}\right)^{-\lambda/2a} & S_1, S_2 \geq 1.4 \\ \text{or} \\ \frac{M_2}{M_1} &= \left(\frac{S_2^2 + 5c/d}{S_1^2 + 5c/d}\right)^{-4\lambda/cd} \left(\frac{S_2^2}{S_1^2}\right)^{-3\lambda/2c} & S_1, S_2 \leq 1.4 \end{aligned}\right\} \quad (8)$$

where for convenience we have written $a = \lambda + 2$, $b = 2\lambda + 2$, $c = 3\lambda + 4$ and $d = 5\lambda + 4$. If the speed range extends over both $S \geq 1.4$ and $S \leq 1.4$ both formulae are required, the range being split accordingly. The loss of accuracy has been discussed in detail in ref. 2, and in the present case is scarcely enough to affect three-figure accuracy.

If we make the more drastic approximation of keeping only the first term in the expansions of the numerator and denominator in equation (6) for large and small S , the loss of accuracy may be greater. The corresponding analytic expressions are, however, very simple, and equation (7) becomes:

$$\left.\begin{aligned} \frac{M_2}{M_1} &= \left(\frac{S_2^2}{S_1^2}\right)^{-\lambda/(\lambda+1)} = \left(\frac{U_2}{U_1}\right)^{-\lambda/(\lambda+1)} & S_1, S_2 \geq 1.4 \\ \frac{M_2}{M_1} &= \left(\frac{S_2^2}{S_1^2}\right)^{-3\lambda/(3\lambda+4)} = \left(\frac{U_2}{U_1}\right)^{-3\lambda/(3\lambda+4)} & S_1, S_2 \leq 1.4 \end{aligned}\right\} \quad (9)$$

As the speed ratio S_2 drops to zero the grain clearly grows without limit. However, our model is restricted: (a) by the size of the grain being small compared with the mean free path of the gas; (b) by the neglect of the thermal motion of the grain itself, which implies a minimum speed ratio of the order of 10^{-5} . In calculating the growth we have therefore confined ourselves to speeds with a lower limit of $S = 10^{-5}$, while for the upper limit we chose $S = 10^3$, as this implies an actual speed of the order of 10^8 cm/sec, which is unlikely to be exceeded.

Tables 1 and 2 show the ratio of the final mass to the initial mass as given by equation (8) for grains slowing down from initial speed ratios $S_1 = 1, 10, 10^2, 10^3$ to final speed ratios $S_2 = 10^{-3}, 10^{-2}, 10^{-1}, 10^{-5}$. Table 1 has been constructed using $\lambda = 1$, and Table 2 using $\lambda = 1/10$, in both cases using the equations (8) resulting from the two-term approximation for the functions in equation (6).

Tables 1 and 2 RATIO OF FINAL TO INITIAL MASSES FOR VARIOUS RANGES OF S , USING EQUATIONS (8)

Table 1, $\lambda = 1$					Table 2, $\lambda = 1/10$				
Initial speed ratios, $\log_{10} S_1$	-1	0	1	2	3	-1	0	1	2
	2.68	7.25	21.9	69.3	219	1.17	1.38	1.68	2.07
	7.20	19.5	58.8	186	588	1.38	1.62	1.97	2.43
	19.3	52.2	158	499	1,580	1.62	1.91	2.32	2.86
	51.8	140	423	1,340	4,230	1.91	2.24	2.72	3.35
						2.24	2.54	3.02	3.52
						3.02	3.35	3.82	4.13
						3.82	4.13	4.52	4.82
Final speed ratios, $\log_{10} S_2$	-2	-3	-4	-5		-2	-3	-4	-5

From Tables 1 and 2 the mass ratio can be calculated for any two speed ratios of the form 10^n where n lies between 3 and -5 . To illustrate this point, we note:

$$\frac{M_2}{M_1} (S_1 = 10^2, S_2 = 10^{-1}) = \frac{\frac{M_2}{M_1} (S_1 = 10^2, S_2 = 10^{-3})}{\frac{M_2}{M_1} (S_1 = 10^{-1}, S_2 = 10^{-3})}$$

The maximum growth obviously occurs when $\lambda = 1$ and the change in the speed ratio is greatest, namely, when it drops from $S_1 = 10^3$ to $S_2 = 10^{-5}$. This gives a ratio of the final to the initial mass of just over 4×10^2 . For the same speed ratios and $\lambda = 1/10$ the corresponding ratio of masses is only 5.

In view of the simplicity and ease of application of the formulae in equations (9) we also calculated the ratio of final to initial masses using these formulae, for the same range of parameters as already mentioned. The percentage differences between these results and those given in Tables 1 and 2 were then calculated. The greatest percentage difference for any of the situations considered was only 1.4 and we therefore deduce that equations (9) are perfectly satisfactory for two-figure accuracy.

Finally, we remark that appreciable growth of a grain moving through interstellar gas cannot occur before its relative motion is lost if less than one in ten of the colliding molecules adhere to it. Moreover, for most astrophysical problems a good estimate of the growth is given by the simple formulae in equations (9).

We thank M. A. Mustafa for assistance with the calculation.

M. J. BAINES
I. P. WILLIAMS

Department of Mathematics,
University of Reading.

¹ Baines, M. J., and Williams, I. P., *Nature*, 205, 59 (1965).

² Baines, M. J., Williams, I. P., and Asebjomo, A. S., *Mon. Not. Roy. Astro. Soc.*, 130, 63 (1965).

³ Magnus, W., and Oberhettinger, F., *Functions of Mathematical Physics* (Chelsea, New York, 1954).

RADIOPHYSICS

Anomalous Intensity Ratios of the Interstellar Lines of OH in Absorption and Emission

WE have previously found¹ intensity ratios of the four microwave lines of OH in disagreement with the theoretical and laboratory ratios² of 1 : 5 : 9 : 1 for the lines at 1,612, 1,665, 1,667 and 1,720 Mc/s. For the strong OH absorption of the radio source Sagittarius A it appeared that the ratios might be attributed to high opacity³. We now report a more detailed series of measurements which shows that high opacity alone cannot account for the observations and that disturbed populations of the levels have to be invoked.

Weaver *et al.*⁴ have recently announced the discovery of narrow-band emission at 1,665 and 1,667 Mc/s in ionized hydrogen regions. The emission at 1,665 Mc/s is usually the stronger, and the excess emission is attributed to an 'unidentified microwave line'. We have observed emission from a number of sources at all four OH frequencies, and conclude that all the emission is produced by OH molecules. Weinreb *et al.*⁵ have expressed the same opinion from observations of three of the four lines in the radio source W3. The intensity ratios of the emission lines are abnormal, but can be explained as extreme cases of perturbation of the populations.

Anomalous intensity ratios for all four OH lines in absorption have been measured with the Australian 210-ft. telescope at many points in the complex region near the galactic centre. The ratios vary widely, as is illustrated by the examples in Table 1; there is one case where the absorption on 1,665 Mc/s exceeds that on 1,667 Mc/s. The marked inequality of the absorption at 1,612 and 1,720

Table 1. INTENSITY RATIOS OF OH ABSORPTION LINES

Position	Cloud velocity km/s	1612	1665	1667	1720
$l=0^\circ$	0	1	2.5	3.3	0.5
0°	+40	1	1.5	1.7	0.5
0°	+8	1	1.9	2.4	0.3
$0^\circ 30'$	+24	1	2.7	3.5	1.7
$0^\circ 40'$	-85	1	2.6	3.7	1.4
$0^\circ 40'$	-94	1	3.4	4.6	2.0
$0^\circ 40'$	+60	1	2.2	2.0	0.9
Sgr. A	+42	1	1.9	2.3	0.7*

* More accurate measurement of value reported earlier¹.

Mc/s is clear evidence for a disturbance of the populations. Recent observations of the nebula RCW 38 have shown weak absorption with ratios of 3 : 3 : 1 for the lines at 1,665, 1,667 and 1,720 Mc/s; thus the anomalous ratios are not confined to the galactic centre region nor to cases of high opacity.

Our first evidence of OH in emission came from observations in the direction of $l=0^\circ 40'$, $b=0^\circ$ in June 1964. Fig. 1 shows the profiles at 1,612, 1,665, 1,667 and 1,720 Mc/s over the radial velocity range -80 to $+120$ km/s, measured with the 37 kc/s filters of the multichannel line receiver. These spectra are blends of absorption lines except for a reversal at about $+70$ km/s on the 1,665 Mc/s profile and possibly on 1,612 Mc/s. That this is emission and not just lack of absorption is clearly demonstrated by observations with 10 kc/s band-width, shown in Fig. 2. In this case the 1,665 Mc/s peak breaks up into at least four components. The principal feature is an emission peak of aerial temperature 36° K and line width not noticeably greater than the experimental band-width. An absorption feature at $+64$ km/s and additional features at $+75$ and $+78$ km/s are visible on three of the profiles in Fig. 2. The source of OH line emission was found to be centred at $l=0^\circ 40' \pm 2'$ and $b=0^\circ 2' \pm 2'$; its angular size is less than the experimental limit of $5'$ of arc.

We have observed nine other thermal radio sources, including three of the sources where emission at 1,665 and 1,667 Mc/s is reported by Weaver *et al.*⁴.

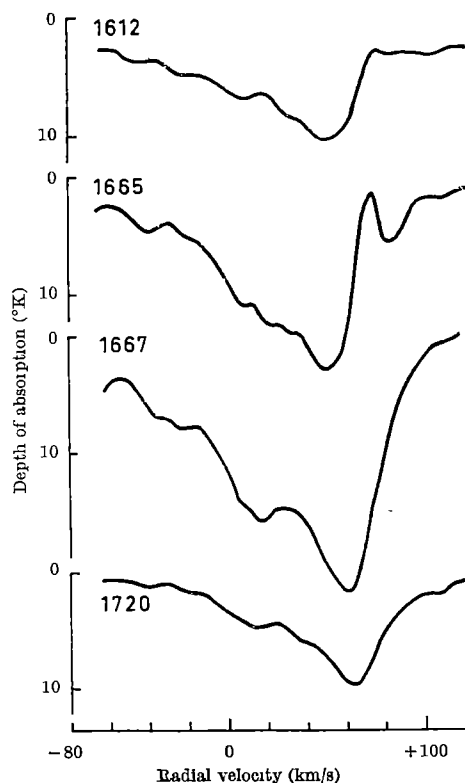


Fig. 1. Line profiles observed at the four OH frequencies in the direction $l=0^\circ 40'$, $b=0^\circ$, with a band-width of 37 kc/s. A sharp emission feature is found at 1,665 Mc/s and possibly at 1,612 Mc/s, on the edge of the broad absorption

For *W49* very strong emission was observed at 1,665 and 1,667 Mc/s, as seen in Fig. 3. Two main peaks were resolved with the 10 kc/s filters, the higher velocity peak being visible on all four OH frequencies. The 1,612 Mc/s profile closely resembles that at 1,665 Mc/s, but the +16 km/s feature is displaced on the 1,667 and 1,720 Mc/s profiles. The intensity ratios of the main emission peaks are 1:19:5:0.3 and 1:11:46:0.4. The line emission source coincides in position with the western (thermal)

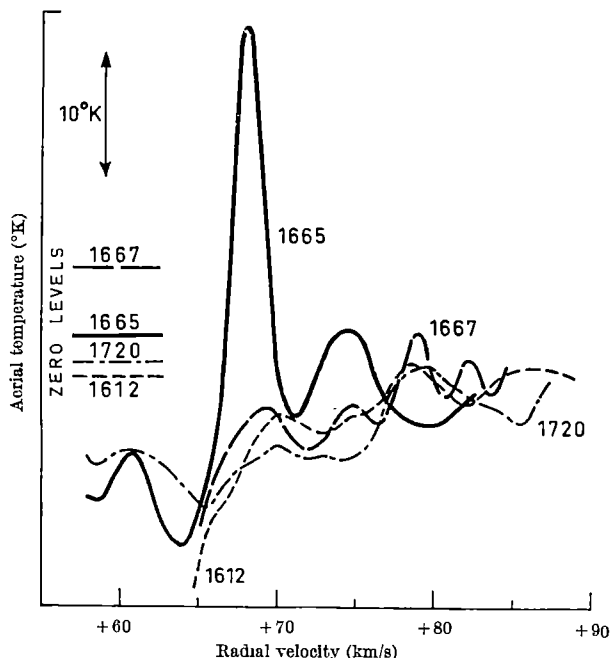


Fig. 2. Detail of the profiles of Fig. 1 over the velocity range 60–85 km/sec. These measurements were made with 15 channels of band-width 10 kc/s. Profiles for each of the OH lines have been superimposed. The zero levels for the four profiles are marked on the ordinate

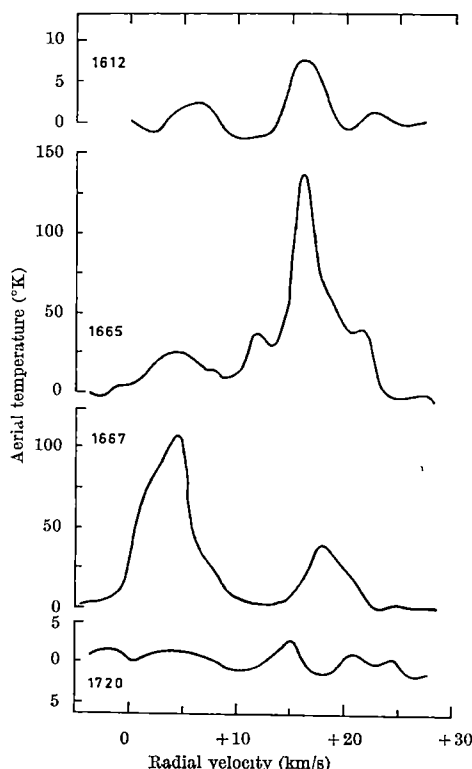


Fig. 3. Emission from the nebula *W49* at each of the four OH frequencies. The aerial temperatures for 1,665 and 1,667 Mc/s reach high values, and the intensity scale for these profiles has been reduced by a factor of 5

component of the double source. The OH emission is not resolved with the 12-min beam, and so must have an angular size less than 5 min of arc. The observed peak antenna temperature of 140° K at 1,665 Mc/s would thus correspond to a brightness temperature of over 800° K.

For *NGC 6334* a complex emission feature was observed near -10 km/s at 1,665, 1,667 and 1,720 Mc/s, the peak temperature being 33° K at 1,665 Mc/s. Pronounced absorption occurred on all four frequencies near -3 km/s. The line emission is clearly displaced by 4 min of arc to the east of the continuum maximum, coinciding with the edge of one of the bright condensations of the nebula. Again the OH source is unresolved with the 12 min of arc beam.

OH emission was seen in the directions of the Orion nebula, η Carinae, *MHR49* (R.A. 16 h 09 m 34 s, Dec. -57° 20' 5) and possibly *NGC 6357*. We found no emission in *RCW38*, *RCW49*, *RCW57*, 30 Doradus or the Crab Nebula.

Weaver *et al.*⁴ have suggested that some or all of the emission near 1,665 Mc/s was an unidentified microwave line. Their argument was based on the finding that the emission at 1,665 Mc/s was stronger than that at 1,667 Mc/s, that the profile shapes on the two lines were very different, and that no counterpart to the 1,665 Mc/s emission in *W49* could be found at 1,612 Mc/s. They explain the anomalous 1,665 Mc/s emission in *W49* and *NGC 6334* as a blend of the OH line and the line of 'mysterium' (with a slightly higher rest frequency).

In *W49* we find that the 1,665 Mc/s profile is closely reproduced at 1,612 Mc/s. Related emission features are also seen on 1,667 and 1,720 Mc/s. We have also observed emission at all four OH frequencies near $l=0^\circ 40'$, $b=-0^\circ 02'$, and Weinreb *et al.*⁵ have observed emission at 1,665, 1,667 and 1,720 Mc/s near the nebula *W3*. The intensity ratios are 'abnormal', but as anomalous ratios are also found in many of our absorption measurements we conclude that both phenomena are produced by OH molecules the populations of which are disturbed from the equilibrium values. The high brightness temperatures of the emission also support the argument for departures from equilibrium.

The Berkeley and Massachusetts Institute of Technology observations show that the emission profiles have many extremely narrow-band components, some as narrow as 1 kc/s. If the intensity ratios are different for each of the components, we would expect the composite profile to have different overall shapes on each of the frequencies.

High molecular densities would be required to explain the observed emission as spontaneous emission from excited molecules. Further, all cases of OH emission are seen against a strong continuum background, when stimulated transitions would be the dominant term in the equation of transfer unless the excitation temperature exceeded the brightness temperature of the continuum. If the populations were inverted, the background could be amplified; then lower OH densities would suffice and narrow lines would be produced.

The anomalous OH emission or absorption has been found only in some ionized hydrogen regions. As the nebulae have appreciable internal motions, the narrow lines imply that the emitting regions are of small size and low kinetic temperature. In *W49* and *NGC 6334* we find that the emitting region is small and displaced to the edge of the nebula. The selective excitation of the molecules may be due to radiation from these bright nebulae. Alternatively the OH molecules might be formed near the ionized region and not all reach equilibrium before they are dissociated or involved in other reactions. A possible mechanism for forming excited OH molecules in the $^2_1I_{3/2}$ state has been proposed by Dr. J. L. Symonds (following communication) involving capture of protons by negative oxygen ions. The energy-levels of the resulting molecules might also be perturbed, thus modifying the

natural rest frequencies. With very weak magnetic fields the molecules so formed could give emission that is polarization sensitive. This could explain the observations⁵ of linearly polarized emission in some features without the need for a magnetic field strong enough to separate the components by Zeeman splitting.

We thank Prof. W. A. Fowler and Prof. V. H. Rumsey for their advice. We also thank Mr. J. D. Murray for modifications to the receiver.

R. X. MCGEE
B. J. ROBINSON
F. F. GARDNER
J. G. BOLTON

C.S.I.R.O.

Radiophysics Laboratory,
Sydney, Australia.

- ¹ Gardner, F. F., Robinson, B. J., Bolton, J. G., and van Damme, K. J., *Phys. Rev. Letters*, **13**, 3 (1964).
² Radford, H. E., *Phys. Rev. Letters*, **13**, 534 (1964).
³ Robinson, B. J., Gardner, F. F., van Damme, K. J., and Bolton, J. G., *Nature*, **202**, 989 (1964).
⁴ Weaver, H., Williams, D. R. W., Dieter, N. H., and Lum, W. T., *Nature*, **208**, 29 (1965).
⁵ Weinreb, S., Meeks, M. L., Carter, J. C., Barrett, A. H., and Rogers, A. E. E., *Nature*, **208**, 440 (1965).
⁶ Rodgers, A. W., Campbell, C. T., and Whiteoak, J. B., *Mon. Not. Roy. Astro. Soc.*, **120**, 1 (1960).

Formation of Hydroxyl Molecules in Interstellar Space

DURING the past year, radio astronomers^{1,2} have been increasingly interested in microwave signals at frequencies of 1,612, 1,665, 1,667 and 1,720 Mc/s, produced by transitions between four energy levels of the OH molecule in the $^2\Pi_{3/2}$ state. Theoretical and laboratory intensity ratios³ for the four microwave lines were found to be 1 : 5 : 9 : 1. It was reported¹ recently that anomalous intensity ratios had been measured in the strong OH absorption lines of the radio source Sagittarius A. McGee *et al.*⁴ have extended their measurements to other radio sources and find emission and absorption at all four frequencies with instances of extremely anomalous intensity ratios.

Emission and absorption lines showing such anomalous intensity distributions indicate unusual populations of energy levels in the OH molecules. The observed distributions are more like those expected of a chemical reaction mechanism, followed by radiative deactivation, rather than those from thermal excitation. Since the gases are tenuous in the regions which produce these signals, the long time-scale between collisions will give the molecules, formed in an excited state, a greater probability of radiative rather than collisional de-activation.

The process of association of two atoms to form a molecule normally has a low probability. Since atomic recombination is known to proceed more rapidly in the presence of a third body, 'dust' grains in space have been suggested as a suitable medium. The tenuous nature of the gas would seem to rule out a three-body process, however, and such reactions do not appear likely to produce the observed intensity anomalies. The processes for production and loss of OH molecules by two-body processes, therefore, deserve closer study.

In relation to the known concentrations of oxygen and hydrogen in our galaxy, the concentration of OH molecules is such as to make it improbable that they are all formed by the direct collision process between atoms. In the study of gaseous processes in flames by spectroscopic means, OH spectra show evidence of pre-dissociation, made possible by radiationless transitions between states of nearly the same energy where the potential energy curves of the states cross or approach closely. The inverse pre-dissociation process⁵ (pre-association) is also possible for forming molecules from a two-body collision. The pre-dissociation of the OH molecule to O and H atoms and its inverse are believed to occur, but with a small

probability, because a normally forbidden transition is involved.

It is suggested that other two-body processes should be investigated for an alternative mechanism. Since the atom recombination is low, a mechanism worthy of consideration is the exothermic association of negative oxygen ions, O⁻, and positive ions of hydrogen, H⁺ (protons).



The presence of O⁻ ions seems assured by the strong electron affinity of oxygen (1.45 eV) and the known presence of oxygen atoms in the regions under study. The anomalous emission and absorption has only been found in ionized regions^{4,6} where large concentrations of electrons and protons must also exist. The pre-association of O⁻ and H⁺ essentially may be a transition from the coulomb potential energy curve to the $^2\Sigma^+$ or, more probably, the $^2\Pi_{3/2}$ state curve, involving charge transfer. The interaction cross-section should have a maximum value when the relative velocity of the two ions produces kinetic energy close to the differences between the binding energy of the OH molecule and the electron affinity of the oxygen atom, that is, $\epsilon = 45 - 1.45 = 3 \text{ eV}$.

In such circumstances, it is possible to form OH molecules in the $^2\Pi_{3/2}$ state with the population of each of the four levels depending greatly on the relative velocity of the O⁻ and H⁺ ions. Whether the molecules emit or absorb energy will depend on the populations of the energy levels and, in bulk, one would not expect to see the theoretical intensity ratios. Situations could arise where the relationships between lines were completely unusual, for example, apparent absorption in some of the four lines and emission in the others. The actual result will depend strongly on the relative velocity distribution of the two ions. Investigation of the intensity ratios may, therefore, give a great deal of information on relative ion velocities.

Since the reaction has a 'resonance' character, it will favour relative velocities producing about 3-eV kinetic energy between the ions. If the O⁻ ion were stationary, the proton velocity would need to be about 25 km/sec, which is certainly in the range found in the ionized regions of the galaxy. The lack of emission or absorption at the four frequencies in galactic regions of lower temperature may be explained in terms of a low cross-section at proton velocities well below 25 km/sec. An accurate calculation of the reaction cross-section and the state of the resulting molecule would be informative.

The process of association between O⁻ ions and protons, or for that matter any similar process between other ions, does not appear to have received great attention. The study of recombination rates in flames⁶ shows evidence of a non-equilibrium condition in the excitation of molecules and atoms in the flame. Unexplained concentrations and intensity ratios exist which relate to OH molecule formations. These conditions may be the result of ionic recombination to form molecules and not atom-molecule reactions. Evidence of strong negative and positive ion concentrations has been found^{7,8}, but the result of their presence on the state of excitation of product molecules has not been elucidated.

If the reaction mechanism is as proposed, radiationless transitions will leave the OH molecule with a velocity similar to that of the O⁻ ion. However, it is not obvious what the 'temperature' of the O⁻ ions will be since their velocity will depend on their mode of formation and their lifetime.

Two effects must be studied. First, the OH line will be shifted by Doppler effects resulting from mass motion relative to the observational point, and the mass motion may not necessarily be related to the motion of the O and H atoms. Secondly, the line broadening may differ from that produced by the 'temperature' of the surrounding ionized gas region because the radiationless transitions occurring in pre-association results in some perturbation

of the rotational levels in the states involved. Corresponding shifts in line positions and changes in the intensity distributions will occur, with widths also being reduced in the process.

Orientation of the magnetic moment of the O⁻ ion by a magnetic field should produce a modification of intensity ratios, possibly an alteration of frequency not associated with a mass movement, and certainly polarization effects. Viewing the OH radiation coming from an ionized region, the proposed mechanism suggests that the radiation will be linearly polarized¹⁰ since the movement of hydrogen appears to be radially outward and the axis of rotation of the OH molecules will tend to be in a plane normal to the proton direction.

The known presence of OH molecules must also lead to the assumption that OH⁻ molecular ions are also present. The electron affinity of the OH molecule is greater than that of the oxygen atom, making possible two reactions of interest. The first is similar to that for the formation of OH and would lead to the formation of the H₂O molecule. The second is the charge transfer process which will result in the production of an atom of hydrogen and an excited OH molecule. Apart from ionizing and other processes which remove OH molecules, it is obvious that there are other modes of OH formation by collision processes between ions, atoms and molecules which must be taken into account.

Finally, the proposed mechanism offers some interesting prospects in relation to the formation of other molecules. Atoms with strong electron affinities are more likely to produce similar reactions with protons. Equally, the rotational states of such molecules will be excited. Atoms of hydrogen, carbon, oxygen, silicon and sulphur would be capable of forming negative ions. Whether the pre-association process is possible is not known for every case. Since nitrogen does not form a stable negative ion, NH may not be observed. Nevertheless, similar reactions may take place between N⁺ and O⁻ or C⁻ to give NO and CN molecules.

The formation of H₂ from H⁻ and H⁺ ions may require higher relative velocities, but the cross-section for H⁻ production from H is four orders of magnitude less than for O⁻ formation. Such a process may not be observable in this count alone, without invoking the question of the frequency range in which signals may be expected.

In summary, the association mechanism appears to offer some prospect of success in accounting for the anomalous intensity ratios; the emitted radiation may well be linearly polarized if a magnetic field is present; some suggestions can be made as to kinds of molecules that might be formed. More theoretical and experimental work on reactions between negative and positive ions appears to be necessary to establish their importance.

I thank Dr. B. J. Robinson and his colleagues of C.S.I.R.O. Radiophysics Laboratory for their advice and for making available the results of their investigations before publication.

J. L. SYMONDS

Australian Atomic Energy Commission
Research Establishment,
Lucas Heights, New South Wales.

- ¹ Gardner, F. F., Robinson, B. J., Bolton, J. G., and van Damme, K. J., *Phys. Rev. Letters*, **13**, 3 (1964).
- ² Robinson, B. J., Gardner, F. F., van Damme, K. J., and Bolton, J. G., *Nature*, **202**, 989 (1964).
- ³ Radford, H. E., *Phys. Rev. Letters*, **13**, 534 (1964).
- ⁴ McGee, R. X., Robinson, B. J., Gardner, F. F., and Bolton, J. G., *Nature* (preceding communication).
- ⁵ Herzberg, G., *Molecular Spectra and Molecular Structure; Spectra of Diatomic Molecules* (D. Van Nostrand Co., Inc., 1959).
- ⁶ Garvin, D., Broida, H. P., and Kostkowski, H. J., *J. Chem. Phys.*, **32**, 880 (1960).
- ⁷ King, I. R., *J. Chem. Phys.*, **37**, 74 (1962).
- ⁸ Miller, W. J., and Calcote, H. F., *J. Chem. Phys.*, **41**, 4001 (1964).
- ⁹ Weaver, H., Williams, D. R. W., Dieter, N. H., and Lum, W. T., *Nature*, **208**, 29 (1965).
- ¹⁰ Weinreb, S., Meeks, M. L., Carter, J. C., Barrett, A. H., and Rogers, A. E. E., *Nature*, **208**, 440 (1965).

PHYSICS

Laminar and Turbulent Flow in Open Channels

IN a paper published in 1884, Prof. Osborne Reynolds¹ expressed the opinion that free surfaces, as found in open channels, tend to be "conducive to direct or steady motion". Thus we should expect the critical Reynolds number for open channels to be, in general, higher than it is for pipes, using the hydraulic radius as the length-parameter throughout.

This view would appear to be borne out by the results of recent work carried out here, in which channels of narrow rectangular and T-shaped cross-sections were used. Further confirmation is supplied by a study of the investigations quoted in Table 1.

It will be appreciated that an analysis of laminar and turbulent flow is considerably more difficult for open channels than for circular sectioned pipes, since (a) only channels having the same width to depth ratio are geometrically similar, while (b) the symmetry of the velocity distribution which occurs in flow through circular pipes cannot be assumed for the more complex pattern of flow encountered in channels. This is particularly true of narrow channels in which the drag of the side-walls has a great influence.

It is tentatively suggested after an examination of Table 1 that the dimensionless group $\frac{\sigma}{\rho^2 \rho} (= \frac{f}{2})$, is

Table 1. CRITICAL REYNOLDS NUMBERS FOR VARIOUS SECTIONS, DETERMINED BY DIFFERENT INVESTIGATORS

Nature of the cross-section	Investigators	Dimension of the cross-section		$R = \frac{\text{width}}{\text{depth}}$	Hydraulic radius 'm' (in)	Critical Reynolds No *	Minimum value of $f/2$
		Width (in.)	Depth (in)				
Circular pipes	Stanton and Pannell (ref. 2)	Pipes of various diameters				525	0.00387
Rectangular pipes	Davies and White (ref. 3)	1.00	0.0059-0.0138	169-72.5	0.00295-0.0069	720	0.00468
" "	Cornish (ref. 4)	0.464	0.159	2.92	0.059	525	0.00412
" "	Allen (ref. 5)	1.00	0.256	3.92	0.102	600	
Rectangular open channels	Allen (ref. 6)	3-15	1.12-8.00	2.81-0.525	0.655-1.25	1,400†	
" "	Horton, Leach and Villet (ref. 7)	5-64	0.12	47.0	0.12	548	0.0035
" "	Straub (ref. 8)	1.7	0.14-2.04	12.15-0.833	0.120-0.60	Approx. 640	0.00375
" "	Owen (ref. 9)	1.5	Max. 0.3	Min. 5.0	0.214	750-800	0.00400
" "	Straub, Silberman, and Nelson (ref. 10)	1.7	0.14	12.1	0.120	—	
			0.23	7.4	0.181	588	0.00375
			0.33	5.1	0.238	775	(average value)
			0.47	3.6	0.303	900	
			0.63	2.7	0.362	663	
" "	Author's results	1.0	4.0	0.25	0.445	657	0.00372
			6.0	0.167	0.461	676	0.00396
			8.0	0.125	0.470	716	0.00401
" "			10.0	0.10	0.475	767	0.00419
" "	U.S. Waterways, Vicksburg (ref. 11)	27.76	0.348	80	0.336	760	
		27.76	0.288	97	0.276	870	
		27.76	0.228	122	0.228	610	

* From the curve of f against Reynolds number where available.

† There seems to be some doubt whether this was a lower critical number.

of paramount importance in determining the laminar or turbulent nature of the flow. Thus it seems that $f/2$ has a reasonably constant critical value irrespective of the shape of the cross-section.

A physical interpretation of the significance of the factor $\sigma/\rho\bar{v}^2$ is that it represents the ratio of the shearing stress at the solid boundary to the momentum per unit area of the section. In the early stages of laminar flow the shearing stress σ is sufficient to ensure that eddies are not created, but if the mean velocity \bar{v} then rises gradually, the denominator, $\rho\bar{v}^2$, increases more rapidly than the numerator σ , thus causing a drop in the value of f which continues until a stage is reached, marking the onset of turbulence, when the shearing stress is insufficient to overcome the effects of the inertia. Further resistance is then developed in the form of eddies.

JOHN ALLEN

Department of Civil Engineering,
Queen Mary College
(University of London),
London, E.1.

¹ Reynolds, Osborne, *Proc. Roy. Inst.*, 11, 44 (1884-86).

² Stanton, T. E., and Pannell, J. R., *Phil. Trans. Roy. Soc.*, A, 214, 199 (1914).

³ Davies, S. J., and White, C. M., *Proc. Roy. Soc.*, 119 (1928).

⁴ Cornish, R. J., *Proc. Roy. Soc.*, A, 120, 691 (October 1928).

⁵ Allen, J., *Phil. Mag.*, Ser. 7, 18, 488 (September 1934).

⁶ Allen, J., *Phil. Mag. and J. Sci.*, 7th Ser., 17, 1081 (June 1934).

⁷ Horton, R. E., Leach, H. R., and Vhet, R. van, *Trans. Amer. Geophys. Union*, 1933-34 (graph on p. 402).

⁸ Straub, Lorenz G., *Trans. Amer. Geophys. Union*, Part 4, 649 (August 1939).

⁹ Owen, W. M., *Trans. Amer. Soc. Civ. Eng.*, 119, 1165 (1954).

¹⁰ Straub, Lorenz G., Silverman, E., and Nelson, H. C., *Trans. Amer. Soc. Civ. Eng.*, 123, 685 (1958).

¹¹ Paper 17, U.S. Waterways Exp. Stat., Vicksburg, Mississippi (January 1935).

ENGINEERING

Detonability of Nitroglycerin contained in Porous Rock

In the course of experiments to determine the feasibility of using liquid explosives to enhance the productivity of oil- and gas-bearing sandstones after hydraulic fracturing, it has been found that dry porous sandstone could imbibe sufficient nitroglycerin-ethylene glycol dinitrate (50-50 NG-EGDN¹) to yield a detonable charge.

The rock used was Berea sandstone of density 2.2 g/cm³ when dry. Imbibition tests with both water and NG-EGDN showed that this rock is capable of holding 11-13 per cent of its own volume of liquid, although comparison of its bulk density with that of crystalline silica indicates that about 17 per cent of the rock volume is void space.

The rock samples were 2 × 2 × 6-in. blocks which were dried at over 100° C for more than 16 h. They were then immersed in NG-EGDN for several hours. It was found that the rock absorbed 6.6-7.4 per cent by weight of the explosive after 2 h immersion, and that this could be increased to 8.2 per cent by immersion for 48 h.

The detonability trials were instrumented with an expendable pressure transducer having a useful range of 1-70 kbar located at the downstream end of the charge and a continuous, pressure-actuated detonation velocity probe for measuring detonation rates along one side of the charge. Data were recorded oscillographically.

The initiator consisted of a No. 8 electric blasting cap and either a 7.5 or 15-g tetryl pellet (0.75 in. diam. × 0.5 in. long or 0.75 in. diam. × 1.0 in. long); in one case which detonated, there was an additional booster consisting of a reservoir of NG-EGDN 2 in. square and 0.75 in. deep, holding 73 g. For each trial using NG-EGDN, a corresponding test was made using water as the imbibed medium in order to determine the response of the instrumentation to the inert shock transmitted from the donor. In no case was this shock sufficient to actuate the instrumentation, although the sandstone, which was quite fragile, was completely shattered.

Samples with up to 7.4 per cent NG-EGDN did not detonate, although it is possible that the booster was inadequate in these cases. With the NG-EGDN reservoir and with a sample that had absorbed 8.2 per cent NG-EGDN, the measured detonation rate was 4.7 mm/μsec. The record from the pressure transducer was slightly obscured by electrical ringing, but a pressure in excess of 25 kbar was indicated.

Thus, it appears that nitroglycerin, absorbed into a solid, porous, inert matrix, is detonable in concentrations as low as 8 per cent by weight; the resulting system, while insensitive, has an unexpectedly high velocity of detonation. This may be compared with the behaviour of gelled NG absorbed in sodium chloride (density 1.37 g/cm³) where the detonation rate with 15 per cent NG in 4.45-cm diam. is 1.55 mm/μsec (ref. 2).

J. EDMUND HAY
FRED H. SCOTT

U.S. Department of the Interior,
Explosive Research Center,
Bureau of Mines,
Pittsburgh, Pennsylvania.

¹ Commercial nitroglycerin usually contains EGDN to depress the freezing point and such mixtures are referred to generically as 'nitroglycerin'.

² Taylor, J., *Detonation in Condensed Explosives*, 179 (Oxford, Clarendon Press, 1952).

CRYSTALLOGRAPHY

Structure of Calcium Oxalate Monohydrate

ALTHOUGH details of the structure of some of the hydrates of calcium oxalate have been reported^{1,2}, the monohydrate is less amenable to crystallographic analysis, since the synthetic salt³ precipitates as a finely divided powder and the naturally occurring mineral whewellite usually contains impurities^{4,5}. Calcium oxalate occurs widely in biological subjects, however, and in plants, where X-ray diffraction powder diagrams have been used to distinguish the various hydrates^{6,7}; crystals of optically visible size are commonplace⁸. In the course of our investigation of the nature of calcium oxalate in plants, we have been able to isolate crystals of the monohydrate of sufficient size to permit complete measurement of the lattice.

The crystals used in the work recorded here were separated from the leaves and stems of *Yucca rupicola* Scheele collected from a native habitat near Oak Hill, Texas. Calcium oxalate monohydrate is present as an intracellular deposit⁹, either in the form of solitary styloids (in the case of the largest crystals) or as smaller needle-shaped crystals in the raphide bundles. The crystals were prepared by triturating the chopped tissue in 95 per cent ethyl alcohol in a high-speed blender, followed by filtration through coarse muslin to remove cellular debris. The filtrate was centrifuged to separate the denser oxalate from the lighter organic substances and the precipitate was resuspended in fresh alcohol and recentrifuged repeatedly until the sediment consisted principally of whole or broken crystals. Samples of the total sediment, and samples of representative groups of the larger crystals selected with a fine glass needle, were examined in a cylindrical X-ray diffraction powder camera. A few specimens of the largest and most perfect crystals, up to 200 μ long, were selected under a low-power polarizing binocular microscope, mounted on a fine glass fibre and subjected to single crystal analysis.

Single crystal X-ray diffraction data obtained with Weissenberg and precession cameras using copper K_α and molybdenum K_α radiation can be indexed on the basis of a monoclinic cell; $a = 6.61 \text{ \AA}$, $b = 14.46 \text{ \AA}$, $c = 10.07 \text{ \AA}$ and $\beta = 116.5^\circ$. The $\theta 0l$ diffraction data permit also the choice of $\beta = 109.4^\circ$ which is closer to the previously reported values of 107° ; the cell parameters then become $a = 6.28 \text{ \AA}$, $b = 14.46 \text{ \AA}$ and $c = 11.10 \text{ \AA}$. There are eight formula weights in the unit cell and the calculated

density of 2.254 compares favourably with the densities recorded elsewhere. Observed systematic extinctions occurred for $h0l$ when l is odd and for $0k0$ when k is odd.

The space group is thus P_{21}^2/c . The $hk0$ Weissenberg diagram shows that festoons of spots whose k index is divisible by 4 are very strong while the spots lying along curves of different k values are either missing or extremely weak. Thus the calcium atom appears to lie near $y = 1/4$. In Table 1 our X-ray diffraction results are compared with axial ratios and β -angles deduced from optical measurements reported previously.

Table 1. COMPARISON OF AXIAL RATIOS AND β -ANGLE OF CALCIUM OXALATE MONOHYDRATE

	a, A	a/b	b, A	b/b	c, A	c/b	β°
Goldschmidt ¹⁰		0.8698		1		1.3695	107.3
Becke ¹¹		0.8628		1		1.3677	107
ASTM No. 13-601 ¹²	6.25	0.430	14.52	1	9.89	0.681	107
Present work	6.61	0.457	14.46	1	10.07	0.696	116.5

The powder diffraction pattern of our material compares closely with the pattern of Montana whewellite reported by Pecora and Kerr¹³. In agreement with these authors, we can find no diffraction lines greater than 5.95 Å in any of our specimens, in contrast to the data recorded by Gude *et al.*⁵ which include a line at 14.3 Å indexed as (101) and a line at 10 Å indexed as (001). Since both these

lines cannot occur in the space group P_{21}^2/c , their presence may be explained by polymorphism, by alteration of space groups by variation in the ratio of water to oxalate, or by impurities such as mica or chlorite.

This work was supported in part by National Science Foundation grant GB 1458 and by National Institutes of Health grant 5T1GM789; a complete structure analysis will be reported in due course.

H. J. ARNOTT

Cell Research Institute,
University of Texas, Austin.

F. G. E. PAUTARD*

Astbury Department of Biophysics,
University of Leeds.

H. STEINFINK

Department of Chemical Engineering,
University of Texas, Austin.

* National Science Foundation Senior Visiting Foreign Scientist, Cell Research Institute, University of Texas.

¹ Honegger, R., *Fortschr. naturforsch. Ges. Zurich. Beh.*, **97**, 1 (1952)

² Sterling, C., *Acta Cryst.*, **18**, 917 (1965).

³ Hammarsten, G., *C. R. trav. lab. Carlsberg.*, **17**, 1101 (1928)

⁴ Frey, A., *Sweiz. mineralog. petrogr. Mitt.*, **4**, 16 (1942).

⁵ Gude, A. J., Young, E. J., Kennedy, V. C. and Riley, L. P., *Amer. Min.*, **45**, 1257 (1960).

⁶ Lecomte, J., Pobegu, T., and Wyart, J., *Phys. Rad.*, **6**, 22 (1945)

⁷ Walter-Levy, L., and Strauss, R., *C. R. Acad. Sci., Paris*, **254**, 1671 (1962).

⁸ Frey, A., *Linsbauer's Handbuch der Pflanzenanatomie*, **3/1a**, 82 (Gebrüder Borntraeger, Berlin, 1929).

⁹ Arnott, H. J., *Third European Symp. on Calcified Tissues*, Davos, 1965 (in the press).

¹⁰ Goldschmidt, V., *Atlas der Kristallformen*, **9** (Winter, Heidelberg, 1923).

¹¹ Becke, F., *Tschermak*, **26**, 391 (1927).

¹² *Power Diffraction File* (Amer. Soc. Testing and Materials, Philadelphia).

¹³ Pecora, W. T., and Kerr, J. H., *Amer. Min.*, **39**, 208 (1954).

CHEMISTRY

Thermoluminescence and Ultra-violet Phosphorescence of the Nucleic Acid Bases

THIS communication reports some preliminary experimental results obtained from a study of:

(1) Thermoluminescence induced in the five nucleic acid bases following irradiation at 77° K in a cobalt-60 γ -ray source.

(2) Ultra-violet phosphorescence from the same materials at the same temperature, excited by a Hanovia fluorescence model 16 mercury arc lamp.

(1) *Thermoluminescence.* The samples were prepared as cylindrical pellets of diameter 1.25 cm and depth 1 mm, formed under pressures of about 4 tons/in². Following irradiation in silicate glass tubes, in the presence of oxygen, glow curves were recorded using a technique identical to that described by Charlesby and Partridge¹. The temperatures at which the peak light intensities occurred were deduced from traces drawn by a recorder connected to a thermocouple in contact with the emitting surface of the sample facing the photomultiplier.

The doses were varied from 200 rads up to 1.25 Mrads, and in all cases the total light output, taken as proportional to the area under the glow curve, was found to vary directly with the dose. In cytosine three intensity peaks were recorded, and in thymine the persistence of thermoluminescence at temperatures close to 273° K was very marked.

The results are summarized in Table 1, and are compiled from at least four separate determinations. The figures given in brackets under the temperature heading are those obtained by Lehman and Wallace², from a similar investigation. The accuracy of the present measurements is $\pm 10^\circ$ K. Thus it will be seen that the two sets of results agree within experimental error, except in the case of cytosine. This discrepancy may be accounted for by the more precisely controlled warming rates pertaining to Lehman and Wallace's work. We found no evidence for the existence of a second (160° K) glow peak in uracil.

	Peak temperature (° K)	Relative thermoluminescent light yield
Adenine	127 (120 \pm 10)	1
Cytosine	100, 145, 200 (115, 130, 180)	0.01, 0.3, 0.05
Guanine	120 (108 \pm 4)	0.012
Thymine	105 (105)	0.02
Uracil	127 (120, 160)	0.03

A determination of the spectral distribution of the glow curves was attempted, having first established by extensive optical filter experiments that the spectral content of each glow peak of the thermoluminescence emission did not vary appreciably with temperature/time. The results are shown, for adenine and cytosine, in Fig. 1. The curves were obtained using a Hilger and Watts E498 ultra-violet quartz spectrograph, in conjunction with the E720 photoelectric scanning unit manufactured by the same company. Since the thermoluminescence emission continued for, at most, 60 sec after the samples had been removed from the irradiation Dewar vessel, the procedure adopted was to set the scanning unit on the required wave-length (at 250 Å intervals) and determine the total intensity of the emission at that wave-length, taken as proportional to the area under the glow curve, suitably corrected for variation of photomultiplier sensitivity with wave-length. Unfortunately, the light collection efficiency of the spectrometer was not sufficiently high to permit the recording of similar spectral curves for

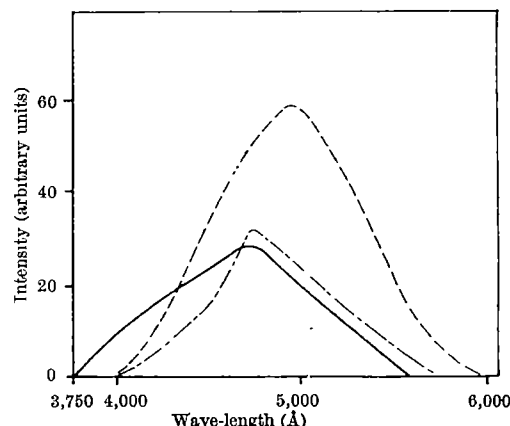


Fig. 1. Thermoluminescent spectra. ---, Adenine; —, cytosine (145° K peak); - - -, cytosine (200° K peak)

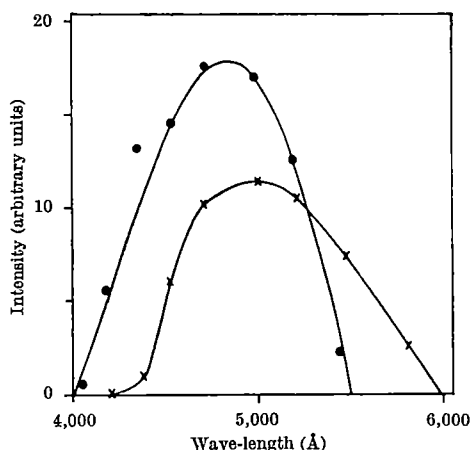


Fig. 2. Phosphorescent spectra. ●, Adenine; ×, thymine

the low intensity emission of guanine, thymine, uracil, and the low temperature (100° K) peak of cytosine.

(2) *Ultra-violet Phosphorescence.* The spectral curves shown in Fig. 2, for adenine and thymine, were obtained using the rotating camera phosphoroscope method first proposed by Lewis and Kasha³. The samples, in crystalline powder form, were contained in continuously rotated quartz test-tubes immersed in liquid nitrogen in a quartz Dewar vessel. The phosphorescence persistence time was approximately 1 sec for adenine, but was less than 0.1 sec in the case of thymine. No phosphorescence was observed from cytosine, guanine, and uracil. These latter results differ from those of Bersohn and Isenberg⁴, obtained for water-glycerine glasses at 77° K. They observed phosphorescence from guanine but not from thymine; the absence of guanine phosphorescence from our experiments may be due to the presence of impurity metal ions in our guanine samples.

The similarity between the thermoluminescence and phosphorescence spectral distribution curves for adenine would appear to indicate that the same luminescence centre is involved in both cases, a conclusion already reached by Singh⁵. Consequently, the absence of phosphorescence in cytosine, contrasting with the relatively strong thermoluminescent glow peaks, is perhaps somewhat surprising. Furthermore, the spectral content differences between the two higher temperature thermoluminescent peaks, at shorter wave-lengths, is the reverse of what might be expected from the greater depth of the electron trap associated with the highest temperature (200° K) glow peak. In the absence of further evidence on the long tail of the thymine glow curve, no explanation of this observation is attempted at present.

R. J. FLEMING
R. M. KERR

Department of Physics,
Royal Military College of Science,
Shrivenham, nr. Swindon, Wiltshire.

¹ Charlesby, A., and Partridge, R. H., *Proc. Roy. Soc., A*, **271**, 170 (1963)

² Lehman, R. L., and Wallace, R., *Electronic Aspects of Biochemistry*, 43 (Academic Press, New York and London, 1964).

³ Lewis, G. N., and Kasha, M., *J. Amer. Chem. Soc.*, **66**, 2100 (1944).

⁴ Bersohn, R., and Isenberg, I., *J. Chem. Phys.*, **40**, 3175 (1964).

⁵ Singh, B. B. (In the press).

Dissociation Energy of the N—H Bond in Hydrazine

THE value of the dissociation energy of the N—H bond in hydrazine is uncertain as there exists a discrepancy between the measured value and that indicated by other chemical evidence.

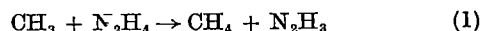
A value may be obtained from electron impact measurements by means of the relationship:

$$D(\text{H—N}_2\text{H}_3) = A(\text{N}_2\text{H}_3^+) - I(\text{N}_2\text{H}_3)$$

provided that the appearance potential, $A(\text{N}_2\text{H}_3^+)$ from hydrazine, involves no kinetic energy and that the ionization potential, $I(\text{N}_2\text{H}_3)$, is close to the adiabatic value. Dibeler¹ obtained 11.3 ± 0.1 eV for $A(\text{N}_2\text{H}_3^+)$ from hydrazine. We have confirmed this value by independent measurements. In our experiments it was possible to obtain parallel ionization efficiency curves for hydrazine and the calibrating gas only after the hydrazine had been present in the mass spectrometer for several hours.

Dibeler derived an indirect value for $I(\text{N}_2\text{H}_3)$ of 6.2 eV which combined with his value for $A(\text{N}_2\text{H}_3^+)$ gave $D(\text{H—N}_2\text{H}_3) = 115$ kcal/mole. This was later revised to 78 kcal/mole by the use of $I(\text{N}_2\text{H}_3) = 7.88$ eV obtained by Foner² from the direct ionization of the N_2H_3 radical. Other chemical evidence has been suggested by Gray³ to give a value of $D(\text{H—N}_2\text{H}_3) = 91$ –93 kcal/mole. Direct values of radical ionization potentials are difficult to assess, however, and the following arguments are offered in justification of Foner's value for $I(\text{N}_2\text{H}_3)$ and hence the lower value of about 78 kcal/mole for $D(\text{H—N}_2\text{H}_3)$.

An indirect value for $I(\text{N}_2\text{H}_3)$ can be obtained in the following manner. Consider the metathetical reaction:



for which Gray⁴ obtained an activation energy, E_0 , of 5.0 kcal/mole. An overall enthalpy of reaction, $\Delta H_{(1)}$, can be calculated by using Semenov's equation⁵:

$$E_0 = 11.5 + 0.25 \Delta H$$

This gives $\Delta H_{(1)} = -26.0$ kcal/mole. The heat of formation of the hydrazyl radical is given by:

$$\Delta H_f(\text{N}_2\text{H}_3) = \Delta H_{(1)} - \Delta H_f(\text{CH}_4) + \Delta H_f(\text{CH}_3) + \Delta H_f(\text{N}_2\text{H}_4)$$

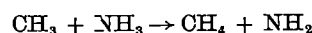
which leads to $\Delta H_f(\text{N}_2\text{H}_3) = 47.1$ kcal/mole. The heat of formation of the hydrazyl ion is given by:

$$\Delta H_f(\text{N}_2\text{H}_3^+) = A(\text{N}_2\text{H}_3^+) - \Delta H_f(\text{H}) + \Delta H_f(\text{N}_2\text{H}_4)$$

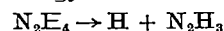
which leads to $\Delta H_f(\text{N}_2\text{H}_3^+) = 230$ kcal/mole. The ionization potential is simply given by the difference:

$$I(\text{N}_2\text{H}_3) = \Delta H_f(\text{N}_2\text{H}_3^+) - \Delta H_f(\text{N}_2\text{H}_3) = 7.9 \text{ eV}$$

in good agreement with Foner's direct value. This procedure for deriving radical heats of formation is somewhat arbitrary but apparently justifiable in that, by similar reasoning, for the reaction:



$I(\text{NH}_2)$ and $D(\text{H—NH}_2)$ so determined are in good agreement with established values. It is interesting to note that, quite independent of any electron impact data, the bond dissociation energy calculated for the reaction:



and given by:

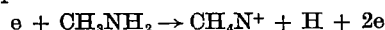
$$D(\text{H—N}_2\text{H}_3) = \Delta H_f(\text{H}) + \Delta H_f(\text{N}_2\text{H}_3) - \Delta H_f(\text{N}_2\text{H}_4)$$

leads to $D(\text{H—N}_2\text{H}_3) = 77$ kcal/mole.

It is possible to calculate the ionization potential of the radical CH_2NH_2 in an analogous manner to $I(\text{N}_2\text{H}_3)$. For the reaction:

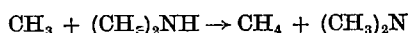


Gray⁴ obtained a value of 8.7 kcal/mole for E_0 . Thus $\Delta H_{(2)} = -11.2$ kcal/mole which leads to $\Delta H_f(\text{CH}_2\text{NH}_2) = 32.5$ kcal/mole and $D(\text{H—CH}_2\text{NH}_2) = 91$ kcal/mole. Mass spectra⁶ of the deuterated amines CH_3ND_2 and CD_3NH_2 indicate that hydrogen loss in the dissociative ionization process:



occurs mainly to give the ion CH_2NH_2^+ and not CH_3NH_2^+ . Collin's data⁷ thus give $\Delta H_f(\text{CH}_2\text{NH}_2^+) = 176$ kcal/mole. The value of $I(\text{CH}_2\text{NH}_2)$ is then 6.25 eV.

A similar calculation for the reaction:



leads to $I((\text{CH}_3)_2\text{N}) = 9.45 \text{ eV}$, compared with a value of about 9.1 eV obtained by Gowenlock⁸.

It is possible to assess Foner's value for $I(\text{N}_2\text{H}_3)$ by calculation of the following differences in ionization potentials of isoelectronic radicals:

$$I(\text{NH}_2) \text{ (ref. 2)} - I(\text{CH}_3) \text{ (ref. 9)} = 11.4 - 9.85 = 1.55 \text{ eV}$$

$$I((\text{CH}_3)_2\text{N}) - I((\text{CH}_3)_2\text{CH}) \text{ (ref. 9)} = 9.45 - 7.9 = 1.55 \text{ eV}$$

$$I(\text{N}_2\text{H}_3) - I(\text{CH}_2\text{NH}_2) = 7.9 - 6.25 = 1.65 \text{ eV}$$

It can be seen that the differences between isoelectronic pairs are practically constant, indicating that Foner's value cannot be greatly in error.

Using our value of $A(\text{N}_2\text{H}_3^+) = 11.3 \text{ eV}$ and $I(\text{N}_2\text{H}_3) = 7.9 \text{ eV}$, a value of $D(\text{H}-\text{N}_2\text{H}_3) = 78 \text{ kcal/mole}$ is obtained.

Unless otherwise stated, thermodynamic values were obtained from the *National Bureau of Standards Circular 500*, Washington, 1952, and *JANAF Thermochemical Tables*, The Dow Chemical Company, Midland, Michigan, up to Quarterly Supp. No. 17, March 1965.

I. P. FISHER
G. A. HEATH

Ministry of Aviation,
Rocket Propulsion Establishment,
Westcott, Buckinghamshire.

¹ Dibeler, V. H., Franklin, J. L., and Reese, R. M., *J. Amer. Chem. Soc.*, **81**, 65 (1959); in *Advances in Mass Spectrometry*, edit. by Waldron, J. D., 443 (Pergamon Press, London, 1959).

² Foner, S. N., and Hudson, R. L., *J. Chem. Phys.*, **29**, 442 (1958).

³ Gray, P., and Thynne, J. C., *Trans. Farad. Soc.*, **60**, 1047 (1964).

⁴ Gray, P., and Thynne, J. C., *Trans. Farad. Soc.*, **59**, 2275 (1963).

⁵ Semenov, N. N., *Some Problems in Chemical Kinetics and Reactivity*, 1 (Pergamon Press, London, 1958).

⁶ Henchman, M. J. (private communication).

⁷ Collin, J., *Bull. Soc. Roy. Sci. Liège*, **21**, 446 (1952); *Bull. Soc. Chim. Belg.*, **62**, 411 (1953).

⁸ Gowenlock, B. G., Jones, P. P., and Majer, J. R., *Trans. Farad. Soc.*, **57**, 23 (1961).

⁹ Lossing, F. P., in *Mass Spectrometry*, edit. by McDowell, C. A. (McGraw-Hill Inc., New York, 1963).

Effect of pH and Addition of Organic Materials on Denitrification Losses from Soil

We have reported¹ that added nitrate-nitrogen was lost by denitrification during incubation of soil at moisture levels as low as 20 per cent saturation (20 per cent of the maximum water-holding capacity). For the soil used this saturation level was equivalent to $pF 4.2$, which is commonly taken as the soil moisture content at which wilting of plants occurs. This loss occurred even in the absence of added organic materials. The results do not agree with a previous report² that denitrification of added nitrate occurred only at moisture levels greater than 60 per cent saturation and then only when decomposable organic materials were added.

In view of the conflicting results it was decided to supplement the results of the previous study¹ by using the same soil and studying the effects of addition of organic materials on the extent of loss of added nitrate over the moisture range from the wilting percentage to waterlogging. In addition, pH was studied as a further variable, as this effect appears to have been studied, as yet, only under waterlogged conditions³.

Samples of the soil were adjusted to different pH levels by addition of varying amounts of calcium carbonate or aluminium chloride, followed by moist storage for 3 months with intermittent leaching. Samples having pH values of 4.7, 6.5 (the original soil) and 8.0 were selected for study. The methods used were as described previously¹, involving 12 weeks' incubation at 28°C after applying the treatments shown in Table 1. Losses of nitrogen were determined by the differences in total nitrogen content initially and at the end of incubation, with due precautions being taken to ensure complete recovery of all organic and inorganic forms of nitrogen in the samples.

Table 1. NITROGEN LOSSES IN SOIL DURING INCUBATION

	$pH 4.7$			$pH 6.5$			$pH 8.0$		
	% saturation	20	50	% saturation	20	50	% saturation	20	50
Percentage loss of applied nitrate-nitrogen									
$\text{NO}_3\text{-N}^\dagger$	2.5	4.4	56.9	10.3	21.7	82.4	2.0	24.5	71.6
" + straw [‡]	22.8	47.0	94.9	23.8	44.3	95.7	43.5	58.5	97.6
" + compost	31.2	29.7	60.8	24.4	48.0	96.1	9.5	40.2	88.9

L.S.D. ($P < 0.05$) = 5.0 per cent.

Each result is mean of duplicate treatments.

* 20 per cent, 50 per cent and 133 per cent saturation equivalent to $pF 4.2$, 2.5 and 0 (waterlogged) respectively.

† 600 p.p.m. (soil basis) as potassium nitrate dissolved in added water.

‡ Ground straw and compost added at 2 per cent w/w (dry soil basis).

The three results obtained in the previous study¹ are included in Table 1 for completeness, namely, losses at $pH 6.5$ at the three moisture levels in the absence of added organic material. In the absence of added organic material significant losses of nitrogen occurred at all pH levels under waterlogging, at $pH 6.5$ and 8.0 at 50 per cent saturation, and only at $pH 6.5$ at 20 per cent saturation. Losses due to addition of organic material (straw or compost) were increased by an average of 26 per cent at 20 per cent saturation, 28 per cent at 50 per cent saturation and 19 per cent under waterlogging. Losses of nitrogen due to addition of straw averaged 13 per cent more than those due to addition of compost, although the differences due to the two materials were consistent only at $pH 8.0$. In the presence of added organic materials nitrogen losses averaged 48, 55 and 56 per cent at $pH 4.7$, 6.5 and 8.0 respectively. With straw additions there were no consistent effects of pH on nitrogen losses at the different moisture levels. With compost additions the only consistent trend due to pH was at 20 per cent saturation, where nitrogen losses decreased with increasing pH .

In general, although greater losses of added nitrate-nitrogen occurred under waterlogging than at the two lower moisture levels, the addition of organic materials increased the extent of losses at all moisture levels, but proportionately more so at the two lower levels. The generally greater loss of nitrogen where straw than where compost was added is presumably due to the higher content of readily decomposable substances in the former material resulting in a greater supply of energy for the activity of the denitrifying organisms.

The high nitrogen losses even at $pH 4.7$ when waterlogged disagree with the results obtained by Bremner and Shaw², who found only small losses below $pH 5.0$. At any moisture level pH had no consistent effect on losses where organic materials were added. This may be related to the ability of denitrifying organisms to operate over a wide pH range³.

The results obtained indicate that losses of nitrogen by denitrification may occur in the field, where heavy applications of nitrate and organic materials are applied, even at low soil moisture levels, and that the losses may occur over a wide range of soil pH . Whether such losses would be of agronomic or economic significance in comparison with losses of nitrate by leaching can only be determined by field trials.

D. M. EKPETE
A. H. CORNFIELD

Department of Chemistry,
Imperial College of Science and Technology,
London, S.W.7.

¹ Ekpete, D. M., and Cornfield, A. H., *Nature*, **201**, 322 (1964).

² Bremner, J. M., and Shaw, K., *J. Agric. Sci.*, **51**, 22, 40 (1958).

³ Waksman, S. A., *Soil Microbiology* (J. Wiley and Sons, Inc., N.Y., 1952).

Absence of Diffusion Control of Reaction Rate in the Thermal Decomposition of Amorphous Polymers

ONE of the difficulties in the determination of decomposition kinetics of solids by dynamic thermogravimetry is the recognized dependence of the results on procedural variables, particularly sample size and rate of heating^{1,2}.

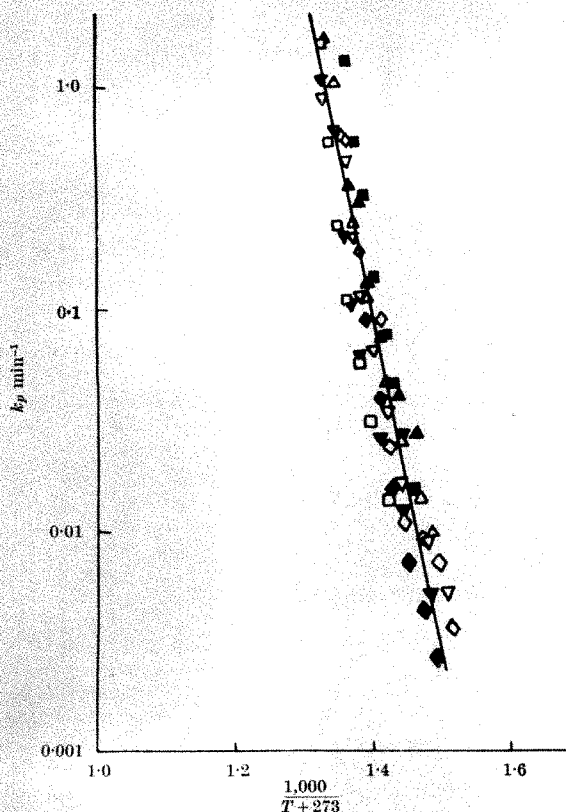


Fig. 1. Arrhenius plot for the thermal degradation of 'Viton A' rubber. Open symbols, 'Viton A', 100-0, mag. oxide 15-0, Diak No. 3, 3-0. Filled symbols, as follows, plus MT carbon black 25-0. (Each compound mould cured for 30 min at 150° C.) Δ , ∇ , 9-0° C min⁻¹/300 mg sample; ∇ , ∇ , 6-0/300; \square , 6-0/50; \diamond , 2-25/300

It is often postulated that slow diffusion of products from the reaction sites is a rate-controlling factor.

The decomposition of rubbers in a stream of inert gas (nitrogen) has been studied using techniques described in previous publications^{3,4}. The materials were mounted so as to permit free release of volatile decomposition products from the sample to the inert gas stream.

In contrast to published results of decomposition studies on crystalline solids, the overall kinetics of pyrolysis of amorphous polymers in the rubbery state do not appear to be influenced seriously by sample size or by heating rate within the ranges of these variables covered in the present study.

The rubbers examined have been ethylene-propylene terpolymers ('Nordel', 'Royalene'), chlorosulphonated polyethylenes ('Hypalon') and vinylidene fluoride-hexafluoropropylene copolymers ('Viton'). Heating rates have been varied between 2° and 10° C min⁻¹ and sample sizes from 20 to 300 mg and, in one experiment, a 1,000-mg single piece of 'EPT' rubber. Fig. 1 gives typical kinetic data for the main stage of decomposition of vulcanized 'Viton A'.

DEREK A. SMITH

National College of Rubber Technology,
Northern Polytechnic, Holloway, London.

¹ Newkirk, A. E., *Anal. Chem.*, **32**, 1558 (1960).

² Duval, C., *Inorganic Thermogravimetric Analysis*, second ed. (Elsevier, 1963).

³ Smith, D. A., *Anal. Chem.*, **35**, 1306 (1963).

⁴ Smith, D. A., *Trans. Inst. Rubber Ind.*, **39**, T275 (1963).

Kinetics of Formation of the Monoacetylacetonato-Iron (III) Complex

Few kinetic investigations on the formation of complexes with β -diketones are found in the literature. Taft and Cook examined the reaction of thenoyltrifluoroacetone with various metal ions and in particular that with Fe(III).

They found the rate-determining step is the reaction of Fe(III) with the enolate anion¹. Celiano and Gentile have investigated the kinetics of the reaction of acetylacetonone, benzoylacetone and its derivatives with Cu(II), and indicated that the rate-determining step is the reaction of Cu(II) with the enol form of the β -diketone and with its enolate anion². In an attempt to evaluate the two types of mechanisms proposed, we have examined the reaction of acetylacetonone, benzoylacetone, trifluoroacetylacetone and thenoyltrifluoroacetone with Fe(III)³. The behaviour is very similar in all cases and under our conditions the product is the mono-chelated complex, Fe(AA)²⁺. The results of experiments on the acetylacetonone/Fe(III) reaction are reported here.

The complex Fe(aca)²⁺ was reported to be formed from acetylacetonone (Haca) reacting with Fe(III) at pH=1.65–2.65 (ref. 4). To avoid the formation of Fe(aca)₂⁺ we have examined this reaction under conditions where the Fe(III)/Haca ratio was maintained at between 100/4 and 300/4, and high acid concentration (0.01–0.1 M). The kinetics were observed by a spectrophotometric method using the absorption band at 485 m μ which is λ_{\max} for Fe(aca)²⁺ (ref. 4). Good linear first-order plots were obtained. Some typical results are shown in Table 1.

Table 1

k_{obs} vs (Haca) (Haca)	$[\text{Fe}] = 2 \times 10^{-2} \text{ M}$, $[\text{H}^+] = 0.1 \text{ M}$, $\mu = 0.5$ $[\text{Fe}]/[\text{Haca}]$	$k_{\text{obs}} (\text{sec}^{-1}) \times 10^2$
1.0×10^{-4}	200	1.90
2.0×10^{-4}	100	1.93
3.0×10^{-4}	66.7	1.89
4.0×10^{-4}	50	1.89
5.0×10^{-4}	20	1.88

By varying the amounts of excess Fe(III) and acid concentration we have found that

$$k_{\text{obs}} = k_1 - \left[k_2 + k_3 \frac{1}{[\text{H}^+]} \right] [\text{Fe}]$$

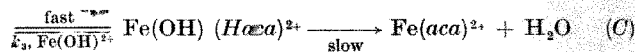
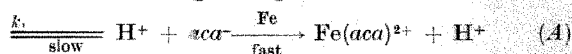
and so the overall rate expression becomes

$$\text{Rate} = k_1[\text{Haca}] - k_2[\text{Haca}][\text{Fe}] + k_3[\text{Haca}] \frac{[\text{Fe}]}{[\text{H}^+]}$$

The values of the k 's at 25° C and $\mu = 0.5$ are:

$$\begin{aligned} k_1 &= 1.22 \times 10^{-2} (\text{sec}^{-1}) \\ k_2 &= 0.26 (\text{sec}^{-1}\text{M}^{-1}) \\ k_3 &= 0.81 \times 10^{-2} (\text{sec}^{-1}) \end{aligned}$$

To interpret this equation we propose that three simultaneous reactions are operating,



If this scheme is correct the first term corresponds to the rate of acid dissociation of the β -diketone: the k_1 value corrected to zero ionic strength ($1.40 \times 10^{-2} \text{ sec}^{-1}$) agrees closely with that reported for such dissociations ($1.67 \times 10^{-2} \text{ sec}^{-1}$ (ref. 5a), $1.32 \times 10^{-2} \text{ sec}^{-1}$ (ref. 5b), $1.40 \times 10^{-2} \text{ sec}^{-1}$ (ref. 5c)).

The reactions (B) and (C) are reminiscent of the reactions of Fe(III) with anions such as SCN⁻, Cl⁻, etc.⁶. Negligible effects of ionic strength on k_2 and k_3 are observed.

The temperature variation of the rate constant has been measured, and the ΔH^\ddagger and ΔS^\ddagger values calculated from the plots of $\log k/T$ versus $1/T$ are shown in Table 2.

Table 2. TEMPERATURE DEPENDENCE OF k_1 , k_2 , k_3

	ΔH^\ddagger	ΔS^\ddagger
k_1	11.9 cal	-27.4 cal deg ⁻¹
k_2	11.7 "	-16.9 "
k_3	14.5 "	-15.1 "

This reaction has been used as an indicator for observing the solvolysis of $M(\text{aca})_3\text{FeCl}_4$, where $M = \text{Si, Ge}$ (ref. 3). These compounds have been shown to be 1:1 electrolytes

with octahedrally co-ordinated cations⁷. The solvolytic behaviour for $\text{Si}(\text{aca})_3\text{FeCl}_4$ and $\text{Si}(\text{aca})_3\text{HCl}_2$ is very similar. The rates are shown in Table 3.

Table 3. SOLVOLYSIS OF $M(\text{aca})_3^+$

	pH	k_{obs} (sec^{-1}) $\times 10^4$		Ref.
		25° C	35° C	
$\text{Si}(\text{aca})_3\text{FeCl}_4$	< 6	2.8	7.7	(3)
$\text{Ge}(\text{aca})_3\text{FeCl}_4$	< 6	2.7	6.4	(3)
$\text{Si}(\text{aca})_3\text{HCl}_2$	< 6	2.7	—	(8)

It is interesting that the solvolytic reactivity of the germanium compound is closely similar to that of silicon. This contrasts with the situation in the four co-ordinated halides in which Ge and Si can show wide differences⁹.

W. K. ONG
R. H. PRINCE

University Chemical Laboratory,
Cambridge.

¹ Taft, R. W., and Cook, E. H., *J. Amer. Chem. Soc.*, **81**, 46 (1959).

² Cellano, A. V., Gentile, P. S., et al., *J. Phys. Chem.*, **65**, 2194 (1961); **66**, 1132 (1962).

³ Ong, W. K., and Prince, R. H. (unpublished results).

⁴ Badoz-Lambling, J., *Ann. Chim. (France)*, **8**, 586 (1953).

⁵ (a) Pearson, R. G., and Dillon, R. L., *J. Amer. Chem. Soc.*, **75**, 2439 (1953).
(b) Bell, R. P., and Cook, S., *Proc. Roy. Soc., A*, **286**, 285 (1965). (c) Eigen, M., *Pure and Applied Chem.*, **6**, 97 (1963).

⁶ Connick, R. E., et al., *J. Amer. Chem. Soc.*, **80**, 2961 (1958); **81**, 6389 (1959).

⁷ Ong, W. K., and Prince, R. H., *J. Inorg. and Nucl. Chem.*, **27**, 1037 (1965).

⁸ Pearson, R. G., Edgington, D. N., and Basolo, F., *J. Amer. Chem. Soc.*, **84**, 3233 (1962).

⁹ Chipperfield, J. R., and Prince, R. H., *J. Chem. Soc.*, 3567 (1963).

Heterogeneity of Titan Yellow demonstrated by Thin-layer Chromatography

SINCE the first observations by Mikkelsen and Toth¹ it has been well established that the reaction between magnesium ions and titan yellow (the sodium salt of methylbenzothiazole (1,3)-4,4'-diazamino benzene (2,2')-disulphonic acid) can be extremely variable due to differences in the composition of the dye. In an attempt to overcome difficulties in the determination of magnesium in plant samples due to such variations Bradfield² recommended a technique for assessing the sensitivity of the dye and later reported the presence of a component which was intensely fluorescent³.

Although atomic absorption spectroscopy has to some extent supplanted the use of titan yellow for magnesium determinations there are many investigators who still rely on this procedure, and during some recent work in this laboratory difficulties were encountered with the determination of plant and soil magnesium employing the dye by published methods^{2,4}. Examination by thin-layer chromatography of some fourteen samples of titan yellow from three manufacturers showed them all to be much more heterogeneous than has previously been described.

Figs. 1 and 2 show a thin-layer chromatogram of a selection of the titan yellows examined from the three sources. The separation was effected on silica gel/alumina (6 g : 12 g in 36 ml. of 5 : 1 water/ethanol mixture) and developed for 6 h with *n*-butanol equilibrated with 1.5 N NH_4OH . The plate was spotted with 20 μl . of a solution containing 5 mg/ml. of dye and was photographed in ultra-violet light using a Kodak Plus X Pan film. The yellow component which is magnesium reactive had an R_F value of 0.25–0.40 with a mean of 0.32 and was the major fraction in samples B, E and H. Samples A and H 22, however, contained very little Mg^{++} -reactive material, but large amounts of yellow non-reactive substances were present which remained at the base line or moved much more slowly than the Mg^{++} -reactive dye. Sample M appeared to be a mixture containing many unreactive components but was also fairly rich in the active fraction. In addition to the multiple yellow fractions, all the samples of titan yellow which were examined contained a varying number of spots which exhibited brilliant blue and yellow-green fluorescence when viewed in ultra-violet light and

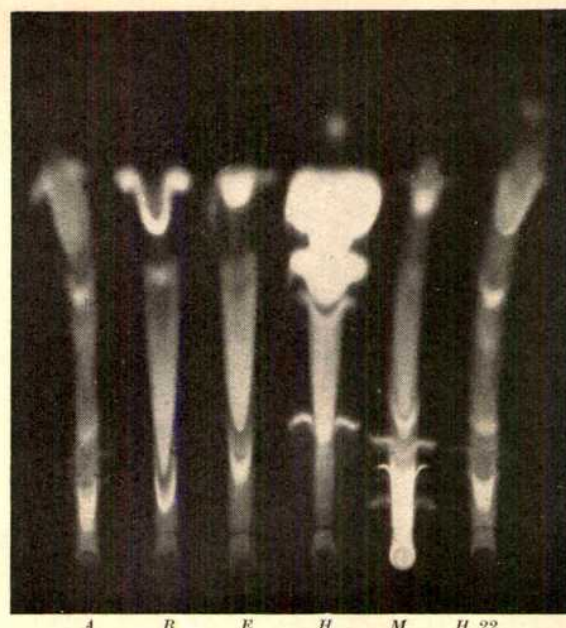


Fig. 1. Thin-layer chromatogram of titan yellows on silica gel/alumina viewed in ultra-violet light

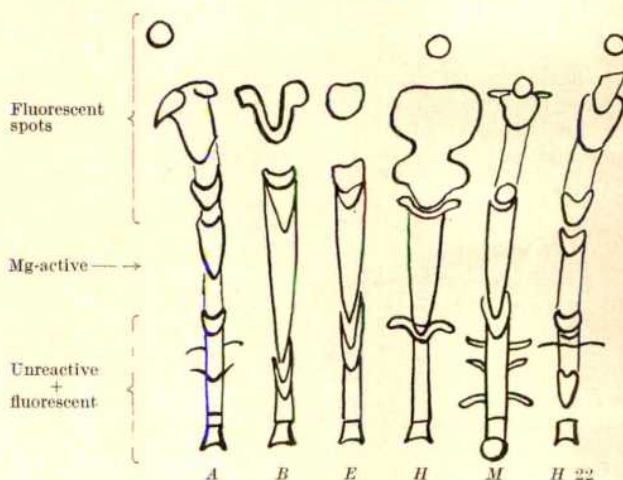


Fig. 2. Key to chromatogram shown in Fig. 1

which tended to travel much faster than the yellow components, although fluorescent spots and bands were seen in some samples interspersed with the main yellow spots. Evidence of the blue fluorescent substances was particularly striking in samples A, H and H 22. In H two large mushroom-shaped components were seen. These particular fluorescent spots were initially invisible to the eye in daylight but after several days' exposure became brown, presumably due to the formation of an oxidation product.

Although colour transparencies demonstrated the complexity of the chromatogram rather better than can be seen in Fig. 1, many more spots were seen in ultra-violet light with the naked eye.

Use has been made of these observations in selecting a titan yellow for magnesium determinations. Samples A, H, H 22 and M were found to be unsuitable for analytical procedures. Further work has now been carried out to establish the presence in all the samples of dye, of a fraction which gives a consistent reaction with magnesium, and the optimum conditions required. It has been possible to develop methods for the determination of total plant magnesium and available magnesium in soil extracts, the results of which show close agreement with those obtained by atomic absorption spectroscopy.

We thank Mr. J. H. White, regional entomologist, National Agricultural Advisory Service, Newcastle upon Tyne, for his help with the ultra-violet photography.

R. J. HALL
L. R. FLYNN

National Agricultural Advisory Service,
Ministry of Agriculture, Fisheries and Food,
Government Buildings,
Kenton Bar,
Newcastle upon Tyne, 3.

Mikkelsen, D. S., and Toth, S. J., *J. Amer. Soc. Agron.*, **39**, 185 (1947).

Bradfield, E. G., *Analyst*, **85**, 666 (1960).

Bradfield, E. G., *Anal. Chim. Acta*, **27**, 262 (1962).

Schachtschabel, P., *Z. Pflanzenernähr. Düng. Bodenk.*, **67**, 9 (1954).

Charge Transfer Properties of *dl*-Thioctic Acid and Other Disulphides

THE ability of organic sulphur compounds to act as donors with electron acceptor molecules is well established. As far as disulphides are concerned, previous studies have been limited to simple aliphatic compounds with iodine as the acceptor¹. In these systems, the charge transfer (CT) band appears between the disulphide absorption and the blue-shifted iodine absorption, and its position appears to be little affected by changing the alkyl groups. We wish to report that alkyl aryl and cyclic disulphides form 1:1 complexes in solution with tetracyanoethylene (TCNE), which show well-separated CT bands in the visible region. Further, the position of these bands clearly reflects steric and electronic factors in the disulphide molecule.

The complexes are readily generated by mixing rigorously purified solutions of the donor and acceptor. Special care must be taken to remove thiols, which are the major impurities in disulphides, since these react with TCNE. Table 1 lists some representative spectral data; several sulphides are included for comparison. It is apparent that (1) simple alkyl disulphides show a progressive decrease in CT transition energy with increasing chain length; (2) branched chain complexes absorb at longer wave-lengths than the corresponding straight chain complexes; (3) for disulphides, ring closure has a larger effect on the CT transition than elongation of the alkyl chain, while the opposite is the case for sulphides. The complexes have low formation constants (K_c , l./mole) and molar extinction coefficients (ϵ). In dichloromethane at 25° typical values are: methyl disulphide $K_c=0.16$, $\epsilon=2,100$; *tert*-butyl disulphide $K_c=0.36$, $\epsilon=4,500$; *dl*-thioctic acid $K_c=1.22$, $\epsilon=6,500$. The corresponding enthalpies of formation are -0.4 , -1.5 , and -7.2 kcal/mole. Based on the photoionization value of 8.46 eV for methyl disulphide², we estimate the ionization potentials of *tert*-butyl disulphide and *dl*-thioctic acid to be 7.78 and 7.53 eV, respectively. The general trend of these data may be rationalized in terms of the increased inductive (electron-releasing)-effect of the larger alkyl groups, and the decrease in the dihedral angle between the adjacent sulphur 3- $p\pi$ orbitals which accompanies an increase in the size of the group or ring closure³. Both factors would be expected to enhance the stability of the complex.

Apart from the CT band, the complexes show absorption arising from the complexed components. Resolution of the spectrum of a TCNE-*dl*-thioctic acid solution (Fig. 1) reveals that the band associated with the —S—S— group

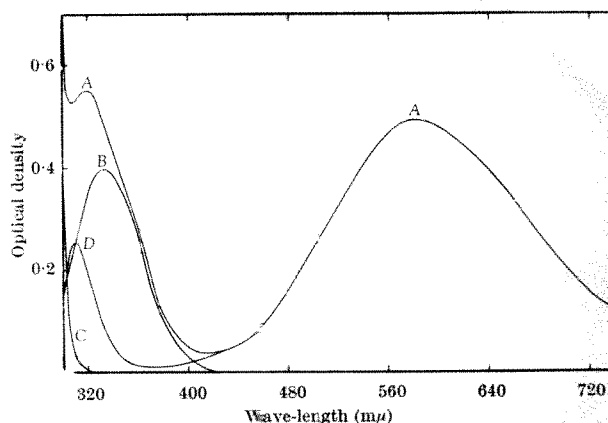


Fig. 1. Resolution of spectrum of dichloromethane solution, 2.786×10^{-2} M in *dl*-thioctic acid and 4.726×10^{-4} M in TCNE. A, Total absorption; B, free —S—S—; C, free TCNE; D, complexed —S—S—

of the complexed acid (curve D) is shifted to shorter wave-lengths by about 23 mμ, relative to that of the free acid (curve B). With TCNE-ethyl disulphide the component absorptions overlap, and the complexed —S—S— band cannot be clearly separated from the complexed TCNE band. The shift is estimated to be 0–6 mμ to longer wave-lengths. For ethyl disulphide, complexation can be envisaged to increase the dihedral angle¹ with consequent lowering of the transition energy. No such structural adaptation occurs with the rigid cyclic disulphide. Consideration of the orientation of the donor orbitals indicates that the two complexes would differ in geometry, particularly with respect to symmetry in the donor-acceptor bond region, and that consequently the perturbation of these orbitals would be different. A forthcoming publication will elaborate this concept.

This work was supported by the Air Force Cambridge Research Laboratories, Office of Aerospace Research, under contracts AF19(604)-7358 and AF19(628)-3836.

WAYNE M. MOREAU
KARL WEISS

Photochemistry and Spectroscopy Laboratory,
Northeastern University,
Boston, Massachusetts.

¹ McGlynn, S. P., Nag-Chaudhuri, J., and Good, M., *J. Amer. Chem. Soc.*, **84**, 9 (1962) (earlier references are cited).

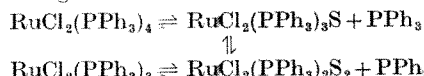
² Watanabe, K., Nakayama, T., and Mottl, J., *J. Quant. Spectrosc. Radiat. Transfer*, **2**, 369 (1962).

³ Bergson, G., *Arkiv Kemi*, **12**, 233 (1957).

Homogeneous Hydrogenation and Hydroformylation using Ruthenium Complexes

WE have shown¹ that the rhodium (I) complex, $\text{RhCl}(\text{PPh}_3)_3$, is an extremely efficient catalyst for the homogeneous hydrogenation of olefins and acetylenes at 25° C and at < 1 atmosphere pressure in benzene solutions. This activity is due, in part, to the dissociation of the complex in solution to give a solvated species $\text{RhCl}(\text{PPh}_3)_2\text{S}$, which has a vacant co-ordination site additional to the solvent-occupied axial positions.

We now find that the ruthenium (II) complexes, $\text{RuCl}_2(\text{PPh}_3)_4$ and $\text{RuCl}_2(\text{PPh}_3)_3$, prepared respectively by the interaction of cold and refluxing methanol solutions of hydrated ruthenium trichloride with excess triphenylphosphine, dissociate in benzene solutions². Molecular-weight investigations show that the dissociation is:



Since the solutions are sensitive to air, the colour changes reported³ for solutions of $\text{RuCl}_2(\text{PPh}_3)_3$ are probably attributable to oxidation.

Table 1. CHARGE TRANSFER MAXIMA OF TCNE-SULPHUR COMPOUND COMPLEXES IN DICHLOROMETHANE SOLUTION

Donor	λ_{max} (mμ)	Donor	λ_{max} (mμ)
Alkyl, aryl disulphides:		Cyclic disulphides:	
Methyl	425	Tetramethylene	535
Ethyl	450	Trimethylene	575
<i>n</i> -Propyl	453	<i>dl</i> -Thioctic acid	583
<i>n</i> -Butyl	460	Sulphides:	
<i>iso</i> -Propyl	465	Ethyl	505
<i>tert</i> -Butyl	530	<i>n</i> -Butyl	530
Cyclohexyl	525	Phenyl	580
Phenyl	515	Pentamethylene	495
Mesityl	430	Tetramethylene	515

Both olefines and acetylenes such as hept-1-ene and hex-1-yne are rapidly hydrogenated at 25° and at 1 atm. by the ruthenium complexes at about 10^{-3} M concentration in benzene-ethanol (1:1) solution. In contrast to the rhodium system, ethanol plays an intimate part in the hydrogenation mechanism; in absence of such a co-solvent, hydrogenation is exceedingly slow. Deuterium investigations, coupled with mass spectroscopic and nuclear magnetic resonance examinations show that: (a) mixed hydro-deutero paraffins are obtained using D_2 ; (b) in the absence of olefine, rapid exchange between the hydroxyl group of ethanol and D_2 occurs. Treatment of concentrated solutions of the complex in ethanol-benzene with H_2 gives a hydrido complex $RuClH(PPh_3)_3$, identified by analysis, infra-red ($\nu_{Ru-H} \sim 2,000\text{ cm}^{-1}$) and nuclear magnetic resonance ($\tau_H = 28.6$) spectroscopy, so that, as before¹, a hydrido species is confirmed as a hydrogenation intermediate.

The rhodium complex $RhCl(PPh_3)_3$ with CO rapidly gives $RhClCO(PPh_3)_2$ and the latter is an effective hydroformylation catalyst for olefines and acetylenes^{1,4}. Although the tetrakis(triphenylphosphine) ruthenium complex gives only low yields of aldehyde from, for example, hept-1-ene (due to the formation of an insoluble carbonyl species $RuCl_2(CO)_2(PPh_3)_2$) the more-soluble complex $RuCl_2(PPh_3)_2CH_3OH$ gives higher yields. However, the most effective ruthenium complex is $Ru(CO)_2(PPh_3)_2$ (ref. 5), which in benzene solution at about 10^{-2} M concentration under mild conditions, for example, 100 atm. total pressure of CO and H_2 (1:1) and 100° C, gives more than 80 per cent yields of hexaldehydes from pent-1-ene, in about 15 h. This appears to be the first case where a well-defined ruthenium complex has been shown to be a hydroformylation catalyst. There have been patents claiming the use of solid catalysts⁶ and a very general claim⁷ covering the use of transition metals in the presence of phosphines, phosphites, etc., but no examples using ruthenium or rhodium were quoted, nor were any stoichiometric complexes of these elements defined.

D. EVANS
J. A. OSBORN
F. H. JARDINE
G. WILKINSON

Inorganic Chemistry Research Laboratories,
Imperial College of Science and Technology,
London, S.W.7.

¹ Young, J. F., Osborn, J. A., Jardine, F. H., and Wilkinson, G., *Chem. Comm.*, 131 (1965). Jardine, F. H., Osborn, J. A., Young, J. F., and Wilkinson, G., *Chem. and Indust.*, 560 (1965).

² Stephenson, T. A., and Wilkinson, G., *J. Inorg. Nucl. Chem.* (in the press).

³ La Placa, S. J., and Ibers, J. A., *Inorg. Chem.*, 4, 778 (1965).

⁴ Osborn, J. A., Wilkinson, G., and Young, J. F., *Chem. Comm.*, 17 (1965).

⁵ Collman, J. P., and Roper, W. R., *J. Amer. Chem. Soc.*, 87, 4008 (1965).

⁶ British Patent 966,482.

⁷ British Patent 988,941.

BIOCHEMISTRY

Lysozymes characterized in Duck Egg-white: Isolation of a Histidine-less Lysozyme

CONTINUING our work on lysozymes from different origins (human; avian egg-white)¹, we have purified, analysed and compared different lysozymes isolated from the duck egg-white.

One l. of duck egg-white was homogenized with 4 l. of water; the pH was adjusted to 4.5 and the solution was mixed for 4 h with 200 ml. of 'Amberlite CG-50 (Type II)' buffered at pH 6.5 with a 0.2 M phosphate buffer. The purification was continued following the procedure of Jollès *et al.*²: elution from 'Amberlite' with a 0.8 M phosphate buffer of pH 6.5; chromatography on CM-cellulose, filtration on 'Sephadex G-25' with water as eluent, and lyophilization. Yield at this stage was about 500 mg of lysozyme (expressed as hen's egg-white lysozyme). It was possible to purify this material further by ion-

exchange chromatography on 'Amberlite CG-50 (Type II)' columns buffered with a 0.2 M phosphate buffer of pH 7.56. Fig. 1 shows that three different lytic enzymes can be obtained in this way, containing respectively 25, 25 and 50 per cent of the initial activity. However, peak No. 3 was not eluted during the ion-exchange chromatography but only with a 0.8 M buffer solution. This lysozyme was desalted again on 'Sephadex G-25' and submitted to a new chromatography in slightly different conditions as indicated in Fig. 2. The purity of these three lysozymes obtained in a chromatographically pure state had been tested again by electrophoresis on 'Cellolog' strips (Chemetron, Milan) (0.1 M acetate buffer of pH 4.5; 110 V; 1.5 h) (Fig. 3). The third active fraction had also been purified by a different procedure by Fujio *et al.*³, but no further analytical investigations were reported by these authors.

Amino-acid analyses were performed after total hydrolyses (6 N hydrochloric acid; 110°; sealed tube) of 18 and 40 h with a Technicon 'AutoAnalyzer'. Cystine was determined after performic acid oxidation and tryptophan following the procedure of Spies and Chambers⁴. As can be seen from Table 1, peak 1 on one side, peaks 2 and 3 on the other, contain analytically quite different substances; the lysozymes from peaks 2 and 3 contain no histidine; it is the first lysozyme devoid of this amino-acid, and this observation seems important, as many investigations were

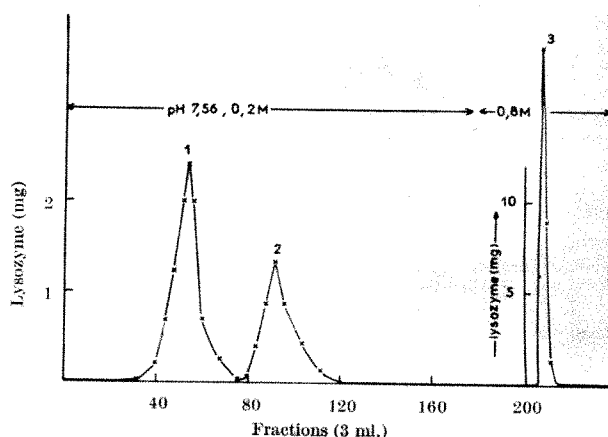


Fig. 1. Ion-exchange chromatography on 'Amberlite CG-50' (67 cm x 1.5 cm) of duck egg-white lysozymes No. 1 and No. 2 (initial activity: 100 mg).

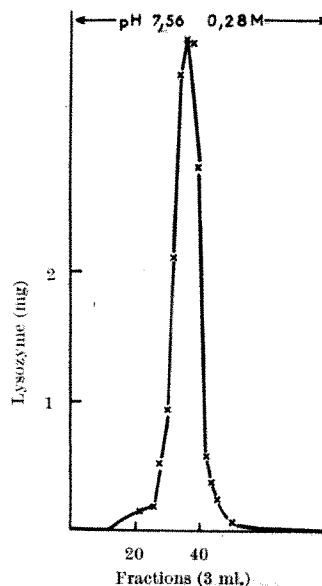


Fig. 2. Ion-exchange chromatography on 'Amberlite CG-50' (67 cm x 1 cm) of duck egg-white lysozyme No. 3 (39 mg).

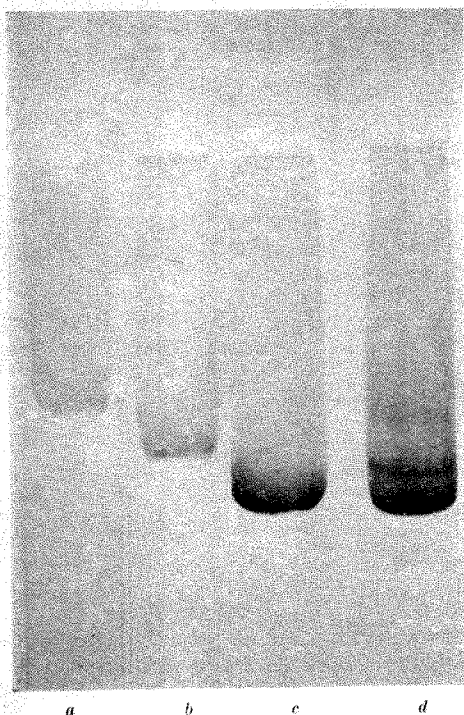


Fig. 3. Electrophoresis on 'Cellolog' strips of duck egg-white lysozymes. a, peak No. 1; b, peak No. 2; c, peak No. 3; d, peaks No. 1 + No. 2 + No. 3 as contained in the material obtained after chromatography on CM-cellulose

devoted to the possible part played by histidine in an eventual active centre⁸⁻⁹. The different chromatographic behaviour of the enzymes contained in peaks 2 and 3 may be due to a different amide-group content.

Digestion experiments with carboxypeptidase allowed to establish that leucine is the C-terminal amino-acid of the duck egg-white lysozyme contained in peak 3.

The new lytic enzymes possess the six properties which allow them to be ranged among the lysozymes^{1,10}: they are basic proteins of low molecular weight, stable at acidic pH even at 100° for 1-2 min, unstable at alkaline

pH, active against suspensions of *M. lysodeikticus* and liberating, by their action on an appropriate substrate, substances which can be detected by specific reagents for amino sugars.

Table 2 summarizes some results obtained during our comparative examination of duck lysozymes, two other birds' egg-white and three human lysozymes^{1,11-14}.

This investigation was supported in part by grant FG-Fr-106 from the U.S. Department of Agriculture.

J. JOLLÈS
G. SPOTORNO
P. JOLLÈS

Laboratory of Biochemistry,
Faculty of Science,
96 Bd. Raspail, Paris 6°.

- ¹ Jollès, P., *Angew. Chem.*, **73**, 20 (1964); *Angew. Chem. (Intern. Ed.)*, **3**, 28 (1964).
- ² Jollès, P., Zowall, H., Jauregui-Adell, J., and Jollès, J., *J. Chromatog.*, **8**, 363 (1962).
- ³ Fujio, H., Saiki, Y., Imanisai, M., Shinka, S., and Amano, T., *Biken's J.*, **5**, 201 (1962).
- ⁴ Spies, J. R., and Chambers, D. C., *Anal. Chem.*, **21**, 1249 (1949).
- ⁵ Jauregui-Adell, J., and Jollès, P., *Bull. Soc. Chim. Biol.*, **46**, 141 (1964).
- ⁶ Horinishi, H., Hachimori, Y., Kurihara, K., and Shibota, K., *Biochim. Biophys. Acta*, **86**, 477 (1964).
- ⁷ Kravchenko, N. A., Kleopina, G. V., and Kaverzneva, E. D., *Biochim. Biophys. Acta*, **92**, 412 (1964).
- ⁸ Jollès, J., Jauregui-Adell, J., and Jollès, P., Paper presented at the Third Intern. Symp. Fleming's Lysozyme, Milan (April 3-5, 1964).
- ⁹ Johnson, L. N., and Phillips, D. C., *Nature*, **206**, 761 (1965).
- ¹⁰ Jollès, P., *Biochim. Biophys. Acta*, **69**, 505 (1963).
- ¹¹ Schellman, C. G., and Jollès, P. (unpublished results).
- ¹² Jollès, P., and Jollès, J., *Nature*, **192**, 1187 (1961).
- ¹³ Petit, J. F., Panigel, M., and Jollès, P., *Bull. Soc. Chim. Biol.*, **45**, 211 (1963).
- ¹⁴ Petit, J. F., and Jollès, P., *Nature*, **200**, 168 (1963).

Evidence discriminating between the Chemical and the Chemiosmotic Mechanisms of Electron Transport Phosphorylation

WE have recently reported that when suspensions of intact rat liver mitochondria hydrolyse ATP, oxidize succinate, or oxidize β -hydroxybutyrate, protons are translocated (written $\rightarrow H^+$) outwards through the mitochondrial membrane with stoichiometries closely approximating to $\rightarrow H^+/P=2$, $\rightarrow H^+/O=4$ and $\rightarrow H^+/O=6$ respectively¹. These observations are in close accord with the requirements of the chemiosmotic hypothesis, according to which electron transport and phosphorylation are coupled by the proton current flowing cyclically through the respiratory chain and reversible ATPase systems across the cristae membrane²; but they could also be explained according to the chemical hypothesis, by invoking a proton pump driven by hydrolysis of $X \sim I$, either directly, or via the 'W complex' suggested by Lardy, Connelly and Johnson³. We have, therefore, sought more crucial experimental tests of the alternative hypotheses.

The fact that fragments of cristae membrane (sonic particles), segregated from sonically disintegrated mitochondria, will catalyse oxidative phosphorylation⁴, energy-linked transhydrogenation⁵, and other reversed electron-transfer reactions⁶ appears to favour the chemical hypothesis, because: (a) the system still works chemically though apparently disorganized morphologically; (b) the membraneous (or other) barrier confining soluble enzymes, co-enzymes and substrates within intact mitochondria, and preventing intermediates such as NAD and NADP from participating in 'energy-linked' electron transport from outside, have apparently disappeared from the sonic particles. It seemed to us that in view of the facts concerning sonic particles, the survival of the chemiosmotic hypothesis would be dependent on the following two rather exacting criteria: (1) the limiting membrane of the sonic particles must be osmotically functional; (2) the membrane of the particles must be inside out so that the side normally accessible only to internal NAD, ATP, substrates, etc., would be directly accessible to NAD, ATP, substrates, etc., in the suspension medium. We have

Table 1. AMINO-ACID COMPOSITION OF DUCK EGG-WHITE LYSOZYMES (RESIDUE/MOLE AFTER 18 h AND 40 h TOTAL HYDROLYSIS)

Amino-acid	Peak 1		Peak 2		Peak 3	
	18 h	40 h	18 h	40 h	18 h	40 h
CysO ₂ H	6.7	n.d.	7.0	n.d.	7.7	n.d.
Asp	19.3	18.35	17.6	15.5	17.2	16.4
Thr	7.35	6.45	5.9	5.5	6.4	6.0
Ser	9.9	8.7	8.6	7.4	9.4	7.25
Glu	5.4	5.0	5.1	4.8	5.0	4.5
Pro	2.9	2.7	2.0	1.6	2.1	1.8
Gly	12.4	11.8	11.4	11.0	11.9	11.8
Ala	11.0	10.85	10.3	9.6	10.4	9.6
Val	4.9	5.7	6.05	6.45	6.1	5.5
Met	1.4	0.95	1.15	0.7	1.4	1.0
Ileu	4.5	4.5	4.7	5.6	4.6	5.0
Leu	7.4	7.8	7.45	8.0	8.1	7.8
Tyr	2.7	2.3	3.65	3.0	4.1	4.3
Phe	3.0	2.9	0.8	1.1	1.0	1.0
Try	6.0	n.d.	5.6	n.d.	5.6	n.d.
Lys	6.1	6.0	5.4	5.0	5.7	5.3
His	1.1	0.85	0	0	0	0
Arg	10.8	10.0	11.5	11.2	12.7	13.0

n.d., not determined.

Table 2. COMPARATIVE DATA CONCERNING SOME LYSOZYMES

Lysozyme	% of initial ion-exchange chromatography on 'Amberlite'		No. amino-acids	C-terminal amino-acid
	activity	pH		
Duck egg-white	Peak 1: 25	7.56	0.20	130 \pm 5
	Peak 2: 25	7.56	0.20	125 \pm 5
	Peak 3: 50	7.56	0.28	125 \pm 5
Hen egg-white ¹	95 \pm 5*	7.18	0.20	129
Turkey egg-white ¹¹		7.47	0.20	125 \pm 5
Human lysozymes ^{1,12-14}				
(milk, saliva, placenta, etc.)	95 \pm 5*	6.98	0.20	122 \pm 5

* A small active peak (≤ 10 per cent of total activity) is eluted before the main peak; it contains probably the same enzyme partially deaminated.
n.d., not determined.

examined these criteria experimentally in sonic particles⁷ from rat liver and from beef heart mitochondria, using techniques similar to those reported on intact rat liver mitochondria¹; these preliminary investigations can be summarized as follows:

(1) The sonic particles behave as a two-phase system when titrated with acid or alkali, and the rate of acid-base equilibration between the internal and external phases is of the same order of magnitude as the rate observed in intact mitochondria.

(2) When the sonic particles hydrolyse ATP, oxidize succinate, or oxidize NADH, protons are translocated inwards through the membrane which separates the inner phase of the particles from the suspension medium, the stoichiometries so far obtained, under conditions that are probably sub-optimal, being respectively $\rightarrow H^+/P=0.53$, $\rightarrow H^+/O=0.83$, and $\rightarrow H^+/O=1.29$. Proton translocation during ATP hydrolysis could be inhibited by oligomycin but not by antimycin A or rotenone, whereas proton translocation during succinate and NADH oxidation was stimulated (about 25 per cent) by oligomycin and inhibited by antimycin A. Rotenone inhibited the NADH-linked proton translocation, but not the succinate-linked proton translocation.

We conclude that the sonic particles possess an osmotically functional membrane, and that, as suggested by Lee and Ernster⁸ in connexion with the effects of Mg^{++} on reversed electron transport, the cristae membrane is effectively turned inside out during sonic disintegration of mitochondria.

Our observations imply that the facts relating to the sonic particles can no longer be regarded as favouring the chemical hypothesis of electron transport phosphorylation. On the other hand, the proponents of the chemical hypothesis could explain the proton translocation in sonic particles, just as in intact mitochondria, by invoking a hypothetical membrane-located proton pump driven by hydrolysis of $X \sim I$.

According to the chemiosmotic hypothesis, protons are translocated across the membrane during oxido-reduction because hydrogen atoms pass across one way via hydrogen carriers and electrons pass across the other way via electron carriers in the membrane-located respiratory chain²; and it follows that the direction of translocation of protons should reverse if electron and hydrogen atom flow reverses. According to the explanation of proton translocation in terms of the chemical hypothesis, however, protons would be pumped through the membrane when forward electron transport synthesized $X \sim I$ (via $C_1 \sim I$, $C_2 \sim I$, etc.); but although, according to this view, the reversal of electron transport would consume $X \sim I$, it would not reverse the flow of protons through the $X \sim I$ -hydrolysing proton pump unless either $X \sim I$ could exist in minus quantities, or the proton pump could become possessed by a Maxwell demon, reversing the thermodynamically natural processes and synthesizing $X \sim I$ from XH , IOH , and H_2O . It is thus evident that the relationship between the change of direction of electron flow and the direction of proton translocation should be diagnostic of the coupling mechanism.

We have observed, by techniques similar to those recently described¹, that the flow of electrons in the reverse, or 'energy-consuming', direction through the transhydrogenase system of sonic particles from beef heart mitochondria (prepared by a method similar to that of Löw and Vallin⁷) causes the outward translocation of protons through the membrane, whereas the protons are translocated inwards during forward electron transport and ATP hydrolysis by these particles. Our present techniques give an observed $\rightarrow H^+/2e$ stoichiometry of 0.21, which, like the forward stoichiometries, can be assumed to be sub-optimal. Further, we have found that reversed electron transport through the succinate oxidase segment of the respiratory chain of beef heart mitochondria between fumarate and the component reacting with

artificial hydrogen donors on the oxygen side of the antimycin A-sensitive site is accompanied by reversed proton translocation.

These observations, which will be reported in detail elsewhere, appear to provide the first crucial evidence against the chemical hypothesis of electron transport phosphorylation. They also provide the most powerful evidence so far obtained in support of the chemiosmotic hypothesis.

PETER MITCHELL
JENNIFER MOYLE

Glynn Research Laboratories,
Bodmin, Cornwall.

¹ Mitchell, P., and Moyle, J., *Nature*, **208**, 147 (1965).

² Mitchell, P., *Nature*, **191**, 144 (1961). Mitchell, P., *Biochem. Soc. Symp.*, **22**, 142 (1962). Mitchell, P., in *Regulation of Metabolic Processes in Mitochondria*, edit. by Quagliariello, E., Slater, E. C., Papa, S., and Tager, J. M., 7, 65 (B.B.A. Library, Elsevier, 1966).

³ Lardy, H. A., Connelly, J. L., and Johnson, D., *Biochemistry*, **3**, 1961 (1964).

⁴ Racker, E., *Adv. Enzymol.*, **23**, 323 (1961).

⁵ Lee, C. P., and Ernster, L., in *Regulation of Metabolic Processes in Mitochondria*, edit. by Quagliariello, E., Slater, E. C., Papa, S., and Tager, J. M., 7 (B.B.A. Library, Elsevier, 1966).

⁶ Löw, H., Vallin, I., and Alm, B., in *Energy-Linked Functions of Mitochondria*, edit. by Chance, B., 5 (Academic Press, 1963).

⁷ Löw, H., and Vallin, I., *Biochim. Biophys. Acta*, **69**, 361 (1963).

Enzymatic Formation of β -Cyanoalanine from Cyanide by *Escherichia coli* Extracts

BLUMENTHAL-GOLDSCHMIDT *et al.*¹ demonstrated that carbon-14 atoms from ^{14}C -labelled HCN supplied to several types of seedling became incorporated almost exclusively into the amide-C of asparagine. Subsequent work^{2,3} indicated that these authors had selected, somewhat fortuitously, mainly asparagine-synthesizing species rather than others which incorporated ^{14}C -cyanide almost entirely into a dipeptide, γ -glutamyl- β -cyanoalanine. This γ -glutamyl peptide is known as a normal constituent only of certain *Vicia* species^{4,5}; its formation in other species is apparently dependent on an exogenous supply of cyanide.

The time-course of ^{14}C -cyanide incorporation by *Chlorella* cultures indicated that β -cyanoalanine was the primary labelled product³; this is afterwards converted either into asparagine by the action of a hydrolysing nitrilase or into γ -glutamyl- β -cyanoalanine by a γ -glutamyl transferring enzyme. These alternative pathways from β -cyanoalanine adequately explain the labelling patterns that develop after ^{14}C -cyanide feeding to a wide variety of species including cultures of *E. coli*, which accumulate radioactivity mainly in asparagine.

Nigam and Ressler⁶ suggested that β -cyanoalanine was formed by a reaction between serine and cyanide, after observing that cyanide stimulated the incorporation of radioactivity into γ -glutamyl- β -cyanoalanine in intact *V. sativa* seedlings that had received ^{14}C -serine. The present communication describes some properties of an enzyme, present in *E. coli* extracts, which catalyses β -cyanoalanine formation from serine and cyanide, while the accompanying letter⁷ presents similar data for an enzyme from *Lotus* seedlings. Since β -cyanoalanine and γ -glutamyl- β -cyanoalanine are not encountered generally in plants, we consider it likely that the catalysis of β -cyanoalanine formation represents a non-specific activity of an enzyme that plays some other indispensable part in living cells. Such an enzyme could be serine sulphydrase⁸, which promotes cysteine biosynthesis from serine and sulphide.

Enzyme preparations were obtained from cells of *E. coli* (strain K12) growing logarithmically on a glucose-mineral salts medium at 30° C. Heavy suspensions of washed cells were disintegrated ultrasonically and the cell-free supernatant obtained after centrifuging at 40,000g for 30 min was dialysed overnight at 1° C against 0.1 M K_2HPO_4 . This dialysed extract (containing 25–50 mg protein/ml.) was used as a source of enzyme. Activities

were measured by following the incorporation of radioactivity into β -cyanoalanine in systems normally containing L-(3- ^{14}C) serine as the labelled substrate. Unlabelled β -cyanoalanine was added to the assay system in amounts sufficient to reduce to negligible proportions the further conversion of the labelled β -cyanoalanine produced into asparagine.

The standard assay mixture had the following composition: serine, 0.5 $\mu\text{c.}$, 11 $\mu\text{g.}$; KCN, 500 $\mu\text{g.}$; β -cyanoalanine, 25 $\mu\text{g.}$; enzyme preparation, 0.1 ml.; total volume, 0.12 ml. Mixtures were incubated at 30° C for various periods and reaction was stopped by the addition of two volumes of ethanol. After the removal of precipitated protein, the supernatant was applied to two-dimensional chromatograms (developed in phenol-ammonia followed by butan-1-ol-acetic acid-water) to separate β -cyanoalanine from other ^{14}C -labelled substances before determination of radioactivity by Geiger scanning. Under these conditions, a pH of 9.0 was optimal for β -cyanoalanine formation. The high concentrations of cyanide present in the standard assay mixture did not fully saturate the enzyme, since a further small increase of rate was observed with amounts of KCN up to 800 $\mu\text{g.}$ (that is, a concentration of about 0.1 M). The K_m value calculated for KCN was about 4×10^{-2} M; this value corresponded to a K_m value of about 1.3×10^{-2} M for cyanide ion.

The dependence of reaction rate on serine concentration was investigated using potassium ^{14}C -cyanide (5 $\mu\text{c.}$, 500 $\mu\text{g.}$) as substrate: serine concentrations in the range 10^{-4} – 7×10^{-2} M were used. The reaction attained its limiting velocity at a serine concentration of 4×10^{-2} M and a K_m value of 8×10^{-3} M was calculated. The *E. coli* enzyme (unlike that from *Lotus* seedling⁷) exhibited a partial dependence on ATP. Fig. 1 illustrates the stimulation of β -cyanoalanine formation by ATP: a four-fold increase of reaction rate was observed in the presence of ATP concentrations greater than 0.01 M. Schlossmann and Lynen⁸ state that serine sulphydrase is ATP-dependent.

The effect of other additions to the standard assay mixture is illustrated by the data in Table 1. The presence of Mg^{2+} or *O*-phosphorylserine resulted in a striking inhibition of radioactive β -cyanoalanine formation (compare with the findings using the *Lotus* enzyme). Since ATP stimulated the reaction, phosphorylserine seemed at first to be a logical intermediate in β -cyanoalanine formation. A biogenetic mechanism of this type would be associated with the incorporation of radioactivity into the phosphoryl derivative, but in practice phosphorylserine remained unlabelled in our experiments. However, it was hydrolysed rapidly, thereby reducing the specific activity

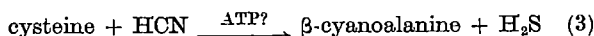
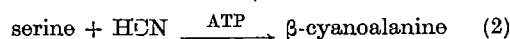
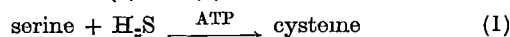
Table 1. SHOWING THE COMPARATIVE RATES OF β -CYANOALANINE FORMATION Determined after the addition of various substances to the standard assay mixture containing L-(3- ^{14}C) serine, 0.5 $\mu\text{c.}$, 11 $\mu\text{g.}$; KCN, 500 $\mu\text{g.}$; β -cyanoalanine, 25 $\mu\text{g.}$; enzyme preparation, 0.1 ml., in a final volume of 0.12 ml

	Per cent of control rate
Standard assay mixture (control)	100
- Cyanide	< 0.1
+ Mg^{2+} (30 mM)	22
+ <i>O</i> -phosphorylserine (8 mM)	26
+ L-Alanine (16 mM)	109
+ L-Threonine (12 mM)	103
+ ATP (10 mM)	434
+ L-Cysteine (4 mM)	5-10
+ Sodium sulphide (2 mM)	4
+ Sodium sulphide (0.5 mM)	~ 80

of the ^{14}C -serine in the assay mixture and so causing an apparent reduction in reaction rate. Neither alanine nor threonine affected the rate of β -cyanoalanine production and so they did not compete as alternative substrates for the active sites of the enzyme.

The addition of cysteine caused a most pronounced reduction in the conversion of ^{14}C -serine into β -cyanoalanine. This observation suggested that cysteine also may act as a substrate for enzymatic formation of β -cyanoalanine. Confirmation was obtained in an experiment in which labelled β -cyanoalanine was formed from ^{14}C -cyanide (5 $\mu\text{c.}$, 500 $\mu\text{g.}$) and cysteine; the K_m value determined for cysteine was approximately 5×10^{-3} M. The presence of sulphide ion also resulted in a striking reduction of β -cyanoalanine formation. Under these conditions radioactivity was introduced into a sulphur-containing amino-acid (presumably cysteine) because ^{14}C -label was detected in cysteic acid present in mixtures that had been oxidized at the end of the reaction period.

If serine sulphydrase, which catalyses reaction (1), can utilize a wider range of substrates than has been appreciated so far, then our observations can be explained in terms of the non-specific action of this enzyme in catalysing reactions (2) and (3).



Most of the experiments described have been aimed at examining reaction (2), but those involving cysteine suggested that this amino-acid can undergo a facile reaction with cyanide as in reaction (3).

PATRICIA M. DUNNILL
L. FOWDEN

Department of Botany,
University College,
London, W.C.1.

- Blumenthal-Goldschmidt, S., Butler, G. W., and Conn, E. E., *Nature*, **197**, 718 (1963).
- Ressler, C., Giza, Y.-H., and Nigam, S. N., *J. Amer. Chem. Soc.*, **85**, 2874 (1963).
- Fowden, L., and Bell, E. A., *Nature*, **206**, 110 (1965).
- Ressler, C., Nigam, S. N., Giza, Y.-H., and Nelson, J., *J. Amer. Chem. Soc.*, **85**, 3311 (1963).
- Bell, E. A., and Tirimanna, A. S. L., *Biochem. J.* (in the press).
- Nigam, S. N., and Ressler, C., *Abst. Sixth Intern. Congr. Biochem.*, New York, 222 (1964).
- Floss, H., and Conn, E. E., *Nature* (following communication).
- Schlossmann, K., and Lynen, F., *Biochem. Z.*, **323**, 591 (1957).

Enzymatic Formation of β -Cyanoalanine from Cyanide

IN 1963 Blumenthal-Goldschmidt *et al.*¹ demonstrated the incorporation of ^{14}C -labelled hydrogen cyanide into asparagine by seedlings of a number of plant species. They showed that the label from H^{14}CN almost exclusively entered the amide carbon of asparagine and obtained preliminary evidence that the other three carbon atoms might be provided by serine or a closely related metabolite. These findings have been confirmed and extended by Tschiersch^{2,3}, Ressler *et al.*^{4,5} and by Fowden and Bell⁶.

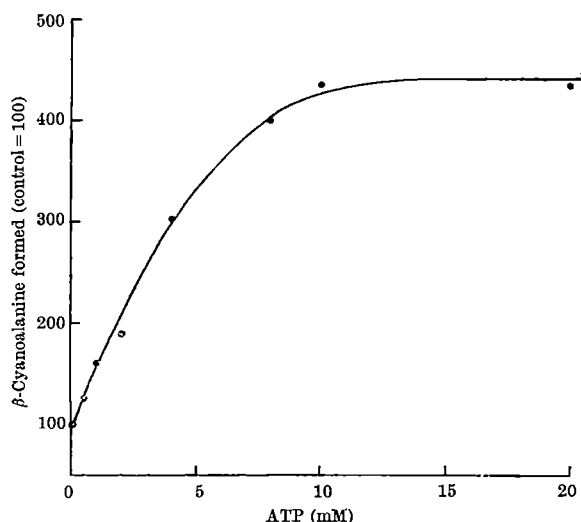


Fig. 1. Effect of ATP formation in the standard assay mixture containing *E. coli* enzyme

The available evidence suggests that HCN reacts with serine to form β -cyanoalanine which then, depending on the plant species used, can either be hydrolysed to asparagine or can be converted into a peptide, γ -glutamyl- β -cyanoalanine. The two latter reactions have been demonstrated in extracts of seedlings⁶, but enzymatic evidence for the first reactions has so far not been reported.

The enzymatic formation of β -cyanoalanine has now been observed by incubating homogenates of *Lotus tenuis* seedlings with $H^{14}CN$ and L-serine. The reaction was followed by measuring the incorporation of ^{14}C from $K^{14}CN$ into β -cyanoalanine. Differential centrifugation of homogenates prepared in the presence of 0.4 M sucrose showed the enzyme to be present mainly in the mitochondria, with some activity in the supernatant solution obtained at 105,000g. The enzyme could be rendered soluble by preparing acetone powders either of whole seedlings or the mitochondria. With $H^{14}CN$ and serine as substrates, the activity has been precipitated from extracts of acetone powders of whole seedlings between 35 and 50 per cent saturation with ammonium sulphate at pH 7.3 and subjected to a heat treatment (10 min at 50°). This preliminary purification gave a five-fold increase in the specific activity.

Both ^{14}C -labelled cyanide and ^{14}C -labelled serine have been used as substrates. On incubation with $K^{14}CN$, 96 per cent of the radioactivity of β -cyanoalanine was found to be located in C-4, while with L-serine- $U^{14}C$, C-4 had 1 per cent, C-1 37 per cent and the two centre carbons had 62 per cent of the radioactivity of cyanoalanine. As shown in Table 1, however, the rate of β -cyanoalanine formation from $H^{14}CN$ and cysteine is about 50 times as rapid as from $H^{14}CN$ and serine. Under identical conditions, for example, 1.80 μM of cyanoalanine were formed without added serine, 2.75 μM on addition of 2.5×10^{-3} M L-serine but 98.0 μM in the presence of 2.5×10^{-3} M L-cysteine. The fact that cysteine apparently can serve as a source of carbon atoms 1, 2 and 3 of β -cyanoalanine was confirmed by the use of cysteine- $1^{14}C$, which gave rise to ^{14}C -labelled β -cyanoalanine carrying most of its label in the carboxyl group. The formation of β -cyanoalanine in the intact plant thus may involve cysteine rather than serine as a 3-carbon precursor, a possibility which so far seemingly has not been investigated by feeding experiments.

Table 1. EFFECT OF POSSIBLE CO-FACTORS AND INHIBITORS ON ENZYMATIC FORMATION OF β -CYANOALANINE

Reaction mixture: enzyme 1.0 ml., potassium phosphate pH 7.3, 70 μ moles, L-serine, 5 μ moles; $K^{14}CN$, 5 μ c. = 0.5 μ moles; final volume 2.0 ml. Incubations were run for 2 h at 33°

	μ moles Cyanoalanine formed	Per cent of controls
Control	2.75	100
- Serine	1.80	66
- Serine + 2.5×10^{-3} serine phosphate	1.96	71
- Serine + 2.5×10^{-3} M cysteine	98.0	3,560
+ 5×10^{-4} M $MgCl_2$	2.70	98
+ 2.5×10^{-4} M pyridoxal phosphate	5.88	213
+ 10^{-3} M ATP	1.93	70
+ 10^{-2} M EDTA	2.68	98
+ 10^{-3} M, α, α' -dipyridyl	0.24	9
+ 10^{-2} M semicarbazide	1.34	49
+ 10^{-2} M hydroxylamine	0.36	13
+ 10^{-2} M isoniazid	4.50	164
+ 10^{-2} M iodacetamide	0.71	26
+ 10^{-3} M <i>p</i> -chloromercuribenzoate	0.36	13
+ 10^{-2} M $HgCl_2$	0.62	23

The reaction leading to β -cyanoalanine had a pH optimum at pH 9. The K_m value for KCN was found to be pH dependent; approximate values were estimated (using serine as a second substrate) to be 4×10^{-4} M at pH 7.3 and 1.5×10^{-5} M at pH 9.0. This shows that CN^- and not HCN is the true substrate of the reaction and the true K_m value for CN^- can be estimated to be in the order of 5×10^{-6} M. For cysteine a K_m of 9×10^{-5} was determined. No K_m value for serine could be obtained because the response of cyanoalanine formation to changes in the serine concentration was too low to give significant data.

The results of some experiments with possible co-factors and inhibitors are also shown in Table 1. The reaction does not require ATP, and serine phosphate cannot replace serine or cysteine as substrates. The enzyme may use pyridoxal phosphate as a prosthetic group, as shown by the stimulation obtained on addition of this compound and by the inhibitory effect of some carbonyl reagents. There seems to be no requirement for, divalent metal ions since Mg^{++} does not stimulate and EDTA does not inhibit the reaction. The enzyme is inhibited by Hg^{++} ions and *p*-chloromercuribenzoate.

The evidence presented classifies the formation of β -cyanoalanine into the group of pyridoxal phosphate catalysed β -replacement reactions of serine and cysteine. Some of the enzymes carrying out such reactions are known to deaminate their amino-acid substrate, particularly serine, in the absence of the second reactant. However, no formation of pyruvate could be detected on incubating cyanoalanine synthetase preparations with either labelled serine or labelled cysteine in the absence of cyanide. Likewise, a preparation of cystathionine synthetase which also deaminates serine did not synthesize β -cyanoalanine.

A number of other organisms was assayed for cyanoalanine synthetase. β -Cyanoalanine formation was demonstrated in an extract of *Vicia sativa* seedlings, and in mitochondria from seedlings of sorghum, barley, safflower, flax and mung beans. In addition to these plant sources, cyanoalanine synthetase activity was found in an extract of *E. coli* (cf. ref. 7), but not in homogenates or mitochondria of rat liver.

The part played by cyanoalanine synthetase in the living cell is not fully understood. It may serve to dispose of HCN in those plants containing cyanogenic glucosides and the location of the enzyme in the mitochondrion is of particular interest. However, its occurrence is not restricted to such plants and the physiological significance of this enzyme in organisms not known to have cyanogenic compounds is obscure.

H. G. FLOSS
L. HADWIGER
E. E. CONN

Department of Biochemistry and Biophysics,
University of California,
Davis.

¹ Blumenthal-Goldschmidt, S., Butler, G. W., and Conn, E. E., *Nature*, **197**, 718 (1963).

² Tschiersch, B., *Phytochem.*, **3**, 365 (1964).

³ Tschiersch, B., *Flora*, **154**, 445 (1964).

⁴ Ressler, C., Giza, Y.-H., and Nigam, S. N., *J. Amer. Chem. Soc.*, **85**, 2874 (1963).

⁵ Nigam, S. N., and Ressler, C., *Biochim. Biophys. Acta*, **93**, 339 (1964).

⁶ Fowden, L., and Bell, E. A., *Nature*, **206**, 110 (1965).

⁷ Dunnill, P. M., and Fowden, L., *Nature* (preceding communication).

Thalidomide as a Possible Biological Acylating Agent

THE teratogenic activity of thalidomide is now well known, but the mechanism by which the drug produces foetal abnormalities is obscure. Recent investigations in this laboratory¹ on the relationship between embryotoxic activity and chemical structure in a series of compounds related to thalidomide (α -phthalimidoglutarimide) suggested that the phthalimide portion of the molecule is important in determining teratogenic activity. Replacement of the phthalimide ring of thalidomide by other ring systems such as those of succinimide and hexahydrophthalimide was found to lead to the loss of embryotoxic activity in the rabbit. However, replacement of the glutarimide ring of thalidomide by other groups did not in every case lead to complete loss of embryopathic activity, for 4-phthalimidobutyramide, phthalimidobenzene and 2-phthalimidoglutaric acid anhydride were still embryotoxic in rabbits but much less so than thalidomide. These observations suggested that an *N*-substituted

phthalimide structure was essential for embryotoxic activity. However, the exact structural requirements in the *N*-substituent for embryotoxicity are not yet clear, but, so far as is known at present, optimum embryotoxic activity is found when the *N*-substituent is α -glutarimide as in thalidomide itself.

These findings raised the question of the possible significance of the *N*-substituted phthalimide structure in biological systems, and this may be considered from two aspects. In the first place, it is possible that the phthalimide structure (1,3-dioxoisindoline) is closely related to some substance involved in the metabolism of the embryo, and thereby interferes in some essential reaction. But, so far as we are aware, the phthalimide structure does not occur naturally. The second possibility is that the embryotoxic activity of certain phthalimide derivatives is a function of their chemical reactivity. It is possible that certain *N*-substituted phthalimides, because of a particular chemical reactivity, are able to interact with certain chemical substances essential to the normal development of the embryo and thereby cause malformations or death. Such an interaction would be a biochemical basis upon which the embryotoxic effects of thalidomide and related *N*-substituted phthalimides could be explained.

In this communication, preliminary evidence is presented which indicates that, under certain conditions, thalidomide behaves as an acylating agent and is able to acylate certain aliphatic diamines of biological interest such as putrescine and spermidine.

Our interest in the acylating activity of thalidomide arose from the discovery, in this laboratory, that thalidomide, at physiological pH values, is hydrolysed and, in effect, the drug acylates water, forming mainly α -(*o*-carboxybenzamido) glutarimide². The ability of thalidomide to acylate the hydroxyl group of water suggested to us that it might also react with hydroxyl or amino groups in other compounds. In fact, it is well known that phthalimide itself will acylate hydrazine to form *o*-carbamylbenzoylhydrazide (ref. 3). The progress of an acylating reaction involving the phthalimide ring of thalidomide can be readily followed spectrophotometrically. The intact phthalimide ring system exhibits an intense ultra-violet absorption at 220 m μ (ref. 4). Cleavage of the five-membered ring of phthalimide results in a marked decrease in this absorption so that the extent of ring-splitting following an acylation reaction can be readily estimated by measuring the decrease in optical density at 220 m μ (ref. 5). Using this procedure we have been able to follow the ability of thalidomide to acylate a number of compounds containing hydroxyl or amino groups in the absence of water.

Thalidomide (4×10^{-5} M) in absolute ethanol containing the test compound (10^{-3} M) was incubated at 37° C for periods up to 10 h. Samples of the incubates were withdrawn at hourly intervals and the optical density at 220 m μ was measured in a Unicam 'SP 500' spectrophotometer. The optical densities so obtained were compared to those of a similar solution of thalidomide in ethanol. In ethanol solution, thalidomide is stable, and no change in optical density at 220 m μ is detectable. In cases where acylation occurred, the disappearance of the thalidomide and the appearance of acylation products were confirmed chromatographically using the solvent systems already described by us⁶.

Table 1 gives a list of ten compounds whose ability to interact with thalidomide was tested. Thalidomide does not, under the conditions of the experiment, react with glucose nor the simple monoamines, *n*-butylamine, *n*-octadecylamine and ethanolamine. However, the aliphatic diamines, putrescine and spermidine, interacted rapidly with thalidomide, while ethylenediamine, cadaverine and spermine reacted less readily. Thus, when thalidomide was incubated with spermidine at 37° C for 2 h, the drug completely disappeared from the incubation medium, as was shown spectrophotometrically and chromatographically.

Table 1. ACYLATING ACTIVITY OF THALIDOMIDE TOWARDS VARIOUS ALIPHATIC AMINES AND OTHER COMPOUNDS

Compound incubated with thalidomide	Percentage thalidomide disappearing in 2 h at 37° C
Glucose	0
<i>n</i> -Butylamine	0
<i>n</i> -Octadecylamine	0
Ethylenediamine	3
1,4-Diaminobutane (putrescine)	72
1,5-Diaminopentane (cadaverine)	27
1-(3'-Aminopropylamino)-4-aminobutane (spermidine)	100
1,4-Di(3'-aminopropylamino)butane (spermine)	28
Ethanolamine	0
γ -Aminobutyric acid	0

Thalidomide (4×10^{-5} M) and the compound under test (10^{-3} M) were incubated together in absolute ethanol at 37° C for periods up to 10 h. Spermine, however, was tested at a concentration of 10^{-2} M.

It is possible that the reactivity of thalidomide towards certain natural diamines may be of significance in relation to the biological properties of the drug. This aspect of the reactivity of thalidomide is being further investigated.

S. FABRO
R. L. SMITH
R. T. WILLIAMS

Department of Biochemistry,
St. Mary's Hospital Medical School,
London, W.2.

¹ Fabro, S., Schumacher, H., Smith, R. L., and Williams, R. T., *Life Sci.*, **3**, 987 (1964).

² Fabro, S., Schumacher, H., Smith, R. L., Stagg, R. B. L., and Williams, R. T., *Biochem. J.*, **90**, 5-6 (1964).

³ Drew, H. D. K., and Hatt, H. H., *J. Chem. Soc.*, **16** (1937).

⁴ Green, J. N., and Benson, B. C., *J. Pharm. Pharmacol.*, **13**, 117T (1961).

⁵ Schumacher, H., Smith, R. L., and Williams, R. T., *Brit. J. Pharmacol.*, **25**, 324 (1965).

⁶ Schumacher, H., Smith, R. L., Stagg, R. B. L., and Williams, R. T., *Pharm. Act. Helv.*, **39**, 394 (1964).

'Buried' Tyrosyl Residues and the Activity of Trypsin

CHEMICAL modifications of enzymes have generally been used to identify catalytically functional amino-acid residues. Thus, inactivation with diisopropylphosphorofluoridate (DFP) has identified a number of 'seryl' enzymes¹ and interaction with alkylhalides or *para*mercuribenzoate (PMB) has defined a group of sulphhydryl enzymes². Similarly, we have used *N*-acetylimidazole to demonstrate a 'tyrosyl' enzyme, carboxypeptidase A (ref. 3).

The categorization of enzymes in terms of catalytically functional, active-centre residues is the direct consequence of the chemical specificity of selective reagents. Such reagents can also serve to identify amino-acid residues which function primarily through maintaining the structure of enzymes rather than participating directly in their catalytic mechanisms. In many enzymes, thiol residues may become reactive only after denaturation³. These unreactive or 'buried' thiol groups are thought to be internally bonded, thereby stabilizing structure in a manner as yet undefined, but distinct from disulphide linkages.

Similarly, 'buried' tyrosyl residues (the terminology here employed has been discussed previously⁴) are thought to maintain structure, for example, through tyrosyl-carboxylate hydrogen bonds, as shown for ribonuclease^{4,5}.

We have now acetylated trypsin with acetylimidazole and the experiments reported here provide evidence that in this enzyme, also, 'buried' tyrosyl residues stabilize tertiary structure. This stabilization is required for the function of the enzyme, presumably by maintaining the critical conformation of the active centre.

Acetylimidazole has been shown to be a relatively specific agent for the *O*-acetylation of 'free' tyrosyl residues of proteins. Experimentally, the degree of acetylation is apparent by a marked decrease in molar absorptivity at 278 m μ ². In a series of proteins, the number of tyrosyl residues acetylated with acetylimidazole at pH 7.5 corresponded quite closely to the number of

'free' residues measured by *pH* titration⁶. Thus, this reagent permits the selective acetylation of those tyrosyl residues of native proteins which are readily accessible to the ambient medium. Reaction of all of the tyrosyl residues of a protein can be achieved subsequent to denaturation⁶.

Previously, we have reported that 6.7 of the 10 tyrosyl residues of trypsin can be acetylated with acetylhydrazole at *pH* 7.5 without loss of enzymatic activity⁸. This number of 'free' tyrosyl residues is in substantial agreement with the data of Inada *et al.*⁷, who have shown, by titration, that six groups are 'free' while four appear to be 'buried'. Since urea denaturation of trypsin is reversible⁸ and exposes the 'buried' tyrosyl residues (see Table 1, column 3), we have acetylated the enzyme in urea with acetylhydrazole and studied the resultant enzymatic changes (the minor discrepancy in the number of 'free' tyrosyls determined by titration and by acetylation might be due to a partially 'buried' residue as discussed elsewhere^{8,9}).

Table 1. EFFECT OF ACETYLATION ON THE ESTERASE AND PEPTIDASE ACTIVITIES OF TRYPSIN

	Peptidase <i>V/V_c</i>	Esterase <i>V/V_c</i>	O-Acetyltyrosyl mole/mole
1. Trypsin	1.0	1.0	0
2. Trypsin + 3.2 M guanidine	0.5	0.5	0
3. Trypsin + acetylhydrazole	1.1	0.9	6.7
4. Trypsin + 3.2 M guanidine + acetylhydrazole	0.1	0.0	10.2

Peptidase activity was determined by the hydrolysis of casein¹⁷ and esterase activity was determined by the hydrolysis of benzoylarginine ethyl ester¹⁸ using a *pH*-stat. Acetylation was carried out with a 120-fold molar excess of acetylhydrazole at 20° and *pH* 7.5 in 0.01 M *tris* buffer ± 3.2 M guanidine HCl.

Twice crystallized salt-free lyophilized trypsin (Worthington Biochemical Corporation) was dissolved in 0.01 M *tris* buffer at *pH* 7.5 and acetylated with a 120-fold molar excess of *N*-acetylhydrazole (K. and K. Laboratories) at room temperature⁸. After 30 min, an aliquot was removed, diluted and assayed for esterase and peptidase activities (Table 1). Compared with the native enzyme, there is no change either in peptidase or esterase activity. However, when the enzyme is acetylated in the presence of 3.2 M guanidine hydrochloride (Table 1) or 8 M urea (Fig. 1), both activities are virtually abolished, though the controls remain active. The abolition of enzymatic activity is accompanied by the acetylation of the buried tyrosyl residues as measured by spectral changes. In 0.01 M *tris* buffer 6.7, but in 8 M urea all 10, tyrosyl residues of trypsin are acetylated (Table 1, column 3). The esterase and peptidase activities of such acetyl-

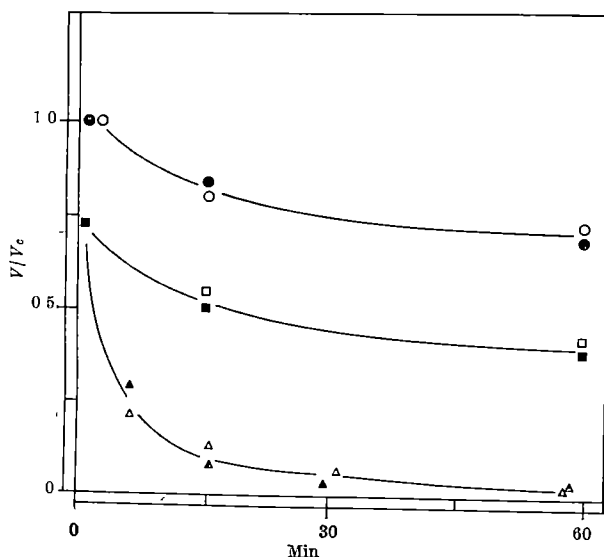


Fig. 1. Changes in esterase (closed symbols) and peptidase (open symbols) activities on acetylation of trypsin: ●, ○, Trypsin in 0.01 M *tris*, *pH* 7.5, and trypsin in 0.01 M *tris*, *pH* 7.5 + 120 molar excess acetylhydrazole; ■, □, trypsin in 0.01 M *tris*, 8 M urea, *pH* 7.5, and (▲, △) trypsin in 0.01 M *tris*, 8 M urea, *pH* 7.5 + 120 molar excess acetylhydrazole.

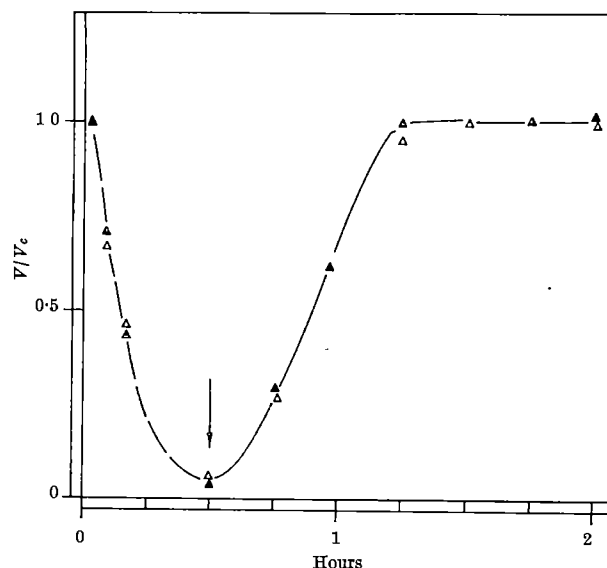


Fig. 2. Inactivation of trypsin by acetylation in 8 M urea and reactivation by deacetylation with 1 M hydroxylamine. A 120-fold molar excess of acetylhydrazole was added to trypsin in urea and activities were measured as a function of time — — —. After 30 min the acetylation mixture was made 1 M in hydroxylamine (indicated by arrow), and esterase (closed symbols) and peptidase (open symbols) activity measurements continued (—). Activities are expressed relative to the control sample measured at the same time.

trypsin, inactive in 8 M urea, are restored to the control values by deacetylation with 1 molar hydroxylamine (Fig. 2). Since the active centre O-acetyl serine is not stable at *pH* 7.5 (ref. 10), the reactivation of the acetylated, inactive enzyme with hydroxylamine suggests that the acetylation and deacetylation of 'buried' tyrosyl residues accounts for the reversible abolition and restoration of activity. These results are consistent with the hypothesis that at least one buried tyrosine is essential to the maintenance of the active configuration of trypsin.

Reversibly denatured trypsin has been inactivated by acetylation in dimethylsulphoxide with acetic anhydride^{11,12}. Under these conditions, however, only lysyl residues were modified and the inactive acetyltrypsin could not be reactivated; hydroxylamine was not used.

A critical structural role of a specific 'buried' tyrosyl residue in trypsin has been proposed on the basis of activity changes which accompany ionization of the enzyme⁷. The alkaline limb of the *pH*-rate profile of trypsin coincided with the ionization curve of the four 'buried' tyrosyl residues, one of which was thought to be essential to the maintenance of the active centre. Ionization of the 'free' tyrosyl residues did not affect activity.

Amino-acid sequence is thought to be a major determinant of secondary and tertiary protein structure¹³. Acetylation of 'buried' tyrosyls in trypsin disrupts the normal sequence and may thereby prevent reformation of one or several specific internal tyrosyl linkages, broken by urea or guanidine. Alternatively, acetylation may preclude proper three-dimensional re-alignment of the molecule, solely through steric effects. The restoration of activity to trypsin by regeneration of internal tyrosyl bonds appears analogous to restoration of activity in ribonuclease by regeneration of disulphide bridges. It may well be that in the latter instance both processes, operating simultaneously, mutually facilitate proper re-alignment. (Similar conclusions regarding the role of 'buried' tyrosyl residues in ribonuclease have been reached by a different approach, that is, inhibition of oxidative reactivation of the reduced enzyme by analogues of tyrosine¹⁴). 'Buried' lysyl residues may also have structural importance in trypsin as suggested by the results of acetylation in dimethylsulphoxide^{11,12}, though acetylation of tyrosyl residues was not entirely ruled out in this instance.

The present observations extend the information concerning the essentiality of tyrosyl residues in the mechanism of enzyme action. Two of the seven 'free' tyrosyl residues of carboxypeptidase have been shown to be essential for the interaction of this enzyme with peptides¹⁴; however, on acetylation of these two tyrosyl residues, the esterase activity of this enzyme increases about seven-fold, while peptidase activity is virtually abolished^{3,15}. Acetylation of trypsin exposed to guanidine or urea abolishes both esterase and peptidase activities by rendering the 'buried' tyrosines accessible to acetylimidazole. Since these critical tyrosyl residues in trypsin are 'buried' they cannot be considered functional in the same sense as the 'free' tyrosines of carboxypeptidase, that is, by interacting directly with substrate. Rather, they appear to be responsible for maintaining the active centre of the enzyme in the configuration required for catalysis.

This work was supported by the Howard Hughes Medical Institute and by grants-in-aid HE-07297 from the U.S. National Institutes of Health.

JAMES F. RIORDAN*
WARREN E. C. WACKER†
BERT L. VALLEE

Biophysics Research Laboratory,
Department of Biological Chemistry,
Harvard Medical School,
and
Division of Medical Biology,
Department of Medicine,
Peter Bent Brigham Hospital,
Boston, Massachusetts.

* Postdoctoral Fellow of the National Foundation
† Investigator of the Howard Hughes Medical Institute.

- ¹ Hartley, B. S., *Ann. Rev. Biochem.*, **29**, 45 (1960).
- ² Boyer, P. D., in *The Enzymes*, edit. by Boyer, P. D., Lardy, H., and Myrback, K., second ed., 511 (Academic Press, Inc., New York, 1959).
- ³ Simpson, R. T., Riordan, J. F., and Vallee, B. L., *Biochemistry*, **2**, 616 (1963).
- ⁴ Broomfield, C. A., Riehm, J. P., and Scheraga, H. A., *Biochemistry*, **4**, 751 (1965).
- ⁵ Riehm, J. P., Broomfield, C. A., and Scheraga, H. A., *Biochemistry*, **4**, 760 (1965).
- ⁶ Riordan, J. F., Wacker, W. E. C., and Vallee, B. L., *Biochemistry*, **4**, 1758 (1965).
- ⁷ Inada, Y. I., Kamata, M., Matsushima, A., and Shibata, K., *Biochim. Biophys. Acta*, **81**, 323 (1964).
- ⁸ Kunitz, M., and Northrop, J. H., *J. Gen. Physiol.*, **17**, 591 (1934).
- ⁹ Herskovits, T. T., and Laskowski, M., Jr., *J. Biol. Chem.*, **237**, 2481 (1962).
- ¹⁰ Stewart, J. A., and Ouellet, L., *Canad. J. Chem.*, **37**, 751 (1959).
- ¹¹ Vratsanos, S. M., Bier, M., and Nord, F. F., *Arch. Biochem. Biophys.*, **77**, 216 (1958).
- ¹² Vratsanos, S. M., *Arch. Biochem. Biophys.*, **90**, 132 (1960).
- ¹³ White, F. H., Jr., and Anfinsen, C. B., *Ann. N.Y. Acad. Sci.*, **81**, 515 (1959).
- ¹⁴ Vallee, B. L., *Fed. Proc.*, **23**, 8 (1964).
- ¹⁵ Riordan, J. F., and Vallee, B. L., *Biochemistry*, **3**, 1768 (1964).
- ¹⁶ Haber, E., and Anfinsen, C. B., *J. Biol. Chem.*, **237**, 1839 (1962).
- ¹⁷ Kunitz, M., *J. Gen. Physiol.*, **30**, 291 (1947).
- ¹⁸ Schwert, G. W., Neurath, H., Kaufman, S., and Snoko, J. E., *J. Biol. Chem.*, **172**, 221 (1948).

Comparison of Salivary Amylase-levels in the Human Male and Female

In 1940, Lacassagne¹ demonstrated sexual dimorphism in the submandibular glands of mice and other rodents. Later, Raynaud and Rebeyrotte^{2,3} found that the amylase-level of saliva from male mice is 50 per cent higher than that of female mice. They also reported that administration of testosterone propionate to female mice raised their salivary amylase-level to that of untreated males and that castration of males reduced their amylase-level to that of normal females. Swigart *et al.*⁴ have reported that the amylase content of the submandibular glands of male mice is three times that of the female, that castration reduced the submandibular gland amylase-level of the male mice to less than 50 per cent of normal and that administration of testosterone to castrated females raised the submandibular amylase-level to a level comparable to that in the male.

The observations reported here were carried out in an attempt to determine whether any sex-linked differences exist in the salivary amylase-levels of man.

Saliva samples for these experiments were collected from each of ten men and ten women aged between 20 and 46. Each subject contributed saliva on three separate occasions, 2-4 weeks apart. In all cases saliva was collected at the same time of day, between 10 and 11 a.m. All subjects had rinsed their mouths thoroughly before collecting saliva and salivary flow was stimulated by chewing gum from which sugar and flavouring was first removed by preliminary chewing.

The saliva samples were centrifuged to remove any solid particles and dilutions of 1:1000-1:5000 were prepared with 0.9 per cent sodium chloride. Amylase activities were determined by the method of Van Loon *et al.*⁵, carrying out each determination in duplicate or triplicate. Values for salivary amylase are given in Van Loon units per 100 ml. Van Loon units are numerically equivalent to Somogyi units.

The value given for each individual subject in Table 1 is the average of the determinations carried out on three different samples, collected as indicated earlier at several weekly intervals. Salivary amylase-levels in these three different samples from the same individual varied from one another by as little as 10 per cent in some cases and as much as 50 per cent in others. The mean of these averages for male subjects was 84,900 and, for the female subjects, 80,400. Using the student 't' test as outlined by Snedecor⁶, the t-value for the difference in these two groups is 0.35. Snedecor's table indicates that the t-value should be at least 2.87 at the 1 per cent level of confidence and at least 2.10 at the 5 per cent level for the difference seen in the salivary amylase-levels of these two groups to be considered statistically significant. The t-value of 0.35 indicates that the difference is not significant. The probability that such a difference will occur by chance is 98 per cent.

Table 1. HUMAN SALIVARY AMYLASE-LEVELS

	Salivary amylase in units/100 ml.	
	Male	Female
(1)	94,700	94,000
(2)	64,600	33,100
(3)	85,600	88,600
(4)	64,600	65,900
(5)	115,100	92,400
(6)	96,800	122,200
(7)	50,300	91,600
(8)	93,500	72,000
(9)	76,800	23,800
(10)	106,500	120,000
Mean	84,900 ± 3,160*	80,400 ± 9,770*

* Standard error of the mean.

The salivary amylase-levels for all three determinations for each female subject were examined in relation to the subject's menstrual cycle and the times of collection of saliva. No correlation could be established. For one female subject, determinations were then undertaken of her salivary amylase-level on samples taken daily throughout seven menstrual cycles. Variations in amylase-level occurred during the cycle studies, but again none of the changes could be correlated with hormonal changes during the cycle.

It is apparent, then, that while there is considerable variation in the salivary amylase-levels of different individuals from week to week, both male and female, there is no statistically significant difference in the salivary amylase-levels of the human male and female and no salivary amylase changes attributable to hormonal changes during the menstrual cycle are seen in the female.

This work was partially supported by research grants (AM 02610 and AM C7944) from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health. One of the authors (P. A. H.) was a participant in the National Science Foundation Under-

graduate Science Education programme in the summer of 1963.

ROBERT L. MCGEACHIN
PATRICIA A. HAY
PATRICIA A. PRELL

Department of Biochemistry,
School of Medicine,
University of Louisville,
Kentucky.

¹ Lacassagne, A., *C.R. Soc. Biol.*, **133**, 180 (1940).

² Raynaud, J., and Rebeyrotte, P., *C.R. Soc. Biol.*, **288**, 433 (1949).

³ Raynaud, J., and Rebeyrotte, P., *C.R. Soc. Biol.*, **288**, 1061 (1949).

⁴ Swigart, R. H., Hilton, F. K., Dickie, M. M., and Foster, B. J., *Endocrinology*, **76**, 776 (1965).

⁵ Van Loon, E. J., Likins, M. R., and Seger, A. J., *Amer. J. Clin. Path.*, **22**, 1134 (1952).

⁶ Snedecor, G. W., *Statistical Methods* (Iowa State College Press, Ames, 1946).

Acid Mucopolysaccharide and Hexosamine Metabolism in Dermal Connective Tissue of Adrenalectomized Rats

SINCE steroids have been reported to be involved in the metabolism of the mucopolysaccharides of the dermis¹⁻³, it appeared interesting to investigate the effect of adrenalectomy on the amount of acid mucopolysaccharides and hexosamines in dermal connective tissue. The investigations were performed on young male rats of the CFE strain. Animals in the experimental group were adrenalectomized when 30 days old; they weighed 95-100 g at this time. Both adrenalectomized and control animals were purchased from Carworth Farms, Inc., New York City, Rockland County, New York. The animals were fed *ad lib.* 'Wayne Lab Blox' (mice and rats) diet. The adrenalectomized rats received 2 per cent sodium chloride in tap water, those of the control group were given tap water alone; both beverages were offered *ad lib.*

The animals were killed when 60 days old (average weight 240 g) by incising the aorta. Their skin was shaved, freed from adherent muscles by scraping with blades, ground into paste in a mill (Wiley laboratory mill, intermediate size) and weighed. One portion of the 'fresh skin' (15-20 g) was defatted with acetone in a Soxhlet extractor, dried at 80° C in a drying oven and used for mucopolysaccharide as well as total hexosamine determination. The other portion of 'fresh skin' (about 1 g) was successively extracted with 0.154 M, 0.5 M sodium chloride and finally with 0.5 M sodium citrate. The hexosamine content was determined (Ehrlich method according to Blix⁴) in these extracts, in soluble residue and in a portion of dry defatted skin after hydrolysis with 6 N hydrochloric acid at 104° C for 4 h.

The acid mucopolysaccharides were extracted, fractionated and determined as described earlier⁵. For this, the dry defatted samples (3-6 g) were digested with papain*, then with trypsin* using respectively 4 and 3 mg of these

* Papain (three times recrystallized) and trypsin (twice recrystallized, free of salt), supplied by Worthington Biochem. Corp., Freehold, New Jersey.

enzymes per gram of dry fat-free tissue. The liberated polypeptides and amino-acids were separated by dialysis. The mucopolysaccharides were precipitated with cetylpyridinium chloride (CPC) and the precipitate washed with 0.03 M sodium chloride. Then the mucopolysaccharide-CPC complex was successively extracted with 0.4, 1.2 and 2.1 M sodium chloride in order to solubilize and separate the three types of mucopolysaccharides, that is, 'hyaluronic acid', 'chondroitin sulphates' and 'heparin' as in the procedure of Schiller *et al.*⁷. The content of various mucopolysaccharides was determined by the carbazole method according to Bitter and Muir⁸ using glucuronic acid as the standard.

The results are given in Tables 1 and 2. The values for mucopolysaccharides are expressed as μ mg of glucuronic equivalent per gram of dry defatted skin. The values for hexosamine obtained for different fractions are given in mg per gram of dry defatted skin. Comparing the results obtained with normal and adrenalectomized animals no significant difference was found in hexosamine content as well as in that of the various mucopolysaccharides. The ratio of total hexosamine to hydroxyproline (Stegemann's method⁹) for dry defatted skin was 0.044 for adrenalectomized and 0.047 for intact rats.

It was previously reported that the hexosamine content accounted for by mucopolysaccharides which contain uronic acid represented only a small portion of the total hexosamine occurring in the dermal tissue¹⁰. Similar results were also reported by Boas¹¹ for subcutaneous tissue. The present study confirms these findings since the total hexosamine content in the skin of both normal and adrenalectomized rats was approximately thrice that of uronic acid (Tables 1 and 2).

These results do not contradict those previously reported on dermal mucopolysaccharides of normal intact animals treated with corticosteroids¹⁻³, since the concentration of these compounds in the dermis of such animals was never determined. (At present Schiller *et al.*¹² have found a decrease in the hyaluronic acid and chondroitin sulphate contents in the dermis of intact rats treated with hydrocortisone.) As recently shown by Schiller¹², the previously reported decreased uptake of sulphur-35 (refs. 1-3) and carbon-14 (ref. 3) by skin mucopolysaccharides of rats pretreated with cortisone and hydrocortisone is not necessarily related to a reduced tissue concentration of acid mucopolysaccharides. Moreover, Bostrom *et al.*⁴ found no significant effect of hydrocortisone on the inhibition of uptake of sulphur-35 by the heart valve mucopolysaccharides.

Boas¹¹ found in adrenalectomized rats, an increase (about 20 per cent) in the total hexosamine content of

Table 1. CONCENTRATION OF ACID MUCOPOLYSACCHARIDES IN THE SKIN OF NORMAL AND ADRENALECTOMIZED RATS

No. of animals	Total concentration μ mg uronic acid/g dry skin	Distribution of uronic acid (%)		
		Hyaluronic acid	Chondroitin sulphates	Heparin
		Normal rats		
6	1,060 \pm 358	54.8 \pm 6.5	30.1 \pm 1.8	6.1 \pm 2.0
		Adrenalectomized rats		
14	1,166 \pm 262	51.2 \pm 1.7	44.1 \pm 2.0	4.8 \pm 2.3

Table 2. CONCENTRATION OF HEXOSAMINE AND NITROGEN IN THE SKIN OF NORMAL AND ADRENALECTOMIZED RATS (MG/G DRY DEFATTED TISSUE)

	No. of animals	Fresh skin				Obtained on defatted dry skin*
		Extracting medium:			Insoluble fraction	
		0.154 M NaCl	0.5 M NaCl	0.5 M Na-citrate		
Hexosamine Nitrogen	9	Normal rats				
		1.14 ± 0.09 20.3 ± 1.3	0.44 ± 0.06 14.3 ± 1.0	0.19 ± 0.02 6.2 ± 0.9	1.65 ± 0.19 112.2 ± 6.4	3.35 ± 0.10 156 ± 3.2
Hexosamine Nitrogen	21	Adrenalectomized rats				
		1.01 ± 0.12 18.2 ± 0.40	0.43 ± 0.07 15.6 ± 0.60	0.18 ± 0.04 5.51 ± 1.2	1.49 ± 0.09 120.7 ± 13.9	3.22 ± 0.22 154 ± 3.3

* Values found by separate determinations on dry defatted skin. The proportion of dry defatted tissue, based on wet weight, was 29.6 \pm 0.8 per cent for normal and 31.9 \pm 0.4 per cent for adrenalectomized rats. The ratio of hexosamine to hydroxyproline was 0.047 and 0.044, respectively, for defatted dry skin of normal and adrenalectomized rats.

subcutaneous tissue. However, because of the different nature of the tissue (subcutaneous versus cutaneous) and the short period of time between adrenalectomy and the test (10 days) used by Boas, the result cannot be compared with present findings.

From the comparison of the present data with those obtained earlier on hypophysectomized rats¹⁴ it is apparent that neither adrenalectomy nor hypophysectomy altered the gross mucopolysaccharide concentration in the skin. Contrariwise, the hexosamine content of the dermis modified by hypophysectomy¹⁰ was not affected in the skin of rats with ablated adrenals.

Adrenalectomy and hypophysectomy were previously reported to affect markedly the activity of compounds altering connective tissue permeability¹⁵. Our earlier *in vivo* and *in vitro* investigations have suggested that hyaluronic acid is an important factor regulating connective tissue permeability^{16,17}. The fact that mucopolysaccharide concentration is not altered by adrenalectomy is in agreement with our previously suggested hypothesis that the effect of substances altering tissue permeability may be related mainly to a different degree of polymerization of hyaluronic acid and seems to be independent of the gross mucopolysaccharide concentration¹⁵.

In conclusion, the results recorded here show that adrenalectomy does not affect mucopolysaccharide or the hexosamine contents of skin of two-month-old rats tested a month after ablation of the adrenals.

The work was supported by the U.S. National Institute for Arthritis and Metabolic Diseases, grant A-4619, and the John Polachek Foundation for Medical Research.

JOHN FABIANEK *
ANTHONY HERP
LEONOR MARTINEZ

Department of Biochemistry,
New York Medical College,
New York.

* Present address: Laboratory of Experimental Metabolic Diseases, Medical Research Programs, Veterans Administration Hospital, Long Beach, California.

- ¹ Layton, L. L., *Arch. Biochem. Biophys.*, **32**, 224 (1951).
- ² Bostrom, H., and Odenblad, E., *Arkiv. Kemi.*, **6**, 39 (1953).
- ³ Schiller, S., and Dorfman, A., *Endocrinology*, **60**, 376 (1957).
- ⁴ Bostrom, H., Moretti, A., and Whitehouse, M., *Biochim. Biophys. Acta*, **74**, 213 (1963).
- ⁵ Blix, G., *Acta Chem. Scand.*, **2**, 467 (1948).
- ⁶ Fabianek, J., Herp, A., and Pigman, W., *Comp. Biochem. Physiol.*, **14**, 21 (1965).
- ⁷ Schiller, S., Slover, G. A., and Dorfman, A., *J. Biol. Chem.*, **236**, 983 (1961).
- ⁸ Bitter, T., and Muir, H. M., *Anal. Biochem.*, **4**, 330 (1962).
- ⁹ Stegemann, H., *Hoppe-Seyler's Z. physiol. Chem.*, **311**, 41 (1958).
- ¹⁰ Fabianek, J., Herp, A., and Pigman, W., *Abst., Section VI*, 508, *Sixth Internat. Congress of Biochem.*, New York City (July 26-August 1, 1964).
- ¹¹ Boas, N. F., *Ann. N.Y. Acad. Sci.*, **72**, 14, 1045 (1959).
- ¹² Schiller, S., *J. Chron. Dis.*, **16**, 291 (1963).
- ¹³ Schiller, S., Rosenkrantz, N., and Dorfman, A., *Biochim. Biophys. Acta* (in the press).
- ¹⁴ Matsumura, G., Fabianek, J., Herp, A., and Pigman, W., *Nature*, **200**, 784 (1963).
- ¹⁵ Fabianek, J., Herp, A., and Pigman, W., *Endocrinology*, **76**, 408 (1965).
- ¹⁶ Fabianek, J., Herp, A., and Pigman, W., *Arch. Intern. Physiol. Biochim.*, **71**, 647 (1963).
- ¹⁷ Fabianek, J., and Herp, A., *Proc. Intern. Symp. Antiinflammatory Drugs*, Milan, September 8-10, 1964 (Excerpta Medica Foundation, International Congress Series No. 82, 35, Amsterdam, 1965).

Occurrence of Odd-numbered Fatty Acids in the Mullet *Mugil cephalus*

ODD-NUMBERED fatty acids (C_{15} , C_{17} , C_{19} and C_{21}) in marine oils are normally found to total 1-4 per cent of the acids of all chain-lengths. This is most reliably indicated by gas-liquid chromatography of hydrogenated ester samples, since minor amounts of unsaturated odd-numbered fatty acids may be overlooked or confused with acids of the predominant even-numbered chain-

lengths. An exception to these typical compositions is the oil of the striped mullet *Mugil cephalus*, which has been found to contain up to 25 per cent saturated, mono-unsaturated and poly-unsaturated odd-numbered fatty acids¹⁻³.

A survey of fatty-acid compositions as determined by gas-liquid chromatography for phytoplankton, zooplankton and other lower marine organisms, including a few molluscs feeding on phytoplankton, does not suggest that odd-numbered fatty acids would occur in the diet in proportions high enough to influence the depot-fat composition of the mullet. The lugworm *Arenicola marina* from European waters is, however, reported⁴ to have an unusually high proportion of odd-numbered fatty acids. Unless the metabolism of the mullet is unusual in some respect, a view which has been discounted⁵, an alternative to a diet high in longer-chain odd-numbered fatty acids must be sought.

It has recently been found in this laboratory⁶ that a culture of the coccolithophore *Syracosphaera carterae* (courtesy of Dr. J. McLachlin, Atlantic Regional Laboratory, National Research Council of Canada) contained equal proportions (about 4 per cent each on a wet weight basis) of extractable lipid and of a material tentatively identified on the basis of its behaviour with cold alkali^{5,6} as dimethyl- β -propiethetin (or dimethyl-2-carboxyethyl sulphonium chloride). This thetin is found in a number of marine algae⁷, although the species investigated have usually been sessile forms and not planktonic. Lesser proportions of the thetin were found in five other species (out of a total of fourteen investigated) of unicellular planktonic algae.

The death of the host alga has been shown in at least one instance to lead rapidly to enzymatic degradation of the thetin with dimethyl sulphide and acrylic acid as the principal products⁷. Propionic acid has also been suggested as a product⁸. In carnivorous fish such as the Pacific chum salmon⁹ or Atlantic cod⁵ the digestive processes apparently do not break down the thetin, but dimethyl sulphide may be found in the fish at certain times^{8,9}, indicating that natural decomposition does occur, probably in a zooplankton¹⁰.

Propionic acid is accepted as a precursor of odd-numbered fatty acids^{11,12}, as has been demonstrated by feeding mice¹³. It has been suggested that acrylic acid may be associated with propionic acid in some metabolic roles^{14,15}, although acrylyl-CoA acts as an inhibitor of normal fatty acid synthesis¹⁶ and acrylic acid inhibits β -oxidation¹⁷. There is no evidence that the basic pathways of fatty acid metabolism in fish differ significantly from those in mammals.

It is therefore suggested that the odd-numbered fatty acids in the mullet may originate in very high proportions of propionic acid or related materials derived from the thetin in the diet, particularly in relation to the total lipid ingested from the same primary phytoplankton food source. The mullet is stated to feed extensively on diatoms and foraminifera, sometimes at the surface, but primarily on mud bottoms, and mud is commonly found in the digestive tract. The omission of zooplankton from the food chain and a digestive system somewhat different from that of the carnivorous species of fish could lead to decomposition of the thetin during digestion by the mullet. The metabolism of intact thetin in fish livers, where it may act as a methyl donor, varies appreciably between chum and red salmon⁸, and may also be markedly different in the mullet.

Syracosphaera is a tropical to subtropical species (the culture was grown at 21°C) and the occurrence of the thetin in one or more species of algae in the gulf of Mexico is entirely probable. At certain times of the year such algae might form a major part of the diet of the mullet, leading to the synthesis of odd-numbered fatty acids in high proportions. Once formed, these acids would be unlikely to be very actively removed from depot fats. The

lugworm analysis may be high in odd-numbered acids for similar reasons.

R. G. ACKMAN

Fisheries Research Board of Canada,
Technological Research Laboratory,
Halifax, Nova Scotia.

- ¹ Reiser, R., Stevenson, B., Kayama, M., Choudhury, R. B. R., and Hood, D. W., *J. Amer. Oil Chemists' Soc.*, **40**, 507 (1963).
- ² Gruger, E. H., jun., Nelson, R. W., and Stansby, M. E., *J. Amer. Oil Chemists' Soc.*, **41**, 662 (1964).
- ³ Sen, N., and Schlenk, H., *J. Amer. Oil Chemists' Soc.*, **41**, 241 (1964).
- ⁴ Cocker, W., Dahl, T., and McMurry, T. B. H., *J. Chem. Soc.*, 1654 (1963).
- ⁵ Ackman, R. G., Tocher, C. S., and McLachlan, J., *J. Fish. Res. Bd. Canada* (in the press).
- ⁶ Challenger, F., *Aspects of the Organic Chemistry of Sulphur* (Butterworths Scientific Publications, London, 1959).
- ⁷ Cantoni, G. L., and Anderson, D. G., *J. Biol. Chem.*, **222**, 171 (1965).
- ⁸ Motohiro, T., *Memoirs of the Faculty of Fisheries, Hokkaido University*, **10**, No. 1 (1962).
- ⁹ Sipos, J., and Ackman, R. G., *J. Fish. Res. Bd. Canada*, **21**, 423 (1964).
- ¹⁰ Sieburth, J. M., *Science*, **132**, 676 (1960).
- ¹¹ Wakil, S. J., *J. Lipid Research*, **2**, 1 (1961).
- ¹² Masoro, E. J., and Porter, E., *J. Lipid Research*, **2**, 177 (1961).
- ¹³ Tove, S. B., *Nature*, **184**, 1647 (1959).
- ¹⁴ Flavin, M., Ortiz, P. J., and Ochoa, S., in *Biochemical Problems of Lipids*, edit. by Popjak, G., and LeBreton, E., 203 (Butterworths Scientific Publications, London, 1956).
- ¹⁵ Rendina, G., and Coon, M. J., *J. Biol. Chem.*, **225**, 523 (1957).
- ¹⁶ Robinson, J. D., Brady, R. O., and Bradley, R. M., *J. Lipid Research*, **4**, 144 (1963).
- ¹⁷ Thijsse, G. J. E., *Biochim. Biophys. Acta*, **84**, 195 (1964).

PHYSIOLOGY

Further Purification and Characterization of a Placental Protein with Immunological Similarity to Human Growth Hormone

Josimovich and MacLaren¹ purified a substance from human placentas which cross-reacted immunologically with antiserum to human growth hormone in Ouchterlony's double-diffusion system. Moreover, they detected this substance in the blood and urine of pregnant women, thereby raising the possibility that it might be a new placental hormone. Investigation of the physiological role of this substance has been hampered by the limited supply of material. The relatively easy method outlined here yields substantially greater quantities of this substance than could be obtained by the previously described purification from fresh-frozen placentas².

Fraction VII, a by-product of the extraction of γ -globulin from human placentas, was obtained from Lederle Laboratories through the courtesy of Dr. P. Bell. When examined by starch-gel electrophoresis and by diffusion in Ouchterlony plates, it was found to contain considerable quantities of the desired substance. Two hundred g, dissolved in 0.05 M ammonium bicarbonate to make a 10 per cent solution (w/v) were stirred with 100 g (wet wt.) of diethylaminoethyl cellulose previously equilibrated for 24 h with 0.05 M ammonium bicarbonate. The ion-exchange medium was collected and washed repeatedly with 0.05 M ammonium bicarbonate until the absorbance of the washings at 278 m μ was less than 0.5. The active component was then eluted with 1.5 l. of 0.5 M ammonium bicarbonate. On freeze-drying, 5–8 g of powder were obtained.

Thirty g of this powder, dissolved in 300 c.c. of 0.1 M ammonium bicarbonate, were fractionated using a column of 'Sephadex G-100' (8 x 80 cm) previously equilibrated with the same buffer. A high concentration of the component which reacted with antiserum to human growth hormone was found in the peak of the retarded fraction (Fig. 1). This was pooled, lyophilized and 2–3 g of white powder were obtained as compared with 150 mg by our earlier method. On examination by starch-gel electrophoresis this fraction was still heterogeneous (Fig. 2), but it appeared similar to our previously purified preparation.

Five mg or more of the final fraction caused a small increase in the body-weight gain of hypophysectomized

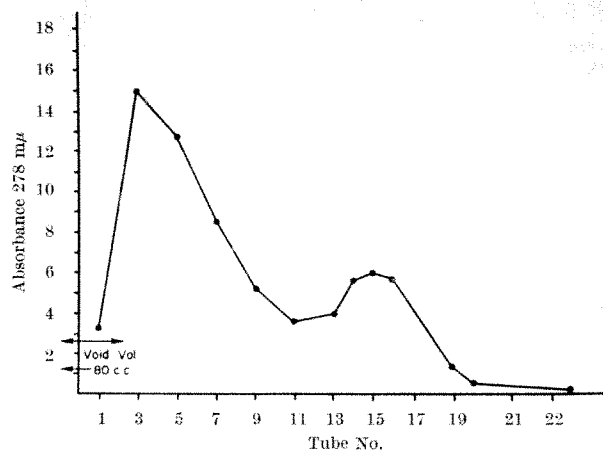


Fig. 1. Absorbance at 278 m μ of effluents from a column of 'Sephadex G-100' 3 x 55 cm. One g of the fraction obtained by elution of DEAE with 0.5 M ammonium bicarbonate was applied. 12 ml./tube

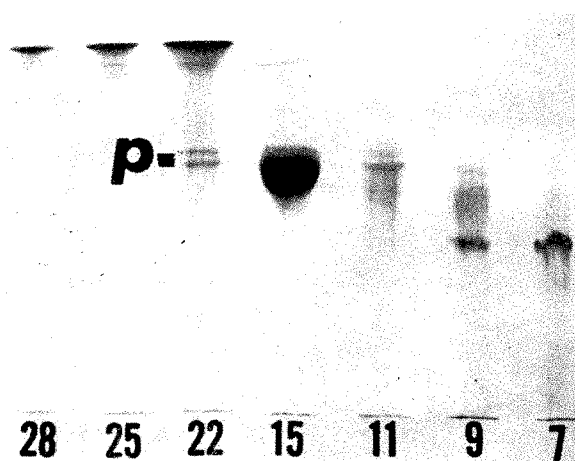


Fig. 2. Starch-gel electrophoresis of concentrates from the effluents of the column shown in Fig. 1. The marker P indicates the immunologically active substance present in highest concentration in channel 15

rats. In some assays 1 mg or more caused a definite but limited increase in the width of the tibial epiphyseal cartilage (Tables 1 and 2). Dr. E. Knobil found no increase in the serum free fatty acids in three hypophysectomized monkeys 4 h after administering 10 mg/kg of the placental protein; human growth hormone is effective at one-twentieth this dose.

In 1953, Astwood³ reported a delay of oestrus in rats if prolactin were given within 24 h of the appearance of cornified cells in vaginal smears. The extension of the cycle equalled the number of days the hormone was injected. Table 3 shows the prolongation of the oestrus cycle of rats by subcutaneous injection of prolactin or of

Table 1. EFFECT OF THE PLACENTAL PROTEIN ON BODY-WEIGHT GAIN OF HYPOPHYSECTOMIZED RATS

	Dose (mg/day*)	No. of rats	Average wt. gain per rat/day (g)
Controls	0	13	-0.08
Placental protein	1	22	+0.08
	2	5	+0.02
	5	5	+0.10
	10	9	+1.0

* The placental protein was injected intraperitoneally for 10 days.

Table 2. EFFECT OF THE PLACENTAL PROTEIN ON THE WIDTH OF THE TIBIAL EPIPHYSEAL CARTILAGE*

	Dose (mg/day)	No. of rats	Tibial epiphyseal width (μ)
Controls	0	10	138 \pm 5
Placental protein	1	10	151 \pm 8
	5	10	167 \pm 8
	10	19	161 \pm 5
	20	10	158 \pm 5

* Injections were given subcutaneously for 4 days.

Table 3. EFFECT OF PROLACTIN AND THE PLACENTAL PROTEIN ON THE OESTRUS CYCLE OF RATS

Total dose (μ g* prolactin)	Total No. of rats	No. of rats in which cycle prolonged†		
		0-2 days	3-5 days	> 5 days
50	5	4		1
100	10	5	2	3
200	12	4	7	1
400	6	0	4	2
Placental protein (mg)				
0.50	6	6		
1.0	16	10	4	2
2.0	14	9	3	2
5.0	11	4	6	1
10.0	10	0	2	8
15.0	11	1	2	8

* Injections were given twice daily subcutaneously. Ovine prolactin, 20 I.U./mg.

† The average duration of the 4 oestrus cycles prior to injection was used as the control period.

Table 4. EFFECT OF PROLACTIN OR THE PLACENTAL PROTEIN ON THE PIGEON CROP SAC

Total dose (mg)	No. of birds	Crop sac wt. (g)	Equivalent (μ g/mg)
Control weight		2.0 \pm 0.2	
Ovine prolactin*			
0.35	0	5.2 \pm 0.6	
1.05	3	9.5 \pm 3.3	
Placental protein			
70	3	9.2 \pm 0.5	
35	3	8.0 \pm 1.0	
17.5	3	10.0 \pm 1.7	
14.0	3	11.3 \pm 1.0	1.14
11.2	3	7.9 \pm 1.2	0.9
7.0	3	6.2 \pm 0.3	1.14
5.6	3	6.3 \pm 1.7	1.4
3.5	3	5.0 \pm 0.2	1.9
2.8	3	2.7 \pm 0.1	1.7

* Ovine prolactin, 10 I.U./mg.

the placental protein. When 200 μ g of ovine prolactin were administered in divided doses for four days, the return of oestrus was delayed by more than 3 days in 6 of 10 rats. It is apparent that, following the injection of placental protein, the results are variable. One mg or less infrequently delayed the return of oestrus, but larger amounts caused prolongation of the cycles in a

greater percentage of rats, and 5 mg or more frequently caused pseudopregnancy.

Dr. R. W. Bates kindly assayed this preparation for prolactin activity using the pigeon crop sac response. By the systemic assay in adult pigeons (Table 4) the placental protein had a potency of 1.4 u/mg compared with 0.25-0.5 u/mg by the local intradermal method in juvenile pigeons⁴. However, there was a quantitative difference between prolactin and the placental protein in the slope of the dose-response curve and also in the mean maximum weight of the crop sac. The latter was only about 10 g for the placental extract, while it is about 20 g with prolactin. Therefore, it is difficult to assign an accurate ratio of potency to the placental protein.

Twelve days after virgin albino rabbits were given 150 u chorionic gonadotrophin intravenously, ovine prolactin or the placental protein was injected into the mammary ducts according to the method of Bradley and Clarke⁵. Five days later the animals were killed and the mammary glands were examined after stripping off the overlying skin. Although no attempt was made to establish the minimum effective dose, 500 μ g of the placental protein caused extensive milk formation in the sector of the mammary gland which was injected (Fig. 3).

Additional biological investigations are now in progress to define the physiological role, if any, of this interesting protein.

I thank Dr. E. B. Astwood for his advice.

This work was supported by grants 2A-5166 and A-612 from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, U.S. Public Health Service.

HENRY FRIESEN

Pratt Clinic—New England Center Hospital
and Department of Medicine,
Tufts University School of Medicine,
Boston, Massachusetts.

¹ Josimovich, J. B., and MacLaren, J. A., *Endocrinology*, **71**, 209 (1962).

² Friesen, H., *Endocrinology*, **78**, 369 (1965).

³ Astwood, E. B., in *Ciba Found. Coll. Endocrinology, Bioassay of Anterior Pituitary Hormones*, **5**, 74, edit. by Wolstenholme, G. E. W. (Little, Brown and Co., Boston, 1953).

⁴ Bates, R. W., Garrison, M. H., and Cornfield, J., *Endocrinology*, **73**, 217 (1963).

⁵ Bradley, T. R., and Clarke, P. M., *J. Endocrinol.*, **14**, 28 (1956).

Influence of the Cerebral Cortex on the Cuneate Nucleus of the Monkey

NEARLY all neurones in the dorsal column nuclei of the cat are either excited or inhibited by stimulation of the pericruciate cortex¹⁻³, the excitation being carried entirely by the pyramidal tract, while inhibition involves an additional route^{2,3}. The cortically excited neurones occupy deep and rostral sites in the dorsal column nuclei, near the pyramidal terminations^{4,5}, while the cortically inhibited neurones are found more superficially. The latter, usually hair-sensitive neurones, possess peripheral inhibitory surrounds⁶; they may be identified with the cluster neurones, receiving large synaptic terminals⁷. The former are more often touch-sensitive or pressure-sensitive neurones possessing peripheral facilitatory surrounds⁶. Thus, neurones excited from one region of cortex are similarly excited from other cortical regions, ipsilateral and contralateral; the same rule holds for inhibition^{1,2}. Few neurones have been observed with both types of cortical influence or with no cortical influence whatever.

By sharp contrast, less than half the neurones in the cuneate nucleus of the monkey seem to be under cortical influence—and those that are often suffer a mixed influence, depending on the site of cortical stimulation. Furthermore, quite unlike that in the cat, cortical excitation in the monkey can often be shown to be monosynaptic.

In this investigation, the dorsal column nuclei and pericruciate cortex were exposed in dial-urethane-anaes-

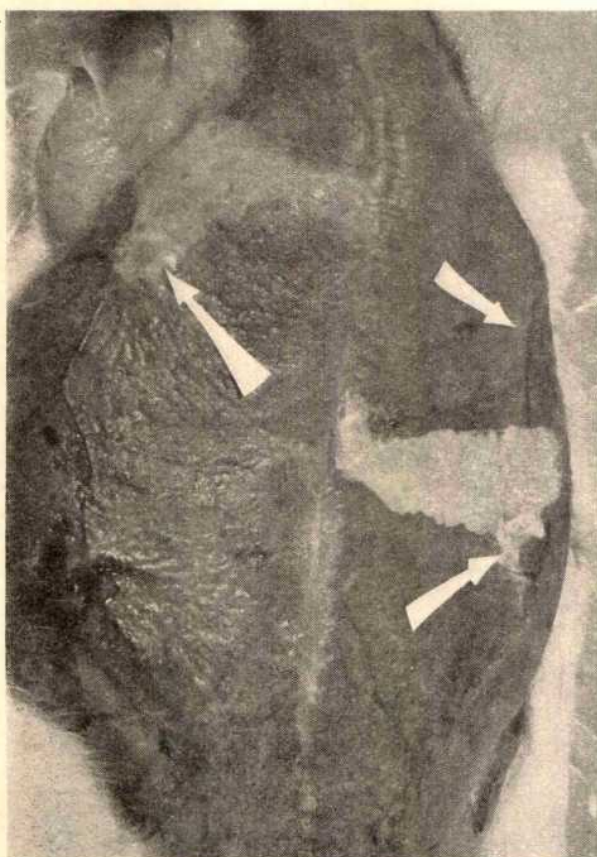


Fig. 3. Photograph of the mammary glands of a pseudopregnant rabbit. The glands in which milk is present (upper left and lower right) were injected with 500 μ g of the placental protein; the control gland was injected with saline.

thetized, curare-paralysed rhesus monkeys. Bipolar stimulating electrodes were placed on both precentral and postcentral arm and leg cortices, and needle electrodes were inserted into the palm and five digital phalanges of the hand ipsilateral to the cuneate recording site. Also, a concentric-needle electrode was stereotactically placed in nucleus ventralis posterolateralis for antidromic activation of certain cuneo-thalamic projection neurones. Micropipettes filled with sodium chloride were driven in a three-dimensional co-ordinate system with the obex as origin. Responses to 'natural' stimulation were also obtained.

Three-fourths of the cuneate neurones were isolated less than $1,000\mu$ in depth, in the lower component of a bimodal distribution. Of about 400 neurones, 44 per cent were measurably influenced by stimulation of one or more of the four cortical sites, and 90 per cent of these were isolated less than one millimetre in depth. Excitation and inhibition were observed equally often—but one in every ten of these neurones displayed both types of influence. Among the latter, nearly every possible combination of excitation and inhibition from the four cortical stimulus sites was observed. In fact, the pattern of cortical influence violated every rule established in the cat. Hair, touch, pressure and joint neurones were scattered throughout the nucleus and revealed all degrees of cortical influence. Cortically driven neurones, half of which could not be fired by any form of peripheral stimulation, were confined to a region⁸ about 1 mm in radius, centred 1.5 mm caudal and 1.5 mm lateral to the obex. Cortically inhibited neurones, on the other hand, were scattered throughout the nucleus. Only 38 per cent of the touch neurones were under cortical influence, while 74 per cent of the hair neurones could be affected. However, there appeared a pronounced tendency for touch neurones to be inhibited, while hair neurones received more than their share of excitation. Also, three in every five cortically affected neurones displayed large receptive fields, while nine in every ten neurones not affected by cortical stimulation possessed very small receptive fields.

The idea⁹ that cortically driven neurones are interneurones mediating inhibition on to the cuneo-thalamic projection neurones does not appear tenable in either the cat⁶ or the monkey. Although one in every four cortically driven neurones was shown to be a projection neurone, the highly restricted thalamic stimulus may have given a limited picture. Several of these projection neurones were monosynaptically driven from the cortex. Fig. 1 shows an intracellular recording of a small field, touch neurone fired antidromically from the thalamus at 0.7 msec latency and orthodromically from postcentral arm cortex in 3.0 msec and postcentral leg cortex in 1.0 msec; it was not affected by precentral stimulation. Evidently mild inhibition descended from the arm cortex, for 100/sec repetitive stimulation decreased the burst length; the decrease could not have resulted from recurrent inhibition, for similar stimulation of leg cortex increased the discharge reliably to two spikes. Alternatively, the route from

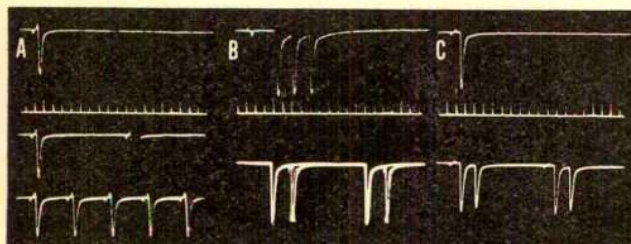


Fig. 1. Intracellular recording of neurone (1.0 lat, 2.0 caud, 2.6 deep) responding to tactual stimulation on ulnar half of dorsum of hand, from knuckles to wrist. A, Upper, response to thalamic stimulation (0.7 msec latency); middle, 100/sec stimulation; lower, 250/sec stimulation. B, Upper, response to 0.5 msec, 7.2-V square pulse to postcentral arm cortex; lower, 100/sec stimulation. C, Upper, response to 0.5 msec, 7.2-V square pulse to postcentral leg cortex; lower, 100/sec stimulation. Time pips are 1 and 5 msec

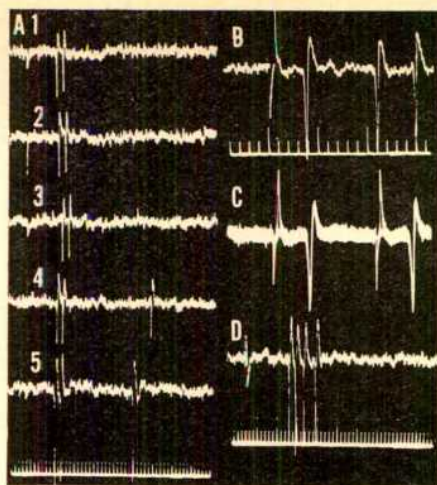


Fig. 2. Extracellular recording of neurone (1.0 lat, 1.0 caud, 1.2 deep) responding to hair deflexion anywhere over entire arm, shoulder, neck, side of head and half-way down torso. A, Response to 0.1 msec, 7.2-V pulse to skin of distal phalanges, from thumb (1) to little digit (5). Neurone responded well to 100/sec stimulation of any digit. B, Response to thalamic stimulation (0.5 msec, 7.2-V). C, Response to 100/sec thalamic stimulation. D, Response to 0.5 msec, 7.2-V pulse to postcentral arm cortex. Neurone followed 50/sec stimulation of cortex. Time pips are 1 and 5 msec

arm cortex to cuneate may not have been monosynaptic, and a cuneate interneurone may have been failing at 100/sec. Fig. 2 shows a wide field, hair neurone responding to electrical stimulation of all five digits, to the thalamus at 3.3 msec and to the postcentral arm cortex at 10.4 msec; all other cortical sites mildly inhibited the neurone. The late spikes produced by thalamic stimulation, a common occurrence, resulted either from excitation of a cortical loop to the cuneate nucleus or spread to some nearby, descending excitatory pathway. The former explanation is more likely, for such late spikes were the rule for cortically driven neurones, whether or not the neurones could be fired antidromically. The presence of these late spikes militates against hypothesizing a recurrent inhibitory loop, keeping brief the response of this neurone to skin stimulation.

The foregoing observations show clearly the hazards of extrapolating across such highly divergent phylogenetic lines as from carnivores to primates. Many of the properties and response patterns of the cat cuneate nucleus are seen in the monkey, but a wide variety of other patterns is also evident. From some point common to both, perhaps in late Cretaceous times, these two somatosensory relay systems have developed separate and distinctive organizations and may perhaps be treating their inputs in slightly different ways.

This work was supported by grants B396 and NB5136 from the National Institute of Neurological Diseases and Blindness, U.S. Department of Health, Education and Welfare.

F. HARRIS
S. J. JABBUR
R. W. MORSE
A. L. TOWE

Department of Physiology and
Regional Primate Research Center,
University of Washington, School of Medicine,
Seattle, Washington.

¹ Towe, A. L., and Jabbur, S. J., *J. Neurophysiol.*, **24**, 488 (1961).

² Jabbur, S. J., and Towe, A. L., *J. Neurophysiol.*, **24**, 499 (1961).

³ Levitt, M., Carreras, M., Liu, C. N., and Chambers, W. W., *Arch. Ital. Biol.*, **102**, 197 (1964).

⁴ Walberg, F., *Brain*, **80**, 273 (1957).

⁵ Kuypers, H. G. J. M., *J. Anat.*, **92**, 198 (1958).

⁶ Gordon, G., and Jukes, M. G. M., *J. Physiol.*, **173**, 263 (1964).

⁷ Rozsos, L., *Acta Morph. Acad. Sci. Hung.*, **8**, 105 (1958).

⁸ Kuypers, H. G. J. M., *Brain*, **83**, 161 (1960).

⁹ Andersen, P., Eccles, J. C., Schmidt, R. F., and Yokota, T., *J. Neurophysiol.*, **27**, 1080 (1964).

Differences in Variability of Discharge Frequency between Primary and Secondary Muscle Spindle Afferent Endings of the Cat

THE frequency of action potentials in a nerve fibre has long been known to be a function of the input intensity. Fluctuations in frequency when the stimulus is held constant have often been considered merely as unwanted 'noise' limiting the accuracy of the experimental results. However, these fluctuations may also limit the amount of information which can be transmitted down a nerve fibre and so are of interest *per se*. In a receptor or an isolated axon, these fluctuations may result from the basic processes of action potential initiation and are of interest in this context. If the variability had the same basis in different cells (for example, resistive noise), one would expect that it would be smaller, the larger the cell or axon, and this is often the case¹. However, one of us (P. B. C. M.) noticed that the discharge initiated from single secondary endings of muscle spindles of the cat seemed to be considerably more regular than that from the primary endings, though these latter have the larger afferent nerve fibres²; later this was also found to have been noticed by others^{3,4}.

In order to check this observation and to compare the predictions of two different mathematical models for the cause of the variability^{5,6}, approximately 200,000 inter-spike intervals from thirteen muscle spindle afferents (seven primaries, six secondaries) from the soleus muscle of the anaesthetized cat were analysed. Fusimotor activity was eliminated by section of the appropriate ventral roots. The methods of dissection⁷, classification of the afferent fibres^{2,7}, and application of stretches⁸ have been described previously. The discharge of single afferent fibres was recorded on magnetic tape for periods of 1-3 min, after stretching the muscle to a new length. Later, a high-speed timer and recorder⁹ measured the intervals between successive discharges to the nearest tenth of a msec and punched this in binary coded form on to eight-hole paper tape. These tapes were afterwards analysed by the English Electric KDF9 computer of the Oxford University Computing Laboratory.

Fifty or 100 intervals were sampled at a time from the longer periods of recording. The distribution of intervals of the second sample was compared to the first using Smirnov's lambda test. If the second sample was not statistically different at the 5 per cent level, it was added to the first sample to form an enlarged comparison sample and the process continued until a statistically different sample was found. Then the distribution of intervals and various statistics for the total comparison sample were printed out by the computer. The new sample, which was statistically different, was used to begin a new comparison sample. Statistically different samples were found more often than expected for a completely stationary process, and this could only in part be explained by the fact that noticeable adaptation often continued for a couple of minutes following a stretch.

Fig. 1 shows four examples of the relation between the variability of the discharge and its mean interval. For each of the endings, the coefficient of variation (the ratio of the standard deviation to the mean interval) is plotted against the mean interval on a log-log scale. Each point represents values for a sample of between 100 and 1,200 successive intervals; within a sample the intervals were normally distributed about the mean at the higher action potential frequencies. For the lowest frequencies the interval distributions were often positively skewed about the mean (more very long than very short intervals) as predicted by various mathematical models^{5,6}. The range of mean intervals was obtained by stretching the muscle to different lengths, all within the normal physiological range. Where, because of adaptation, the mean intervals of the discharges elicited by different length stretches overlapped, the coefficients of variation were similar. It will be observed for three of the endings (10-2, 10-5, 3-3)

that the coefficient of variation tended to fall to a constant value as the mean interval decreased. This was a typical finding and occurred for twelve of the thirteen endings. One primary ending (10-1, Fig. 1), however, behaved in almost the opposite manner and the reason for this is unknown. Such behaviour is not covered by the theories to be discussed and will not be further considered here.

The variability of all the primary and secondary endings was compared at a mean interval of 40 msec, which fell within the range of discharge of all the units examined. The average coefficient of variation for the seven primaries was 0.048 and the range was 0.035-0.068 (that is, the standard deviation was 3.5-6.8 per cent of the mean, and at the mean interval of 40 msec the average standard deviation was \pm msec). When the atypical primary ending was excluded the average was 0.045, and the range was 0.035-0.056. The average coefficient of variation for the six secondaries was 0.020, and the range was 0.018-0.023 (that is, the average standard deviation was 0.8 msec). In no case was the coefficient of variation for a secondary ending higher than it was for a primary ending at this particular interval (40 msec). The same was true for comparisons at shorter intervals. The greater variability of the primary endings was not due to their greater dynamic sensitivity² causing them to respond to small random movements of the electro-magnetic stretcher, for their variability did not change when the stretcher current was turned off and its position maintained by mechanical clamping (the filled circles of Fig. 1). Nor were the primary endings examined being significantly influenced by vascular pulsation, for this gives a rhythmic disturbance of their discharge, which is readily recognizable on the reciprocal pulse interval display⁸ which was observed throughout the recording; rather, the variation in their discharge appeared to be random. Thus it may be concluded that at frequencies above about 20/sec the secondary endings fire appreciably more regularly than do the primary endings. It therefore appears that they provide the more accurate signal of the length of the muscle, at least in the absence of fusimotor activity; for a given length of the muscle the absolute frequency of discharge of the two kinds of ending may be very similar². In addition, it may be noted that the length response of the secondary endings is much less disturbed by small dynamic stimuli than is that of the primary endings².

It is also interesting to consider the results in terms of the mathematical models of Verveen and co-workers^{1,5,10} and of Stein⁶ for the variability of neuronal discharge. Both models predict that the coefficient of variation should tend to a constant value at short intervals of discharge. This was indeed the observed behaviour. At very long

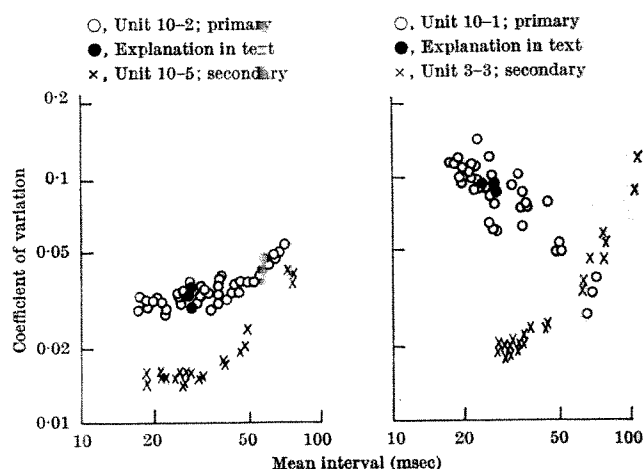


Fig. 1. The fractional variability of the discharge of single muscle spindle afferents plotted as a function of the mean inter-spike interval on a log-log scale.

intervals both models predict that the coefficient of variation should tend towards unity, the value for a random exponential distribution, but sufficiently long intervals for this situation have not been studied here. The region in which the coefficient of variation increases with increasing mean interval may be called the 'transition region', and here the two models lead to different predictions.

Verveen assumed that the disturbing noise which caused the variability was Gaussian in its distribution. This led him to conclude that in the transition region the standard deviation should increase as the square of the mean interval. The coefficient of variation should thus increase linearly with the mean interval, so that the right-hand side of plots such as those of Fig. 1 should tend to a straight line with a slope of 1. For the primary endings, the mean value of the slope in the transition region was indeed 0.98; but that of the secondary endings was 1.56, which seems to be too great a deviation to be readily accommodated by the theory. Stein's model⁶ assumes that there is a quantum or unit of excitation, and that these unit excitations occur randomly with a frequency determined by the stimulus and then decay exponentially in size with time. Summation and decay of unit excitations continue until threshold is reached. By computer simulation experiments (as in ref. 6) it has been found that in the transition region the coefficient of variation increases roughly as a power function of the mean, and that the power exponent increases as the number of quanta required to reach threshold increases. In addition, as the number of quanta increases, so the asymptotic value of the coefficient of variation decreases⁶. On both these criteria (high exponent, low asymptotic value) the excitation of the secondary ending would appear to depend on more quanta than that of the primary ending. The asymptotic values suggest that about 700 quanta are required to excite the primary ending to discharge, and that about 3,600 are required for the secondary endings. These figures also agree with computations based on the rather less accurate values of the slopes.

The nature of the quantal unit is, however, quite uncertain. One possibility is that it corresponds to the opening of a single pore in the receptor membrane, thus permitting the ionic flux which generates the receptor potential. A difficulty with this interpretation is that to give the best fit to our results the time constant for decay in the effectiveness of each quantum has to be 30–50 msec, which is far too long to correspond to the time-constant of the receptor membrane. Preliminary calculations indicate that this difficulty can be overcome if it is assumed that the closing of pores is also a random process and that the effectiveness of the pores is constant while open. This model yields very similar predictions and would fit the experimental results well if the mean time a pore stays open is of the order of 50 msec. It is possible that some of the variability may be due to other as yet unconsidered causes, such as the interaction of abortive impulses occurring in the different terminals of a single afferent ending¹¹.

R. B. STEIN
P. B. C. MATTHEWS

Laboratory of Physiology,
University of Oxford.

¹ Verveen, A., *Acta Morphologica Neerlandico-Scandinavica*, **5**, 79 (1962).

² Matthews, P. B. C., *Physiol. Rev.*, **44**, 219 (1964).

³ Eldred, E., and Tokizane, T., *Amer. J. Physiol.*, **183**, 612 (1955).

⁴ Hongo, T. (personal communication).

⁵ Ten Hoopen, M., and Verveen, A. A., in *Nerve, Brain and Memory Models*, edit. by Wiener, N., and Schade, J. P. (Elsevier, Amsterdam, 1963).

⁶ Stein, R. B., *Biophysic. J.*, **5**, 173 (1965).

⁷ Harvey, R. J., and Matthews, P. B. C., *J. Physiol.*, **157**, 370 (1961).

⁸ Matthews, P. B. C., *J. Physiol.*, **169**, 58P (1963).

⁹ Stein, R. B., *J. Physiol.* (1965).

¹⁰ Verveen, A. A., and Derksen, H. E., *Kybernetik*, **2**, 152 (1965).

¹¹ Katz, B., *J. Physiol.*, **111**, 248 (1950).

Squid Giant Axon: Repetitive Responses to Alternating Current Stimulation

SINUSOIDAL alternating currents were used by Hill, Katz, and Solandt¹ to stimulate the frog sciatic nerve. At low frequencies of stimulation, repetitive responses caused discrepancies between the observed threshold values of current and the values predicted by Hill's theory of excitation². Teorell³ used triangular-wave alternating currents to excite action potentials in the *Nitella* cell. At low stimulus frequencies, two spikes per current cycle were occasionally observed. Repetitive all-or-none responses to constant currents were reported by Katz⁴ in frog sciatic nerve, by Hodgkin⁵ in single crustacean axons, and by Hagiwara and Oomura⁶ in the squid giant axon. Hagiwara and Oomura⁶ also stimulated the squid giant axon with linearly rising currents. In these experiments, single-action potentials were followed by a steadily increasing depolarization of the membrane, but repetitive action potentials did not occur. Tasaki⁷ obtained similar results when he used linearly rising currents to excite single toad-nerve fibres. The present research was undertaken to determine whether the squid giant axon could produce repetitive all-or-none responses to triangular-wave and sinusoidal alternating-current stimuli and to observe the responses at various current amplitudes and frequencies.

Giant axons 300–500 μ in diameter were isolated from the squid *Loligo pealii*. Stimulating and recording electrodes made of 50 μ silver wire were inserted longitudinally in an axon as it lay in a shallow pool of sea-water at a temperature of approximately 21° C. The stimulating electrode extended 7 mm in each direction from the recording electrode, which was 1 mm in length. Sinusoidal and triangular-wave alternating currents were supplied by a Hewlett Packard 202A function generator in series with a resistance of 0.5, 1, or 2 M Ω . A Tektronix 502 differential-input oscilloscope was used to measure the potential difference between the recording electrode in the axon and a return electrode in the sea-water pool. It is assumed that the observed potential differences largely appeared across the membrane.

Membrane potential was recorded as a function of current at stimulation frequencies in the range 20–1,000 c/s and with peak currents up to 37 μ amp. Typical data are presented in Fig. 1. Before and after alternating-current stimulation, each axon was tested for all-or-none excitability with short current pulses. Thirty-eight axons yielded acceptable data.

When the squid axon was stimulated by sub-threshold currents, the recorded voltage increased continuously with the current amplitude. The current and voltage waveforms were related in approximately the same way as the current through a series RC circuit is related to the voltage across the capacitor. Thus a sinusoidal current yielded a sinusoidal voltage, while a triangular current produced a voltage waveform consisting of consecutive curved segments. This behaviour is to be expected, since the resting membrane can be represented by an RC ladder network.

All-or-none action potentials appeared when the membrane was excited by the outward-flowing or positive phase of the alternating current. Threshold values of the stimulating current and of its amplitude were frequency-dependent. However, the threshold membrane potential was constant to within ± 10 mV when the axon was excited by sinusoidal and triangular current waves of all amplitudes and frequencies that were tested.

A train of repetitive action potentials was observed during each current cycle when the axon was stimulated at frequencies of 20–80 c/s. All-or-none responses rarely occurred at frequencies less than 20 c/s. The spike trains always began before the stimulating current reached its peak value and often continued after the current had

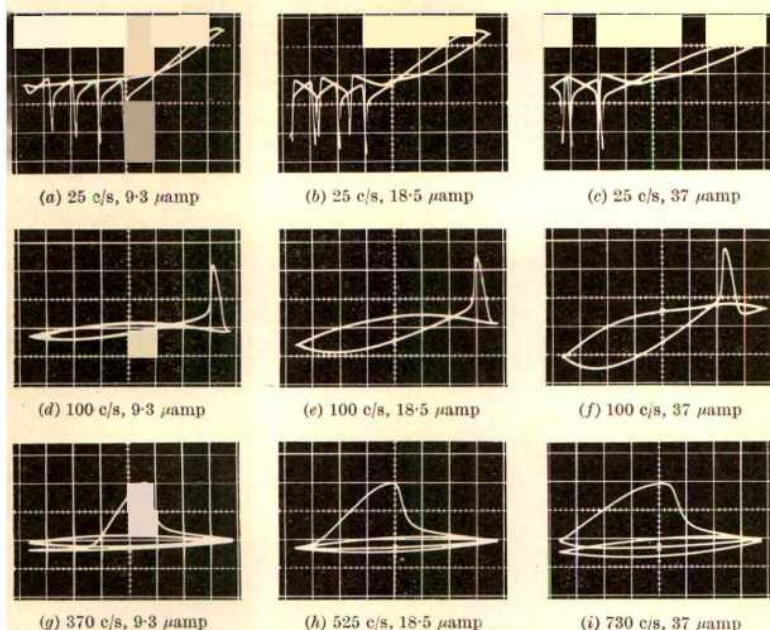


Fig. 1. Membrane potential as a function of triangular-wave current. Frequency and peak current of stimulus are indicated. Vertical scale: 50 mV/cm. Horizontal scales: (a), (d), and (g), 2.5 μ amp/cm; (b), (e), and (h), 5 μ amp/cm; (c), (f), and (i), 10 μ amp/cm. Positive current flows out of the axon.

begun to decrease. The threshold potentials of the individual spikes were approximately equal.

The trains of repetitive action potentials decreased in length as the stimulation frequency was increased. Typical lengths were four to six spikes at 25 c/s, two spikes at 50 c/s, and just one spike at 100 c/s. With frequencies in excess of 100 c/s, a single response occurred when the stimulating current had passed its peak and was decreasing. Above 150–300 c/s, a spike followed every second, third or fourth current maximum.

The preceding results may be compared with the data obtained by Hagiwara and Oomura⁶ when they stimulated the squid giant axon with linearly rising currents. In both studies, responses appeared at a critical membrane potential that was independent of the current and its time derivative. The alternating current, unlike the linearly rising current, was capable of eliciting repetitive responses, each of which occurred at approximately the same critical membrane potential.

This research was performed at the Marine Biological Laboratory, Woods Hole, Mass., while I was an employee of the National Institute of Mental Health, Bethesda, Maryland.

H. R. HIRSCH

Department of Physiology and Biophysics,
University of Kentucky, Lexington, Kentucky.

¹ Hill, A. V., Katz, B., and Solandt, D. Y., *Proc. Roy. Soc., Lond.*, B, **121**, 74 (1936).

² Hill, A. V., *Proc. Roy. Soc. Lond.*, B, **119**, 305 (1936).

³ Teorell, T., *Acta Physiol. Scand.*, **53**, 1 (1961).

⁴ Katz, B., *J. Physiol.*, **88**, 239 (1936).

⁵ Hodgkin, A. L., *J. Physiol.*, **107**, 165 (1948).

⁶ Hagiwara, S., and Oomura, Y., *Japan. J. Physiol.*, **8**, 234 (1958).

⁷ Tasaki, I., *Japan. J. Physiol.*, **1**, 1 (1950).

PHARMACOLOGY

Teratogenic Effects of Synthetic Compounds related to Trypan Blue: the Effect of 1,7-Diamino-8-naphthol-3,6-disulphonic Acid on Pregnancy in the Rat

In 1948 Gillman *et al.*¹ observed that the injection of the disazo dye, trypan blue, into pregnant rats produced congenital abnormalities in their offspring. Since then a number of papers dealing with the teratogenic

activity of several closely related dyes and chemical compounds, in various species, have been published, and attempts have been made to correlate molecular structure with teratogenic activity. Beaudoin and Pickering² tested several synthetic disazo compounds, and it is suggested, on the basis of their results, that a naphthalene nucleus with an NH_2 group in position 1, and two HSO_3 groups, preferably in positions 3 and 6, is at least necessary for teratogenicity. This communication reports the results of an investigation of the effect of such a compound—namely, 1,7-diamino-8-naphthol-3,6-disulphonic acid—on pregnancy in the rat.

Twenty female rats of a Wistar strain (average weight 200 g) were used. Preliminary experiments in non-pregnant does had shown that the LD_{50} (Kärber³) of the compound to be tested (hereafter referred to as compound A) was 66 mg/kg rat, so 5 mg in a distilled water solution were injected subcutaneously on day 8.5 of pregnancy. Control animals were injected with saline. The does were killed at 24-h intervals from 10.5 to 12.5 days of gestation, as pregnancies persisting beyond that time tended to be totally aborted. The uterine horns, liver, kidney, lung and spleen were removed and fixed in Bouin's fixative. After 24 h the

embryos were dissected out of their membranes in 70 per cent alcohol under a dissecting microscope, examined for abnormalities, and classified into developmental stages according to Christie⁴. The other tissues, including the placenta and yolk sac, were embedded in paraffin wax, and sectioned at 10 μ . Unstained sections, and sections stained with haematoxylin and eosin, were examined for the presence of dye granules, and for histological alterations, and compared with sections from the control pregnant animals.

Ascending chromatography of the compound tested, and of trypan blue, was carried out in *n*-butanol-pyridine-water (1:5:4 by volume) as described by Beck and Lloyd⁵.

Table 1. EFFECTS ON PREGNANCY OF AZO DYES OR RELATED COMPOUNDS INJECTED ON OR ABOUT DAY 8.5 OF PREGNANCY

Compound	No. of mothers	Pregnancies terminating before killing day	% Total resorption	% of all pregnancies	No. of implantations	% Survivors re-sorbed	% Survivors mal-formed
Control	11	0	0	100	113	6.2	0
Trypan blue	45*	4	16	80	407	44	49
Niagara blue 4B	15*	7	13	80	126	15	4
Compound 8	8†	0	0	100	76	14	3
α -Toluidine	10*	0	0	100	109	8	0
Compound A	20	35	35	30	61	13	0

* Data from Wilson (ref. 5).

† Data from Beaudoin and Pickering.

As can be seen from Table 1, the quantity of compound A injected (5 mg) produced an incidence of 35 per cent each for maternal death and total resorption before the killing day, these figures being higher than those quoted for any of the other compounds listed. In the surviving pregnancies, however, the average number of embryos per litter was no different from that in other experimental groups or controls. Some increase in the percentage resorption (13 per cent) was present, but was no greater than with the least toxic of the dyes tested.

Enlargement of the adrenals and spleen (as has previously been reported by Gillman *et al.*¹ for trypan blue) and of the kidney was present in the injected rats. Histological examination showed lymphoid hyperplasia in the liver, spleen and lungs. Degeneration of the cells of

the entire proximal convoluted tubule of the kidneys was found with accumulation of eosinophilic material within the remaining basement membrane and in the tubules in the outer medulla, in which cellular debris including droplets of basophilic material was present. The glomeruli, distal convoluted tubules and remaining kidney structure appeared normal. The placenta appeared normal in structure. No dye was found in the unstained sections of any of the tissues examined.

Table 2 shows the stage of development attained by the embryos at the time of killing. All exhibited general developmental retardation of the order of one half to one stage⁴, but no abnormalities were present. As the entire litter was either aborted or retarded in development, it seems probable that this was related to the degeneration of the renal proximal tubule in the mother, rather than to a direct effect of the dye on the embryos.

Chromatography of compound A showed that it was not identical with either of the commonly described red or purple contaminants of trypan blue.

Table 2. DEVELOPMENTAL STAGE OF EMBRYOS AT TIME OF KILLING IN INJECTED GROUP AS COMPARED WITH CONTROL RATS

Day of killing	Developmental stage	Normal stage reached
10-5 (1)	8 at stage 16	18-19
	7 at stage 17	
10-5 (2)	4 at stage 16	18-19
	4 at stage 17	
11-5 (1)	3 at stage 20 (early)	20 (late)-21
11-5 (2)	2 at stage 19	20 (late)-21
	9 at stage 20 (early)	
11-5 (3)	3 at stage 19	20 (late)-21
	5 at stage 20 (early)	
12-5	8 at stage 22 (early)	22 (late)

The chemical formulae of trypan blue and the other known teratogenic disazo dyes tested in Table 1 include two 8-naphthol rings (one in the case of compound 8) substituted in the 1 and 7 positions with amino groups, and with two sulphonic acid groups in the 3,6 positions, linked through di-*o*-toluidine, or di-*o*-anisidine. As *o*-toluidine has been found not to have teratogenic activity, it seemed likely that reduction of trypan blue *in vivo* over the disazo linkage could release the active part of the molecule, that is, compound A. However, this investigation has clearly shown that this is not so, and it would appear that it is to the whole molecule rather than parts of it that one must look for teratogenic activity.

I thank the Clayton Aniline Company for supplying the compound tested in this investigation.

GEORGE A. CHRISTIE

Department of Anatomy,
University of Glasgow.

¹ Gillman, J. C., Gilbert, C., Gillman, T., and Spencer, I., *S. Afr. J. Med. Sci.*, **13**, 47 (1948).

² Beaudoin, A. R., and Pickering, M. J., *Anat. Rec.*, **137**, 297 (1960).

³ Kärber, G., *Arch. Exp. Path. Pharmacol.*, **162**, 480 (1931).

⁴ Christie, G. A., *J. Morph.*, **114**, 263 (1964).

⁵ Wilson, J. G., *Anat. Rec.*, **123**, 313 (1955).

⁶ Beck, F., and Lloyd, J. B., *J. Embryol. Exp. Morphol.*, **11**, 175 (1963).

Biphasic Dose-response Relationship with Acetylcholine on the Heart of the Mollusc *Tapes turgida*

THE heart of the mollusc *Tapes turgida* has been shown to be very sensitive to acetylcholine¹ and provides a satisfactory alternative to the heart of *Venus mercenaria*, a species not found in Australia, for the assay of acetylcholine.

In the experiments described here, only the ventricle was used, and this was suspended between threads tied at the anterior and posterior vessels, in an organ bath containing sea-water. In most experiments, oxygen containing 5 per cent carbon dioxide (carbogen) was bubbled through the bath, though in some, no aeration at all was provided. Under the conditions of assay,

when the bath fluid was changed frequently, the heart continued to beat constantly without aeration.

Acetylcholine produces a negative inotropic effect on the hearts of both *Venus* and *Tapes* and the dose-response relationship is particularly steep in both species. A concentration of acetylcholine between 10^{-10} and 10^{-9} g/ml. is generally sufficient to produce complete block.

During an examination of the effect of concentrations of acetylcholine less than 10^{-10} g/ml. we have found that the heart showed another inhibitory phase, often proceeding to a complete block, but only when aeration with carbogen was used. The dose-response curve over the range of acetylcholine concentrations studied showed a biphasic effect (Fig. 1).

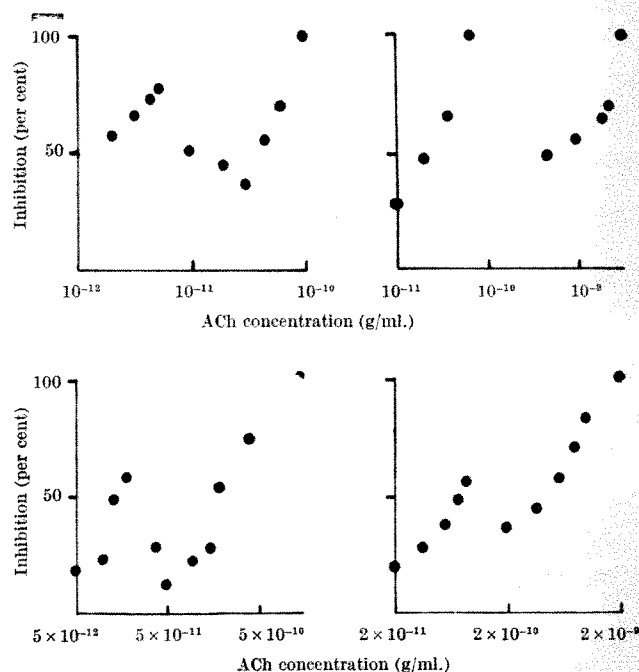


Fig. 1. Graphs showing the relationship between concentration of acetylcholine and the percentage reduction in the amplitude of the heart beat in four hearts of the mollusc, *Tapes turgida*

Preliminary experiments without aeration failed to reproduce inhibition at the lower concentrations of acetylcholine.

A similar biphasic dose-response relationship with acetylcholine has been described recently for the inhibitory effect on the perfused frog heart², and for the stimulation of the frog rectus abdominus muscle³.

This biphasic relationship presents an inherent source of error when the *Tapes* heart, aerated with carbogen, is used as an assay preparation for acetylcholine. Particularly is this so if the 'matching' technique is used, where the concentration of the unknown solution is estimated by matching a response on the heart (generally about 50 per cent inhibition) with that produced by a standard solution of acetylcholine.

With this technique it is possible to match responses produced by the unknown and standard solutions on different slopes of the biphasic dose-response curve.

However, preliminary experiments suggest that this difficulty does not arise if the heart is allowed to beat in sea-water without aeration.

P. R. CARROLL
G. B. CHESHER

Department of Pharmacology,
University of Sydney, Australia.

¹ Ladd, R. J., and Thorburn, G. D., *Austral. J. Exp. Biol. and Med. Sci.*, **33**, 207 (1955).

² Boyd, I. A., and Pathak, C. L., *J. Physiol.*, **176**, 191 (1965).

³ Teitel, A., *Arch. intern. Pharmacodyn.*, **152**, 1 (1964).

HAEMATOLOGY

Synthesis of the α_1 -Glycoprotein (Darcy) of Rat Serum by the Liver

AN α_1 -glycoprotein present in normal rat serum has been observed to increase markedly in concentration in conditions characterized by tissue injury or cell death¹. Based on the reduced response of the protein to tissue injury in partially hepatectomized rats, it has been suggested that the liver is the tissue of origin^{2,3}. The present communication reports the demonstration of the synthesis of α_1 -glycoprotein (Darcy) by the liver during the acute phase of an inflammatory response.

An anaesthetized, adult, male rat of Sprague-Dawley origin weighing approximately 400 g was injected with 1.0 ml. of a sterile solution of spirits of turpentine, N.F., in corn oil (v/v); 0.5 ml. subcutaneously in the scapular area and 0.25 ml. intramuscularly in each thigh. Twenty-four hours later, the rat was exsanguinated by cardiac puncture under ether anaesthesia and the liver removed aseptically.

The tissue culture and radio-immuno-electrophoretic techniques described by Hochwald *et al.*⁴ and by Williams *et al.*⁵ were followed. The liver was minced and duplicate 500 mg (wet weight) portions were incubated in 25-ml. Erlenmeyer flasks containing 3 ml. of Hanks's buffered salts solution at 37°C for 30 min to deplete the system of amino-acids. The salts solution was removed and replaced by 3 ml. of a medium consisting of the vitamin mixture recommended by Eagle *et al.*⁶, 600 units penicillin, 600 μ g streptomycin, 0.5 per cent ovalbumin, and the following compounds (mmole $\times 10^{-3}$): glucose, 48; L-asparagine, 0.9; L-glutamine, 4.5; DL-tryptophan, 0.09; and L-hydroxyproline, 0.45. As a source of other essential amino-acids and the radioactive label, 10 μ c. of ¹⁴C-algae hydrolysate (1.4 mc./mg) (New England Nuclear Corporation, Boston, Massachusetts) were added to each culture flask. The

cultures were incubated for 6 h at 37°C in an atmosphere of 95 per cent oxygen and 5 per cent carbon dioxide. Following incubation, the contents of the flasks were frozen and thawed once, centrifuged, and the supernatant dialysed against four changes of 0.14 M sodium chloride over a 48-h period. The dialysand was concentra-

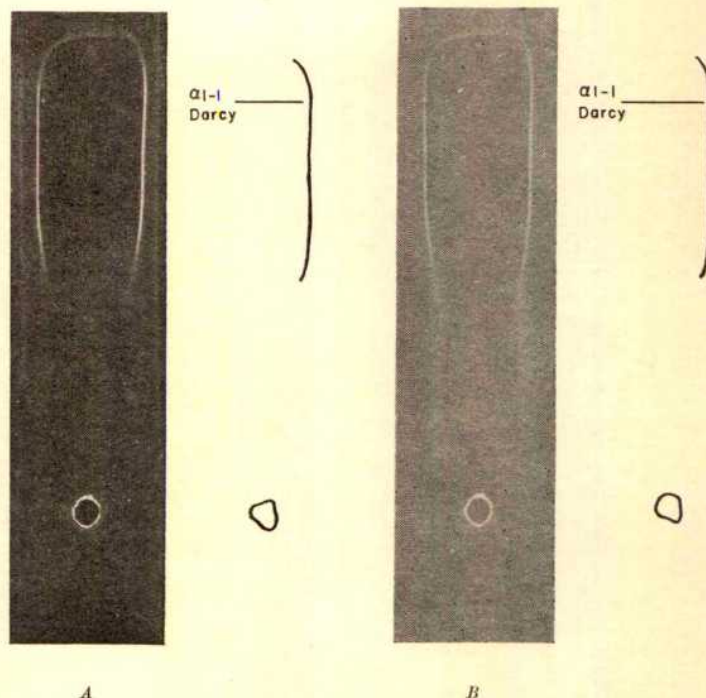


Fig. 2. A, Photograph of results of immunoelectrophoretic analysis of tissue culture fluid and serum using monospecific antiserum. B, Autoradiograph of same slide

ted 20-fold by dehydration with 'Aquacide' (Calbiochem, Los Angeles, California). The concentrated culture fluid was analysed by immunoelectrophoresis in 0.05 M veronal buffer, pH 8.2, according to Scheidegger⁷, using a 1:1 dilution of serum from the donor rat as a protein carrier. After washing and drying the slides, autoradiography was carried out by the method of Hochwald *et al.*⁴, using Kodak 'Royal Ortho' sheet film and an exposure time of 2 weeks. The preparation of the anti-whole serum and the specific antiserum in rabbits has already been described^{8,9}.

Fig. 1A shows the photograph of the results of the immunoelectrophoretic analyses of the concentrated tissue culture fluid and carrier proteins. Fig. 1B is an autoradiograph of the same slide. Radioactive labelling of the α_1 -glycoprotein (Darcy) indicative of synthesis by the liver was unequivocally demonstrated. Also of interest was the incorporation of radioactivity into 12 other proteins. These are depicted schematically and designated according to a system of nomenclature previously reported⁸. Results of similar analyses using the monospecific antiserum are presented in Figs. 2A and B, respectively. These results provide independent confirmation of the findings shown in Fig. 1 with respect to the α_1 -glycoprotein (Darcy).

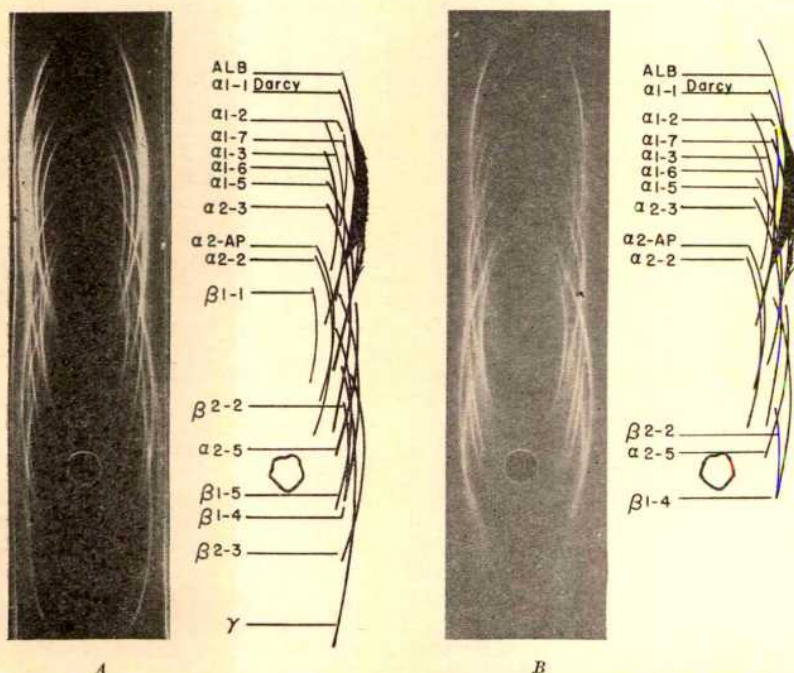


Fig. 1. A, Photograph and schematic representation of results of immunoelectrophoretic analysis of mixture of tissue culture fluid and carrier serum proteins from donor rat. B, Photograph and schematic depiction of autoradiograph of same slide

This work was supported by grant CA-02368 from the U.S. Public Health Service.

H. E. WEIMER
D. C. BENJAMIN

Department of Medical Microbiology and Immunology,
Center for the Health Sciences,
University of California, Los Angeles.

D. A. DARCY

Chester Beatty Research Institute,
Royal Cancer Hospital, London.

¹ Darcy, D. A., *Brit. J. Exp. Path.*, **45**, 281 (1964).

² Neuhaus, O. W., and Liu, A., *Proc. Soc. Exp. Biol. and Med.*, **117**, 244 (1964).

³ Darcy, D. A., *Brit. J. Exp. Path.*, **46**, 155 (1965).

⁴ Hochwald, G. M., Thorbecke, G. J., and Asofsky, R., *J. Exp. Med.*, **118**, 315 (1961).

⁵ Williams, C. A., jun., Asofsky, R., and Thorbecke, G. J., *J. Exp. Med.*, **118**, 315 (1963).

⁶ Eagle, H., Oyama, V. I., Levy, M., and Freeman, A. E., *J. Biol. Chem.*, **226**, 191 (1957).

⁷ Scheidegger, J. J., *Intern. Arch. Allergy*, **7**, 103 (1955).

⁸ Benjamin, D. C., and Weimer, H. E., *J. Immunol.*, **91**, 331 (1963).

⁹ Darcy, D. A., *Immunology*, **3**, 325 (1960).

A New Bisalbuminaemic Family

THIS communication reports the first bisalbuminaemic family found in France. As the family tree shows (Fig. 1), the anomaly seems to be due to a co-dominant, autosomal

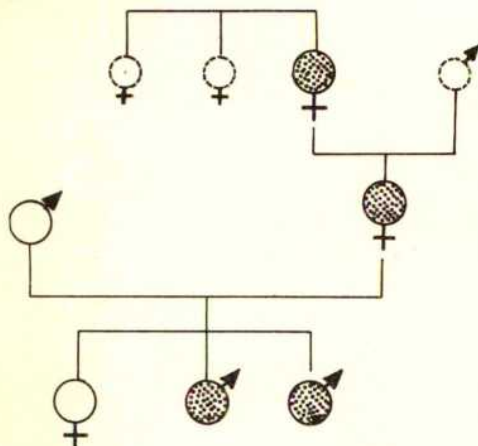


Fig. 1. The family tree. Positive cases, black spotted circles; negative cases, blank circles; cases not examined, broken circles



Fig. 2. Cellulose-acetate strip electrophoretogram of a positive case

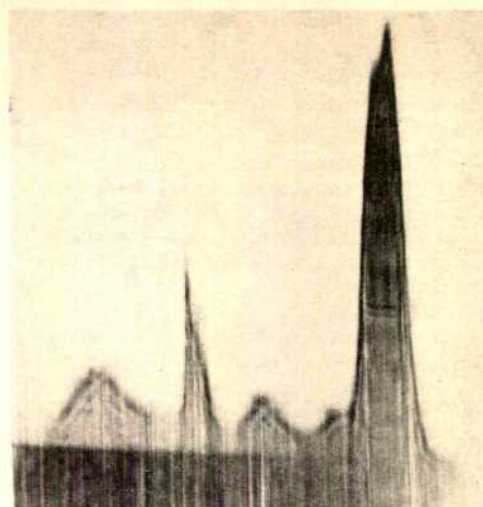


Fig. 3. Free electrophoresis of one positive case



Fig. 4. Immunoelectrophoretic analysis of a positive case. Development with equine anti-human serum. The serum of the subject is diluted to 1/30th; so the α_1 -globulin traits are distinctly separated from the albumin trait

allele at the albumin locus; we have confirmed this general conclusion. The separation of the two albumins was excellent on a cellulose acetate strip (Fig. 2) and shows, without any doubt, that the anomalous albumin is the slower one. But free electrophoresis using a Perkin-Elmer apparatus disclosed only a weak 'shoulder' on the cathodic side of the albumin band (Fig. 3). Immunoelectrophoresis and the Ouchterlony test using anti-human serum, or specific anti-human albumin serum, did not show any difference between the two albumins (Fig. 4). The anomalous albumin, evaluated after dye elution on the cellulose acetate strip, represents in all cases about 40 per cent of the total albumin. The subjects presenting the anomalous trait were normal in all other respects.

A recent check disclosed that about fifteen bisalbuminaemic families have been reported in the literature¹. Nearly always, the anomalous albumin demonstrated the same characters. Only Tarnoky *et al.*¹ and Wieme² conclude that the anomalous albumin was the faster-moving one. But in these cases, due perhaps to technical imperfections, the separation of the two bands was very poor and so any comparison with normal sera is not likely to lead to any clear-cut conclusion. We agree with Harris³ that bisalbuminaemia could be a unique hereditary trait. Taking into account the chemical results obtained by Gitlin *et al.*⁴, it may be attributed to a mutation involving a specific point in the albumin polypeptide chain.

GEORGES SANDOR
LOUIS MARTIN
MICHEL PORSIN
ALAIN ROUSSEAU
RENÉ MARTIN

Hospital and Protein Physico-chemistry
Laboratory,
Pasteur Institute,
Paris.

¹ Tarnoky, A. L., and Lestas, A. N., *Clin. Chim. Acta*, **9**, 55 (1964).

² Wieme, R. J., *Clin. Chim. Acta*, **5**, 443 (1960).

³ Harris, H., *Brit. Med. Bull.*, **17**, 217 (1961).

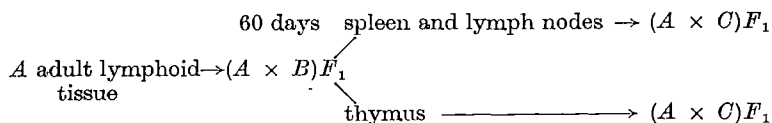
⁴ Gitlin, D., Schmid, K., Earle, D. P., and Gevillier, H., *J. Clin. Invest.*, **40**, 820 (1961).

IMMUNOLOGY

Bone Marrow as the Major Source of Potential Immunologically Competent Cells in the Adult Mouse

PREVIOUS work from this laboratory¹⁻⁴ has suggested that immunologically competent cells are derived from foetal liver during embryonic life and that their 'functional maturation', or proliferation, or both, is dependent on the residence of these cells in thymic tissues. The source of these lymphoid precursor cells during adult life is not known. Using chromosomally marked cells, it has been shown, however, that when cell suspensions of various adult lymphoid tissues are injected into lethally irradiated mice only those cells derived from bone marrow are found in the thymus of the host in significant numbers; while the cells from other lymphoid tissues readily proliferate in the peripheral lymphoid tissues of the host, they are only rarely found in the thymus⁵. In experiments involving the restoration of immunological competence in lethally irradiated thymectomized adult mice, Miller *et al.*⁶ concluded that "marrow cell suspensions lack adequate numbers of immunologically competent cells but contain precursor cells" which mature under the influence of the thymus. Furthermore, Feldman and Globerson⁷ have reported work which suggests that the immunological reactivity manifested by thymectomized mice which have been lethally irradiated, but protected with bone marrow cells and restored to immunological competence with an allogeneic thymic graft, may be due to the donor marrow cells rather than to cells from the thymic graft. Taken together, these results suggest that bone marrow is the source of potential immunologically competent cells in the adult. It is the purpose of this communication to present data which suggest that bone marrow may be the major, or perhaps sole, source of lymphoid precursor cells in the adult mouse.

The presence of potential immunologically competent cells was demonstrated by a modified parental- F_1 hybrid, 'graft-versus-host', method. When adult lymphoid tissues from a homozygous donor are injected into sub-lethally irradiated F_1 hybrids, one parental strain of which is identical to that of the donor, a significant number of deaths will occur as a result of an immunological reaction by the donor cells against the transplantation antigens of the second parent of the hybrid. Survivors can be obtained, however, by transplanting small numbers of donor cells. If it is postulated that mature immunologically competent cells are consumed, that is, 'allergic death', during the course of this graft-versus-host reaction⁸, then surviving mice which were injected with mature cells only should no longer contain lymphoid cells of donor origin. On the other hand, immature lymphoid cells (potential immunologically competent cells) are incapable of responding to the foreign antigens of the host¹⁻⁴ and, as was found with foetal liver cells¹⁻⁴, should survive in the primary host in significant numbers. On this basis, graded numbers of cells from various lymphoid tissues of adult homozygous mice were injected into groups of sub-lethally irradiated F_1 hybrids. The spleen and lymph node cells and cells from the thymus of the mice which survived 60 days were then injected into a second F_1 hybrid (one parental strain identical to that of the donor but the second parental strain differing from the second parent of the first F_1 hybrid). Death of the secondary recipients was taken as evidence for the presence of lymphoid precursor cells in the original inoculum. The experimental design may be summarized as follows:



The lymphoid organs used were spleen and lymph node, thymus, bone marrow, liver and peripheral blood. These tissues were obtained from 12-14-week-old A/HeJ male and female mice. Where necessary, the tissues were gently disrupted in a glass homogenizer, and the cells were suspended in Tyrode's solution. Graded numbers of viable nucleated cells were injected into ($BALB/c \times A$) F_1 mice which had just received 500-rad whole-body X-radiation. After 60 days, the survivors were killed individually; their spleen and lymph nodes and thymus were gently disrupted, and the resultant cell suspensions were injected into one thymus) or two (spleen and lymph node) sub-lethally irradiated (500 rads) ($C57L \times A$) F_1 mice. In addition, lymphoid tissues were taken from adult $CBA-T6T6$ mice and, as already described, injected into sub-lethally irradiated ($C57BL/10 \times CBA-T6T6$) F_1 mice. After 80 days these mice were killed and their tissues (thymus, lymph node, spleen and bone marrow) prepared for chromosome analyses⁹. $CBA-T6T6$ mice contain two distinct minute chromosomes; their presence among the metaphase plates of the F_1 hybrid host would provide direct evidence of the survival of the donor cells.

Table 1 SIXTY-DAY MORTALITY IN SUB-LETHALLY X-IRRADIATED (500 RADS) PRIMARY AND SECONDARY F_1 HYBRID RECIPIENTS OF ADULT LYMPHOID TISSUES. THE SECONDARY HOSTS RECEIVED EITHER SPLEEN AND LYMPH NODES OR THYMUS FROM THE PRIMARY RECIPIENTS

Adult tissue injected	No. of cells $\times 10^6$	60-day mortality (No./total)		
		Primary hosts	Spleen + lymph node	Thymus
Spleen and lymph node	0.9	7/10	0/6	0/3
	1.0	8/15	1/10	
	2.5	10/10		
Thymus	2.1	0/10	0/20	
	2.5	3/10	0/8	
	7.0	0/10	4/20	1/10
Peripheral blood	0.2	1/10	0/18	0/10
	0.2	8/10	0/4	
	0.5	9/10	0/2	0/1
	1.0	8/10	0/4	
Bone marrow	2.1	0/10	5/20	
	6.0	0/10	11/20	0/10
	7.0	3/10	9/14	
Liver	1/40 adult liver	0/10	0/14	0/7
None	1/4 "	3/10	0/20	0/10
		0/36	3/49	0/17

Table 2. $CBA-T6T6$ CELLS IN THE LYMPHOID TISSUES OF SUB-LETHALLY X-IRRADIATED ($C57BL/10 \times CBA-T6T6$) F_1 HOSTS 80 DAYS AFTER THE INTRAPERITONEAL INJECTION OF VARIOUS ADULT $CBA-T6T6$ LYMPHOID TISSUES

Adult tissue injected	No. of cells $\times 10^6$	$CBA-T6T6$ cells/total mitotic figures			
		Bone marrow	Spleen	Lymph nodes	Thymus
Spleen and lymph nodes	1.0	0/50	0/50	* 23/69	0/50
Thymus	3.0	* 23/200	0/77	* 21/118	0/39
Peripheral blood	0.3	* 23/125	0/38	0/14	0/28
Bone marrow	7.0	2/34	1/21	2/15	3/23
Liver	8.0	* 24/125	* 21/50	* 21/50	0/50

* These mitotic figures contained one typical $T6$ minute chromosome and another small somewhat atypical minute chromosome. As there was no other evidence of radiation damage to the chromosomes in these plates, they were classified as possible $T6T6$.

A significant number of deaths occurred among the secondary hosts only when they had received spleen cells from mice which had been injected with adult bone marrow (Table 1). There were no deaths among the mice which received thymus cells from the primary hosts. Further, the $CBA-T6T6$ chromosomal marker was found consistently and unequivocally only in those mice which had received bone marrow cells (Table 2). While there were cells which might have been of $CBA-T6T6$ origin in the peripheral lymphoid tissues of mice given other adult lymphoid tissues, $CBA-T6T6$ cells were found in the thymus of the host only when bone marrow had been given.

While it is known that bone marrow contains relatively few mature immunologically competent cells, taken together, these results, based both on functional and on morphological criteria, support the thesis that bone marrow serves the adult mouse

as a major, or perhaps as the sole, source of potential immunologically competent cells. Furthermore, these results imply that the thymus is not the source of lymphoid precursor cells, but rather that it provides a critical site for the maturation of these cells.

M. L. TYAN
L. J. COLE

Biological and Medical Sciences Division,
U.S. Naval Radiological Defense Laboratory,
San Francisco, California.

¹ Tyan, M. L., and Cole, L. J., *Transplantation*, **1**, 847 (1963).

² Tyan, M. L., and Cole, L. J., *Transplantation*, **2**, 241 (1964).

³ Tyan, M. L., *Science*, **145**, 934 (1964).

⁴ Tyan, M. L., and Cole, L. J., *Transplantation* (in the press).

⁵ Ford, C. E., and Micklem, H. S., *Lancet*, **1**, 359 (1963).

⁶ Miller, J. F. A. P., Leuchars, E., Cross, A. M., and Dukor, P., *Ann. N.Y. Acad. Sci.*, **120**, 205 (1964).

⁷ Feldman, M., and Globerson, A., *Ann. N.Y. Acad. Sci.*, **120**, 182 (1964).

⁸ Gorer, P. A., and Boyse, E. A., *Immunology*, **2**, 182 (1959).

Immunogenic Activity of Lipids of *M. tuberculosis*

LIPIDS of mycobacteria are known to be immunogenically active components of tubercle bacilli¹. First of all, it was surprising that mycobacteria contain lipids up to 35 per cent of their dry weight². This fact did not escape the notice of immunologists. It seemed highly improbable that this fraction of the mycobacteria should not contain antigens or—more likely—haptens. Since alcohol-soluble lipids form stable aqueous suspensions, they can be examined relatively easily. However, the majority of mycobacterial lipids consists of waxes which form no stable aqueous suspensions and usually exhibit an anti-complementary action. By means of a newly developed microprecipitin technique³ it became possible to investigate all kinds of lipids, independently from their solubility in organic solvents.

The extracts were prepared according to the method described by Aebi *et al.*⁴. Seven fractions were obtained: fats, phosphatides, waxes A, B, C (cord-factor) and D, and firmly bound lipids. It has been shown that only three of these fractions contained compounds capable of combining with antibody⁵. These fractions were the phosphatides and the waxes B and D. All other lipids were serologically inactive. The phosphatides and the waxes B showed no strain-specificity. However, the wax D might have strain-specificity⁶. It is interesting to note that C (cord-factor), the toxic substance of mycobacteria, did not combine with antibody. Consequently, antitoxic immunity might be impossible.

The waxes B did not always contain serologically active substances. This might be due to minor variations of the fractionating procedures. The active substance of the phosphatide fraction consisted of phospholipids, which contained inositol and 2–5 mannoses⁷. After saponification, phospholipids gave no precipitation, while with the water-soluble part of the wax D a weak precipitation could be observed. (Pure phospholipids and many other fractions were kindly supplied by Prof. E. Lederer and Mme. E. Vilkas, Gif-sur-Yvette, France.) The hydrophobic part of the molecules (for example, mycolic acid) had no antibody-combining capacity. Evidence available at present indicates that the determinant groups of lipid haptens are hydrophilic.

The immunogenic activity of mycobacterial lipids was experimentally tested in guinea-pigs. Groups of 10 guinea-pigs each of mixed colour and sex, approximately 500 grams in weight, were used. Groups of guinea-pigs were inoculated subcutaneously with phosphatides (0.5 mg phosphatide per ml.), wax B (0.25 mg wax B per ml.), wax D (0.5 mg wax D per ml.) with and without bovine serum albumin (0.5 mg/ml.) in 1 ml. of Freund's adjuvant. Control animals received Freund's adjuvant alone (1 ml., 'Arlacel A', Paraffinöl, Merck, saline), heat inactivated bacteria (1 mg/ml.) of the strain H 37 Rv, and living bacteria of the BCG strain (1 mg/ml.).

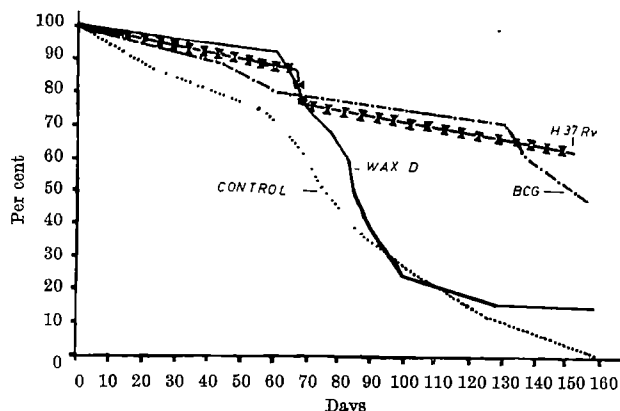


Fig. 1. Survival-time of guinea-pigs (wax D). Day 0 represents the day of challenge (4 months after immunization)

In order to avoid non-specific reactions, the animals were infected with 1 mg of virulent bacteria of the strain H 37 Rv 4 months after immunization. They were observed for 160 days. After that period all control animals inoculated with Freund's adjuvant alone died. The tuberculosis was diagnosed macroscopically.

Fig. 1 shows the results of a typical experiment. The survival time of the control group and of the test group (wax D) did not differ significantly. The results of the other control experiments (heat-killed H 37 Rv and BCG) indicated a partial protection against tuberculosis; the difference between the survival time of these groups is significant. The phosphatides, waxes B and waxes D failed to provide measurable immunity, even when administered together with bovine serum albumin.

B. STOSS
R. HERRMANN

Robert Koch Institute,
Berlin.

¹ Crowle, A. J., *Bact. Rev.*, **22**, 183 (1958).

² Asselineau, J., *Fortschr. Tök.-Forsch.*, **5**, 1 (1952).

³ Stoss, B., *Zbl. Bakt. I Orig.*, **179**, 72 (1960).

⁴ Aebi, A., Asselineau, J., and Lederer, E., *Bull. Soc. Chim. Biol.*, **35**, 661 (1953).

⁵ Stöss, B., and Herrmann, R., *Z. Immun.-Forsch.*, **123**, 492 (1962).

⁶ Stoss, B. (unpublished results).

⁷ Vilkas, E., and Lederer, E., *Bull. Soc. Chim. Biol.*, **42**, 1013 (1960).

PATHOLOGY

Cutaneous Elastin in Ehlers-Danlos Syndrome

EHLERS-DANLOS syndrome (cutis hyperelastica, india-rubber skin), a rare heritable generalized disorder of the connective tissues, is characterized by hyperextensibility of the skin, hyperlaxity of the joints, fragility of the skin, and, not infrequently, by one or a combination of such internal manifestations as cardiac anomalies, dissecting aneurysm of the aorta and diaphragmatic hernia¹. Previous investigations directed towards identifying the fundamental defect in the connective tissues, using the methods of light and electron microscopy, have led to contradictory results. Many investigators have described an increase in the amount of elastin in the corium while others have observed a normal or even a decreased amount of elastin¹. Similar controversy exists over the possibility that the collagen is either quantitatively or qualitatively abnormal in this disease^{1,2}. The purpose of the work reported here was to isolate elastin quantitatively from the skin of patients with this disease and from the skin of normal, control subjects, and to examine chemically the purified elastin isolated from these two sources.

Normal human skin was obtained as necropsy specimens from the thighs of Caucasian females. The skin from four female patients with Ehlers-Danlos syndrome (E-D) was obtained as biopsy material from the same site as in

the control subjects. All test subjects demonstrated the complete syndrome. Hair and subcutaneous fat were removed mechanically as completely as was possible. The skin, after being cut into small pieces and frozen in liquid nitrogen, was crushed by percussion in a small metal mill. The powdered tissue was weighed both before and after drying in a desiccator over phosphorus pentoxide at room temperature. When the weight of the tissue became constant it was treated with a 5.0 M sodium chloride solution at 4° C for 48 h. After removal of the salt by washing with water, the material was treated with acetone for 72 h. The dried, defatted tissue was autoclaved in a 2 per cent aqueous solution of acetic acid (50 ml./g of dried tissue) at 15 lb./in.² for 2 h. The remaining insoluble material, washed free of acetic acid, was exposed to a 0.1 N sodium hydroxide solution for 15 min at 98°. The treatment with sodium hydroxide was repeated for a second 15-min interval using a fresh aliquot of alkali. The insoluble product, after suitable washing and drying, was weighed and designated purified elastin. Amino-acid analyses were carried out on the Technicon auto-analyser after hydrolysis of the purified elastin with 6 N hydrochloric acid for 24 h.

The elastin content of skin from patients with E-D and from normal subjects was essentially the same (Table 1). The 40-year-old patient with E-D had demonstrated marked clinical improvement in the elasticity of her skin over the years, and at the time the biopsy was taken the skin was much less extensible than that of her 9-year-old daughter with the disease. The observation that the elastin content of their respective skin biopsies from corresponding sites was so similar, suggested that improvement in the condition of the skin with age was independent of the amount of elastin present. It was of interest that the elastin content of the skin from the knee and from the thigh of one of the patients was almost the same, as it has been suggested that, in this disease, excessive and repeated stretching of the skin at articular sites might stimulate the production of excessive amounts of elastin¹. Germane to our biochemical findings was the observation made with the electron microscope that teased preparations of skin from all four patients with E-D and from normal control subjects demonstrated similar amounts of elastin in the skin of test and control subjects. This observation conflicts with the findings of an early paper by Tunbridge *et al.*² but confirms the work of Jansen⁴. We found no consistent difference in the amount of elastica staining material seen by light microscopy in the skins of patients with E-D and of normal individuals. The amount of such material varied greatly even in a given case and depended to a large extent on the exact angle at which the tissue was cut.

The amino-acid composition of elastin from the skin of patients with E-D and from that of normal subjects was almost identical. Typical results are shown in Table 2. The polyfunctional, isomeric amino-acids, desmosine and isodesmosine⁵, involved in the cross-linking of the polypeptide chains found in the elastin of aorta and bovine ligamentum nuchae also appeared to be present in the elastin of human skin and in similar amounts as in elastin from aorta and ligament. The elastin in the skin of patients with E-D contained a normal amount of desmo-

Table 2. AMINO-ACID COMPOSITION OF ELASTIN FROM NORMAL SKIN AND FROM A PATIENT WITH EHLERS-DANLOS SYNDROME

Amino-acid	Elastin from skin of normal subject* g amino-acid/100 g protein	Elastin from skin of patient with E-D* g amino-acid/100 g protein
Aspartic acid	0.4	0.4
Threonine	0.6	0.8
Serine	0.6	0.8
Glutamic acid	2.4	2.5
Proline	18.0	15.9
Glycine	23.4	24.2
Alanine	22.0	21.9
Valine	13.0	13.3
Isoleucine	2.8	3.0
Leucine	7.6	7.7
Tyrosine	3.4	3.2
Phenylalanine	3.7	3.8
Quartern-desmosine	0.7	0.6
Quartern-desmosine	0.9	0.8
Lysine	0.6	0.6
Histidine	trace	trace
Arginine	0.7	0.7
Hydroxyproline	0.9	0.6

* The normal subject is the one designated N 15 while the patient with E-D is the one identified as E-D 15.

sine and isodesmosine. As the lysine content of elastin varies inversely with the desmosine content⁶, the normal lysine content of the skin elastin from these patients provided further evidence that the elastin in E-D was normally cross-linked.

However, another type of cross-linking in elastin, one involving sialic acid, has been described⁷. This type of cross-linking was not examined by us, as the small quantity of elastin isolated from the biopsy material was insufficient to permit us to measure quantitatively the minute sialic acid content of purified elastin.

As the quantity, amino-acid composition and desmosine cross-linking of elastin in the skin of these patients was normal, one can conclude that elastin is most probably not the defective element in the connective tissues in E-D. The observation made by Rollhäuser that the tensile strength of the skin of these patients was only approximately 1/5 that of normal skin⁸ makes it even more likely that the fundamental defect does not lie in the elastin. The tensile strength of skin is attributable, for the most part, to the collagen present by virtue of the quantity (70 per cent of the dry weight of skin) and nature of its fibres⁹. Any marked decrease in tensile strength in skin is thus more likely to be secondary to a change in the quantity and/or quality of the collagen or in factors such as water and polysaccharide content which influence collagen strength, than to abnormalities in the elastin. On theoretical grounds alone, it is difficult to understand how an increase in the elastin content of skin without a proportional decrease in collagen could account for the observed increased extensibility of the skin. The hyperlaxity of non-cutaneous structures in this disease, such as fascia and joint capsules composed almost wholly of collagen with no elastin, further supports the hypothesis that the collagen rather than elastin is defective in E-D.

We thank Dr. R. Reed for doing the electron microscopy, Miss Rosalind Slater for the preparation of specimens for light microscopy, Dr. L. Rodén for the amino-acid analyses and Prof. R. E. Tunbridge for his assistance in obtaining the pathological skin specimens

DAVID P. VARADI*
DAVID A. HALL

Department of Medicine,
University of Leeds

* Present address: Department of Medicine (Section of Dermatology), University of Chicago, Illinois

¹ McKusick, V. A., *Heritable Disorders of Connective Tissue*, second ed. (C. V. Mosby and Co., St. Louis, 1960).

² Wechsler, H. L., and Fisher, E. R., *Arch. Path.*, **77**, 613 (1964).

³ Tunbridge, R. E., Tattersall, R. N., Hall, D. A., Astbury, W. T., and Reed, R., *Chin. Sci.*, **11**, 315 (1952).

⁴ Jansen, L. H., *Dermatologica*, **110**, 108 (1955).

⁵ Thomas, J., Elsdon, D. F., and Partridge, S. M., *Nature*, **200**, 651 (1963).

⁶ Miller, E. J., Martin, G. R., and Piez, K. A., *Biochem. Biophys. Res. Comm.*, **17**, 248 (1964).

⁷ Czerkawski, J. W., *Nature*, **194**, 869 (1962).

⁸ Rollhäuser, H., *Gegenbaurs Morphol. Jahrb.*, **90**, 249 (1950).

⁹ Glagov, S., and Wolinsky, H., *Nature*, **199**, 606 (1963).

Table 1. ELASTIN CONTENT OF SKIN FROM NORMAL SUBJECTS AND FROM PATIENTS WITH EHLERS-DANLOS SYNDROME

Subject*	Dry weight of skin (mg)	Purified elastin (% of dry wt.)
N 15	155.2	3.17
N 6	140.1	2.78
E-D 15	145.3	3.32
E-D 24		
thigh	138.3	3.00
knee	121.6	2.81
E-D 40	120.0	2.50
E-D 9	153.7	2.63

* N designates a normal subject while E-D represents a patient with Ehlers-Danlos syndrome. The figure following the letters gives the age of the subject. All subjects were female and except in E-D 24 the skin samples were removed only from the lateral aspect of the midthigh.

Prevention of Cholesterol-induced Cholelithiasis by Neomycin

RABBITS receiving 0.25–1 per cent dietary cholesterol (5 α -cholestan-3 β -ol) for 3–6 weeks develop gall-stones which have been shown to consist predominantly of calcium and sodium glycoallodeoxycholate (3 α -, 12 α -dihydroxy-5 α -cholanoic acid)^{1,2}. Gall-stone formation in cholesterol-fed rabbits has been postulated to occur as follows: Cholesterol is absorbed from the intestinal tract of the rabbit and is transported to the liver where some of the cholesterol is metabolized to allocholic acid (3 α -, 7 α -, 12 α -trihydroxy-5 α -cholanoic acid). The latter is conjugated with glycine, excreted into the bile and eventually reaches the intestinal tract where it is hydrolysed and dehydroxylated by the intestinal bacterial flora to form allodeoxycholic acid (3 α -, 12 α -dihydroxy-5 α -cholanoic acid). This dihydroxy bile acid is reabsorbed and returns to the liver where it is conjugated with glycine and re-excreted into the bile as glycoallodeoxycholate. The mixed calcium and sodium salt of the latter is relatively insoluble in bile², and therefore is thought to be the cause of gall-stones in cholesterol-fed rabbits. In contrast, the calcium-sodium salt of glycodeoxycholate is relatively soluble in aqueous media.

Since a major step in the formation of gall-stones appears to be the bacterial 7-dehydroxylation of allocholic acid, it is reasonable to assume that the reduction or elimination of the bacteria responsible for the conversion might interfere with gall-stone formation. Because of its well-known anti-microbial effect against the normal intestinal flora, neomycin seemed to be a suitable antibiotic for testing this hypothesis.

The experiments were carried out as follows. Two groups of six rabbits each were fed 1 per cent cholesterol (kindly donated by the Schering Corporation) in their diet of Purina rabbit chow pellets for a period of four weeks. One group was given drinking water containing 0.5 per cent (w/v) neomycin sulphate (kindly donated by E. R. Squibb and Sons). The control animals received ordinary water. During the experimental period food and water intake was measured daily, and the animals were weighed weekly. None of the animals suffered any diarrhoea. Specimens of faeces were collected at the start of the experiment, at the end of two weeks and on the final day of experiment. The number of coliform organisms per gram dry faeces was determined using eosin-methylene blue plates³ and lactose broth in the five-tube method³. The animals were killed at the end of four weeks and autopsies were performed. The contents of the gall bladders, either as liquid bile or gall-stones, and samples of the liver were collected from each animal; the livers were analysed for total sterol and cholesterol⁴. Gall-stones, when present, were dried, weighed and analysed for allo (5 α -) and normal (5 β -) bile acids². Bile, when present, was treated with hot ethanol to precipitate the proteins. The ethanol supernatant was analysed for bile acids by the methods applied to the gall-stones. The results are summarized in Table 1.

None of the animals that received cholesterol and neomycin developed gall-stones. In contrast, all the animals that received cholesterol alone developed gall-stones; the average dry weight of the stones was 211 mg

per animal. The neomycin-treated group ate 16 per cent less food and drank 28 per cent less water than the cholesterol group, and, as a result, the rabbits in the neomycin-cholesterol group gained only 150 g each in four weeks compared with 300 g gained by the cholesterol group. However, all animals appeared healthy throughout the experiment. The average food intake of 75 g/day in the neomycin-cholesterol group corresponded to the ingestion of 750 mg of cholesterol daily which should have been adequate to induce cholelithiasis^{1,5}.

Bacteriological analysis of the faeces disclosed that the administration of neomycin generally resulted in an almost complete abolition of the coliform organisms. However, in one animal there was a thousand-fold increase of coliform bacteria resistant to neomycin, but, like the other animals of the group, it did not develop gall-stones.

Histological examination of the gall bladder revealed little difference between the two groups. One small collection of inflammatory cells was seen in two of the six animals with cholelithiasis. Necrosis, ulceration of the mucosal surface, oedema, or marked inflammation were not observed. These findings confirm those of Borgstrom *et al.*⁶ and suggest that cholesterol-induced cholelithiasis can occur without antecedent injury to the wall of the gall bladder. No abnormalities were found in other organs.

The average liver total sterol content and the relative proportion of cholesterol to cholesterol in the liver sterols were lower in the neomycin-treated group than in the control group which received no antibiotic. However, the results listed in Table 1 show that there was considerable overlap among the two groups. For example, one rabbit in the neomycin group (which had no gall-stones) had a higher proportion of cholesterol in its liver sterol fraction than any of the controls.

Analysis of bile and gall-stones by thin-layer and gas-liquid chromatography² revealed the following: (1) The gall-stones of the rabbits receiving dietary cholesterol but no antibiotic consisted of glycoallodeoxycholate (75 per cent), and small amounts of glycodeoxycholate and glycocholates, as described previously². (2) The bile of the animals treated with cholesterol plus neomycin (in which gall-stones were absent) contained only traces of glycoallodeoxycholate. Glycodeoxycholate and glycocholate were present in approximately equal proportions together with lesser amounts of glycoallocholate and unidentified cholanoic acids.

The oral administration of neomycin to cholesterol-fed rabbits prevented gall-stone formation because it reduced the biliary concentration of glycoallodeoxycholic acid, which is the major component of cholesterol-induced gall-stones. The absence of allodeoxycholate from the bile of rabbits treated with cholesterol plus neomycin is thought to be due to the antibiotic action of neomycin on the intestinal micro-organisms which carry out the 7-dehydroxylation of allocholic acid.

These findings are compatible with the following possibilities: (a) the bacteria acting on 5 α -(allo) bile acids are different from those acting on the normal 5 β -bile acids; (b) the bacteria acting on 5 α -(allo) bile acids are extraordinarily sensitive to neomycin; (c) neomycin, in reducing the bacterial flora, reduces the amount of enzyme available for the 7-dehydroxylation of the bile acids. If the enzyme has a greater affinity for the normal 5 β -bile acids, little

Table 1. EFFECT OF NEOMYCIN ON THE BIOLOGICAL DISPOSITION OF CHOLESTANOL

Regimen	Food intake (g/day)	Liquid intake (ml/day)	Average weight of rabbits		Average dry weight of gall-stones (mg)	Liver sterols, average	
			Initial (kg)	Final (kg)		mg/g (dry weight)	% DHC†
1% cholesterol	89	175	2.76	3.08	211 (34–269)*	18.8 (14.9–27.9)*	53.5 (43.6–63.9)*
1% cholesterol plus neomycin (0.5% of drinking water)	75	125	2.80	2.95	0	12.1 (10.6–15.3)*	45.9 (31.6–65.5)*

* Range. † DHC = 5 α -cholestan-3 β -ol.

enzyme may be available for the dehydroxylation of the 5 α -(allo) bile acids; (d) neomycin acts by preventing the re-absorption of allodeoxycholic acid from the intestinal tract.

E. H. MOSBACH
V. BOKKENHEUSER
D. P. CHATTOPADHYAY

Public Health Research Institute,
New York.

M. SCHMIDT
R. L. HIRSCH

Columbia University Research Service,
Goldwater Memorial Hospital,
New York.

A. F. HOFMANN

Rockefeller Institute,
New York.

¹ Bevens, M., and Mosbach, E. H., *Amer. Med. Assoc. Arch. Path.*, **62**, 112 (1956).

² Hofmann, A. F., and Mosbach, E. H., *J. Biol. Chem.*, **239**, 2813 (1964).

³ *Standard Methods for the Examination of Water and Waste Water*, eleventh ed. (Amer. Public Health Assoc., 1960).

⁴ Chattopadhyay, D. P., and Mosbach, E. H., *Anal. Biochem.*, **10**, 435 (1965).

⁵ Mosbach, E. H., Bevens, M., Kaplan, R., and Halpern, E., *Arch. Path.*, **66**, 72 (1958).

⁶ Borgström, S., Fajers, C. M., and Van der Linden, W., *Acta Chir. Scandinav.*, **126**, 329 (1963).

RADIOBIOLOGY

In vitro Effect of Gamma-radiation on Different Cholinesterase Preparations

THE interaction of high-energy radiation with proteins has received a great deal of attention and in the past few years much has been published on this subject.

Although the interactions with proteins undoubtedly play a vital part, the biochemical effects are frequently ambiguous, and this is the case in the results described for the variation of the cholinesterase activity after γ -irradiation.

Experiments were designed to investigate the *in vitro* effect of γ -rays on cholinesterase from various sources and of differing grades of purity. Irradiations were performed at 18° C in a thermostatically controlled cylindrical 2,000-c. cobalt-60 source. The dose-rate used was 2.5×10^5 r./h. Ten samples were simultaneously irradiated in sealed glass ampoules with a head space of 6 ml., containing air. Controls were handled in the same fashion, but they were not placed within the cobalt source. Dosimetry for experiments was performed by the method of Weiss¹.

The preparations exposed to γ -radiation were: (1) bovine red blood cell cholinesterase (Sigma Chemical Co.); (2) common fly-brain cholinesterase preparations at different stages of purification. These stages were: whole heads; homogenate; and a purified fraction. Heads were collected by the Moorefield method² and the homogenate had 60 mg of fresh tissue per ml. distilled water.

The purified fraction was obtained according to the following method: a homogenate was adjusted to pH 8.5 with 0.1 N sodium hydroxide and allowed to stand 20 h at 5° C, after which time it was centrifuged in the cold at 100,000*g* for 30 min. The supernatant was brought to 41 per cent saturation with solid ammonium sulphate and centrifuged again at 15,000*g* for 15 min. The supernatant was brought to 78 per cent saturation. Centrifugation at 15,000*g* yields a purified cholinesterase preparation which was used as a suspension in distilled water³.

The enzymatic activities of the samples were controlled within 1 h of irradiation. Cholinesterase activity was measured potentiometrically using as substrate a solution of 0.5 M sodium chloride-0.04 M magnesium chloride-

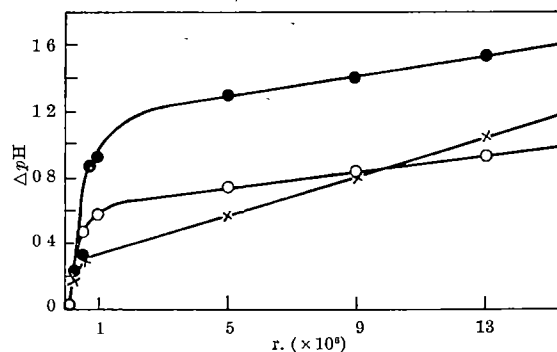


Fig. 1 pH decrease (ΔpH) following several doses of γ -radiation. \times , Whole heads; \bullet , purified samples of common fly brain cholinesterase; \circ , red cell cholinesterase

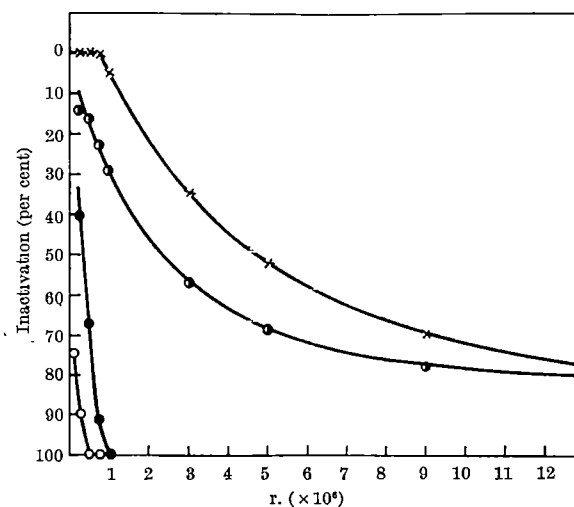


Fig. 2 Effect of irradiation on the activity of cholinesterase preparations. \times , Whole heads; \bullet , homogenate; \bullet , purified preparation, \circ , red cells

0.002 M acetylcholine bromide in 0.5 M phosphate buffer pH 7 and 38° C. The acetic acid liberated was automatically determined by titration with 0.005 N sodium hydroxide. Enzymatic activity was expressed as ml. consumed alkali per min.

Fig. 1 gives the extent to which the pH of the samples was affected by the γ -irradiation.

Experimental results were evaluated by plotting the percentage of the remaining enzymatic activity as a function of irradiation dose (Fig. 2).

From these results it is clear that the more purified the samples were, the more was the inactivating effect of the irradiation. In the cholinesterase preparations from common fly, the order of inactivation was: purified sample > homogenate > whole heads. Assays carried out with red blood cells and cholinesterase from the electric eel (Sigma Chemical Co.) showed a high grade of inactivation (100 per cent of inactivation in a 50 u/ml. solution receiving a 10^6 r. dose). Some data on the nature of this radio-resistance were obtained. The homogenate completely lost its cholinesterase activity by heating it at 100° C for 2 min; when the filtrate was added to the purified sample, a clear protection could be shown ranging from 80 to 40 per cent. Nevertheless, the supernatant from the homogenate precipitated by addition of trichloroacetic acid did not show any protective properties.

R. E. CATALAN
A. M. MUNCIO

Institute of Chemistry,
Madrid.

¹ Weiss, J., *Nucleonics*, **10**, 7 (1952).

² Moorefield, H., *Contrib. Boyce Thompson Inst.*, **18**, 413 (1957).

³ Barberan, C., Ph.D. thesis (Madrid, 1964).

BIOLOGY

Evolutionary Tendency of Relative Growth in Arthropods and Man

IN a series of investigations dealing with the comparative studies of relative growth in various groups of arthropods and man¹⁻⁷ I have confirmed a tendency that the growth ratios of rapidly growing parts of the body tend to be more similar than those of slowly growing parts when two or more closely related forms (such as geographically isolated populations, subspecies, parthenogenetic and bisexual strains of the same arthropod species, interspecific-hybrid and very closely related species in arthropods, Negro and Caucasian children) are compared. This indicates that in incipient stages of structural divergence during evolution the slowly growing parts would tend to be more affected than rapidly growing parts.

This tendency appears, *a priori*, to be possible (Fig. 1). If the growth ratios of rapidly growing parts differ greatly between very closely related forms, the final (adult) sizes and shapes of these parts would tend to be greatly different, and these forms would scarcely appear to be closely related. Therefore, the growth ratios of rapidly growing parts are expected to be similar among very closely related forms. In the comparison of Negro and Caucasian children's growth (see ref. 9), the growth ratios of five out of six most rapidly growing parts (bitrochanteric breadth, upper arm length, lower arm length, thigh length, lower-leg length) are nearly identical between the two races. The same tendency may well hold true in similar comparisons in other groups of animals besides the arthropods and man.

If the growth ratios of slowly growing parts are considerably different (Fig. 1) while those of rapidly growing parts remain much the same, the adults of the closely related forms would tend to look similar in general size

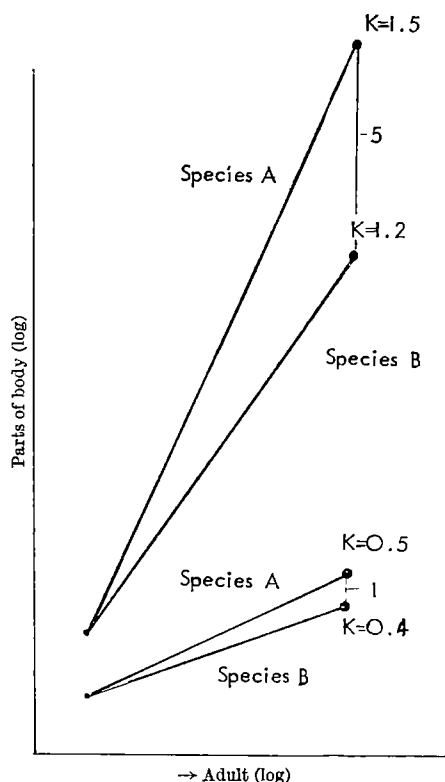


Fig. 1. A simplified model showing the difference in adult sizes in rapidly and slowly growing parts between two closely related species A and B. K is the growth ratio of allometric growth equation. The growth ratios of both rapidly and slowly growing parts in species B are assumed to be 80 per cent of the corresponding parts in species A. The final difference between rapidly growing parts here is five times greater than that between slowly growing parts.

and shape while showing minor yet distinct structural divergences. This is exactly what taxonomists would expect to see in comparing closely related forms. This tendency has been abundantly proved in several groups of arthropods and the human races.

The modification of growth ratios of rapidly growing parts would tend to lead to the divergence at higher taxonomic units. The evolution of relative growth at higher levels of taxonomic unit in arthropods has been discussed earlier⁸.

RYUTCHI MATSUDA

Museum of Zoology,
University of Michigan,
Ann Arbor.

¹ Matsuda, R., *Univ. Kansas Sci. Bull.*, **41**, 25 (1960).

² Matsuda, R., *Ann. Ent. Soc. Amer.*, **54**, 4, 578 (1961).

³ Matsuda, R., *J. Kansas Ent. Soc.*, **34**, 5 (1961).

⁴ Matsuda, R., *Univ. Kansas Sci. Bull.*, **33**, 113 (1962).

⁵ Matsuda, R., *Kontyû*, **30**, 152 (1962).

⁶ Matsuda, R., *Growth*, **26**, 129 (1962).

⁷ Matsuda, R., *Proc. Roy. Ent. Soc., London*, **A**, **38**, 86 (1963).

⁸ Matsuda, R., *Z. Wiss. Zool.*, **169**, 64 (1963).

⁹ Matsuda, R., *Growth*, **27**, 271 (1963).

¹⁰ Matsuda, R., and Rohlf, J., *Growth*, **25**, 211 (1961).

Phytotoxin Production by *Verticillium albo-atrum* Reinke et Berthold

THE physiology of vascular wilt diseases caused by *Fusarium* and *Verticillium* species has received considerable attention during recent years¹⁻⁴. Of these organisms, less work has been carried out on *Verticillium*, and certain aspects of the disease syndrome caused by this fungus are not fully understood. Studies on symptom initiation have for the most part been concerned with the production by the fungus of pectolytic enzymes⁵⁻¹¹. Numerous authors have mentioned the possible role of toxins in disease initiation, but little supporting experimental evidence has so far been produced. Moreover, from the lack of a precise definition, the toxins of some authors^{12,13} might include extracellular pectolytic enzymes. Porter and Green¹⁴ and Green¹⁵ published the first authentic accounts of toxins produced in *Verticillium* culture filtrates. Bioassay of nearly all suspected disease-inducing agencies has been on cut shoots, in an attempt to simulate wilt conditions, in the naturally infected plant. In Nature, however, wilting symptoms usually develop after about 14 days, and experiments in this laboratory have shown that, in the case of seedling infection, growth inhibition rather than overall wilting is a more valid symptom of disease. Up to five weeks after infection in these plants wilting is a gradual basipetal desiccation while the leaf area of apparently turgid leaves is reduced by 80 per cent. This symptom is quite different from the general collapse of turgor common in mature infected plants and may be independent of it.

Experiments reported in this communication represent the preliminary findings of an attempt to isolate from *Verticillium* culture filtrates a growth-inhibiting substance other than a large protein molecule which could account for some of the naturally occurring stunting symptoms. A virulent strain of *V. albo-atrum* Reinke et Berth. was used, originally isolated from a wilting tomato plant by Dr. H. H. Glasscock of the National Agricultural Advisory Services, Wye, Kent. Still cultures consisting of 300 ml. of 'Seitz'-filtered tomato xylem exudate, supplemented with 1 per cent sucrose in horizontally placed 1-l. Thompson flasks, were grown at 22° C for 21 days in a dark incubator. The xylem exudate collected from tomato, cultivar 'Victory', had previously been stored at -20° C until required. Culture solutions were filtered once through several layers of fine cheese muslin and then twice through Whatman No. 1 paper. The filtrate was centrifuged at 16,000g for 15 min at 5° C and then reduced to small volume in a cyclone evaporator at 23° ± 5° C. The pH of the filtrate was adjusted to 2.5 with N hydrochloric

acid and partitioned against three equal volumes of 'AnalaR' redistilled ethyl acetate. The individual solvent phases were extracted on the counter-current principle with a single equal volume of 5 per cent sodium bicarbonate (pH 8.2). The residual solvent phases were bulked and regarded as the neutral fraction and the aqueous alkaline phase was adjusted to pH 2.5 with 4 N hydrochloric acid and extracted as before with ethyl acetate, this latter phase being regarded as the acidic fraction. The original aqueous phase after the preliminary partition was adjusted to pH 8.2 with an excess of solid sodium bicarbonate and extracted with ethyl acetate as before. The extracts were dried by freezing out the water at -20°C and then reducing to dryness at $23^{\circ} \pm 5^{\circ}\text{C}$. The three dry or oily residues were dissolved in a minimal quantity of ethyl acetate and chromatographed on Whatman 3 MM solvent-washed paper for about 15 h with the solvent isopropanol-ammonia-water, 10:1:1 v/v descending. Active regions were eluted and rechromatographed on Whatman No. 1 paper and run with the same solvent ascending. Papers were dried and segmented into 10 strips parallel with the origin, each about 6.4×2 cm corresponding to an R_F value of 0.1. These were eluted with 1.5 ml. M/100 phosphate citrate buffer and incubated with 1 cm segments of etiolated tomato hypocotyl tissue in a test designed to measure growth inhibitors¹⁶. The final lengths of extended hypocotyl

segments after 48 h incubation corresponding to each R_F value were expressed as a percentage of the control segments incubated in buffer only. Fig. 1 illustrates the results of assays of acidic, basic and neutral extracts of culture filtrates. In the acidic fraction, two large peaks of inhibition are evident at R_F 's 0.0-0.2 and 0.3-0.6, each of about 30 per cent of the control values. Tissue in these tubes was brown and flaccid and incapable of further growth when washed and placed in pure buffer. In the assay of the basic fraction, inhibition was detected at R_F 0.8-0.9 with a peak at R_F 0.85. A single peak was seen similar to the basic at R_F 0.85 in the histogram of the neutral fraction with, in addition, a minor peak at R_F 0.35. Tests with various substances using the foregoing method of extraction have shown that weakly acidic substances may partition imperfectly between ethyl acetate and water at pH 2.5 and very small quantities of acidic substances may escape into the neutral fraction. The neutral substance at R_F 0.35 might therefore represent escaped acidic material.

Wilting tests carried out on cut shoots of six-week-old tomato plants cultivar 'Potentate' using aqueous eluates of chromatographed acidic, basic and neutral extracts showed that the acidic extracts were not capable of inducing wilt symptoms. Neutral and basic extracts, however, both induced wilt in the tomato shoots only in the eluate corresponding to R_F 0.75. The neutral eluate at R_F 0.35 was inactive as a wilt inducer.

These results from *in vitro* studies on *Verticillium albo-atrum* suggest that wilting and stunting in infected plants may result from different causes. It is unlikely that the disease syndrome could result from a single causative factor, and indeed, by the time of symptom appearance, disordered host metabolism, quite apart from the initiating effect, may account for a great part of the visible symptoms.

Substances such as those described in this communication, however, could be instrumental in initiating pathogenesis in *Verticillium*-infected tomato plants.

G. F. PEGG

Department of Biological Sciences,
Wye College,
University of London.

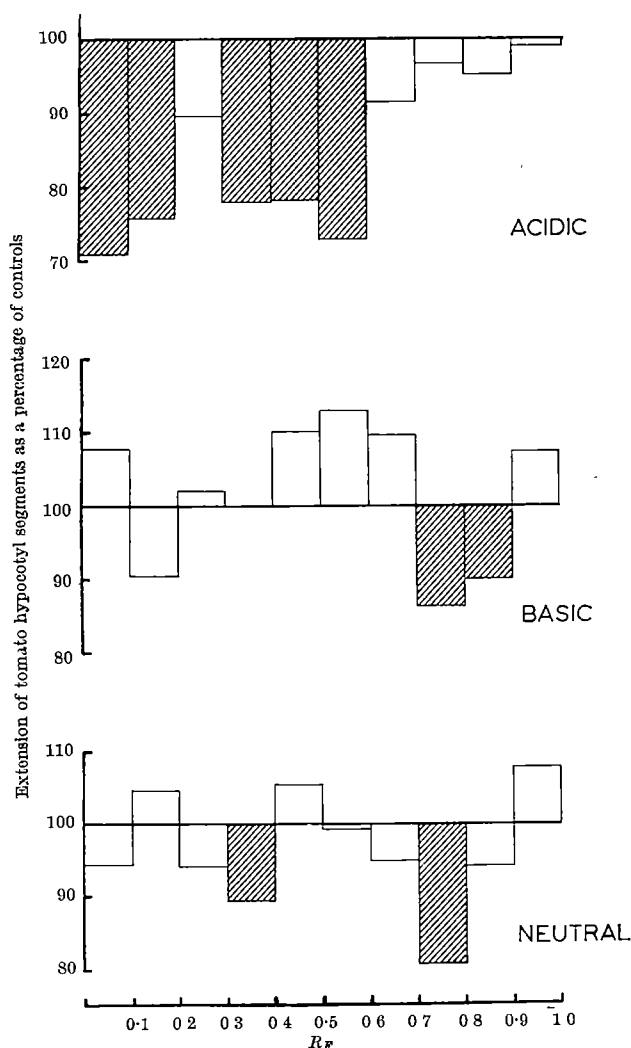


Fig. 1. Histograms of acidic, basic and neutral ethyl acetate extracts from 21-day-old cultures of *Verticillium albo-atrum*, chromatographed in isopropanol-ammonia-water, 10:1:1 v/v, and bioassayed on etiolated tomato hypocotyl segments. Shaded portions represent inhibition statistically significant. $P < 0.05$.

¹ Dimond, A. E., *Ann. Rev. Plant Physiol.*, **6**, 329 (1955).

² Dimond, A. E., *Trans. N.Y. Acad. Sci.*, Ser. II, **27**, 609 (1959).

³ Sadasivan, T. S., *Ann. Rev. Plant Physiol.*, **12**, 449 (1961).

⁴ Wood, R. K. S., *Ann. Rev. Plant Physiol.*, **11**, 299 (1960).

⁵ Kamal, M., and Wood, R. K. S., *Nature*, **175**, 264 (1955).

⁶ Scheffer, R. P., Gothoskar, S. S., Plerson, C. F., and Collins, R. P., *Phytopath.*, **46**, 85 (1956).

⁷ Leal, J. A., and Villanueva, J. R., *Microbiol. Espanola*, **15** (3), 199 (1961).

⁸ Wood, R. K. S., *Ann. App. Biol.*, **49**, 120 (1961).

⁹ Blackhurst, F. M., and Wood, R. K. S., *Ann. App. Biol.*, **52**, 89 (1963).

¹⁰ Deese, D. C., and Stahmann, M. A., *Phytopath. Z.*, **46**, 1, 53 (1962).

¹¹ Matta, A., *Phytopath. Medit.*, **2**, 55 (1963).

¹² Weir, G. M., *Mycopath. Mycol. Appl.*, **18** (3), 194 (1962).

¹³ McLeod, A. G., and Smith, H. P., *N.Z. J. Agric. Res.*, **4**, 123 (1961).

¹⁴ Porter, G. L., and Green, R. J., *Phytopath.*, **42**, 472 (1952).

¹⁵ Green, R. J., *Phytopath.*, **44** (8), 433 (1954).

¹⁶ Pegg, G. F., *Ann. Bot. N.S.*, **26**, 207 (1962).

Establishment of a Pure Infection of the Nematode *Ostertagia circumcincta* in the Sheep

THE four smaller nematode parasites of the abomasum of the sheep, *Ostertagia circumcincta*, *O. trifurcata*, *Teladorsagia davidi* and *O. mentulata*, commonly occur as mixed infections. A search of the literature revealed only three instances¹⁻³ in which it was claimed that pure infections of *O. circumcincta* had been used. In all three, the evidence on which the claim was based was not provided. However, Horak (personal communication) states of his pure strain that he has "examined thousands of male worms and all have been *O. circumcincta*". In Australia,

and elsewhere, mixed infections have been reported to be the common finding by Gordon (personal communication); Sommerville¹; Armstrong⁵ in New Zealand; Todd⁶ in the United States; Parnell⁷ in Great Britain, the proportions of the different species being between 80 and 90 per cent *O. circumcincta*^{4,8}, and the remainder one or a mixture of the others, the predominant species being *O. trifurcata*.

In an examination of the immunological reactions of sheep to ostertagiosis, and possible antigenic differences between the four species and of their validity as distinct species, an attempt was made to establish a pure infection of *O. circumcincta*.

Morphologically, the four species resemble each other so closely that females cannot be differentiated; but males are distinguishable by the length of their spicules together with certain less prominent differences in structure of their genital cone. On the basis of spicule length the nematodes fall into three groups—long spicules (*O. mentulata*), medium length spicules (*O. circumcincta*) and short spicules (*T. daviani* and *O. trifurcata*).

Three hundred living adult *O. circumcincta* males were collected individually from the abomasal contents of freshly killed sheep, using a dissecting microscope, and placed via a laparotomy incision in the abomasum of a worm-free lamb together with 300 fourth-stage mixed larval females of unidentifiable species. These latter came from a sheep in which 88 per cent of the males were *O. circumcincta* and 12 per cent of the short-spicule group, mainly *T. daviani* and a few *O. trifurcata*. The experimental lamb was kept in a metabolism crate in a bird-proof pen which was washed daily. The resulting eggs were collected and infective larvae obtained from them. Ten thousand larvae were then given intra-uminally to another worm-free lamb and the resulting worm burden examined six weeks later. The proportion of the short-spicule group had fallen to 2.9 per cent; of these *T. daviani* was predominant.

The procedure was repeated, again using adult males of *O. circumcincta*, but this time fourth-stage larval females came from the first experimental lamb in which *O. circumcincta* constituted 97.1 per cent of the male population. The second selection further reduced the short-spicule group to 0.82 per cent of the total male population, all of which were *T. daviani*. The third and final selection produced 100 per cent *O. circumcincta* males. Throughout the experiment controls remained free from nematode infection.

Although 'cross-mating' apparently occurred between *O. circumcincta* males and females of the short-spicule group resulting in apparently normal *T. daviani* and *O. trifurcata* males together with a few aberrant and unclassifiable males (having dissimilar and unequal spicules and present only in the first selection), it would appear that a distinct fertility barrier is present, suggesting *O. circumcincta* to be a true species. This supports Trach's hypothesis⁹ that some of the more rare and unusual 'species' of the genus *Ostertagia* may be the result of interspecific variation of *O. circumcincta*, *O. trifurcata* and *T. daviani*. It is interesting to note that hybridization between two members of a closely related genus *Cooperia* has already been observed experimentally⁹.

J. W. COPLAND

George Aitken Pastoral Research Fellow,
Department of Veterinary
Pathology and Bacteriology,
University of Sydney.

¹ Threlkeld, W. L., *Va. Agric. Exp. Sta. Bull.*, 52 (1934).

² Turner, J. H., *et al.*, *Proc. Helminth. Soc. Wash.*, 20, 210 (1962).

³ Horak, I. G., *et al.*, *Onderstepoort J. Vet. Res.*, 31, 163 (1964).

⁴ Sommerville, R. I., *J. Parasit.*, 49, 698 (1963).

⁵ Armstrong, M. C., *N.Z. J. Agric.*, 97, 241 (1958).

⁶ Todd, A. C., *Vet. Med.*, 46, 186 (1951).

⁷ Parnell, I. W., *Brit. Vet. J.*, 116, 9 (1960).

⁸ Trach, V. N., *Helminth. Ab.*, 31, 371 (1961).

⁹ Isentzen, R. S., *J. Parasit.*, 50 (2), 36 (1964).

Gametogenesis of *Plasmodium vinckei*

MANY authors in the past have observed the disappearance of gametocytes of certain blood protozoa as a result of their continuous and prolonged mechanical transmission. This phenomenon was especially studied by Vincke *et al.*¹, Rodhain *et al.*², Jadin, Yoeli and Pierreux³, and by Michiels⁴ in strains of *Plasmodium berghei* maintained in the laboratory for years by 'syringe passages'. The rodent malaria parasite *Plasmodium (Vinckei, from Garnham⁵) vinckei*⁶, isolated in 1952, behaves similarly.

It has recently been observed by Vincke, Scheepers-Biva and Bafort⁷ that *P. berghei* which has lost its ability to produce gametocytes could recover this power if kept in a state of 'quiescence'. This 'quiescence' may be obtained by transmitting the infection to adult albino rats in the meta-critical phase, where it survives in a mild, latent stage, and also by keeping the parasite at low temperatures (-75°C).

We wondered if the same phenomenon could be observed with *P. vinckei*. This parasite has been maintained since its isolation by blood transfers and has long lost its ability to produce gametocytes. We received the strain on September 14, 1962, from Algeria, through the courtesy of Prof. Fabiani. No precise information was available on the number of mechanical transmissions undergone by the strain prior to its arrival in our laboratories. Our estimation is that the strain had, at the time, passed at least 400 times, assuming that one blood transfer was carried out every ten days between the time of its discovery by Vincke in January 1952 and November 1962.

We maintained the strain by weekly transfers in mice and rats. On May 20, 1965, the strain, about thirteen years old, had been passed 68 times in 934 days in our laboratory: 59 times in mice, four times in albino rats, and in five transfers in tree rats, *Thamnomys surdaster*. Two transfers were also carried out in deep freeze at -75°C during this period.

Tables 1 and 2 summarize the details of the successive blood transfers in the two lines of animals which originated from the *P. vinckei*-infected animals received from Prof. Fabiani in September 1962.

It must be stressed that the blood of all *P. vinckei*-infected animals was regularly checked for the appearance of gametocytes and for exflagellation, but none was observed. However, after keeping *P. vinckei*-infected blood from rat 362 at low temperature (-75°C) and later passing it twice through albino rats, few gametocytes and scarce exflagellation were observed in the blood of mouse 734. More numerous exflagellation occurred in the blood of mouse 901 after the strain was maintained for a second period in deep freeze.

Table 1. SUCCESSIVE TRANSMISSIONS AFTER RECEIPT OF THE STRAIN ON NOVEMBER 14, 1962* MOUSE 901

Date	No of passage	Animal	Observations
14/11/62-20/11/62	1-2	Young mice	Weekly transfers
27/11/62-4/1/63	3-7	<i>Thamnomys</i>	Weekly transfers
2/1/63-20/9/63	8-41	Young mice	Weekly transfers
27/9/63	42	Albino rat 362	Blood in deep freeze (-75°C) tenth day after inoculation. Kept at low temperature for 67 days (8/10/63-12/12/63)
12/12/63-20/2/64	43-53	Young mice	Weekly transfers
27/2/64	54	Albino rat	Weekly transfers
13/3/64-8/5/64	55-63	Young mice	Weekly transfers
15/5/64	64	Rat reservoir	Rat clinically cured on the 30th day after inoculation and sub-inoculated on the 107th day
31/8/64	65	Rat reservoir	Rat clinically cured on the 21st day after inoculation and sub-inoculated on the 60th day
20/10/64	66	Young mouse	
30/10/64	67	Young mouse 734	Few exflagellations of microgametocytes observed in the blood on 5/11/64, the 6th day after inoculation. The blood was kept at -75°C from 6/11/64 to 13/5/65, 190 days
13/6/65	68	Young mouse 901	Exflagellation observed on 20/5/65, the 7th day after inoculation

Table 2 SUCCESSIVE TRANSMISSIONS AFTER RECEIPT OF THE STRAIN ON NOVEMBER 14, 1962 MOUSE 931

Date	No. of passage	Animal	Observations
14/11/62-20/11/62	1-2	Young mice	Weekly transmissions
27/11/62-4/1/63	3-7	<i>Thamnomys</i>	Weekly transmissions
2/1/63-20/9/63	8-41	Young mice	Weekly transmissions
27/9/63	42	Albino rat 362	Blood in deep freeze on 10th day after inoculation. Kept at low temperature for 414 days (8/10/63-26/11/64)
26/11/64	43	Young mouse	
3/12/64-11/12/64	44-45	Albino rat	Weekly transmissions
18/12/64-29/5/65	46-68	Young mice	Weekly transmissions
28/5/65	69	Young mouse	Few exflagellations observed in the blood on 3/6/65, 6 days after inoculation

We observed also exflagellation in the transfers originating from mouse 931 (Table 2). This animal was also infected with *P. vinckei* blood originating from rat 362, but the infected blood was kept for 414 days at -75°C and afterwards passed in successive transfers to mice. Exflagellation of microgametocytes in small numbers was observed at the 69th blood transfer. In the five observations in which exflagellation was hitherto observed, all were made on the sixth and seventh days after inoculation.

It may, therefore, be concluded (or at least assumed) that in *P. vinckei*, as in *P. berghei*, a period of 'quiescence' at low temperature (-75°C) favours the later re-appearance of viable gametocytes. This phenomenon, however, must be confirmed by further observations as it conflicts with investigations made in human malaria in which gametogony is present in acute cases of malaria. However, it is important that more attention should now be given to the possibility of transmission of infection by asymptomatic cases.

We thank Prof. M. Yoeli for his help in correcting our translation.

J. BAFORT
I. H. VINCKE
G. TIMPERMAN

Institut de Medecine Tropicale
Prince Leopold,
Anvers, Belgium.

¹ Vincke, I. H., Peeters, E., and Frankie, Gh, *Ann. Soc. Belge Med. Trop.*, **33**, 269 (1953).

² Rodhain, J., and Vincke, I. H., *Ann. Soc. Belge Med. Trop.*, **2**, 297 (1951).

³ Jadin, J., Yoeli, M., and Pierreux, G., *Ann. Soc. Belge Med. Trop.*, **39**, 847 (1959).

⁴ Michiels, G., *Ann. Soc. Belge Med. Trop.*, **43**, 1, 67 (1963).

⁵ Garnham, P. C. C., *Ann. Soc. Belge Med. Trop.*, **44**, 2 (1964).

⁶ Rodhain, J., *Ann. Soc. Belge Med. Trop.*, **32**, 275 (1952).

⁷ Vincke, I. H., Scheepers-Blva, M., and Bafort, J., *Ann. Soc. Belge Med. Trop.*, **45**, 1 (1965) (in the press).

Phosphorus-deficiency Symptoms in Tobacco and Transpirational Water Loss

PHOSPHORUS-DEFICIENCY symptoms have been described for tobacco¹ and have been duplicated here in solution culture with the 'Virginia Gold' variety. The symptoms, however, do not develop until a weather condition permits a high rate of transpiration. It is under this condition that the spotting and scorching of the lower leaves of tobacco plants occur. Until there was a stress there were no visual symptoms present.

The water loss per unit of leaf area from plants to which no phosphorus was added was about twice as great for phosphorus-sufficient plants as for the phosphorus-deficient plants (Table 1). The kerosene spot test indicated that the stomata of most of the lower leaves of phosphorus-deficient plants not having any visual symptoms were closed when stomata of phosphorus-sufficient plants were fully open. Stomata on leaves on top of the plant were open in either case. The reason for this latter effect is that phosphorus in deficient plants is retranslocated to

new growth. Hence, the low water use of the phosphorus-deficient plants was related to the failure of stomata to open. When the top third of both kinds of plants was cut off, the differential transpirational water loss per unit area was about 2.5-3 times greater for the phosphorus-sufficient plants.

The classical explanation for regulation of stomatal opening is a photosynthetic decrease of carbon dioxide in the guard cell, which decreases pH. This in turn stimulates amylase which converts starch to sugar, which leads to an increase in osmotic pressure which results in opening of the stomata². More recently, the metabolism of glycolic acid has been implicated³. Oxidative phosphorylation seems to be associated in some manner, in that appropriate inhibitors prevented stomatal opening³.

Table 1. YIELDS, WATER LOSS, AND PHOSPHORUS CONTENTS OF PLANTS

Measurements	No phosphorus	0.003 M phosphorus
	Whole plants	
Dry wt. of tops (g)	24.3	70.5
Dry wt. of roots (g)	5.0	5.6
Leaf area (cm ²)	5,570	13,300
Water loss in 6 days per cm ² leaf (ml.)	0.50	0.91
Water loss in 6 days per g root (ml.)	560	2,160
P in top leaves (% dry wt.)	0.17	0.71
P in middle leaves (% dry wt.)	0.07	0.82
P in bottom leaves (spotted and scorched without P) (% dry wt.)	0.07	0.65
	Plants with tops removed	
Water loss in 6 days per cm ² leaf (ml.)	0.30	0.80
Water loss in 6 days per g root (ml.)	290	791

The role of phosphorus in metabolism is so pronounced that it is quite understandable that phosphorus deficiency can upset the functions of guard cells. The interesting point is that the reactions in guard cells are more sensitive to phosphorus deficiency than many other plant reactions in that the plants continued to grow under the conditions of the experiments. It can thus be expected that the phosphorus nutrition of at least this one plant species can regulate to a certain extent its water relations.

An intriguing question relates to the function of transpiration. In tobacco, injury occurred when transpiration could not proceed at the usual rate. This was very pronounced where the kerosene had been applied to the leaves. In phosphorus-sufficient plants the kerosene entered the stomata and there was no subsequent injury. In the phosphorus-deficient plants, the kerosene did not penetrate but formed a layer on the leaves and evidently decreased even more the exchange of gases between the leaf and atmosphere. These kerosene spots developed the usual phosphorus-deficient leaf spots for this species. The logical inference is that transpiration, or at least open stomata, serves a beneficial role. It could be that of regulating the temperature of the leaf, although the loss of a toxic volatile compound through open stomata may offer a better explanation of all the results.

The phosphorus deficiency in this plant species resulted in decreased yield of tops but in little decrease for that of roots (Table 1). This behaviour is observed for nitrogen⁴ but has not been reported for phosphorus. The volume of water transpired per gram of roots was about three times as high for the phosphorus-sufficient plants as for the phosphorus-deficient plants (Table 1).

This work was supported in part by a grant, G-23841, from the National Science Foundation.

A. WALLACE
E. FROLICH

Department of Agricultural Sciences,
University of California, Los Angeles,
California.

¹ Bahrt, G. H., in *Hunger Signs in Crops*, edit. by Hambridge, G., 327 (American Society of Agronomy, Washington, D.C., 1941).

² Miller, E. V., *Within the Living Plant*, 66 (Blakiston Co., New York, 1953).

³ Zelitch, I., *Stomata and Water Relations in Plants*, edit. by Zelitch, I., *Conn. Agr. Exp. Sta. Bull.*, No. 664, 18 (1963).

⁴ Rosemark, N. O., *Physiol. Plantarum*, **7**, 497 (1954).

Undernutrition and Early Embryonic Mortality in Merino Ewes

UNTIL recently there was little evidence that the plane of nutrition in early pregnancy could affect ovine foetal growth or survival¹⁻³. In the past year or so, however, it has been shown that maternal undernutrition during the first 90 days of gestation may influence foetal weight at 90 days⁴ and possibly embryo survival⁵.

In a recent investigation in this Department, short-term, severe nutritional stresses during the first 20 days of pregnancy have resulted in increased embryonic mortality. Groups of mature Merino ewes (mean body-weight, 96 lb.) were submitted to 7-day periods of sub-maintenance feeding from mating (day 0) to day 20 post-mating. Numbers of ova shed were recorded by counting corpora lutea at laparotomy, and subsequent embryonic loss or fertilization failure was assessed by return to service, pregnancy test by laparotomy about day 40, and lambing performance. The imposed stress consisted of 7 days in a bare yard, with a ration of 100 g/head/day lucerne hay, plus water. A mean body-weight loss of 3 lb. per sheep resulted.

Oestrus was synchronized, using progesterone, in 150 Merino ewes, and the ewes were hand-mated to fertile rams at the second post-synchronization oestrus. They were then divided at random on the basis of body-weight into groups of 30, 30 and 90 ewes. One group of 30 received sub-maintenance treatment from day 0 to 7 (Group 1); the second group (2), their control, grazed normally. Ewes in Groups 1 and 2 were laparotomized and their corpora lutea counted on day 12.

Laparotomy was performed on the remaining 90 ewes on day 6, and these were then randomized according to body-weight and number of ova shed into three groups of 30 ewes. Group 3 received sub-maintenance treatment from day 6 to 13 and Group 4 from day 13 to 20; Group 5 was a control. Except during sub-maintenance treatment, all ewes were paddock-grazed together on adequate pasture. During pregnancy their mean body-weight gain was 22 lb.

Following mating, the ewes were run with raddled fertile rams for 7 weeks, after which raddled infertile rams were introduced. Returns to service were recorded daily to day 60, and thereafter thrice weekly. On day 40 (± 2), ewes which had not returned to service were tested for pregnancy by laparotomy. Just prior to lambing, the groups were separated so that an accurate count of lambs born in each group was obtained.

Table 1 shows the experimental layout and the results in terms of ova shed and their eventual fate. Significantly ($P < 0.05$) less lambs were born in the treatment groups than in the controls.

Table 1. OVULATION, LAMBING AND EMBRYONIC MORTALITY DATA BY GROUPS

Group	Treatment	No. of ewes	No. of ova	No. of lambs born	Ova not represented by lambs	
					No.	Per cent
1	Day 0-7	29	44	26	18	40.9
3	Day 6-13	30	43	22	21*	48.8
4	Day 13-20	29	41	20	21	51.2
Sub total		88	128	68	60†	46.9
2	Control	30	43	31	12	27.9
5	Control	30	44	29	15	34.1
Sub total		60	87	60	27	31.0
Total		148	215	128	87	40.5

* Groups 3 and 4 versus group 5. $P < 0.10 > 0.05$

† Treatments versus controls. $P < 0.05$.

There was a high level of homogeneity within treatment groups and within controls, but differences between individual treatment and control groups did not reach significance.

Further analysis of the data showed that the loss of potential lambs was significantly higher ($P < 0.01$) among ova shed as twins than as singles.

The apparent embryonic deaths in Group 1 did not result in prolonged oestrus cycles, but in each of Groups

3 and 4, following nutritional stresses during the second and third weeks after mating, respectively, five ewes experienced cycles exceeding 22 days in length. Each of the control groups had one such ewe. This is considered to be strong supporting evidence that embryonic deaths have, in fact, resulted from the treatments.

The effect of early embryonic death on subsequent cycle-lengths is now being subjected to detailed investigation, and further work on the effect of nutritional stress in the pre-implantation period is in progress.

T. N. EDEY

Department of Livestock Husbandry,
University of New England,
Armidale, New South Wales.

¹ Wallace, L. R., *J. Agric. Sci.*, **38**, 93, 243, 367 (1948).

² El-Sheikh, A. S., Hulet, C. V., Pope, A. L., and Casida, L. E., *J. Anim. Sci.*, **14**, 919 (1965).

³ Coop, I. E., *Proc. Ruakura Fmrs. Conf.*, 69 (1964).

⁴ Everett, G. C., *Nature*, **201**, 1341 (1964).

⁵ Bennett, D., Axelsen, A., and Chapman, H. W., *Proc. Austral. Soc. Anim. Prod.* (in the press)

Human Lymphocytes cultured in Microplates

LYMPHOCYTES from peripheral blood can be stimulated to undergo blast-like transformation when cultured *in vitro*¹. This reaction is revealed by many techniques including direct morphological observations, the appearance of mitotic figures in dividing cells, and specific staining or autoradiography for the various biochemical processes involved in cell division². Although any of these techniques is satisfactory, the percentage of transforming cells in many specific immune reactions is low³, making standardization both tedious and difficult. In addition, in culture, a small percentage of lymphocytes undergo blast transformation in unstimulated controls either spontaneously or possibly because of prior sensitization of the individual to one of the products in the tissue-culture medium⁴.

We attempted to avoid some of these difficulties by using microtechniques which enabled us to test an individual's cells against a series of antigens on successive days and to have each determination matched by at least one control. So far we have only used the method of counting cells in metaphase (mitotic index) to standardize our observations. We shall report initially promising results as well as details of the method of growth of lymphocytes in microplates and some data of individual variation in responses to phytohaemagglutinin (PHA).

A disposable glass 10-ml. syringe was pre-wetted with heparin, leaving about 0.2 ml. in the syringe. Exactly 10 ml. of venous blood was drawn and the syringe was placed upright at room temperature to allow the red cells to sediment. When red cells had settled, leaving 4 ml. of plasma above the cell pack (30 min–3 h depending partially on room temperature), the needle was bent into a U-shape and by gentle pressure on the plunger the plasma was ejected into a collecting tube. This plasma contained some red cells, and the white cell counts on numerous occasions ranged from 6 million to 10 million cells per cubic ml. The cells were agitated to assure equal distribution in the plasma and one drop of the plasma-cell suspension was placed in each well of plastic disposable U-shaped microplates (Cooke Engineering Co. 220-24). Initially, we used micropipettes, but found that disposable Pasteur pipettes were sufficiently accurate for our needs. To the drop of plasma-cell suspension we added three drops of tissue culture medium, either alone as a stock solution or containing phytohaemagglutinin (PHA) or a known antigenic product. Our basic medium was Medium 199 with inactivated foetal calf serum, and penicillin, streptomycin, acromycin and mycostatin. In order to determine the optimum concentration of PHA, old tuberculin (OT), purified protein derivative (PPD) or

brucellargin, dilutions of these products were prepared and tested against several people's lymphocytes. We used a minimum concentration of PHA which produced blastogenesis in the majority of cells by the fifth day. For all other reagents we used the maximum concentration which did not produce obvious toxicity when compared to cells in control wells. All solutions were approximately 37° C at time of inoculation.

For short-term cultures, up to 6–8 days, it was not necessary to use foetal calf serum in Medium 199, the homologous plasma being sufficient to maintain growth. If we wished to culture cells in the absence of homologous serum, it was possible to centrifuge the original plasma suspension and wash and resuspend the lymphocytes in growth medium. We were not successful in growing the cells without serum in dextran-glucose as has been reported⁵. For maximum longevity of lymphocytes in culture, 40 per cent foetal calf serum in the medium plus the homologous plasma were optimal. As revealed by the colour indicator in Medium 199, this solution was quite acid. Acidity, however, did not appear to affect the cells or, possibly, had a beneficial effect. Cells did equally well in the standard incubator or in a carbon dioxide chamber at 37° C. The carbon dioxide was able to penetrate through the mineral oil seal (described below) or perhaps directly through the plastic plate, since, within several hours of being placed in the carbon dioxide chamber, the indicator in the medium assumed a more acid colour.

After the four drops of solutions were added to the wells, the latter were sealed by the addition of two drops of mineral oil. In later investigations we have found that the mineral oil could be dispensed with if plates were maintained in a moist chamber. Cells remained viable for up to 4 weeks although the numbers gradually diminished. When the experiment called for the addition of colcemid, one drop of a stock solution of colcemid was added directly to the wells the evening before the readings were to be made. The colcemid solution passed through the mineral oil without agitation within 5 or 10 min because of its relative density.

The microplates were not pre-treated or cleaned before use. The Pasteur pipettes were clean but not sterile, and the mineral oil was not autoclaved. In no instance was contamination apparent in the cultures.

All readings were made as wet mounts. Using gentle suction from a water pump, a Pasteur pipette was immersed below the fluid level of the wells and as much liquid as possible was removed without agitating the small white button of cells on the bottom. To the cells in the well, one drop of a stain consisting of 2 per cent orcein in 45 per cent glacial acetic acid was added. Cells and the stain were removed with a cotton plugged plastic disposable 1-ml. pipette. The pipette was introduced to the bottom of the well and rotated gently in order to free the cells from the plastic surface. We were surprised at the sticking properties of white cells in the U-shaped plastic wells. After several revolutions of the pipette the stain and cells were vigorously sucked up into the pipette and blown on to one pre-wiped glass slide. Immediately a cover slip (22 mm × 50 mm) was placed on the drop and this caused even spreading of the lymphocytes. Occasionally when too much mineral oil remained in the well there were mineral oil islands on the slide which interfered with the reading. Slides were sealed with nail polish and could be read for at least 8 h when desired. In general, however, they were not sealed and were studied immediately. We attempted to read two to five slides per determination, counting 200 cells and disregarding polymorphonuclear leucocytes.

When PHA was added to the cultures we had no difficulty in observing the morphological changes previously described¹. Blast-like cells were observed in cultures containing OT, PPD and brucellargin, but when these cultures were read blindly with control cultures we were unable to demonstrate differences in percentages and types

of cells which would consistently distinguish them morphologically from the controls.

In cultures to which PHA had been added, up to 7 per cent of cells were in metaphase after addition of colcemid. This percentage varied considerably among the three individuals sampled repeatedly. In one individual, ten cells in a thousand were in metaphase on day 2 (48 h after inoculation) with a peak of 78 per thousand on day 4 and still having four per thousand on day 17. This individual consistently had a few cells in metaphase even without the addition of colcemid from day 2 to day 17. A second individual had cells in metaphase only from day 4 to day 7 with a peak of 65 per thousand on day 6. On days 6 and 7, cells in metaphase were seen in cultures which did not receive colcemid. The third individual had cells in metaphase from day 5 to day 8 with a maximum of 35 per thousand on day 6. This individual had spontaneous (no colcemid added) cells in metaphase from day 5 to day 7.

Examination of cells in metaphase in the presence of OT after addition of colcemid was conducted on a tuberculin positive and tuberculin negative individual. The tuberculin negative individual did not show cells in metaphase during 25 days of observation, either in the wells with OT or in the controls. The tuberculin positive individual showed no cells in metaphase for the first 14 days. Thereafter, metaphases appeared through day 23 but never more than one per hundred cells counted. (In order to prolong the number of days of observation we only made one slide per day and occasionally we could not count more than 100 cells per slide.) On day 23, one cell in metaphase was observed in the control preparation of 160 cells counted.

The microtechnique has the advantage of providing numerous preparations from a single specimen of blood which can be tested against several antigens or against varying concentrations of reagents and under different conditions. Further, the technique permits serial sampling of a given specimen. Utilizing the mitotic index to demonstrate changes, our results were encouraging and we were able to demonstrate marked variations in responses by different individuals. We are now examining the use of various differential stains or techniques for demonstrating RNA or DNA or acid phosphatase or glycogen, all of which are reported to accumulate during different phases of blastogenesis^{2,6,7}.

JACOB A. BRODY
BOB HUNTLEY

Arctic Health Research Center,
945 Sixth Avenue, Anchorage, Alaska.

¹ Nowell, P. C., *Cancer Res.*, **20**, 462 (1960).

² Robbins, J. H., *Science*, **146**, 1648 (1964).

³ Oppenheim, J. J., and Perry S., *Proc. Soc. Exp. Biol. and Med.*, **118**, 1014 (1965).

⁴ Mellman, W. J., *Human Peripheral Blood Leucocyte Cultures*, 22 (Academic Press, New York, 1965).

⁵ Hutton, W. E., and Watson, E. D., *Proc. Soc. Exp. Biol. and Med.*, **116**, 924 (1964).

⁶ Quaglini, D., Hayhoe, F. G. J., and Flemans, R. J., *Nature*, **196**, 338 (1962).

⁷ Hirschhorn, R., Kaplan, J. M., Goldberg, A. F., Hirschhorn, K., and Weissmann, G., *Science*, **147**, 55 (1965).

A Well-preserved Grizzly Bear Skull recovered from a Late Glacial Deposit near Lake Simcoe, Ontario

THE results of a carbon-14 collagen test performed on a portion of a long bone collected in association with the skull of a grizzly-type bear of the *Ursus arctos-horribilis* complex have just been received which indicate that this remarkably well-preserved specimen died some 11,700 ± 250 years ago. The specimen was discovered in a load of gravel removed from approximately 30 ft. below the local grade of a commercial gravel pit in November 1964.

The geology of the general area has recently been investigated and mapped¹. According to the map pro-

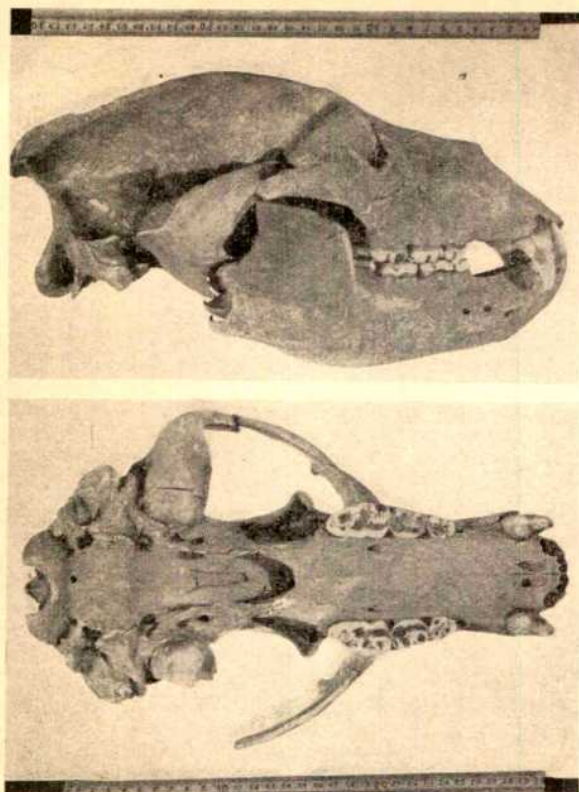


Fig. 1

vided, this site is associated with beach sand and gravel deposits near the shore of Lake Algonquin. More recent investigations have suggested that the North Bay outlet of Lake Algonquin opened about 10,000–11,000 years ago². Further excavations and examinations of the site are planned as soon as the frost is thawed sufficiently to allow work.

Cranial measurements (mm) of this specimen (R.O.M. 34226) are as follows: condylobasal length, 322; zygomatic breadth, approximately 302; mastoid breadth, 136.6; length of palate, 164; canine-molar length of maxillary tooth row, 128; length of last three upper cheek teeth (P^4-M^2), 75.8; greatest width of palate including last molars (M^2), 87.8; width across upper canines, 70.2; height of occiput, 96.7; least interorbital breadth, 76.5; width across postorbital processes, 110.6; greatest length of mandible, 320; canine-molar length of mandibular tooth row, 145.3; length of last four lower cheek teeth (P_3-M_2), 82.8; width across lower canines, 61.7. The dentition is complete except for the incisors and the anterior or first premolars, both above and below.

There appear to be no valid records of the grizzly bear in eastern Canada (east of Manitoba) in historical times. However, there have been persistent rumours of such an animal in the Ungava region of northern Quebec, although no specimen or portion of specimen is known to exist. A large bear of grizzly size has been suspected in the interglacial deposits of the eastern Great Lakes region on the basis of post-cranial fragments, but this appears to be the first cranial specimen to be recovered from the late glacial deposits east of the Great Lakes and

provides a particularly good specimen of confirmed age.

R. L. PETERSON

Department of Mammalogy,
Royal Ontario Museum,
University of Toronto,
Ontario, Canada.

¹ Deane, R. E., *Geol. Surv. Canada, Mem.*, 256.

² Terasmae, J., and Hughes, O. L., *Science*, 131, 1444 (1960).

Direct Action of Thyroxine on the Trout Pituitary *in vitro*

ALTHOUGH it is established that a negative feed-back system operates between the thyroid and pituitary in teleosts¹, the mechanism controlling the activity of the pituitary thyrotrophs has scarcely been examined. In mammals where the problem has been extensively investigated, a normal or high level of thyrotrophic activity seems to depend on stimulation from the hypothalamus and requires an intact hypothalamo-pituitary link²; a reduction of thyrotrophic activity can largely be attributed to the inhibitory action of thyroxine acting both on the hypothalamus and also directly at the pituitary level^{3,4}. In the teleost *Poecilia (Mollienesia) formosa*, experiments suggest, on the contrary, that the thyrotrophs possess an intense autonomous activity which is normally suppressed by the hypothalamus⁵. Whether or not thyroxine also has a direct inhibitory effect on their activity is not known. The present work investigates the nature of the hypothalamic influence and the effect of thyroxine on the trout pituitary, using an organ-culture technique.

Adult trout (*Salmo gairdneri*), 27–30 cm in length and weighing 200–230 g, were used for these experiments.

In experiment 4, carried out in November, the pituitaries were cut in half sagittally. One half, acting as a control, was fixed immediately in sublimated Bouin-Hollande for histological study; the other half was cultured on a hormone-free medium with an agar base, as described

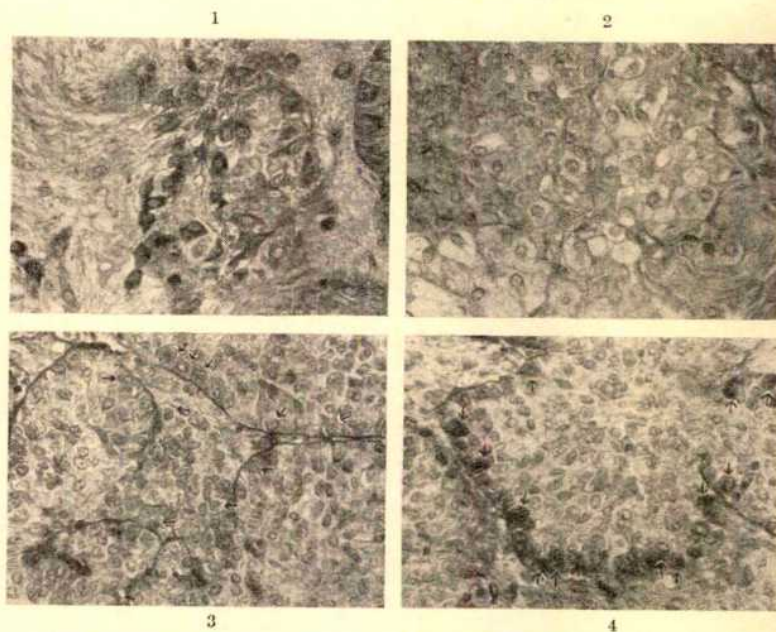


Fig. 1. Uncultured pituitary; rostral basophils are darkly stained

Fig. 2. Opposite half of same pituitary as Fig. 1, cultured for 28 days on a hormone-free medium; rostral basophils are degranulated, hypertrophied and vacuolated

Fig. 3. Pituitary cultured for 8 days on a hormone-free medium; rostral basophils are degranulated

Fig. 4. Opposite half of same pituitary as Fig. 3, cultured for 8 days on a medium enriched with 20 µg/ml. thyroxine; rostral basophils are not degranulated

Arrows indicate rostral basophilic cells. All sections stained with Heilant's tetrachrome ($\times c. 95$)

Table 1

No. of pituitaries	Thyroxine concentration	Period of culture
4	40 µg/ml.	6 days
8	20 µg/ml.	8 days
2	40 µg/ml.	14 days

previously⁶. Pituitaries were cultured for 6 days (sixteen pituitaries) or 28 days (eight pituitaries) after which they were fixed, sectioned at 4µ and stained with Herlant's tetrachrome, A.F., or periodic acid-Schiff and haemalum.

In experiment B, carried out in June-July, the control half of the pituitary was cultured on a hormone-free medium while the other half was cultured on a medium to which 8.0 µg or 16.0 µg of thyroxine (Roche) was added, making a final concentration of 20.0 µg/ml. or 40.0 µg/ml. of medium. These concentrations are of the same order as those found effective *in vivo* to inhibit thyrotrophic activity in the eel⁷ or cause hypothalamic modifications in *S. gairdneri*⁸ when used as an ambient solution. Pituitaries were cultured for 6, 8, or 14 days (Table 1) and then fixed and stained as in experiment A.

Experiment A. In the control pituitary halves, rounded or angular basophilic cells with dense, homogeneous cytoplasm, staining intensely with aniline blue, periodic acid-Schiff or A.F., were always found rostrally, near the junction of the pro- or meso-adenohypophysis. These cells occurred between the other cell-types bordering the neurohypophysis and were also found scattered within the neurohypophysis itself. Similar cells were less frequently found throughout the meso-adenohypophysis, again bordering the neurohypophysis. These cells seem to correspond in position and staining reaction to those identified by Oliverneau *et al.* in *S. gairdneri* as thyrotrophs⁹. Their identification as thyrotrophs is supported by the fact that in fish with rudimentary gonads they were the only basophils present; in fish with well-developed gonads, other basophils were also present, but these were usually distinguishable from the rostral basophils by differences in position, staining intensity and size of granules.

After 6 days of culture, the staining reaction of the rostral basophils is much weaker than in the controls. The cytoplasm is less homogeneous and may be slightly vacuolated. After 4 weeks of culture, their histological appearance suggests an intense secretory activity: the cells are hypertrophied and rounded, with degranulated and very vacuolated cytoplasm; the nuclei are usually rounded with a distinct nucleolus (compare Figs. 1 and 2).

Experiment B. In ten of the twelve pituitaries cultured for 6-8 days on a thyroxine-enriched medium, the rostral basophils were less degranulated and more intensely stained than in the control half of the same pituitary, cultured on a hormone-free medium. (Compare Figs. 3 and 4.) The degree of difference between the staining reaction of the two halves was very variable: for some pituitaries the difference was very striking, in others it was much less intense; in two pituitaries, any difference between the two halves was doubtful. These variations were not correlated with the different doses of thyroxine used. In the two pituitaries cultured for 14 days, the basophils of the control cultures were completely degranulated and very vacuolated; those of the thyroxine-treated cultures were only slightly more granulated but very much less vacuolated. No consistent difference between the appearance of the nuclei in the control and thyroxine-treated halves could be observed. None of the other cell-types showed any histological changes in response to thyroxine and both the experimental and control cultures appeared very healthy, with very little or no central necrosis. It therefore seems improbable that the response of the rostral basophils is a pathological one.

In conclusion, these results indicate that the rostral basophils of the trout pituitary become hyperactive after removal from hypothalamic control; this activity can be modified by thyroxine. If the identification of these basophils as thyrotrophs is correct, then:

(a) their response to separation from the hypothalamus is comparable with the increased thyrotrophic activity observed in pituitary grafts in *Poecilia*⁵;

(b) the results would suggest that thyroxine may normally inhibit thyrotrophic activity in teleosts, as in mammals, by acting directly at the pituitary level.

This work was supported in part by the award of a N.A.T.O. fellowship and by a grant from the C.N.R.S., Paris.

BRIDGET I. BAKER

Department of Zoology,
University of Liverpool.

¹ For bibliography see: Pickford, G. E., and Atz, J., *The Physiology of the Pituitary Gland of Fishes* (New York Zool. Soc., New York, 1957). Oliverneau, M., *Gen. Comp. Endocrin.*, **3**, 312 (1963).

² Szentagothai, J., Flerko, B., Mess, B., and Halasz, B., *Hypothalamic Control of the Anterior Pituitary* (Akademiai Kiado, Budapest, 1962).

³ Yamada, T., and Greer, M. A., *Endocrinology*, **64**, 559 (1959).

⁴ Bogdanove, E. M., and Crabill, E. V., *Endocrinology*, **69**, 581 (1961).

⁵ Ball, J. N., Oliverneau, M., and Kallman, K. D., *Nature*, **199**, 618 (1963).

⁶ Baker, B. I., *C. R. Acad. Sci., Paris*, **256**, 3356 (1963).

⁷ Oliverneau, M., *Anat. Anz.*, **109**, 468 (1961).

⁸ Arvy, L., Fontaine, M., and Cabe, M., *J. Physiol. (Paris)*, **49**, 635 (1957).

⁹ Oliverneau, M., La Roche, G., and Woodall, A. N., *Ann. Endocrinol.*, **25**, 481 (1964).

MICROBIOLOGY

Differences in the Antigenic Pattern of *Azotobacter* grown on Different Nitrogen Sources

THE presence of hydrogenase in nitrogen-fixing bacteria is ascribed a specific significance, as this enzyme is considered to take part in the fixation of gaseous nitrogen¹. This assumption is based on several observations. First, a significantly higher hydrogenase activity has been demonstrated in *Azotobacter* cells assimilating molecular nitrogen instead of combined forms of nitrogen, for example, ammonium ions, nitrate or glutamic acid. Secondly, *Azotobacter* mutants which have lost their capacity for nitrogen fixation also displayed a low hydrogenase activity as compared with the wild type. Thirdly, an extract of *Clostridium pasteurianum* with a retained capability for nitrogen fixation was also shown to contain hydrogenase activity. A fraction containing the hydrogenase activity which is separated from the extract loses its ability to fix nitrogen. Recombination of the fractions restored the activity¹. The site of the hydrogenase activity in *Azotobacter* has been located to the surface components of the cell^{2,3}.

This paper comprises a study of the antigenic structure of *Azotobacter* cells grown with either nitrogen gas or ammonium ions as the sole source of nitrogen. The organism used, *Azotobacter vinelandii*, strain ATCC 7492, was cultivated in Erlenmeyer flasks on a rotary shaker at 30° C in a nitrogen-free medium⁴, or in the same medium to which ammonium chloride (1 g/l.) had been added. In the latter medium the sodium molybdate was omitted. Antigens prepared from the cells of these cultures were studied by gel precipitin tests, which were performed according to the techniques described by Feinberg⁵. The supernatants of cell suspensions, subjected to sonic treatment, were used as precipitating antigens.

Antisera were prepared in rabbits. Antigens for immunization consisted of washed suspensions of whole cells, grown in the two different media. Each type of antigen was injected into a group of three rabbits. Immunization was performed by intravenous injections of 0.5 mg of the antigen twice a week for ten weeks. The rabbits were then exsanguinated and sera collected. The globulins precipitating at half saturation with ammonium sulphate in the cold were prepared from each antiserum. The globulin precipitate was dissolved in distilled water to approximately one-tenth of the original volume of the serum.

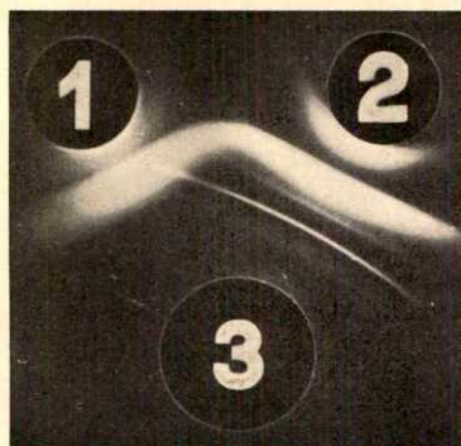


Fig. 1. Gel-precipitin test illustrating different precipitation patterns obtained with antigens from *Azotobacter* cells grown in a medium containing ammonium ions and devoid of sodium molybdate (1) and in a nitrogen-free medium (2). Antiserum against the latter type of antigen was placed in the serum well (3)

The results are illustrated in Fig. 1. Cells grown in nitrogen-free medium displayed an antigenic pattern with five precipitation lines. Cells prepared from a culture grown in the medium containing ammonium ions developed two lines, one of which showed a reaction of identity with one of the lines of the other system. The six different antisera gave similar precipitation patterns.

The fact that the rabbits immunized with cells grown in the medium containing ammonium ions develop antibodies against all antigens detected indicates the presence of these antigens also in ammonium-grown cells. The concentration of these antigens, however, is apparently much lower in the ammonium-grown cells, since they were not detected in the gel-precipitin tests. Hydrogenase activity is regularly observed in ammonium-grown cells, although at a much lower level than in cells grown with molecular nitrogen.

As can be seen in Fig. 1, a number of additional antigens were demonstrated in the system using an extract of cells grown in a nitrogen-free medium. These antigens might possibly represent different enzymes of the nitrogen-fixing system. We hope that the technique demonstrated in this report might be of value in investigations concerned with the mechanism of nitrogen fixation.

This work was aided by a grant from the Swedish State Technical Research Council.

TORD HOLME
BENGT ZACHARIAS

Department of Bacteriology,
Karolinska Institutet,
Stockholm, Sweden.

¹ Mortenson, L. E., *The Bacteria*, 3, 119, edit. by Gunsalus, I. C., and Stanier, R. Y. (Academic Press, New York, 1962).

² Marr, A. G., and Cota-Robles, E. H., *J. Bact.*, **74**, 79 (1957).

³ Cota-Robles, E. H., Marr, A. G., and Nilsson, E. H., *J. Bact.*, **75**, 243 (1958).

⁴ Nicholas, D. J. D., Fisher, D. J., Redmond, W. J., and Wright, M. A., *J. Gen. Microbiol.*, **22**, 191 (1960).

⁵ Feinberg, J. G., *Proc. Fourth Intern. Congr. Biol. Stand.*, Brussels, 194 (1959).

Human Indigenous Microflora in Gnotobiotic Rats

WHILE the existence of a symbiotic relationship between man or animals and micro-organisms was recognized as early as the times of Pasteur and Metchnikoff¹, little is known concerning the evolution, complete identification, survival factors and the mechanisms which control the beneficial or pathogenic activities of the intestinal flora. Recently, however, model systems for examination of the intestinal flora have been made available in the establishment of germ-free², gnotobiotic (known flora)³ and specific pathogen-free (SPF) animals⁴.

Using the germ-free rat, we have established known microfloras composed of 1-4 predominating micro-organisms of human intestinal origin. Our work complements that of Gibbons *et al.*⁴ who established bacteria indigenous to the gingival crevice area of the mucous membranes of man in germ-free mice. Four of the organisms we used were originally isolated by Gall *et al.*^{5,6} from the billionth and higher dilutions of fresh faeces of young men participating in space investigations. These bacteria are obligate anaerobes which far outnumber the aerobes. They are only tentatively identified but have been separated into faecal anaerobe (FA) 'groups' by well-defined differences based on morphology and reaction to various media⁷. The one other pure culture used was a *Lactobacillus acidophilus* isolated from human faeces after ingestion of a commercial culture for one week. The test micro-organisms are listed in Table 1.

A semi-synthetic diet was prepared in agar-gel form by the method of Miller and Allison⁸, sealed in No. 2 cans and sterilized by irradiation to 4 Mrads in the U.S. Army Natick Laboratories cobalt-60 source. Diet composition on a dry basis in per cent is as follows: casein (22), sucrose (18), dextrose (18), dextrine (19), lard (7), corn oil (3), Wesson salt mix (4), vitamin mix (1), cellulose (4) and agar (4). One kg of the vitamin mix contained 9,750 i.u. vitamin A palmitate, 975 i.u. vitamin D₂, 2,000 mg ascorbic acid, 125 i.u. vitamin E acetate, 100 mg menadione, 60 mg thiamine hydrochloride, 30 mg riboflavin, 100 mg niacinamide, 2,000 mg choline chloride, 300 mg calcium pantothenate, 1,000 mg inositol, 50 mg pyridoxine hydrochloride, 10 mg folic acid, 1 mg biotin, 50 mg para-aminobenzoic acid, 25 mg vitamin B₁₂ and glucose diluent to make up the balance of the kilogram. Agar was dissolved in hot water before being added to a dry mix of the other dietary components. Ratio of dry ingredients to added water was 1:1.

After sterilization, the above diet was fed to weanling germ-free rats of the Charles River Breeding Laboratories strain maintained in Trexler-type⁹ flexible film isolators. At the start of the experiments, animals were inoculated with desired micro-organisms which were brought into the isolators in sealed ampoules and then introduced into the diet which had been withheld from the rats for 12 h. The experiment consisted of two runs conducted two months apart with experimental groups as shown in Table 1. Germ-free rats and gnotobiotics inoculated with normal rat caecum contents served as controls. There were five rats per group. Each experiment was of three weeks duration.

Establishment of inoculated organisms was determined by culturing of fresh faecal samples obtained in the isolators and of the contents of the gastrointestinal tract at the termination of the runs. Samples were cultured aerobically and anaerobically in thioglycollate broth and Gall's medium⁷. In addition, Gram stain smears were examined microscopically.

At the end of the first run, cultures taken from the jejunum, caecum, and colon showed that the mono-contaminants FA 1, 9, 13 and 15 were established in greatest concentration in the colons and caeca. Very few micro-organisms were found in the jejunum as compared with the caecum and colon. Therefore, when the second run was terminated, only the caeca were sampled for bacteriological culturing. As shown in Table 1 (run No. 2), mono-inoculated FA 13 was found to be associated with a contaminant which was not intentionally introduced. Also, in the tetra-inoculated group FA 13 and FA 15 were identified as being present, but morphological similarities made it difficult to determine definitely whether both FA 1 and FA 9 were established. While there was no quantitation, it was noted that FA 15 predominated in the tetracontaminated rats.

Early growth as measured by weight gain was the overall measure of physiological response to the artificially

Table 1. WEIGHT GAIN, CAECUM SIZE AND CHOLESTEROL-LEVELS OF GNOTOBIOTIC RATS MAINTAINED GERM-FREE OR INOCULATED WITH ORAL DOSES OF HUMAN AND RAT ORIGIN MICRO-ORGANISMS. ALL ANIMALS WERE FED THE SAME STERILE SEMI-SYNTHETIC DIET FOR THREE WEEKS

Micro-organisms introduced *	Three-week body-weight gain (g)†	Caecum as % of body weight	Plasma cholesterol‡ (mg/100 ml.)	Liver cholesterol‡ (mg/g)
Run No. 1				
Germ-free	74 ± 3 (c)§	7.1 (a)	95 ± 5 (a)	2.52 ± 0.11 (ab)
FA 1 (<i>Sphaerophorus</i> or <i>Catenabacter</i>)	95 ± 5 (a)	6.8 (ab)	97 ± 10 (a)	2.78 ± 0.28 (ab)
FA 9 (<i>Bacteroides</i>)	80 ± 5 (bc)	6.2 (ab)	124 ± 5 (b)	3.23 ± 0.16 (b)
FA 13 (<i>Yelloneilla</i> sp.)	98 ± 2 (a)	5.6 (ab)	99 ± 5 (a)	2.16 ± 0.10 (a)
FA 15 (<i>Fusiformis</i> sp.)	91 ± 2 (ab)	5.1 (b)	158 ± 3 (b)	2.64 ± 0.18 (ab)
Run No. 2				
Germ-free	83 ± 6 (a)	7.1 (a)	88 ± 3 (b)	2.41 ± 0.19 (a)
Rat caecum organisms	101 ± 6 (b)	2.4 (c)	78 ± 15 (a)	2.21 ± 0.17 (a)
FA 1, 9, 13, 15	83 ± 4 (a)	6.6 (ab)	95 ± 5 (b)	2.54 ± 0.08 (a)
FA 13 + <i>Lactobacillus acidophilus</i>	77 ± 3 (a)	6.1 (b)	73 ± 3 (a)	2.11 ± 0.13 (a)
FA 13 + contaminant	87 ± 2 (ab)	7.3 (a)	71 ± 15 (a)	2.14 ± 0.20 (a)
<i>Lactobacillus acidophilus</i>	90 ± 3 (ab)	7.3 (a)	74 ± 15 (a)	2.17 ± 0.06 (a)

* FA (faecal anaerobic) micro-organisms only tentatively identified.

† Average body-weight gains and standard errors, not corrected for caecum weights.

‡ Cholesterol determined by the fluorometric procedure of R. W. Albers and O. H. Lowry (*Anal. Chem.*, 27, 1329; 1955).

§ Common letters denote statistically similar group values by analysis of variance.

established microfloras. As shown in Table 1, mono-established organisms varied in their weight-gain promoting ability. The weight gains of monocontaminated rats were 8–32 per cent greater than those obtained in the flora-free animals. In run No. 2 the greatest weight gain was obtained in the group polycontaminated with normal rat caecum organisms. Since the average gain of 4.8 g/day compares favourably with that of conventional rats, it may be assumed that the sterile diet used was of high nutritional value. Animals with the other induced microfloras in run No. 2 were not statistically different in body weight from germ-free controls. The failure of micro-organisms which increased weight gain as monocontaminants to do so when associated together indicates that physiological response is governed not only by the type of micro-organisms present but also by the outcome of complex competitive inter-relationships.

Enlarged caeca, characteristic of the germ-free state in rodents², were found in all rats except those polycontaminated with rat caecum organisms. Omitting this group, the caeca which varied from 5.1 to 7.3 per cent of body weight were not as large as those obtained by some workers. This may be a diet-influenced effect. Nevertheless, the presence of enlarged caeca indicates that none of the human-origin micro-organisms established in the germ-free rat, either singly or in combination, were physiologically adequate.

Plasma and liver total cholesterol-levels were also influenced by the introduced micro-organisms. In this respect, our results are not in agreement with those of Graber *et al.*¹⁰ who found that differences in plasma cholesterol of conventionally maintained rats were the result of dietary factors rather than of alteration in bacterial flora. Average plasma cholesterol of rats monocontaminated with FA 15 organisms was significantly higher than that of the germ-free rats and two other monocontaminated groups. Also, liver cholesterol-levels in FA 9 rats were statistically greater than those of FA 13 animals.

The reported pattern of higher cholesterol-levels in germ-free rats over conventional rats¹¹, probably due to a decrease in cholesterol catabolism in the germ-free rats¹², was observed in run No. 2. However, there was one exception in that the tetracontaminated group had the highest plasma cholesterol levels. An explanation for the cholesterol elevation may be that FA 15, which predominated in the tetracontamination, by itself results in a high level of blood cholesterol. Thus, our results appear to indicate that, while some intestinal micro-organisms may help to eliminate cholesterol, others may contribute directly or indirectly to the body cholesterol pool.

While we have established human indigenous micro-organisms in germ-free rats, we emphasize that differences exist between the two species. Two important differences are that the rat has a caecum and practices coprophagy. Our results demonstrate that the germ-free rat can

maintain specific organisms native to other species, with subsequent physiological changes.

H. A. DYMSZA

G. S. STOEWAND

J. J. ENRIGHT

Nutrition Branch, Food Division,

U.S. Army Natick Laboratories, Natick, Massachusetts.

P. C. TREXLER

Gnotobiotic Research Foundation,
251 Hallardvale Street,

North Wilmington, Massachusetts.

L. C. GALL

Life Sciences and Space Environment Laboratory,
Republic Aviation Corporation,
Farmingdale, Long Island, New York.

¹ Metchnikoff, E. and others cited by Rettger, L. F., and Cheplin, H. A., in *A Treatise on the Transplantation of the Intestinal Flora with Special Reference to the Implantation of Bacillus acidophilus*, 1 (Yale University Press, New Haven, 1921).

² Luckey, T. D., *Germfree Life and Gnotobiology* (Academic Press, New York, 1963).

³ Dubos, R., Schaedler, R. W., and Costello, R., *Fed. Proc.*, **22**, 1322 (1963).

⁴ Gibbons, R. J., Socransky, S. S., and Kapsimalis, B., *J. Bact.*, **88**, 1316 (1964).

⁵ Gall, L. S., and Helvey, W. M., *Bact. Proc. Abst.*, **G84**, 44 (1963).

⁶ Gall, L. S., Riely, P. E., Cardenas, R. R., and Albright, G. A., *Bact. Proc. Abst.*, **M148**, 72 (1964).

⁷ Gall, L. S., and Riely, P. E., *Determination of Aerobic and Anaerobic Microflora of Human Faeces* (Office Tech. Services, Dept. Commerce, Washington, D.C., 1964, Publ. No. AMRL-TR-64-107).

⁸ Miller, S. A., and Allison, J. B., *J. Nutrition*, **64**, 493 (1958).

⁹ Trexler, P. C., *Ann. N.Y. Acad. Sci.*, **78**, 29 (1959).

¹⁰ Graber, C. D., O'Neal, R. M., and Rabin, E. R., *J. Bact.*, **89**, 47 (1965).

¹¹ Westmann, B. S., and Wiern, M., *Amer. J. Physiol.*, **201**, 1027 (1961).

¹² Westmann, B. S., *Proc. Indiana Acad. Sci.*, **72**, 83 (1964).

PSYCHOLOGY

Neural Volleying: Upper Frequency Limits detectable in the Auditory System

THE discovery that nerve fibres exhibit a refractory period which limits the number of impulses produced in a given time dealt a death blow to early 'telephone' theories, which postulated that information concerning the frequency of a tone (or a Fourier component of a complex sound) was faithfully reproduced by the rate of discharge of an individual fibre. Simple 'telephone' theories of neural encoding of pitch were replaced by 'volley' theories¹ in which stimulus frequency was encoded in the auditory nerve as a whole. According to 'volley' theory, different fibres firing at sub-multiples of the stimulating frequency produce a composite discharge that faithfully reproduces the frequency of the stimulating wave-form.

Evidence for 'volleying' in the auditory system comes primarily from electrophysiological experimentation. Wave activity of the eighth nerve (presumed an envelope of a large number of individual fibre discharges) was observed by Derbyshire and Davis² to be synchronous with the stimulus tone up to a limiting frequency of about 4 kc/s. More recent studies³ utilizing computer averaging

of eighth nerve activity failed to demonstrate synchronous activity beyond 3 kc/s. A report⁴ on wave activity in the trapezoid body indicated an upper frequency limit of synchronous activity about 2.5 kc/s.

In the present experiment the upper limits of volleying were investigated by recording the wave activity generated in the superior olivary complex (SOC) in anaesthetized cats⁵. Five chronically implanted animals and five acute preparations were used. The animals were placed in a head-holder equipped with hollow ear bars into which were introduced tone bursts, 15–20 msec in duration with a 1-msec rise time and a fixed phase of onset. Stimulation was to the ear contralateral to the SOC because the amplitude of the wave response was larger and the possibility of picking-up volume-conducted cochlear microphonics, eighth nerve potentials or possible cochlear nuclei potentials was minimized.

Recording was monopolar with respect to an indifferent electrode on the bone over the frontal sinus or to a flap of skin. Insulated insect pins were used and the recording site histologically verified. The SOC potentials were amplified, passed through a band-pass filter⁶, and then either fed directly into a computer of average transients or recorded on magnetic tape for averaging when replayed at a slower speed⁷. The computer-averaged wave-forms were written out with an x-y recorder.

Earlier observations of SOC potentials in which a computer was not employed had usually failed to detect synchronous wave activity above 3 kc/s⁴. With response-averaging, no difficulty was encountered in demonstrating synchronous activity well beyond 3 kc/s, although amplitude declined markedly with higher stimulus frequencies. As the frequency of the stimulating tone increased above 3 kc/s, more and more intense tones were required to raise the signal above the noise-level.

Averaged responses to stimulus tones of different frequency are presented in Fig. 1. In this figure it is readily apparent that the frequency of the stimulating tone is reliably reproduced in the SOC wave up to 4.3 kc/s. The upper limits for demonstrating synchronous activity varied from animal to animal and this was probably a result of differing electrode positions. In all cases it was above 3.5 kc/s. A definite upper limit could not be established. Other than electrode position, the criterion for determining whether the averaged potentials were generated in the SOC was that the latency of the response to a high-intensity tone was greater than 2 msec. About 4 kc/s the amplitudes of the SOC potentials were so small that the limits of resolution of the recording system were approached, and it became difficult to separate SOC potentials from possible artefacts. These artefacts included volume conducted potentials from other areas, 'broadcasting' from the earphone and possible mechanical vibration of the head-holder with high intensity tones.

That computer-averaged SOC responses show an upper frequency limit higher than any demonstrated in the eighth nerve is possibly due to differences in experimental conditions and the large amplitude of the potentials generated in the SOC. The surprising fact is that the introduction of a synapse at the cochlear nuclei and probably another at the accessory nucleus (the method of recording cannot distinguish presynaptic from postsynaptic potentials) apparently does nothing to disturb the temporal relationship of inter-fibre volleying. This fact implies extremely accurate temporal resolution at the cochlear nuclei relay to maintain stable inter-fibre volleying relationships. Temporal variability of inter-fibre discharge can also be introduced by minute variation in the length or diameter of the trapezoid body fibres innervating the accessory nucleus, but this variability is either minimal or somehow counterbalanced.

There is evidence from single-unit investigations that extensive re-coding of auditory information often occurs in the cochlear nuclei⁸. It is evident from the present study, however, that the temporal information encoded in the

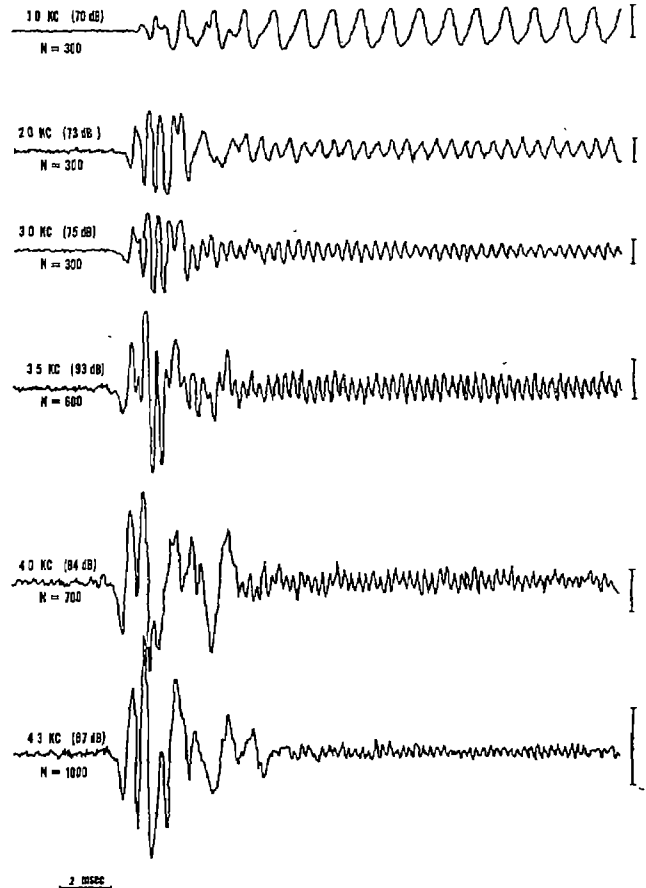


Fig. 1. Computer-averaged responses recorded from the superior olivary complex of an anaesthetized cat. Stimuli were tone bursts of different frequency delivered to the ear contralateral to the SOC. The number of responses averaged is *N*. Stimulus intensity expressed in decibels above 0.0002 dynes/cm². Vertical calibration line is 0.1 mV

eighth nerve by means of inter-fibre volleying is encoded in the same way by the trapezoid fibres innervating the SOC. That the detectable upper frequency limits of the wave activity recorded from the eighth nerve and SOC are about equal implies little or no loss of the temporal information encoded by inter-fibre volleying. Apparently, the accessory nucleus of the SOC, which is evidently primarily involved in binaural localization⁹, finds it convenient to receive temporal information in a form relatively unmodified from that found in the eighth nerve.

JAMES C. BOUDREAU

Division of Psychology,
U.S. Army Medical Research Laboratory,
Fort Knox, Kentucky.

¹ Wever, E. G., and Bray, C. W., *Psychol. Rev.*, **37**, 365 (1930). Troland, L. T., *J. Gen. Psychol.*, **2**, 28 (1929).

² Derbyshire, A. J., and Davis, H., *Amer. J. Physiol.*, **113**, 476 (1935).

³ Peake, W. T., *Tech. Rep. 363, Res. Lab. Electron., M.I.T.* (1960). Peake, W. T., Goldstein, M. H., jun., and Kiang, N. Y.-S., *J. Acoust. Soc. Amer.*, **34**, 562 (1962).

⁴ Kemp, E. H., Coppes, G. E., and Robinson, E. H., *Amer. J. Physiol.*, **120**, 304 (1937).

⁵ Previous reports from this laboratory have described in greater detail the wave-forms of the SOC and the various techniques used. Interested readers are referred to Tsuchitani, C., and Boudreau, J. C., *J. Neurophysiol.*, **27**, 814 (1964); Boudreau, J. C., and Rohwer, J. W., *U.S. Army Med. Res. Lab. Rep. No. 696* (1964); and Boudreau, J. C., *J. Acoust. Soc. Amer.* (in the press).

⁶ A Krohn-Hite band-pass filter, model 330 MR, was used with variable bandwidth. The half amplitude cut-off points for the records in Fig. 1 were 0.5 kc/s and 11 kc/s.

⁷ A computer of average transients (CAT 400B, Technical Measurements Co.) and an enhancer 1024 (Nuclear Data, Inc.) were used on different occasions. The latter had the advantage of on-line averaging of wave forms above 3 kc/s because of the greater number of memory channels available.

⁸ Hall, S., and Whitfield, I. C., *J. Physiol.*, **122**, 158 (1953).

⁹ Galambos, R., Schwartzkopf, J., and Rupert, H., *Amer. J. Physiol.*, **197**, 527 (1959). Hall, J. L., *Mass. Inst. Tech. Res. Lab. Electron. Tech. Rep.*, **416** (1964). Moushegian, G., Rupert, A., and Whitcomb, M. A., *J. Neurophysiol.*, **27**, 1174 (1964).

FORTHCOMING EVENTS

Monday, December 20

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Mr. D. G. Drummond: "Recent Developments in the Control of Commensal Rodents".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT LECTURER (with a good honours degree in botany and/or zoology and a special interest in cell biology and microbiology) in BIOLOGY—The Secretary, University of Exeter, Northcote House, The Queen's Drive, Exeter, Devonshire (December 31).

ASSISTANT LECTURER (with special qualifications in experimental physics, aeronomy or astronomy) in APPLIED MATHEMATICS (GEOPHYSICS)—The Secretary, The Queen's University, Belfast, Northern Ireland (December 31).

CHAIR IN AND HEADSHIP OF THE DEPARTMENT OF METALLURGY AND MATERIALS TECHNOLOGY—The Secretary, Battersea College of Technology, Battersea Park Road, London, S.W.11 (December 31).

LECTURER IN EDUCATION COMBINED WITH GEOGRAPHY—The Assistant Registrar (Establishment), University of Sussex, Stanmer House, Stanmer, Brighton, Sussex (December 31).

LECTURER IN EDUCATION with special reference to the teaching of science or engineering or mathematics in Technical Colleges and Sixth Forms—The Registrar (Room 39, O.R.B.), The University, Reading (December 31).

LECTURER OR ASSISTANT LECTURER IN MATHEMATICAL SCIENCES—The Secretary, University of Exeter, Northcote House, The Queen's Drive, Exeter, Devonshire (December 31).

LECTURER (preferably with an interest in physical chemistry) in CHEMISTRY at the University of the West Indies—The Inter-University Council, 33 Bedford Place, London, W.C.1 (December 31).

LECTURER (with an Hons. B.Sc. degree in biochemistry or equivalent qualification, and preferably a dental qualification) in DENTAL BIOCHEMISTRY—The Secretary of the University Court, The University, Glasgow (December 31).

LECTURERS OR ASSISTANT LECTURERS (with interest in inorganic chemistry, physical chemistry, or theoretical chemistry) in CHEMISTRY—The Registrar, University of East Anglia, Earlham Hall, Norwich, NOR 88C (December 31).

RESEARCH STUDENT IN THE DEPARTMENT OF PHYSICS to carry out research with a small group working on certain aspects of vacuum breakdown within the Research School of Ionization Physics—The Registrar, University College of Swansea, Singleton Park, Swansea (December 31).

SENIOR RESEARCH FELLOW IN THE DEPARTMENT OF METALLURGY to carry out research in the technology of primary metal production either by the conventional or powder routes—The Registrar, University College of Swansea, Singleton Park, Swansea (December 31).

ASSISTANT EDITORS AND EDITORIAL ASSISTANTS (preferably with a good honours degree in chemistry and some postgraduate experience) for various duties with the Society's scientific publications—The General Secretary, The Chemical Society, Burlington House, Piccadilly, London, W.1 (January 1).

ASSISTANT LECTURER IN STATISTICS IN THE DEPARTMENT OF APPLIED STATISTICS (formerly the Unit of Biometry), for duties which will involve teaching, consulting and research—The Registrar (Room 39, O.R.B.), The University, Reading (January 1).

HEAD (with high academic qualifications and considerable research experience in genetics and plant breeding, not necessarily with vegetables) OF THE PLANT BREEDING SECTION—The Secretary, National Vegetable Research Station, Wellesbourne, Warwick (January 3).

SENIOR LECTURER IN CLINICAL MEDICINE; a LECTURER IN PATHOLOGY, and a LECTURER IN SURGERY at the University of Singapore—The Inter-University Council, 33 Bedford Place, London, W.C.1 (January 7).

SENIOR SCIENTIFIC OFFICER/SCIENTIFIC OFFICER (with first- or second-class honours degree in chemistry or microchemistry with suitable postgraduate experience; or an honours degree in agricultural chemistry with postgraduate experience) in THE CHEMICAL RESEARCH DIVISION, MINISTRY OF AGRICULTURE, to undertake research work in animal nutrition (successful candidate may be required to undertake teaching duties in the Faculty of Agriculture in Queen's University, Belfast)—The Secretary, Civil Service Commission, Stormont, Belfast, 4, Northern Ireland (January 7).

LECTURER IN ANCIENT PHILOSOPHY—The Secretary of Faculties, University Registry, Oxford (January 8).

SENIOR LECTURER/LECTURER IN PHARMACOLOGY IN THE DEPARTMENT OF MATERIA MEDICA AND THERAPEUTICS—The Secretary, The University, Aberdeen (January 8).

SENIOR LECTURER IN VETERINARY MEDICINE at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, January 14).

CHAIR OF PHYSICAL CHEMISTRY—The Secretary, The Queen's University, Belfast, Northern Ireland (January 15).

CHAIR OF PSYCHOLOGY at the University of Cape Town—The Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1; and The Registrar, University of Cape Town, Private Bag, Rondebosch, Cape Town, South Africa (January 15).

PROFESSOR OF GEOBOTANY—The President of the Board of the Swiss Federal Institute of Technology, Leonhardstrasse 33, CH-8006, Zurich, Switzerland (January 15).

SENIOR LECTURER OR LECTURER (with a higher degree in psychology and preferably with specialization in personality and social psychology) in PSYCHOLOGY at the Australian National University—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 17).

ASSISTANT LECTURERS OR LECTURERS (2) (preferably with interests in either theoretical chemistry, chemical crystallography or physical organic chemistry, and research experience at post-doctoral level) in THE DEPARTMENT OF CHEMISTRY—The Registrar, University of Essex, Wivenhoe Park, Colchester, Essex (January 22).

FELLOW/LECTURER IN ANTHROPOLOGY within the Centre for Middle Eastern and Islamic Studies—The Registrar and Secretary, University of Durham, Old Shire Hall, Durham (January 28).

ASSISTANT OR ASSOCIATE PROFESSORS (with research experience in biochemistry, plant physiology or experimental biology) in BIOCHEMISTRY AND BIOLOGY—Head of the Bioscience Department, Brock University, St Catharines, Ontario, Canada (January 31).

LECTURER OR SENIOR LECTURER (suitably qualified graduate in science, medicine or veterinary science, and preferably with some research experience) in PHYSIOLOGY at Massey University of Manawatu, Palmerston North, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall London, S.W.1 (New Zealand and London, January 31).

LECTURER/SENIOR LECTURER (with a suitable, preferably a higher degree in science or agricultural science and experience in the field of plant physiology) in AGRONOMY (Plant Physiology) in THE FACULTY OF AGRICULTURE AND HORTICULTURE, Massey University of Manawatu—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, January 31).

OFFICIAL FELLOW IN ENGINEERING SCIENCE—The Principal, Brasenose College, Oxford (January 31).

SCIENTIFIC OFFICER OR SENIOR SCIENTIFIC OFFICER (botanist with a good honours degree) to study the physiology of sucrose storage in sugar beet—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (January 31).

SENIOR LECTURER IN METEOROLOGY at Victoria University of Wellington, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, January 31).

SENIOR LECTURER IN STATISTICAL MATHEMATICS and/or NUMERICAL MATHEMATICS at Victoria University of Wellington, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, January 31).

LECTURER (preferably graduate in sociology and social anthropology) in SOCIOLOGY OF THE MIDDLE EAST in THE DEPARTMENT OF SOCIAL THEORY AND INSTITUTIONS—The Registrar and Secretary, University of Durham, Old Shire Hall, Durham (February 1).

LECTURER (with a good honours degree in metallurgy or physics and preferably post-doctoral experience in the application of X-ray diffraction methods to the study of metals) in METALLURGY—The Registrar, The University, Sheffield (February 15).

CHAIR OF BIOCHEMISTRY—The Registrar, University of Strathclyde, Glasgow, C.1 (February 28).

ANDRÉ DOREMAN FELLOW FOR RESEARCH IN MINING ENGINEERING and/or MINING TECHNOLOGY—The Registrar, Imperial College of Science and Technology, London, S.W.7 (March 31).

LECTURER (with a degree in pure science or agricultural science) in ENTOMOLOGY in THE DEPARTMENT OF AGRICULTURAL ZOOLOGY, Lincoln College (University of Canterbury), New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, March 31).

ASSISTANT DIRECTOR (with a degree in agriculture, agricultural botany or pure science, a reading knowledge of two or more modern languages, the ability to write good concise English, and preferably some editorial experience) OF THE COMMONWEALTH BUREAU OF PASTURES AND FIELD CROPS, Hurley, near Maidenhead, Berks—The Secretary, Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, near Slough, Bucks.

GRADUATE ASSISTANT (science or biology graduate) in THE CYTOLOGY DEPARTMENT of a large modern group laboratory, for duties which will include routine cytodiagnostic and research work under the guidance of the Consultant Pathologist in charge—The Director or Consultant in charge of the Cytodiagnostic Unit, Group Laboratory, St. Stephen's Hospital, Fulham Road, Chelsea, London, S.W.10.

LECTURER IN INORGANIC CHEMISTRY in THE DEPARTMENT OF CHEMICAL TECHNOLOGY—The Registrar, Bradford Institute of Technology, Bradford, 7.

LECTURER OR ASSISTANT LECTURER in GEOGRAPHY—The Registrar, The University, Hull.

LECTURER (with qualifications in communication and control theory, operational research or stochastic processes) in MATHEMATICS—The Registrar (Room 39, O.R.B.), The University, Reading.

RESEARCH ASSISTANT to work on radio-biological and oncological problems using histological, histochemical, autoradiographic and electron-microscope techniques—The Deputy-Director, Strangeways Research Laboratory, Worts Causeway, Cambridge.

RESEARCH ASSISTANT (with a good honours degree in physics or a good general honours degree including physics) in PHYSICS for research in the field of either radiation physics or vacuum physics, and would lead to the higher degree of either M.Sc. or Ph.D.—Head of the Department of Physics, The Polytechnic, Regent Street, London, W.1.

RESEARCH ASSISTANT (with H.N.C. in physics or chemistry, or an equivalent qualification) to join a small group working on high temperature chemistry and physics—Dr. A. J. Croft, Clarendon Laboratory, University of Oxford, Oxford.

RESEARCH FELLOW (young research worker, either post-Ph.D., or about to submit a Ph.D. dissertation) in THEORETICAL PHYSICS in one of the following fields: elementary particle physics, nuclear physics or solid state physics—The Assistant Registrar (Establishment), University of Sussex, Stanmer House, Stanmer, Brighton, Sussex.

RESEARCH LABORATORY TECHNICIAN to assist in a study of the physiology and biochemistry of isolated mammalian nerve cells—The Chief Laboratory Technician, Battersea College of Technology Annex, 14 Falcon Road, London, S.W.11.

SCIENTIFIC INFORMATION OFFICER (with a degree in agriculture or related subject and a flair for languages, including English) to join a team engaged in publishing the latest research findings on temperate and tropical grasslands and annual field crops in two journals of international repute—The Director, Commonwealth Bureau of Pastures and Field Crops, Hurley, nr. Maidenhead, Berks.

SENIOR LABORATORY TECHNICIAN (preferably with some experience in metallurgical analysis) to supervise the chemical laboratories of the Department of Metallurgy, Sir John Cass College, Whitechapel High Street, London, E.1—The Secretary, Sir John Cass College, Jewry Street, London, E.C.3.

SENIOR PHYSICIST to take charge of the Physics Department, Radiotherapy Centre, St. William's Hospital, Rochester, Kent—The Group Secretary, Medway and Gravesend Hospital Management Committee, Medway Hospital, Gillingham, Kent.

SENIOR TECHNICIAN (CHEMICAL PATHOLOGY) for research projects, mainly enzymological—Dr. D. M. Matthews, Deputy Director, Vincent Square Laboratories of Westminster Hospital, 124 Vauxhall Bridge Road, London, S.W.1.

SENIOR TECHNICIAN (preferably with experience of working with an electron microscope and/or electronics) in THE CRYSTALLOGRAPHY RESEARCH DEPARTMENT—The Administrative Assistant, Birkbeck College (University of London), Malet Street, London, W.C.1, quoting Ref. C.S.T.

TECHNICIAN (with a good G.C.E. in science subjects including chemistry, and preferably previous experience in biochemical techniques) FOR A RESEARCH UNIT—The Administrator, Department of Biochemistry, The University, South Parks Road, Oxford.

TECHNICIAN (with previous experience of chromosome work) to work in the Chromosome Laboratory of the Department of Clinical Research at the Royal Marsden Hospital, Fulham Road, London, S.W.3—The Secretary, Institute of Cancer Research, 34 Sumner Place, London, S.W.7, quoting Ref. 301/B/229.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

- Ditch or Crash-Land? By B. W. Townshend. Pp. vii+60. (Farnham, Surrey: B. W. Townshend, Little Gables, Crooksbury Road, 1965.) [311]
- Tavistock Pamphlet No. 12: Towards a Theory and Practice of Social Architecture: The Building of Indispensable Institutions. By Dr. Howard V. Perlmutter. Pp. vii+60. (London: Tavistock Publications, 1965.) 12s. 6d. net. [311]
- Manchester Literary and Philosophical Society. Memoirs and Proceedings. Vol. 107, 1964-65. Pp. 142+hi. (Manchester: Manchester Literary and Philosophical Society, 1965.) 31s. 6d. [311]
- The British Cast Iron Research Association. Forty-fourth Annual Report for the year ending 30th June 1965. Pp. 111. (Alvechurch, Birmingham: The British Cast Iron Research Association, 1965.) [311]
- The West of Scotland Agricultural Colleges. Research Bulletin No. 84: Comparison of Production of Four Varieties of White Clover. By I. V. Hunt, R. D. Harkess and T. W. Martin. Pp. 39. (Auchincruive, by Ayr: The West of Scotland Agricultural College, 1965.) 1s. [311]
- University of Oxford. Annual Report of the Curators of the Bodleian Library for 1963-64. Pp. 28. (Supplement No. 9 to the *University Gazette*, August, 1965.) (Oxford: The University, 1965.) 2s. 6d. [311]
- Medical Research Council Monitoring Report Series, No. 11: Assay of Strontium-90 in Human Bone in the United Kingdom, Results for 1964, Part 2. Pp. v+17. (London: H.M. Stationery Office, 1965.) 1s. 9d. net. [311]
- Queen Elizabeth College (University of London). The Development of Experimental Botany. By Prof. J. Sidelman. (Inaugural Lecture delivered 19th January, 1965.) Pp. 17. (London: Queen Elizabeth College, 1965.) [311]
- Marine Biological Station, University of Liverpool. Annual Report No. 77 for 1964. Pp. 55. (Liverpool: Liverpool University Press, 1965.) [311]
- The Overseas Development Institute, Ltd. The Decade of Development—a Study in Frustration? (A lecture delivered in London under the auspices of the Overseas Development Institute on May 3, 1965.) Pp. 23. (London: The Overseas Development Institute, 1965.) 3s. 6d. [311]
- Scottish Society for Research in Plant Breeding: Scottish Plant Breeding Station. Record 1965. Pp. 236. (Pentlandsfield, Roslin, Midlothian: Scottish Society for Research in Plant Breeding, 1965.) [311]
- Publications List of the Commonwealth Agricultural Bureaux 1965-66. Agriculture; Animal Health; Forestry. Pp. 42. (Farnham Royal: Commonwealth Agricultural Bureaux, 1965.) [311]
- The Institute of Personnel Management, 1964-1965. Pp. 41. (London: The Institute of Personnel Management, 1965.) [311]
- Proceedings of the Society for Psychical Research, Vol. 54, Part 197 (August, 1965): The Blue Vase. By G. W. Lambert. Time and ESP. By H. A. C. Dobbs. Pp. 233-361. (London: The Society for Psychical Research, 1965.) 12s. 6d.; 2 dollars. [311]
- Ministry of Technology. Research for Industry 1964. (A Report on work done by industrial research associations in the Government scheme.) Pp. iii+72+20 plates. (London: H.M. Stationery Office, 1965.) 8s. net. [311]
- Friends of the Lake District. Report and News Letter, September 1965. Pp. 18. (Ulverston: Friends of the Lake District, 1965.) [311]
- Procter and Gamble, Ltd. Financial Statement for the year ended 30th June 1965. Pp. 16. (Newcastle upon Tyne: Procter and Gamble, Ltd., 1965.) [311]
- National Union of Teachers. What Chance Has Your Child? By Kathleen Gibberd. (A Study of the Effects of Overcrowded Classes in Our Schools.) Pp. 15. (London: National Union of Teachers, 1965.) 6d. [311]

Other Countries

- Republic of South Africa: Department of Commerce and Industries. Division of Sea Fisheries. Investigational Report No. 52: Ocean Currents and Water Masses at 1,000, 1,500 and 3,000 Metres in the South-West Indian Ocean. By G. A. Visser and M. M. Van Niekerk. Pp. 40. Investigational Report No. 53: The Rock Lobster of the South African West Coast, *Jasus lalandii* (H. Milne-Edwards). 1: Notes on the Reproduction Biology and the Determination of Minimum Size Limits for Commercial Catches. By A. E. F. Heydorn. Pp. 32. (Sea Point, Cape Town: Division of Sea Fisheries, 1965.) [411]
- Bilan des Maladies et Infirmités Découlées dans les Classes de Milice 1953-1960 en Belgique. Par F. Twisselmann, J. François, P. Moureau et S. Vrydagh. (Extrait de *Population et Famille*, No. 5.) Pp. 81. (Brussels: National Center for Radiobiology and Genetics, 1965.) [411]
- New Zealand. Report of the Board of Trustees of the National Art Gallery and Dominion Museum for the year ended 31 March 1965. Pp. 28. (H. 21.) (Wellington: Government Printer, 1965.) 1s. 6d. [411]
- Salamandra*, Band 1, Heft 1/2 (September 1965). Herausgegeben von der Deutschen Gesellschaft für Herpetologie und Terrarienkunde. Pp. 52. (Frankfurt/Main-Niederrad: Deutsche Gesellschaft für Herpetologie und Terrarienkunde, 1965.) [411]
- Annals of the New York Academy of Sciences. Vol. 105, Article 18: *In Vivo* and *In Vitro* Behavior of Clotting Factors in Blood and Tissues. By F. Nour-Eldin. Pp. 983-1004. 2 dollars. Vol. 118, Article 13: Further Approximations to the Blastus Function. By Ernest D. Kennedy. Pp. 575-584. 1.50 dollars. Vol. 118, Article 14: Application of Electrodynetic Phenomena in Civil Engineering and Petroleum Engineering. By S. A. Anbar, O. M. Beeson and G. V. Chillingar. Pp. 585-602. Vol. 118, Article 15: Mercenene: *In Vivo* Effects of Mollusk Extracts on the Sarcoma 180. By M. Rosarli Schneer and Cecilia V. Huala. Pp. 603-610. 1.50 dollars. Vol. 118, Article 23: The Formation of Noble Gas Compounds. By Leland G. Allen. Pp. 883-898. 1.50 dollars. (New York: New York Academy of Sciences, 1964 and 1965.) [411]
- The Research Institute of Applied Electricity, Hokkaido University Monograph Series. No. 11: Network Synthesis with Multiwire Lines. By Prof. A. Matsumoto. Pp. 98. No. 12: Developments in Multiwire Networks. By Prof. A. Matsumoto. Pp. 107. No. 13: Dipole, Molecule and Chemistry. By Prof. K. Higasi. Pp. 67. (Sapporo, Japan: The Research Institute of Applied Electricity, Hokkaido University, 1963, 1964 and 1965.) [411]
- Regeneratie, Volumetrische en Numerieke Hypertrofie van Skeletspieren bij Kikker en Rat. Door Walter Reitsma. Pp. 118. (Amsterdam: Vrije Universiteit, 1965.) [411]

- Institut for Atomenergi, Kjeller Research Establishment. Kjeller Report No. 96: Correlation Between Calculated and Measured Mechanical Properties of Thin-Walled Fuel Element Canning Tubes of Stainless Steel. By Erik Brodahl and Steinar Aas. Pp. 14+13 figures. (Kjeller, Norway: Institut for Atomenergi, Kjeller Research Establishment, 1965.) [411]
- Food and Agriculture Organization of the United Nations. Indo-Pacific Fisheries Council. Proceedings—11th Session, Kuala Lumpur, Malaysia, 16-21 October, 1964, Section 1. Pp. iii+90. (Bangkok: IPFC Secretariat, FAO Regional Office for Asia and the Far East, 1965.) [411]
- Indonesia: Ministry of Air Communications. Meteorological and Geophysical Service. Earthquakes in Indonesia 1962. Pp. 13. (Jakarta: Ministry of Air Communications, Meteorological and Geophysical Service, 1966.) [411]
- Transactions of the American Philosophical Society. New Series, Vol. 55, Part 6: The Pyramid of the Sun at Teotihuacán: 1959 Investigations. By René Millon, Bruce Drewitt and James A. Benneyhoff. Pp. 93. (Philadelphia: The American Philosophical Society, 1965.) 3 dollars. [411]
- Publications of the Dominion Observatory, Ottawa. Vol. 30, No. 3: Bibliography of Seismology, Items 17127-17944 (September-December, 1963). By F. E. Langill and J. H. Hodgson. Pp. 75-117. (Ottawa: Queen's Printer, 1965.) 1 dollar. [411]
- United States Department of the Interior. Geological Survey. Water-Supply Paper 1475-Q: Present and Future Water Supply for Mammoth Cave National Park, Kentucky. By R. V. Cushman, R. A. Krieger and John A. McCabe. Pp. v+601-647. 25 cents. Water-Supply Paper 1662-B: Specific Yield—Laboratory Experiments Showing the Effect of Time on Column Drainage. By R. C. Prill, A. I. Johnson and D. A. Morris. Pp. iv+55. 25 cents. Water-Supply Paper 1682: Magnitude and Frequency of Floods in the United States. Part 8. Western Gulf of Mexico Basins. By James L. Patterson. Pp. xvi+506+plate 1. Water-Supply Paper 1787: Ground-Water Conditions and Storage in the Central Sevier Valley, Utah. By Richard A. Young and Carl H. Carpenter. Pp. vi+95+plates 1-6. Water-Supply Paper 1792: Ground Water in Permafrost Regions—An Annotated Bibliography. By John R. Williams. Pp. iii+294+plate 1. 1.25 dollars. Water-Supply Paper 1794: Ground Water Resources of Pavant Valley, Utah. By R. W. Mower. Pp. v+73+plates 1-10. Water-Supply Paper 1802: Fluvial Sediment of the Mississippi River at St. Louis, Missouri. By Paul R. Jordan. Pp. vii+89. (Washington, D.C.: Government Printing Office, 1965.) [411]
- United States Department of the Interior. Geological Survey. Water-Supply Paper 1804: Drought of the 1950's, with Special Reference to the Midcontinent. By R. L. Nace and B. J. Plunowski. Pp. v+38+plates 1 and 2. Water-Supply Paper 1809-D: An Evaluation of Aquifer and Well Characteristics of Municipal Well Fields in Los Alamos and Gaje Canyons, Near Los Alamos, New Mexico. By R. L. Cushman. Pp. v+50+plates 1-4. 1 dollar. Water-Supply Paper 1809-L: Ground Water Pumpage and Water-Level Changes in the Milwaukee-Waukesha Area, Wisconsin, 1950-61. By J. H. Green and R. D. Hutchinson. Pp. iii+19+2 plates. Water-Supply Paper 1809-J: Water-Resources Reconnaissance of the Ouachita Mountains, Arkansas. By Donald R. Albin. Pp. iii+14+plate 1. 45 cents. Water-Supply Paper 1809-M: Elimination of Thermal Stratification in Reservoirs and the Resulting Benefits, with special emphasis on Study of Lake Whittford, California. By Gordon E. Koberg and Maurice B. Ford, Jr. Pp. iv+28. 15 cents. Water-Supply Paper 1809-N: Effects of Waste Water from A.E.C. Plant on the Hydrology of Glowegee Creek at West Milton, New York, 1958-61. By F. H. Pauszek and F. H. Ruggles. Pp. iv+29. 20 cents. (Washington, D.C.: Government Printing Office, 1965.) [411]
- Annals of the New York Academy of Sciences. Vol. 127, Article 1. Comparative Cardiology. By A. M. Albina and 80 other authors. Pp. 1-876. (New York: New York Academy of Sciences, 1965.) [511]
- Smithsonian Miscellaneous Collections. Vol. 148, No. 8: Middle and Late Turonian Oysters of the Lophia lugubris Group. By Erle G. Kaufmann. (Publication No. 4602.) Pp. i+92+8 plates. (Washington, D.C.: Smithsonian Institution, 1965.) [511]
- Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Bulletin 115: Chemical Analyses of Canadian Rocks, Minerals, and Ores, Compiled from the Records of the Geological Survey of Canada for the period 1846-1965. By J. A. Maxwell, K. R. Dawson, Margaret E. Tomlinson, Dorothy M. E. Pocock and Diane Tetreault. Pp. 476. (Ottawa: Queen's Printer, 1965.) 6.50 dollars. [511]
- Records of the Australian Museum. Vol. 26, No. 15 (3rd March, 1965): Cestoda (Tetraphyllidae and Trypanorhyncha) from Marine Fishes of New South Wales. By E. S. Robinson. Pp. 341-348. (Sydney: The Australian Museum, 1965.) 2s. [511]
- Occasional Papers of the California Academy of Sciences. No. 50 (September 15, 1965): A Review of *Kittoria*, a Genus of Diatoms. By A. L. Brigger and G. Dallas Hanna. Pp. 10. Proceedings of the California Academy of Sciences, Fourth Series. Vol. 30, No. 12 (September 15, 1965): New Species of Plants from Baja California, Mexico. By Prof. Ira L. Wiggins. Pp. 243-256 (5 plates). (San Francisco: California Academy of Sciences, 1965.) [511]
- Bulletin of the Museum of Comparative Zoology, Harvard University. Vol. 134, No. 4 (September 30, 1965): The Species *Bufo granulatus* Spix (Sallentia: Bufonidae) and Its Geographic Variation. By José M. Gallardo. Pp. 107-138. Vol. 134, No. 5 (September 30, 1965): The Mesopelagic Fishes Collected During Cruise 17 of the R/V *Chain*, with a Method for Analysing Faunal Transects. By Richard H. Backus, Giles W. Mead, Richard L. Haedrich and Alfred W. Ebeling. Pp. 139-157. (Cambridge, Mass.: Museum of Comparative Zoology, Harvard University, 1965.) [511]
- Current Tissue Culture Literature. A Key to the World Periodicals and Abstract Indexes, Vol. 5, Nos. 1 and 2 (June 1, 1965). By Margaret R. Murray and Gertrude Kopeck. Pp. 71. Annual subscription: 25 dollars. (New York, N.Y.: October House, Inc., 1965.) [511]

Editorial and Publishing Offices of "NATURE"

MACMILLAN (JOURNALS) LIMITED

4 LITTLE ESSEX STREET, LONDON, W.C.2

Telephone Number: Temple Bar 6633. Telegrams: Phusis London W.C.2

Annual subscription: Inland £14, Overseas £13 15s., payable in advance, postage paid to any part of the world.

Advertisements only should be addressed to

T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2

Telephone Number: Holborn 4743

Registered as a newspaper at the General Post Office

Copyright © Macmillan (Journals) Limited, December 18, 1965

NATURE

No. 5017

SATURDAY, DECEMBER 25, 1965

Vol. 208

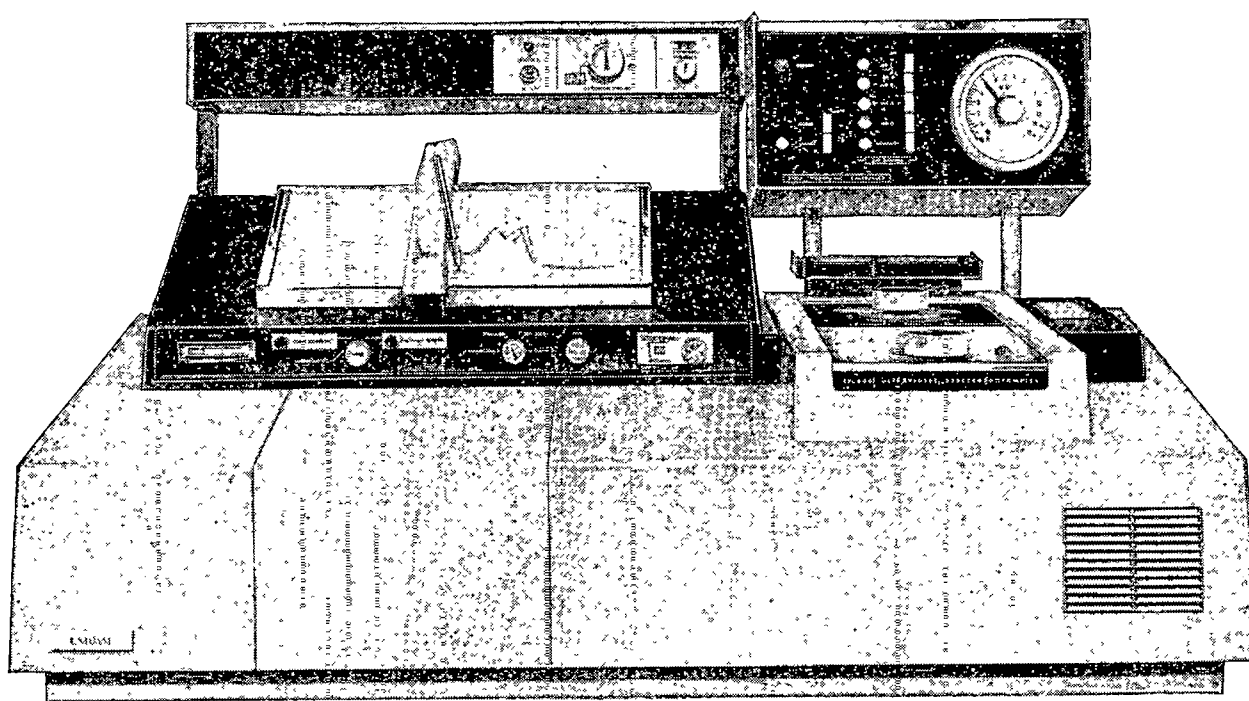
Published by
MACMILLAN (JOURNALS) LTD.

CONTENTS

	<i>Page</i>
CONSERVING THE NATURAL HERITAGE	1241
THE ROCKEFELLER INSTITUTE UNTIL 1953. By The Lord Adrian, O.M., F.R.S.	1244
EXPANDING HORIZONS IN OPERATIONAL RESEARCH. By S. J. Morrison	1245
SHAPE AND ARRANGEMENT IN LIPIDS. By Dr. F. D. Gunstone	1246
COGNITIVE DEVELOPMENT OF THE YOUNG CHILD. By Dr. J. H. Duthie	1246
SEROTONIN. By Dr. G. B. West	1247
FIBRINOLYSIS. By Dr. D. Ogston	1247
NEUROTROPHIC PROCESSES. By Prof. K. Krnjević	1248
1965 NOBEL PRIZE FOR MEDICINE. By Prof. M. R. Pollock, F.R.S.	1250
BIOCHEMISTRY AND MENTAL FUNCTION. By Prof. Seymour S. Kety	1252
CHEMICAL EDUCATION	1257
OBITUARIES:	
SIR RONALD HATTON, C.B.E., F.R.S. By Prof. H. G. H. Kearns, C.B.E.	1258
PROF. E. J. DIJKSTERHUIS. By Prof. H. Freudenthal	1258
PROF. R. H. HOPKINS. By Dr. F. W. Norris	1259
NEWS AND VIEWS	1259
THE COUNTRYSIDE IN 1970. By E. M. Nicholson, C.B.	1265
MALARIA AND THE OPENING-UP OF CENTRAL AFRICA. By R. Weatherall	1267
APPLIED SCIENCE IN THE SCHOOLS. By T. A. Burdett	1269
CHEMICAL PHYSIOLOGY OF THE ACID MUCOPOLYSACCHARIDES. By J. E. Scott	1270
POSSIBLE CONTRIBUTION OF JUPITER'S MAGNETOSPHERIC TAIL TO THE RADIO EMISSIONS OF THE PLANET IN THE DECAMETRIC REGION. By G. M. Gruber	1271
WELDING BY HIGH-VELOCITY PARTICLE IMPACT. By Dr. Robert F. Rolsten, Alan K. Hopkins and Harold H. Hunt	1273
SEISMICITY AND OTHER PROPERTIES OF GEOLOGICAL PROVINCES. By Prof. E. R. Kanasevich	1275
CARBON ISOTOPE RATIOS AND PALAEO-SALINITIES OF PURBECK-WEALDEN CARBONATES. By Prof. P. Allen and Prof. M. L. Keith	1278
RETURN OF LEUCOCYTES TO THE BONE MARROW IN CHRONIC MYELOGENOUS LEUKAEMIA. By Dr. John H. Moxley, Prof. Seymour Perry, Prof. George H. Weiss and Dr. Marvin Zelen	1281
EFFECTS OF CYCLIC ADENOSINE MONOPHOSPHATE AND MELANOCYTE-STIMULATING HORMONE ON FROG SKIN <i>in vitro</i> . By Dr. M. W. Bitensky and S. R. Burstein	1282
BIOSYNTHESIS OF 2-AMINOETHANEPHOSPHONIC ACID: A PHOSPHORAMIDIC ACID RE-ARRANGEMENT? By Dr. W. Segal	1284
IMMUNITY TO MALARIA: ANTIGENIC VARIATION IN CHRONIC INFECTIONS OF <i>Plasmodium knowlesi</i> . By Dr. K. N. Brown and I. N. Brown	1286
A PHARMACOLOGICAL INVESTIGATION OF HUMAN ISOLATED ILEUM. By Dr. A. Bennett	1289
CENTRAL STIMULANT PROPERTIES OF COMPOUNDS WITH PERIPHERAL MUSCARINIC PROPERTIES. By G. B. Leslie	1291
REGIONAL CHANGES IN BRAIN SEROTONIN AFTER HEAD X-IRRADIATION AND ITS SIGNIFICANCE IN THE POTENTIATION OF BARBITURATE HYPNOSIS. By Dr. V. Nair	1293
CATION PERMEABILITY OF PHOSPHOLIPID MODEL MEMBRANES: EFFECT OF NARCOTICS. By Dr. A. D. Bangham, Dr. M. M. Standish and Nigel Miller	1295
MOLECULAR WEIGHT OF CHICK INTERFERON. By Dr. D. C. Burke and Dr. J. Ross	1297
ARGININE AND UREA BIOSYNTHESIS IN THE LAND PLANARIAN: ITS SIGNIFICANCE IN BIOCHEMICAL EVOLUTION. By Prof. James W. Campbell	1299
CHLORAMPHENICOL-, DIHYDROSTREPTOMYCIN-, AND KANAMYCIN-INACTIVATING ENZYMES FROM MULTIPLE DRUG-RESISTANT <i>Escherichia coli</i> CARRYING EPISOME 'R'. By Suehiko Okamoto and Yoshiaki Suzuki	1301
RECENT SCIENTIFIC AND TECHNICAL BOOKS	dexxxxii

(Continued overleaf)

Progress in Spectroscopy



Unicam SP800 Spectrophotometer with automation accessories

*Save operator time, increase speed
and efficiency with Unicam
automation for:*

ENZYME REACTION RATES

**LIQUID COLUMN
CHROMATOGRAPHY**

**ALL ROUTINE ULTRAVIOLET
& VISIBLE SPECTROSCOPY**

Send for descriptive brochure.

Among the many important applications for the Unicam SP800 Spectrophotometer none is a better example of its potential than the measurement of enzyme reaction rates. For this the Automation Accessories are vital components. Other widely acclaimed features are the Flat Bed Recorder, the Beam Balance Smoothing Cam and the unique Second Sample Position, all of which mark the SP800 as the outstanding U.V. and Visible Recording Spectrophotometer. The Automatic Equipment provides:

AUTOMATIC interchange of up to 4 sample and 4 reference cells,
AUTOMATIC re-cycling over any pre-selected wavelength range,
AUTOMATIC recording at predetermined time intervals.

UNICAM

Precision Spectrophotometers

LETTERS TO THE EDITOR

	Page		Page
ASTRONOMY		Involvement of the Adrenal Cortex in the Appearance of Rat Slow Alpha ₂ -Globulin.—Prof. Werner G. Heim and Sandra R. Ellenson	
Stability of Rotating Massive Stars in General Relativity.—B. Durney and I. W. Roxburgh	1304		1330
PHYSICS		HISTOCHEMISTRY	
Effect of Ultra-violet Irradiation on the Electrical Conductivity of Zinc Oxide Single Crystals.—Dr. R. B. Lal and Dr. G. M. Arnett	1305	Vital Staining of the Granulated Juxtaglomerular Cells in the Mouse Kidney.—V. Szokoly, Sz. Gomba and M. B. Soltész	1331
Tschebychev Approximation to the Equation of Thermogravimetric Data.—Dr. Eric L. Jones	1306		
Calculations with Two Nearly Equal Quantities.—Dr. J. H. Fremlin; Dr. Francis T. Worrell	1306	IMMUNOLOGY	
METALLURGY		Thymus and the Production of Antibody-plaque-forming Cells.—Dr. J. F. A. P. Miller, P. M. de Burgh and G. A. Grant	
Mechanism of Oxidation of Chromium Diffusion Coatings on Iron.—Dr. I. A. Menzies and D. Mortimer	1307	Decay of Immunological Responsiveness after Thymectomy in Adult Life.—R. B. Taylor	
Mechanism of Oxidation of Chromium Diffusion Coatings on Plain Carbon Steels.—Dr. I. A. Menzies and D. Mortimer	1309	Delayed Effect of Thymectomy in Adult Life on Immunological Competence.—Dr. Donald Metcalf	
GEOLOGY		Effect of Thymectomy in Adult Mice on Immunological Responsiveness.—Dr. J. F. A. P. Miller	
Organic Matter in Recent and Ancient Limestones and its Role in their Diagenesis.—D. J. Shearman and Sir Patrick A. d'E. Skipwith	1310	Rapid Detection and Titration of Antihuman Serum Albumin Antibody.—Dr. Mark S. Tobin	
GEOCHEMISTRY		PATHOLOGY	
Abundances of Rare Earth Elements in Eclogitic Rocks and Basaltic Achondrites.—J. W. Morgan and Dr. J. F. Lovering	1311	Virus-like Particles in Sub-acute Sclerosing Encephalitis.—Dr. Nicholas E. Gonatas and Prof. G. Milton Shy	
CHEMISTRY		Differential Inhibition of Deoxyribonucleic Acid and Ribonucleic Acid Synthesis induced in Cultured Mammalian Cells by 7:12-Dimethylbenzanthracene —Lawrence J. Alfred	
Cation Sites in Synthetic Zeolites.—Dr. T. I. Barry and L. A. Lay	1312	RADIOBIOLOGY	
Quantitative Chromatographic Analysis of Organic Acids by Tritiating Methylation.—G. K. Koch and [the late] G. Jurriens	1312	Effect of Sodium Arginate in inhibiting Uptake of Radiostromium by the Human Body.—R. Hesp and B. Ramsbottom	
Mass Spectrometric Fragmentation of Nitrophenylhydrazones.—Prof. Carl Djerassi and Shelia D. Sample	1314	BIOLOGY	
Electronic Absorption Spectrum of the CH ₃ O-radical.—J. F. Ogilvie	1315	Production of Carotenoid by a Green Alga.—Prof. F. Dentice di Accadìa, Olga Gribanovski-Sassu, A. Romagnoli and L. Tuttobello	
Equation of State of Liquid-vapour Equilibrium with the Same Constants for Both Phases.—Dr. István Kuhn	1315	A Functional Relationship between Neurosecretory Fibres and Pituitocytes in the Eel.—Sir Francis Knowles and Lutz Vollrath	
Organochlorine Pesticides in the Atmospheric Environment.—Dr. D. C. Abbott, R. B. Harrison, J. O'G. Tatton and Dr. J. Thomson	1317	A Dual Neurosecretory Innervation of the Pars Distalis of the Pituitary of the Eel.—Sir Francis Knowles and Lutz Vollrath	
BIOCHEMISTRY		Control of Furunculosis in Impounded Adult Salmon.—Angela C. Leaman	
Separation of High-molecular Deoxyribonucleic Acid and Ribonucleic Acid.—Dr. Olaf Klammerth	1318	Action of Gibberellic Acid on Auxin Transport.—Prof. P. B. Pilet	
Optical Rotatory Dispersion and Secondary Structure of Ribonucleic Acid in Mammalian Ribosomes.—A. Blake and Dr. A. R. Peacocke	1319	Growth Responses of Mature Endosperm in Cultures.—Prof. B. M. Johri and S. S. Bhojwani	
Sulphocysteine in the Urine of the Blotched Kenya Genet.—John C. Crawhall and Dr. Stanton Segal	1320	Ascent of Sap in Trees: Effect of Negative Absolute Pressure on Flow Resistance.—Prof. Bruce Johnson and Prof. John R. Dixon	
Biogenesis of Ethylene in <i>Penicillium digitatum</i> .—Dr. B. A. Sprayberry, Prof. W. C. Hall and Dr. C. S. Miller	1322	Respiratory Changes in the Cacao Seed Cotyledon coincident with Seed Death.—Iván A. Casas, Edward S. Redshaw and Manuel L. Ibáñez	
Characterization of Glucose-6-phosphate Dehydrogenase among Chinese.—Paul W. K. Wong, Ling-yu Shih, Prof. David Yi-Yung Hsia and Dr. Y. C. Tsao	1323	VIROLOGY	
Separation Method for Melatonin in Pineal Extracts.—Dr. F. C. G. van de Veerdonk	1324	Mixed Infection of African Green Monkey Kidney Cells by Adenovirus 7 and SV 40.—Dr. Richard K. Wertz, Cynthia C. O'Connor, Dr. Alan S. Rabson and Gregory T. O'Connor	
Distribution of Collagenase in Rat Tissues —John F. Woods and Prof. George Nichols, jun	1325	CYTOLOGY	
Cystine Content of Cattle Hair.—P. H. Springell and S. J. Leach	1326	Isolation of Macronuclei from the Ciliate <i>Tetrahymena pyriformis</i> GL.—Dr. Y. C. Lee and Prof. O. H. Scherbaum	
PHYSIOLOGY		GENETICS	
Energy Requirements for Relaxation from Tonic Contractions ('Catch') in an Invertebrate Muscle.—Dr. Kathleen Minihan and Prof. R. E. Davies	1327	Aggressive Behaviour, Mental Sub-normality and the XYY Male.—Patricia A. Jacobs, Muriel Brunton, Marie M. Melville, Lr. R. P. Brittain and Dr. W. F. McClelland	
HAEMATOLOGY		PSYCHOLOGY	
Hereditary Ovalocytosis and Haemoglobin E-Ovalocytosis in Malayan Aborigines —Dr. Lie-Injo Luan Eng	1329	Tactile-vision: Thermal and Texture Cues in the Discrimination of Black and White.—C. N. French	
Antigen in Leukaemic Patients.—Dr. J. Ducos, J. Ruffie, P. Colombies, Y. Marty and E. Ohayon	1329		



*microbiological reagents
and media*

THE ONLY COMPLETE LINE OFFERED IN U.K.

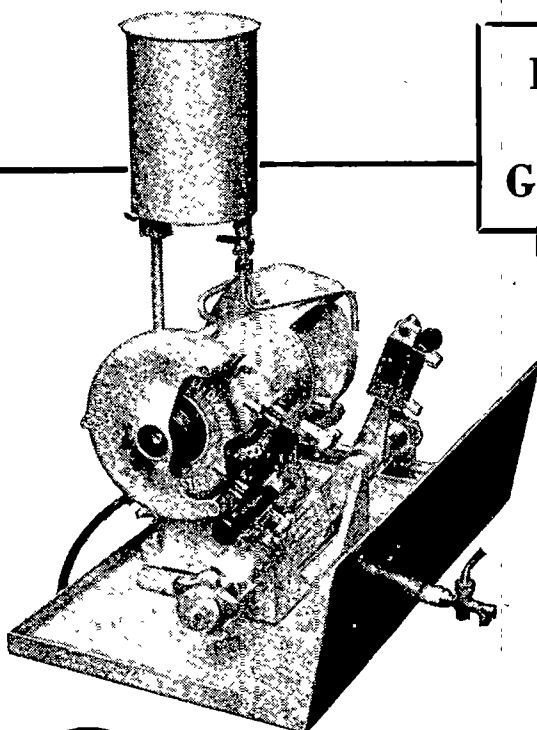
<i>Culture Media</i>	<i>Peptones</i>
<i>Microbiological Assay Media</i>	<i>Hydrolysates</i>
<i>Tissue Culture Media</i>	<i>Amino Acids</i>
<i>Serological Reagents</i>	<i>Enzymes</i>
<i>Antisera</i>	<i>Enrichments</i>
<i>Diagnostic Reagents</i>	<i>Dyes</i>
<i>Sensitivity Disks</i>	<i>Indicators</i>
<i>Unidisks</i>	<i>Carbohydrates</i>
	<i>Biochemicals</i>

Requirements of the Bacteriologist, Biochemist, Biologist, Pathologist and Pharmacologist can usually be met promptly from our extensive stocks. We shall always be pleased to obtain other items specially to order.
Please send for the latest technical information.



BAIRD & TATLOCK (LONDON) LIMITED, CHADWELL HEATH, ESSEX, ENGLAND.
Branches in London, Birmingham, Manchester and Glasgow. Member of the Derbyshire Stone Group.

TAS/DO.11A



DIAMOND WHEEL CUTTING AND GRINDING MACHINE

THE MARK II UNICUTTA

The thin section machine quickly and efficiently produces thin sections for microscope study. Fitted with 6-in. dia. diamond cutting and grinding wheels.

Accessories for the machine:

- CAST IRON POLISHING PLATE
- NYLON SIMPLEX POLISHING PLATE COVERS
- EMERY DISC CLAMPING RINGS TO FIT EMERY DISCS TO POLISHING PLATE
- EMERY DISCS AND LOOSE ABRASIVES (Carborundum and Aloxite)



For full information, write or telephone:

CUTROCK ENGINEERING CO. LTD., 35 BALLARDS LANE, LONDON N.3
Telephone: FINCHLEY 5978/9

CONSERVING THE NATURAL HERITAGE

AN impressive feature of the First Study Conference, "The Countryside in 1970", held in November 1963, was the range of the papers presented and of the reports of its several working parties and panels. This is equally true of the Second Study Conference held in London during November 10-12, 1965 (see p. 1265 of this issue). Its twelve study groups, whose work was initiated and co-ordinated by the Nature Conservancy, covered the training and qualifications of planners; the training and qualifications of the professions concerned with land and water; technology in conservation; countryside planning practice; outdoor recreation, both active and passive; traffic and its impact on the countryside; legislation; the preservation of natural, historic and other treasures; information and the countryside; living and working in the countryside; and the reclamation and clearance of derelict land. One panel was specifically concerned with the planning and development of the countryside in Scotland. The membership of the panels was representative and authoritative. Mr. E. M. Nicholson's review of the preparatory studies they presented shows how thoroughly the problems considered were tackled and that particular attention was given to the selection of priorities, to practicabilities and to timing in the light of further developments and enquiries since the previous conference.

The thoroughness of the preparation for this Conference is unmistakable, not merely from this review of the preparatory studies, but equally from the terms of reference of the study groups. This constitutes a major distinction from the First Conference two years ago, though it is possibly unfair to over-emphasize the very hurried preparation for that first conference, as is done in the review. Be that as it may, the first conference brought together for the first time many interested parties and made some advance towards a common understanding and a sense of common concern and a recognition of the opportunities for fuller co-operation. It did not lead to any adequate programme of action, and continuing reports in the interval from the Nature Conservancy and the National Parks Commission have indicated no decrease in their concern over particular threats to their responsibilities in the countryside. On the contrary, it has been substantiated by recommendations from the Town and Country Planning Association, and by the call for a Buchanan code to protect the countryside which came recently from a conference on roads and traffic in the National Parks, organized by the Friends of the Lake District, at Buxton. The reaction of the Botanical Society and the Northumberland and Durham Naturalists' Trust to the threat to Upper Teesdale inherent in the proposals of the Tees Valley and Cleveland Water Board, and very notably in the forthright report and newsletter, September 1965, issued by the Friends of the Lake District, have also added weight to the recommendations of the First Conference.

Throughout all this material there is a remarkable consensus of opinion as to the two critical needs of finance and of effective authority and power, failure in which respects has from the start intensified defects in organization that might otherwise have been less important or

insignificant. It is scarcely surprising, however, that this consensus of opinion should be reflected so largely and authoritatively in the conclusions and recommendations which the study groups made to the Second Conference. This is the more significant in that, as Mr. Nicholson explains, the subjects which the study groups tackled were limited in number, and priority was accorded to problems on which several different interests and organizations were ready to work with some prospect of producing realistic conclusions which might command widespread acceptance and thus be likely to be followed up effectively. Other factors which determined the selection of problems were suitable timing, the need for tackling issues of strategic importance for advances all along the line, the need to give the greatest possible impetus to improved communications, and co-ordination of the many diverse efforts within a more coherent and gradually emerging national policy for the countryside. The emphasis is on a series of simultaneous practical advances, expressing a new attitude and enabling it to develop faster and further in future.

Accordingly, this Second Study Conference began with an impressive stock of facts, of ideas and of well-considered proposals endorsed by people of impressive experience widely representative of the leading interests concerned. As suggested already, the varied and balanced membership of the study groups added greatly to the weight which their agreed reports could command on their inherent merits. Mr. Nicholson is fully justified in claiming that it can no longer be said that nothing can be done pending further investigations, or that the whole matter is too controversial for agreement among the main parties to be practicable. The main difficulty now appears to be not in reaching agreement but in getting action taken on the established facts.

Endorsement by the Conference of the proposals of the various study groups will create a new situation, in that the many and diverse interests in the countryside will have begun to come together, in something much larger than any of them, to ensure a fair and acceptable future for the countryside as a whole, within which each interest can find its place. If adequately sustained by public understanding and support, the new awareness, the new sense of common purpose and the new intimate contacts created by the Conference could maintain a new dynamic, leading to countless constructive actions, by individual or co-operative efforts. They must be supported, however, by vigorous and comprehensive measures in Parliament and by the provision of adequate resources by the Government, and no public protestations by the Government will dispense with its responsibility for finding Parliamentary time in spite of the heavy pressure of business.

Here may well lie the acid test of sincerity, and, welcome as was the presence at the Conference of one of the Ministers concerned—the Minister of Land and Natural Resources—that test has still to come. Its importance is demonstrated by the specific measures recommended for legislation not merely by the study group concerned with this aspect but also by those concerned with planning practice, the preservation of natural, historic and other national treasures and the Nuffield Enquiry into Common

Lands. They include, for example, the re-enactment, on a comprehensive basis in a new Town and Country Planning Act, of the so-called 'amenity clause', to be renamed the 'conservation clause', the removal of exemption from planning control of all 'sites of special scientific interest', and its modification in respect of agricultural and forestry buildings; the removal of any obligation for the Ministry of Agriculture to make ploughing grants in respect of such sites, and conferment on the Nature Conservancy of powers to make agreements with, and payments to, their owners. Also proposed are an amending Act to the National Parks and Access to the Countryside Act, 1949, incorporating the proposals of the National Parks Commission, but with the major new provision that the Commission should be reconstituted with nation-wide responsibilities for conserving fine landscape, co-ordinating surveys of outdoor recreation resources, designating a new category of "areas of special value for open-air recreation", and for ensuring the development of use of these resources with due regard to conservation and other interests. There are more than a dozen further specific proposals, but in sum they amount to the creation out of the National Parks Commission of a much stronger and more adequately supported (financially and in other ways) Countryside Commission, armed with more comprehensive powers.

These recommendations for legislation have been mentioned in some detail because they indicate the fundamental responsibility which rests on the Government and the key importance of adequate finance and of adequate powers if the Commission is to be effective. They also show that the Minister of Land and Natural Resources is only one of several Ministers directly involved and possibly not even the most important. Besides the Minister for Agriculture, those for Transport, for Power, for Housing and Local Government, for Education and Science, and for Technology, are deeply involved. Furthermore, the position has not been made the easier for the Minister of Land and Natural Resources by some of the recent changes in the structure of Government. Time has still to show how they will work in practice, but ostensibly the Ministry appears to be ill-served in the vital matter of advice. While the National Parks Commission falls within his responsibility, the Nature Conservancy now enjoys special status as a statutory Committee of the Natural Environment Research Council, which together with the other Research Councils falls within the Department of Education and Science.

The scientists working with the Nature Conservancy do not appear to be unduly disturbed by the change, and it is the quality of the advice on which the Minister appears to be relying that calls attention to the fact that a logical place for the Natural Environment Research Council would be within his Ministry. This weakness has, however, been highlighted by the Minister's own statement to the Second Conference, "The Countryside in 1970", and by the ineptitude of the second annual report of the Water Board, which was published by the Minister within a week of the Study Conference. Mr. Willey can surely not be unaware that his Ministry has already been strongly criticized, and not just by political opponents, for being unresourceful. He might have been expected to be on his guard against any further such charge, particularly as, when questioned in the House of Commons on November 1, he said that he hoped to indicate to the Conference the lines along which the Government was thinking. He had also stated on that occasion that due account would be taken of the results of the Conference and of the informal

consultations with representatives of the local authorities before submitting proposals to Parliament.

This was repeated by Mr. Willey in his statement to the Conference on November 12, but although he also said that he had been most impressed by what he had seen of the work leading up to the Conference, his statement gave no indication that either he or his advisers had paid any attention to that work or even to the results of the First Study Conference or to the present representatives of the Nature Conservancy or the National Parks Commission in their latest annual reports. The review of the work of the study groups was available at a press conference on November 3 and presumably to the Minister's advisers also, but there was no hint in his remarks that the crucial issues were recognized, either as presented there or in the many reports coming from other bodies.

It is in all these circumstances that the Minister's statement should be considered, and it says much for the restraint and good manners of the Conference that his statement should have been received without violent protest. Either Mr. Willey did not trouble to examine the evidence submitted to him, or he failed to appreciate the significant issues, for all that they have been clearly and emphatically reiterated in successive reports from the Nature Conservancy, the National Parks Commission and other bodies, independently of those coming from the working groups of the Conference itself.

The Minister's statement begins with the admission that Britain is now in a new situation which could not have been foreseen in 1949. His assertion that the National Parks Act of 1949 has been singularly effective in securing its objectives is certainly open to question. He admits, also, that there is a general consensus of opinion on the need for change, but that the Government is concerned merely to find the largest common measure of agreement on what ought to be done now and to limit its action to measures already long overdue, dealing with matters where continued inaction is causing harm. Even within this frame of reference, his statement is self-condemning: he limits his proposals to the care of the countryside and its enjoyment by the general public. By excluding consideration of the system of planning control and of the provision of the increase in financial support, which all are agreed is imperative, his proposals are ineffective and unworthy of serious positive discussion.

This is demonstrated by the paragraph which expressly excludes any major change in the administrative structure of the National Parks. In its endeavour to secure the co-operation of the local authorities to develop the National Parks, which undoubtedly is a cardinal condition of success, the Government omitted to write into the 1949 Act the establishment, for each Park, of a fully independent Planning Board, even when the Park lay within the area of several local authorities. Although, notably in the Peak District, this arrangement has sometimes worked surprisingly well, it was recognized from the start to be an administrative weakness, which was intensified by the failure to provide adequate financial support from national resources. This fundamental weakness was revealed in a glaring anomaly during the Manchester Water Inquiry, to which the report and newsletter of the Friends of the Lake District directed attention. Although the Lake District National Park Planning Board was whole-heartedly against Manchester's proposals, none of the Board's three planning officers was called to give evidence at the Inquiry in support of the Board's policy. These three planning officers are on the staffs of the three County Councils constituting the Board, and because

the county authorities differed in their opposition or objection to the proposed Order, none of the officers was called.

This example alone demonstrates the absurdity of taking seriously any legislative proposals which do not remove such a long-standing anomaly and provide the Planning Boards with their own planning officers, if not complete autonomy. Mr. Willey, however, appears to favour the continuance of the system of Joint Planning Boards where more than one county is involved. Although this system is fraught with weaknesses and drawbacks, he now proposes to extend the responsibilities of the Boards. Even on the financial side, he offers no more than that the additional administrative expenses directly involved in creating new facilities or improving the landscape should qualify for grant aid. The fundamental point is ignored that national issues and national responsibilities should be a prime charge on national resources and not, as at present, on local authorities, the financial resources of which are, in the nature of things, among the most limited of all the County Councils. Not even the legislative question, which was so clearly set out by a special study group of the Conference, is considered.

It is in this context that renders the Minister's statement and its proposals so derisory. He cannot even claim that his major proposal that the National Parks Commission should be reconstituted as a Countryside Commission with a new function of encouraging the provision of opportunities for enjoying the countryside generally has the support of the study groups of the "Countryside in 1970" Conference. The Countryside Commission there recommended would be much stronger and more adequately supported than the National Parks Commission and would be armed with more comprehensive powers and with effective administration at both national and local levels. These qualifications, which refer explicitly to legislative action, cannot be ignored without destroying the basis of the whole proposal.

Mr. Willey's concept of 'Country Parks', to be created by local authorities with Exchequer assistance in the countryside or on the coast, seems to pay no attention to the fundamental and vital issues on which urgent action is imperative if much of the countryside is not to be irreparably destroyed; this applies as much to the country parks now proposed as to the national parks and nature reserves. Nor does he show any wide vision, or deeper understanding, when he refers to forestry, to reservoirs, or to waterways. He promises that the Government will examine the findings of the report of the British Waterways Board on the future of the waterways, with reference to preserving for recreation and amenity those waterways which are no longer of importance for transport and commercial use; however, he gives no hint that the importance of waterways as a national resource in transport is generally appreciated. Even on derelict land and eyesores he says no more than that the Government has in mind legislation providing that expenditure by local authorities on derelict land should qualify for Exchequer aid, and that a programme should be drawn up, by consultation between the Countryside Commission and local authorities, to deal with places where removal of eyesores is specially urgent and where expenditure could restore a landscape of great attractiveness.

On traffic, Mr. Willey said that the powers in the 1949 Act to regulate road traffic in National Parks were out of date, and that it was proposed to amend the Act to bring these powers into line with the present needs of the

National Parks and to apply them, where necessary, to the Country Parks. In spite, however, of the wide and growing support for the policy of the Friends of the Lake District, who have urged the Minister of Transport and the Minister of Housing and Local Government to commission a species of rural Buchanan report on the traffic problems of National Parks, Mr. Willey gave no hint of any check to the present process of piecemeal 'improvement' and lack of consultation. The closure of the passenger train services between Ulverston and Lakeside, meanwhile, not only sacrifices an opportunity to relieve road congestion but aggravates the present severe congestion on the roads in the Lake District.

Mr. Willey made more encouraging remarks about the creation of long-distance footpaths and public access to open country, and hinted at the possibility of legislation to improve the situation. His Ministry is also considering the better use of our common land without waiting for further legislation, the lines of which are also being considered. He referred also to the need for further research into potential demands, and into the techniques of conservation and management, but, apart from the points already noted, the trend of the statement raises doubts as to how well the Minister is served by his advisers. These doubts are intensified by the recent annual report from his Water Resources Board, as well as by the unsatisfactory reply of the Secretary of State for Scotland to a recent question in the House of Commons regarding the feasibility study of the Solway Firth Barrage scheme. The Water Resources Board is itself in charge of the feasibility studies for the Morecambe and Solway barrage schemes and it was presumably on its advice that Mr. Willey based his reply. Even in its narrow view of them as potential sources of water supply, it appears to be far behind the times and unaware of the advice of the Nature Conservancy against locating resources in the hills and tapping water at the source. The advantages in transport, land reclamation, power and economics generally are ignored, not to mention the overriding importance of the results of such investigations being available at the earliest possible date if censured and unjustifiable decisions are to be avoided. The Board's shallow and obsolete thinking on the economic benefits of barrage schemes is paralleled by its inept remarks about the national parks.

These doubts are scarcely dispelled by the reply which Lord Mitchison, Joint Parliamentary Secretary to the Ministry of Land and National Resources, gave to a question in the House of Lords on November 16 regarding the feasibility survey of the Morecambe Bay Barrage scheme. Lord Mitchison said that the preliminary study now authorized to discover the quantity of water likely to be made available, the best way of making it potable, and the probable cost of making it available for use where required would take about 6 months and was unlikely to cost more than £15,000. The proposals for feasibility studies had been estimated by the consultants in July as costing £324,000 in the present and 3 subsequent years, or £80,000, £100,000, £90,000 and £54,000 in 1965, 1966, 1967 and 1968, respectively.

Lord Mitchison's reply makes nonsense of the reason given by Mr. W. Ross on November 3 for not proceeding with the full-scale trials immediately, and only brings to bear on the Secretary of State for Scotland the same criticisms which have already been made against the Minister of Land and Natural Resources. Whatever the extent to which his advisers have failed him, he cannot be entirely absolved from responsibility. He must, indeed, ask some of them to think again and quickly—especially

the Water Resources Board—for there is a strong case for bringing in others with a wider outlook and keener appreciation of the possibilities that exist to-day, alike for the utilization and the preservation of natural resources. Seldom, however, can a Cabinet Minister have confronted a meeting of the standing and authority of the Second Study Conference "The Countryside in 1970" with a brief so ill-prepared and irrelevant to the critical issues. For his own sake, to clear himself of the charge of incompetence and neglect, Mr. Willey must do some rapid and hard thinking before he prepares and presents his promised White Paper, or Parliament and the nation will be entitled to demand a change not merely of advisers but of Minister.

THE ROCKEFELLER INSTITUTE UNTIL 1953

A History of the Rockefeller Institute 1901–1953

Origins and Growth. By Dr. George W. Corner. Pp. 635+41 illustrations. (New York City: The Rockefeller Institute Press, 1965.) n.p.

FOR students of the history of science in the twentieth century *A History of the Rockefeller Institute 1901–1953* should be obligatory reading. For all of us who like to be reminded of great advances made in our life-time, it tells a fascinating story in which different parts of the puzzle are gradually fitted together to reveal a coherent picture. Dr. Corner has written a masterly account of a great Research Institute which was founded in New York in 1901 and has been intimately concerned with the spectacular advance of the 'life sciences' in recent years. He is an author distinguished for his scientific work as well as for his writing. He can describe the wide range of problems investigated at the Rockefeller Institute, without confusing us by the technical language of pathology, bacteriology, immunology and biochemistry, and he can interest us in the men as well as in their achievements. The typography and illustrations are worthy of the contents.

The Rockefeller Institute deserves such a volume, for it has played an essential part in the development of the natural sciences in the United States and of medical and biological science everywhere. The names of those who worked there and the subjects of their research recall many of the major advances in medical science in this century. It is of great interest, too, to learn how the Institute came to be founded, how its particular organization has suited different periods and different types of problem and of investigator, how it fared in the two great wars and how it has faced the recent expansion of scientific research and the tendency to substitute the team for the individual worker. In fact, Dr. Corner's history has lessons for all who may be concerned in the advance and the application of scientific knowledge, by research and by teaching, and under the aegis of Governments, universities or private institutions.

The Institute was founded by John D. Rockefeller, senior, advised by his almoner, Frederick Gates, and by his son, John D. Rockefeller, junior. Gates had been a Baptist minister for eight years and had then shown his great talents for organization as secretary of the Baptist Education Society. He came to Mr. Rockefeller in 1891, and soon acquired a poor opinion of current medical practice, of the efficacy of the medicines which were prescribed and of the theories on which they were based (whether by homeopaths or allopaths). He found that most of the medical schools in the United States had nothing to match the active research going on in universities or institutes in Europe. Here, then, was an opportunity for Mr. Rockefeller to use his great wealth in a most worthy

cause, for the relief of pain and sickness in the United States and throughout the world.

The proposal was accepted in 1901 and it was only natural to seek the help of Dr. Welch of Baltimore, already recognized as the leading authority on medical science and scientists in the United States. A board of directors was set up in 1902 and one of them, Simon Flexner, was made director of the Institute. There was still a farm of 13 acres for sale in the heart of New York beside the East River. It was bought for 650,000 dollars to provide a site for the permanent buildings: they were started in 1904 and Flexner began work in that year in temporary laboratories with the group of scientists he had collected with Welch's advice. It included Opie, Meltzer, Noguchi and Levene the biochemist, with some able people in junior posts and several voluntary workers, including Auer and Newton Richards.

Flexner made no attempt to follow the continental pattern of a research institute occupied exclusively on one kind of investigation. His staff were free to choose their own problems. Noguchi investigated snake venoms, Levene identified and analysed nucleic acids, Flexner worked on bacillary dysentery and on an epithelial tumour which he and Jobling had found in a rat and were able to transplant from one rat to another; he was soon to be widely recognized as the author of an improvement in the treatment of cerebro-spinal meningitis.

The new building, opened in 1906, had rooms for more men in the senior posts. Flexner and his advisers appointed Alexis Carrel, already well known for his successful vascular surgery, and Jacques Loeb, who came in 1910 to start a division of experimental biology. Meanwhile, the poliomyelitis epidemic in New York in 1907 had led Flexner to search for the source of infection, and in 1909, following a clue discovered by Landsteiner in Vienna, Flexner and his assistant, Lewis, succeeded first in infecting monkeys with material from fatal human cases and then in showing that the infectious substance could be drawn through a Berkefeld filter without losing its potency; that in fact it must be what is described as a filterable virus. Landsteiner and Levaditi made the same important discovery only a few days after Flexner and Lewis. It was not long before other discoveries were announced, for in 1910 Noguchi succeeded in detecting the spirochaete of syphilis in the brain and cord of patients with Tabes and general paresis, and Peyton Rous, appointed in 1909, found a transmissible chicken sarcoma which was caused by a filter-passing virus. In the same year the Hospital of the Institute was opened with a group of able young residents and a programme of research into five diseases: poliomyelitis, lobar pneumonia, syphilis, heart disease and 'intestinal infantilism' (coeliac disease). Judged by its immediate effects on the treatment of these diseases, the work done at the hospital may seem unrewarding, but we can now realize that some of it has played an essential part in the general advance.

The story of the attack on lobar pneumonia could, in fact, be used to point a variety of morals. It seemed for a time to be a story of wasted effort, when pneumonia could be cured by the use of antibiotics, but much of it can now be seen as having led to, or at least given strong support to, some of the recent developments of molecular biology.

The chemical investigation of the pneumococcus, conducted by Avery and his associates, showed that the immune reactions of the four known types of pneumococcus depend on the presence of particular polysaccharides in the capsule of the bacterium. In 1928, however, it was reported by Griffith in England that a harmless strain of living pneumococci became virulent when placed in contact with the dead remains of a virulent strain. Avery and his group found that the transforming agent appeared in a cell-free extract of the dead bacteria. By systematic fractionation they arrived at a practically pure substance of very high potency and this, in the end, proved to be a

nucleic acid of a type already studied at the Institute by Landsteiner and Jacobs and identified as deoxyribonucleic acid (DNA). It seemed, therefore, that a nucleic acid could be the effective agent in inducing heritable changes in a living organism, and, since this work was done, many investigators have shown that DNA is present in the chromosomes of higher animals and is a constant ingredient in the genes.

Avery's work on the pneumococcus was not completed until after his retirement in 1943, but much earlier the Rockefeller Institute had set up a branch at Princeton for the study of animal diseases, and here another chapter of the nucleic acid story had been added by J. H. Northrop and Wendell Stanley (who shared a Nobel Prize for it in 1946). Northrop had studied the chemistry of enzymes and found methods of crystallizing and purifying them. In 1936 he turned to the bacteriophages and was able to isolate a highly purified nucleoprotein which would invade a culture of streptococci and ultimately destroy it. He found that his phage material was actually a nucleoprotein containing ribonucleic acid, and it is now agreed that the bacteriophages are aggregates of nucleoproteins which replicate their substance by drawing on the host bacteria for the necessary ingredients. By that time Stanley, also at Princeton, had already succeeded in obtaining crystals of the tobacco mosaic virus and in showing that they were formed of some kind of protein molecules of very high molecular weight. Within a few years, Bawden and Pirie had shown that the active substance was not a simple protein but a nucleoprotein, with a nucleic acid fraction containing ribose.

These researches have helped to build up the present conception of living matter and the story of DNA and RNA, familiar to every schoolboy but still a wonder to his teachers. They have done so by introducing new techniques as well as by the facts they have established. But the Institute has many other investigations to its credit. Dr. Corner gives a full account of these and is able to show how the whole research programme of the Institute was helped by the progress in different departments. The two World Wars have chapters to themselves, with accounts of research on blood and blood substitutes, wound infection, shock and anti-malarial drugs. Another chapter deals with the retirement of Simon Flexner from his position as director in 1935 and the appointment of Herbert Gasser to succeed him. Dr. Corner's account of these two able and devoted scientists will show the reader why both were so well supported by their staff. Gasser's appointment came at a time when the effects of the Depression ruled out a policy of further expansion, but his many friends will be glad to realize how much was achieved in many fields of research under his direction. His retirement in 1953 brings Dr. Corner's history of the Institute to a close. It is a fine history: the world should be grateful to Mr. Rockefeller and to those who helped him to found and to run his Institute. It has increased our understanding of living things. The University which it has now become is increasing it still further and we can be sure that it will continue to do so. ADRIAN

EXPANDING HORIZONS IN OPERATIONAL RESEARCH

Proceedings of the 3rd International Conference on Operational Research, Oslo 1963

Edited by G. Kreweras and G. Morlat. Pp. xiv + 952. (London: English Universities Press, Ltd.; Paris: Dunod, 1964.) 130s.

IT is scarcely possible to mention operational research in general conversation without provoking the question "What is operational research and what does it do?" Many people will identify operational research with management science, but usually only a few well-informed

individuals will be able to distinguish it clearly from other branches of management science or to identify areas of research or types of problem to which the techniques of operational research can be applied.

The publication of the *Proceedings of the 3rd International Conference on Operational Research, Oslo, 1963*, may help to extend acquaintance with the subject among those who are not yet familiar with it. The book is intended primarily for the operational research specialist, both as a report of the conference and as a work of reference, but it also provides a wealth of informative reading for workers in other fields who are aware of the impact of operational research and who wish to keep abreast of the most recent developments. The volume runs to 982 closely printed pages and contains the text of more than 80 papers presented at the conference and reports of discussion. The international character of the conference is borne out by papers from fourteen different countries but the tendency for such conferences to be dominated by one or two countries remains. In this case more than half the papers came from the United States, France and Great Britain.

Operational research is characterized by the application of mathematical techniques to management problems. It is therefore not surprising to find an appreciable mathematical content in the book, but this should not deter the non-mathematician from reading it. Only a few of the sessions were devoted to specialized mathematical topics, while the greater part of the conference was devoted to the application of operational research in a great variety of civil, economic and social problems. One does not have to be a mathematician to appreciate this publication.

There is said to be a firm of operational research consultants whose definition of the subject is: "If we do it, it is operational research". This might have been the principle adopted by the organizers of this conference, for not only were there papers on well-established areas of operational research activity such as programming, scheduling, simulation, and forecasting, but also on less familiar operational research topics such as public health, traffic, research planning, long-term investment, national planning, agriculture and forestry and social organization. For this reason the book should appeal to a much wider readership than the specialist in operational research. It will serve to help workers in other fields to formulate their own empirical definition of operational research and to inform them of the impact which operational research is likely to make on their problems. The book should certainly appear as a reference work on the shelves of libraries catering for a great variety of interests.

In reviewing a book of this sort it is impossible to deal with the subject-matter and treatment of all the individual papers in sufficient detail to do each one justice. Also it is difficult to find an impartial basis for sampling the contents. In a review addressed to scientists it may prove acceptable to discuss only those papers presented at a session on "Research Planning" since this is a topic in which all scientists have an interest.

In the first of these papers, Eric Rhenman (Sweden) discusses the theories of planning and decision-making and observes that the most refined methods have only been applied to simple routine decisions, and not in important complex situations. He suggests a reason for this and urges closer contact with the emerging modern theory of organization. He warns operational research workers against the danger of attempting to derive algorithms when the problems are too complex for an analytical solution, and recommends an examination of the heuristics of practising research managers. In a much more mathematical paper, H. Theil (The Netherlands) makes a mathematical analysis of an idealized situation and considers the interplay of a decision-maker and research manager, when the goal of the former is to maximize a quadratic objective subject to linear con-

straints. In a paper by B. V. Dean (United States), on group research as an educational tool, the author discusses the advantages and disadvantages of group research (as distinct from research done by individual students). This will be of special interest to educationalists and others responsible for planning courses in which some form of applied research is an essential part of the curriculum. K. W. Webb (United States) reports on an exploratory operational research study of the United States national oceanographic programme, and makes the point that the experience that operational research has gained in the field of industrial management can be applied to good effect in the field of national management. He makes specific recommendations for work of an operational research nature to be continued in certain specific fields of oceanographic research. Perhaps one of his most important recommendations relates to the setting up of a scientific information system, with special attention to the information explosion that will take place because of continuous recording devices and sensor satellites. A paper by A. H. Rubenstein and M. Radnor (United States) deals with an investigation of the organization of research and development in decentralized companies and focuses attention on the relationship between top management and divisional management in the area of research and planning. Data from two field studies are presented. Finally, there is a paper by B. T. Price (United Kingdom) on the national control of scientific and technical resources, which takes the form of a contribution by someone who has not specialized in operational research, but wishes to find out whether it can help in the difficult problem of managing research and development at Government level.

This small sample of the subject-matter and content of papers presented at the conference should serve to attract the interest of scientists who are not directly concerned with operational research.

S. J. MORRISON

SHAPE AND ARRANGEMENT IN LIPIDS

The Structure of Lipids by Spectroscopic and X-ray Techniques

By D. Chapman. Pp. xu+323. (London: Methuen and Co., Ltd.; 1965.) 63s. net.

IT has frequently been observed that the investigation of lipids, once carried out by only a few patient investigators, has been revolutionized during the past ten years by the application of chromatographic techniques in general and of thin-layer and gas-liquid chromatography in particular. These procedures have solved many of the problems of separation and isolation which all lipid investigators have to face, and in recent years rapid advances have been made on problems where progress had been slow and a beginning has been made on problems which previously could not be started. These important developments must not be allowed to overshadow the less spectacular, but nevertheless important, developments recently made in our understanding of the shape and arrangement of the fascinating lipid molecules in crystals and in biological systems. Dr. D. Chapman has contributed to these advances and is well placed to describe for us the present situation.

Though it is now possible to isolate a lipid in a relatively pure state and to determine its structure and fatty-acid composition without too much difficulty, this tells us little about the arrangement of lipid molecules *in vitro* or *in vivo* systems, and advances in this area have developed from the application of those spectroscopic and X-ray methods which occupy the greater part of *The Structure of Lipids by Spectroscopic and X-ray Techniques*. It is made clear that there remain large areas of uncertainty and ignorance. This follows, for example, from the comparison between what is known about the long-chain

acids and their simple glycerides and the paucity of information concerning the more complex lipids. It is, of course, in the nature of science to start with simple and controllable systems, and to move from these to more complicated systems. Dr. Chapman has placed us in his debt by summarizing so effectively the answers to the simpler (relatively speaking) questions and by indicating the more difficult questions which still have to be solved. It is to be hoped that he will provide a sequel when further advances have been made.

After a brief chapter outlining the field of lipids, Chapter 2 (29 pp.) is devoted to the major separation techniques, including the use of the ultracentrifuge for lipoproteins, counter-current distribution, and the usual battery of chromatographic procedures. It seems to me that this chapter could have been omitted without serious loss. Apart from the final chapter ("Future Developments and Other Techniques", 5 pp.), the remainder of the book consists of six chapters covering the use of five spectroscopic techniques and of X-ray crystallography. The space given to each topic (ultra-violet spectroscopy, 15 pp.; infra-red and Raman spectroscopy, 81 pp.; mass spectrometry, 47 pp.; nuclear magnetic resonance spectroscopy, 48 pp.; electron spin resonance spectroscopy, 13 pp.; and X-ray diffraction, 95 pp.) reflects the present-day value of these various techniques. In each of these chapters a description of the basic theory is followed by a brief account of the experimental technique. Thereafter its application to carboxylic acids and related aliphatic compounds, glycerides, and, where relevant, sterol esters, phospholipids and other complex lipids, is described.

Figures and tables are liberally distributed throughout the book and add considerably to its value. Selected references are given at the end of each chapter and the index seems adequate but not over-long. Only a few minor errors were noted. There is no doubt in my mind that this is a valuable addition to lipid literature which chemists, biologists and medical men will want to have and will consult frequently.

F. D. GUNSTONE

COGNITIVE DEVELOPMENT OF THE YOUNG CHILD

The Early Growth of Logic in the Child

Classification and Seriation. By Bärbel Inhelder and Jean Piaget. Translated from the French by E. A. Lunzer and D. Papert. Pp. xxv+302. (London: Routledge and Kegan Paul, Ltd., 1964.) 40s. net.

THE gulf which, for forty years, has separated the work of Jean Piaget from the mainstream of psychological thought in the United States and in Britain is at last being bridged. Evidence of this is to be found in the new books which are appearing in Britain and in the United States, summarizing Piaget's work so far and attempting to synthesize it with recent developments in American and British psychology; and in Piaget's attempt to take account of the objections which are commonly, if sometimes rather uncomprehendingly, made to his methodology.

In such a context, translations of recent books by Piaget are to be welcomed, especially when the translation is of an unusually high standard and the book itself makes an important contribution to Piaget's theory of cognitive development. Not that *The Early Growth of Logic in the Child* is an easy book to read, even in its elegant translation. Nor does it make a good introduction to Piaget's work. (It is far too specialized for that, and the recent American texts by J. McV. Hunt and J. H. Flavell—read in that order—make a better introduction than anything written by Piaget himself.) But it is an important book since it provides a highly detailed description and explanation of the ways in which, according to Piaget, the logical processes of classification and seriation develop

in the young child: processes which are central to the attainment of the concept of number.

Piaget's orientation is a developmental one (or, as it is often termed, rather misleadingly, 'genetic'). He takes the position—contrary to that of American developmental psychologists such as Gesell—that, at any stage of development, abilities can be fully understood only by referring to their growth out of behaviour at an earlier stage. Thus, in the systematic errors, failures and omissions of the younger child are to be discovered the beginnings of the mechanism (or 'structure') which eventually enables the child to classify and to seriate without 'trial and error': abilities which are so facile in the average seven-year-old as to delude many non-Piagetians into taking them as given.

In their preface to the present book, Piaget and Inhelder imply that a reading of the conclusions section is virtually all that is required. "The main body of the work can be consulted as he [the reader] thinks fit, to find the justification for this argument or that. Finally (but only if he feels obliged to read the whole) he may turn to the introduction, to find out why these problems have been chosen." Even for the reader experienced in Piaget's writings, such a course of action is, I think, scarcely feasible. Piaget's habitual failure to define new terms in the appropriate place sees to that. (*A fortiori*, the reader new to Piaget will find that many terms peculiar to the system are defined only in the author's earlier texts.) Nevertheless, it might be wise to read the conclusions as a preliminary to tackling the main body of the work since experimental evidence on a number of investigations is presented in enormous detail.

A summary of these experimental studies is, of course, beyond the scope of a review such as this. Piaget and Inhelder not only trace the development of simple classification and seriation more thoroughly than any previous investigator but also investigate in detail the ability to deal with complementary classes, multiplicative classification (matrices) and multiple seriation. In each of these studies (most of which are broken down into a number of sub-problems) Piaget and Inhelder display considerable ingenuity in providing the child with meaningful problems which require physical manipulation for their solution and hence avoid the dangers inherent in relying on a merely verbal response.

One of the features of this book is that in it Piaget for the first time represents his findings in the form of statistical tables. This is certainly an improvement on the previous texts, in which it was impossible to tell how many cases Piaget had investigated before drawing his conclusions. But this innovation is—or should be—a long way from satisfying Piaget's critics. Just as many of them have failed to see the need to derive their hypotheses by first looking to see what is actually there (that is, Piaget's clinical technique), so Piaget still fails to come to grips with their basic criticism, the need for proper statistical tests of clinically derived hypotheses as distinct from the merely descriptive statistics contained in the book. Meanwhile, *The Early Growth of Logic in the Child* provides for those research workers such as Smedslund and Lovell, who are already attempting such a programme of testing, an unparalleled source of hypotheses concerning the development of classification and seriation.

J. H. DUTHIE

SEROTONIN

Serotonin

By Prof. S. Garattini and Prof. L. Valzelli. Pp. x+392. (Amsterdam, London and New York: Elsevier Publishing Company, 1965.) 140s.

SEROTONIN is a comprehensive review of the available literature on serotonin, an amine usually referred to as 5-hydroxytryptamine in most scientific journals. As

such, it is a fine reference book which should be on the shelves of all who work with biogenic amines. Although serotonin was only truly discovered and synthesized about fifteen years ago, no fewer than three thousand six hundred literature references are included in this volume. Not only that—the book contains more than 30 tables in which are given the serotonin concentrations in various animal and vegetable tissues. The appendixes are also extensive—the first, on the distribution of the amine in Nature, occupies 30 pages; the second and third, on the effects of drugs and various treatments in relation to amine levels in animals, cover nearly 60 pages; the fourth, on drugs affecting the actions of serotonin, is contained in 16 pages; while the fifth (a very useful one) describes in 20 pages the distribution of many amines and enzymes forming or inactivating them in various parts of the brain of different animal species.

The first part of the book covers the biochemistry, physiological behaviour, localization and turnover of serotonin, and the pharmacology of the amine is contained in the second part. After reading the text, one has the impression that the authors have attempted to be critical in several sections; yet in other chapters this approach is entirely lacking and the reader is left bewildered by the mass of confused reports on a particular subject. Further, although the presence of serotonin in the nervous system is undoubtedly important, this should not completely overshadow the fact that serotonin and its metabolism are vitally concerned in the patient with a carcinoid tumour and even in the normal process of peristalsis.

The production of such a vast volume of data on serotonin has been no easy task, so one must not comment too much on presentation. However, references are sometimes confusing to the reader—for example, the reference numbers are in large size print in the tables yet in the text they appear as small upgrades. And this is not all the story, as reference numbers also appear in large size print in the text. An example is on page 112, lines 13 and 14, where reference 214 appears twice, each in a different form. It is more complicated when many reference numbers are listed (as on page 214, lines 12–14), and suggests that consistency has not been considered in this respect.

The book is printed in The Netherlands and is published on good paper. It will remain the major reference book on serotonin for some time to come. G. B. WEST

FIBRINOLYSIS

Fibrinolysis

By George R. Fearnley. Pp. xii+191. (London: Edward Arnold (Publishers), Ltd., 1965.) 35s. net.

FOLLOWING a lengthy period in the scientific wilderness, the fibrinolytic enzyme system now attracts the interest of biochemist, physiologist, pathologist, and physician. The resultant voluminous literature is scattered over a multitude of scientific journals covering a variety of disciplines. A monograph on fibrinolysis is therefore timely. The crescendo of interest in the fibrinolytic system would not be surprising if its function were so well-defined as that of the coagulation system. Its exact physiological role remains speculative, however, despite an accumulation of information on many of its facets. Its possible role in the prevention of fibrin deposition in blood vessels is one of the major reasons for the interest of the clinician in fibrinolysis and, as a clinician, the author has orientated his monograph, *Fibrinolysis*, in this direction.

After an account of the known components of the fibrinolytic system and their inter-relationships, the author discusses the methods used to assess overall blood fibrinolytic activity and the measurement of the individual components of the system. There follows an account of

the mechanism whereby the fibrinolytic system may remove deposited fibrin, and a discussion on the relationship between fibrinolysis and occlusive vascular disease. The changes in blood fibrinolytic activity produced by exercise, stress, fat meals, etc., are described. It may be argued that much of the quoted work in this section and, indeed, throughout the book has methodological weaknesses, but there can be little doubt that the use of these methods has contributed to knowledge about fibrinolysis. As the author says, "they are the tools of clinical rather than of fundamental research". There is a persuasive argument for the use of pharmacological enhancement of blood fibrinolytic activity in the treatment of thromboembolic disease, although evidence of therapeutic efficacy has still to be sought. No such enthusiasm is apparent for the use of thrombolytic therapy, and the author outlines the reasons why such therapy may be of little therapeutic benefit, particularly in occlusion of the coronary and cerebral vessels. There is a suitably brief account of the rare fibrinolytic states which occur in various pathological conditions. A chapter on the relationship between fibrinolysis and coagulation reinforces frequent pleas throughout the book that the two processes should be considered together; their separation into distinct entities loses sight of their probable interplay in a state of dynamic balance. A hint of the protean functions of the fibrinolytic system is provided by a chapter on fibrinolysis and inflammation and a chapter with sections on such topics as its relationship to cancer, renal disease, and immunology. The author finally discusses future developments by pointing out some areas of deficient knowledge and suggesting that their elucidation will require the participation of workers of several disciplines.

This unpretentious monograph is more than a mere aggregation of facts; the author adds a stimulating and sometimes provocative commentary. As befits one who has maintained an active interest in this subject for a decade and a half, he has drawn extensively on his observations. It is pointed out that the investigation of fibrinolysis has become so diverse that it is outside the scope of one individual to be competent and informed on all its aspects. For the non-clinical investigator this monograph will perhaps be disappointing since it is not, nor was it intended to be, a complete reference manual on fibrinolysis. It can, however, be warmly recommended to the general physician who wishes a readable guide to a relatively new subject. As an introduction to those intending to take up the study of fibrinolysis, it provides a valuable survey of practically every aspect of the subject, and its many suggestions for future research will help to channel effort into productive areas. D. OGSTON

NEUROTROPHIC PROCESSES

The Effect of Use and Disuse on Neuromuscular Functions

Edited by Ernest Gutmann and Pavel Hnik. (Proceedings of a Symposium held at Liblice, near Prague, September 18-23, 1962.) Pp. 576. (Amsterdam, London and New York: Elsevier Publishing Company, 1963.) 100s.

Mechanisms of Neural Regeneration

Edited by M. Singer and J. P. Schade. (Progress in Brain Research, Vol. 13.) Pp. v + 241. (Amsterdam, London and New York: Elsevier Publishing Company, 1964.) 80s.

IT has long been known that many organs tend to atrophy when deprived of their nerve supply; this has naturally led to the concept of a trophic influence of nerve fibres, which is thought to be conveyed either by special trophic fibres, or by the ordinary motor and sensory innervation. This kind of influence, whatever its true nature, acts in a very slow and intangible manner, and it was therefore somewhat neglected by experi-

mentalists while the more readily distinguishable electrical events accompanying nerve conduction and synaptic transmission were being analysed in great detail.

In recent years, however, the trophic processes have become of great interest to many biologists; if anyone should doubt this, he need only glance at these two large books. In the first symposium (first book), held in Czechoslovakia, sixty-six participants contributed nearly fifty short articles, covering a great variety of subjects. They have been classified basically into two main groups. The first covers the trophic function of the nerve cell, as reflected in long-term interactions between nerve and muscle. Here, one finds articles by Eccles, Gutmann, Mileti, Thesleff, Hnik and others, dealing with the properties of denervated and re-innervated muscle; further articles by Eccles, Szentagothai, Buller, etc., describe changes in the properties of muscle which follow re-innervation by foreign nerve fibres; and finally there is a discussion of axonal flow by Weiss and by Lubinska, as well as a somewhat less obviously relevant description by Hyden of neuroglial interactions.

The second main group is concerned with the effects of use and disuse on nerve and muscle cells. Most of the observations on nerve cells were made on spinal motoneurons (Asratyian, Eccles, Kostyuk, Beranek, etc.), but three articles by Hyden, Malcolm and Desmedt also describe experiments on cerebral neurones; in the final section, various aspects of muscle function and metabolism are considered by several authors, including Feng, Lissak and Shamarina. This book is full of fascinating information and interesting ideas; as usual, many questions are raised, the most glaring perhaps being that of the identity of the specific trophic factors which are generally assumed to be released by nerve fibres. There is, at present, scarcely any evidence about the kind of substances which are likely to be involved.

Although the second book is part of the now well-established series published under the general title of *Progress in Brain Research*, it is mainly concerned with changes observed in peripheral nerves. The approach here is much more didactic; there are only nine authors and their articles are based on lectures given at a summer school on brain research. Most of these articles are comprehensive reviews of selected subjects and they include extensive lists of references. Thus, although there is a substantial overlap with the Czech symposium, both in subject-matter and in authors (five authors are found in both books), the emphasis is mainly on processes of regeneration, and the tone and presentation are quite different.

There are three long reviews, by Lubinska on axoplasmic streaming, by Gutmann on neurotrophic relations and by Zelena on the degeneration and regeneration of receptor organs. Other substantial articles deal with intraxonal movements of phospholipids (Miani), the nerve growth factor and spinal regeneration (Scott and Liu), demyelination and remyelination (Webster) and some other aspects of the mechanism of regeneration (Trampush). Apart from a singularly obscure and unhelpful introduction (by the presumably overworked editors of this prolific series), most of the material has been clearly presented and it should be of much value to anyone needing detailed information in this field.

Both books have been well produced, with very legible print and with plenty of figures and tables, and each has an author index and a subject index; moreover, unlike the *Proceedings* of many other symposia, they have been published with commendable speed. Both books report a good deal of the informal discussions held during the meetings. Most readers will no doubt have their own views about the desirability of this rather general practice; to me, it seldom seems to add much substance to the main presentations, yet it must often contribute appreciably to the bulk and the cost and the delays of publication.

K. KRNEJVIĆ

Solid Circuits and Microminiaturization

Edited by G. W. A. Dummer. (Proceedings of the Conference held at West Ham College of Technology, June 1963.) Pp. 346. (London and New York: Pergamon Press, 1964.) 60s. net.

THE conference held at West Ham in 1963 made many sections of the British electronics industry more aware of the coming revolution in equipment design and technology. But too much must not be made of the implication, in the title of the conference, that the main driving force in the development of integrated circuits is the desire for large reductions in the size of equipment; reduced cost and increased reliability will often, singly or together, be greater incentives.

The *Proceedings* of the conference, informally written and informally presented to the reader, show a fair balance between the extension of the planar transistor technology to solid circuits, the relative merits of various logic circuits, the problems confronting the designer of analogue circuits, some new technologies such as electron beam machining, and several other important aspects of the subject. Packaging and interconnexions, however, are less well dealt with.

American developments have long dominated the subject and were given attention mainly by authors working in the European laboratories of the subsidiaries of American firms. But the *Proceedings* must now be seen against a recent issue (December 1964) of the *Proceedings of the Institute of Electrical and Electronics Engineers* which devoted about 340 pages to integrated circuits, giving a fairly comprehensive picture for mid-1964. Although even now the scope for improvements in materials, processing and assembly, for new active devices and for new outlets is very large, the stage has been reached when the customers of the industry can sample the products of the already extensive development programmes and study on an adequate scale the advantages accruing.

The rapid rate of advance in the field makes fairly frequent conferences very desirable, but greatly reduces the period over which any conference *Proceedings* continue to be reasonably up to date. This volume may, therefore, already be of little more than historical value.

J. R. TILLMAN

A Review of Sterilization and Disinfection as Applied to Medical, Industrial and Laboratory Practice

By Prof. Sydney D. Rubbo and Dr. Joan F. Gardner. Pp. xiii + 250. (London: Lloyd-Luke (Medical Books), Ltd., 1965.) 35s. net.

DURING the past ten years it has become increasingly obvious that many time-honoured methods used for sterilization in medical and hospital practice are either intrinsically inefficient or are rendered so by ignorance or inefficiency on the part of the operator. Nevertheless, very few hospital bacteriologists possess the necessary background knowledge to detect and correct these errors. It is for this reason that *A Review of Sterilization and Disinfection as Applied to Medical Industrial and Laboratory Practice* by Prof. Sydney Rubbo and Dr. Joan Gardner is all the more welcome.

Following chapters on the theoretical basis of sterilization, tests for its efficiency and the preparation of articles for sterilization, each of the usual sterilizing agents such as hot water, steam, hot air, radiation, filtration, gases and chemicals are described in considerable detail. The remainder of the book deals with special cases such as treatment of the hospital environment, the preparation of the skin or mucous membranes before operation, the treatment of pharmaceutical products and the sterilization of glassware and media used in bacteriological laboratories.

A great deal of useful information not easily obtainable is summarized in this way. This includes such diverse matters as tables giving the various indicators that may be used

to detect inefficiencies and the various faults that may develop in steam sterilizers, the methods used for the sterilization and preservation of pharmaceutical preparations, how to deal with discarded culture media and even a conversion table, which will be of particular value to anyone dealing with engineers who still persist in using the Fahrenheit scale.

Some of the opinions expressed by the authors may not be acceptable to everyone: this is perhaps inevitable, but adequate references are given to previous work so that anyone who is prepared to take the trouble can form his own.

The book is well produced, with a large number of diagrams and tables, together with an adequate index. It can confidently be recommended. R. HARE

Germes and Ideas

Routes of Epidemics and Ideologies. By Andre Siegfried. Translated by Jean Henderson and Mercedes Clarasó. Pp. viii + 98. (Edinburgh and London: Oliver and Boyd, Ltd., 1965.) 15s.

THIS book is based on a lecture given by Dr. Siegfried at the Clinique Médicale Propédeutique of Paris in 1958. It is an interesting example of the new outlook brought to a well-known subject by a wide and lively intelligence which had received no previous formal training in this field. Siegfried lectured on the routes followed by germs in the process of infection. In the first part he considers the geographical factors which dictate the routes, by caravan, by sea or by aeroplane, along which men, goods and germs travel. The germs tend to start from centres of infection in India, China or South America and to travel outwards, assisted by the speed of modern transport, hindered by modern hygienic and public health control. The spread of infection is then illustrated by accounts of the world-wide epidemics of cholera, Asian 'flu, plague and yellow fever. These are clearly and interestingly described for non-medical readers (probably medical readers would doubt whether there is danger of yellow fever being spread by cargoes of bananas bitten by infected mosquitoes). In the fourth part, Dr. Siegfried makes an interesting development by passing from the spread of germs to the spreading of ideas and propaganda. There is almost a suggestion that these are equally pernicious, and if they cannot be exterminated in their primary focus (as Simon de Montfort exterminated the Albigenian heretics) then they can often be kept out by an effective quarantine at the frontier (as practised in the U.S.S.R. and China).

This booklet contains interesting ideas, set out with French clarity and brevity. Besides students of medicine it should interest students of geography, history and sociology, bringing to their notice medical information in an attractive and comprehensible guise. The translation is good and lucid. F. HAWKING

Native Vegetation of Nebraska

By Prof. J. E. Weaver. Pp. 185. (Lincoln: University of Nebraska Press, 1965.) 4.75 dollars.

IN his latest book, Prof. Weaver, who is well known for his researches on prairie ecology, outlines for "the widest possible audience" the vegetation of the State in which he has worked for half a century. The native communities are straightforwardly described in turn, with a rather fuller ecological commentary for grasslands than for forests, and a final chapter is concerned with the cultivated crops of grassland soils. Vernacular names are used throughout, and their botanical equivalents are not given, not even in an appendix. There is no vegetation map, no index, and no list of the numerous illustrations.

D. R. HUNT

1965 NOBEL PRIZE FOR MEDICINE

By PROF. M. R. POLLOCK, F.R.S.

Department of Molecular Biology, University of Edinburgh

THE Nobel Prize for Medicine this year acknowledges the achievements over the past 15 years of three great French biologists: Prof. André Lwoff, Prof. Jacques Monod and Prof. François Jacob. It will be especially welcomed by molecular biologists as a highly appropriate recognition of the brilliant contributions of these scientists to our understanding of the mechanism of gene action in cell physiology. In this respect, it can be regarded as complementary to, and in some degree dependent on, the great discoveries of Dr. F. H. C. Crick, Prof. W. Watson and Prof. M. H. F. Wilkins, recognized by their Nobel Award of three years ago, which solved—at the molecular level—the problem of gene replication and laid the foundation for the formal solution of the nucleic acid code for sequences of amino-acids in proteins.

One need be no expert in this subject to appreciate the impact of the work of the 'Pasteur group', if one remembers that well-known terms in constant current use such as 'prophage', 'temperate', 'virulent bacteriophages', 'episome', 'structural', 'regulatory genes', 'permease', 'operon', 'messenger-RNA', 'allosteric proteins', etc., owe their origin to these three workers. Indeed, the concepts these terms represent, if not in every case entirely new, were defined, developed and made precise by them and their collaborators.

But, of course, their achievement ranges further and deeper than this by itself might imply. Its greatness is based, to a large extent, on the unique type of co-operation and cross-fertilization of ideas (perhaps 'complementation' might be a more appropriate description) arising from their three very different personalities.

Each of them would rank on his own as a scientist of outstanding talent. Though originally in the same department at the Pasteur Institute, they have seldom worked together in the same laboratory, and, unlike many successful research collaborators, only rarely plan the minutiae of experiments in continuous consultation.

Indeed, the circumstances and basis of how the members of this group (which was never a 'team' in any sense of the word) matured and developed towards an intellectual symbiosis make a fascinating story. It has often seemed that they came together almost by chance; certainly their co-operative work did not appear to arise from long-term conscious planning. Yet, it is difficult to believe that it could have happened without the encouragement and help originally provided, no doubt very consciously, by André Lwoff. François Jacob, trained in medicine, might perhaps be lingering in frustration in some antibiotics production unit (though one doubts it) and Jacques Monod might have fulfilled his earlier dreams and become an orchestral conductor. France might have had one more talented physician or even her Toscanini; but molecular biologists the world over would have remained the poorer for many years.

Lwoff, assisted by his wife Marguérite, had already, of course, achieved an international reputation in protozoology from work begun before the Second World War. This included their joint discovery of phosphopyridine nucleotide as the 'V' growth factor for *Haemophilus*, his theories on the independent genetic continuity of ciliate cinetosomes, and his thesis on evolution through loss of function.

Monod had discovered the phenomenon of biphasic growth ('diauxie') of bacteria growing on twin sources of carbon and energy in 1942. This, he found, was due to

the sequential utilization of the two carbohydrates present in the medium. His tentative interpretation had been in terms of the repression, followed by the induction, of the enzymes metabolizing the compound utilized last. From then on, he and his colleagues devoted themselves more and more completely to the elucidation of the fascinating problem of enzymic adaptation in micro-organisms. In 1950 it was still an open question whether this phenomenon was due to activation (or minor metabolic conversion) of a pre-existing inactive protein molecule, or to true *de novo* synthesis of enzyme from amino-acids. By 1954 he had succeeded (in collaboration with Cohn and Hogness) in showing that the process did indeed involve the biosynthesis of the whole enzyme molecule.

The 'great period' of the Pasteur group began only a little more than 15 years ago, following Lwoff's discovery that the genes of many bacteriophages could exist and multiply in two different alternative states: freely in the bacterial cytoplasm (leading to phage maturation and cell lysis) or integrated (as 'prophage') at a specific locus in the bacterial chromosome. It is encouraging for middle-aged research workers to realize, as they approach their half-century, that Lwoff was beginning his study of lysogenic bacteria (which previously had been little more than a biological curiosity) only a year or so before he reached his sixth decade. This has undoubtedly been his greatest scientific achievement so far. From it, the trail led, albeit somewhat tortuously, towards the formulation of two important biological concepts.

The first was that of 'episomes'—genetic entities which could be either extra-nuclear and partially autonomous or integrated as part of the chromosome, according to circumstances. This was developed by Jacob together with Elie Wollman, primarily on the basis of the phage/prophage interrelationship and their own observations on the semi-autonomous sex factor in *E. coli* (see later).

The second concept was that of 'cytoplasmic repressors'—a class of molecule responsible for specifically inhibiting the expression of a variety of genes. This was based, in the first instance, on two closely analogous phenomena: (a) the so-called 'zygotic induction' of prophage to free-growing active phage (discovered by Jacob and Wollman), which occurs after transfer of prophage, during conjugation, into a (non-immune) female cell not carrying prophage; and (b) the spontaneous production of β -galactosidase (for a limited period at maximal rate) which occurs immediately after transfer of the *inducible* gene during conjugation into a female cell carrying the 'constitutive' allele (discovered by Pardee, Jacob and Monod). In both instances reciprocal crosses had no such effect. This led to the notion that the donor cells, in both cases, contained in their cytoplasm an inhibitor responsible for blocking phage and β -galactosidase production respectively. The idea was developed mainly by Jacob and Monod.

In the early 'fifties, however, Jacob worked mainly with Wollman, whose critical mind contributed essentially to their remarkable success during the remainder of that decade.

In initial investigations of the autonomous, cytoplasmic *F* (sex) factor in *E. coli* (discovered independently by Hayes and by the Lederbergs and Cavalli in 1953), Jacob and Wollman elucidated its episomal nature by showing that it could be integrated into the chromosome and was thereby responsible for promoting the process of chromo-

some transfer during sexual conjugation. Their demonstration that the chromosome of *E. coli* is circular (broken only just before conjugation at the point where the sex factor had been incorporated) was really part of this discovery, since one of the two chromosomal 'ends' thus formed proved to be the leading point in its migration from the male to the recipient female, in which recombination occurs. By mechanical interruption of the mating process at different times after its initiation, they further demonstrated that there was a fixed order of entry of the various genes into the female cell. This order corresponded almost exactly to the order determined by the completely different method of measuring relative recombination frequencies of the chromosomal genes. It was thus a further confirmation of the validity of the classical mapping techniques which had been in use in all types of organism for several decades. Moreover, taken in conjunction with the demonstration that the chromosome was transferred from male to female at constant speed, it enabled the distance between genes to be measured in absolute terms (time units). Knowing the total amount of DNA present, this could be translated directly into lengths of DNA double helix.

During this period, Monod and his group were concerned primarily with defining and characterizing the so-called 'permeases'—specific enzyme-like entities, often inducible like ordinary enzymes and similarly controlled by genes with precise loci on the chromosome. They appear to function as energy-requiring pumps for transporting small molecules through the otherwise poorly permeable barriers of the cell envelope.

Although permeases have not yet been isolated or characterized at the molecular level, the idea that they could be regarded as entities in their own right has proved stimulating and useful. Moreover, the study of permeaseless ('cryptic') strains and mutants clarified a number of perplexing problems in cell enzymology.

It was not until 1957 that Jacob and Monod began their famous collaboration, with the demonstration (together with Pardee) that in *E. coli* strains diploid with respect to the β -galactosidase locus the wild-type character of inducibility usually completely suppressed the (mutant) character of constitutivity, and never vice versa. This was powerful evidence against the frequently discussed possibility that enzyme production might be due to direct stimulation of gene function by a specific inducer (endogenous in constitutive strains). It strongly indicated that enzyme induction, just like the induction of prophage (in lysogenic bacteria) to the free-growing, active phage state was essentially a release from inhibition. The fact that the R^+ genome (showing the property of inducibility) inhibited the expression of the R^- (constitutive) mutant allele in the *trans* position (that is, across the physical gap presumed to separate the two homologous chromosomes of the diploid) suggested that the suppressing effect was due to a diffusible molecule. The idea of specific cytoplasmic repressor substances being primarily responsible for control of gene expression was thus further developed.

During the next few years, Jacob and Monod defined, studied and characterized (one might almost say 'promoted') the following important biological entities, many of which have since received further experimental substantiation from other laboratories.

(1) *Regulatory genes*, specifically controlling the rates of formation of proteins, as distinct from *structural genes*, responsible for determining their amino-acid sequences.

(2) The *operon*, consisting of a cluster of genes, arranged contiguously along the chromosome, being concerned with one particular metabolic function (such as the formation of enzymes catalysing the biosynthesis of an amino-acid) and expressing themselves co-ordinately, being switched on and off together, under the control of a single regulator gene.

(3) *Messenger-RNA*. Not entirely a new idea, of course, and investigated by many other molecular biologists,

but illuminated by the work of Monod and Jacob and their associates in a characteristically impressive fashion. According to them, it embodies the properties of a relatively labile and rapidly produced carrier of information from the gene to the rest of the cell, by its action as a template for protein synthesis.

Their work together culminated in the classic paper published in 1961 in the *Journal of Molecular Biology* on "Genetic Regulatory Mechanisms in the Synthesis of Proteins". In this they outlined their famous hypothesis on the mechanism of control of genetic expression through the action of a series of specific cytoplasmic repressor substances. In the case of induction of a particular enzyme, it is supposed that the regulatory gene produces a repressor molecule which, by combining with a specific chromosomal locus (the 'operator') located at one end of the operon, inhibits transcription of the operon genes into messenger-RNA, so that synthesis of the relevant proteins is suppressed. The role of the inducer is to inactivate the repressor. With repressible enzymes, the production of which is normally inhibited by the compound they synthesize, the product of the regulatory gene is supposed to become activated as a repressor only after combination with the end-product ('co-repressor') molecule.

The essence of this theory is that the information provided by the small effector molecules involved in enzyme induction or repression is a 'switch impulse', transmitted through the activation or inactivation of a specific pre-formed inhibitor of the expression of a predetermined genetic sequence. Thus, one of the most important mechanisms by which the environment can 'mould' the physiology of the cell towards more effective performance in a given situation is by the 'uncovering' of genes in their role as blue-prints for the synthesis of the relevant enzymes. The pattern that is changed by an alteration in the environment is thus the relative amounts of different enzymes present in the cell and not their chemical structure. This is now generally accepted as one of the fundamental bases of biological adaptation processes at the cellular level.

However, the hypothesis, in its detailed form, is far from proved. It must be remembered that cytoplasmic 'repressors' have not yet been isolated in any form, nor have their effects been directly demonstrated. Like permeases, they do not yet have molecular reality. Moreover, it is uncertain how complete the model is, even for *E. coli* β -galactosidase. And it is still largely a matter for conjecture how far it may be extended to living systems beyond the bacteria and certain other species of micro-organism. For example, it could clearly be applied to embryonic differentiation; but even if differentiation is based on a sequential expression of various portions of the genome, triggered individually by inducers and repressors, the theory does not by itself indicate by what mechanism the 'control' genes are themselves controlled. Is it simply the structural genes themselves—acting through the inducing or repressing properties of the metabolites the production of which they activate? Or is there some new entity still waiting to be discovered—a master regulatory gene, perhaps, with an endogenous rhythm or maturation-pace 'of its own'? The speculative 'regulatory circuits' outlined by Monod and Jacob are interesting attempts to give some idea of how interrelated control systems might be built up on the basis of their main hypothesis. The irreversibility of differentiation processes can indeed be thus modelled. But the authors would probably be the first to admit that they have not yet provided a satisfactory answer to the problem of the time sequencing that is inevitably involved.

On the other hand, there is no doubt that the theory has had a profound influence on the trend of biological thought during the past four years and has been of immense importance in stimulating much valuable experimentation, as well as in providing a model for workers investigating regulation processes in many types of biological system.

Monod, together with Changeux and Wyman, has now plunged deeply into the problem of enzyme function, in elaborating the concept of 'allostericity' in proteins. This principle is based on the Bohr effect and O_2 -dissociation curves of haemoglobin, and on the more recently observed phenomenon of 'feed-back inhibition' of certain enzymes by the end-products of the metabolic chain which they have initiated (discovered by Novick and Szilard, and by Umbarger). The essential idea, which has already received considerable support, is that the functioning of the active site in an enzyme can be specifically modified by combination with another type of molecule (chemically unrelated to the normal substrate) at a different site. In this way, the function of an enzyme can be specifically controlled, with immediate effect, by direct action and not only by influences on its rate of biosynthesis. The mechanism of what Monod has called 'allosteric transitions' involves reversible switches between alternative conformational states in the structure of the enzyme. He believes it must often, if not always, depend on the molecule being oligomeric and having bilateral symmetry. Certainly, most enzymes that show the phenomenon do consist of a number of (often identical) polypeptide chains. It offers a reasonable teleological explanation of why enzymes are often such large, polymeric molecules.

Furthermore, as Monod and Jacob have often emphasized, their hypothetical 'cytoplasmic repressor' must also possess the property of 'allostericity' in order to exhibit the character of interrelated reactivity with both the effector molecule and the operator locus. Indeed, it is difficult to avoid the conclusion that it was just this particular character, with which the repressor molecule had to be endowed in order for it to fulfil its role in the Monod-Jacob scheme, that led them to search for analogous properties in other, more concrete, types of molecule. If so, it is a fascinating example of the ironic way by which man can make his great discoveries. A purely logical necessity involving the postulate of a new type of property in a purely hypothetical molecule leads to the search for, and discovery of, just this property—observed but hitherto not adequately recognized—in a whole range of concrete and well-defined substances. But it is probably best not to try to recapitulate the mental processes involved. The results by themselves justify the method, whatever it may be. Although in some ways less complete and less precise than the other hypotheses, this may ultimately prove to be the most useful of them all.

But this cannot be the end of the story. There is still plenty of time ahead for further discoveries, and one wonders what new natural phenomenon—observed, no doubt, but not yet properly appreciated or understood—will be the next to blossom under such patronage.

All this work has rightly been regarded as typical of the 'French' approach at its best: what has been called the 'hypothetico-deductive' type of reasoning, which many believe to be the only satisfactory method of argument in science. But in their case (as in so many others) it is

surely the method of presentation, rather than the method of thinking or reasoning, that stands out so clearly as 'hypothetico-deductive'. It is difficult to believe that something akin to a process of induction from observations that have often been made fortuitously has not contributed importantly to their discoveries.

The work of the 'Pasteur group' has, inevitably, been subjected to the criticism that would be expected from those who do not understand what molecular biology is about, or who are envious of its great achievements. They have been accused, by many old-fashioned morphologists and by neo-vitalists of the twentieth century, of having an oversimplified or mechanistic approach.

On the other side, chemists, and even some biochemists, have been distrustful of interpretations made at the molecular level, which often do not involve identification and isolation of a known type of compound, or the specific demonstration of its action in a chemically defined system.

More seriously, there has developed in some quarters a resentment at what has been taken to be an inflexibly dogmatic attitude which is too impatient of criticism or alternative hypotheses. Certainly the devastatingly brilliant disposal of "objections from the floor" in open conference, which is characteristic of this outlook, would scare off all but the most confident contestant. But in private discussions among themselves, and in *tête-à-tête* conversations with colleagues, there is often so sweet a reasonableness that criticism is disarmed. Few visitors to their laboratories in the old days failed to fall under the spell of the charmed atmosphere in the Service du Physiologie Bactérienne—though it might have been only a matter of minutes since they had been hacked to pieces in one of those well-known seminars.

Nevertheless, there is perhaps substance in some of this anxiety—in the sense that too great an intellectual dominance and too brilliant an advocacy in promoting a theory can dazzle the originality out of other workers and stop people thinking. Far too much may then be taken for granted. But it would have to be admitted that, great experimentalists though they may be, it is just this ability to clarify an issue, define the problems, contrast and evaluate alternative interpretations, and finally to offer a precise all-embracing hypothesis (not necessarily entirely original or totally satisfying: how could it be?) that Lwoff, Monod and Jacob have been able to offer most to science. It is interesting that this has, indeed, been specifically recognized in their Nobel Award citation.

Moreover, they usually expound their ideas in papers written in an English that (it must reluctantly be conceded) is not only grammatically impeccable, but has a beauty of style and clarity of expression that an Englishman would be almost ashamed to contemplate, were it not such a pleasure to see our native language prove itself to be so fine a vehicle for the communication of ideas.

BIOCHEMISTRY AND MENTAL FUNCTION*

By PROF SEYMOUR S. KETY

Chief, Laboratory of Clinical Science, National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland

IF we are ever to understand and rationally to meliorate the disturbed processes which underlie mental illness, it will be by investigation of the clinical problems themselves and examination of the mental, social, neural and biological elements which comprise behaviour. It is all of these which the Americans call 'psychiatric research',

and in Great Britain, the Mental Health Research Fund has added much to its vigour for more than a decade.

Although they are not alone in their importance to psychiatry¹, the biological sciences have a significance which is not attenuated by community with the social and psychological sciences, nor is their power less real by having been only partially demonstrated. It is the area, tentative as yet, between biology and human psychology

* Substance of the Third Mental Health Research Fund (38, Wigmore Street, London, W.1) Lecture delivered on February 26.

that I have chosen to dwell on, and I shall do so largely in terms of the work of my associates and myself in the Laboratory of Clinical Science at the National Institutes of Health, Bethesda, without any pretension that this will constitute an adequate review of a growing field.

The time is not yet at hand, if, in fact, it will ever be reached, when one can speak meaningfully of the biochemistry of mental state. There are, however, a few areas where one can see the beginnings of correlations and significant interrelationships and these include consciousness, intellectual function and affect.

My interest in consciousness goes back to the Science and Philosophy Club at the Central High School in Philadelphia, a club which bore the brave motto "*Felix qui potuit rerum cognoscere causas*", and in which a great teacher, Edwin Landis, introduced us to Berkeley, Mach, Eddington, and the fathomless problem of the nature of consciousness. Many years later I was introduced to the cerebral circulation by Carl Schmidt, through his definitive work in the rhesus monkey, and began to feel how much might be learned from measurements in man, whose brain, with its subjective wealth, and whose diseases could not be replicated in animals. Making use of some fundamental principles of inert gas exchange, the Fick principle, and a little calculus, which there is no time to discuss in any detail, we were eventually able to make what still appear to be satisfactory measurements of blood flow, oxygen and glucose consumption in the conscious human brain under a variety of physiological and pathological conditions. In Table 1 are presented some normal values representing, in fact, the average of the first investigations of healthy volunteers of about twenty-five years of age². Measurement of glucose consumption followed later, confirming the thesis that the major substrate for oxidative energy in the brain is glucose, the utilization of which represented an almost stoichiometric equivalent of the oxygen consumed. From these measurements it was possible to compute the rate of energy utilization by the human brain which turned out to be close to 20 W. In comparison with the enormous expenditure of energy which modern computers require, this represents a remarkable degree of efficiency and miniaturization.

Table 1. OVER-ALL BLOOD FLOW AND ENERGY METABOLISM OF THE NORMAL HUMAN BRAIN

Blood flow ml./100 g/min	54
Oxygen consumption ml./100 g/min	3.3
Respiratory quotient (CO ₂ /O ₂)	0.99
Glucose consumption mg/100 g/min	4.9

We were anxious to examine states markedly different from the normal in functional level and chose states of altered consciousness (Table 2). It was quite apparent that there was a rough correlation between level of consciousness and over-all oxygen and energy utilization by the brain³. In anaesthesia, for example, where the cerebral oxygen consumption was reduced by 40 per cent, there appeared to be support *in vivo* to the earlier *in vitro* investigations of Quastel⁴, who had shown that anaesthetics interfere with the oxygen consumption of brain slices. But all these data merely tell us that the oxygen consumption and energy-level of the brain are reduced in states of depressed consciousness; they do not explain the coupling between function and metabolism which is one of the most interesting topics of present concern. One could argue that the primary effect in any of these conditions was on the metabolic 'power supply' of the brain necessary for the maintenance of consciousness. An alternative hypothesis, however, would be that the primary site of interference was in the interaction between neurones at the synapses, which once inhibited, depressed both the functional activity and the energy requirements of the system. This interesting problem of the coupling between function and metabolism must await clarification by the work of those like McIlwain, Rodnight, Larrabee and Chance, among others.

Table 2. CEREBRAL OXYGEN CONSUMPTION IN STATES OF DEPRESSED CONSCIOUSNESS (EXPRESSED AS PERCENTAGE OF THE VALUE IN HEALTHY YOUNG MEN)

Senile psychosis	82
Diabetic acidosis	82
Insulin hypoglycaemia	79
Surgical anaesthesia	64
Insulin coma	58
Diabetic coma	52

Table 3. CEREBRAL OXYGEN CONSUMPTION IN VARIOUS MENTAL STATES (EXPRESSED AS PERCENTAGE OF THE VALUE IN NORMAL CONTROL STATES)

Normal sleep	97
Schizophrenia	100
LSD psychosis	101
Mental arithmetic	102

There are states of altered consciousness, however, in which such a neat correlation with total cerebral metabolism and energy does not exist (Table 3). Normal sleep is one such state; the poetic description of the wakening brain by Sir Charles Sherrington in "Man on His Nature" is well known:

"Suppose we choose the hour of deep sleep. Then only in some sparse and out of the way places are nodes flashing and trains of light-points running . . . the great knotted headpiece of the whole sleeping system lies for the most part dark. . . Should we continue to watch the scheme we should observe after a time an impressive change which suddenly accrues. In the great head end . . . spring up myriads of twinkling stationary lights and myriads of trains of moving lights of many different directions. . . the great topmost sheet of the mass, that where hardly a light had twinkled or moved, becomes now a sparkling field of rhythmic flashing points with trains of travelling sparks hurrying hither and thither. The brain is waking and with it the mind is returning."

Not only did our results⁵ force the rejection of a simple cerebral ischaemic theory for sleep which dated back to Alcmaeon; they challenged as well the generally accepted 'Sherringtonian' notion which equated sleep with neuronal inactivity. More recent neurophysiological findings are more consonant with what we learned about the nature of sleep. Evarts⁶, in very elegant investigations of the activity of individual neurones which he has observed through microelectrodes chronically implanted in the cortex of unanaesthetized cats, has found no net decrease in cortical neuronal activity during natural sleep. He has, on the other hand, demonstrated characteristic alterations in the activity of individual neurones or groups of neurones, some showing inhibition when the animal sleeps, but others coming into greater activity at that time.

The results in schizophrenia⁷, during LSD psychosis⁸ or in mental arithmetic⁹ all reinforce the concept that the brain, unlike the heart or the liver or kidney, is an organ for computation and communication. In such functions there is no necessary correlation between the energy utilized and the efficiency of the process or the quality of the output. To differentiate these alterations of consciousness in terms of the cerebral oxygen consumption would be like trying to correlate the nature of a radio programme with the power used.

Some of our more recent investigations have attempted to examine the energy utilization of many structures within the living brain. The first approach to measurement of oxygen consumption is in defining the local perfusion rates. Using basic principles similar to those of the nitrous oxide method, we have related the quantity of an inert diffusible substance taken up by a small tissue region to its perfusion¹⁰. If the tracer is radioactive, one can measure its uptake during a standard time interval in the various structures of the brain by autoradiography. In the autoradiogram, density is related to the concentration of tracer which in turn can be related to the blood flow during the physiological state just prior to the abrupt killing of the animal. Under most physiological conditions there is reason for believing that the blood flow is determined by the oxygen consumption, so that in a rough way the autoradiographic density gives information

on the differential energy utilization in various structures of the brain. Such investigations have revealed a remarkable differentiation of cortical blood flow in the unanaesthetized brain with the primary sensory areas showing far greater activity¹¹. This differentiation does not appear to be present in the brain of the foetus or the neonate. Anaesthesia obscures this differentiation, reducing the areas of greater cortical oxygen consumption to a relatively homogeneous average value, while there is evidence that sensory stimulation results in a recognizable increase in blood flow and, presumably, oxygen consumption along the appropriate sensory pathways¹².

The maturation of intellectual function and its maldevelopment depends on many processes in addition to oxygen consumption. In 1949, when Sokoloff first became associated with us, we undertook an investigation of cerebral blood flow and oxygen consumption in patients with hyperthyroidism. This resulted in the quite surprising finding that although the total oxygen consumption of such patients was markedly elevated, there was no significant increase in the oxygen consumption of the brain¹³. These results demonstrated that the effects of thyroid hormone were not uniformly applied to the metabolism of all cells in the body. A finding such as this requires an explanation and Sokoloff set about to find one. Using radioactive thyroxine he learned that the hormone crossed the blood-brain barrier and was available to the cells of the brain. He knew also that the brain was peculiar, in that its oxidative processes were almost entirely confined to a single substrate, glucose, and that, although Richter, Waelsch and others had demonstrated an active protein synthesis in the mature brain, that process was still considerably slower in brain than in liver and could scarcely account for a significant fraction of the cerebral oxygen consumption. These considerations led him to the hypothesis that thyroxine neither stimulated oxidative metabolism directly nor uncoupled it from phosphorylation (which were the prevailing concepts) but acted on some specialized process such as protein synthesis.

In 1954 he came to the National Institute of Mental Health and began a highly productive collaboration with Kaufman. In 1959 they were able to report that L-thyroxine, administered to normal animals *in vivo* or added directly to the incubation medium *in vitro*, stimulated the rate of amino-acid incorporation into protein in cell-free, rat liver homogenates. This stimulation of protein synthesis *in vitro* occurred in the absence of changes in oxygen consumption or oxidative phosphorylation. They further suggested that the characteristic effects of thyroxine on energy metabolism were secondary to the stimulation of reactions which required energy such as protein biosynthesis¹⁴.

The mitochondria are clearly involved in this process since it is these structures alone rather than the microsomes or the cell sap which differentiate hyperthyroid rats from euthyroid controls in the ability to stimulate amino-acid incorporation into protein¹⁵. Thyroxine in the presence of mitochondria and an oxidizable substrate apparently produces a soluble factor that can be isolated and which will in itself stimulate protein synthesis. The evidence indicates that this factor stimulates the transfer of sRNA-bound amino-acids into microsomal protein^{16,17}. Much remains to be done in identifying the factor and further defining its action on the microsome and the precise step in protein synthesis at which it occurs. The demonstration of a stimulation of protein synthesis by thyroxine, however, clearly defined as it is by *in vitro* investigations and confirmed *in vivo*, appears to be the fundamental mechanism of action of this hormone and explains much of its physiological effects.

Recently, Sokoloff, in collaboration with Klee¹⁸, has re-examined the effects of this hormone on the brain. Although thyroxine does not stimulate protein synthesis in mature brain, explaining its failure to increase cerebral oxygen consumption in adult man, it does so significantly

in neonatal brain, and again it is the mitochondria which differentiate the two.

These findings help to explain the well-known clinical effects of the thyroid hormone on the development of the brain and of intellectual function in infants compared with its relatively minor effects on these functions in the adult. They corroborate and offer a mechanism for Eayrs's finding of the requirement for thyroxine in the dendritic proliferation of immature cerebral cortex. Thus, this work forms a crucial link between the absence of thyroid hormone and the retarded cerebral development in cretinism.

In 1956, soon after the Laboratory of Clinical Science, National Institute of Mental Health, was organized, some of us became interested in the cluster of thought disorders which is called schizophrenia, and gave attention to the hypothesis which attempted to explain many of the mental symptoms of schizophrenia on the basis of an abnormal degradation of circulating epinephrine to abnormal oxidation products such as adrenochrome or adrenolutin¹⁹. That hypothesis seemed especially plausible because it took cognizance of the evidence for genetic factors as well as the importance of stressful life experiences in the pathogenesis of the mental disorder. The difficulty in testing the hypothesis lay in the lack of knowledge concerning the normal metabolism of epinephrine, let alone its possible abnormality in schizophrenia. In 1956 one could account for some 5 per cent of administered epinephrine which was excreted unchanged in the urine, while the remaining 95 per cent was disposed of by unknown mechanisms.

Isotopic techniques, which had been so valuable in the tracing of other metabolic pathways, were not readily applied to this problem because the pharmacological potency of epinephrine prevented the administration of enough of the hormone labelled with carbon-14 which was then available to permit characterization of its products. It was apparent that to use isotopic techniques to advantage for studies of the metabolism of epinephrine, especially in man, would require an isotopically labelled epinephrine of unheard-of specific activity. We were finally successful in having a few millicuries of 7-³H-epinephrine synthesized. The tritium label made possible the high specific activities required, while its position at C7 met our expectation that the label would be retained through the various possible metabolic degradations.

In 1957, Armstrong, McMillan and Shaw identified the first major metabolite of epinephrine (vanillylmandelic acid, VMA, or 3-methoxy-4-hydroxymandelic acid) in the urine of a patient with pheochromocytoma and in normal urine²⁰.

A few years before, Julius Axelrod had joined the Laboratory, bringing with him great interest and competence in the catecholamines. Although the metabolism of adrenaline to VMA was generally regarded as involving first deamination by monoamine oxidase and then *O*-methylation, Axelrod, on the basis of pharmacological and biochemical evidence, postulated the existence of an alternative pathway with *O*-methylation as the first step followed by deamination. He then proceeded to demonstrate in the urine the existence of that hypothetical compound which he designated "*O*-methylepinephrine" or "metanephrine", a second major metabolite of epinephrine²¹. He described and characterized the enzyme responsible for this conversion (catechol-*O*-methyltransferase) and the requirement of *S*-adenosylmethionine as the methyl donor²². He suggested that *O*-methylation rather than deamination was the principal enzymatic process involved in the inactivation of circulating epinephrine and later went on to show that norepinephrine was metabolized through completely analogous pathways by the same enzymes²³. Fig. 1 shows the present state of knowledge of the metabolism of these two catecholamines with a number of additional minor metabolites which Axelrod *et al.* have identified. Together all these metabolic pro-

ducts account for some 98 per cent of administered epinephrine or norepinephrine and, presumably, a similar accountability would hold for these substances when they are released into the circulation under physiological conditions.

With the background of information on the normal degradation of the hormone which was thus provided, it was then possible to examine the metabolism of epinephrine in schizophrenic patients and normal volunteers using the tritium-labelled substance^{24,25}. We were unable to find any evidence for a significant abnormality in the metabolism of intravenously administered epinephrine among the schizophrenics either qualitatively or quantitatively, the four normal metabolites and the unchanged hormone accounting for 98 per cent of the tritium in the urine in both groups of subjects.

The synthesis of tritiated epinephrine which was stimulated by that hypothesis but, most important, the work of Axelrod *et al.* have, however, had important implications for psychiatry. The identification of the metabolites of epinephrine and the development of methods for their estimation in urine make it possible to obtain information on the secretion of this hormone in a variety of physiological and pathological states and in response to drugs. Investigations by Axelrod and Kopin,

among others, with norepinephrine were a logically related step, and in the past few years the storage and release of norepinephrine at the sympathetic nerve endings and the factors which control these processes have become one of the most exciting fields of pharmacology²⁶. The insights which such investigations have given us into the possible actions of drugs which affect mood will be discussed later.

Thirteen years ago Osmond and Smythies, in conjunction with Harley-Mason²⁷, advanced the interesting hypothesis that there was an accumulation of an abnormal methylated compound with hallucinogenic properties in schizophrenia. They were led to this possibility by the fact that the potent psychotomimetic drug, mescaline, was almost identical with trimethylated dopamine. In the same communication Harley-Mason pointed out that the dimethyl derivative (3,4-dimethoxyphenylethylamine), which had interesting behavioural effects, could possibly be formed by transmethylation *in vivo*. In 1961, Pollin, Cardon and I²⁸ tested this hypothesis by observing the mental effects of methionine given orally to a small number of chronic schizophrenic patients who had been maintained on a monoamine oxidase inhibitor. We reasoned that under those conditions it was conceivable that the levels of *S*-adenosylmethionine, which Cantoni²⁹ had shown to be an important methyl donor, could be increased and the biological transmethylation of amines facilitated. In some of the patients there was a temporary but quite obvious exacerbation of psychotic symptoms associated with methionine administration. These observations have now been confirmed by several other groups²⁹⁻³²; in addition, Brune and Himwich found similar effects with betaine, another methyl donor³⁰.

Further work by investigators in our laboratory has tended to support some of this reasoning. The absence of information regarding tissue levels of *S*-adenosylmethionine led Baldessarini and Kopin³³ to devise an ingenious assay of high specificity. By means of this they found a considerable elevation of *S*-adenosylmethionine in brain and liver of rats following methionine feeding. Axelrod³⁴ demonstrated the presence in normal mammalian tissue of an enzyme capable of methylating normal metabolites, that is, tryptamine and serotonin, to their dimethyl derivatives in the presence of *S*-adenosylmethionine. Dimethyltryptamine has been shown to be a potent psychotomimetic agent³⁵.

In 1962, Friedhoff and Van Winkle³⁶ detected a substance which behaved like 3,4-dimethoxyphenylethylamine in the urine of a substantial fraction of schizophrenic patients and which appeared to be absent from normal urine. That finding, which has had substantial confirmation by Bourdillon and Ridges³⁷, lends further support to the hypothesis of Osmond, Smythies and Harley-Mason, especially since it was that compound to which Harley-Mason had directed attention ten years previously.

Our observations of the effect of methionine in schizophrenic patients, as well as the findings of Friedhoff and Van Winkle, are open to a number of alternative explanations which have not been ruled out. Nevertheless, the hypothesis that the accumulation of one or more methylated compounds plays a significant part in some forms of schizophrenia remains a plausible and parsimoni-

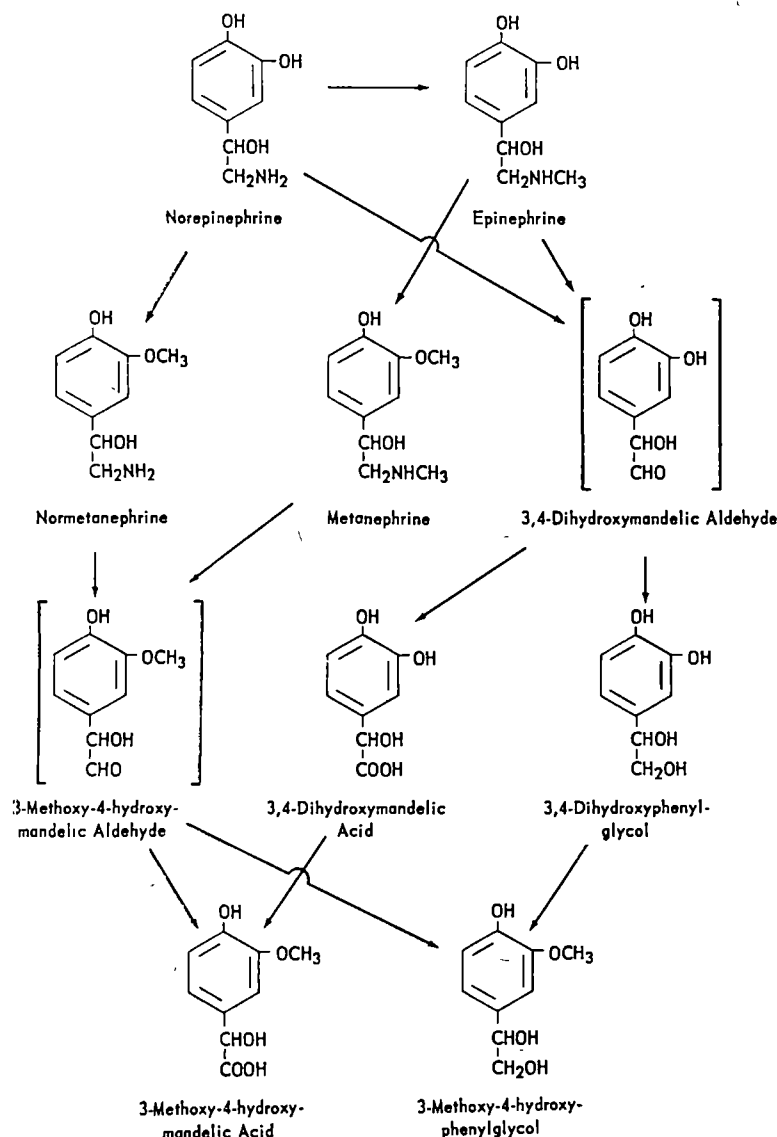


Fig. 1. Present knowledge of the metabolism of epinephrine and norepinephrine (after Axelrod and Kopin)

ous explanation of a number of different and independent observations and seems worthy of further evaluation.

The possible interrelationships between the biogenic amines and affective states have become a subject of lively interest and productive investigations in the relatively few years which have elapsed since the pioneering studies of Gaddum and Vogt in Great Britain, Erspamer in Italy, Rapport and Woolley in the United States. Interest centred at first on serotonin after the remarkable demonstration by Shore *et al.*³⁸ of a depletion of that amine from the brain during reserpine-induced depression and its elevation following the antidepressant monoamine oxidase inhibitors. As evidence accumulated, however, it was learned that these drugs also affected noradrenaline- and dopamine-levels in the brain and that the catecholamine precursor, dopa, promptly and effectively reversed the depressant actions of reserpine in animals, suggesting to some an equally important role for catecholamines in the action of these drugs and possibly in affective states. It has been difficult to explain, however, the action of two effective antidepressant drugs in terms of the central biogenic amines. These agents, amphetamine and imipramine, are not especially active as monoamine oxidase inhibitors and have not been shown to elevate the levels of norepinephrine or serotonin in the brain.

Recently, Kopin was able to demonstrate, in the isolated, perfused heart, a differential metabolism of tritiated noradrenaline released under different circumstances³⁹. When the catecholamine was liberated in a manner which did not provoke its characteristic effects on the heart, that is, by reserpine, it appeared largely as deaminated products in the perfusate. On the other hand, when its release was accompanied by cardiac stimulation as with stimulation of the cardiac sympathetic nerves or by tyramine, *O*-methylated products appeared in the perfusate. These observations have suggested the generalization that catechol-*O*-methyl transferase is the enzyme normally involved in the degradation of norepinephrine which is released physiologically and perhaps its *O*-methylated metabolites are indicative of adrenergic activation, at least in the periphery.

The release and metabolism of noradrenaline in the brain, however, remained quite a mystery, since the blood-brain barrier prevented the uptake by the brain of radioactive norepinephrine and the amount of label which could be applied through synthesis from tagged tyrosine was hardly enough for fractionation. In 1964, Glowinski, who had applied Feldberg's technique to injection into the lateral ventricles of rats, joined Axelrod and Kopin and succeeded in developing what appears to be a valid technique for labelling, at a high specific activity, the norepinephrine stores within the brain by injecting the tritiated form intraventricularly⁴⁰. The label distributes itself quite rapidly in a pattern similar to that of endogenous norepinephrine and shows the same intracellular localization. Furthermore, it follows a curve of disappearance from the brain similar to that of ¹⁴C-norepinephrine endogenously produced from ¹⁴C-tyrosine.

With convincing evidence that they were studying the metabolism of endogenous norepinephrine in the brain, they examined the effects of a number of psychoactive drugs. Reserpine caused a rapid depletion and the predominant formation of deaminated products as it did in the heart. On the other hand, monoamine oxidase inhibitors, amphetamine and imipramine, all of which are antidepressant or euphoriant drugs, were followed by an increase in *O*-methylated norepinephrine products in the brain⁴¹. If one may generalize from Kopin's findings in the heart and infer physiological activity from an increase in norepinephrine *O*-methylation, these findings are compatible with the thesis that the drugs which induce depression or elevation of mood do so by depressing or facilitating the release of physiologically active nor-

epinephrine in the brain or altering its availability at effector sites.

Such a hypothesis as well as the possibility that normal and abnormal changes in mood are dependent on alterations of catecholamines in the brain remain to be validated. The possibility of labelling norepinephrine in the brain has overcome a major obstacle in the way of elucidating its physiological role there.

Another recent development in this laboratory has some clear-cut implications, this time for cardiology. Despite the expectation that monoamine oxidase inhibitors should elevate the levels of the sympathetic neurotransmitter, these agents are found to have hypotensive and other sympatholytic effects which, though undesirable in psychiatry, have been found useful in the treatment of hypertensive disease and angina pectoris. An explanation of this paradoxical effect has been advanced by Kopin *et al.*⁴², who presented evidence for the normal synthesis and accumulation of octopamine, the β -hydroxylated derivative of tyramine, in the region of the sympathetic nerve endings, its enhancement by monoamine oxidase inhibitors and its release by sympathetic stimulation. Their hypothesis that this relatively inactive amine may replace norepinephrine and act as a false neurochemical transmitter appears capable of explaining the partial sympathetic blockade observed after chronic inhibition of monoamine oxidase.

Much of what I have outlined is illustrative of an important generalization from the history of science, a principle which, though taken for granted by most scientists, nevertheless requires reinforcement to-day. The most practical way to attack a major medical problem or to bridge a great hiatus is not usually head-on, but by strengthening and extending the foundations on both sides and narrowing the gap which lies between. This is best accomplished when the scientists themselves choose their logical next steps, which each will do from his knowledge of the state of the field, the feasibility of an approach, the likelihood and significance of its being successful.

Nearly a hundred years ago in England, Thudichum, whom many regard as the father of modern neurochemistry, advanced a hypothesis that many forms of insanity were the result of toxic substances produced within the brain by faulty metabolism. But, more important, he went on to suggest that these processes, then quite obscure, would be obvious when we understood the biochemistry of the brain to its utmost detail⁴³. It was in the latter area that he spent the next ten years in what was to become the classical isolation, description and characterization of the chemical constituents of the brain.

It is not difficult to predict what would have resulted had Thudichum spent those years and the funds made available to him by the Privy Council in a premature search for the toxins of insanity. With the tools, techniques and knowledge available to him at that time, it is extremely unlikely that he would have found any of those hypothetical substances; it is equally unlikely that he would have made his fundamental contributions to our present knowledge of the biochemistry of the brain.

¹ Kety, S. S., *Science*, **132**, 1861 (1960).

² Kety, S. S., and Schmidt, C. F., *J. Clin. Invest.*, **27**, 476 (1948).

³ Kety, S. S., in *Metabolism of the Nervous System*, edit. by Richter, D., 221 (Pergamon Press, London, 1957).

⁴ Quastel, J. H., *Anesthes. Analg.*, **31**, 151 (1952).

⁵ Mangold, R., Sokoloff, L., Conner, E., Kleinerman, J., Therman, P. G., and Kety, S. S., *J. Clin. Invest.*, **34**, 1092 (1955).

⁶ Everts, E. V., Bental, E., Bhari, B., and Huttlenlocher, P. R., *Science*, **135**, 726 (1962).

⁷ Kety, S. S., Woodford, R. B., Harmel, M. H., Freyhan, F. A., Appel, K. E., and Schmidt, C. F., *Amer. J. Psychiat.*, **104**, 765 (1948).

⁸ Sokoloff, L., Perlman, S., Kornetsky, C., and Kety, S. S., *Ann. N.Y. Acad. Sci.*, **66**, 468 (1957).

⁹ Sokoloff, L., Mangold, R., Wechsler, R. L., Kennedy, C., and Kety, S. S., *J. Clin. Invest.*, **34**, 1101 (1955).

¹⁰ Kety, S. S., in *Methods in Medical Research*, edit. by Bruner, H. D., **8**, 228 (Year Book Publishers, Chicago, 1960).

- ¹¹ Landau, W. M., Freygang, W. H., Rowland, L. P., Sokoloff, L., and Kety, S. S., *Trans. Amer. Neurol. Assoc.*, **80**, 125 (1955).
- ¹² Sokoloff, L., in *Regional Neurochemistry*, edit. by Kety, S. S., and Elkes, J., 107 (Pergamon Press, Oxford, 1961).
- ¹³ Sokoloff, L., Wechsler, R. L., Mangold, R., Balls, K., and Kety, S. S., *J. Clin. Invest.*, **32**, 202 (1953).
- ¹⁴ Sokoloff, L., and Kaufman, S., *Science*, **129**, 569 (1959).
- ¹⁵ Sokoloff, L., and Kaufman, S., *J. Biol. Chem.*, **236**, 795 (1961).
- ¹⁶ Sokoloff, L., Kaufman, S., Campbell, P. L., Francis, C. M., and Gelboin, H. V., *J. Biol. Chem.*, **238**, 1432 (1963).
- ¹⁷ Sokoloff, L.: The action of thyroid hormones on protein synthesis, as studied in isolated preparations and in the whole rat. *Proc. Second Intern. Congr. Endocrinol.*, London, Aug. 17, 1964 (Elsevier Publishing Co., Amsterdam; in the press).
- ¹⁸ Klee, C. B., and Sokoloff, L., *J. Neurochem.*, **11**, 709 (1964).
- ¹⁹ Hoffer, A., Osmond, H., and Smythies, J., *J. Ment. Sci.*, **100**, 29 (1954).
- ²⁰ Armstrong, M. D., McMillan, A., and Shaw, K. N. F., *Biochim. Biophys. Acta*, **26**, 422 (1957).
- ²¹ Axelrod, J., *Science*, **126**, 400 (1957).
- ²² Axelrod, J., and Tomchick, R., *J. Biol. Chem.*, **233**, 702 (1958).
- ²³ Axelrod, J., *Physiol. Rev.*, **39**, 751 (1959).
- ²⁴ LaBrosse, E. H., Axelrod, J., and Kety, S. S., *Science*, **128**, 593 (1958).
- ²⁵ LaBrosse, E. H., Mann, J. D., and Kety, S. S., *J. Psychiat. Res.*, **1**, 68 (1961).
- ²⁶ Kopin, I. J., *Pharmacol. Rev.*, **16**, 179 (1964).
- ²⁷ Osmond, H., and Smythies, J., *J. Ment. Sci.*, **98**, 309 (1952).
- ²⁸ Pollin, W., Cardon, P. V., and Kety, S. S., *Science*, **133**, 104 (1961).
- ²⁹ Cantoni, G. L., *J. Biol. Chem.*, **204**, 403 (1953).
- ³⁰ Brune, G. G., and Himwich, H. E., in *Recent Advances in Biological Psychiatry*, **5**, 144 (Pleum Press, New York, 1963).
- ³¹ Alexander, F., Curtis, G. C., Sprince, H., and Crosley, A. P., *J. Nerv. Ment. Dis.*, **137**, 135 (1963).
- ³² Park, I. C., Baldessarini, R. J., and Kety, S. S., *Arch. Gen. Psychiat.*, **12**, 346 (1965).
- ³³ Baldessarini, R. J., and Kopin, I. J., *Anal. Biochem.*, **6**, 289 (1963).
- ³⁴ Axelrod, J., *Science*, **134**, 343 (1961).
- ³⁵ Szara, S., *Fed. Proc.*, **20**, 355 (1961).
- ³⁶ Friedhoff, A. J., and Van Winkle, E., *J. Nerv. Ment. Dis.*, **135**, 550 (1962).
- ³⁷ Bourdillon, R. E., and Rieges, A. P., in *Amine Metabolism in Schizophrenia*, edit. by Himwich, H. E., Smythies, J. R., and Kety, S. S. (to be published).
- ³⁸ Shore, P. A., Pleischer, A., Tomich, E. G., Carlsson, A., Kuntzman, R., and Brodie, B. B., *Ann. N.Y. Acad. Sci.*, **66**, 609 (1957).
- ³⁹ Kopin, I. J., and Gordon, E., *J. Pharmacol.*, **140**, 207 (1963).
- ⁴⁰ Glowinski, J., Kopin, I. J., and Axelrod, J., *J. Neurochem.*, **12**, 25 (1965).
- ⁴¹ Glowinski, J., and Axelrod, J., *J. Pharmacol.* (in the press).
- ⁴² Kopin, I. J., Fischer, J. E., Musacchio, J. M., Horst, W. D., and Weise, V. K., *J. Pharmacol.*, **1-7**, 186 (1965).
- ⁴³ Thudichum, J. L. W., *A Treatise on the Chemical Constitution of the Brain*, 13 (Baillière, Tindall and Cox, London, 1884).

CHEMICAL EDUCATION

THE Royal Institute of Chemistry is launching an appeal for £100,000 with which to develop and extend its work in the educational field over the next five years. Already the Institute is spending £10,000 a year out of its limited resources on this work, but it is considered that a greatly increased effort is now urgently necessary if the Institute is to meet its national obligations to ensure an adequate supply of properly trained entrants to the profession.

With the present trend away from science in the schools and the consequent large number of vacant places in university science departments, the main emphasis for utilizing increased funds is placed on improving the teaching of chemistry at school-level. This, it is believed, is where the root of the problem lies, for only by presenting chemistry in a sufficiently attractive way to encourage boys and girls to adopt it as a career can the problems of the universities and the British chemical industry be solved. In fact, the marked decline in interest in chemistry on the part of school-leavers may be associated with lack of stimulation during their formative years at school, often due to out-dated methods of presenting the subject.

The trend away from chemistry in the school is disturbing in itself, but it is made more serious by the continuing shortage of qualified chemists in industry and elsewhere. It has been estimated by the secretary of one of the major university appointments boards that by 1970 there will be a shortage of some 10,000 qualified chemists in industry alone. This estimate is based on the best available figures, including those from the 1962 *Report of the Scientific Manpower Committee* and the 1961 *Census*. A particularly disturbing feature is that, on average, only 41 per cent of university graduates in chemistry enter industry—either directly or after staying at university for a higher degree. Indeed, in 1963 only 32 per cent of such graduates entered industry.

Present educational work of the Institute. The interest that the Institute now takes in educational matters stems from its functions as a qualifying and examining body, and in 1957 a Fund for the Development of Education in Chemistry was established to finance educational activities. With the exception of a repayable grant of £10,000 generously provided by the Nuffield Foundation for the launching of *Education in Chemistry* the Fund has been financed solely by the Institute, essentially out of its subscription income.

In addition to the new journal, *Education in Chemistry*, which was launched in 1964 and is now issued six times a year, the Institute, through its Trust Fund, publishes a series of *Monographs for Teachers* on aspects of chemical principles and arranges a number of symposia, or refresher

courses for teachers, as well as summer schools in chemistry. More recently it has organized, jointly with the Chemical Society, the first two of a series of review symposia for practising chemists.

Proposed future programme. The main emphasis of the Institute's five-year programme of work is on the teaching of chemistry in schools, because it is at this level that the need is greatest and also because experience over the past four or five years has demonstrated the ability of the Institute to help substantially in this sector. Moreover, the impetus given by the Nuffield Foundation to curriculum reform in science education provides a valuable opportunity for professional institutions to play their part in supporting complementary projects for the mutual benefit of the profession and the schools.

Among the projects to be sponsored for school teachers are: (a) Refresher courses; a flexible series of one-day symposia and one-week courses aimed at all teachers of chemistry (not just the keener ones) and also provision for married women wishing to return to the profession. (b) Bursaries; to enable a selected number of teachers to be seconded to a university or college of education, for a period of up to one year, in order to study modern aspects of chemical education and to take part in research projects in the field of science education. (c) Teaching aids; a series of teaching charts, filmstrips and other visual aids. (d) Surveys; to follow the progress of the projects outlined earlier and to use the information to modify the programme accordingly.

In addition to the problems of the schools there are very real difficulties to be tackled in the re-education of practising chemists and in the teaching of chemistry at university-level. The Institute intends to devote some of its funds to these problems.

Indeed, the importance of providing extensive courses, of short and long duration, for bringing practising chemists up to date in their subject and its applications cannot be over-emphasized. The expansion of scientific knowledge may mean that within fifteen years of graduation a scientist is ignorant of half the total knowledge in his field. This has given rise to a great deal of waste of older men and women and the 'too-old-at-forty' prejudice is re-establishing itself in British industry, at least for new appointments. The younger scientists are less out of date than their senior colleagues and the solution of retiring men early and replacing them by scientists under thirty is one that an increasing number of companies are using. That such a solution is unjust, wasteful and unsatisfactory goes without saying, and it may well defeat its own object by discouraging sixth-form pupils from choosing science as a career.

To combat this situation, a systematic and massive programme of refresher courses is required. Such a programme could be provided in the long vacations at selected universities and technical colleges and, because of its close liaison with these academic institutions and with industry, the Institute would be well placed to administer it for chemists.

Finally, the Institute proposes to devote a proportion of its funds to assisting the teaching of chemistry in the developing countries, especially the newly independent countries of the British Commonwealth. Help that might be given includes the provision of symposia and refresher courses for teachers, the secondment of teachers from the

United Kingdom, and the provision of all kinds of visual aids and of programmed learning techniques.

By acting as a pioneer and 'pump-primer' in the ways suggested in this article, the Institute feels that it can best exercise its function as an independent professional body in promoting the development of chemical education and ensuring an adequate supply of properly trained chemists to meet national needs in the United Kingdom and in the developing countries of the Commonwealth.

Further details about the proposals are contained in an appeal booklet available from the Royal Institute of Chemistry, 30 Russell Square, London, W.C.1.

OBITUARIES

Sir Ronald Hatton, C.B.E., F.R.S.

SIR RONALD HATTON died on November 11, 1965, in his eightieth year at his home in Benenden, Kent.

On leaving Balliol College, Oxford, he went to the South Eastern Agricultural College, Wye, Kent, and soon afterwards became a member of the horticultural staff there. Later he was transferred to the newly established Wye College Experimental Station at East Malling, where he assisted Wellington, the first director. After the First World War, Hatton was appointed director and remained so until his retirement in 1949. Under his guidance, despite numerous financial difficulties, the Station rapidly developed and attained a world-wide reputation. During the formative period he was whole-heartedly supported by the fruit industry of the United Kingdom and a devoted staff. When he retired he had the satisfaction of knowing that horticultural science was fully recognized as an essential service to the fruit-growing industry.

Hatton's own research in the 'twenties on rootstocks was a major turning-point in fruit culture. There were then wide variations within the same orchard in tree size and fruit quality: Hatton aimed at obtaining uniformity in both, and his research resulted in a series of rootstocks which imparted to the scion standardized growth characteristics. This was a major, if not the major, development for fruit culture throughout the world. Order was created out of chaos, for his work made possible a planned pattern for orchards and prepared the way for future trends in orchard practice, including, in particular, extensive mechanization. As a result, orchards of the future will be planted for high yields at the lowest possible costs.

Although pomological research remained his great interest, Hatton was fully alive to the importance of developing at the Research Station ancillary subjects such as plant physiology, applied entomology, plant pathology and statistics.

His delightful and friendly personality inspired confidence and was a constant stimulus to everyone, both in the Station and outside. His staff were known as "Hatton's boys" and there was among them a wonderful productive team spirit. His appetite for work was enormous and he was never satisfied that enough progress had been made. Perhaps his only fault—a forgivable one—was that he failed to realize anyone grew older, and although he knew that progress must require additional staff, he dreaded the Station expanding beyond a scientific "family circle".

Among his many achievements was the major part he took in the establishment of the Imperial Bureau of Fruit Production at East Malling, of which he was consultant director. His interests went far beyond his own Station: he was generous-minded to all and he had innumerable fruit-grower friends, who took his advice because it was so forward-thinking and practical. Undoubtedly there was in him a most happy blending of science and horticultural technology, which plays such a big part in the

well-being of a crop research station. The fruit-growing industry will for ever owe a great debt of gratitude to Sir Ronald Hatton.

H. G. H. KEARNS

Prof. E. J. Dijksterhuis

IN 1924, Eduard Jan Dijksterhuis, an obscure mathematics teacher in the Tilburg secondary school, published a book, *Val en Worp*, sub-titled "Contribution to the History of Mechanics from Archimedes to Newton", which was actually a summary of all that history could tell on this subject at that time, written by somebody who was obviously a master in this art.

Although the history of mathematics had flourished in The Netherlands for many years around the monumental work of editing Christian Huygens's works and the discovery and publishing of Isaac Beekman's journal, Dijksterhuis as a historian was an autodidact. He had attended the secondary school in Tilburg, where his father was the principal. Then, in order to be admitted to a university, he prepared for the final examination of a grammar school; this he passed after one year, at the age of seventeen. Still considered by his parents to be too young to study at a university, he spent one more year at home, during which he immersed himself in the classics, with which he had fallen in love during the preparations for his examination. Nevertheless, at the University of Groningen he chose his older favourite, mathematics, as his major subject. In 1918 he gained a doctor's degree for a thesis on a geometrical subject. Meanwhile, he had already taught at a Groningen girls' school, but in 1919 he agreed to fill a vacancy at the Tilburg school where he had been a student. This commitment did not end until 1953, when he was appointed a professor extraordinary in the history of mathematics and exact sciences at the University of Utrecht; from 1954 he also held an equivalent post at the University of Leiden. From 1960 until his retirement in 1963 he was a professor ordinary at the University of Utrecht.

From his marriage in 1920 with Johanna K. E. Niemeyer until his departure to Utrecht in 1953 he lived in the lovely Brabant town of Oisterwijk. In this rustic climate he matured as a scholar, and he wrote the greater part of his work. But he did not lead a retired life. For many years he was a 'privaat-docent' at the Universities of Amsterdam and Leiden—an activity which was interrupted by the Second World War. He played a great part in commissions on the teaching of mathematics and mechanics, and from 1934 until 1958 was a member of the Government Education Council (Onderwijsraad). For nearly a quarter of a century he was an honorary secretary to the most outstanding Netherlands literary periodical, *De Gids*. But, first of all, he was admired as a lecturer because of his firm grasp of the subject and the easy command of the language in which he expressed his thoughts.

His *Val en Worp* was followed in 1929–30 by his commentary (in Dutch) on Euclid's *Elements*. His *Archimedes* was written before the War; its English translation appeared in 1956. In 1943 his *Simon Stevin* appeared, and in 1950 the best known of his works, *De Mechanisering van het Wereldbeeld*, was published; the latter has been translated into German and English. Besides these books, he published a large number of papers, lectures and addresses on historical, educational and philosophical subjects. His longest-lasting activity was that of chairman of the Royal Netherlands Academy Commission on the Edition of Works of Simon Stevin, which still met at his home when he had lost his speech and had to rely on gestures to conduct its meetings.

Educated people, and above all scholars of the humanities, were struck by his style. He abstained completely from metaphors, puns, oratorical and dramatic effects, and from any choice of words that would excite emotions rather than thoughts. He was a lover of art and poetry, but in his own work he never gave way to the spell of rhetoric. He put his thoughts into a form which matched their contents, and above all matched his character. He wished to grip the reader by the subject rather than by the form—though just for this reason it was the form which fascinated.

For his *De Mechanisering van het Wereldbeeld* he was awarded a high literary honour; he was a member of the Royal Netherlands Academy of Sciences, a bearer of the Karl Sudhoff Medal of the Deutsche Gesellschaft für Geschichte der Medizin, Naturwissenschaften und Technik, the George Sarton Medal of the American History of Science Society and many other honorary awards.

H. FREUDENTHAL

Prof. R. H. Hopkins

PROF. R. H. HOPKINS, who was formerly Adrian Brown professor of brewing and applied biochemistry in the University of Birmingham, died recently after a short illness at his home in Bournemouth, aged seventy-four.

His association with Birmingham was a long one, as he graduated in 1910, after which he gained experience in various analytical laboratories. He returned to the University towards the end of the First World War and became assistant to the first professor of brewing, Prof. Adrian Brown.

He became a Fellow of the Royal Institute of Chemistry in 1918, and served on the Council for two periods.

In 1920 he took up an appointment in the Department of Chemistry of the Heriot-Watt College, Edinburgh, and

was in charge of courses in malting and brewing and biochemistry. In 1926 he was awarded an M.Sc., and in 1928 a D.Sc. of Birmingham. When the Adrian Brown chair of malting and brewing fell vacant in Birmingham, his record and qualifications rendered him a very appropriate candidate, and he was appointed in 1931.

Hopkins always, and rightly, insisted that the biochemical principles on which the art of malting and brewing rested were of fundamental importance, and he strongly resisted attempts to isolate the School of Brewing. He was able to maintain the dual nature of the Department as a School of Brewing and Department of Industrial Fermentation, later of Applied Biochemistry. There is little doubt that without this steady pressure over the years there would have been no Department of Biochemistry as it exists to-day.

He was primarily a man of academic interests, but he combined this to a very unusual degree with a knowledge and appreciation of the technical processes and problems involved in malting and brewing. This is clearly demonstrated in the large number of papers which he contributed to the *Journal of the Institute of Brewing*, the *Biochemical Journal* and other journals of a more technical nature. There is scarcely any fundamental aspect of malting and brewing which he failed to deal with in his publications.

A topic which held his sustained interest over a number of years was the nature of starch and the complex amyolytic actions involved in its breakdown. He did much to bring order and clarity into a field of research where confusion reigned, and his claim to recognition will doubtless be confirmed in future perspective.

He directed a team of research workers in the Department under the aegis of the Institute of Brewing from 1934 until 1950, when it became absorbed in the Brewing Industry Research Foundation in Surrey. During the Second World War he sponsored work by the teaching staff on the qualitative and quantitative aspects of vitamins in beer. Most of the water-soluble vitamins came under scrutiny, and the large and useful amounts of riboflavin which occurred naturally in the beverage were reported for the first time.

He was a frequent participator in symposia and congresses throughout the Western world, and made several lecture tours of the United States.

His other interests included a deep appreciation of many branches of music, and he was particularly fond of holidays in the mountains and by the lakes of the Continent. He will be missed by a host of colleagues, students and friends.

F. W. NORRIS

NEWS and VIEWS

European-American Committee on Reactor Physics

DR. V. RAIEVSKI, head of the Reactor Physics Department at the Ispra Joint Nuclear Research Centre of Euratom, has been elected chairman of the European-American Committee on Reactor Physics for the next 2 years. Dr. Raievski succeeds Mr. Peter Mummery of the Atomic Energy Establishment, Winfrith, U.K. At the same time, Dr. H. Kouts, of Brookhaven National Laboratory, has been elected scientific secretary of the Committee in succession to Mr. E. Critoph of Atomic Energy of Canada, Ltd., Chalk River. The European-American Committee on Reactor Physics is a group of fifteen reactor physics experts from member countries of the Organization for Economic Co-operation and Development, and Euratom (nine from Western Europe, three from the United States, one each from Canada, Japan and Euratom). It was set up in 1962 by the Steering Committee of the European Nuclear Energy Agency,

which recently confirmed its mandate for a further 4-year period. The work of the Committee consists of reviews of national programmes in reactor physics, periodical examination of research on selected topics, and exchange of information on new research results. The Committee also advises on priorities for new research activities.

Analytical Chemistry in the National Bureau of Standards:

Dr. David H. Freeman

DR. DAVID H. FREEMAN recently joined the Analytical Chemistry Division of the Institute for Materials Research at the National Bureau of Standards. His work will be concerned with the prediction, control and reproducibility of analytical separations, and he will deal especially with the relationships between chemical specificity and the structure of ion-exchange derivatives of synthetic organic copolymers. Dr. Freeman was formerly an assistant professor at Washington State University, where his

research activities were supported by a contract from the Atomic Energy Commission. From 1958 until 1960 he worked as a research associate at the Massachusetts Institute of Technology. Born in Rochester, New York, Dr. Freeman completed his B.S. in chemistry at the University of Rochester in 1952; he then went to the Carnegie Institute of Technology for his M.S. in 1954, and gained his Ph.D. at the Massachusetts Institute of Technology in 1957. His professional affiliations include membership in Phi Lambda Upsilon (president of the Massachusetts Institute of Technology Chapter in 1958-1959), American Chemical Society, American Institute of Physics, American Optical Society, American Association for the Advancement of Science, and Sigma Xi.

Preserving the Lake District

SOME of the points raised in the *Report and Newsletter*, September 1965, of the Friends of the Lake District are noted elsewhere in discussing the "Countryside in 1970" Conference (see pp. 1241, 1265 of this issue of *Nature*). It is obvious from the report, however, that the Friends of the Lake District are concerned that considerations of amenity are being pushed into a minor place in proposed amendments to the National Parks Act. They feel that without the expression of a widely based and well-informed body of public opinion the objects for which National Parks were created are unlikely to be achieved. Much of the criticism in this report centres on the water plans of Manchester, particularly the powers sought to construct a duplicate in Longsleddale of the Mardale tunnel, although it is recognized that the Order has been most carefully framed and that the initial quantities of water to be abstracted are so calculated that it is extremely difficult to estimate the effect on the two lakes. It is the ultimate effects and the immediate physical effects on Longsleddale that are the cause of misgivings, and the report does well to emphasize the viable alternative of extraction from rivers rather than lakes, and of the possibilities of desalination: consideration of our water resources in terms of nineteenth-century thought is no longer valid. One welcome feature of the report is an indication of some improvement in the litter position, although constant effort and vigilance are still necessary.

Information Activities of the U.S. National Academy of Sciences

THE scientific information activities of the National Academy of Sciences—National Research Council of the United States are surveyed in a booklet recently issued by the Office of Documentation (National Academy of Sciences—National Research Council. Publication 1291: *Scientific Information Activities of the National Academy of Sciences—National Research Council*. A Report of the Office of Documentation. Revised 1965. Pp. 116. Washington, D.C.: National Academy of Sciences—National Research Council, 1965). This is a revision of a booklet issued under the same title in 1962. It covers such activities as the preparation of abstracting periodicals and literature digests, bibliographies, codes and classifications, tables of data, glossaries, handbooks, indexes, lists of publications, reviews, translations, the maintenance of information centres and the provision of information services, as well as studies and analyses in these fields. Original research publications are not included unless concerned with these fields. The information is arranged alphabetically by the several divisions of: behavioural sciences; biology and agriculture; chemistry and chemical technology; earth sciences; engineering and industrial research; mathematics; and physical sciences. Within these are given, alphabetically by title, details of the committees, boards, offices or similar units.

Scientists in the 'New Deal'

THE summer issue of *Minerva* contains an interesting article by L. E. Auerbach, "Scientists in the New Deal",

reviewing the relations between science and Government in the United States at the time of the formation of the Science Advisory Board. The article is essentially an account of the establishment and work of the Science Advisory Board under the National Research Council during its brief existence from July 1933 to December 1935. The Board sought to broaden the perspectives of the Government and to increase its awareness of foreign scientific achievements, but Mr. Auerbach considers that many of its recommendations were made largely in a political vacuum and that its attempt to formulate a comprehensive national science policy was essentially a failure, although the Board itself was rather a failure in adaptation to a new situation. This is attributed largely to conflict between the National Academy of Sciences and the Science Advisory Board. Dr. K. T. Compton held that if the National Academy of Sciences had been more involved with the social sciences, many of the difficulties would have been avoided. Mr. Auerbach also suggests that the Board would have been more influential if the scientific community as a whole had been able to agree on a united stand on the need for a national science policy. To-day its most valuable contribution to national welfare and towards raising the support and status of basic research appears to be the political experience it gave to men like K. T. Compton, I. Bowman and Vannevar Bush.

Science and Economic Growth

AN article by Dr. C. E. Chastain, "Science, Technology and Economic Growth", in *Impact of Science on Society* (14, No. 4; 1964) reviews some recent studies of the relation of science and technology to economic growth and emphasizes the need for further understanding of those relations, which require studying at three levels. We need to know more about the diffusion of technological knowledge for the development of new products and processes at the level of the individual firms in an industry. We need, next, a detailed study of some major industries, emphasizing the capacity of firms to adopt new technologies and analysing what motivates or hinders their advance. Thirdly, studies of the effect of technological change on the overall economy would also illustrate how technological change promotes new industries and new sectors in the economy. The Industrial Development Research Programme of the Institute of Science and Technology at the University of Michigan has recently completed an investigation, at the firm level, of engineering and technical developments and their business implications in the Michigan machine-tool industry, and a similar investigation of the electronics industry will shortly be completed.

The Royal Society of Edinburgh

THE *Year Book of the Royal Society of Edinburgh, 1965*, covering the session 1963-64 (Pp. 133. Edinburgh: Royal Society of Edinburgh, 1965. 20s.; 3.50 dollars), contains the usual calendar, obituary notices, proceedings of the ordinary meetings and of the statutory general meeting, together with laws of the Society, accounts for the year ended July 21, 1964, and lists of Fellows of the Society, members of Council and recent awards. There are also lists of additions to the Library and of papers recently published in the *Proceedings* and *Transactions* of the Society. Among the obituary notices are those of W. M. Ames, Prof. R. N. Arnold, L. E. Bayliss, Prof. E. Finlay-Freundlich, A. H. R. Goldie, Prof. I. A. Preece and Sir James Wallace Peck.

The Manchester Literary and Philosophical Society

THE *Memoirs and Proceedings of the Manchester Literary and Philosophical Society* (107; 1964-65. Pp. 142+lii. Manchester: Manchester Literary and Philosophical Society, 1965. 31s. 6d.) includes, besides the account report of the Council, accounts, lists of presidents of the Society,

Council members, awards of the Dalton Medal and special lectures, Sir William Slater's Joule Memorial Lecture, "The State as the Patron of Science". Other papers in the *Memoirs* include those by K. F. Bowden on progress in oceanography; N. L. Hanson on "The Architect's Dilemma"; G. Polanyi on "North Sea Gas—What will it Mean?"; F. Oldfield and D. C. Statham on "Stratigraphy and Pollen Analysis on Cockerham and Pilling Mosses, North Lancashire"; R. H. Kantorowich on "Choice and Chance—Prospects for the North-West"; Brian Rodgers on "Making Social Policy"; and an autobiographical sketch of J. B. Dancer, with a foreword by W. Browning.

National Art Gallery and Dominion Museum, New Zealand

THE year 1965 marks the centennial year of the Dominion Museum and is being celebrated by evening functions, special exhibitions and the publication of a history of the Museum. In addition to various curatorial activities, the extra-mural influence of the institution is particularly impressive and includes circulation of works of art and museum specimens, public lectures, scientific excursions and advice on conservation. Exhibition effort has been concentrated mainly in the Maori Hall, new cases of foreign aviary birds, early silver, and the completion of a reconstruction of an Egyptian tomb (a life-sized X-ray negative of the mummy was added to this exhibit). The report for the year ended March 31, 1965, also records the opening of the Hall of Memories in 1964 and comments on the beauty and artistry of the interior and the manner in which the symbolic message is conveyed (New Zealand. Report of the Board of Trustees of the National Art Gallery and Dominion Museum for the year ended 31 March, 1965. Pp. 28. (H.21.) Wellington: Government Printer, 1965. 1s. 6d.).

French Oceanography

THE September 1965 issue of *Le Progrès Scientifique* (No. 88) is devoted to a survey of the situation and perspectives of French oceanography, with, for comparison, an estimate of expenditure on oceanography in Great Britain and in the United States programme for 1966. Some 596 scientists and engineers are estimated to have been engaged on oceanographic research in France in 1964, equivalent to 450 full-time workers; the corresponding expenditure is estimated at 90–100 million francs. There is also a brief account of three recent French contributions to the advance of oceanographic technique in the Bouée laboratory, the submarine seismograph, and the Télénaut.

Chemical Analyses of Rocks and Minerals

THE latest of a recent succession of regional compilations of chemical analyses of rocks and minerals, prepared to facilitate the more ready use of data in the files of Government departments, emanates from the Geological Survey of Canada and is the first part of a project designed to bring together all available Canadian geochemical information (Canada: Department of Mines and Technical Surveys. *Geological Survey of Canada. Bulletin 115: Chemical Analyses of Canadian Rocks, Minerals, and Ores*. By J. A. Maxwell and others. Pp. 476. Ottawa: Queen's Printer, 1965. 6.50 dollars). It comprises analyses, with petrographical and topographical annotations, made by the Survey from the time of its foundation in 1846 up to the end of 1955. This follows closely on a similar monograph based on sixty years of records of the Geological Survey of Malaya (*Geological Survey of Malaya Professional Paper E-64: Chemical Analyses of Malayan Rocks, Commercial Ores and Mineral Concentrates*. Compiled by J. B. Alexander and others. Pp. 295. Ipoh: Malaysian Ministry of Lands and Mines, 1964. 10.00 Malayan dollars). In the U.S.S.R., an important regionally based collection of

some 2,000 full (but not annotated) chemical analyses of rocks and rock-forming minerals, mostly executed in recent years, has appeared under the aegis of the Institute for Ore-Deposit Geology, Petrography, Mineralogy and Geochemistry (*Khimicheskie analizy izverzhennykh gornykh porod i porodoobrazuyushchikh mineralov*. Compiled by V. F. Morkovkina. Pp. 250. Nauka, Moscow, 1964. 3r. 66k). It carries useful calculations of the average composition of the more important rock-types in the several regions of the U.S.S.R. A fourth recent work of this kind is a compilation by Miss Germaine A. Joplin of the analyses in Australian literature (*Chemical Analyses of Australian Rocks*. Part 1: *Igneous and Metamorphic. Bureau of Mineral Resources, Geology and Geophysics Bulletin, 65*. Pp. 4+6. 1963). Part 2 of the Australian monograph, on sedimentary rocks, is in preparation. These several works bring together data from a wide range of sources and they will save petrologists much tedious searching of the literature.

Chiswick–Langley Section of the M.4 Motorway

THE Chiswick–Langley section of the M.4 motorway opened by Mr. T. Fraser, the Minister of Transport, on March 24, 1965, is 12.5 miles long, and contains the longest stretch of elevated road in Europe; incidentally, it also incorporates a much-needed link road to London Airport. Some interesting details of the design and construction of this highway, with its viaducts, overbridges, underbridges and footbridges, some of which are illustrated, are contained in an article, "Chiswick–Langley Section of the M.4" (*Concrete Quarterly*, No. 65; April–June, 1965. Cement and Concrete Association, London). For about the first 2 miles from the Chiswick end, this motorway is a viaduct with two twin-lane carriageways. The first mile or so is constructed with pre-cast, pre-stressed concrete deck beams carried on T-shaped piers; this follows the line of the Great West Road. The remainder of the viaduct swings away from the Great West Road and is of steel and concrete construction. There are thirty-one bridges between Chiswick and Langley, including the link road to London Airport; these owe their lightness of form and speed of construction to the use of pre-cast concrete units and pre-stressing techniques. Twelve bridges carry roads over this new section of the M.4; sixteen carry the motorway itself over roads, railways, canals and rivers; three footbridges are available for pedestrians, and sixteen pedestrian subways have been constructed at various points along it. At the end of the elevated section, where it descends to ground-level, the carriageways widen to accommodate three 12-ft. lanes; outside these carriageways are constructed hard shoulders, 10 ft. wide, bounded by grass verges of similar width. The length of approximately 7 miles between Boston Manor and London Airport is in concrete; between the Airport and Langley interchanges there are approximately 4 miles of flexible construction on various bases; this section was purposely laid for practical specification trials by the Road Research Laboratory. It is stated that the concrete road slab is 38 ft. wide, 11 in. thick, with welded steel mesh reinforcement 3 in. below the top surface. The consolidated granular sub-base is between 7 in. and 12 in. thick. Expansion joints are provided every 240 ft. and contraction joints at 80-ft. centres. The illustrations in this article, including one of the elevated section of the motorway with slip roads at the Brentford interchange, and one of an elegant pre-stressed footbridge with spiral ramp at the Langley interchange, are particularly good.

Shrinking Aggregates a Cause of Failure in Concrete

THERE exists an extensive literature on research into failure of concrete ascribed to instability of aggregates involved, both physical and mineralogical. Aggregates known to exhibit excessive dimensional change on drying out and ageing of concrete would normally be inad-

missible, but there are cases on record where such materials have been used, apparently with disastrous results. Thus there is every reason for investigating those rocks which are potentially vulnerable to dimensional instability when emplaced as aggregates in concrete, and especially the role of the mechanism set up by shrinkage in causing distress and ultimate breakdown. This problem has recently been successfully tackled by H. Roper, J. E. Cox and B. Erlin, in a joint paper entitled "Petrographic Studies on Concrete Containing Shrinking Aggregate" (*Journal of the P.C.A. Research and Development Laboratories*, 6, No. 3; September 1964. Pp. 2-18. Portland Cement Association Research and Development Laboratories, Skokie, Illinois). As the title implies, the approach to the problem of shrinkage of aggregates is essentially petrographic; it has been shown that this technique is useful in detecting and diagnosing distress in concrete, also in supplying new information leading to a more complete understanding of the phenomena involved.

Two extreme cases of aggregates known to possess high-shrinkage properties resulting from water loss were chosen for this investigation, both from Karroo System of South Africa. These materials were used because their physical properties have been thoroughly studied; they were used in a distressed building, which initiated investigations on excessive dimensional instability, and it was considered important to use materials which had given rise to the largest shrinkages recorded. The material used as coarse aggregate was the Adendorp Sandstone, from a quarry in Cape Province, Union of South Africa, with a water absorption of 5.78 per cent; the fine aggregate was Kruidfontein Sand, with a water absorption of 4.17 per cent. Petrological studies of concretes made from these aggregates show that internal cracking of the mass has resulted from shrinkage of the aggregate; cracking occurs around aggregate particles and is sometimes modified by the relative positions of adjacent particles. The various stages of cracking and disruption of concrete due to this mechanism are convincingly illustrated in this paper in a series of ground sections of specimens examined, accompanied by interpretative diagrams showing the precise relationships between peripheral, to a lesser extent radial, crack developments and the aggregate particles causing the trouble. An important general observation is made in this paper; it appears from the examination of many concrete aggregates that rocks prone to excessive shrinkage when used for this purpose often contain clay minerals, which may occur as matrix materials, as alteration products of minerals due to weathering, or to the formation of secondary unstable minerals under hydrothermal conditions (for example, chlorophaeite in the case of some basic igneous rocks).

Reactivation of the Hijaz Railway, Saudi Arabia

ONE of the world's most famous railways, the Hijaz Railway, linking Esh Sham (Damascus) in Syria, Amman in Jordan, and Medina in Saudi Arabia, and its contemporary reactivation, is the subject of an interesting and well-illustrated article by Daniel da Cruz, entitled appropriately "Pilgrim's Road", and published in a special issue of *Aramco World* (16, No. 5; September-October, 1965. Arabian American Oil Company, New York, 10022). This was originally an Ottoman Empire project designed to link Damascus with Medina and Mecca, and destined to follow the centuries-old pilgrims' route through the then forbidding country of Hijaz, bordering the Red Sea. Construction of the Hijaz Railway commenced in May 1900; by September 1901 the first section, Damascus-Der'a, was completed; a year later the Der'a-Zarqua section was opened, to be followed by the extension to Amman in 1903, thence to Ma'an in 1904, Tabuk in 1906, Mada'in Salih in 1907, reaching Medina in 1908. For various political reasons it was not prolonged to Mecca. Thus, in the course of eight years the Turks

had put down 808 miles of rail, a remarkable engineering feat considering the nature of the country, the climate, and the attitude of often hostile inhabitants. But the aspirations of the promoters of this worthy project were doomed, largely because of the advent of the First World War when Turkey sided with the Central Powers and her occupation of the Middle East threatened the Suez Canal, along which passed the vital lifeline between Britain and her maritime bases in the Far East. Following the Allied capture of Aqaba, many Turkish locomotives and many miles of track were destroyed; travelling on this railway became an uncertain adventure; civilian traffic languished, and with the termination of hostilities, ceased altogether. After 1918 the Allies set about repairing as much of the line as possible; so much so that despite many vicissitudes, a fairly regular service on the Damascus-Ma'an section has been maintained up to the present day. But rehabilitation of this famous railroad was inevitable, especially on the long-abandoned Ma'an-Medina section. Following the independence of Syria and Jordan, an Executive Committee for Recommissioning the Hijaz Railroad Line was established, representing Syria, Jordan and Saudi Arabia; a re-survey was undertaken in 1956, culminating with a contract being placed for the necessary reconstruction in 1963. Work began in March 1964, and if the projected schedule is met, there is every reason to anticipate this reactivation towards the end of 1966. Apart from the details of this Hijaz Railway scheme, this number of *Aramco World*, entirely devoted to Saudi Arabia, contains other articles, and a remarkably fine series of coloured and black-and-white photographic illustrations which bring to life glimpses of "Arabia the Beautiful", as it is herein described, but equally leave little to the imagination of what the founding and now the revitalizing of the Hijaz Railway have really meant in terms of human effort and achievement.

National Institute of Agricultural Engineering: Component Failure Investigation

A 'COMPONENT failure investigation' service has been started at the National Institute of Agriculture to enable a manufacturer who is investigating the cause of failure of a component in an agricultural machine to draw on experience accumulated in the course of machinery testing and research and on the facilities available at the Institute. The work done by the Institute, which is on a confidential basis, can include laboratory examinations, calculations and possible rig testing, but any extensive field tests or collection of information about machines in service will normally be carried out by the manufacturer. A consultation fee of from £5 to £20 will be charged for the initial examination, which will be based on written evidence and samples supplied by the manufacturer. If further work is needed, a programme and cost estimate will be agreed between the manufacturer and the Institute, and the closest possible liaison will be maintained throughout the work. The new service, which has been welcomed by the Agricultural Engineers' Association, will be operated by the Materials Engineering Section of the Engineering Division, National Institute of Agricultural Engineering, Silsoe, Bedfordshire, from which further details can be obtained.

Pollen Studies in New York State

AN ambitious and well-planned attempt to reconstruct the vegetational and climatic events from late glacial into post-glacial and recent times is recorded in Bulletin 397 of the New York State Museum and Science Service (*Pollen Studies in the Cruso Lake Area of Prehistoric Indian Occupation*. Donald D. Cox and Donald M. Lewis. Pp. iii + 29. Albany, New York: The University of the State of New York—The State Education Departments, 1965). Prehistoric Indian sites are widely distributed in the immediate area, and the authors have also tried to incorporate this occupational evidence into the climatic

sequence, although their success in this aspect is rather limited, because no indications of cultivated plants were found in any of the pollen profiles taken from three different areas. Of some interest is the tentative recognition of three episodes within late glacial times, representing the pre-Valders ice retreat, Two Creeks interstadial and Valders ice advance, although until now it was believed that the Valders re-advance in fact covered the area. The detailed geological evidence for this is not discussed, but clearly some further consideration is required to resolve the problem. The subsequent post-glacial vegetational history is well documented, and radiocarbon dates from the area and elsewhere combine to present a closely detailed picture of the environmental conditions which influenced the prehistoric Indian groups attested here from about 3000 B.C.

Traditional Celtic Customs

THE latest issue of *Folk Life* (3; 1965), the journal of the Society for Folk Life Studies, contains a number of papers recording traditional customs and technological processes of certain areas within the British Isles. It is noteworthy that almost all the areas selected for study are in the 'Highland Zone', the Celtic fringe of north-western Europe. A well-illustrated article on "Pennine Houses" records a number of constructional features ranging from cruck-framed houses and fourteenth-century aisled halls to stone buildings of the eighteenth century. There follow papers dealing with Cornish migrants to America, customs and traditions in Glamorgan and Ulster, and elver fishing on the Severn. Of particular interest is the publication of a plan, with descriptive text, of the multiple-tenancy farm at Auchindrain in mid-Argyll. The fine preservation of this complex is due to a continuity of occupation down to relatively recent years, and the linear settlement, of mainly cruck-framed buildings, is expected to be maintained in its present or restored state. Of more widespread interest may be the paper on holed stone amulets and their traditional use as protective or recuperative agencies against such misfortunes as nightmare, bewitching and disease of horses and cattle, rheumatism, and night-riding. The volume also contains a number of notes and reviews.

Commentariolus of Copernicus

Dr. J. Dobrzycki (Institute for the History of Science, Polish Academy of Science, Warsaw) and Dr. W. P. D. Wightman (Department of History and Philosophy of Science, King's College, Aberdeen) have written to the Editor as follows: "It is fairly generally known that early in his life, and some considerable time before the publication of his epoch-making *De Revolutionibus*, Copernicus circulated a few MS. copies of a brief work (commonly referred to as the *Commentariolus*) in which the idea of a heliocentric system was sketched out. Before the end of the sixteenth century Tycho Brahe had difficulty in obtaining a copy; thereafter it disappeared until the nineteenth century when two copies—an apparently complete one in Stockholm, and a somewhat defective one in Vienna—were brought to light. A few years ago a single page of the *Commentariolus* was discovered in the interleaved copy of the Basle (1566) edition of the *De Revolutionibus* in the hand of Dr. Duncan Liddel (whose library passed to the Marischal College and University of Aberdeen on his death in 1613) (*Science and the Renaissance*, 2, item No. 172; Edinburgh, 1962). A further search recently gave promise of a complete transcript of the *Commentariolus* on inserted folios widely scattered throughout the book. As a result of a more detailed collation we are now convinced that these MS. pages comprise a complete transcript corresponding closely to, but differing in some significant features from, the Stockholm MS. of the *Commentariolus*. A new revision of the *Commentariolus* based on all extant transcripts will be prepared in the Institute for the History of Science

(Polish Academy of Science) in Warsaw with the view of publication, probably in the course of 1966".

Albert Lasker Medical Research Awards

THE twentieth annual Albert Lasker Medical Research Awards have been presented to Prof. A. B. Sabin, professor of research pediatrics at the University of Cincinnati College of Medicine, in recognition of his fundamental contribution to the understanding of the nature of poliomyelitis, and the development of a live vaccine that can be taken by mouth, and to Prof. R. W. Holley, professor of biochemistry at Cornell University, in recognition of his determination of the chemical structure of alanine tRNA.

Feldberg Foundation Award

THE British prize-winner for 1965 of the Feldberg Foundation Award is Prof. B. Katz, of the Department of Biophysics, University College, London, and the German prizewinner is Dr. G. Braunitzer of the Max-Planck-Institut für Biochemie, Munich. The Feldberg Foundation for German-English scientific exchange was established in 1961 through the generosity of Prof. W. Feldberg, a member of the staff of the Medical Research Council at the National Institute for Medical Research, London. The Foundation provides for a prize to be awarded each year to one German and one British scientist. In recognition of the gift the German recipient gives a lecture in Britain and the British prizewinner lectures in Germany.

Davissson-Germer Prize

THE American Physical Society and Bell Telephone Laboratories have announced the establishment of a new Prize for outstanding contributions in the fields of electron and atomic physics. The award will be given in honour of the late C. J. Davissson and of L. H. Germer, former members of the research staff of the Bell Laboratories, and pioneers in the study of the wave-like quality of electrons. The award will be presented every other year by the American Physical Society until 1973, and will consist of a 2,500 dollar cash prize and a citation describing the accomplishments of the recipient.

New Shell Research Fellowship

A NEW series of post-doctoral research fellowships, each worth £1,300 per annum, has been instituted by Shell International Petroleum Co. in extension of the considerable support it already gives to research and education. This is announced in the November issue of Shell's *Science and Technology Newsletter*. These awards, tenable for 2 or 3 years in any university, college of technology or other recognized research establishment in Britain, are intended to assist Fellows to pursue research on a subject of their own choice lying within the spheres of engineering, mathematics, physics, chemistry, biochemistry, botany, zoology or microbiology—disciplines with which Shell's own research in the United Kingdom is concerned. Two awards will be made each year. Candidates may be of any nationality but must hold a Ph.D. degree or equivalent, and be less than thirty years old on October 1 of the year of appointment. Application forms for the 1966-67 research fellowships can be obtained from the Secretary, Shell Grants Committee, Shell Centre, London, S.E.1, and should be returned by February 1, 1966.

South-East Asia Treaty Organization: Research Fellowships

THE British Council, on behalf of the South-East Asia Treaty Organization (SEATO), announces that SEATO research fellowships for 1966-67 are available to scholars whose academic standards are of approximately doctorate level. The research projects for which the SEATO grants will be given should relate to social, economic, political,

Science Reporter, published by the Council of Scientific and Industrial Research, New Delhi, has published a "Haldane Number" (2, No. 11; November 1965. 50 p.). This is devoted entirely to assessments of the late Prof. J. B. S. Haldane by numerous European and Indian scientists. As might be expected, it deals more fully with the Indian period of his life, but other aspects are not neglected.

Announcements

MR. E. B. TEESDALE has been appointed director of the Association of the British Pharmaceutical Industry as from December 1.

Dr. A. R. COLLIES has been appointed director of the Civil Engineering Research Association as from January 1, following the resignation of Dr. M. F. Kaplan, who has been appointed professor of civil engineering at the University of Cape Town.

DR. B. R. NIJHAWAN, director of the National Metallurgical Laboratory, India, has been awarded the 1964 Shanti Swarup Bhatnagar Memorial Award for his contributions in the field of engineering sciences by the governing body of the Indian Council of Scientific and Industrial Research.

DR. F. AYLWARD has been appointed director and Dr. T. G. Gillespy deputy director of the Fruit and Vegetable Canning and Quick Freezing Research Association.

The seventh international Transplantation Conference, sponsored by the New York Academy of Sciences, will be held in New York City during February 14-16. Further information can be obtained from Felix T. Rapaport, New York University Medical Center, 560 First Avenue, New York 16, N.Y.

A SEMINAR on "The Role of a Ministry of Technology", arranged by the Science of Science Foundation, will be held at the Ciba Foundation, London, on February 21. Further information can be obtained from the Science of Science Foundation, c/o the Ciba Foundation, 41 Portland Place, London, W.1.

A MEETING of the Midlands Section of the Society for Analytical Chemistry will be held in the University of Birmingham on January 5. During the meeting the Elwell Award will be presented. Further information can be obtained from the Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1.

CORRIGENDUM. In the article entitled "Transpiration as related to Internal Water Content", by Dr. E. R. C. Reynolds, which appeared on p. 1001 of the August 28, 1965, issue of *Nature*, the quantities of water, where expressed as equivalent depths of water, are 6.1 times too large.

ERRATUM. In the article entitled "Relationships between Constituents of Cones and Male Flowers of the Hop (*Humulus lupulus*, L.)", by Dr. R. D. Hartley and Dr. R. A. Neve, which appeared on p. 804 of the November 20, 1965, issue of *Nature*, the seventh word on the second line should read 'brewing', not 'breeding'.

THE NIGHT SKY IN JANUARY

	All times are in Universal Time						
	MOON		CONJUNCTIONS WITH THE MOON				
	New Moon	21d 16h	Venus	—			
	Full Moon	7d 05h	Mars	23d 12h, 4° N.			
			Jupiter	5d 17h, 2° S.			
			Saturn	25d 05h, 8° N.			
PLANETS							
	Times of rising (<i>R</i>) and setting (<i>S</i>) during the month						
Name	<i>R/S</i>	Beginning	Middle	End	Mag.	<i>D_g</i> (10⁴ miles)	Zodiacal position
Mercury	<i>R</i>	6h 40m	7h 25m	Unfavourable	—	126	—
Venus	{ <i>S</i> <i>R</i> }	19h 00m	18h 10m	—	-3·8	26	—
Mars	<i>S</i>	18h 15m	18h 20m	18h 30m,	+1½	207	Capricornus
Jupiter	<i>S</i>	7h 05m	6h 00m	5h 00m	-2·2	396	Taurus
Saturn	<i>S</i>	21h 35m	20h 45m	19h 55m	+1·3	959	Aquarius
<i>D_g</i> is the distance of planet from the Earth on the 15th of the month							
OCCULTATIONS OF STARS BRIGHTER THAN MAGNITUDE +6 AT GREENWICH							
	Star	<i>R/D</i>	Time	Mag.			
	η Leo	<i>D</i>	9d 20h 53·5m	+3·6			
	γ Leo	<i>R</i>	9d 21h 19·7m	+3·6			
	51 Tau	<i>D</i>	31d 20h 53·8m	+5·6			
	53 Tau	<i>D</i>	31d 21h 01·8m	+5·4			
(D, disappearance; R, reappearance)							

METEORS

Name	Active period	Date of maximum	Radiant	Remarks
Quadrantids	3d-4d	3d 20h	222° R. A., + 50° Dec.	Unfavourable

OTHER PHENOMENA: 8d 05h, Venus 4° N. of Mars

THE COUNTRYSIDE IN 1970

By E. M. NICHOLSON, C.B.

Director-General, The Nature Conservancy, 19 Belgrave Square, London, S.W.1

CONFERENCES nowadays follow one another so rapidly that those most involved in them may find it hardest to assess their real objectives in relation to what they actually do and what, if any, further results flow from them. Yet the fact that conferences monopolize so much time of busy people and cost so much to organize and attend casts a moral obligation on their promoters not only to see that these resources are well spent, but also to provide their quota towards the necessary data for developing the science and art of holding conferences, as well as towards whatever may be the subject-matter of their own exercise. This is borne in mind in the following discussion of the Duke of Edinburgh's Second Study Conference on "The Countryside in 1970", held in London during November 10-12, 1965.

Conflicts between developers accused of despoiling the countryside and champions of its preservation go back at least to Wordsworth, and arguably to John Evelyn, who succeeded three centuries ago in promoting what would now be termed a highly successful conservation campaign against the ironmasters who were laying waste the English woodlands. Thus vanquished, they found necessity the mother of invention to the extent of devising means of substituting coal for charcoal and thus, under conservationist pressure, finding the way to creating the steel industry. During the nineteenth century, however, development in its crudest forms was increasingly permitted, and the well-meaning voluntary bodies formed to combat such tendencies never won enough support or attained enough cohesion to reverse the trend. This was dramatically shown in the main London exhibition for National Nature Week in 1963. Following a visit to the exhibition the Duke of Edinburgh resolved to bring the parties together at a study conference in the autumn of 1963 to seek a means of agreeing on both a diagnosis and an approach to a remedy before, as he feared, the national inheritance in the countryside became irrevocably damaged by about 1970.

Hurriedly improvised and patchily documented, the 1963 Conference was nevertheless judged a considerable success. It brought together representatives, mainly at a high level, of some ninety national organizations including Government departments and public concerns, professions, scientific and technical organizations, and bodies representative of industry, agriculture, landowning, recreation, natural history, amenity and other interests concerned.

Many of these were hitherto scarcely aware of the existence of one another, and it proved a revelation for them to meet face to face in the three large working parties and to begin to learn of their respective aims, problems, policies and points of view. By the time the Conference ended, it had initiated many fruitful contacts, harnessed much vague goodwill into a definite, if frankly empirical, common movement embracing hitherto conflicting parties, and prepared the way for a more scientific and educational treatment of the whole vast complex of problems.

Such treatment was facilitated by a contribution from the Nature Conservancy of a chart of "Human Impacts on the Countryside", prepared by A. W. Colling and myself. This chart listed and analysed the incidence and effects of all traceable activities and operations (numbering more than 160) involving distinguishable types of impact by a human group or organization on the country-

side. The Conference directed much attention to requirements in terms of research and investigation leading to a better understanding of the problems. Following publication of the *Proceedings* of the Study Conference (H.M.S.O., 1964), an exhaustive analysis was made of all the suggestions, and recommendations for action or for further study. Three follow-up conferences were arranged, on "Agricultural Chemicals—Progress in Safe Use" held in London in March 1964, and on Scottish aspects held at Inverness in April 1964, and on educational aspects at the University of Keele in March 1965. Special account was also taken of a number of allied projects, including the National Trust's "Enterprise Neptune" for saving key areas of coastline, the Nature Conservancy working party and report on "Broadland", the Civic Trust's recreational plan for the Lea Valley, and the Nuffield-Cambridge survey of common lands.

While these conferences and projects contributed encouragingly towards giving practical shape to the ideas outlined in November 1963, it was felt that a renewed effort in more depth was needed. This took the form of a second conference, two years after the first, prepared by twelve study groups working intensively within four broad areas; legislation and administration; users and uses of the countryside; technology and impacts on land; education, training and qualifications; and information.

With the formation of these study groups a new departure began, since their terms of reference were based on needs brought to light by the first conference, while their membership included more than 180 men and women selected partly for their specialized knowledge and partly for their close association with a wide range of bodies most intimately and continuously concerned. Each study group was serviced by a different organization, official or unofficial, including the National Parks Commission (4, countryside: planning practice); the Scottish Development Department (9, countryside: planning and development in Scotland); the Forestry Commission (3, technology in conservation); the National Coal Board (12, reclamation and clearance of derelict land); the Lincolnshire (parts of Lindsey) County Council (8, preservation of natural, historic and other treasures); the Town Planning Institute (1, training and qualifications of planners); the Royal Institute of Chartered Surveyors (2, training and qualifications of the professions concerned with land and water); the Council for Nature (5, review of legislation); the National Council of Social Service (11, living and working in the countryside); the Automobile Association (7, traffic and its impact on the countryside); the National Benzole Co., Ltd. (10, information and the countryside).

All these study groups worked simultaneously to complete their reports by July 1965. Their devoted chairmen and convenor secretaries formed, together with the organizing committee of vice-presidents, sessional chairmen, joint secretaries and others, a central steering group, served administratively by the Royal Society of Arts and on matters of programme and content by the Nature Conservancy. Thus, of about 360 who participated in the final plenary sessions of the second conference, some 50 had actively assisted in its organization, and nearly 150 more had worked on the preparatory study groups from many different points of view, and had taken part in the lively and critical review of the findings as a

whole during the first two days (November 10 and 11). It appeared, as was to be expected, that this stiffening of seasoned members gave the eventual conference a markedly greater depth, cohesion and focus than that held in 1963, when the majority came new to the subject and to one another.

It had been expected that some study groups would not merely overlap in their coverage, as they rightly did, but would reach conflicting or inconsistent findings, which, with negligible exceptions, they did not. The difficulties which occupied the conference were of a different order, arising from differences in underlying assumptions or emphasis regarding the right scope of the enquiry and the true importance of different elements or interests. Differences also arose regarding the means of fulfilling aims on which all were broadly agreed, and the safeguards for other national or sectional interests affected.

Originally it had seemed that the most urgent and intractable problems might be to resolve differences between voluntary bodies within the naturalist and conservation movement, and between that movement and its potential allies in recreation and among the other 'guardians of amenity', so as to enable the 'conservationists' to speak with one voice to the 'developers'. This presupposed that the 'developers' themselves would become familiar with the issues and be organized not only to express views but also to be able to play an active part in improving liaison, planning machinery and procedures, so as to transform the often negative and time-wasting encounters into a positive and constructive process of reconciling, so far as practicable, conservation with development.

Somewhat surprisingly, it seemed, subject to later confirmation, that these difficulties had been overrated. Since, and no doubt partly owing to, the first conference, it had become fashionable to pay a good deal more than mere lip-service to the overriding need for rapid joint action, and many good examples became manifest. These included the voluntary agreement for withdrawal of toxic chemicals known to be injurious to wild life under certain conditions; the joint policies and practices agreed on by the Forestry Commission; the National Parks Commission and the Nature Conservancy; the surprising unanimity reached by authorities and interests concerned with the Lea Valley and the Norfolk Broads; the progressive outlook of the 'land-linked professions' revealed in Study Group 2; and the almost universal welcome for a broad-based 'Countryside Commission'. The merger of representative bodies for employers and industry in the new Confederation of British Industry opened fresh possibilities of liaison. There was also a marked and most welcome growth in the readiness of planners and planning authorities to envisage substantial changes.

Welcome as were these and other signs of probable progress, they were still outnumbered and outweighed by more adverse factors. The new Regional Boards, too lately constituted to be reliably evaluated yet, were clearly liable to exercise great influence over large-scale development which could fail to take adequate account of the findings of the conference. Nationally it was clear that, in the absence of such impetus as the conference provided, recent tendencies to neglect countryside factors and interests in national planning were likely to continue. It seemed misguided on this basis to argue, as some did, that the scope should be widened to include the whole of urbanization and town planning, the effect of which could only be to relegate countryside problems and interests to a minor place in the only effective forum available to them. On the other flank some, although by no means all, spokesmen of country-dwellers and rural occupations adopted an attitude by no means consistent with the growing trends and impacts, which they seemed to underrate or misunderstand, and which called for much more energetic and thoughtful efforts on their part to reach a new *modus vivendi* with the towns and their recreational, technological and accommodation pressures before it is

too late. While it is only too easy to attract letters to the editor and impromptu speeches and articles from the angle of the country-dweller, there seems to be a severe dearth of thorough study and critical thought by country-dwellers themselves about present-day and impending impacts on the countryside. While this situation persists it helps no one to coin slighting descriptions of academic and other contributors who try, as best they can, to throw some light on these dangerously neglected problems.

Another problem area in this regard is education, and some feeling was aroused by the suggestion that the educational world had not been approached to participate, or that it had been approached in the wrong way. In fact, although every other major national interest approached, from administration (national and local) and angling to women's institutes and youth hostels, responded to the call, it proved impossible, even with patient, tactful and persistent efforts in many quarters, to obtain leading representatives of the teachers who bear so great a responsibility and dispose of such great resources and potentialities regarding the future of the countryside. It is to be hoped that the leaders of this great profession will soon awaken, and that meanwhile they will discourage suggestions that their help has not been invited, which are very far from the truth. Meanwhile, fortunately, such bodies as the Field Studies Council and a number of local education authorities and training colleges are demonstrating how much can be done where the vision and the will exist.

Among problems which remain to be seriously faced are the study of resource economics, including the adequate development of cost/benefit analysis in relation to recreational uses, the future relation of industry to the countryside, tourism in relation to domestic, educational and recreational policies, and the implications of a national land-use policy based on multi-purpose use and zoning principles. Others include land requirements for defence purposes and the role of research in both the natural and the social sciences on human impacts on the natural environment. It is to be hoped that the Natural Environment Research Council and the Social Sciences Research Council will be able to stimulate more adequate work in these fields.

So far as practicable, the Countryside Conferences have been based on assembly of data from research and investigations, supplemented by fresh work wherever time and resources permit, and brought into focus on emerging problems as a basis for the disciplined discussion of the issues and of possible recommendations by selected groups deliberately drawn from a wide range of professions and interests. Much of the knowledge used was either new, unused or under-used previously, and it therefore led to an unexpectedly wide and rapid advance in understanding of the problems. For example, the report of Study Group 3 assembles a range of selected factors in technology relevant to the countryside which make possible a new and more comprehensive view of modern environmental management. Not only the results of the Conference, but the means by which they were reached, will repay investigation.

No other country has yet attempted such an approach. The nearest has been President Johnson's White House conference on "Natural Beauty" in May 1965, but this, although most impressive of its kind, was very lightly documented and was preoccupied with urgent legislation and administrative action, much of which has long been taken in Britain. It is, however, to be borne in mind that documentation on such aspects as recreational pressures and water resources is vastly fuller and more advanced in the United States than in Britain. There is, however, no scientific organization elsewhere in the world looking systematically at problems of the natural environment and the impact of man on it. Without such a basis anything quite like the conferences on "The Countryside in 1970" would not be possible.

MALARIA AND THE OPENING-UP OF CENTRAL AFRICA

IN his inaugural address to the University College of Rhodesia and Nyasaland, Prof. Michael Gelfand took the opportunity to review the part which malaria played in delaying the opening-up of Central Africa to European traders and settlers¹. His original paper is illustrated with reproductions of some fine contemporary pictures which add to the interest of the text.

The earliest in the field were the Portuguese, who were setting up trading posts along the west coast of Africa as early as 1443. Somewhat later they were establishing footholds along the east coast too, but the 'Angel of Death' effectively blocked their penetration into the interior. When quinine became available, the Portuguese took to it more readily than the British and put it to more effective use. Its introduction, although spread over a longer period of time, "had an impact on medicine similar to that of antibiotics to-day".

The legend that cinchona bark was introduced to Europe by the Countess of Chinchon, wife of the Viceroy of Peru, has been convincingly disproved. What is clear is that Jesuit missionaries were in contact with Indians who were aware of the medical properties of cinchona bark, at Loxa, in Brazil, and in Peru, about the beginning of the seventeenth century. Its first recorded importation into Europe was made by Barnabe de Cobo, after exploring parts of Mexico and Peru in 1632. News of it soon spread from Spain to Italy and the Netherlands, and it was introduced to England by James Thomson of Antwerp in 1650.

In many places its acceptance by doctors was very varied, in part perhaps on religious grounds, but in England, resort to its use was stimulated by some serious epidemics of ague, so that the weekly publication *Mercurius Politicus* could report that, by 1658, the bark was on sale by several London chemists. Its fame was heightened by Robert Talbot, who among many others treated King Charles II and the Dauphin Charles of France. He made a fortune out of its secret use. Sydenham was convinced of its efficacy as a medicine, and administered the powdered bark mixed with red wine. As early as 1659 Willis found that the bark relieved acute attacks of ague, although relapses were common; and it was not until 1768 that James Lind observed that, in cases of fever, the drug was most effective if given early in large doses.

In 1745, Claude Touissant de Cagarage attempted to produce an extract of quinine, as did Bernadino Antonio Gomes, of Lisbon, in 1810. Ten years later, Pierre Joseph Pelletier and Joseph Caventou were the first to isolate two of the four alkaloids in the substance. This discovery so stimulated the demand for cinchona bark that exploitation of the forests of Peru, Ecuador and Bolivia was carried to such an extent that fears arose about their exhaustion; the English and the Dutch took up its cultivation, and by 1862 the Dutch had almost established a monopoly in its supply, mainly based on Java.

In comparison with the Portuguese, the British were slow in making attempts to trade with or settle in Central Africa. One of the earliest schemes for settlement was made by William Bolts; it was sponsored by Maria Theresa of Austria. The expedition set out from Leghorn in 1776. It consisted of 152 Europeans, who set up stations along the Masoomo River, in Delagoa Bay, but malaria soon made its presence felt; local Africans rose against the enfeebled party, which was later attacked by the Portuguese, and within three years the entire scheme had collapsed.

These experiences might be taken as representative of a series of disasters which were to follow. About that time some people thought that the Niger joined up with

the Nile, others that it had a confluence with the Gambia River. To explore the Niger, the British African Association sent out a Major Houghton, but he, after travelling through the kingdom of Bamouk, was robbed of his possessions and later died. His task was taken over by a Dr. Mungo Park, who left England with two servants in 1795. He reached the Niger in 1796, followed it for 300 miles, and then, emaciated with fever, returned home.

In 1803 Mungo Park led a fresh expedition. This time he asked for a mosquito net and two pairs of trousers for each man. The party left England in 1805; and, after having to contend with malaria, dysentery, incessant rain, swollen rivers and mud, they reached the Niger. There they built a schooner, and sailed down the Niger past Timbuktu, after which the boat capsized and all were drowned. Of the 44 Europeans in the expedition, 35 died of malaria.

An expedition financed by the Navy and led by Captain James Kingston Tuckey set out from England in 1816. The party entered the mouth of the Congo, and some members worked their way up the River as far as Soondy Nsanga, 280 miles from Cape Padron, where, stricken by disease, they had to abandon all hope of progressing farther. Of the 44 Europeans in the party, 18 died of malaria. Dr. McKerrow, a member of the team, gave a good description of the symptoms of malaria. He noticed that the men most seriously affected were those who had visited African villages or slept in the open. As a medicine he made some use of cinchona bark, but only as a last resort, and the results were unpromising.

Another expedition, sponsored by the Admiralty, left England in 1822, with the view of exploring the east coast of Africa, Madagascar and parts of Arabia. A small detachment attempted to make its way up the Zambesi, reaching as far as Senza. Of the three Europeans in the group, one had already died, and the other two died on the journey back to their base.

The failure of the Congo expedition, sent out in 1816, intensified the desire to solve the problem of the Niger. In 1822 Hugh Clapperton with two companions set out to cross the desert from Tripoli. They discovered Lake Chad; then Oudney, one of the group, died, leaving Clapperton to struggle as far as Sokoto by way of Kano, whence he was able to return alive.

Clapperton made another attempt in 1825, with five Europeans in the party. From the Bight of Benin, one of the group reached Yaourie before being murdered by his followers. The remainder went on to Jannah, where they were all ill with fever; but against formidable odds, Clapperton reached Eatunga, crossed the Niger, and moved on to Kano. Weakened with dysentery and malaria he died at Sokoto, so that, of the Europeans, four died.

At about the same time, a further expedition set out to reach the Niger by way of Tripoli and Timbuktu. It was led by Major Alexander Gordon, who was the first known European to reach Timbuktu, but he was murdered by Arabs soon afterwards. In 1827, a Frenchman, René Caillié, starting from Freetown, reached the Niger and became the second European to see Timbuktu; he succeeded in returning to France alive.

The riddle of the Niger still remained unresolved. To solve it, Richard Lander, who had been a member of Clapperton's expedition, and his brother, John, offered their services to the British Government. They left Portsmouth in 1830, followed Clapperton's route as far as Bussa, and set sail down the Niger with four negroes as a crew. They reached Eboe, near the Atlantic, but were then captured by Ibu traders and held by different people

until finally released, when they found their way to the Atlantic along one of the subsidiary channels of the Niger delta. A boat picked them up and took them to Rio de Janero before they could return to England. They had been advised to take two to five grains of quinine every six hours.

Interest in the Niger rose still higher. A company at Liverpool financed an expedition which was led by Richard Lander and had the use of three small steam-boats. Special attention was paid to the physique and fitness of the crews. But before the ships reached the Niger, fever began to take its toll. Moving up to Ebboe, two of the boats were surrounded by dense vegetation and swamps on either side, and the men were exposed to relentless rain and "torrents of sandflies and mosquitoes".

Further up stream, one of the boats, the *Quorra*, ran aground and remained stuck fast from November until the following March. At the end of March, MacGregor Laird penetrated up stream as far as Fundah, but got no further, and what had been the supply ship, the *Alburkah*, reached Raba and then turned round. At the end of two years, of the total European complement of the three ships, numbering 82, 64 were dead.

By this time the British Navy was active along the west coast of Africa, attempting to suppress the slave trade. Some indication of the health risks to which the crews were exposed is conveyed by the fact that in 1834, of 792 men serving in seven British ships, 204 died. Nevertheless, in 1841 the British Government and members of the public jointly financed the Great Niger Expedition. Three special ships were built, with the *Wilberforce* joining them later. Each boat had a special system of ventilation: during the night as few men as possible were to remain on deck, and when up river, all the white crew had to sleep below. Special clothing was provided, and dry clothing was to be readily available. The men selected were all robust and in the prime of life.

The ships left Woolwich in 1841, and in August, entered the Niger. On September 4 a virulent attack of fever struck the crew of the *Albert*; soon after, the *Soudan* was sent down stream with all the sick. In October the plan to reach Raba was given up. Sickness and deaths continued so that the British Government decided to recall the expedition. Of a total of 145 Europeans, 42 died—almost all of them of malaria.

In his book on this expedition, M'Williams, surgeon of the *Albert*, reported that the practice of blood-letting, which had been the procedure of first choice for almost all the earlier expeditions, was of no value whatsoever, while quinine given at a late stage and in large doses was of some benefit; but he missed the point, which his own records would have shown, that quinine was more effective if given early. He gave details of eight autopsies in each of which he found the gall bladder distended with bile, "the colour and consistence of tar". The colon was generally empty except for "dark pultaceous matter viscid and tenacious".

By this time it was obvious that Africans were much less susceptible to malaria than Europeans, although these too became more resistant if they lived in the country for long periods. This realization, along with a better appreciation of the value of quinine, marked the turning-point in the opening-up of Central Africa. Quinine had been put to medicinal use in the Navy by Sir William Burnett, although it was not given a fair trial. But Alexander Bryson, who was later to become Director General of the Naval Medical Service, recommended that it should be used as a prophylactic and administered to all members of crews on going ashore and on their return, as well as to those who remained on board in swampy places.

With considerations like these in mind, a new vessel, the *Pleiad*, set sail from Plymouth in 1854, under the command of Mr. Beecroft—later succeeded by Dr. William Blaikie. With 12 Europeans and 53 coloured men on

board, it entered the mouth of the Niger and travelled as far as Tshomo; scurvy broke out among the crew, yet although the Europeans were subject to great fatigue and went ashore in unhealthy places, while some of them slept on deck, none of them died. They took three to four grains of quinine every morning, and sometimes in the afternoon.

At this stage David Livingstone came into the picture. It was through reading M'Williams's account of the Niger expedition that he devised his famous pill, consisting of quinine and purgatives. That was in 1850. With it he first treated an English party and members of his own family. His procedure was to give doses large and early; by means of it he was able to cross Africa from coast to coast. His confidence in the pill was so great that he severed connexion with the London Missionary Society, so that he could operate on a wider scale.

By then a national hero, Livingstone had no difficulty in persuading the Foreign Office to sponsor an expedition to ascend the Zambesi as far as Chobe and plant a mission somewhere near the Batoka plateau. When the party reached Africa the members took two grains of quinine every day. Livingstone doubted if that was enough, but he felt that he could stave off serious attacks of fever by extra doses. Altogether, at first things went fairly well in spite of personal dissensions, and in spite of the fact that with a good deal of malaria about, some individuals became more seriously affected. Livingstone also believed in the therapeutic value of physical exertion.

In April 1859, he discovered the Shire Highlands, and in September, Lake Nyasa. Soon afterwards he heard of the fate of the Helmore-Price expedition to Linyanti—an 'unhealthy place'. There was no medically trained person in the group, and out of nine Europeans, six lost their lives.

Further experience of malaria, to which Europeans made a varying response, induced Livingstone to abandon the prophylactic use of quinine for a time. He ran into difficulties over the U.M.C.A. Mission at Magomero, and a series of disasters followed, including the death of Bishop Mackenzie, who lost his supply of quinine when a boat capsized. Worse was to follow: Livingstone's wife died of malaria; the Mission at Magomero, which had been moved to Chibisa, had to be closed down, and he was recalled to England.

The two doctors, John Kirk and Charles Meller, now with extensive experience of malaria, realized that the disease was not so simple as Livingstone had imagined. They identified the dysenteric kind called 'blackwater fever'. Meller distinguished the asthenic and hepatic forms, and experience convinced him of the prophylactic value of quinine, with doses of up to five grains taken daily.

The next steps in the conquest of malaria are more widely known. In 1880, Laveran, a French Army doctor, discovered the cyst-like bodies of the protozoon in the red corpuscles of human blood. This observation was only slowly taken up, but it was confirmed by Marchiafava in 1884; and in 1889, the tertian, quartan and malignant types of the disease were distinguished. Following the discoveries of Manson and Theobald Smith, that insects can act as vectors of disease, on Manson's suggestion, Ross, a British Army doctor, started work in India, and in 1897 found the oocyst in the outer wall of a mosquito's stomach. He worked out the life-cycle of the avian type of the plasmodium in the following year.

Up to 1914, quinine was the only drug known to be effective against *Plasmodium falciparum*, *P. vivax* and *P. malariae*, but not against the gametocytes. The outbreak of the First World War stimulated the search for other preventatives, particularly in Germany where supplies of quinine might be cut off. An observation by Guttman and Ehrlich, in 1891, that methylene blue had some action against the plasmodium served as a starting-point for Schulemann, who after a series of trials synthesized plasmoquin in 1925, the first artificial anti-malarial.

The discovery of atebirin soon followed. This proved to be a valuable drug for prophylactic use in the Second World War. Still more potent drugs, chloroquine and amodiaquine, were isolated shortly afterwards. These two are excellent, having a complete prophylactic action against almost all forms of malaria, while producing no side-effects. Two further anti-malarials to be discovered were proguanil and pyrimethamine ('Daraprim'), the latter being particularly long-lasting. It was discovered by George Hitching of the Burroughs Wellcome Laboratories.

The conquest of malaria has also been greatly assisted through the use of insecticides, such as pyrethrum, Paris green, benzene hexachloride (BHC) and DDT. In the case of some of these there was long delay between their first discovery and exploitation. BHC, for example, was isolated by Faraday in 1825, yet its insecticidal properties were only discovered in the United States in 1933; and a German chemist, Zeidler, synthesized DDT in 1874, yet its properties as an insecticide were first noticed by Paul Müller in the Geigy Laboratories, in Basle, in 1939. The Second World War did a great deal to stimulate the production and exploitation of these compounds. As an example of insecticidal potency, one might mention BHC, which six months after application on mud walls is capable of killing 80 per cent of *Anopheles gambiae*.

Taken together, all these developments have completely transformed the situation in relation to the conquest of malaria. In Nyasaland, for example, as late as 1897, the death rate among European settlers averaged between 9 and 10 per cent, mainly from fever, in a young population. In Northern Rhodesia, during 1907-08, the death rate from malaria and blackwater fever combined was 30.4 per 1,000; in 1925, even before the aforementioned developments, it had fallen to 2.8.

Such results can be compared with those associated with the construction of the Kariba Dam, which was started in 1956 and completed by 1960. Not once was work held up or even interrupted because of disease. This huge under-

taking involved the importation of enormous numbers of African workers from many parts of the country and the employment of many Europeans who had not previously built up any immunity through exposure to malaria. For health and comfort, the living quarters were placed on high ground. All workers were informed about the dangers of heat stroke, and employers were encouraged to allow their workers a period for acclimatization. A survey of the locality showed a spleen rate of 80 per cent, and a parasite rate of more than 30 per cent among the original, local community. All the interior walls of dwellings were sprayed with BHC, and this procedure was repeated three times a year.

As a preliminary measure, an attack was made against mosquito breeding-places through spraying with 'High Spread Malariol' (Shell). Survey counts showed that the operation was effective, so that there was no need to repeat it later. All workers were required to take the prophylactic drug with which they were provided—at first, 0.4 g of camoquin weekly for the Europeans, and 100 mg of mepacrin for the Africans. Later, daraprim was substituted at the rate of 25 mg each per week.

All windows of the European houses were screened, and this helped to keep away millions of other insects as well as mosquitoes. As a consequence, there was not one death from malaria among Europeans living on the site during the first two years. There were two deaths, however, of men living in temporary camps outside the recognized limits, and nearly all the European cases of fever gave a history of irregular prophylaxis, or fishing or hunting at night beyond the controlled area.

These results show that apart from human factors, in Central Africa the conquest of malaria is now almost complete. They also show that in Africa it is a mistake to think of medicine in terms of individual territories.

R. WEATHERALL

¹ Gelfand, M., *Rivers of Death* (Supplement to *The Central African Journal of Medicine*, 11, No. 8, August, 1965).

APPLIED SCIENCE IN THE SCHOOLS

IN recent years a recurrent theme in discussions of science teaching has been the inability of courses in applied science and engineering at universities to attract the best students. This has led to a good deal of self-analysis and exposition of their aims by engineers, and to attempts to acquaint science and careers masters with the nature and purposes of these courses. More fundamental questions are those concerned with changes in the nature of school science teaching itself, and some of the great interest in the new pure science syllabuses of the past few years has now shifted to the possible introduction of applied science courses at school level. This concern led the Institution of Mechanical Engineers* to ask Mr. G. T. Page to assemble a report on the present situation in schools, and in a very short time he produced a mass of information and ideas, obtained both by direct visits and by a postal survey, collated, compared and summarized into an urgent, highly relevant volume.

A conference in October, to discuss this report, showed a very high degree of interest in the whole subject and also produced very complimentary estimates of the report itself, and it is certain that it is at present exercising headmasters and many educational authorities throughout the country. Mr. Page labels his book as descriptive rather than prescriptive, and a large part of the 328 pages is taken up with detail of what is done in a number of schools, both in terms of actual teaching of applied science and 'application-conscious' teaching of physics and chemis-

try, with syllabuses, details of projects, costing and much more important information, both in the text and in appendixes. A lot of space is devoted to the best known experiments in the field, such as the Technical Activities Centre at Sevenoaks School, the examined project work at Dauntsey's School, and the sixth-form courses in applied science such as that at Ealing Grammar School. However, the report is much better regarded as a digest of many different opinions about, and aspects of, the whole subject. There are, for example, chapters on 'Engineers as Teachers' and 'Workshop and Laboratory Technical Assistance', and a provocative section called 'What the Universities Really Think', in which the widely different views of many authorities are contrasted. Throughout, one finds figures, data of all kinds, and tables and photographs of typical laboratories as a basis for further discussion and investigation. In the final chapter, thirty-one suggestions of possible definite actions are made, ranging from joint industrial/school associations to long-term curriculum research. Two particularly interesting lines of argument emerge. One is that it was everywhere insisted by schools who have begun applied science courses that this was done for educational reasons rather than vocational reasons (although some comment in Scotland was less altruistic). The Crowther Report suggested that for many schoolchildren, the 'alternative road' to interest in scientific and technological matters is the most attractive one, and in many cases it has been stated that applied science has been introduced to make school science in some way less dull. Generally, it is true to say that there has been in the past, and still is, in school courses, a pre-

* The Institution of Mechanical Engineers' *Engineering Among the Schools: Activities in Applied Science and Engineering—a Survey of 290 Schools*. Pp. 328. (London: The Institution of Mechanical Engineers, 1965.)

ponderance of analytical rather than synthetic or creative work, a tendency to measure an effect rather than to make use of it; new courses, especially, say, those of the Seven-oaks type, can be more properly regarded as introduced to redress this lack of balance. This kind of response may automatically help to foster the kinds of attitude one would look for in successful engineers, but has not specifically been introduced for this purpose.

Yet, secondly, a good deal of play is made of the reluctance of headmasters to accept that the 'alternative road' into a scientific career might be the best one for some of the best students. This necessarily brings to mind many thoughts about educational attitudes in the country, and the concern of engineers with their own 'public image', especially in the eyes of science teachers counselling boys and girls when they consider university courses. If one considers the diversity of activities under the broad head of engineering and technology, it is perhaps not too surprising that this image is a little out of focus, but the present careful expositions of the need for flexibility, breadth and social and economic awareness in an engineer may alter this. If a teacher is able to consider an engineer as a bridge-builder between two cultures, rather than a glorified technician, he can then be more inclined to satisfaction at the prospect of the best intellects entering engineering careers. The objections by a number of authorities to the idea of A-Level examinations in applied science make good sense when one considers the present-day attempts to ask for more flexible university entrance requirements, and the talk of a single chemistry-

physics A-Level subject. At the same time, the investigations of approaches to technological studies at sixth-form level and the centralization of such work by the Schools Council are very welcome. What seems vital is that any teaching institution should be concerned, within the general framework of science courses which its size and resources allow it to offer, to present as wide a picture of what the whole effort of scientific and technological work consists of, as is possible, whether this be through complete courses in large schools, or project work, or re-orientation of existing pure science into application-conscious treatment. This is particularly true, if like the Nuffield Scheme co-ordinator, one regards a sixth-form course as being a final chance to sample disciplines; it will be of very great interest to see the eventual content of the Nuffield A-Level courses. Help which can be obtained, through curriculum research of any kind, for example, is obviously most important in all circumstances.

In any event, the Page Report, making available detailed information, opinion and informed suggestion, is a key document for anyone concerned in decisions about school science courses. In addition, it is eminently readable, and, despite the rapidity of its publication, excellently produced. It is possible that it may be the preliminary to further substantial changes in school work, and it will certainly help the careful thinking about this rather complex subject which is needed while school science is altering at the present rapid rate.

T. A. BURDETT

CHEMICAL PHYSIOLOGY OF THE ACID MUCOPOLYSACCHARIDES

ALTHOUGH there have been several excellent symposia on connective tissues, it is now too big a subject to cover adequately in a meeting of manageable size, and its various compartments tend to be treated separately. The acid mucopolysaccharides have been quite fully discussed from the metabolic and chemical aspects, but not often from the point of view of the chemical physiologist. This is not surprising, since the state of combination of acid mucopolysaccharides in tissues was unknown, their localization depended on primitive and non-specific techniques, and practically nothing was known of their molecular biology. Considerable progress is being made on all three problems, and it was thought that the results would support a three-day meeting, with ample discussion.

The symposium was organized by Dr. Giuliano Quintarelli, of the University of Alabama, and held under the auspices of the Carlo Erba Foundation in Milan during September 23-25. Travel grants were made available by American Chicle Co. and Colgate-Palmolive Co. through Dr. J. Volker, vice-president of the University of Alabama Medical Center.

Seventeen speakers were invited, several of whom comprehensively reviewed their subjects in addition to presenting new data. Dr. Helen Muir (St. Mary's Hospital, London) opened the proceedings with a paper on "Protein-Polysaccharides", and was followed by Dr. L. Rodén (University of Chicago) on "The Protein-Carbohydrate Linkages of Acid Mucopolysaccharides". Dr. Rodén presented the evidence that chondroitin sulphate A is linked to protein by means of a glucuronosyl-galactosyl-galactosyl-xylosyl-serine bridge, with much detail on positions of glycosidic bonds, etc. Dr. P. Hoffman (Research Institute for Skeletomuscular Diseases, New York) discussed threonine, serine and asparagine as alternative linkage points for the polysaccharide at the protein. Dr. Hoffman took up a theme introduced by Dr. Muir (frequently mentioned during the meeting) as to how heterogeneous the cartilage protein-polysaccharide

is, and showed that his preparations contained chemically dissimilar fractions, separable on electrophoresis.

Papers by Dr. Castellani (Pavia) and Drs. Pedrini and Pedrini-Mille (University of Iowa) dealt with the keratan-sulphate-protein complex from epiphyseal plates and human costal cartilage, respectively. Dr. Pedrini reported three components in his water extracts, as shown by electrophoresis on cellulose acetate, the slowest being predominantly the keratansulphate complex, and the fastest the chondroitin sulphate complex. The ratio of the slowest to the fastest increased with age. They differed significantly in their amino-acid content. Dr. J. D. Gregory (Rockefeller University, New York) described countercurrent fractionation of the hyaluronidase digested cartilage protein-polysaccharide. Two components were isolated, one containing the keratansulphate, and the other remnants of chondroitin sulphate attached to protein. Dr. N. di Ferrante (University of Texas), in the course of a review of the "Antigenicity of the Protein Polysaccharide Complexes from Cartilage", presented evidence that the keratansulphate-protein fragment of Dr. Gregory possessed the antigenic properties of the original protein-polysaccharide. The second fraction of Dr. Gregory, containing remnants of chondroitin sulphate, was not antigenic.

The papers of Dr. S. M. Partridge (Low Temperature Research Station, Cambridge) and Dr. D. Dziwiatkowski (Rockefeller University, New York) dealt with the effects and the isolation, respectively, of an enzyme present in cartilage which proteolytically degrades the protein-polysaccharide. The implications of these findings to the study of the complex are great, and probably much of the work already done will have to be reassessed. Dr. Cifonelli (University of Chicago) rounded off this section by pointing out areas in which chemical investigations had turned up conflicting data, and discussed degradative techniques which could resolve the difficulties.

Dr. T. C. Laurent (University of Uppsala, Sweden) opened the 'molecular biology' section with a demon-

stration of the importance of the 'excluded volume' and 'available volume' concepts in this field. The interpretation of the physiological role of the acid mucopolysaccharides is likely to draw heavily on this work. Dr. E. A. Balazs (Retina Foundation, Boston) considered the role of polyanions in the transformation of chemical to mechanical energy in a paper entitled "The Mechanochemical Function of Mucopolysaccharides in the Organization of the Intercellular Matrix". Dr. J. E. Scott (Taplow, England) reviewed "Ion Binding in Solutions containing Acid Mucopolysaccharides" and suggested that Coulombic complexes of proteins and acid mucopolysaccharides could be 'ion-buffers'. Work on the "Interaction of Collagen and Acid Mucopolysaccharides" was presented by Dr. M. B. Mathews (University of Chicago).

The microlocalization section was introduced by Dr. G. Quintarelli (University of Alabama) in a review of relevant histochemistry which demonstrated that little reliance could be placed on previously accepted techniques. Dr.

J. E. Scott (Taplow, England) described how the "critical electrolyte concentration" phenomenon could be used to investigate and improve on the specificity of cationic dyes. In the final paper, Dr. S. Gardell (University of Lund, Sweden) compared the results of the best available mucopolysaccharide fractionation techniques for the analysis of microgram quantities of tissues obtained by microdissection.

The chairman of the concluding session, Dr. E. A. Balazs, thanked the organizers and particularly Dr. Sirtori and the Carlo Erba Foundation for their magnificent hospitality; the participants joined in complete agreement. This was the first full three-day symposium in English to be held at the Carlo Erba Foundation, and everyone present will wish for the opportunity to be present at others in the future.

The *Proceedings* will be edited by Dr. Quintarelli and published in book form by Little and Brown, Boston.

J. E. SCOTT

POSSIBLE CONTRIBUTION OF JUPITER'S MAGNETOSPHERIC TAIL TO THE RADIO EMISSIONS OF THE PLANET IN THE DECAMETRIC REGION

By G. M. GRUBER

Department of Physics, Rhodes University, Grahamstown, South Africa

SINCE Bigg¹ showed that the satellite Io influences the decametric emission it seemed promising to look for dependences which are not related to the rotation of the planet Jupiter. Prof. J. A. Gledhill suggested to me that it might be interesting to investigate the possibility that radiation from Jupiter's magnetospheric tail contributes towards the decametric noise.

Since the data obtained at our research station were insufficient for any large-scale investigation, the obvious choice was the data of J. W. Warwick and W. T. Kreiss² compiled from observations during January 1, 1960–December 31, 1963. In addition, Drs. Warwick and Dulk supplied me with the data obtained during January 1, 1964–May 31, 1965, for which I thank them.

In their data they recorded the U.T. start and finish of each observing period and of each Jupiter event, its frequency range, intensity (on a scale of 1, 2 and 3), the burstiness as weak, moderate or strong, and the System III CML (Central Meridian Longitude) of each event.

To find whether any influence of the magnetospheric tail existed, the following procedure was adopted. The period between two superior conjunctions was divided into 27 intervals each of 15 days, except for the first and the last which have less than 15 days. The intervals were numbered from -13 to +13; interval 0 was centred on the day of opposition. For each of these intervals the total time for which Jupiter was observed was computed as well as the total time during which Jupiter events occurred. The ratio of the latter to the former gives the percentage probability of occurrence for that particular interval. (No notice was taken of the frequency distribution of the bursts.) The number of events during the five apparitions were 57, 247, 354, 429 and 229 respectively. The results for each apparition are shown in Figs. 1–5.

On combining the values for the five apparitions, that is, calculating the average percentage probability for correspondingly numbered intervals, a most interesting result is obtained (Fig. 6).

From the graph it appears that the percentage probability of observing decametric emission is much higher before opposition than after opposition, the ratio being approximately 3:2. Furthermore, a sudden 'cut-off' after opposition is evident.

G. A. Dulk³ has drawn up histograms for the first and second half of the apparitions of 1963 and 1964. The higher probability of occurrence is quite noticeable, and he suggested that the difference might be attributed to the

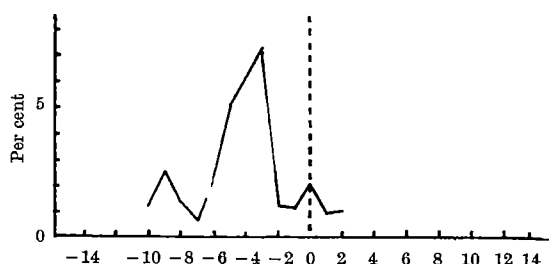


Fig. 1. Percentage probability versus interval number for 1960

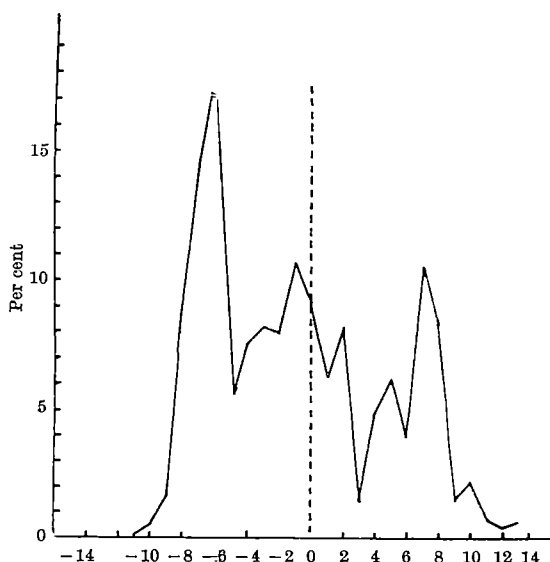


Fig. 2. Percentage probability versus interval number for 1961

influence on Jupiter reception of the maximum usable frequency of the Earth's ionosphere.

To check whether the cut-off frequency of the ionosphere has any noticeable influence, the 'mean lowest frequency' observed was calculated for each interval for the five

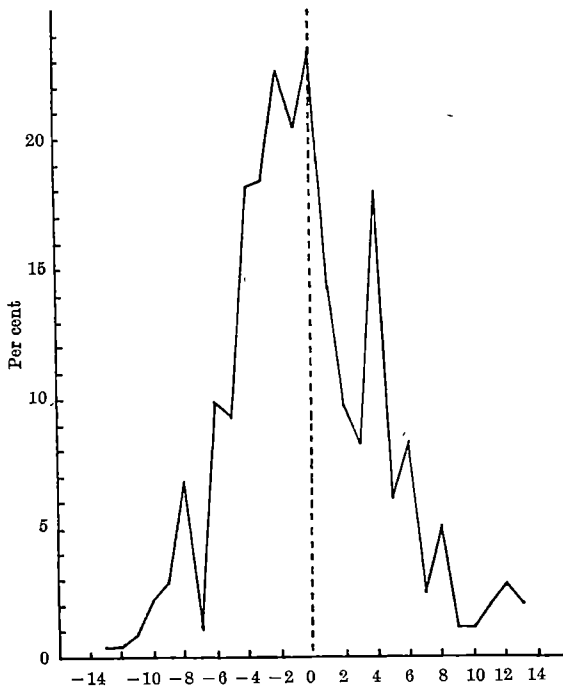


Fig. 3. Percentage probability versus interval number for 1962

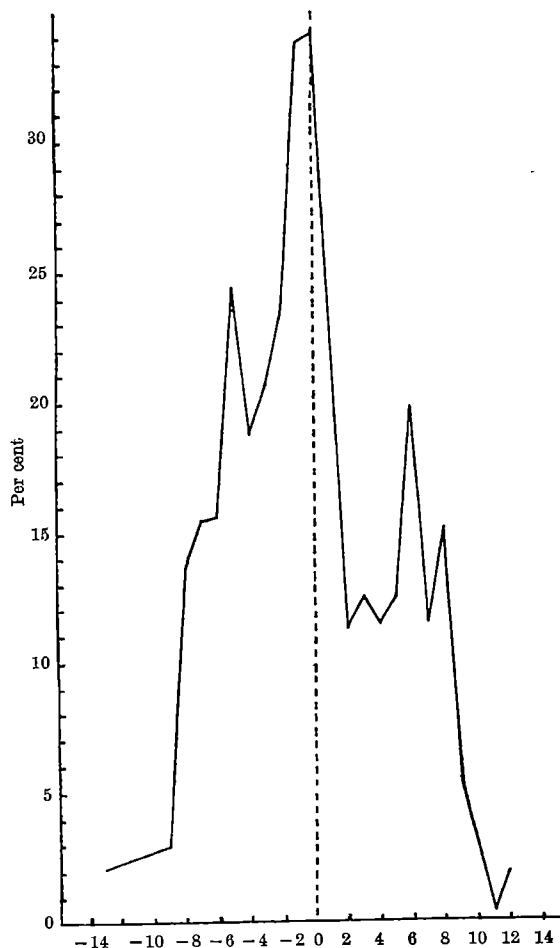


Fig. 4. Percentage probability versus interval number for 1963

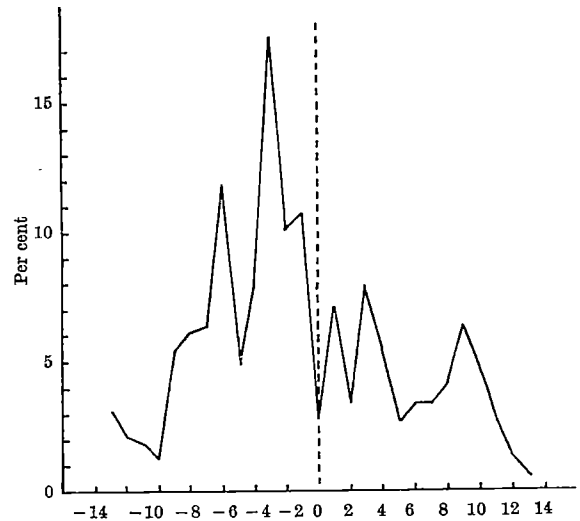


Fig. 5. Percentage probability versus interval number for 1964

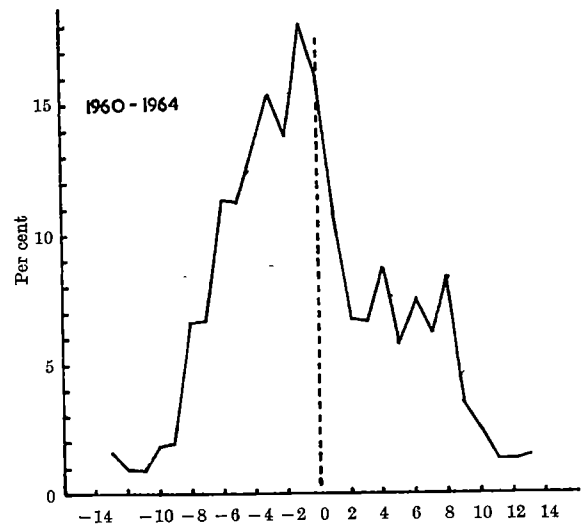


Fig. 6. Percentage probability versus interval number for the five-year period 1960-1964

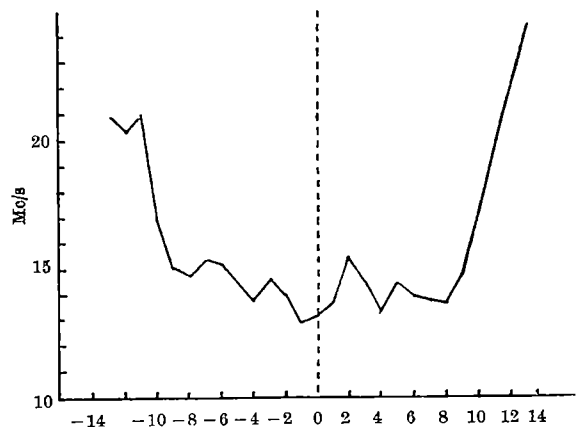


Fig. 7. 'Mean lowest frequency' versus interval number for the five-year period 1960-1964

apparitions. This was done by noting the lowest frequency observed and totalling over the fifteen days interval, the sum then being divided by the number of events in the interval. The results were plotted as Fig. 7.

From Fig. 7 it seems obvious that the variation of the mean lowest frequency observed does not account at all for the large drop in percentage probability after opposition. The steady level reached in Fig. 6 from interval 2 to interval 8 emphasizes the discrepancy.

Furthermore, it can be seen by comparing Figs. 6 and 7 that the rise in percentage probability starting at interval -10 and the fall of percentage probability starting at interval +8 can be explained by the rise in cut-off frequency.

The asymmetry of Fig. 6 suggests that the emission from the planet Jupiter does, in a certain way, depend on the relative position of the Sun, Earth and Jupiter, the higher percentage probability of receiving Jupiter noise being before opposition. Such a pattern of radiation can be produced by a magnetospheric tail. The tail, assuming it exists, would be swept back from the Sun-Jupiter line by an amount depending on the ratio of Jupiter's velocity in its orbit to the velocity of the solar wind in the vicinity of Jupiter. This means that the angle which the tail sub-

tends at the Earth would vary with the position of the Earth relative to the Sun-Jupiter line, the tail subtending a larger angle before opposition, the maximum value being reached three months before. If this source is anisotropic a sudden cut-off, such as shown in Fig. 6, might be expected.

Fig. 6 can therefore be explained if one assumes two sources, although they might have similar mechanisms, one closely related to the rotation of Jupiter, and Io related, the other having its origin in a Jovian magnetospheric tail.

¹ Bigg, E. K., *Nature*, **203**, 008 (1964).

² Warwick, J. W., and Kreiss, W. T., *IGY Solar Activity Report Ser. No. 28* (1964).

³ Dulk, G. A. (private communication).

WELDING BY HIGH-VELOCITY PARTICLE IMPACT

By DR. ROBERT F. ROLSTEN*

Technical Operations Research, Burlington, Massachusetts

ALAN K. HOPKINS

Air Force Materials Laboratory, Wright-Patterson Air Force Base, Dayton, Ohio

AND

HAROLD H. HUNT

5965 Manon Street, La Mesa, California

STACKS of target plates have been spot-welded by the hypervelocity impact of a microparticle¹. Parts of the plate surfaces became plastic under the impulsive load and were welded. Metallographic examination of the cross-section of the weld indicated that the structure was rippled². The ripples show a definite crest structure, which may explain the mode of their formation; that is, the movement of the plastic surfaces at different velocities while the surfaces are held together under the high pressure produced by the projectile impact. The ripple effect associated with the welding may be described as "surface jetting", which enhances welding because of the maximum surface friction and the increased contact area at the interface.

In an investigation of these impact spot welds we have conclusively related the weld-ripple phenomenon to the explosive welding of metals as described by Rinehart and Pearson³ and Cowan and Holtzmann⁴ and to the surface jetting described by Walsh *et al.*⁵, Allen *et al.*⁶, and Abrahamson⁷.

Hypervelocity particle impact welding was obtained by firing 0.005-g 'Mylar' cylinders into stacked targets of



Fig. 2. Cross-section of welds. Area indicated by arrow in Fig. 1

aluminium or copper plates or a combination of aluminium and copper plates. In one experiment a target of 1100-0 aluminium plates alternating in thicknesses of 0.064 in. and 0.010 in. was impacted in vacuum by a 'Mylar' projectile at a measured velocity of 28,200 ft./sec. A profile of this target is shown in Fig. 1. Welds were produced between the third (0.064-in. thick) and fourth (0.010-in. thick) plates and between the fourth and the fifth (0.064-in. thick) plates. The wavy, or rippled, interfaces of these welds, shown in Fig. 2, have been attributed to the phenomenon of surface jetting.

Welding did not always occur during hypervelocity impact on a stack of plates. In some experiments the rippled interface and weld were produced, but the weld bond was ruptured by shock in the parent metal plates, and the plates became separated. This forceful separation of a once smooth, rippled and welded structure resulted in a sawtooth and free surface, as seen in Fig. 3. In other experiments a weld did not form or was exceptionally weak, but the mating surfaces, plastic and moving at different velocities, were left with a rippled surface, as shown in Fig. 4.

* Consultant, 5141 Willow Crest Road, Dayton, Ohio.

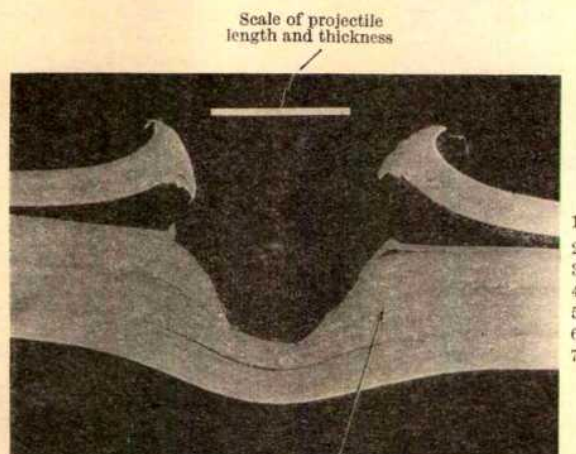


Fig. 1. Welded 1100-0 aluminium plates. Impact velocity, 28,200 ft./sec. Section at arrow shown in Fig. 2

The target shown in Fig. 4 was a two-plate target consisting of a 0.090-in.-thick plate of 6061-T6 aluminium in contact with a 0.375-in.-thick plate of the same material. It was impacted in air at 15,000 ft./sec by a 0.25-in.-diam. right circular steel cylinder of 0.5-g mass. Fig. 5 is a diagram of the sequence of events which we believe occurred during impact on the target in Fig. 4, which punctured the two-plate target system and produced concentric ripples around the puncture. In Figs. 4 and 5 we have designated the outer boundary of a series of concentric ripples as *A*; the outer boundary of a galled, or chaffed, ring-shaped area, evidently caused by relative motion of the two surfaces while they were tightly compressed, as *B*; and the outer edge of the upward bulge of the surface produced by lateral displacement of material surrounding the puncture as *C*.

The outer radius of the chaffed ring (*B*, Fig. 4) in a two-plate system, by our hypothesis, should be a measure of the time for penetration of the first plate according to the following relation:

$$R_r - R_p = f(V_w, T, E, V_p)$$

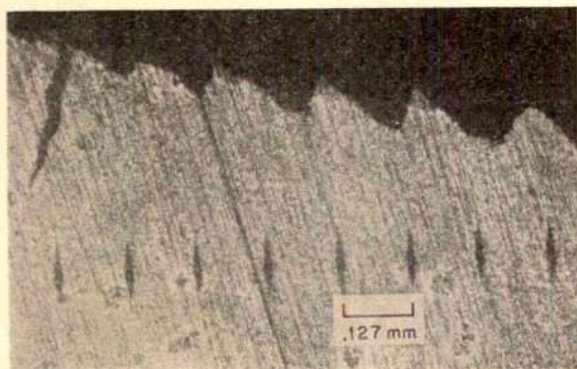


Fig. 3. Sawtooth structure produced by forceful separation of welded plates

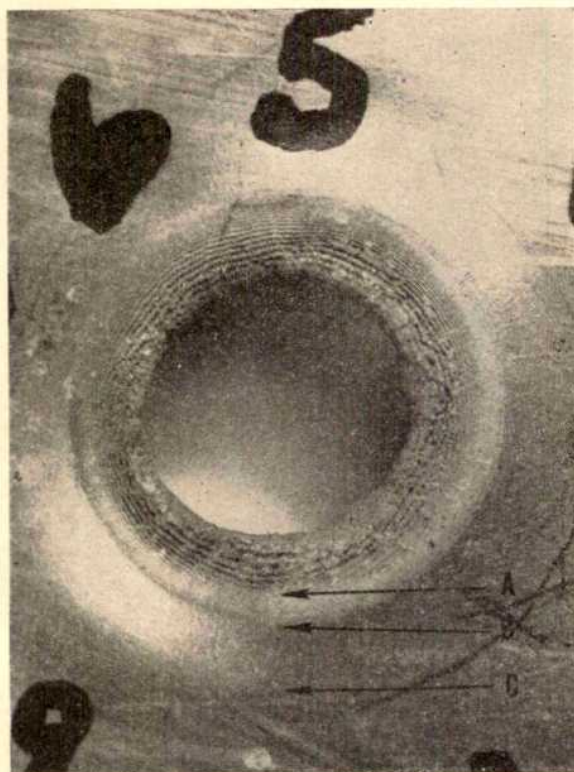


Fig. 4. Second, or bottom, plate with concentric ripples (*A*), galled ring (*B*), and distorted surface (*C*) referred to in Fig. 5

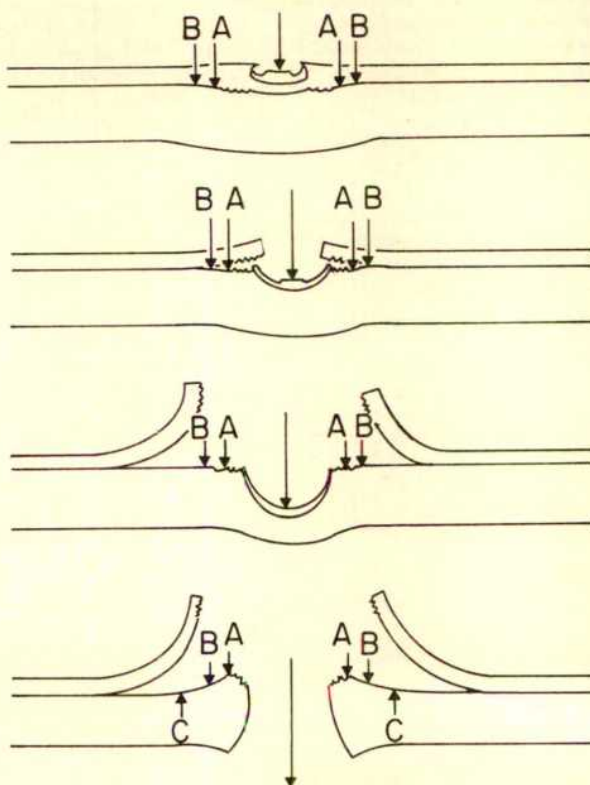


Fig. 5. Puncture of a two-plate system

Top to bottom:
A shock-wave in the first plate from lateral pressure of the projectile reduces to an elastic wave at *A*, the outer boundary of the ripple pattern. The elastic wave reaches *B*, the outer boundary of the galled ring, as the projectile punctures the first plate. The pressure holding the plates in contact is reversed by elastic rebound and ejecta now moving from the developing crater in the second plate.
The first plate 'petals' under pressure of ejecta from the second plate. Lateral plastic and shock waves are set up in the second plate.
The lateral elastic wave reaches *C*, the outer edge of the upward bulge of the surface as the second plate is punctured.

where R_r is the outer radius of the chaffed ring, R_p is the radius of the projectile on impact, V_w is the wave velocity, T is the thickness of the first plate, E is the elastic recoil of the first plate, and V_p is the velocity of penetration. Data to establish the functional relation are lacking. However, the available data do show that the length of the outer radius of the galled ring is a function of the thickness of the first plate and an inverse function of impact velocity. This has been determined from the experiments with right circular cylindrical steel projectiles 0.25 in. in diameter with a 0.5-g mass fired in air into a stack of two 6061-T6 aluminium plates (Table 1).

We postulate that the ripple structure in Fig. 4 resulted from dynamic instability of the shock wave in the first (top) plate at the interface of the two plates. Material in the first plate was being forced outward radially. The chaffing, or galling, in the area between *A* and *B* was caused by the same shock wave after it had been reduced to an elastic wave. The welling up of the surface, which extends from *B* to *C*, resulted from shock and plastic waves produced in the second (bottom) plate during its puncture. The area between *B* and *C* is not related to the rippled or the galled area since the surfaces of the first and the second plates were no longer in close contact.

Table 1. SIZE DEPENDENCE OF CHAFFED RING ON IMPACT VELOCITY AND THICKNESS OF FIRST (TOP) PLATE

Measured impact velocity, ft./sec	Thickness of first plate (in.)	Radius of chaffed ring (<i>B</i> , Fig. 4) (in.)
9,400	0.090	0.55
15,000	0.090	0.53
14,700	0.1875	0.578

The first plate was forced away from the second plate and deformed in a direction opposite that of the projectile movement by the force exerted by the relief wave sweeping over the upper free surface of the first plate and by the ejecta from the crater in the second plate and to a lesser extent by its elastic reaction to the release of impact pressure. No evidence of welding was noted in this experiment, although the same rippled structure is associated with welding.

In some stacked targets welding has been observed to occur between the first and second plates of the structure and in successive plate interfaces; in other targets the welds did not occur before the second plate and third plate interface. If either the relief wave or the crater ejecta from the projectile and the second and successive layers force the first plate away from the structure, then a criterion for the welds to occur in this first interface must be the tensile strength of the first plate material. A high-ultimate tensile strength material (6061-T6 or 2024-T3) will resist this upward deformation and therefore may enhance the conditions for weldment; whereas a low-strength material (1100-0), being more ductile, may easily deform and tear the interlocked ripple crests and form sawteeth.

The impact energy in the experiment referred to in Fig. 1 was 1.85×10^9 ergs. Complete deceleration of the projectile was accomplished in 0.8475 cm, giving a force of 2.185×10^9 dynes. A circle with a radius of 0.565 cm will include the welds, which yields a surface area of 1 cm². If we assume that the entire impact pressure was applied normal to the target face simultaneously and was evenly distributed over 1 sq. cm, the pressure would be 2.185×10^9 dynes/cm², or 31,750 lb./in.², a direct pressure which we believe to be far below the threshold necessary for welding. The very high pressure contained within the thin expanding circle at the shock-wave front must be the cause for the welding that occurred⁸.

The impact pressure at the surfaces of a two-plate target system forces the target interface surfaces into intimate contact during the time the projectile is moving through the first plate. At this same time, the projectile with the target material directly under the point of impact expands radially and exerts a lateral thrust (cavitation) tending to enlarge the hole in the first plate. The lateral disturbance is propagated as a shock-wave. To permit sufficient heat transfer to induce all parts of the shock-wave to propagate at the same velocity, it is necessary

for the whole wave front to be very thin, or 'steep'. Material in this shock front is highly compressed. The high-pressure wave front sweeping radially outward in the first plate can act across the interface since the surfaces of the two plates are in intimate contact. The inertia of the materials responding to the pressure across the interface creates a dynamic instability in the pressure at the interface due to the alternation of jetting and non-jetting collisions. This causes a rippling of the surfaces as the shock-wave sweeps outward.

The rippling of the surface structure increases the surface area and produces maximum shear, which opens wide fissures in the surface layers of adsorbed gases, reactants, and foreign materials. This dilution and shear effect, combined with the induced turbulence and the high pressures within the wave-front, promotes intimate contact and permits the linking of metal atoms across the interface. If the surfaces are reasonably free of foreign substances and entrapped air, mate well, and are held in firm contact, then bonding may result. This bonding may be enhanced by an interlocking action of the ripples.

The experiments reported here suggest that satisfactory welds can be made in a stacked target with a minimum amount of energy (where the surfaces of the target plates are held in intimate contact under high pressure) by projecting a strong shock-wave to one of the parts more or less parallel to the interface. The conditions for the production of the desired weld are dependent on the plate material, surface condition and thickness and on the strength and propagation velocity of the shock-wave.

We thank the Utah Research and Development Company for performing the hypervelocity impact experiments at the Air Force Materials Laboratory, and Lieutenant W. A. Dean, who conducted the metallurgical analyses of all specimens.

¹ Rolsten, R. F., Dean, W. A., and O'Neil, R. W., *AIAA J.*, **3**, 1966 (1965).

² Rolsten, R. F., Hunt, H. E., and Meyer, K. H., *J. App. Phys.*, **35**, 1655 (1964).

³ Rinehart, J. S., and Pearson, J., *The Explosive Working of Metals*, 305 (Macmillan Co., New York, 1963).

⁴ Cowan, G. R., and Holtzmann, A. H., *J. App. Phys.*, **34**, 928 (1963).

⁵ Walsh, J. M., Shreffler, R. G., and Willig, R. J., *J. App. Phys.*, **24**, 349 (1953).

⁶ Allen, W. A., Mapes, J. M., and Wilson, W. G., *J. App. Phys.*, **25**, 675 (1954).

⁷ Abrahamson, G. R., *J. App. Mech.*, 519 (1961).

⁸ A shock-wave also results in an embossed surface. A scratch placed across the surface of one plate was observed in raised relief in the mating face. This reproduction of the scratch did not extend into the elastic region; that is, not in the region of BC, Fig. 4.

SEISMICITY AND OTHER PROPERTIES OF GEOLOGICAL PROVINCES

By PROF. E. R. KANASEWICH

Department of Physics, University of Alberta, Edmonton, Alberta

MUCH effort has been expended in sub-dividing the Canadian Shield into suitable units of study. Early classifications¹⁻⁴ were based primarily on stratigraphic, petrological and structural similarities. Features such as parallel fold belts, foliation trends and truncation of ancient orogenic centres by younger mountain belts were invaluable aids in this work. Lately, the classification has been carried out with the aid of geochronological data⁵⁻¹⁴. The classification is now based on the repeated recurrence of certain dates over widespread areas when K/Ar, Rb/Sr and Pb/U dating methods are used. These dates are related to periods of severe metamorphism during which minerals crystallize or recrystallize. Often they can be correlated with similar events on widely separated regions of the Earth.

Most of the geochronological data for North America have been assembled on a large-scale map and the continent has been sub-divided according to the latest available information. References used but not mentioned in the text are listed at the end of this article. The

compilation now consists of nearly 2,000 dates, with the majority being potassium-argon determinations on biotites, muscovites and hornblende. Rubidium-strontium whole-rock ages and lead-uranium concordia and discordia¹⁵ ages were included wherever available. A small-scale copy showing the geological provinces and the general location of the data is reproduced in Fig. 1.

The classification into geological provinces in Fig. 1 and Table 1 is based on the oldest reliable dates present in each area. A certain amount of subjective judgment cannot be avoided and particular points of difficulty are discussed below. When considering the K/Ar data alone, the boundaries proved to be surprisingly sharp considering the presence of over-thrusting, tectonic inliers and the effect of multiple metamorphism along adjacent orogenic belts. When model lead and strontium whole rock ages are also considered the boundaries are less distinct.

So far as possible, secondary features, such as miogeosynclinal mountains, basins and troughs known to be

Table 1. CLASSIFICATION OF GEOLOGICAL PROVINCES BY LIMITS ON RADIOACTIVE AGE DETERMINATIONS

Geological province	Range of age determinations (m.y.)
Superior	3,300-2,300
Slave	2,900-2,200
Churchill	2,300-1,700
Penokian	1,900-1,400
Ozarkian	1,450-1,200
Grenville	1,200- 600
Innuvian	600- 200
Appalachian	550- 200
Cordilleran	350- 0

underlain by older crystalline rocks were not considered in this map. Neither were such igneous bodies as the Duluth gabbro, which has a well-established age of 1,000 million years on the basis of K/Ar, Rb/Sr (ref. 9) and strontium whole-rock analyses¹⁶. This large sill occurs among rocks metamorphosed and folded about 1,700-1,800 m.y. ago and was emplaced in a series of intrusions into these older rocks through fractures formed in response to the severe 1,000-m.y. event which had its greatest effect in the Grenville province 500 miles to the east.

The nomenclature closely follows that used by Stockwell in the Geological Survey of Canada^{12,13}. Gastil¹⁰ recognized that the data indicate the necessity of at least two new geological provinces. The first of these is here called the Penokian⁹ province and includes the large strip from eastern California to Sudbury. It appears to extend into the region of the Purcell Mountains in British Columbia on the basis of model lead ages¹⁷ in the Kimberley, Coeur D'Alene and Kootenay are districts and two K/Ar dates^{18,19}. The Beltian rocks may be underlain by an older crystalline basement, but there is no evidence for this at the present time.

The second new province includes the area between the Penokian and Grenville and is here termed the Ozarkian after the Ozark uplift in south-eastern Missouri where the Pre-Cambrian basement rocks are exposed. The dated outcrops occur mainly in Missouri, Kansas, Texas, New Mexico and Arizona. The major tectonic activity occurred here between 1,300 and 1,450 m.y. ago.

The sub-division of the continental crust is based largely on K/Ar data. A certain amount of caution must be shown in using the results, since the mathematical equations may not model the physical environment of the mineral closely enough. All minerals are susceptible to a certain loss of daughter products during episodes of metamorphic activity. Furthermore, the present Pre-Cambrian surface represents such a deep erosional level that what we now sample in many places are the old mountain roots which were at an elevated temperature during much of geological time. A small but continuous amount of diffusion cannot be discounted without corroborating evidence.

Loss of daughter products will cause the age to be too low. However, a mineral may also incorporate some argon in the lattice during the time of crystallization and produce a measured age that is too high. Richards and Pidgeon²⁰ have observed that some biotites from the Broken Hill district, Australia, yielded ages twice the Rb/Sr ages. Evans and Tarney²¹ report more than 20 whole-rock K/Ar dates on dolerites in Scotland between 1,550 and 3,860 m.y. The Rb/Sr ages on a biotite and a whole-

rock sample are 2,190 m.y. Workers from the Geological Survey of Canada²² have dated a biotite in Newfoundland as 3,730 m.y. with the K/Ar method and 900 m.y. with the Rb/Sr method. These and other reports^{13,23,24} indicate that K/Ar data must be checked carefully. If there are a few dates which are 400 per cent too large, there may be more that are only 10 per cent too large.

A further difficulty which may be recognized is that rocks of clastic origin may occasionally be dated. Thus Burwash and Baadsgaard²⁵ give K/Ar dates of 2,400 m.y. on the basal Nonacho Conglomerate. These lie on a gneissic complex of the Churchill province with biotite dates of 1,800 m.y. The interpretation of dates from rocks which may have been transported long distances is not unambiguous and must be evaluated carefully when considering theories on continental growth^{6,26}.

The difficulties mentioned in the preceding two paragraphs are common to all dating methods to a certain degree. Research workers carrying out the geochemistry and mass spectrometry have recognized these and other difficulties and interpreted their results with caution. Usually a representative suite of minerals is dated and consistent results on cogenetic minerals are given more weight. Samples which satisfy the assumptions built into the mathematical formulae are used in the interpretation. Such caution is not evident in many statistical compilations to determine the 'peaks of orogenic activity'. Histograms showing peaks of K/Ar results are particularly susceptible of misinterpretation since the peak may lag the period of most intense metamorphism due to argon loss in subsequent episodes. Samples from contact metamorphic zones²⁷ are often dated but are difficult to

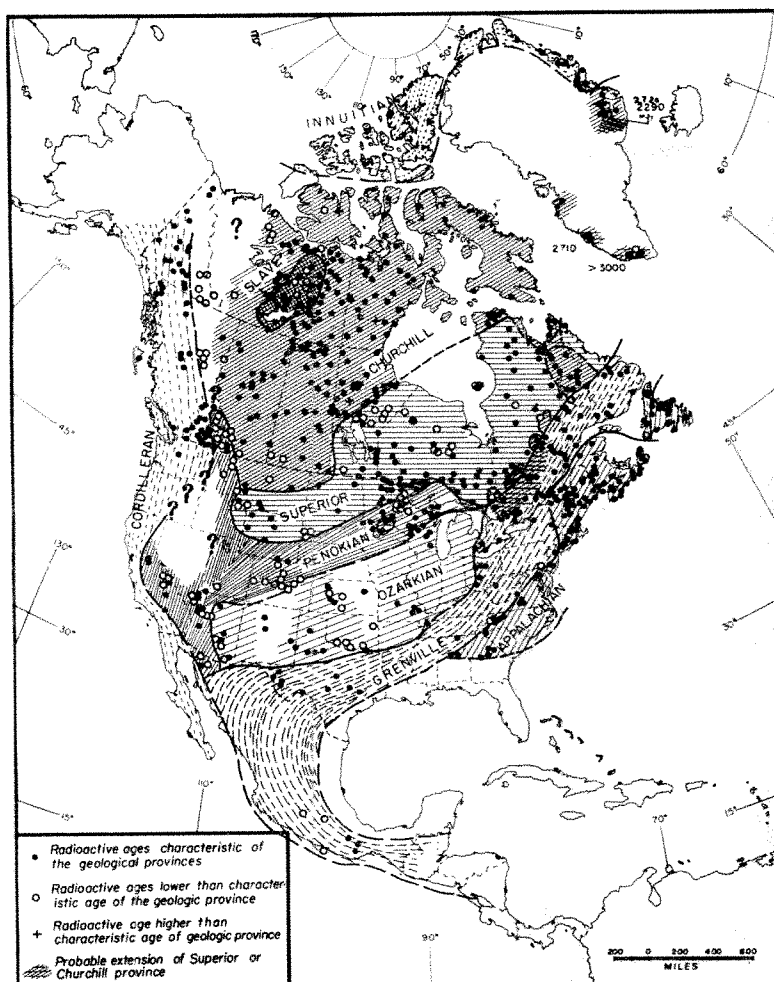


Fig. 1. Geological provinces of North America

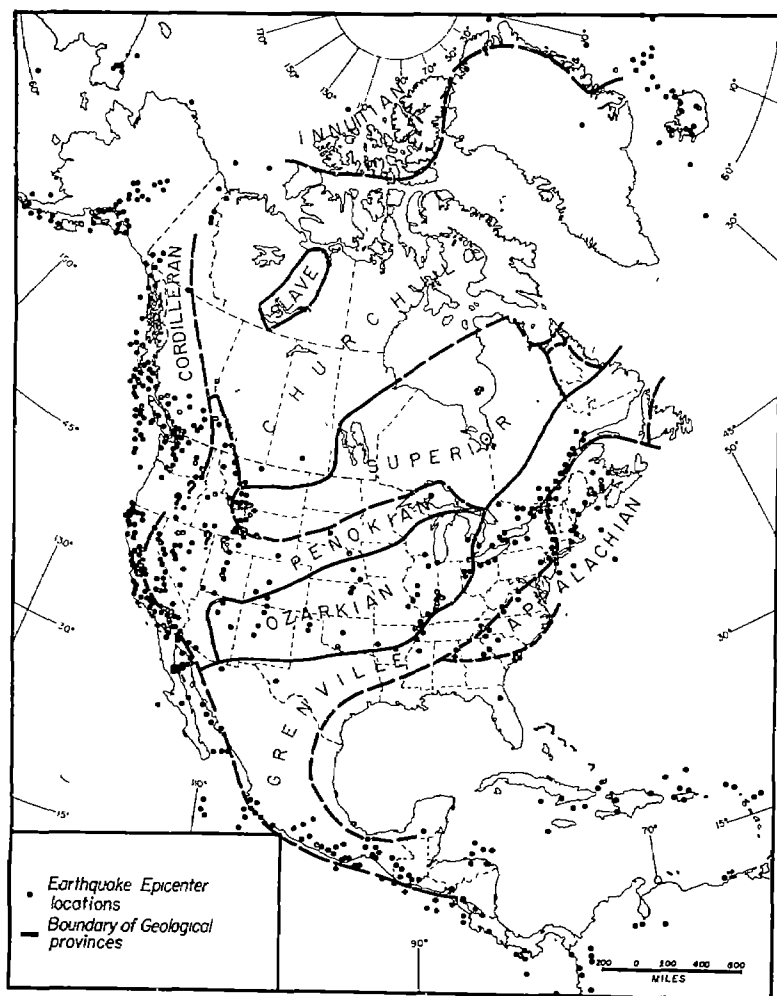


Fig. 2. Instrumentally determined earthquake epicentres in North America showing the correlation with some boundaries between geological provinces

recognize. Until a large amount of Rb/Sr and Pb/U data is available the K/Ar results from the Pre-Cambrian era should be used only in resolving broad regional structure. In particular, caution is necessary in subdividing geological provinces or the Pre-Cambrian time-scale on the basis of such low-resolution data.

An extreme example of the complexity that is possible is illustrated along the southern margin of the Superior province in Ontario and Quebec. There is a group of well-established ages between 1,600 and 2,300 m.y.²⁸⁻³⁰ which may be correlated with early orogenic activity in the Churchill province or late events in the Superior province. From lead isotope evidence³¹ and whole rock strontium results³² it appears that these ages extend into the region classified as the Grenville province on the basis of potassium-argon dates. An attempt was made to indicate the complexity in this area by cross-hatching in Fig. 1. From Fig. 1 it is also clear why the Sudbury area has had such a complicated pattern of ages. It forms the focal point of five geological provinces and has consequently been affected to some extent by orogenic activity in each province.

Several boundaries are the site of rapid and large-scale variations of physical properties. For example, the north-western boundary of the Superior province is bounded by a prominent group of gravity anomalies called the Nelson River high³³. The pattern of arcuate alternating positive and negative Bouguer anomalies is suggestive of the isostatic anomalies along the Indonesian Trench³⁴. Another prominent group of anomalies follows the St. Lawrence River³⁵ and into the United States³⁵ along the

entire length of the boundary between the Grenville and Appalachian provinces. The boundaries of the Slave and Grenville provinces also correlate with aeromagnetic trends. These correlations indicate that the boundaries between geological provinces have played an important part in the development of the continents.

A characteristic of the oldest parts of the continent is obtained from seismic evidence. The area has a very small amount of earthquake activity at the present time. This would seem to strengthen the arguments for continental growth. It is difficult to see why tectonic activity should avoid the oldest portions of North America unless this is a natural sequence of events in the evolution of the continental crust and upper mantle. Similar remarks apply to the moderately sized continents of South America, Australia and Antarctica. The pattern is more complex for the two largest continents of Africa and Asia. Africa, in particular, appears to be breaking apart along the Rift Valleys.

A compilation of instrumentally determined epicentres as reported by the Dominion Observatory, Canada, and the United States Coast and Geodetic Survey is presented in Fig. 2. The depth of the foci under North America is generally less than 70 km, but no attempt has been made to classify the shocks by depth or magnitude. The location of the epicentres may be uncertain by one degree of latitude and longitude for small earthquakes. With the new seismograph network and digital computation facilities now being established, it will soon be possible to prepare comprehensive maps with depth, magnitude and *P* nodal solutions from first motion studies.

The boundaries between several geological provinces have a large amount of earthquake activity. This is more evident on the eastern side of the continent where the level of activity is not so great. Concentrations of earthquakes occur along the boundaries between the Ozarkian, Grenville and Appalachian provinces. There is little information on crystalline basement rocks in the western United States and British Columbia. Consequently the boundaries of the provinces are largely undetermined. The postulated correlation between earthquake epicentres and geological boundaries suggests that elastic stresses may be relieved along the system of faults and fractures which may parallel the boundaries. These fractures probably extend to great depth in the crust and have possibly existed since the inception of orogenic activity along these boundaries.

The North American continent may be divided into nine geological provinces on the basis of the oldest K/Ar, Rb/Sr, Pb/U and model lead ages in the crystalline basement rocks. On this basis it is evident that only a minor amount of continental growth has occurred in the past billion years. The central continental nucleus, called the Superior province, extends from Greenland to Wyoming and was formed more than three billion years ago. The Superior, Slave and Churchill provinces have negligible seismic activity at the present time, and these very old stable blocks must have an upper mantle in which the radioactive elements have been depleted through transfer into the crust to a greater extent than the rest of the continent. Surface wave investigations³⁶ and long-range observations on longitudinal waves³⁷ also indicate that there are significant differences in the upper mantle

under the Canadian Shield as compared with other parts of the continent. Over North America the pattern of ages is defined sufficiently to test the possibility of continental drift. Unfortunately there are insufficient data on other continents to make an evaluation of this hypothesis.

A tentative correlation has been found between the boundaries of geological provinces and zones of earthquake activity. It appears that elastic stresses may be relieved along fractures which extend to great depths in the crust and have existed since the inception of orogenic activity along these boundaries.

- ¹ Wilson, M. E., *Geol. der Erde, Geology of North America*, 1, 232 (1939).
- ² Gill, J. E., *Eighteenth Intern. Congr.*, 13, 97 (1940).
- ³ Gill, J. E., *Trans. Roy. Soc. Canad.*, 43, 61 (1949).
- ⁴ Wilson, J. T., *Trans. Canad. Inst. Mining Met.*, 52, 231 (1949).
- ⁵ Wilson, J. T., *Trans. Roy. Soc. Canad.*, 43, 157 (1949).
- ⁶ Wilson, J. T., *Trans. Amer. Geophys. Union*, 33, 444 (1952).
- ⁷ Cumming, G. L., Wilson, J. T., Farquhar, R. M., and Russell, R. D., *Geol. Assoc. Canad., Proc.*, 1, 27 (1955).
- ⁸ Farquhar, R. M., and Russell, R. D., *Roy. Soc. Canad. Spec. Publ.* No. 2, 28 (1957).
- ⁹ Goldich, S. S., Nier, A. O., Baadsgaard, H., Hoffman, J. H., and Kreuger, H. W., *The Precambrian Geol. and Geochronology at Minnesota* (Univ. Minnesota Press, Minneapolis, 1961).
- ¹⁰ Gast, G., *Amer. J. Sci.*, 258, 1 (1960).
- ¹¹ Burwash, R. A., Baadsgaard, H., and Peterman, Z. E., *J. Geophys. Res.*, 67, 1617 (1962).
- ¹² Stockwell, C. H., *Geol. Survey Can., Paper* 61-17, 108 (1961).
- ¹³ Stockwell, C. H., *Geol. Survey Can., Paper* 64-17 (1964).
- ¹⁴ Engel, A. E. J., *Science*, 140, 143 (1963).
- ¹⁵ Tilton, G. R., *J. Geophys. Res.*, 65, 2933 (1960).
- ¹⁶ Faure, G., *Ninth Ann. Progr. Rep., M.I.T.*, 225 (1961).
- ¹⁷ Sinclair, A. J., *IUGG, Intern. Assoc. Volcanol.* (Abstr.), Berkeley, 7 (1963).
- ¹⁸ Hunt, G. H., *Geol. Soc. Amer.*, 1960 Meeting (Abstr.), 125 (1960).
- ¹⁹ Hunt, G. H., Ph D. thesis (Univ. Alberta, 1961).
- ²⁰ Richards, J. R., and Pidgeon, R. T., *J. Geol. Soc. Austral.*, 19, 243 (1963).
- ²¹ Evans, C. R., and Tarney, J., *Nature*, 204, 638 (1964).
- ²² Leech, G. B., Lowdon, J. A., Stockwell, C. H., and Wanless, R. K., *Geol. Survey Can., Paper* 63-17 (1963).
- ²³ Lovering, J. F., and Richards, J. R., *J. Geophys. Res.*, 69, 4895 (1964).
- ²⁴ McDougall, I., and Green, D. H., *Norsk. Geol. Tidsskr.*, 44, 183 (1964).
- ²⁵ Burwash, R. A., and Baadsgaard, H., *The Tectonics of the Canadian Shield*, 22 (Univ. Toronto Press, 1962).
- ²⁶ Lawson, A. C., *Bull. Geol. Soc. Amer.*, 43, 353 (1932).
- ²⁷ Davis, G. L., Tilton, G. R., Aldrich, L. T., Hart, S. R., Steiger, R. H., and Kouvo, O., *Carnegie Inst. of Wash. Ann. Rep.*, Washington, D C, 218 (1963).
- ²⁸ Fairbairn, H. W., Hurley, P. M., and Pinson, W. H., *Eighth Ann. Prog. Rept. M.I.T.*, 7 (1960).
- ²⁹ Ulrych, T. J., and Russell, R. D., *Geochim. Cosmochim. Acta.*, 28, 455 (1964).
- ³⁰ Kanasevich, E. R., and Farquhar, R. M., *Canad. J. Earth Sci.*, 2, 361 (1965).
- ³¹ Farquhar, R. M., *Trans. Amer. Geophys. Union* (Abstr.), 43rd Annual Meeting, Washington (1962).
- ³² Grant, J. A., *Science*, 146, 1049 (1964).

- ³³ *Gravity Anomaly Map of Canada*, Dominion Observatory, Ottawa (1957).
- ³⁴ Vening Meinesz, F. A., *Publ. Neth. Geol. Comm., Waltman, Delft*, 2 (1934).
- ³⁵ Woolard, G. P., *Bouguer Gravity Anomaly Map of the United States* (U.S. Geological Survey, Washington, D C, 1964).
- ³⁶ Brune, J., and Dorman, J., *Bull. Seis. Soc. Amer.*, 53, 167 (1963).
- ³⁷ Cumming, G. L., and Kanasevich, E. R., *Nature*, 206, 248 (1965).

THE FOLLOWING REFERENCES WERE USED IN COMPILING FIG 1. OLDER REFERENCES ARE SUMMARIZED IN KANASEVICH (1962)

- Armstrong, R. L., *Geol. Soc. Amer. Bull.*, 74, 1189 (1963).
- Baadsgaard, H., Cumming, G. L., Folinsbee, R. E., and Godfrey, S. D., *Geochronology in Canada*, 20 (Univ. Toronto Press, 1964).
- Banks, P. O., and Silver, L. T., *Trans. Amer. Geophys. Un. (Abstr.)*, 44, 107 (1963).
- Brookins, D. G., *Amer. J. Sci.*, 263, 1 (1965).
- Catanzaro, E. J., *J. Geophys. Res.*, 68, 2045 (1963).
- Catanzaro, E. J., and Kulp, J. L., *Geochim. Cosmochim. Acta*, 28, 87 (1964).
- Fairbairn, H. W., Hurley, P. M., and Pinson, W. H., *J. Geophys. Res.*, 69, 4889 (1964).
- Fairbairn, H. W., Hurley, P. M., and Pinson, W. H., *Geol. Soc. Amer. Bull.*, 75, 253 (1964).
- Ferris, jun., C. F., and Krueger, H. W., *Geol. Soc. Amer. Bull.*, 75, 1051 (1964).
- Gabriele, H., and Reesor, J. E., *Geochronology in Canada*, 96 (Univ. Toronto Press, 1964).
- Godfrey, J. D., and Baadsgaard, H., *The Tectonics of the Canadian Shield*, 30 (Univ. Toronto Press, Toronto, 1962).
- Haller, J., and Kulp, J. E., *Meddelelser om Gronland*, 171, 1 (1962).
- Hills, A., and Gast, P. W., *Geol. Soc. Amer. Bull.*, 75, 759 (1964).
- Kanasevich, E. R., Ph D. thesis, Univ. British Columbia, Vancouver (1962).
- Lamphere, M. A., Wasserburg, G. T., Albee, A. L., and Tilton, G. R., *Isotopic and Cosmic Chemistry*, 269 (North-Holland Publ. Co., Amsterdam, 1963).
- Lamphere, M. A., MacKevett, jun., E. M., and Stern, J. W., *Science*, 145, 705 (1964).
- Lamphere, M. A., *J. Geol.*, 72, 381 (1964).
- Laphan, D. M., and Bassett, W. A., *Geol. Soc. Amer. Bull.*, 75, 661 (1964).
- Leech, G. B., Lowdon, J. A., Stockwell, C. H., and Wanless, R. K., *Geol. Survey Can., Paper* 63-17 (1963).
- Lowdon, J. A., Stockwell, C. H., Tipper, H. W., and Wanless, R. K., *Geol. Survey Can., Paper* 62-17 (1963).
- Morse, S. A., *Nature*, 203, 509 (1964).
- Poole, W. H., Kelley, D. G., and Neale, E. R. W., *Geochronology in Canada*, 61 (Univ. Toronto Press, 1964).
- Silver, L. T., and Green, J. C., *Trans. Amer. Geophys. Union (Abstr.)*, 44, 107 (1963).
- Slawson, W. F., Kanasevich, E. R., Ostic, R. G., and Farquhar, R. M., *Nature*, 200, 413 (1963).
- Snelling, N. J., *Geol. Survey Can. Bull.*, 85 (1962).
- Tilton, G. R., Wetherill, G. W., and Davis, G. L., *J. Geophys. Res.*, 67, 4011 (1962).
- Van Schmus, W. R., Wetherill, G. W., and Bickford, M. E., *J. Geophys. Res.*, 68, 5589 (1963).
- Wager, L. R., and Hamilton, E. I., *Nature*, 204, 1079 (1964).
- Wasserburg, G. J., Wetherill, G. W., Silver, L. T., and Flawn, P. T., *J. Geophys. Res.*, 67, 4021 (1962).
- Williams, H., *Geol. Survey Can., Paper* 64-17, 22 (1964).
- Zartman, R. E., Norton, J. J., and Stern, T. W., *Science*, 145, 479 (1964).
- Zartman, R. E., *J. Geophys. Res.*, 70, 965 (1965).

CARBON ISOTOPE RATIOS AND PALAEO-SALINITIES OF PURBECK-WEALDEN CARBONATES

By PROF. P. ALLEN

Sedimentology Research Laboratory, Department of Geology, University of Reading
AND

PROF. M. L. KEITH

Department of Geochemistry and Mineralogy, Pennsylvania State University

BIOLOGICAL and lithological evidence leads to the conclusion that the Purbeck-Wealden beds of southern England were deposited in a variable-salinity environment. Salinities apparently ranged from supersaline in the lower Purbeck (evaporites), to near-marine in the mid-Purbeck and upper Wealden (for example, 'cinder bed' members with oysters, echinoids, etc.), to freshwater in parts of the lower and upper Purbeck and lower and upper Wealden (Unio beds, horsetail soils, etc.).

Lateral and vertical fluctuations occurred at these extremes. Thus faunal gradients seem to exist in the Purbeck Cinder Bed¹⁻³, and many individual Wealden laminae are dominated by one genus at a time. On Wealden bedding planes *Unio*, *Viviparus* and *Physa*

tend to be 'incompatible' with *Neomiodon* and *Filosina*, especially among assemblages containing articulated shells. Only *Physa* is known 'at home' among the Wealden freshwater plant communities⁴. If the Wadhurst and Grinstead Clay sequences are partly transgressive they might be expected to show slightly elevated salinities⁵. But the macropalaeontological evidence, though purposely sought, has not been found⁶.

Geochemical evidence of depositional environment is difficult to interpret and unique solutions seem equally unlikely. We are not so sure as Walker⁷ that equivalent boron⁸ is a 'realistic index of palaeosalinity'. Quantitative interpretation of the Purbeck-Wealden environments is complicated by provenance of the sediments. Through

much of southern England they contain derived material (including illite-rich clay) from marine Upper Jurassic sources⁹. Variable proportions of mixed-layer clays¹⁰ and of associated boron could have resulted from Purbeck-Wealden recycling.

The carbon isotope ratios of limestones and some fossil carbonates have been shown^{11,12} to be affected by the proportional contributions of carbon-13-deficient carbon from land plants. They therefore provide additional evidence for differentiating marine and freshwater carbonate rocks. However, one must recognize that isotopic criteria, as well as trace-element criteria, fail to provide a clear-cut separate category for sedimentary rocks formed in transitional variable-salinity environments¹³. In deltas, estuaries and marginal bays, spatial and temporal changes of the carbon-13 : carbon-12 ratio of bicarbonate, food web and carbonates, may result from short-term changes in rainfall or the patterns of bars and distributaries, as well as from long-term advances and retreats of the sea. In shallow bays with restricted connexions to the open ocean, there may be additional complications due to variable amounts and isotopic compositions of the organic matter¹⁴ and of the carbon dioxide from its local decomposition^{15,16}.

Possible misinterpretations are to be expected in the palaeontological records as well, for example, where mass mortality of organisms results from abrupt salinity change, or where mollusc shells are transported, broken or size-sorted due to a change from static to dynamic hydraulic conditions.

The foregoing qualifications apply to the isotopic data here presented and to comparisons with other criteria used for environmental interpretation. Probably one should resist the temptation to sub-divide ancient variable-salinity environments except where (a) sufficient samples have been analysed to show lateral variation, and (b) there is a concordance of several indexes of environment.

Isotopic data are presented in Table 1, and the carbon isotopic compositions are shown diagrammatically in Fig. 1, for a stratigraphic sequence of Purbeck-Wealden limestone samples from southern England.

The graph of $\delta^{13}\text{C}$ variation (Fig. 1) can be interpreted, with reservations, as mainly representing relative variations in the influence of marine and continental waters. Reservations are based chiefly on the observation^{13,16} that $\delta^{13}\text{C}$ of modern organic carbonates exhibits an imperfect correlation with salinity of the water. The correlation is reasonably good in most transitional environments, but does not extend to shallow supersaline bays¹³.

As expected from their palaeontology, the Purbeck-Wealden carbonates show wide variations in carbon-13 content. An interesting upward trend is suggested: each succeeding carbon-13 minimum (and presumed minimum of marine influence) is more negative than the preceding. Stratigraphical horizons of the carbon-13 minima correspond with the virtually exclusive dominance of *Hydrobia* and *Valvata* (sample S8102), *Neomiodon* (sample S8114), *Equisetites in situ* (sample S8119) and ostracods (sample S8127). *Neomiodon* occurs in beds exhibiting a wide range of $\delta^{13}\text{C}$ and presumed salinity (as inferred by Casey⁴⁰ and confirmed by Hudson⁴¹), and its diagnostic significance should be questioned.

Maxima of carbon-13 content (and of presumed marine influence) also suggest, though less clearly, a general upward decline. They occur in the basal

Purbeck (sample S8101), again in the range Cinder Bed-Broken Shell Limestone (consistent with the presence of *Liostrea* and marine ostracods²², and the conclusion of Walker⁷) and near the base of the Wadhurst Clay (samples S8109, S8110, S8111: bed almost exclusively of *Neomiodon*). Successively smaller carbon-13 maxima occur in the lower Weald Clay above the Horsham Stone (*Filosina* beds: S8120 and S8121) and in the upper Wealden Shales (sample S8126, with oysters (*Liostrea*?), echinoids, ?*Viviparus*). The figure from the basal Purbeck limestones is consistent with Brown's recent conclusion that some of the basal deposits are marine or supersaline²⁴.

As a basis for comparison with prior data from elsewhere, an index mark is shown at $\delta^{13}\text{C} = -2$ parts per thousand (Fig. 1), the approximate mid-point between the means of marine and freshwater limestones analysed by Keith and Weber¹². We do not intend an arbitrary division of our present samples at $\delta^{13}\text{C} = -2$ parts per thousand, nor recommend that limestones from transitional environments should be more finely classified on the basis of carbon isotope ratios.

The oxygen isotope ratios (Table 1) do not show a strong correlation with $\delta^{13}\text{C}$, and there appears to be no advantage in using the 'Z' index of environmental discrimination based on both $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ (ref. 12). However, eight of the present samples with $\delta^{13}\text{C}$ greater than -2 parts per thousand have a mean $\delta^{18}\text{O}$ of -4.5 parts per thousand, whereas twenty-two samples with $\delta^{13}\text{C}$ less than -2 parts per thousand have a mean $\delta^{18}\text{O}$ of -6.7 parts per thousand. The original oxygen isotope record seems not to have been entirely destroyed by recrystallization.

We conclude that the new carbon isotope data: (1) confirm the complex variability of Purbeck-Wealden carbonate environments; (2) suggest an underlying tendency for carbon-13, and presumed salinity, to decline with time; (3) indicate horizons of possible maximum and minimum marine influence where more intensive palaeontological, petrological, sedimentological and geochemical work seems likely to be rewarding.

We thank John Kaufman for assistance with the isotopic analysis. The work was supported by

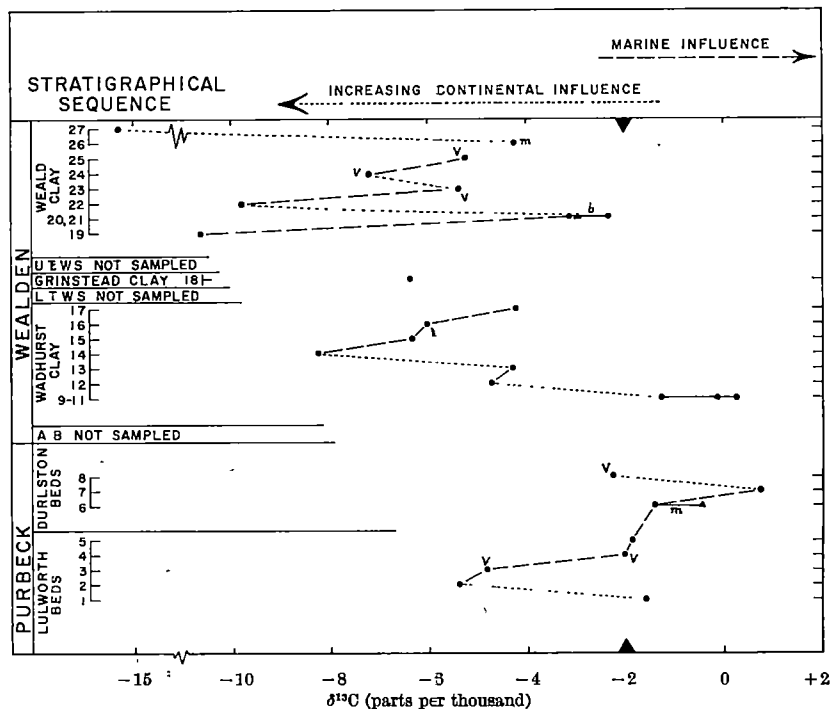


Fig. 1. Variation in carbon isotopic composition of carbonates from the Purbeck-Wealden of southern England. Formations not sampled: A.B., Ashdown Beds; L.T.W.S., Lower Tunbridge Wells Sand; U.T.W.S., Upper Tunbridge Wells Sand. Analyses: ●, bulk carbonate, ▲, separated fossil shells; m, near-marine fauna (oysters, echinoids) presumed indigenously; V, *Viviparus* dominant, presumed indigenously. Sample numbers: Reading prefixes (S81-, see Table 1) omitted for simplicity.

Table 1. PURBECK-WEALDEN ISOTOPIC DATA

University specimen Nos.		Stratigraphical horizon		Fossils in sample	$\delta^{13}\text{C}$ in subsample (a) (‰)	$\delta^{18}\text{O}$ in subsample (a) (‰)
Sample (Reading)	Subsample (Penn. St.)					
S8127	66-382	Wealden	Weald Clay (b)	ostracods	-15.33	-4.71
S8128	66-385				-4.27	-5.09
S8125	66-251 (f)		middle (e)	Oysters, echinoids, <i>Viviparus</i>	-5.26	-3.64
S8124	66-252 (f)				-7.20	-3.76
[S8123]	66-626 (f)		middle (Petworth Marble) (e)	large <i>Viviparus</i>	-5.41	-6.04
S8122	66-383				-9.78	-9.05
S8121	66-261		middle (Clock House Sandstone Bed 2 ^{17,18})	large <i>Viviparus</i>	-2.32(-2.97)	-7.83(-8.14)
S8120	66-380				-3.13	-8.09
S8119	66-381		lower	<i>Filosina</i> (g)	-10.61	-9.20
S8118	66-379		lower (Horsham Stone (Shinfold Soil Bed)) (h)	<i>Equisetites</i> (in situ)	-6.39	-7.56
S8117	66-378		Grinstead Clay	<i>Neomiodon</i> (g)	-4.21	-7.68
S8116	66-610 (f)		Lower ²¹	<i>Equisetites</i> (in situ)	-6.02	-7.34
S8115	66-253 (f)		Wadhurst Clay	<i>Viviparus</i>	-6.34	-9.16
S8114	66-254 (f)		upper (High Brooms Soil Bed) (h)	<i>Neomiodon</i> (g)	-8.26	-10.00
S8113	66-256 (f)		upper	<i>Umo</i>	-4.29	-8.68
S8112	66-608 (f)		Hog Hill Sand (Telham Pebble Bed)	<i>Neomiodon</i> (g)	-4.72	-6.84
S8111	66-255 (f)		Hog Hill Sand (middle)	<i>Neomiodon</i> (g)	-1.27	-4.10
S8110	66-377		" " " (base)	<i>Neomiodon</i> (g)	-0.14	-4.33
S8109	66-376		lower (Sharpthorne Shell Bed) (i)	<i>Neomiodon</i> (g)	+0.21	-4.24
[S8108]	66-625 (f)	Purbeck	Durlston Beds	<i>Viviparus</i>	-2.24	-4.92
S8107	66-629 (f)			ostracods, ? <i>Viviparus</i>	+0.72	-5.80
S8106	66-375			<i>Loostrea</i>	-1.40(-0.46)	-4.77(-3.59)
S8105	66-624 (f)		Lulworth Beds	? freshwater gastropods	-1.89	-5.62
S8104	66-257 (f)			<i>Viviparus</i>	-2.01	-5.26
S8103	66-258 (f)			<i>Viviparus</i>	-4.84	-4.22
S8102	66-384			<i>Hydrobia</i> (l), <i>Valvata</i> (l)	-5.40(-5.39)	-4.71(-4.60)
S8101	66-374		basal limestone (m)	algal structures, <i>Serpula</i>	-1.60	-3.30

(a) Data are for calcitic shelly and calcitic sandy or silty limestones, except that figures in parentheses represent separated fossil shells. Sample treatment and analyses were made by standard procedures¹¹. Isotopic compositions are expressed, in the conventional δ notation, as differences (in parts per thousand) from the PDB carbon dioxide standard.

(b) Tentative sub-divisions of Weald Clay after Worssam, B.C. (in litt.).

(c) 3 ft. from top. Carbonate almost entirely ostracod valves; little cement.

(d) Upper beds of Wealden Shales.

(e) Exact relations of these three horizons uncertain.

(f) Ref. 12, pp 1809-10.

(g) Identified by Dr. R. Casey.

(h) Carbonate (cement in quartzose sandy siltstone) probably penecontemporaneous because *Equisetites* tissue preserved^{19,20}.

(i) 30-40 ft. above base of Wadhurst Clay. Minor aragonite. Carbonate almost entirely *Neomiodon* valves; virtually no cement.

(j) Marine ostracods reported by Anderson²²; freshwater gastropods by Arkell²³.

(k) Walker²⁴ reported 405 p.p.m. "equivalent boron" in illite from the marine Cinder Bed. Our results (sample S8106) are consistent with his, and the overlying Broken Shell Limestone (sample S8107) seems to be dominantly marine. The same author also assigned a marine origin to the upper Durlston *Viviparus* beds on the basis of 390 p.p.m. equivalent boron in their illite fraction. Our results from the Purbeck-Wealden generally (see also Fig. 1, — V) do not suggest that *Viviparus* was marine.

(l) Identified by the late Dr. W. J. Arkell.

(m) Marine algae reported by Brown²⁵.

Localities and references of specimens: S8101—Perryfield Quarry, Isle of Portland, Dorset²⁶; S8102—Holworth House, Ringstead Bay, Dorset²⁶; S8103-5—Durlston Bay, Swanage, Dorset²⁷; S8106—Nuthanger Farm (boring), Kingsclere, Hampshire²⁸; S8107, S8108—Durlston Bay, Swanage, Dorset²⁶; S8109-11—Sharpthorne brickyard, West Hoathly, Sussex²⁹; S8112—Baldalow Lane, Westfield, Sussex³⁰; S8113—Silverhill Quarry, Hollington, Hastings, Sussex³¹; S8114—Post Office (cesspool excavation, 10 ft. depth), Brede, Sussex³²; S8115—Telgh Farm, Stone, Kent³³; S8116—0.75 mile S.S.E. railway station, Balcombe, Sussex³⁴; S8117—Brambletye, 1.75 miles S.E. East Grinstead, Sussex³⁵; S8118—Philpota Quarry, West Hoathly, Sussex³⁶; S8119—0.25 mile N.W. Theale Farm, Slinfold, Sussex³⁷; S8120, S8121—Geerings, Warnham, Sussex; S8122—Clock House Brickworks, Capel, Surrey³⁸; S8123—'Horsham, Sussex' (museum label); S8124—'Nr. Redhill', Surrey; S8125—Kilnbrow, Earlswood, Redhill, Surrey³⁹; S8126—Yaverland Cliff, Isle of Wight; S8127—Nr. railway station, Berwick, Sussex⁴⁰. Sample S8101 was supplied by Mr. N. E. Butcher (Reading); S8105, S8107, S8108, and S8123 by Dr. J. D. Hudson (Oxford); S8112 and S8116 by Dr. J. Yen (Villanova, U.S.A.).

grant GP-5109 from the U.S. National Science Foundation.

¹ Casey, R., *Proc. Malacol. Soc. Lond.*, **31**, 220 (1955).

² Casey, R., *Bull. S.E.U.S.S.*, No. 117, 7 (1963).

³ Casey, R., and Bristow, C. R., *Geol. Mag.*, **101**, 124 (1964).

⁴ Allen, P., *Phil. Trans. Roy. Soc.*, **242**, 295 (1959).

⁵ Allen, P., *Phil. Trans. Roy. Soc.*, **242**, 342 (1959).

⁶ Allen, P., *Proc. Geol. Assoc., Lond.*, **73**, 226 (1962).

⁷ Walker, C. T., *Geol. Mag.*, **101**, 189 (1964).

⁸ Walker, C. T., and Price, N. B., *Bull. Amer. Assoc. Petrol. Geol.*, **47**, 833 (1963).

⁹ Allen, P., *Nature*, **202**, 585 (1965).

¹⁰ Tank, R. W., *Geol. Mag.*, **101**, 539 (1964).

¹¹ Keith, M. L., Anderson, G. M., and Eichler, R., *Geochim. Cosmochim. Acta*, **28**, 1757 (1964).

¹² Keith, M. L., and Weber, J. N., *Geochim. Cosmochim. Acta*, **28**, 1787 (1964).

¹³ Keith, M. L., and Parker, R., *Marine Geology*, **3**, 115 (1965).

¹⁴ Parker, P. L., *Geochim. Cosmochim. Acta*, **28**, 1155 (1964).

¹⁵ Landergrén, S., *Deep Sea Res.*, **1**, 98 (1954).

¹⁶ Lloyd, R. M., *J. Geol.*, **72**, 84 (1964).

¹⁷ Kirkaldy, J. F., and Bull, A. J., *Proc. Geol. Assoc. Lond.*, **59**, 80 (1948).

¹⁸ Allen, P., in ref. 17, 59, 81 (1948).

¹⁹ Whybrow, P. J., in ref. 6, 73, 230 (1962).

²⁰ Watson, J. (in litt.).

²¹ Gallois, R. W., *Proc. Geol. Assoc.*, **75**, 364 (1964).

²² Anderson, F. W., *Liverpool and Manchester Geol. J.*, **3**, 28 (1962).

²³ Arkell, W. J., *Quart. J. Geol. Soc. Lond.*, **97**, 123 (1941).

²⁴ Brown, P. R., *Geol. Mag.*, **100**, 585 (1963).

²⁵ House, M. R., *Geol. Assoc. Centenary Guide*, No. 22, 13 (1958).

²⁶ Arkell, W. J., *Mem. Geol. Surv.*, **142** (1947).

²⁷ Arkell, W. J., *Mem. Geol. Surv.*, **135** (1947).

²⁸ Lees, G. M., and Tait, A. H., *Quart. J. Geol. Soc. Lond.*, **101**, 258 (1946).

²⁹ Arkell, W. J., *Mem. Geol. Surv.*, **137** (1947).

³⁰ Allen, P., *Proc. Geol. Assoc., Lond.*, **73**, 239 (1962).

³¹ Topley, W., *Mem. Geol. Surv.*, **61** (1875).

³² Allen, P., *Proc. Geol. Assoc., Lond.*, **60**, 279 (1949).

³³ Allen, P., *Geol. Assoc. Centenary Guide*, No. 24, 13 (1960).

³⁴ Allen, P., *Geol. Assoc. Centenary Guide*, No. 24, 25 (1960).

³⁵ Allen, P., *Proc. Geol. Assoc., Lond.*, **73**, 229 (1962).

³⁶ Allen, P., *Proc. Geol. Assoc., Lond.*, **73**, 241 (1962).

³⁷ Terrell, F. H. J., in ref. 4, 242, 313 (1959).

³⁸ Topley, W., *Mem. Geol. Surv.*, **107** (1875).

³⁹ White, H. J. O., *Mem. Geol. Surv.*, **29** (1926).

⁴⁰ Casey, R., *Proc. Malacol. Soc., Lond.*, **31**, 213 (1955).

⁴¹ Hudson, J. D., *Palaeontology*, **6**, 322, 324, 334 (1963).

RETURN OF LEUCOCYTES TO THE BONE MARROW IN CHRONIC MYELOGENOUS LEUKAEMIA

By DR. JOHN H. MOXLEY* and PROF SEYMOUR PERRY

Medicine Branch

AND

PROF GEORGE H. WEISS and DR. MARVIN ZELEN

Biometry Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

IT is the purpose of this article to present evidence that immature leucocytes in the peripheral blood of patients with chronic myelocytic leukaemia (CML) may return to the bone marrow and mature. Our findings offer a possible explanation for the prolonged peripheral-blood disappearance time of CML leucocytes^{1,2}.

infused into the peripheral blood return to the bone marrow.

One other important possibility to consider for the presence of radioactivity in the bone marrow aspirate is peripheral blood contamination. In the evaluation of this possibility, the observed intensity of radioactivity in the aspirate can be written:

$$C_{\text{obs}} = p C_{\text{marrow}} + (1 - p) C_{\text{peripheral blood}}$$

where the C s refer to intensities and p is a parameter depending on the (volume) contamination and the ratio of densities of white blood cells per unit volume associated with the peripheral blood and marrow. Specifically it is:

$$p = \frac{1 - a}{1 - a + a \rho}$$

where a is the percentage contamination, and

$$\rho = \frac{\text{white blood cell density in peripheral blood}}{\text{white blood cell density in marrow}}$$

Measurements on the white blood cells in both the peripheral blood and marrow resulted in values of $\rho = 2/7$ and $1/5$ for patients A. S. and E. R., respectively. Estimates of peripheral blood contamination of bone marrow aspirate are in the neighbourhood of $a = 20$ per cent⁴. Substituting the foregoing values of ρ with $a = 0.2$ in the formula for p gives $p = 0.93$ (A. S.) and $p = 0.95$ (E. R.). If one assumes no radioactivity in the marrow ($C_{\text{marrow}} = 0$), then $C_{\text{obs}} = 0.07 C_{\text{peripheral blood}}$ for A. S. and $C_{\text{obs}} = 0.05 C_{\text{peripheral blood}}$ for E. R. This means that we would expect the observed radioactivity in the aspirate to be 7 per cent (A. S.) and 5 per cent (E. R.) of that in the peripheral blood. Even if the contamination

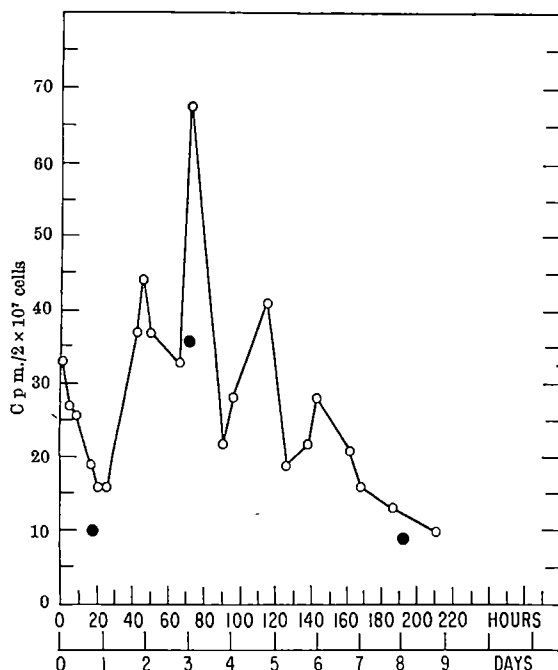


Fig. 1. Patient E. R., 58 years old, N, ♀. Ph_1^+ CML relapse, *in vitro* labelling. ○—○, White blood cells; ●, marrow

We have investigated the leucocyte kinetics in CML using techniques of liquid scintillation counting and autoradiography following *in vitro* leucocyte labelling with tritiated thymidine. Two CML patients, Ph_1 chromosome positive, were leucophoresed and their leucocytes labelled *in vitro* with 4 mc. of tritiated thymidine (sp. act. 1.9 c./mmole). After excess radioactivity was removed by two washings with autologous cell-free plasma, the labelled leucocytes were re-infused. At time zero and at intervals after labelling 5 ml. of blood and 2 ml. of bone marrow aspirate were obtained and processed by a method previously described³. The isolated leucocytes were washed, counted, hydrolysed, and their radioactivity determined in a liquid scintillation spectrometer. Autoradiographs of the whole-blood samples and of the separated leucocytes were prepared in the usual manner using Kodak AR 10 stripping film. Four thousand cells were counted for each autoradiograph. Details of the methods used will be included in a later publication.

Results of the liquid scintillation counting are presented in Figs. 1 and 2. Leucocyte radioactivity was followed in both the peripheral blood and bone marrow for 120 h in each investigation. The radioactivity in the marrow strongly suggests that some of the *in vitro* labelled cells

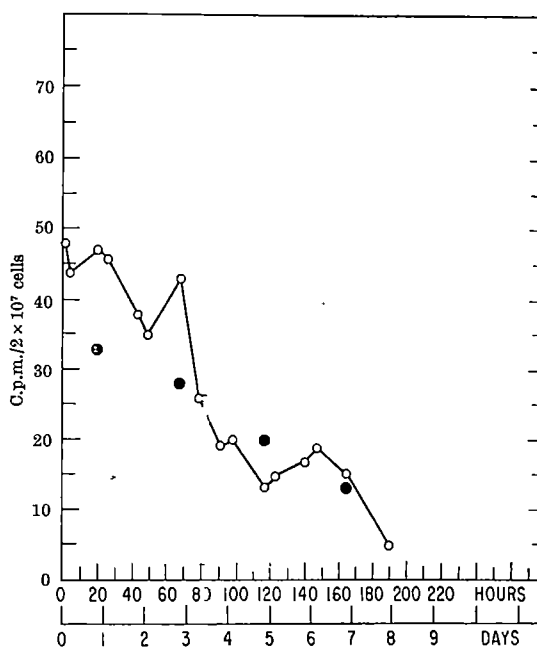


Fig. 2. Patient A. S., 47 years old, W, ♂. Ph_1^+ CML relapse, *in vitro* labelling. ○—○, White blood cells; ●, marrow

* Present address: Peter Bent Brigham Hospital, Boston, Massachusetts.

were as much as 50 per cent, the observed aspirate radioactivity would be 22 per cent (A. S.) and 17 per cent (E. R.) of that in the peripheral blood. Therefore the observations in Figs. 1 and 2 cannot be accounted for by peripheral blood contamination.

Corresponding evidence of return of CML leucocytes to the bone marrow was found in analysis of the bone marrow autoradiographs from one of the two patients (E. R.) (Tables 1 and 2). Seven per cent of the leucocytes obtained in the marrow aspirate on day 3 were significantly labelled. The labelled cells included 32 metamyelocytes and 19 mature granulocytes. This finding suggests that cells not only return to the bone marrow but also, while there, a portion of them divide and mature.

It is highly unlikely that immature leucocytes are able to mature during the 1-h *in vitro* incubation period. Consequently, for the purposes of assessing the significance of the results in Tables 1 and 2, we have assumed that all grains overlying mature cells in Table 1 represent background labelling. We will now show that the labelled mature neutrophils found in the bone marrow (Table 2) cannot be accounted for by the re-infusion of these background labelled mature cells. For this calculation:

L = total number of re-infused labelled leucocytes;

N_{PB} = total number of leucocytes in the peripheral blood;

N_m = total number of leucocytes in bone marrow;

E_g = labelling index;

E = leucophoresis efficiency.

(The leucophoresis efficiency is defined as the ratio of total number of leucocytes extracted from a given amount of whole blood to the total number of leucocytes in the given amount of whole blood.) Since 150 out of 946 labelled cells were mature neutrophils labelled *in vitro* by background, an average of $150/946 L = 0.16 L$ mature labelled neutrophils would be infused. Assuming that all these labelled mature neutrophils return to the marrow, we would expect to find:

$$(4,000) (0.16 L) / N_m$$

labelled mature cells in a bone marrow autoradiograph where the number of cells counted is 4,000. The total number of re-infused labelled leucocytes from a sample of 1,000 ml. of whole blood is:

$$L = \frac{1,000}{5,000} (E) (E_g) (N_{PB})$$

where 5,000 ml. is taken as the blood volume. Consequently, the expected number of labelled mature neutrophils counted in a bone marrow autoradiograph can be written as:

$$(4,000) (0.16) (0.2) (E) (E_g) (N_{PB}/N_m)$$

Table 1. *In vitro* LABELLING OF CML LEUCOCYTES WITH ³HTDR* (PATIENT E. R.)

Cell type	No. of cells	Grain count			
		< 5	5-10	11-15	16-20 > 20
Segmented neutrophils	513	40	8		
Band neutrophils	365	24	2		
Metamyelocytes	567	72	4		
Myelocytes	1,921	393	40	16	18 96
Promyelocytes	402	115	20	9	5 34
Myeloblasts	143	35	4	2	
Eosinophils	82	5			4
Basophils	9				
Lymphocytes	22				
Erythroid precursors	16				

* Sample taken from bag just prior to infusion into the patient.

Table 2. RESULTS OF BONE MARROW AUTORADIOGRAPHS ON DAY 3 AFTER INFUSION OF LEUCOCYTES LABELLED *in vitro* (PATIENT E. R.)

Cell type	No. of cells	Grain count			
		< 5	5-10	11-15	16-20 > 20
Segmented neutrophils	982	14	1		
Band neutrophils	379	4			
Metamyelocytes	635	23	2	1	1 5
Myelocytes	1,497	156	13	5	1 1
Promyelocytes	342	48	9	5	1 1
Myeloblasts	129	21	2		
Eosinophils	98	3			
Basophils	1				
Lymphocytes	1				
Erythroid precursors	84				

Yoffey cites (N_{PB}/N_m) to be 0.01 for normal individuals⁵. Yankee has found values of E between 0.35 and 0.70 in 25 CML patients⁶. Taking E to be 0.50 and $N_{PB}/N_m = 0.01$, the expected number of mature labelled neutrophils on a bone marrow autoradiograph should be $0.64 E_g$. The proportion of labelled cells to total cells counted gives an $E_g = 946/4,000 = 0.24$. Thus we estimate the expected number of mature labelled neutrophils to be 0.15. We observed 51 labelled mature cells, which clearly is a highly significant result.

Since tritiated thymidine labels cells only during the DNA synthesis phase, the presence of labelled mature granulocytes in the bone marrow indicates that immature cells which took up the label *in vitro* must have undergone maturation. The absence of labelled erythroid precursors in the bone marrow aspirate supplies strong evidence that our observations were not influenced by the re-utilization of the tritiated thymidine.

These preliminary results from examination of two patients with acute myelogenous leukaemia (AML) indicate that leucocytes do not return to the bone marrow in this disease.

¹ Athens, J. A., Raab, S. O., Haab, O. P., Boggs, D. R., Ashenbrucker, H., Cartwright, G. E., and Wintrobe, M. M., *J. Clin. Invest.*, **44**, 756 (1965).

² Galbraith, P. R., Valberg, L. S., Gruner, P. H., Brown, G. M., *Ann. Intern. Med.*, **62**, 1085 (1965) (abst.).

³ Marsh, J. C., and Perry, S. J., *J. Clin. Invest.*, **43**, 287 (1964).

⁴ Osmond, D. G., and Everett, N. B., *Quart. J. Exp. Physiol.*, **50**, 1 (1965).

⁵ Yoffey, J. M., *Quantitative Cellular Hematology*, 48 (Springfield, Illinois, Charles C. Thomas, 1960).

⁶ Yankee, R. (personal communication).

EFFECTS OF CYCLIC ADENOSINE MONOPHOSPHATE AND MELANOCYTE-STIMULATING HORMONE ON FROG SKIN *IN VITRO*

By DR. M. W. BITENSKY and S. R. BURSTEIN

Department of Medicine, New York University School of Medicine, New York

IN 1957 Sutherland and Rall¹ described the function of cyclic-AMP in the control of glycogenolysis, thus providing insight into one of the mechanisms of hormonal regulation of enzymatic activity. These investigators have since characterized the enzymes involved in the synthesis and degradation of cyclic-AMP as adenylyl cyclase and phosphodiesterase, respectively². Davoren and Sutherland found that adenylyl cyclase is located on the cell membrane in the pigeon erythrocyte³. Sutherland and Rall and other investigators have provided evidence to support the role of adenylyl cyclases as intermediaries in the action of ACTH⁴, TSH⁵, glucagon¹, and epinephrine¹. Each of

these hormones has been shown to stimulate selectively an adenylyl cyclase in its respective target organ. Furthermore, Orloff and Handler⁶ demonstrated that the effects of ADH on the toad urinary bladder could be mimicked by cyclic-AMP. Subsequent work has shown that ADH increases the levels of cyclic-AMP formed in homogenates prepared from dog kidney⁷. Mansour *et al.*⁸ have reported activation of adenylyl cyclase by serotonin in *Fasciola hepatica*, and Rizaack⁹ has demonstrated that a lipolytic activity in rat epididymal fat pads is stimulated by cyclic-AMP, as well as by epinephrine. Although cyclic-AMP has been shown to control glucose utilization through

its action on phosphorylase kinase and on phosphofructokinase, and to affect glycogen synthesis through its action on transglucosylase¹⁰, little is known about the manner in which cyclic-AMP regulates other types of cellular activities.

Sutherland and Rall and their co-workers have thus described a common mechanism of interaction between a certain class of hormones and their target tissues. Although adenylyl cyclase and cyclic-AMP are involved in all these systems, there is a high degree of specificity both in the activation of a specific adenylyl cyclase by its appropriate hormone and in the way that the differentiated target cell responds to increasing levels of cyclic-AMP.

Skin darkening in the frog has been demonstrated with methyl xanthines such as caffeine and theophylline^{11,12}. Since these reagents are known to inhibit competitively the hydrolysis of cyclic-AMP by phosphodiesterase¹³, it seemed reasonable to enquire whether MSH causes pigmentary changes in amphibian skin by increasing cellular levels of cyclic-AMP.

The effects of various concentrations of cyclic-AMP were determined on excised strips of frog skin. Since it is known that cell membranes are relatively impermeable to this cyclic nucleotide, it was necessary to use higher concentrations than are believed to be present intracellularly¹⁴.

Summer *Rana pipiens*, stored up to two weeks at 4° C, were used. Strips of dorsal skin relatively free from spots were mounted vertically in 4-ml. cuvettes on a polystyrene frame (Fig. 1). All test reagents were dissolved in modified Ringer's solution (110 mmoles NaCl, 2 mmoles CaCl₂, 2 mmoles NaHCO₃ and 2 ml. KCl).

The time required for the full expression of hormonally induced pigmentary changes in amphibian skin was 1-2 h. By using the Gilford spectrophotometer, it was possible to record pigmentary changes in four skin specimens simultaneously. Optical density measurements were recorded at 530 mμ using a chart speed of 0.25 in. per min with a full-scale deflexion set equal to 0.500 O.D. unit. This method is based on the assumption that the optical density of frog skin is a function of the skin area occluded by dispersed pigment granules¹⁵. In practice, the method proved to be sensitive and precise, and the recorded changes in optical density correlated well with gross and microscopic observations. In addition, the smoothness of the curves obtained indicated that the optical density measurements were made from the same portion of each specimen during the experimental period.

At the time the frogs were taken from the cold room, their skins were usually dark. Before each experiment,

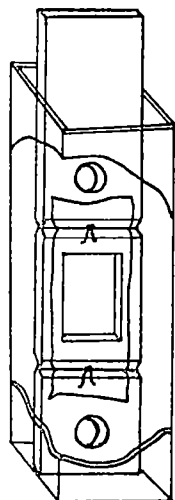


Fig. 1. Strips of frog skin approximately 5 mm × 25 mm were mounted on a polystyrene frame with 4-0 surgical silk and inserted in a 4-ml. cuvette. Optical density was measured at room temperature in a Gilford spectrophotometer at 530 mμ.

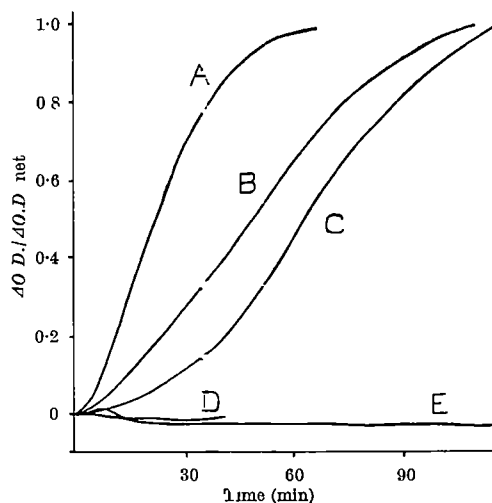


Fig. 2. Changes in optical density of frog skin caused by 5'-AMP and cyclic-AMP. A, 1.0×10^{-3} M cyclic-AMP; B, 5.0×10^{-3} M cyclic-AMP; C, 3.0×10^{-3} M cyclic-AMP; D, 5.0×10^{-3} M 5'-AMP; E, Ringer's solution alone. Results for cyclic-AMP are expressed as fraction of total net change, data from a single representative experiment are shown. Results for 5'-AMP and Ringer's are expressed as change in optical density from time zero divided by average net change for seven experiments with cyclic-AMP.

skin specimens were rinsed with Ringer's solution and allowed to equilibrate at room temperature in the cuvette. The skins lightened slowly and, after about half an hour, reached a constant optical density. The Ringer's solution was then replaced by various experimental media.

Aminophylline, β -MSH and ACTH produced an increase in optical density, and the time required and the magnitude of the net change achieved depended on the nature and concentration of the compound tested (Table 1). The results obtained with various concentrations of cyclic-AMP are shown in Fig. 2. In order to compare results with different skin samples, the optical density readings during the experiments are expressed as fractions of total change.

Compound concentration	Maximal increase in O.D. at 530 mμ	Time required for maximal 40 D. (min)
Aminophylline 1.0×10^{-3} M	0.177	38
β -MSH 0.10 μg/ml.	0.442	104
ACTH 0.30 mg/ml	0.418	54
Cyclic-AMP 1.0×10^{-3} M	0.298	64
5.0×10^{-3} M	0.419	112
3.0×10^{-3} M	0.381	123

The data represent average values for three or more experiments.

Although the rate of darkening of the specimens was related to the concentration of cyclic-AMP, the final net increase in optical density was approximately the same for all effective concentrations of the cyclic nucleotide. The lowest concentration of cyclic-AMP which produced a significant response was 3.0×10^{-3} M. In contrast, 5'-AMP did not significantly alter the optical density of the specimen at all concentrations used. Neither 5'-AMP nor cyclic-AMP caused any change in the optical density of the non-pigmented abdominal skin.

The spotted skin pigmentation characteristic of *Rana pipiens* caused some variability in the fraction of the total area of skin capable of undergoing a maximal degree of colour change. The results were surprisingly reproducible, considering that the areas of skin observed were heterogeneous and that experiments were made comparing skin specimens from different frogs.

Although oxygenation of the medium or the presence of physiological concentrations of glucose increased the magnitude of the optical density changes, these factors did not alter the rate or the direction of the relative response.

ACTH, α -MSH and β -MSH have all been shown to induce skin darkening in the frog¹². The common heptapeptide sequence shared by ACTH and the MSHs is thought to be responsible for their skin-darkening properties. The heptapeptide and, indeed, a derived tetrapeptide residue¹³ produce darkening in amphibian skins, but at much higher concentrations than are required for the parent hormones. Oxytocin and ADH, on the other hand, do not darken amphibian skin, and they do not contain this amino-acid sequence¹³. Interaction between the melanocyte and the active hormone and hormonal fragments could thus depend on the adenylyl cyclase system. Such interaction would imply that the melanocyte adenylyl cyclase system has a specific means of recognizing this particular amino-acid sequence.

Lerner *et al.*¹² have found that acetylcholine causes lightening of amphibian skin. This same compound causes a decrease in the formation of cyclic-AMP in cardiac muscle¹⁷. Paradoxically, epinephrine and serotonin induce lightening of amphibian skin¹². Although epinephrine and serotonin can stimulate the formation of cyclic-AMP in some mammalian tissues², it is not known what effects these compounds have on frog melanocyte cyclic-AMP levels.

The demonstration of darkening with cyclic-AMP *in vitro* provides some evidence that the mediation of pigmentary changes in frog melanocytes could involve an adenylyl cyclase. Further investigations on the adenylyl cyclase of melanocytes are needed, however, in order to evaluate directly the effect of MSH on intracellular levels of cyclic AMP.

We thank Dr. Aaron B. Lerner for a generous gift of purified β -MSH. This work was supported by the New

York Research Council and by a U.S. Public Health Service departmental research grant.

- ¹ Rall, T. W., and Sutherland, E. W., *J. Biol. Chem.*, **232**, 1065 (1958).
- ² Rall, T. W., and Sutherland, E. W., *Cold Spring Harbor Symp. Quant. Biol.*, **27**, *Cellular Regulatory Mechanisms*, Long Island Biological Assoc., Cold Spring Harbor, L.I., N.Y., 347 (1961).
- ³ Davoren, P. R., and Sutherland, E. W., *J. Biol. Chem.*, **238**, 3016 (1963).
- ⁴ Haynes, R. C., Jun., *J. Biol. Chem.*, **233**, 1220 (1958).
- ⁵ Klatner, L. M., Chi, Y.-M., Friedberg, S. L., Rall, T. W., and Sutherland, E. W., *J. Biol. Chem.*, **237**, 1239 (1962).
- ⁶ Orloff, J., and Handler, J. S., *J. Clin. Invest.*, **41**, 702 (1962).
- ⁷ Brown, E., Clarke, D. L., Roux, V., and Sherman, G. H., *J. Biol. Chem.*, **238**, PC852 (1963).
- ⁸ Mansour, T. E., LeRouge, N. A., and Mansour, J. M., *Fed. Proc.*, **20**, 226 (1961).
- ⁹ Rizzack, M. A., *J. Biol. Chem.*, **239**, 292 (1963).
- ¹⁰ Rosell-Perez, M., and Lerner, J., *Biochemistry*, **3**, 81 (1964).
- ¹¹ Wright, M. R., and Lerner, A. B., *Endocrinology*, **66**, 599 (1960).
- ¹² Lerner, A. B., *Nature*, **184**, 674 (1959).
- ¹³ Butcher, R. W., and Sutherland, E. W., *J. Biol. Chem.*, **237**, 1244 (1962).
- ¹⁴ Davoren, P. R., and Sutherland, E. W., *J. Biol. Chem.*, **238**, 3009 (1963).
- ¹⁵ The following equation is suggested to describe the relationship between the light which is incident on the frog skin and that transmitted through it:

$$\frac{I_t}{I_i} = 1 - (R + C) \quad (1)$$

I_i is the intensity of the incident light, I_t is the intensity of the transmitted light, R is the fraction of light absorbed by a variable pigment system, and C is the fraction of light not transmitted because of constant optical properties of the skin. R can be expressed in terms of the ratio of the total area blocked by opaque pigment particles, x , to the total area illuminated, A :

$$R = \frac{x}{A} \quad (2)$$

It can then be shown that:

$$A.O.D. = -\log \left(1 - \frac{Ax}{B} \right) \quad (3)$$

where $B = A - A_0 - x_0$ and x_0 is equal to the initial area covered by opaque pigment particles.

¹⁶ Otsuka, H., and Inoue, K., *Bull. Chem. Soc. Japan*, **37**, 289 (1964).

¹⁷ Murad, F., Rall, T. W., and Sutherland, E. W., *Fed. Proc.*, **19**, 296 (1960).

BIOSYNTHESIS OF 2-AMINOETHANEPHOSPHONIC ACID: A PHOSPHORAMIDIC ACID RE-ARRANGEMENT?

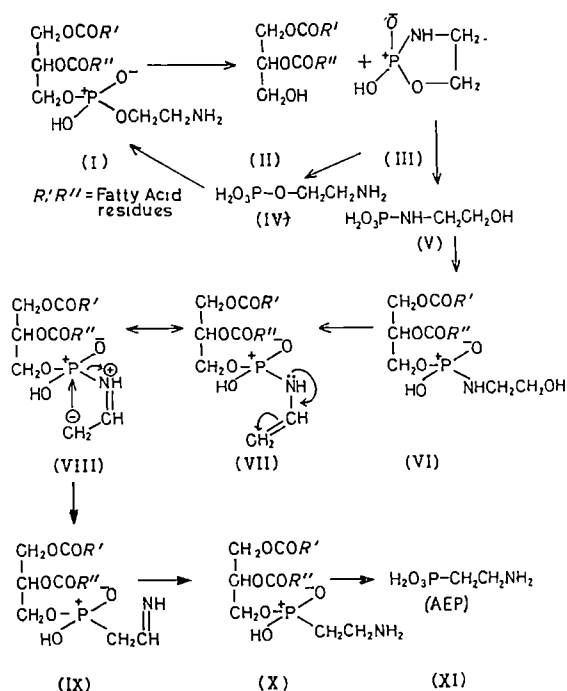
By DR. W. SEGAL

Department of Biochemistry, University of Western Australia, Nedlands, Western Australia

RECENT papers have reported the presence of free and bound 2-aminoethanephosphonic acid (AEP) in protozoa^{1,2} and the sea anemone³. This substance possesses a C-P bond and represents the first example of such a bond in biological materials, but hitherto there has been no published information or theory on the mode of biosynthesis of AEP.

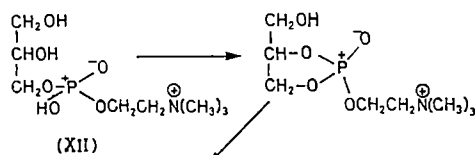
Free, lipid-bound, and protein-bound² AEP have been found and evidence for a glycerol ester of AEP has been presented³. In ³²P₄-labelling experiments Rosenberg³ found that in *Tetrahymena* cells in log phase, the uptake of label was greatest in the lipid-bound AEP, least in the free AEP, and intermediate in the protein-bound AEP remaining after solvent extraction. These results are compatible with the assumption that the lipid-bound AEP is the precursor of the free and protein-bound AEP. There is abundant evidence^{4,5} that lipid-bound AEP occurs together with, and is closely related to, the phospholipids. Rouser *et al.*⁴ have described the isolation and characterization of ceramide aminoethanephosphonate—a deoxy analogue of sphingomyelin—from sea anemone lipid. Baer and Stanacev⁵ and, contemporaneously, Rosenthal and Pousada⁶ have synthesized AEP analogues of phosphatidyl ethanolamine. The properties of these compounds correspond so closely to those of the normal cephalins that it was suggested^{5,6} that AEP may occur in nature as a cephalin analogue.

The purpose of this article is to propose, on the basis of the published data³⁻⁶ and the properties of phospholipids, ribonucleic acids, and model compounds, that AEP (XI) is derived from phosphatidyl ethanolamine (I) by the following mechanism:



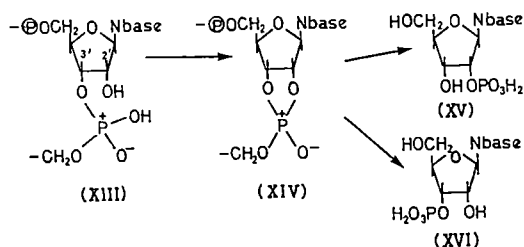
There are numerous examples of phosphate migration in the chemical and biochemical literature. Baer and Kates⁷ have shown that cholestyl α -glyceryl phosphate (XII)

undergoes acid and alkaline hydrolysis liberating choline and forming α - and β -glyceryl phosphates via an intermediate cyclic phosphate.



α - and β -glyceryl phosphates + choline

The alkaline hydrolysis of ribonucleic acid (for example, XIII) affords 2'- and 3'-mononucleotides (XV) and (XVI), explained by Brown and Todd⁸ in terms of a cyclic phosphate intermediate (XIV). Furthermore, cyclic nucleotides of the type (XIV) have been isolated from the ribonuclease digestion of ribonucleic acids⁹.



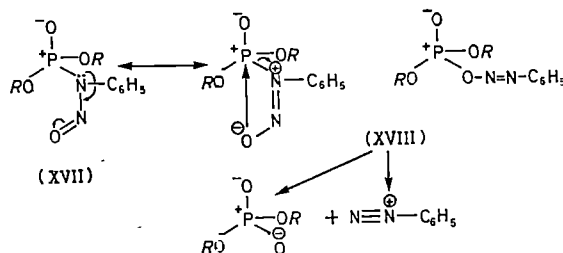
The feature that these substrates have in common is the presence of an α -glycol half-phosphate ester system. Todd¹⁰ has pointed out that the phosphate residue remains attached to the glycol moiety in ribonucleic acid hydrolysis, and this is also the case for cholesteryl α -glyceryl phosphate (XII) hydrolysis. In the case of α -glyceryl inositol phosphate there are two different α -glycol systems available and the products of alkaline hydrolysis are inositol and α - and β -glyceryl phosphates and glycerol and two isomeric inositol phosphates¹¹.

Phosphatidyl ethanolamine (I) represents the amino analogue of an α -glycol half-phosphate ester. By a similar mechanism to cyclic ester formation (nucleophilic attack) cyclic ester-amide (III) formation is postulated together with the release of free α : β diglyceride (II)—the phosphate retaining the glycol analogue as in the examples above. Again, as in cyclic ester cleavage, the ester-amide (III) is postulated to produce phosphoryl ethanolamine (IV) and the isomeric *N*-phosphoethanolamine (V). The presence of phosphatases may be a factor determining the survival of the P-N bond in (V) and it is noteworthy that the P-N bond occurs in biological material as *N*-phosphocreatine and *N*-phosphoarginine. The phosphoryl ethanolamine (IV) is available for conversion to phosphatidyl ethanolamine (I) through the intermediate cytidine diphosphoethanolamine and α : β -diglyceride¹². The *N*-phosphoethanolamine (V) may be considered an antimetabolite of phosphoryl ethanolamine (IV)—possessing similar constitution and geometry. The differences in solubility and melting behaviour of phosphatidyl ethanolamines and the corresponding lecithins¹³ indicate that the zwitter-ionic form of (IV) would be relatively unimportant at physiological pH and that the polarity of (IV) and (V) would be similar. (Note added in proof. A recent study of the infra-red spectra of phospholipids (Abramson, M. B., *et al.*, *J. Biol. Chem.*, 240, 2389; 1965) assigns an intramolecularly hydrogen-bonded structure—involving amino and hydroxyl groups—to phosphatidyl ethanolamine and phosphatidyl serine rather than the zwitter-ionic character of lecithin.) As such the enzymatic systems may be insufficiently discriminating and likewise convert

(V) to the phosphoramidic acid derivative (VI) isomeric with the cephalin (I).

The *N*-phosphoethanolamine portion of the compound (VI) bears a relationship to phosphoglyceric acid (see XIX). The latter is enzymatically dehydrated (enolase) to phosphoenolpyruvic acid. The postulated dehydration of (VI) to the *N*-vinyl phosphoramidic acid (VII) is analogous to the formation of phosphoenolpyruvic acid and generates a potential carbanion which becomes activated by the electromeric displacement (VII) \leftrightarrow (VIII).

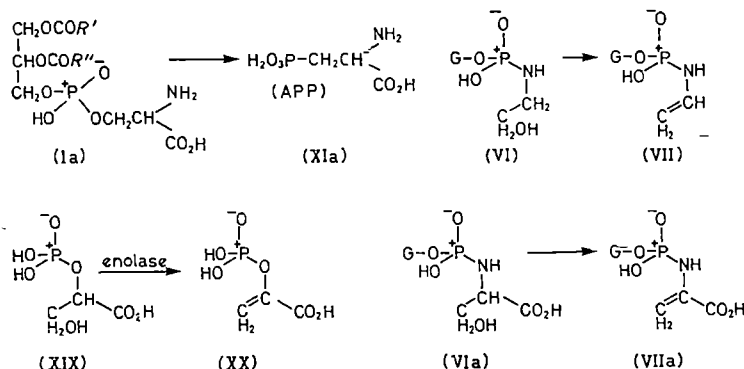
A Stuart model of (VII) is such that the *P*-vinylamino grouping is planar and lies symmetrically between two of the phosphate oxygen atoms while the third oxygen lies in the *P*-vinylamino plane. Furthermore the methylene carbon is in close proximity to the electron-deficient phosphorus. The intramolecular re-arrangement of (VIII) is postulated to account for the C-P bond formation (IX) and involves nucleophilic attack by a carbanion and heterolysis of the P-N bond. In this regard, Gilman and Robinson¹⁴ have shown that triethyl phosphate undergoes nucleophilic attack by the phenyl carbanion of phenyl magnesium bromide affording esters of phenyl- and diphenyl-phosphonic acids. A further indication that the intramolecular re-arrangement of (VIII) to (IX) is feasible is derived from the work of Bunyan and Cadogan¹⁵, who readily obtained diazonium salt derivatives and phosphate ester from the *N*-nitroso-*N*-phenylphosphoramidate (XVII), postulating the intramolecular mechanism shown as follows:



This re-arrangement is highly analogous with that postulated for (VIII) \rightarrow (IX).

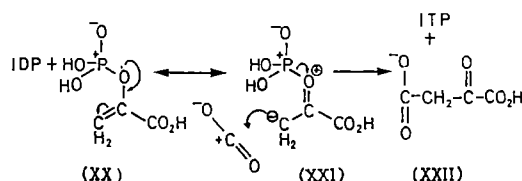
The remaining steps in the biosynthesis involve hydrogenation of the imino-compound (IX) to the 'phosphonolipid' (X) which is considered (from the data of Rosenberg² and Kittredge *et al.*³) to be the precursor of free AEP (XI) and of protein-bound AEP.

Since the proposed mechanism (I) \rightarrow (XI) was derived, my attention has been directed to the recent isolation of α -amino- β -phosphonopropionic acid (APP) (XIa) from *Zoanthus sociatus* and *Tetrahymena pyriformis*¹⁶. This metabolite (APP) bears the same relationship to AEP as serine bears to ethanolamine, and it is considered that APP biosynthesis follows an analogous course involving phosphatidyl serine (Ia) in place of phosphatidyl ethanolamine (I).



G = α Diacyl glyceryl

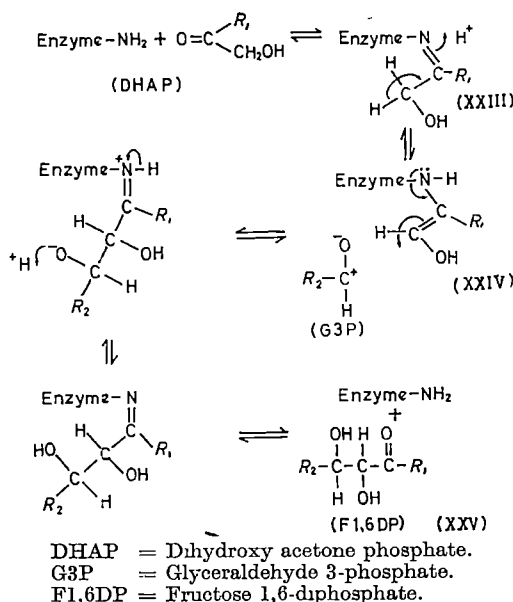
The dehydration of (VI) to (VII) was compared with the enolase-induced dehydration of phosphoglyceric acid (XIX) to phosphoenolpyruvic acid (XX). The proposed mechanism, applied to phosphatidyl serine (Ia), affords the intermediate (VIa), the structure of which is more closely related to phosphoglyceric acid (XIX) than the initially compared intermediate (VI). The co-occurrence of APP and AEP suggests that APP may indeed be a precursor of AEP, similar to the precursor relationships of serine and ethanolamine and phosphoserine and phosphoryl ethanolamine¹⁷. Such a relationship between APP and AEP, however, is in accord with the proposed mechanism of their biosyntheses. It is noteworthy that phosphoenolpyruvate (XX) by electromeric shifts similar to that postulated (VII \leftrightarrow VIII) leads to the carbanion (XXI) which fixes carbon dioxide forming oxaloacetate (XXII) and inosine triphosphate (ITP) in the presence of inosine diphosphate (IDP).



The apparent absence of an intramolecular re-arrangement in phosphoenolpyruvate (XXI) forming a C-P bond as in VIII \rightarrow IX is explained in terms of the relatively diminished positivity of the phosphorus atom due to the dissociation of the phosphate residue of XXI.

The formation of the C-P bond VII \rightarrow IX postulates the intramolecular re-arrangement of an enamine (VII) which is a nitrogenous analogue of phosphoenolpyruvic acid (XX). In this regard it is significant that Rutter¹⁸ has postulated a similar mechanism involving an enzyme-enamine complex to account for the action of class I aldolases. Rutter's mechanism involves an initial Schiff base formation (XXIII) and protonation and deprotonation to produce the enamine (XXIV). Electromeric displacement (similar to that postulated in AEP synthesis (VII)) and nucleophilic attack on the aldehyde group of a second substrate leads to aldol synthesis (XXV).

The exchange of one of the protons of the methylene carbon atom with those of the medium as shown in (XXIII) \rightleftharpoons (XXIV) and the invariable *trans* relationship



of the hydroxyl groups on the carbon atoms of the newly formed bond¹⁹ is explained by the formation of the enamine intermediate (XXIV).

- ¹ Horiguchi, M., and Kandatsu, M., *Nature*, **184**, 901 (1959), *Bull. Agr. Chem. Soc. (Japan)*, **24**, 565 (1960), *Agric. Biol. Chem. (Tokyo)*, **26**, 721 (1962).
- ² Rosenberg, H., *Nature*, **203**, 299 (1964).
- ³ Kittredge, J. S., et al., *Biochemistry*, **1**, 624 (1962).
- ⁴ Rouser, G., et al., *J. Amer. Oil Chem. Soc.*, **40**, 425 (1963).
- ⁵ Baer, E., and Stanacev, N. Z., *J. Biol. Chem.*, **239**, 3209 (1964).
- ⁶ Rosenthal, A. F., and Pousada, M., *Proc. Chem. Soc.*, 358 (1964).
- ⁷ Baer, E., and Kates, M., *J. Biol. Chem.*, **175**, 79 (1948); **185**, 615 (1950).
- ⁸ Brown, D. M., and Todd, A. R., *J. Chem. Soc.*, 52 (1952).
- ⁹ Markham, R., and Smith, J. D., *Biochem. J.*, **52**, 552 (1952).
- ¹⁰ Lord Todd, *Proc. Chem. Soc.*, 199 (1962).
- ¹¹ Hawthorne, J. N., *J. Lipid Res.*, **1**, 255 (1960).
- ¹² Kennedy, E. P., in *Proc. Fifth Intern. Congr. Biochem.*, **7**, 120. *Biosynthesis of Lipids*, edit. by Popjak, G. (Pergamon Press, 1963).
- ¹³ Dervichian, D. G., in *Progress in Biophysics and Molecular Biology*, edit. by Butler, J. A. V., and Huxley, H. E., **14**, 328.
- ¹⁴ Gilman, H., and Robinson, J., *Rec. Trav. Chim.*, **48**, 328 (1929).
- ¹⁵ Bunyan, P. J., and Cadogan, J. I. G., *J. Chem. Soc.*, 1304 (1962).
- ¹⁶ Kittredge, J. S., and Hughes, R. E., *Biochemistry*, **3**, 991 (1964).
- ¹⁷ Kometiani, P. A., in *Proc. Fifth Intern. Congr. Biochem.*, **7**, 136. *Biosynthesis of Lipids*, edit. by Popjak, G. (Pergamon Press, 1963).
- ¹⁸ Rutter, W. J., *Fed. Proc.*, **23**, 1253 (1964).
- ¹⁹ White, A., Handler, P., and Smith, E. L., *Principles of Biochemistry*, third ed., 371 (McGraw-Hill, 1964).

IMMUNITY TO MALARIA: ANTIGENIC VARIATION IN CHRONIC INFECTIONS OF *Plasmodium knowlesi*

By DR. K. N. BROWN and I. N. BROWN

National Institute for Medical Research, Mill Hill, London, N.W.7

SOME parasitic protozoa can survive and multiply in host animals which show high levels of parasiticidal antibody developed as a result of infection¹⁻⁴. In trypanosomiasis, repeated parasitaemias in the presence of lytic antibodies are produced by a succession of antigenic variants. Each variant stimulates a specific trypanocidal antibody and at the same time remains unaffected by antibodies to other variants; more than twenty variants have been recorded from one strain⁵. In *Brucei* group trypanosomes, the variants appear to differ in the antigenic constitution of at least two groups of soluble proteins⁶⁻⁸.

There is indirect evidence that some antigenic variation can occur in rodent malaria⁹, but repeated antigenic changes, of an order which might account for chronic simian or human malaria, have not previously been demonstrated. Alternative explanations of chronicity in

malaria include the suggestions that the parasite is only poorly immunogenic, or that, being intracellular, it is not accessible to antibodies¹⁰. On the other hand, it is unlikely that such a complex foreign organism would be poor in antigens, and the intracellular schizonts at least are known to react with antibodies¹¹. These considerations suggest that frequent antigenic variation similar to that occurring in trypanosomes is the most likely explanation of chronic malaria. Using a parasite agglutination test¹¹, direct evidence has now been obtained for repeated antigenic variation in *Plasmodium knowlesi* infections of monkeys. These experiments, and some of their implications for the immunology of malaria, are summarized here; full details will be published elsewhere.

A syringe-passed derivative of the 'Nuri' strain of *P. knowlesi*¹² was used, which is highly virulent in rhesus monkeys (*Rhesus mulatta*); death occurs within 4-6 days

of patency, with up to 90 per cent of the erythrocytes infected. Sub-curative chemotherapy produces a chronic relapsing infection with parasitaemias rarely rising above 1 per cent. A frozen stabilate¹³ of this strain, designated K1, was isolated by freezing citrated blood of a monkey 6 days after infection. Stabilates of four relapses, K1A, K1B, K19 and K21, were also isolated. K1A and K1B were successive relapses occurring 10 and 21 days after sub-curative proguanil treatment of a K1-infected monkey. Stabilate K19 was collected from a monkey infected with K1A, cured with sulphadiazine when the infection was patent, and then reinfected with K1A. Reinfection produced without further drug treatment a series of spontaneously terminating parasitaemias, of which stabilate K19 was the fourth; it appeared 34 days after reinfection. Another stabilate (K21) was collected at the fourth patency in a second chronically infected monkey. When nearly all the stock of a stabilate had been used, a direct derivative was isolated by inoculating the stabilate into a monkey from which further blood samples were frozen down soon after patency. These secondary isolations were designated K1A2 and K1B2.

Antisera to parasites derived from specific stabilates were obtained from monkeys cured with sulphadiazine, or sulphadiazine plus proguanil, when the infection had become patent following inoculation of the stabilate; sera were taken before infection and 7-14 days after drug treatment. Serum samples were also collected at intervals from the chronically infected monkeys. All the sera were stored at -20° C and inactivated at 56° C before use.

For the agglutination test, schizont- or trophozoite-infected erythrocytes were separated by centrifugation from heavily parasitized blood taken 6-7 days after infection with a stabilate. Five-fold dilutions of the serum in diluent (1 per cent normal monkey serum in saline) were set out in W.H.O. haemagglutination trays, and equal volumes (0.4 ml.) of a saline suspension of either schizont- or trophozoite-infected cells containing approximately 4×10^7 cells per ml. were added to each well. The trays were incubated at 20°-22° C for at least 3 h and then examined macro- and microscopically for agglutination. One unit of antibody was arbitrarily taken as the maximum dilution producing agglutination; serum titres were recorded in units/ml.¹⁴

The results of testing schizont-infected erythrocytes against antisera to homologous and heterologous parasites are summarized in Table 1, which shows both the high specificity of the reaction produced by each relapse type, and the titre of agglutinins detected. The antigenic specificity of each relapse indicated by these results was further emphasized when K19-derived parasites, which had failed to react with the antisera to other relapses, were tested against sera from the chronically infected monkey from which it was isolated. Two serum samples taken during the infection, but before the K19 relapse, had no effect on K19 parasites at 1/50 dilution, although the first sample agglutinated K1A, and the second sample agglutinated both K1A and K1B, at titres greater than 1,250 units/ml. In contrast, K19 parasites were agglutin-

ated by serum collected 6 days after the isolation of K19 at a titre of 6,250, and by a sample collected 67 days later at 31,250 units/ml. A similar result was obtained with sera and the stabilate K21 obtained from the other chronically infected monkey, and these K21 parasites appeared to be antigenically related to K19 (Table 1).

Schizonts derived from K1A2 and K1B2 (secondary isolations of K1A and K1B) were not appreciably less specific than K1A and K1B, but parasites at the second monkey passage of K1A2 reacted with both K1A2 and K1B2 sera at high titre, indicating that an increase in antigenic heterogeneity had occurred with passage.

Later relapses in the chronically infected monkeys rarely showed more than one parasite per hundred erythrocytes. The stabilates (K19 and K21) derived from late relapses produced a lethal infection when inoculated into normal monkeys, indicating that the low parasitaemias in the chronically infected animals were due to immune suppression of the parasites and not to a loss of virulence by these later variants.

As judged by the titres of agglutinins detected in our tests, *P. knowlesi* and presumably, therefore, other species of malaria, are quite strongly immunogenic. Natural antibodies were not involved in these reactions, since sera collected before infection were without effect. In some instances, artificial dissociation of the parasites by anti-malarials may have enhanced the immune response, but comparable titres of agglutinins were also detected after the spontaneous disappearance of the K19 and K21 relapses. Parasites derived from one relapse stabilate agglutinated at high titres only with antisera obtained from monkeys infected with the same stabilate, but sera from monkeys cured of infection with parasites from other relapses had little or no effect. It is conceivable that the difference shown by the K1A and K1B stabilates (that is, of successive relapses) were drug-induced, but this cannot apply to the other relapses, so that the specificity of the reaction seems explicable only on the basis of antigenic variation analogous to that occurring in trypanosomes. Also, in trypanosomiasis, drug- and antibody-induced relapse variants are antigenically indistinguishable (K. N. B., unpublished results). Eaton has shown that trophozoite-infected erythrocytes are not agglutinated¹¹, and we have found this to be so with antisera from monkeys infected with both homologous and heterologous stabilates. In a comparison of schizont- and trophozoite-infected cells isolated at the same time from a K1-infected monkey, the schizont-infected cells gave titres of > 1,000 units/ml. for anti-K1 serum, but the trophozoite-infected cells were not agglutinated by this serum or by antiserum to any other relapse. The absence of agglutination with homologous antiserum implies either that the trophozoites and schizonts are antigenically different, or that the trophozoites, unlike the schizonts, are protected from antibody by the containing erythrocyte. In exoerythrocytic *P. gallinaceum* the host cell becomes progressively damaged as the parasite matures¹⁵, and similar damage by the mature schizont to erythrocytes may render the schizonts more accessible to antibodies.

Table 1. TITRES OF AGGLUTININS AGAINST SCHIZONT-INFECTED CELLS IN SERA FROM MONKEYS IMMUNIZED WITH *P. knowlesi*

Serum												
Immunized with stabilate		K1		K1A			K1B		K1B2		K19	K21
Monkey		1	2	1	2	3	1	2	1	2	1*	1*
Antigen	K1	> 500	6,250	10	50	—	10	—	< 10	250	—	—
	K1A	—	< 10	1,250	6,250	> 1,250	< 10	< 10	—	< 10	—	—
	K1A2	—	—	6,250	—	—	< 10	—	50	—	—	—
	K1B	—	—	< 10	—	< 10	1,250	1,250	—	—	—	—
	K1B2	—	—	10	10	—	1,250	—	31,250	31,250	—	—
	K19	—	—	< 10	—	—	< 10	—	< 10	—	31,250	—
	K21	—	< 10	50	—	—	10	10	—	10	1,250	6,250

* Serum taken from chronically infected monkey after isolation of the stabilate.

So far, the present experiments have shown only relapse-specific agglutinins and not the protective antibodies of similar specificity which are implied by the relapsing nature of chronic malaria. The appearance of aberrant schizonts and the decrease in the proportion of mature to immature schizonts which occurs at crisis^{10,16} indicate the possible protective role of antibodies reacting with schizont-infected cells, but whether the cells are destroyed by lysis or by phagocytosis following agglutination or opsonization is not clear.

In our experiments, relapses in chronic infections usually produced low parasitaemias only, but when stabilates of these relapses were inoculated into normal 'non-immune' monkeys, virulent fatal infections followed. Thus, there appears to be another partially protective immune response transcending antigenic variation, although purely variant-specific antibodies are probably decisive in terminating each relapse. The nature of the more general immunity is not known but it seems to be effective against all relapse variants, and may be a response to antigens common to all variants (unpublished gel-diffusion experiments have shown that common antigens occur). Alternatively, an adjuvant action by parasite constituents¹⁷ may produce a non-specific heightening of the immune response. Some of the splenomegaly and liver and bone marrow changes characteristic of malaria^{10,18} are perhaps associated with an adjuvant action^{17,19}, although in one investigation malarious children of three years of age showed no better response to tetanus toxoid than controls²⁰; auto-immune destruction of erythrocytes may also play a part in reducing parasitaemias²¹.

Exoerythrocytic infection was unlikely to have occurred with our blood-induced *P. knowlesi* infections²², but in other species, for example, *P. vivax*, erythrocyte-infecting relapses may also arise from the exoerythrocytic pool²³. There may be separate and distinct immunity to the two phases, erythrocytic and exoerythrocytic²⁴, and the persistence of exoerythrocytic infection as in *P. vivax* may itself involve antigenic variation. In infections like *P. falciparum*, a variant-transcending immunity in both the liver and blood may limit the duration of the infection.

The implications of antigenic variation for trypanosomiasis have been discussed elsewhere⁶, and some of these possibilities may be applicable to malaria. Insect transmission has a stabilizing effect on antigenic variation in trypanosomes, as all variants generally return to a common parent type following development in the vector^{25,26}. The possible occurrence of this reversion in malaria is of considerable biological interest, particularly as this parasite is known to have a sexual stage in the vector, and it may also be important in the interpretation of epidemiological and immunological data. The fluorescent-antibody and haemagglutination tests^{27,28} are currently used in malaria epidemiology but these reactions are not species specific and probably normally measure 'common' antigens. Nevertheless, when human malaria parasites are used as a source of antigen, possible reactions with the 'variable' antigens should be taken into account.

Frequent antigenic variation by the parasite may also be a factor contributory to the persistent macroglobulinaemia which sometimes accompanies malaria²⁹, as each successive variant antigen is likely to stimulate the production of a new 19S antibody³⁰ to give the raised IgM levels observed during continuously patent infections and at each relapse; a similar macroglobulinaemia occurs in trypanosomiasis³¹.

The simultaneous occurrence of two levels of immunity, one specific for each relapse variant, and the other transcending antigenic variation and only partially inhibiting all relapses, may account for the inconsistent results that have been obtained by artificial immunization^{10,32}, where the possibility of antigen variability within the strain used has not been allowed for. The development of an anti-malarial vaccine, particularly against *P. falciparum*, now seems to depend on the following considerations: (a) the

successful *in vitro* cultivation of the human parasite as a source of dead antigen or of attenuated live material³³; (b) the absence of appreciable differences between strains; (c) the ability of humans to develop an immunity transcending antigenic variation. Geographically separated strains may not be as different as once thought³⁴, and human adults do develop reasonable immunity to *P. falciparum*³⁵. Children exposed to endemic malaria, however, show high parasitaemias up to 6–10 years of age, and this susceptibility may mean that resistance depends on an experience of a wide but finite range of antigenic variants occurring in one locality. Alternatively, children, unlike adults, are perhaps incapable of developing a generalized immunity effective against all variants. Children do appear to become blood-positive earlier than adults after prophylactic antimalarial treatment^{36,37}, and young rats infected with *P. berghei* relapse more frequently and with higher parasitaemias than older animals (I. N. B., unpublished results). Thus, the young of some hosts may be constitutionally incapable of developing a generalized immunity, and if this should prove to be so, immunization is unlikely to be effective. Much more evidence is needed on this point.

To summarize, frequent antigenic variation has been demonstrated in blood-induced *P. knowlesi* infections, which implies that it probably occurs in human malaria also. This variation can explain the chronicity of the disease and the inconsistent results obtained with artificial vaccines; it may possibly account for, or contribute to, the persistently raised IgM levels which accompany malaria. Some degree of immunity transcending antigenic variation also occurs, but its potential value as a possible basis for the development of a vaccine is uncertain.

- ¹ Coggeshall, L. T., and Kumm, H. W., *J. Exp. Med.*, **66**, 177 (1937).
- ² Remington, J. S., Melton, M. L., and Jacobs, L., *J. Immunol.*, **87**, 578 (1961).
- ³ Mesnil, F., and Brimont, E., *Ann. Inst. Pasteur*, **23**, 129 (1909).
- ⁴ Andersen, F. L., Lowder, L. J., Hammond, D. H., and Carter, P. B., *Exp. Parasit.*, **16**, 23 (1965).
- ⁵ Lourie, E. M., and O'Connor, B. J., *Ann. Trop. Med. Parasitol.*, **31**, 319 (1937).
- ⁶ Brown, K. N., British Soc. Immunology Symp. Immunity to Protozoa, 1961, in *Immunity to Protozoa*, edit. by Garnham, P. C. C., Pierce, A. E., and Rolitt, I., 204 (Blackwell, Oxford, 1963).
- ⁷ Williamson, J., and Brown, K. N., *Exp. Parasitol.*, **15**, 44 (1964).
- ⁸ Brown, K. N., and Williamson, J., *Exp. Parasitol.*, **15**, 69 (1964).
- ⁹ Cox, H. W., *J. Immunol.*, **82**, 209 (1959).
- ¹⁰ Taliaferro, W. H., in *Malariaology*, edit. by Boyd, M. F., **2**, 935 (Saunders, Philadelphia, 1949).
- ¹¹ Eaton, M. D., *J. Exp. Med.*, **67**, 857 (1938).
- ¹² Jaswant Singh, Ray, A. P., and Nair, C. P., *Nature*, **172**, 122 (1953).
- ¹³ Lumsden, W. H. R., and Hardy, G. T. C., *Nature*, **205**, 1032 (1965).
- ¹⁴ Humphrey, J. H., and White, R. G., *Immunology for Students of Medicine*, 193 (Blackwell, Oxford, 1963).
- ¹⁵ Meyer, H., and Musacchio, M. de O., *J. Protozool.*, **12**, 193 (1965).
- ¹⁶ Taliaferro, W. H., and Taliaferro, L. G., *Amer. J. Hyg.*, **20**, 1 (1934).
- ¹⁷ Munoz, J., *Adv. Immunol.*, **4**, 397 (1964).
- ¹⁸ Clark, H. C., and Tomlinson, W. J., in *Malariaology*, edit. by Boyd, M. F., **2**, 874 (Saunders, Philadelphia, 1949).
- ¹⁹ Havens, Jun, W. P., *Intern. Arch. Allergy Appl. Immunol.*, **14**, 75 (1959).
- ²⁰ McGregor, I. A., and Barr, M., *Trans. Roy. Soc. Trop. Med. Hyg.*, **56**, 368 (1962).
- ²¹ Zuckerman, A., *Exp. Parasit.*, **15**, 138 (1964).
- ²² Garnham, P. C. C., Lalson, R., and Cooper, W., *Trans. Roy. Soc. Trop. Med. Hyg.*, **51**, 384 (1957).
- ²³ Garnham, P. C. C., *Brit. Med. Bull.*, **8**, 10 (1961).
- ²⁴ Shortt, H. E., and Garnham, P. C. C., *Trans. Roy. Soc. Trop. Med. Hyg.*, **41**, 785 (1948).
- ²⁵ Broom, J. C., and Brown, H. C., *Trans. Roy. Soc. Trop. Med. Hyg.*, **34**, 53 (1940).
- ²⁶ Gray, A. R., *Intern. Sci. Committee Trypanosomiasis Research*, ninth meeting, 361 (Conakry, 1962).
- ²⁷ Tobie, J. E., *Amer. J. Trop. Med. Hyg.*, **13**, 195 (1964).
- ²⁸ Stein, B., and Desowitz, R. S., *W.H.O./Mal/393* (1963).
- ²⁹ Tobie, J. E., in *Progress in Protozoology*, 164 (Excerpta Medica Foundation, London, 1965).
- ³⁰ Haurowitz, F., *Physiol. Rev.*, **45**, 1 (1965).
- ³¹ Mattern, P., Masseyeff, R., Michel, R., and Peretti, P., *Ann. Inst. Pasteur*, **101**, 352 (1961).
- ³² *Progress in Protozoology* (Excerpta Medica Foundation, London, 1965).
- ³³ Weiss, M. L., *Progress in Protozoology*, 168 (Excerpta Medica Foundation, London, 1965).
- ³⁴ McGregor, I. A., Carrington, S., and Cohen, S., *Trans. Roy. Soc. Trop. Med. Hyg.*, **57**, 170 (1963).
- ³⁵ McGregor, I. A., *Trans. Roy. Soc. Trop. Med. Hyg.*, **58**, 80 (1964).
- ³⁶ Kilgler, I. J., and Mer, G., *Trans. Roy. Soc. Trop. Med. Hyg.*, **27**, 269 (1963).
- ³⁷ Clyde, D. F., *W.H.O./Mal/508* (1965).

A PHARMACOLOGICAL INVESTIGATION OF HUMAN ISOLATED ILEUM

By DR A. BENNETT

Department of Surgery, King's College Hospital Medical School, Denmark Hill, London, S.E.5

A PART from a report of the action of 5-HT on isolated strips of circular muscle from the human ileum¹, the *in vitro* pharmacology of this tissue has been little investigated. The experiments recorded here are a preliminary investigation of the pharmacology of the circular and longitudinal muscle of human isolated ileum.

Strips of muscle were cut from specimens resected at operation and in most instances the mucosa was removed. Some of the specimens were from mid-ileum but most were from terminal ileum; a few of the circular muscle strips were from the ileo-caecal junction. The preparations were suspended in an organ bath containing Krebs's solution at 37° C and movements of the muscle were recorded on a revolving smoked drum by a frontal writing lever. The load on the tissue was usually 1 g. The details of the method and the drugs used were as described by Bucknell and Whitney² in their examination of human isolated taenia coli.

Most preparations exhibited moderate spontaneous activity throughout the experiment. Acetylcholine (0.01–0.5 µg/ml.) caused a contraction in each of the 21 experiments on circular muscle and in each of the 15 experiments on longitudinal muscle. This contraction

was either unaffected (Fig. 1) by hexamethonium (20–50 µg/ml.) or was occasionally reduced a little; the contraction was prevented by hyoscine (20–200 ng/ml.) and potentiated by eserine (0.3–1 µg/ml.). These results indicate that acetylcholine acts on muscarinic receptors and that none or only a little of its effect at these concentrations is due to stimulation of parasympathetic ganglia.

The sympathomimetic amines phenylephrine, nor-adrenaline, adrenaline and isoprenaline relaxed both the circular and longitudinal muscle strips (including those from the ileo-caecal junction), and inhibited spontaneous activity in each of seventeen experiments. In five of these, the muscle 'tone' was not high enough to show a relaxation, and the inhibitory effects were demonstrated by a reduction in the response to acetylcholine. In seven experiments, the α -receptor antagonist 'Hydergine' (1 µg/ml.) prevented the relaxation caused by phenylephrine but not that caused by isoprenaline; the β -receptor antagonist pronethalol (10 µg/ml.) prevented the relaxation caused by isoprenaline but not by phenylephrine (Fig. 2). The simultaneous presence of both antagonists prevented the relaxation produced by any of the sympathomimetic amines. As phenylephrine acts mainly on α -adrenergic

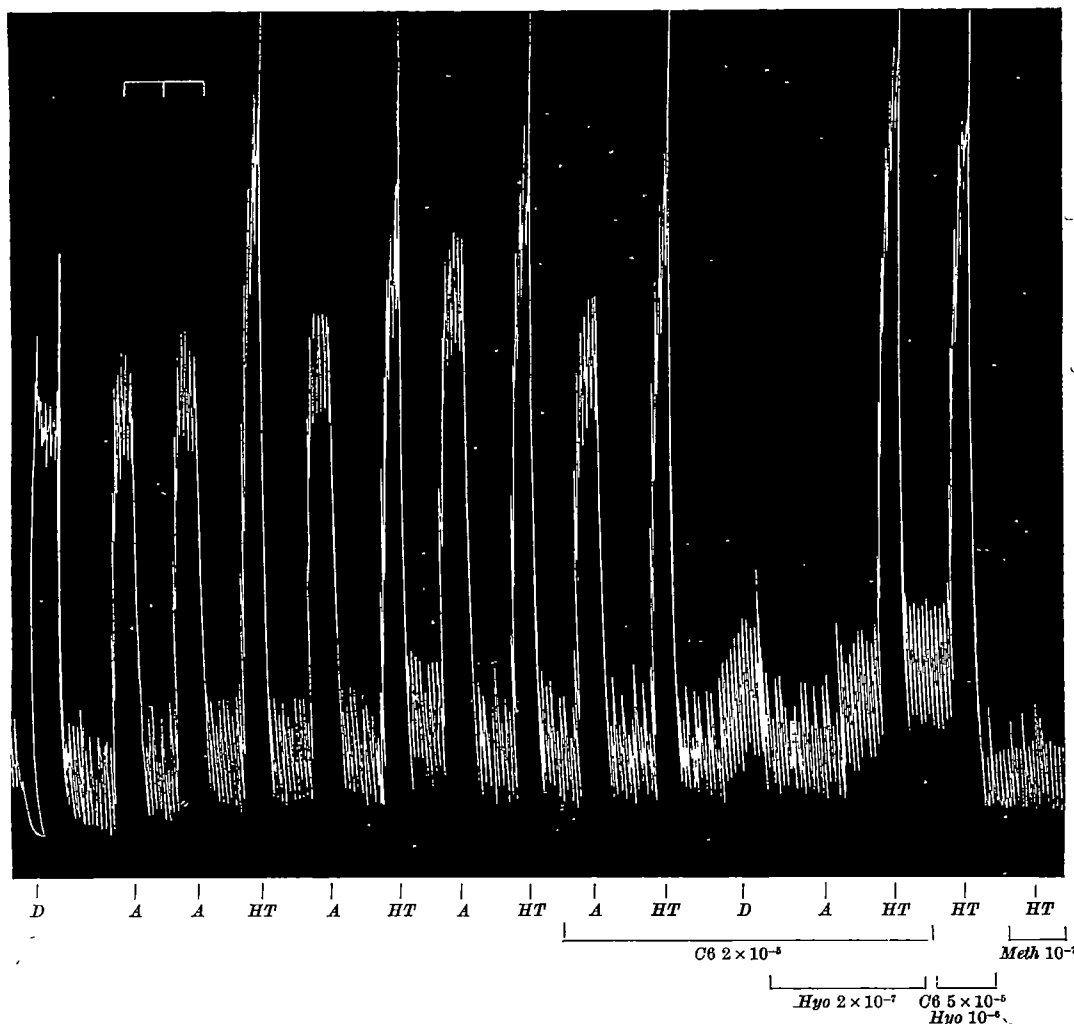


Fig. 1. The effects of hexamethonium (C6), hyoscine (Hyo) and methysergide (Meth) on the responses of a circular muscle strip of ileum to DMPP (D; 10 µg/ml.), acetylcholine (A; 20 ng/ml.) and 5-hydroxytryptamine (HT; 20 ng/ml.). Hexamethonium blocks DMPP but acetylcholine and 5-HT are unaffected. Hyoscine blocks acetylcholine but 5-HT is unaffected. Methysergide blocks 5-HT. Time-signal 1 min.

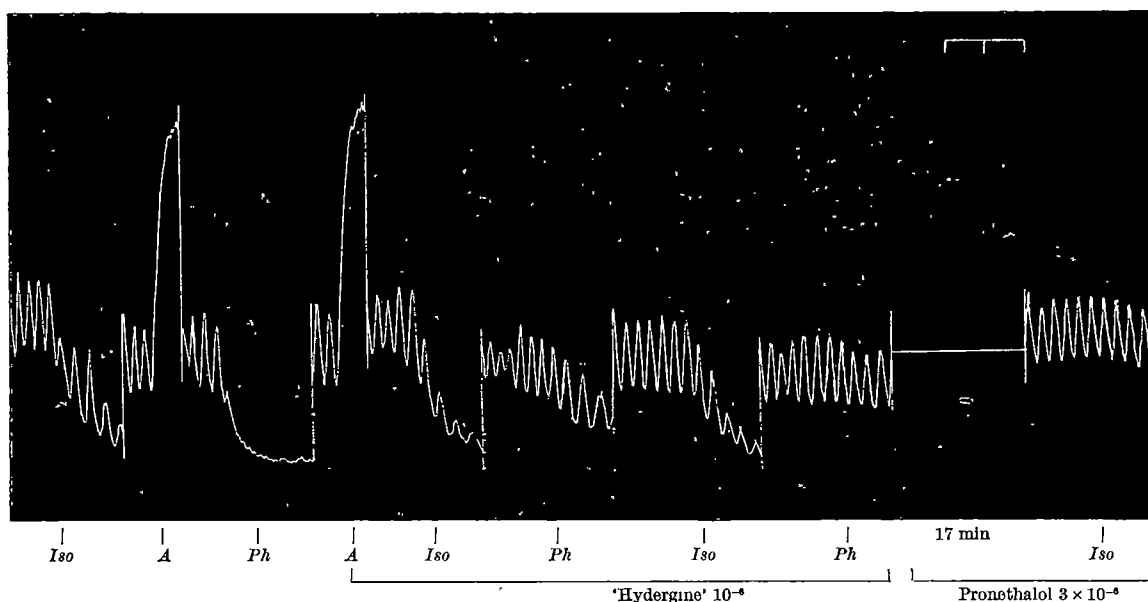


Fig. 2. The effects of 'Hydergine' and pronethalol on the relaxations produced by phenylephrine (Ph, 17 $\mu\text{g}/\text{ml}$.) and isoprenaline (Iso; 0.7 $\mu\text{g}/\text{ml}$.) 'Hydergine' blocks phenylephrine but not isoprenaline. Pronethalol blocks the effect of isoprenaline. Time-signal 1 min

receptors, and isoprenaline acts mainly on β -adrenergic receptors^{3,4}, these results indicate that both α - and β -adrenergic receptors are present in human ileum and that stimulation of either produces a relaxation. Similar results have been obtained with human colon² and jejunum⁵. Some investigators have found that in laboratory animals adrenaline contracts the ileocolic sphincter^{6,7} and terminal ileum⁸. The opposite findings in the present experiments that the circular muscle strips from the region of the human ileocolic sphincter were relaxed by sympathomimetic amines agrees with the *in vivo* observation⁹ on the ileocolic sphincter of a patient.

The ganglion stimulant DMPP (20–40 $\mu\text{g}/\text{ml}$.) was used in fourteen experiments on circular muscle strips and in thirteen experiments on longitudinal muscle strips. In every experiment on the circular muscle strips, including those from the region of the ileo-caecal sphincter, DMPP produced a relaxation; in one experiment this relaxation was followed by a contraction and in another experiment the first dose of DMPP caused a contraction, the second dose caused a relaxation followed by a small contraction, and thereafter DMPP only relaxed the tissue. Hexamethonium (20–40 $\mu\text{g}/\text{ml}$.) was used in eight of the fourteen experiments (Fig. 1) and blocked the response to DMPP in every case. In the presence of pronethalol (10 $\mu\text{g}/\text{ml}$.) or eserine (1 $\mu\text{g}/\text{ml}$.) DMPP caused a contraction. The effects of DMPP on the longitudinal muscle strips were less consistent. There was a relaxation in six experiments, a contraction in four experiments and no effect in one experiment. In the remaining three instances, the longitudinal strips were initially relaxed by DMPP, but as the experiment continued, DMPP produced only a contraction in one experiment, and produced a small relaxation followed by a larger contraction in two others. Hexamethonium (20–40 $\mu\text{g}/\text{ml}$.) prevented both the relaxant and contractile effect of DMPP. Hyoscine (0.1 $\mu\text{g}/\text{ml}$.) inhibited the contractile effect of DMPP and in one experiment converted it into a relaxant effect, while in other experiments 'Hydergine' (1 $\mu\text{g}/\text{ml}$.) and eserine (0.5 $\mu\text{g}/\text{ml}$.) converted the relaxation into a contraction.

Since adrenergic receptor blockade inhibited the relaxant effect of DMPP, and hyoscine abolished its contractile effect, it appears that DMPP can release both an adrenaline-like substance and acetylcholine. The finding that hexamethonium blocked the contractile effect of DMPP indicates that DMPP stimulates parasympathetic ganglia in the wall of the ileum. On the other hand, the possi-

bility that DMPP relaxes the ileum by acting on sympathetic ganglia conflicts with the general view that there are no sympathetic ganglia in the bowel wall. It is possible that the relaxant effect of DMPP is due to an action on sympathetic nerve endings, or on stores of adrenaline-like substances, or on inhibitory nerves in the intrinsic nerve plexuses.



Fig. 3. The effects of hexamethonium (C6), hyoscine (Hyo) and mepyramine (Mep) on the biphasic response to histamine. Hexamethonium and hyoscine have no effect but mepyramine blocks the relaxant and the contractile action of histamine. Time-signal 1 min

Human jejunum⁵ is usually contracted by DMPP, the ileum is usually relaxed, and the colon² always relaxed by DMPP. It appears, therefore, that this drug has a greater tendency to produce a relaxation in the more distal parts of the bowel.

Histamine (0.3–4 μ g/ml.) caused either a relaxation (twelve experiments) or a contraction (seven experiments) of strips of circular and longitudinal muscle; it had a biphasic effect in three instances and had no effect (0.4–2 μ g/ml.) in another. Both the relaxant and the contractile responses were unaffected by hexamethonium (20–40 μ g/ml.; Fig. 3) but were blocked by mepyramine (5–100 ng/ml.). Sympathetic receptor blockade did not affect the relaxation produced by histamine, and its contractile effect was not altered by hyoscine in doses which abolished the response to acetylcholine. Histamine, therefore, appears to have only a direct action on the longitudinal and circular muscle. This contrasts with the finding in guinea-pig ileum¹⁰ that histamine has a direct action on the longitudinal muscle but has an indirect action on the circular muscle.

5-Hydroxytryptamine (0.01–1 μ g/ml.) contracted both the circular and longitudinal muscle strips in each of twenty-four experiments. The contractions were prevented by methysergide (0.1 μ g/ml.; seven experiments) but were unaffected by hexamethonium (10–50 μ g/ml.; five experiments), hyoscine (0.1–1 μ g/ml.; seven experi-

ments) or mepyramine (0.1–0.2 μ g/ml.; four experiments) in doses which abolished the responses to DMPP, acetylcholine and histamine, respectively (Fig. 1). Fishlock¹ found that lysergic acid diethylamide completely blocked the effect of 5-HT on human ileum, and that hexamethonium and hyoscine partly blocked the effect. He concluded that 5-HT had both an indirect action (that is, through nerves) and a direct action on the tissue. Since lysergic acid diethylamide¹¹ and methysergide¹² have no effect on the indirect action of 5-HT, and since, in the experiments recorded here, hexamethonium and hyoscine did not affect the response to 5-HT, it appears that 5-HT acts directly on human ileal muscle.

I thank the many surgeons from this and other hospitals for their co-operation in making available the specimens of ileum.

¹ Fishlock, D. J., *J. Physiol.*, **170**, 11 (1964).

² Bucknell, A., and Whitney, B., *Brit. J. Pharmacol.*, **23**, 164 (1964).

³ Ahlquist, R. P., and Levy, B., *J. Pharmacol. Exp. Ther.*, **127**, 146 (1959).

⁴ Levy, B., and Ahlquist, R. P., *J. Pharmacol. Exp. Ther.*, **133**, 202 (1961).

⁵ Whitney, B., *J. Pharm. Pharmacol.*, **17**, 465 (1965).

⁶ Elliot, T. R., *J. Physiol.*, **32**, 401 (1905).

⁷ Hinrichsen, J., and Ivy, A. C., *Amer. J. Physiol.*, **96**, 494 (1931).

⁸ Munro, A. F., *J. Physiol.*, **112**, 84 (1951).

⁹ White, H. L., Rainey, W. R., Monaghan, B., and Harris, A. S., *Amer. J. Physiol.*, **108**, 449 (1934).

¹⁰ Brownlee, G., and Harry, J., *Brit. J. Pharmacol.*, **21**, 544 (1963).

¹¹ Gaddum, J. H., and Hamed, K. A., *Brit. J. Pharmacol.*, **9**, 240 (1954).

¹² Day, M., and Vane, J. R., *Brit. J. Pharmacol.*, **20**, 150 (1963).

CENTRAL STIMULANT PROPERTIES OF COMPOUNDS WITH PERIPHERAL MUSCARINIC PROPERTIES

By G. B. LESLIE

Research Laboratories, May and Baker Ltd., Dagenham, Essex

MOST drugs with an excitatory or stimulant action on the central nervous system (other than convulsants) have peripheral actions on adrenergic mechanisms. Imipramine-like anti-depressants and cocaine enhance the contractions of the cat nictitating membrane in response to noradrenaline or electrical stimulation of the pre-ganglionic sympathetic nerve¹. Monoamine oxidase inhibitors potentiate the actions of some sympathomimetic amines and cause an increase in the amounts of amines found in the tissues. Amphetamine has sympathomimetic actions. It was suggested by Rothballer² that a central adrenergic mechanism may be involved in the stimulant actions of these drugs. We know of few reports of central stimulant activity of compounds with peripheral muscarinic properties. Borison³ observed that pilocarpine administered into the cerebral ventricles of cats occasionally caused a rage reaction characterized by pilo-erection, hissing and unsheathing of the claws. A similar response to intraperitoneal injection of pilocarpine was reported by Zablocka and Esplin⁴. Another muscarinic compound, tremorine, was shown by Baker, Hosko, Rutt and McGrath⁵ to produce a similar state of rage, and George, Haslett and Jenden⁶ observed this following administration of oxotremorine, a metabolite of tremorine. Sabelli and Toman⁷ produced rage in cats with tremorine, arecoline, morphine and lysergic acid diethylamide.

We have examined the actions on the central nervous system of a number of drugs with muscarinic properties. The drugs examined were oxotremorine, pilocarpine, arecoline, carbachol and acetylcholine, which are known to cause bradycardia, hypotension, increased intestinal tonus, salivation and sweating, and are antagonized by atropinic drugs. These compounds were found to have marked central action of a stimulant nature.

The activity of these muscarinic drugs has been compared with that of several standard drugs in four tests for central nervous system stimulation. The tests used

were: (1) production of rage-like states in the cat; (2) behavioural changes in week-old chicks; (3) reversal of reserpine-induced sedation in the rabbit; (4) reversal of chlorpromazine-induced sedation in the rabbit.

(1) *Production of rage in the cat.* Drugs were injected into the cephalic vein or into the cerebral ventricles via an implanted cannula as described by Feldberg and Sherwood⁸. Cats were premedicated with a quaternary derivative of atropine (methyl bromide salt, 1 mg/kg) to prevent peripheral muscarinic effects, or with hyoscine hydrobromide (1 mg/kg) or ethopropazine (10 mg/kg) to determine whether the rage effect was mediated by a central cholinergic mechanism.

The cats were judged by two independent observers for the presence of rage characterized by pilo-erection, unsheathing of the claws, hissing, spitting, growling, crouching, flattening of the ears and attacking other cats or a moving object introduced into the cage. The results are summarized in Table 1. A number of muscarinic agents caused rage when administered intravenously. Rage was produced with physostigmine only at doses

Table 1. ACTIVITY OF SOME MUSCARINIC AND OTHER DRUGS IN PRODUCING RAGE IN CATS

Minimal dose (mg/kg intravenous) producing rage in cats premedicated 1 h previously with either atropine methylbromide, hyoscine hydrobromide or ethopropazine
Figures in parentheses refer to doses producing rage when injected into cerebral ventricles

Drug	Atropine methylbromide 1 mg/kg (i.p.)	Hyoscine hydrobromide 1 mg/kg (i.p.)	Ethopropazine 10 mg/kg (i.p.)
Morphine	10	10	10
Arecoline	0.2	>10	>10
Oxotremorine	0.1 (0.02)	>10 (>0.08)	>1.0
Pilocarpine	25	>200	>200
Physostigmine	0.1	>0.8	
Prostigmine	>0.4		
Acetylcholine	0.25 (>0.3)		
Carbachol	>0.25 (0.005)		
Nicotine	>1.0 (>0.02)		
Dimethylphenyl- piperazinium	>0.1		

which caused convulsions after the rage. When administered into the cerebral ventricle, carbachol caused a rage response, but acetylcholine and nicotine did not. Acetylcholine caused convulsions and loss of consciousness. Nicotine caused vomiting and twitching of the ears.

Rage produced by muscarinic drugs in the cerebral ventricles was preceded by tremors and ear-twitching and was followed by stupor.

Rage produced by muscarinic agents was similar to that caused by morphine, but unlike that produced by morphine it was prevented by premedication with hyoscine or ethopropazine. A further difference in the rage state was that cats treated with muscarinic rage-producing drugs would attack one another and any moving object. Morphine-treated cats made little attempt to bite or attack. Cats treated with muscarinic drugs also appeared hallucinated and more ataxic.

(2) *Behavioural effects in week-old chicks.* Since chicks at one week old have no effective blood-brain barrier⁹, it is possible to observe the central actions of compounds which do not normally reach the brain when injected intravenously. The effects of a number of muscarinic and other drugs are shown in Table 2. The drugs were injected in warm saline solution into the jugular vein. Arecoline, pilocarpine, oxotremorine and physostigmine produced a behavioural response similar to that observed by Key and Marley¹⁰ with amphetamine and methylphenidate—spreading of the wings, raising the head, feather erection, increased cheeping and increased ambulation followed by ataxia. Unlike amphetamine, the action of the muscarinic stimulant drugs were completely antagonized by hyoscine, ethopropazine and other atropinic drugs. Arecoline caused sedation in chicks premedicated with hyoscine.

Table 2. BEHAVIOURAL EFFECTS OF SOME MUSCARINIC AND OTHER DRUGS IN THE WEEK-OLD CHICK

Figures in parentheses refer to activity after premedication with hyoscine (1 mg/kg)

Drug	Groups of 5 chicks used at each dose-level	
	Dose (mg/kg i.v.) producing stimulation in 50% of animals	Dose (mg/kg i.v.) producing sedation in 50% of animals
Amphetamine	0.5 (0.5)	—
Adrenaline	> 1.0	0.02 (0.02)
Arecoline	0.05 (> 0.25)	> 0.1 (0.05)
Oxotremorine	0.025 (> 0.05)	> 0.1 (> 0.1)
Pilocarpine	25 (> 100)	> 100 (> 100)
Physostigmine	> 1.0 (> 1.0)	0.25 (0.25)
Prostigmine	> 1.0 (> 1.0)	0.20 (0.20)
Acetylcholine	> 1.0	0.8
Carbachol	> 0.2	0.05
Nicotine	> 1.0	0.025
Dimethylphenyl- piperazinium	> 0.025	0.0125

Acetylcholine, carbachol, nicotine and prostigmine caused no excitatory effects but produced sedative behaviour similar to that described by Key and Marley with adrenaline. The chicks became sedated, the head dropped, motor activity was much reduced and the eyes closed. These effects were not blocked by premedication with hyoscine nor with mecamlamine or pempidine.

(3 and 4) *Antagonism of drug-induced sedation in the rabbit.* The drugs were infused in dilute solution into the marginal ear veins of rabbits sedated with reserpine¹¹ or chlorpromazine¹². Central stimulants such as amphetamine, cocaine or lysergic acid diethylamide (LSD) and anti-depressant drugs such as imipramine and amitriptyline are able to reverse the sedation (as measured by reversal of ptosis) due to both chlorpromazine and reserpine. The activities of some muscarinic and other drugs in these tests are shown in Fig. 1. Arecoline, pilocarpine, oxotremorine and physostigmine were highly active in these tests, while nicotine, acetylcholine, carbachol and prostigmine were inactive. The activity of the active muscarinic drugs was abolished by premedication with hyoscine. That of amphetamine and LSD was not significantly altered by hyoscine.

The results obtained in our experiments indicate the presence of excitatory mechanisms in the central nervous

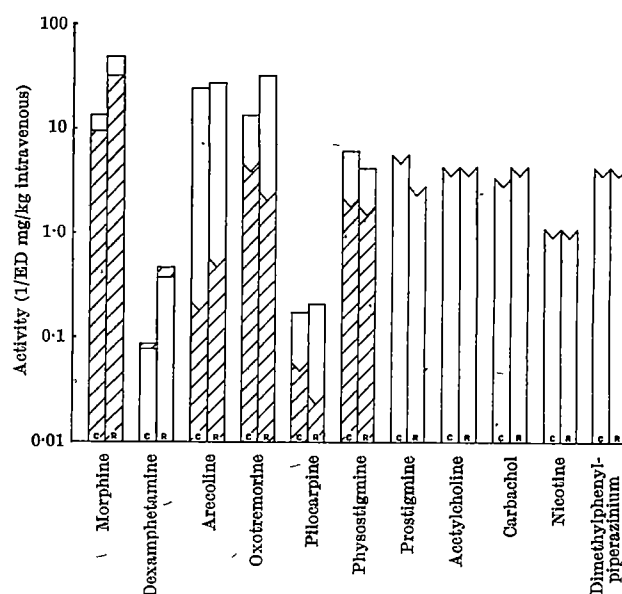


Fig. 1. Activity of some muscarinic and other drugs in reversing reserpine- and chlorpromazine-induced ptosis in the rabbit. White, activity after premedication with atropine methyl bromide (5 mg/kg); hatched, activity after premedication with hyoscine hydrobromide (1 mg/kg). C, Activity against chlorpromazine, R, activity against reserpine

system which are stimulated by muscarinic drugs. It is probable that the three actions we have investigated—rage in the cat, stimulation in the chick and reversal of drug-induced sedation in the rabbit—have quite different mechanisms of action.

The rage response elicited in cats by muscarinic drugs resembles that caused by electrical stimulation of the hypothalamus, described by Nakao¹³ and by Wasman and Flynn¹⁴, and it is possible that hypothalamic rage is mediated through a muscarinic mechanism and that central stimulation and rage caused by intravenous injection of oxotremorine, pilocarpine and arecoline are due to an action of these agents on the hypothalamus. A similar site of action could be postulated for the effects produced by carbachol injected into the cerebral ventricles. All these drugs were specifically blocked by non-quaternary atropine agents. It is surprising that acetylcholine did not cause rage when injected into the cerebral ventricles, since physostigmine was able to do so when given intravenously. It may be that other cholinergic receptors in the central nervous system, which caused stupor, masked the effect on the hypothalamus. There is evidence for cholinergic mechanisms involved in sleep. George, Haslett and Jenden¹⁵ demonstrated that micro-injections of oxotremorine or carbachol into the reticular formation of the pons or caudal mesencephalon of the cat caused effects indistinguishable from physiological sleep.

The fact that some muscarinic agents cause sedation, while others cause stimulation, in the chick may be due to some drugs penetrating more easily to parts of the brain where inhibitory or excitatory mechanisms are present. It is of interest that while we were able to antagonize the excitant effects with atropinic drugs, we could not antagonize the sedative effects with atropine, nor were these sedative effects blocked by drugs antagonizing the nicotinic effects of acetylcholine, for example, mecamlamine, pempidine and hexamethonium.

Our results in rabbits confirm that an excitatory cholinergic mechanism is present also in this species. These stimulant properties were specifically blocked by atropinic drugs which reach the central nervous system. Some of these muscarinic compounds (oxotremorine, arecoline) showed a more potent central stimulant action than any other drugs we have tested in the rabbit. The discovery by Shute and Lewis¹⁶ of cholinergic innervation in the ascending reticular activating system of the rat

provides evidence of a possible site of action for this stimulant effect of muscarinic drugs.

- ¹ Sigg, E. B., Soffer, L., and Gyermek, L., *J. Pharmacol. Exp. Therap.*, **142**, 13 (1963).
- ² Rothballer, A. B., *Pharm. Rev.*, **11**, 494 (1959).
- ³ Borison, H. L., *J. Physiol.*, **147**, 172 (1959).
- ⁴ Zablocka, B., and Esplin, D. W., *Arch. Intern. Pharmacodyn.*, **147**, 490 (1964).
- ⁵ Baker, W. W., Hosko, M. J., Rutt, W. J., and McGrath, J. R., *Proc. Soc. Exp. Biol. and Med.*, **104**, 214 (1960).
- ⁶ George, R., Haslett, W. L., and Jenden, D. J., *Life Sci.*, **8**, 361 (1962).
- ⁷ Sabelli, H. C., and Toman, J. E. P., *Excerpta Med.*, **11**, 1229 (1962).
- ⁸ Feldberg, W., and Sherwood, S. L., *J. Physiol.*, **120**, 3P (1953).
- ⁹ Waelsch, H., *Biochemistry of Developing Nerve System*, 187 (Academic Press, New York, 1955).
- ¹⁰ Key, J., and Marley, E., *Electroenceph. and Clin. Neurophysiol.*, **14**, 90 (1962).
- ¹¹ Maxwell, D. R., and Palmer, H. T., *Nature*, **191**, 64 (1961).
- ¹² Maxwell, D. R., *Neuropsychopharmacology*, **3**, 501 (Elsevier Publishing Co., Amsterdam, Netherlands, 1964).
- ¹³ Nakao, H., *Amer. J. Physiol.*, **194**, 411 (1958).
- ¹⁴ Wasman, M., and Flynn, J. P., *Arch. Neurol.*, **6**, 220 (1962).
- ¹⁵ George, R., Haslett, W. L., and Jenden, D. J., *Intern. J. Neuropharmacol.*, **3**, 541 (1964).
- ¹⁶ Shute, C. C. D., and Lewis, P. R., *Nature*, **189**, 1060 (1963).

REGIONAL CHANGES IN BRAIN SEROTONIN AFTER HEAD X-IRRADIATION AND ITS SIGNIFICANCE IN THE POTENTIATION OF BARBITURATE HYPNOSIS

By DR. V. NAIR

Neuropharmacology and Biochemistry Laboratories, Michael Reese Hospital Psychiatric Institute and Department of Pharmacology, Chicago Medical School

It has been shown earlier in rats that X-irradiation of the head at high doses reduces the susceptibility to seizure with maximal electroshock¹. It was also observed that the pharmacological actions of central nervous system drugs such as anticonvulsants² and barbiturates³ were enhanced in these head-X-irradiated animals. The faster onset of drug action after irradiation may be explained by the blood brain barrier permeability changes revealed in the above studies. With regard to the prolongation of drug action after irradiation, several factors, in theory, may be implicated. Some of these are: (1) sensitization of brain by irradiation; (2) an abscopal effect (inhibition) on drug-metabolizing enzymes; (3) interference with the transport of drug from within the brain to blood; (4) specific changes in the neuro-chemical transmitters, that is, on their relative balance and/or distribution within the brain. The first three as they affect the barbiturate hypnosis have been considered in another publication⁴. The work recorded here deals with the effects of cephalic X-irradiation on brain serotonin (5-hydroxytryptamine).

Previous investigators have examined the effects of total body X-irradiation on the serotonin content of brain and other tissues. Their findings differ widely. Royal *et al.*⁵ found that in mice, one week after exposure to 900 r. X-irradiation, serotonin levels in brain, lung, kidney, spleen and liver were reduced below that of non-irradiated controls. Melching *et al.*⁶, however, failed to detect a reduction in the serotonin content of brain, liver or spleen of mice killed up to 2 h after exposure to 810 r. whole-body X-irradiation. Ershoff and Gal⁷ found no significant difference in the brain serotonin content between X-irradiated, γ -irradiated and control rats. They exposed the rats to 900 r. total-body X-irradiation and examined the brain at six days' post-irradiation. However, Ershoff *et al.*⁸ afterwards detected a reduction in the serotonin content of the brain and small intestine of rats exposed to 450 or 900 r. at 1, 3 and 6 days' post-irradiation. Speck⁹ examined the effects of massive total body X-irradiation on rat brain serotonin and found a small decrease at 18 h after exposure to 4,500 r. and immediately after 9,000 r. followed by a return to normal values. Whole-body radiation studies with 900, 4,000, 8,000 and 12,000 r. were performed in rats by Randic and Supek¹⁰. Immediately after irradiation, serotonin content was determined. No change was found after doses of 900 and 4,000 r. However, after the higher doses of radiation, a significant increase in brain serotonin was noted. The details of their work were not available. Differences in radiation factors, time of examination and method of serotonin estimation may account for the divergence in results among these investigators.

In the work recorded here, a systematic investigation of brain serotonin changes were made at various predeter-

mined intervals after irradiation, until the animals reached the terminal stage. Since we were primarily interested in the effects of radiation on the central nervous system, irradiation was restricted to the cephalic region with the rest of the body shielded. We feel that for studying the effects of radiation on the central nervous system, total-body radiation is less suitable, in view of the varied effects of radiation on other organ systems and their interaction with the central nervous system, which complicates the interpretation of results. Serotonin was determined in the whole brain as well as in the hypothalamus and medulla.

Male Sprague-Dawley rats ranging in weight from 200 to 230 g were used in these investigations. The irradiation conditions were the same as described in an earlier publication¹¹. Rats received a single dose of 10,000 r. or 500 r. to the head at a dose-rate of 295 r./min. The radiation source was a 250-kV 30-m.amp GE 'Maxitron' X-ray machine (Argonne Cancer Hospital, University of Chicago). After irradiation, the rats were housed two in a cage in air-conditioned quarters. Rats exposed to the high dose radiation were examined at 1, 24, 48, 72, 96, 120, 144 or 168 h post-irradiation while those exposed to the low dose were examined at the 72-h interval. They were killed by decapitation, and the brain quickly removed (visible blood being removed by means of filter paper) and weighed. Serotonin was determined according to the method of Bogdanski *et al.*¹². For the determination of serotonin in the hypothalamus and medulla, brain removed from the decapitated head was frozen on a bed of dry ice in a refrigerated room (-10°C). The part of the brain anterior to the pons was sectioned free-hand with a razor blade and 1-2 mm thick coronal sections were obtained. Hypothalamus was dissected out from these sections and tissue from 3-4 brains was pooled for each experiment. For medulla, pooled tissue from 2 brains was adequate. The procedure for the determination of serotonin was the same as that for whole brain except that the tissue was always homogenized in 2 ml. of 0.1 N hydrochloric acid irrespective of the weight of the sample. Since there was a progressive weight loss in heat-irradiated animals³, it was pertinent to examine the serotonin changes in starved controls also. For this purpose, rats were deprived of food and water and their brain analysed at varying intervals.

The effect of X-irradiation on the serotonin content of whole brain is shown in Table 1. It is noteworthy that the amine level began to rise about 48 h post-irradiation, reached a peak value about 72-96 h and subsequently returned to normal value by 144 h post-irradiation. That this is a specific effect of irradiation unrelated to the weight loss or nutritional changes is suggested by the absence of any such increase in the brain amine following total food and water deprivation for 72 h. In fact, star-

Table 1. RAT BRAIN SEROTONIN CONTENT

Time of examination (h)	Control	X-irradiation (10,000 r)	Food and water deprivation	X-irradiation (500 r.)
1-2	0.55 ± 0.02 (34)	0.58 ± 0.02 (4)		
24	—	0.54 ± 0.03 (7)		
48	—	0.81 ± 0.04 (7)	0.54 ± 0.01 (6)	
72	—	*0.70 ± 0.04 (10)	†0.47 ± 0.03 (6)	‡0.47 ± 0.02 (5)
96	—	*0.68 ± 0.03 (8)		
120	—	0.48 ± 0.05 (3)	†0.71 ± 0.06 (7)	
144	—	0.62 ± 0.008 (2)	0.50 ± 0.01 (3)	
168	—	0.59 ± 0.02 (2)		

The values are expressed as $\mu\text{g/g}$ wet weight tissue \pm S.E. The number in parentheses refers to the number of animals.

* $P < 0.01$

† $P < 0.05$

‡ $P < 0.02$

Table 2. REGIONAL CHANGES IN SEROTONIN CONTENT OF RAT BRAIN

	Hypothalamus	Medulla
Control	1.98 ± 0.31 (7)	0.76 ± 0.07 (10)
X-irradiated (10,000 r.)		
48 h post-r.	2.15 ± 0.24 (5)	0.75 ± 0.13 (3)
72 h post-r.	2.07 ± 0.26 (5)	*1.01 ± 0.07 (8)
96 h post-r.	2.00 ± 0.41 (4)	0.82 ± 0.10 (6)

The values are expressed as $\mu\text{g/g}$ wet weight tissue \pm S.E. The number in parentheses refers to the number of experiments, each experiment being composed of 3-4 animals in the case of hypothalamus and 2 animals in the case of medulla.

* $P < 0.05$

vation produced a fall in brain serotonin at 72 h ($P < 0.05$) and a rise at 120 h ($P < 0.05$). The significance of this bimodal effect of starvation is not understood. In contrast to this divergence in serotonin changes, the pattern of weight loss was about the same in both the 10,000-r. X-irradiated and totally starved animals. In both these groups, body-weight decreased linearly and almost to the same extent with time³.

The results of regional investigations are summarized in Table 2. It is interesting that hypothalamus, the region normally richest in serotonin, showed no increase in the amine level at 72 h post-irradiation, while significant increase in serotonin was detected in the medulla at this radiation interval. It may be recalled here that enhancement of barbiturate hypnosis was also maximal at this irradiation interval in animals exposed to 10,000 r. of X-ray. For example, the duration of thiopental hypnosis was increased about five-fold in the high dose irradiated animals. That this was a specific central nervous system effect of irradiation, unrelated to dehydration, nutritional factors, or fall in temperature, was established by appropriate controls³. In animals exposed to low dose cephalic X-irradiation (500 r.), serotonin level of the brain decreased at 72 h post-irradiation ($P < 0.02$). In these animals, in contrast to the high-dose irradiated ones, the barbiturate effects were also decreased, as evidenced by the shorter duration of hypnosis. Even though the decrease in serotonin seems to be the same in both the starved and low-dose irradiation groups, again this does not appear to be related to the body-weight changes. The loss in weight resulting from complete starvation was about 4-5 times that accompanying 500 r. cephalic X-irradiation³. The demonstration of an association of serotonin and enhancement of barbiturate hypnosis, although not of sufficient evidence by itself to suggest a cause and effect relationship, certainly raise some questions regarding the functional role of serotonin.

It is known that the reticular activating system (RAS) is very sensitive to the depressant effects of barbiturates and other sedative hypnotics¹³. The inability to maintain wakefulness under barbiturate medication is attributed to its depressant effects on the RAS. Brain stem reticular formation contains both inhibitory and excitatory fibres, and it has been postulated that they are dispersed diffusely throughout the reticular activating system¹⁴. The distribution of serotonin in brain parallels that of norepinephrine and, like the latter, is present in high amounts in areas controlled by the RAS. On the basis of his work on the reticular activation produced by epinephrine and structurally similar compounds, Dell has favoured the concept of an adrenergic mechanism at the reticular level¹⁵. Monnier has considered that the high-voltage, slow-wave

activity resulting from low-dose serotonin and 5-hydroxytryptophan was due in part to reticular depression¹⁶. It has been suggested that these two amines may have opposing functional roles, norepinephrine by association with the excitatory fibres and serotonin by association with the inhibitory fibres¹⁴. Other hypotheses and evidences concerning the neurohumoral regulation of reticular activity have been presented in the excellent review article by Killam¹⁷.

Many investigators have reported the potentiation of barbiturate hypnosis by serotonin¹⁸. The potentiation of barbiturate hypnosis in head-irradiated rats and its association with increased brain serotonin-levels revealed in this study are consistent with the above observations. Particularly interesting is the finding that, although whole brain analysis of head-irradiated rats revealed an increase in serotonin content, no such increase was seen in the region normally richest in the amine, that is, the hypothalamus. A significant increase was, however, detected in the medulla. It has been noted that low doses of ionizing radiation produce increased activity of the RAS^{19,20}. On theoretical grounds, it is thus not unreasonable to expect additive effects with central nervous system stimulants whose action is mediated through the RAS and antagonistic effects with central nervous system depressants. Indeed, the decreased barbiturate effects noted by us in the low dose irradiated group support this belief. Furthermore, as might be expected, the serotonin content of the brain decreased in the low-dose irradiated animals. These findings, viewed together, appear to reinforce the possibility that serotonin has a functional role (possibly by its association with the inhibitory fibres of the RAS) in the regulation of wakefulness. Further investigations under different experimental conditions should reveal whether or not the phenomenon described here—association of medullary rise in serotonin and suppression of wakefulness—is a general one. These investigations also re-emphasize the importance of regional examination of the brain with due regard to its anatomical and functional heterogeneity in biochemical studies of the central nervous system.

This work was supported in part by a grant from the State of Illinois Mental Health Fund 1711. I thank the University of Chicago Argonne Cancer Hospital and the Department of Radiology for use of the radiation facilities and Mr. James Bland for his assistance with the radiation procedure. I also thank Messrs. D. Shah and S. Finer for technical help.

¹ Nair, V., Sugano, H., and Roth, L. J., *Proc. Soc. Exp. Biol. and Med.*, **112**, 273 (1963).

² Nair, V., Sugano, H., and Roth, L. J., *Rad. Res.*, **23**, 265 (1964).

³ Nair, V., Finer, S., and Shah, D., *Proc. Soc. Exp. Biol. and Med.*, **120**, 246 (1965).

⁴ Nair, V., and Finer, S., *Proc. Soc. Exp. Biol. and Med.* (in the press).

⁵ Royal, G. W., Congdon, C., and Royal, G. C., *Rad. Res.*, **11**, 464 (1959).

⁶ Melching, H. J., Ernst, H., and Rosier, H., *Strahlentherapie*, **110**, 505 (1959).

⁷ Erschoff, B. H., and Gal, E. M., *Proc. Soc. Exp. Biol. and Med.*, **108**, 160 (1961).

⁸ Erschoff, B. H., Hellmers, R., and Wells, A. F., *Proc. Soc. Exp. Biol. and Med.*, **110**, 538 (1961).

⁹ Speck, L. B., *J. Neurochem.*, **9**, 573 (1962).

¹⁰ Bandie, M., and Suppek, Z., *Proc. Second Intern. Congr. Rad. Res.* (Abst.), **146**, Harrogate (August, 1962).

¹¹ Nair, V., and Roth, L. J., *Rad. Res.*, **23**, 249 (1964).

¹² Bogdanski, D. F., Pletscher, A., Brodie, B. B., and Udenfriend, S. F., *J. Pharmacol.*, **117**, 82 (1956). Udenfriend, S. F., Weissbach, H., and Brodie, B. B., *Methods of Biochemical Analysis*, edit by Glick, D., **6**, 95 (Interscience, New York, 1958).

¹³ (a) Arduini, A., and Arduini, M. G., *J. Pharmacol.*, **110**, 76 (1954). (b) French, J. D., Verzeano, M., and Magoun, H. W., *Arch. Neurol. Psychiat.*, **69**, 519 (1953).

¹⁴ Brodie, B. B., Sulser, F., and Costa, E., *Ann. Rev. Med.*, **12**, 300 (1961).

¹⁵ Dell, P., in *Reticular Formation of the Brain*, edit. by Jasper, H. H., Proctor, L. D., Knighton, R. S., Noshay, W. C., and Costello, R. T., 365 (Little, Brown, Boston, 1958); in *Ciba Found. Symp. Adrenergic Mechanisms*, edit. by Vane, J. R., Wolstenholme, G. E. W., and O'Connor, C. M., 393 (Churchill, London, 1960).

¹⁶ Monnier, M., *Arch. Intern. Pharmacodyn.*, **124**, 281 (1960).

¹⁷ Killam, E. V., *Pharmacol. Rev.*, **14**, 188 (1962).

¹⁸ Page, I. H., *Physiol. Rev.*, **38**, 321 (1958).

¹⁹ Garcia, J., Buchwald, N. A., Bach-y-Rita, G., Feder, B. H., and Koelling, R. A., *Science*, **140**, 289 (1963).

²⁰ Cooper, G. P., and Kimmelhof, D. J., *Science*, **143**, 1040 (1964).

CATION PERMEABILITY OF PHOSPHOLIPID MODEL MEMBRANES: EFFECT OF NARCOTICS

By DR. A. D. BANGHAM, DR. M. M. STANDISH* and NIGEL MILLER

Agricultural Research Council Institute of Animal Physiology, Babraham, Cambridge

IT would appear that spontaneously formed liquid crystals of egg lecithin (phosphatidyl choline), when swollen in salt solutions, exhibit many of the ion and water diffusion properties of biological membranes¹. Thus, if the unit structure of the liquid crystal is accepted as being that of a bimolecular leaflet and the concentrically arranged leaflets are assumed to form closed surfaces, the diffusion rate of anions and water across the membranes is found to be several orders of magnitude greater than the diffusion rate of cations¹. Furthermore, the diffusion rate of cations is very significantly controlled by the sign and magnitude of the surface charge at the lipid/water interface. For example, there is a decrease of the diffusion rate of Na^+ and K^+ as the negative charge on the membrane is decreased—diminishing to zero for a positively charged membrane¹. In contrast, the diffusion rates of Cl^- and water appear to remain high irrespective of the surface charge. Although such membranes do not appear to distinguish between different cations, the diffusion rate of anions is, with certain exceptions, related to their hydrated ion radius¹ ($\text{Br}^- = \text{Cl}^- = \text{I}^- = \text{HCO}_3^- > \text{NO}_2^- > \text{F}^- > \text{HCO}_3^- > \text{H}_2\text{PO}_4^-$).

Evidence has also been presented² which indicates that membrane-active steroids can modify the diffusion rate of cations in such a model-membrane system in a direction which is compatible with the hypothesis that cell or cell-organelle (lysosomes) lysis is preceded by an increase in cation permeability. While these investigations give some encouragement to the idea that the model system is a valid one, it was felt that they should be tested more rigorously against compounds the interaction of which with biological membranes had been more quantitatively investigated. To this end, the effects of a variety of narcotic compounds have been investigated.

The two most common groups of compounds that produce narcosis in excitable cells are the organic solvents, for example the *n*-alkyl alcohols, chloroform and ether, and the local anaesthetics characterized by the presence of secondary or tertiary amino-groups or both. The precise mechanism of action is not known for either group, but for the purposes of this investigation, the thermodynamic treatment proposed by Ferguson³, Brink and Posternak⁴ and Clements and Wilson⁵ relating narcotic activity to a free energy of adsorption of the compounds to a lipid phase and the discussion on the mode of action of local anaesthetics by Ariens and Simonis⁶ will form the basis of the discussion in the present article.

Experimental details together with a description of the molecular structure of the phospholipid liquid-crystals have been given by Bangham, Standish and Watkins¹. In principle, a mixture of egg phosphatidyl choline and dicetylphosphoric acid (90:10 mole/mole) in chloroform was taken to dryness *in vacuo*, the dried lipids were then swollen in a small volume (1.0 ml./30 μmole lipid) of 0.145 M potassium chloride containing about 100 μc . of $^{42}\text{K}^+$ and the milky dispersion dialysed against isotonic, non-isotonic, lightly buffered (pH 7.0) potassium chloride until the dialysate was free of $^{42}\text{K}^+$. Equal portions (0.6 ml.) of the dialysed dispersion were then pipetted into 0.6 ml. of the narcotic compounds already dissolved in 0.145 M potassium chloride (pH 7.0), and the mixture incubated for 30–90 min at 22° C. Finally, 1.0 ml. of the mixture was pipetted into a small, moistened dialysis bag which was then placed into 10.0 ml. of 0.145 M potassium chloride. A further dialysis was then carried

out for 30 min and the radioactivity of the final dialysate measured.

The organic solvents were tested at concentrations (*c*), which were varying fractions (*c/c₀*) of a standard state (*c₀*), that is, solvent saturated solutions of 0.145 M potassium chloride at 22° C. At the values of *c/c₀* used, the ideality of the solution is assumed and the *c/c₀* term is referred to as thermodynamic activity. Local anaesthetics were tested at various concentrations expressed as moles/l. Zeta potential measurements of the liquid crystals were carried out in an apparatus described by Bangham, Flemans, Heard and Seaman⁷.

Two important facts emerge from these investigations. First, the model membranes show a measurable response to all the compounds at or about their effective *in vivo* concentrations. Secondly, the cation diffusion behaviour is different for the two principal types of narcotic agent.

Organic Solvent Anaesthetics. At constant thermodynamic activity, the alcohols of the *n*-alkyl series clearly permit an increase in the diffusion rate of K^+ as the chain length decreases (Fig. 1). This result is completely in accord with a variety of well-documented *in vivo* systems tested against a similar series of alcohols. Indeed, if the ratios of the activities required for any one *in vivo* system are plotted against the ratios of the activities required to produce an equal diffusion rate in the present model system, the points are evenly distributed about the 1:1 correlation line. The maximum deviation corresponding to a $\Delta\Delta G$ of ~44 cal per mole on a total calculated free energy of ~230 cal per mole $-\text{CH}_2^-$. (Obtained from the activity values in Fig. 1 by calculating $\Delta G_{\text{heptanol}} - \Delta G_{\text{hexanol}}$, where ΔG is the free energy change required to produce equal K^+ diffusion rates.) Thus, the *n*-alkyl alcohol series exhibit a reasonable quantitative agreement between the model system and a constant end-point in

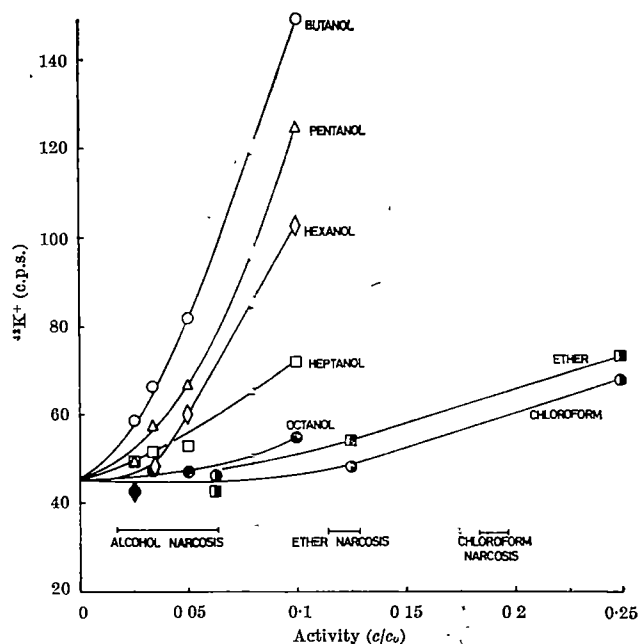


Fig. 1. The leakage rate of $^{42}\text{K}^+$ out of egg phosphatidyl choline/15 per cent dicetylphosphoric acid liquid crystals in the presence of varying concentrations (expressed as *c/c₀*) of long chain alcohols, chloroform and diethyl ether. Range of activities producing narcosis in biological systems is indicated by bars

* Visiting scientist: Unilever Research Laboratories, Port Sunlight, Cheshire.

Table 1. PENETRATION OF EGG PHOSPHATIDYL CHOLINE MONOLAYERS BY VARIOUS ALCOHOLS AT AN INITIAL PRESSURE OF 30 DYNES/CM

Alcohol	Bulk concentration (moles/l.)	$\Delta\pi$	Area/alcohol molecule (\AA^2)	No. lipid molecules per alcohol in surface phase
$C_1 - C_8$ (Extrapolated)	e.g. CHCl_3		12,750	213 (0.5%)
C_1	0.0379	3.5	7,953	133
C_2	0.0135	4.2	4,884	81.4
C_3	0.0038	4.65	3,677	61.1
C_7	0.0014	6.1	1,996	33.2

Areas per alcohol molecule were calculated from the expression⁹

$$\frac{d\pi}{d \log_{10} c} = 2.303 \frac{A_1}{A_1 - A_\pi} kT\Gamma$$

where: $\frac{d\pi}{d \log_{10} c}$ is the gradient of the penetration pressures (π)—versus ($\log_{10} c$) curve at the particular concentration; A_1 is the initial area/egg phosphatidyl choline molecule in the constant area monolayer (\AA^2); A_π represents the area occupied by a phosphatidyl choline molecule in a pure phosphatidyl choline monolayer at a given pressure (π); k is the Boltzmann constant, T is the absolute temperature; Γ is the surface density of penetrant molecules (as molecules/ \AA^2).

an *in vivo* system. Such quantitative agreement appears to extend beyond *n*-alkyl alcohol series because, if one compares the diffusion rate of K^+ in the model system at the activities for butanol and propanol to that for ether and chloroform at an activity required to produce narcosis in a non-synaptic nerve fibre⁴, the agreement is also remarkably good (Fig. 1). It would not be unreasonable to conclude that narcosis induced by these types of compound could act by increasing the permeability to cations.

On the other hand, if the activity coefficients of a series of *n*-alkyl alcohols are similar in the hydrocarbon region of the phospholipid molecules, one might expect equal numbers of alcohol molecules to be present there at equal bulk phase activity. To test this explicitly for the membrane-forming lipids used in the model system, the pressure increase produced in a constant area monolayer, initially at 30 dynes/cm, was measured for a range of bulk concentrations of the different alcohols. The results, presented in Fig. 2, seem to support the idea that at equal thermodynamic activity an equal number of molecules of the *n*-alkyl alcohol series penetrate into the membrane. But the results already presented in Fig. 1 show that at equal thermodynamic activities the diffusion rate of K^+ increases as the chain length decreases; thus the shorter the chain length the more effective is the molecule. The conclusion extends the suggestion made by Haydon and Taylor⁸ that when the chain length of an

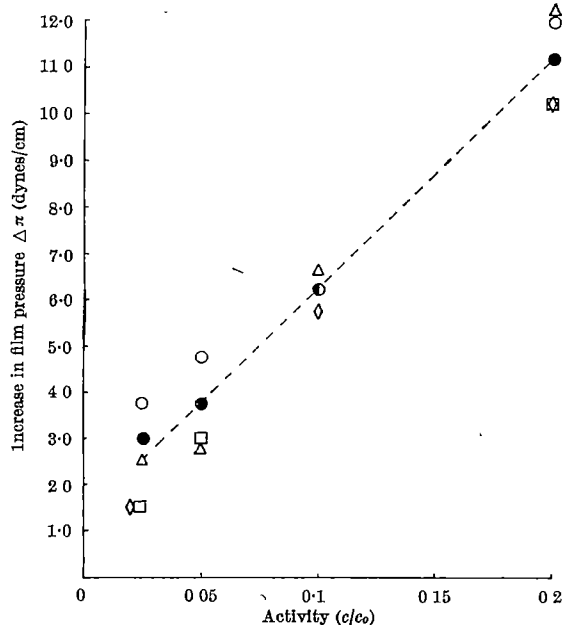


Fig. 2 Penetration pressures of egg phosphatidyl choline/15 per cent diethylphosphoric acid monolayers in equilibrium with varying bulk concentrations (expressed as c/c_0) of $C_1 - C_8$ alcohols. ---, Mean of the experimental points which lie on lines of approximately equal gradient. ○, Butanol; Δ, pentanol; ◇, hexanol; □, heptanol; ●, octanol

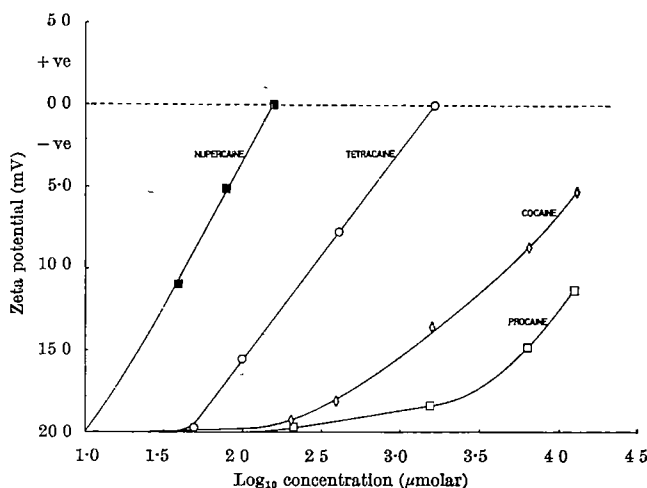


Fig. 3. Zeta potentials in mV of egg phosphatidyl choline/15 per cent diethylphosphoric acid liquid crystals in the presence of various concentrations of local anaesthetics. ■, Nupercaine; ○, tetracaine; ◇, cocaine; □, procaine

adsorbing molecule is small, say less than half the length of the lipid chain, the leaflet breaks up due to the formation of space in the hydrocarbon region above the short inserted chain. Their prediction, therefore, that in a homologous series of straight chain molecules the ratios of the aqueous concentrations producing break-up for members differing by one $-\text{CH}_2-$ would be:

$$\exp\left(\frac{-\Delta G_{\text{CH}_2}}{RT}\right): 1$$

is not entirely valid in the present system.

From the investigations of monolayer penetration (Fig. 2) it is possible to calculate⁹ thermodynamically the number of membrane molecules per narcotic molecule at aqueous concentrations capable of producing a state of narcosis in excitable cells (Table 1). Extrapolation of these values towards a chain length $n_{\text{CH}_2} = 1$, equivalent approximately to a small gas molecule such as CHCl_3 , would suggest that there would be as many as 213 phospholipid molecules per CHCl_3 (0.5 moles per cent CHCl_3)—a value in close agreement with that computed by Clements and Wilson⁵ from their investigations using inert gases.

Local Anaesthetics. Whereas there had been no obvious *a priori* reason why the model membrane system should demonstrate a measurable and parallel response to the organic solvent group of narcotics, the established relationship between the cation exchange diffusion rate and the sign and magnitude of the membrane/water interface¹ would predict a diminution of such rates if the local anaesthetic molecules were active in their cationic form. These compounds penetrate orientated lipid structures as shown by Skou¹⁰, and the results presented in Fig. 3 confirm and amplify the measurements of Bangham, Rees and Schotland¹¹ and show that all four of the local anaesthetic compounds—namely, nupercaine, tetracaine, cocaine and procaine—reduce the zeta potential of a negatively charged membrane system. Moreover, if one compares the ratios of the concentrations producing an equal reduction in zeta potential to the ratios of the concentrations producing a reversible nerve block¹⁰, the values are in reasonable agreement (Table 2). Too much emphasis should not be laid on this correlation, since the concentration ranges over which a nerve remains reversibly

Table 2

	Highest concentrations still producing reversible block (Skou) (mM)	Relative blocking potency	Concentrations for 5-mV reduction in zeta potential (μM)	Relative concentrations
Nupercaine	0.7	1.0	20.0	1.0
Tetracaine	2.5	3.57	100.0	5.0
Cocaine	44.0	63.0	891.0	44.5
Procaine	137.0	195.0	5,012.0	250.0

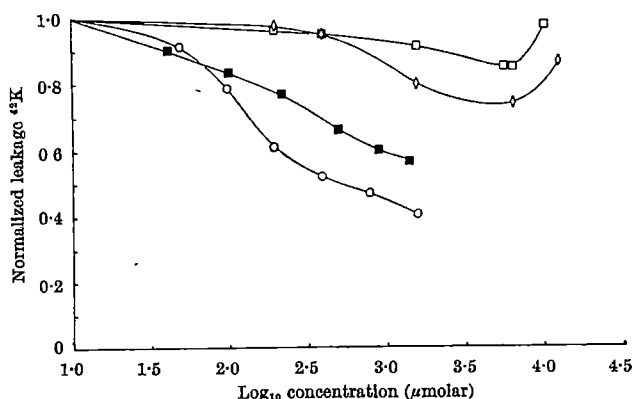


Fig. 4 Normalized leakage rates of $^{42}\text{K}^+$ out of egg phosphatidyl choline/15 per cent didecylphosphoric acid liquid crystals as a function of bulk local anaesthetic concentrations. ■, Nupercaine; ○, tetracaine; ◇, cocaine; □, procaine

blocked are considerable. It is encouraging, however, that the model membrane systems show significant reductions in zeta potential at the minimum blocking concentrations¹³.

As indicated earlier¹, the diffusion rate of cation is very significantly controlled by the sign and magnitude of the surface charge prevailing at the lipid/water interface. It was therefore with no surprise that we were able to establish a systematic reduction in the diffusion rate of cation out of the liquid crystals in the presence of the local anaesthetic compounds (Fig. 4).

That there is a poor quantitative agreement between the percentage reduction in cation leakage and the reduction of zeta potential for the two more potent anaesthetics, namely, nupercaine and tetracaine, may be ascribed to the experimental necessity of reacting a large number of lipid molecules with a limited small volume of anaesthetic solution. The trend throughout the series clearly suggests that the cation diffusion is reduced as the zeta potential becomes less negative. If this correlation reflects the mode

of action of local anaesthetics *in vivo* then it supports the view¹² that these compounds are primarily active in the ionized form. Two of the less potent compounds, however, have the further effect of increasing permeability at high concentrations, even though the zeta potentials continue to fall. It is tempting to suggest that this effect may be attributed to the presence of a higher proportion of un-ionized molecules since it was not observed when the system was studied at a lower pH.

Thus the model membrane system described broadly mimics the behaviour of biologically excitable membranes in their response to two chemically unrelated groups of reagents. Both groups manifest changes in cation permeability across a bimolecular lipid membrane but in opposite directions and explicable by different physical mechanisms.

Inherent in these investigations is the belief that narcosis represents a transient, reversible increase in membrane permeability to cation; higher concentrations resulting in a degree of permeability (Fig. 1) which the energy-driven pumps in biological membranes cannot keep up with. A Donnan redistribution of ions will develop due to the presence inside the cell or cell organelle of non-diffusible proteins, and the concomitant rise in osmotic pressure will cause swelling and rupture of the membrane^{3,13}.

¹ Bangham, A. D., Standish, M. M., and Watkins, J. C., *J. Mol. Biol.*, **13**, 238 (1965).

² Bangham, A. D., Standish, M. M., and Weissmann, G., *J. Mol. Biol.*, **13**, 253 (1965).

³ Ferguson, J., *Proc. Roy. Soc., B*, **197**, 387 (1939).

⁴ Brink, F., and Posternak, J. M., *J. Cell. Comp. Physiol.*, **32**, 211 (1948).

⁵ Clements, J. A., and Wilson, K. M., *Proc. U.S. Nat. Acad. Sci.*, **48**, 1008 (1962).

⁶ Ariëns, E. J., and Simons, A. M., in *Molecular Pharmacology*, **1**, edit. by Ariëns, E. J. (Academic Press, 1964).

⁷ Bangham, A. D., Flemans, E., Heard, D. H., and Seaman, G. V. F., *Nature*, **182**, 642 (1958).

⁸ Haydon, D. A., and Taylor, J., *J. Theoret. Biol.*, **4**, 281 (1963).

⁹ Pethica, B. A., *Trans. Faraday Soc.*, **51**, 1401 (1955).

¹⁰ Skou, J. C., *J. Pharm. Pharmacol.*, **13**, 204 (1961).

¹¹ Bangham, A. D., Rees, K. R., and Schotlander, V., *Nature*, **193**, 754 (1962).

¹² Ritchie, J. M., and Greengard, P., *J. Pharmacol. Exp. Therap.*, **133**, 241 (1961).

¹³ Skou, J. C., *Acta Pharmacol. Toxicol.*, **10**, 292 (1954).

MOLECULAR WEIGHT OF CHICK INTERFERON

By DR. D. C. BURKE and DR. J. ROSS

Department of Biological Chemistry, Marischal College, University of Aberdeen

THE molecular weight of interferon has been investigated by a number of workers. Burke¹ purified chick interferon, prepared in chick chorioallantoic membranes, twenty-fold to obtain material of molecular weight 63,000, which was homogeneous in the analytical ultracentrifuge. This figure for the molecular weight was consistent with that of less than 80,000 obtained by Porterfield *et al.*², based on the rate of diffusion of interferon through agar. However, several workers using chick interferon, prepared *in ovo* in chick embryo cells, found, by indirect methods, that the molecular weight of chick interferon was in the 13,000–45,000 range^{3–8}. Lampson *et al.*³ achieved 4,500-fold purification of interferon prepared *in ovo* to give a product of molecular weight 20,000–34,000 as determined in the ultracentrifuge. Thus, with two exceptions^{1,2} all the reported values for the molecular weight of interferon have been in the range 13,000–45,000. Since interferon is adsorbed by agar³, the value of less than 80,000 obtained by the diffusion method is of doubtful significance. The difference between our earlier results¹ and those of other workers^{4–8} could be due to the different methods of preparation of interferon that were used, that is, in chorioallantoic membranes rather than in chick embryo cells or *in ovo*. We have now found that the molecular weight of interferon prepared in chorioallantoic membranes is about 30,000, and that the preparation of molecular weight 63,000 is

heterogeneous, containing at least two other proteins apart from interferon.

Interferon was prepared in chorioallantoic membranes (CAM), and assayed by a plaque-inhibition method in chick embryo cells by methods previously described^{1,9}. Interferon was also prepared in chick embryo cells¹⁰, and *in ovo*³. Sucrose-gradient centrifugation was carried out by Dr. P. A. Charlwood, as previously described⁴. Gel-filtration was carried out, essentially by the method of Andrews¹¹, using a column 105 cm × 4 cm diameter, and 0.1M phosphate buffer, pH 7.7. A series of purified proteins were used to calibrate the column. The proteins in the eluant were estimated spectrophotometrically and, where possible, enzymatically. The elution volume of a protein was defined as the volume of eluate at which the maximum amount of protein was measured. In all cases, spectrophotometric and enzymatic assays gave identical values for the elution volume.

An estimate of the molecular weight of chick interferon was obtained by sucrose-gradient centrifugation of crude interferon. Concentrated CAM interferon was centrifuged in a sucrose gradient, together with radioactively labelled lysozyme and ovalbumin as markers⁴. The contents of each tube were then assayed for radioactivity, and for interferon activity by the plaque-inhibition assay. The results (Fig. 1) show that chick membrane interferon sediments at a rate between that of ovalbumin and lyso-

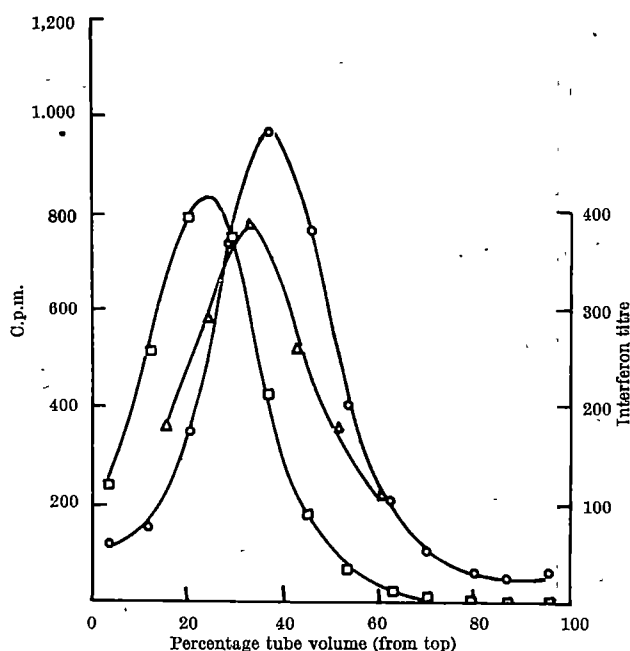


Fig. 1. The centrifugation of CAM-interferon in a sucrose gradient, using radioactively labelled lysozyme and ovalbumin as markers. Δ , Interferon titre, \square , lysozyme radioactivity; \circ , ovalbumin radioactivity

zyme, although closer to that of ovalbumin. Since Charwood¹² has shown that the rate of sedimentation of a protein in a sucrose gradient is a function of its sedimentation coefficient, these results suggest that chick membrane interferon has a sedimentation coefficient somewhat lower than that of ovalbumin (3.4 S). This corresponds to a molecular weight of about 30,000–40,000 for chick membrane interferon.

A second estimate of the molecular weight of chick interferon was obtained by gel filtration through 'Sephadex G-100'. The elution volumes of a series of proteins, with molecular weights in the range 20,000–65,000, were determined. When the elution volumes of the proteins were plotted against the logarithms of their molecular weights, a straight line was obtained (Fig. 2), although the points for two of the proteins did not lie on this straight line. One of these proteins, carboxypeptidase, was filtered using 10 per cent lithium chloride as eluant. When albumin and ovalbumin were filtered in 10 per cent lithium chloride, it was found that the elution volume of each protein was 100 ml. greater than the elution volume using 0.1M phosphate buffer, pH 7.7, as eluant. The elution volume of carboxypeptidase used in Fig. 2 was obtained by subtraction of 100 ml. from the elution volume found when 10 per cent lithium chloride was used as eluant, and is therefore an approximate figure. No explanation of the large elution volume of trypsin can be given, although there appears to be some doubt about its homogeneity¹³. Gel-filtration of concentrated CAM interferon, under identical conditions, gave an elution volume of 680–750 ml. These figures correspond to a molecular weight of 27,000–35,000. The recovery of biological activity was very low in early experiments, but by using larger amounts of interferon, working at 2° and adding 'Tween 80' to the eluting buffer, the recovery was raised to about 40 per cent. The biological activity of the eluate was unstable, presumably due to its low protein content. The product was still impure since it gave two precipitin lines on immuno-diffusion with an anti-interferon antiserum (see later). Samples of interferon prepared *in ovo*, or in chick embryo cells, were, after suitable concentration, filtered through the 'Sephadex' column. The elution volumes of 710–730 ml. and 710 ml., respectively, correspond to molecular weights of 29,000–31,000. Thus within the limits of this method, no differ-

Table 1. COMPARISON OF ELUTION VOLUMES IN 0.1M PHOSPHATE pH 7.7, AND IN 0.1 M CHLORIDE BUFFER, pH 2.0

Protein	Elution volume at pH 7.7 (ml.)	Elution volume at pH 2.0 (ml.)
Albumin	510	430
Ovalbumin	610	630
Pepsin	670	870

The elution volume of each protein was measured as described in the text.

ence was detected in the molecular weights of the interferon from the three sources.

The elution volumes of several proteins were also determined using a 0.1 M-chloride, pH 2.0, buffer as eluant (Table 1). The relationship between molecular weight and elution volume, determined for filtration at pH 7.7, was not applicable at pH 2.0. The elution volume of albumin was small at pH 2.0, indicating that the molecule was larger, and several authors¹⁴ have shown that albumin expands at low pH values. The elution volume of pepsin was smaller at pH 7.7 than at pH 2.0, and this is consistent with the elongated structure of pepsin at neutral pH values¹⁵. The elution volume of ovalbumin was virtually unchanged. The elution volume of CAM interferon was greater at pH 2.0 than at pH 7.7 and, by analogy with pepsin, this may be due to a more compact structure at pH 2.0.

The results show that the molecular weight of CAM interferon lies within the range 27,000–35,000, and are at variance with the molecular weight of 63,000 obtained for purified CAM interferon¹. It was therefore of interest to re-examine the product obtained by Burke's procedure. A 5-l. batch of crude CAM interferon was taken through this purification procedure, with results very similar to those previously described. The product, which had been purified about twenty-fold, gave a single band on starch-gel electrophoresis and a single symmetrical peak of protein when chromatographed on DEAE-cellulose at pH 5.8 with an increasing phosphate gradient. However, when the product was examined by agar-gel double-diffusion analysis or by immuno-electrophoresis using an anti-interferon rabbit serum, it was shown to be heterogeneous.

The antiserum had been prepared by repeated injection of rabbits with crude CAM interferon, and neutralized the biological activity of CAM interferon (J. Ross and D. C. Burke, unpublished results). It also contained antibodies to the other protein components of crude CAM interferon, including ovalbumin and albumin, immuno-electrophoresis showing that the serum reacted with seven of the ten components detectable in crude CAM interferon by starch-gel electrophoresis. Both agar-gel double-diffusion analysis and immuno-electrophoresis showed the presence of four components in the purified CAM interferon. One of these was identified as a trace of chick albumin (which could not be detected by starch-gel electrophoresis), and another was shown to be present in

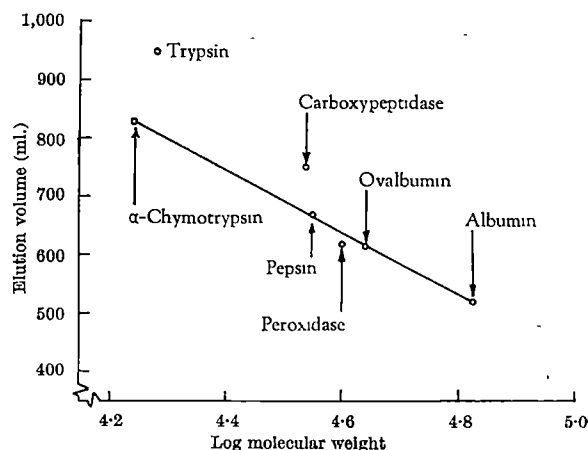


Fig. 2. Relationship between the molecular weights of proteins and elution volumes from a column of 'Sephadex G-100', in 0.1M phosphate buffer, pH 7.7, plus 'Tween 80' (20 μ g/ml) at 2° C

control interferon preparations (that is, CAM interferon made as usual, but without exposure to ultra-violet-inactivated virus), and is therefore derived from the host cells. The other two components were only found in purified preparations, but it is not known if either was interferon. These components could not be resolved by starch-gel electrophoresis, a diffuse single band being obtained. The product obtained by Burke¹ is therefore impure, and the observed molecular weight of 63,000 must be that of the major component, which is not interferon.

We have attempted to estimate the purity of Burke's material by comparison of the specific activity of the different preparations. Our crude CAM material has a specific activity of about 10 M.R.C. interferon units/mg protein and the most purified preparation about 2×10^2 M.R.C. interferon units/mg. The best crude interferon that we have been able to prepare *in ovo* has a specific activity of 3 M.R.C. interferon units/mg protein, and this material has been purified 4,500-fold (ref. 3) and 6,500-fold (ref. 8), giving a specific activity for the products of 1.35×10^4 M.R.C. interferon units/mg and 1.95×10^4 M.R.C. interferon units/mg, respectively. Thus it would appear

that our earlier purified preparation¹ contained at most 1 per cent of pure interferon, and this amount of a second protein would not have been detected by the methods used.

We thank the Medical Research Council, Glaxo Laboratories, Ltd., the Pharmaceuticals Division of Imperial Chemical Industries, Ltd., and the Wellcome Research Laboratories for grants which supported this work.

¹ Burke, D. C., *Biochem. J.*, **78**, 556 (1961).

² Porterfield, J. S., Burke, D. C., and Allison, A. C., *Virology*, **12**, 197 (1960).

³ Lampson, G. P., Tytell, A. A., Nemes, M. M., and Hilleman, M. R., *Proc. Soc. Exp. Biol. Med.*, **112**, 468 (1963); **118**, 441 (1965).

⁴ Rotem, Z., and Charlwood, P. A., *Nature*, **198**, 1066 (1963).

⁵ Kreuz, L. E., and Levy A. H., *Nature*, **200**, 883 (1963), *J. Bact.*, **89**, 462 (1965).

⁶ Phillips, A. W., and Wood, R. D., *Nature*, **201**, 819 (1964).

⁷ Jungwirth, C., and Bodo, G., *Biochem. Z.*, **339**, 382 (1964).

⁸ Merigan, T. C., *Science*, **145**, 811 (1964); *J. Mol. Biol.* (in the press).

⁹ Burke, D. C., and Buchan, A., *Virology*, **26**, 28 (1966).

¹⁰ Ruiz-Gomez, J., and Isaacs, A., *Virology*, **19**, 8 (1963).

¹¹ Andrews, P., *Biochem. J.*, **91**, 222 (1964).

¹² Charlwood, P. A., *Analyt. Biochem.*, **5**, 226 (1963).

¹³ Whitaker, J. R., *Anal. Chem.*, **35**, 1950 (1963).

¹⁴ Porter, J. F., in *The Plasma Proteins*, edit. by Putnam, F. W., **1**, 179 (Academic Press, Inc., New York, 1960).

¹⁵ Edelhoch, H., *J. Amer. Chem. Soc.*, **79**, 6100 (1957).

ARGININE AND UREA BIOSYNTHESIS IN THE LAND PLANARIAN: ITS SIGNIFICANCE IN BIOCHEMICAL EVOLUTION

By PROF. JAMES W. CAMPBELL

Department of Biology, Rice University, Houston, Texas

THE most widely accepted theory of the origin of life on this planet is that of Oparin¹. According to this theory, the first living systems were heterotrophic and utilized the organic compounds then present in primaeval waters as their source of free energy. The acquisition of biosynthetic function—primitive autotrophy—came as these substrates were gradually depleted from the nutrient environment. During this period, catalysed biosynthetic reactions had a high positive survival value for those systems possessing them and selection led to the establishment of complete biosynthetic pathways². This sequence of events is implicit in the concept of biochemical unity in accounting for the occurrence of several major metabolic pathways in diverse living forms.

One such biosynthetic pathway which has received considerable attention in this connexion is that for the synthesis of the amino-acid arginine, a universal protein constituent which is especially abundant in some nucleoproteins. This pathway is present, in whole or part, in bacteria, blue-green and green algae, yeast, fungi, higher plants, and most major vertebrate classes. Besides being the source of a necessary protein constituent, the arginine pathway has additional significance in biochemical evolution in serving as the main route for ammonia detoxification in ureotelic vertebrates. Urea synthesis is only one step removed from that of arginine, and it has been suggested that ancestral vertebrates utilized this pre-existent pathway for ammonia detoxification during the invasion of the land³. The utilization of the arginine pathway for ureotelism required mainly a change in the nature of the carbamyl phosphate-forming enzyme which enabled it to function efficiently at low ammonia concentrations.

While this has been recognized as an important step in evolution for the vertebrates, the exploitation of the arginine pathway for ureotelism by invertebrates during their invasions of the land habitat has not previously been considered. This was due, perhaps, to the lack of documentation of this pathway in any extant invertebrate species. From a consideration of the theoretical aspects of the establishment of metabolism², there is little reason to suspect that the pathway would not occur among the

invertebrates. The alternative would be that the pathway was not continuous in its evolution; that is, that it arose in primitive cells, was not present during the long geological period of the invertebrates, and then reappeared in ancestral provertebrates. In this alternative, the selective pressures which brought about the redevelopment of the pathway would seem more obscure, since they would not at first appear to be nutritional ones. On the other hand, the presence of the arginine pathway, in whole or part, among the invertebrates is more in keeping with the general concepts of biochemical unity since it indicates that the pathway was continuous in its evolution.

Urea cycle enzymes are of common occurrence among terrestrial invertebrates. Arginase has long been known to occur in the land snail⁴, the earthworm⁵, insects⁶, and more recently along with ornithine transcarbamylase, in the land planarian⁷. All urea cycle enzymes have now been demonstrated in the earthworm^{8,9} and it has been shown in another report that they function *in vivo* to synthesize both protein arginine and excretory urea¹⁰. Except for carbamyl phosphate synthetase, all other urea cycle enzymes have also been demonstrated in the land snail¹¹, and the incorporation of bicarbonate-¹⁴C into citrulline¹², protein arginine¹³, and urea^{12,14} indicates that the pathway is complete. Its role in excretion or nutrition in the snail is not, however, as yet clear. Although arginase occurs in some insects, ornithine transcarbamylase apparently does not¹⁵, and arginine is required in the diet¹⁶. Insects thus seem to lack the capacity to synthesize citrulline although it can partially replace arginine in the diet¹⁶. In this respect, they occupy a similar position among the invertebrates to birds among the vertebrates¹⁷.

Because of the land planarian's unique phylogenetic position among the terrestrial invertebrate species, it was of considerable interest to examine it for the presence of the arginine pathway. The origin of the Metazoa is not agreed on, but some feel that primitive flatworms bear certain similarities to the hypothetical first metazoans¹⁸. In any event, the land planarian, although itself a specialized species, may well represent the first successful invasion of the land by animals. The present report of

the urea cycle in the land planarian indicates, first of all, that the arginine pathway was probably present in the most primitive invertebrates, thus supporting the idea that the pathway was continuous in its evolution from unicellular organisms through to the vertebrates. Secondly, it once again demonstrates the importance of the arginine pathway and the development of ureotelism in the invasion of the land by animals.

The land planarians were collected in a local greenhouse and were identified as *Bipalium kewense* on the basis of external characteristics and with reference to Hyman¹⁹. They were kept until their guts emptied and were washed in 0.017 per cent 'Zephrian chloride' (Wintthrop Laboratories trade mark for alkyl dimethylbenzylammonium chloride) to free the external surface of bacteria. They were not checked for symbionts such as pleuropneumonia-like organisms, and I have assumed that the metabolism measured is attributable to the flatworms. Arginine is rapidly degraded by pleuropneumonia-like organisms (PPLO)²⁰, and in infected cells arginine synthesis from bicarbonate apparently does not take place²¹. Urease activity, which is widely associated with bacteria²², could not be detected in *Bipalium*²³ and this suggests that contamination may not have been a factor. For incubation, the flatworms were placed on filter paper moistened with 0.004 per cent 'Zephrian chloride' in sterile 225-ml. Warburg vessels. The side arms contained 3 μ mole $\text{NaH}^{14}\text{CO}_3$ (20 μC , ^{14}C). The vessels were sealed and the $^{14}\text{CO}_2$ was liberated into the chamber by adding sulphuric acid into the side arm through a rubber seal. The worms were kept in the $^{14}\text{CO}_2$ atmosphere for 4–5 h at room temperature (23°–24° C) and were then homogenized in 9 vol. 5 per cent trichloroacetic acid (TCA). The homogenate was centrifuged and the supernatant fluid was collected. The residue was washed twice with TCA and these washings were combined with the supernatant fluid and stored at –20° C until analysed.

The incorporation of $^{14}\text{CO}_2$ into protein arginine, aspartic acid and glutamic acid is shown in Table 1. To isolate the protein amino-acids, the residue from the foregoing was washed three times with TCA, twice with hot TCA (kept in a water bath at 68° C), twice more with TCA, twice with ethanol, twice with hot 3:1 ethanol-ether (52° C), twice with 2:2:1 ethanol-ether-chloroform (52° C), and finally with anhydrous ether. The dried residue (an average of 126.5 mg/g fresh wt.) was suspended in 100 vol. constant-boiling, peroxide-free hydrochloric acid and was hydrolysed in a sealed tube for 20 h at 115° C. The hydrolysate was freed of hydrochloric acid *in vacuo* and was decolorized with 'Nortit-A'. The amino-acids in portions of the hydrolysate representing 1 mg protein were separated by two-dimensional chromatography on Whatman 3MM paper using 2-butanone-formic acid-water (75:15:10, by vol.) in the first dimension and 75 per cent (w/w) phenol in an ammoniacal atmosphere in the second. The radioactive compounds were located by autoradiography and were eluted from the paper with water. A portion of the eluate was plated on aluminium planchets for radioactivity measurements and another portion was used to determine the amount of amino-acid present. For arginine, a modified Sakaguchi method was used, and for aspartic and glutamic acids, the picryl sulphonic acid method²⁴. No correction was made for absolute recoveries: arginine made up 4.88 per cent of the protein when determined as Sakaguchi-positive material in the protein hydrolysate, and 4.26 per cent by the chromatographic method. With this method, incorporation of carbon-14 was detected only in those amino-acids listed in Table 1.

To isolate arginine from the protein hydrolysate, carrier arginine was added to that remaining after chromatography to give a total of 20 mg. The arginine was then precipitated as the difluoride which was recrystallized to constant specific radioactivity²⁵. The difluoride was

Table 1. INCORPORATION OF $^{14}\text{CO}_2$ INTO PROTEIN AMINO-ACIDS OF *Bipalium*

Amino-acid	Protein content (%)	Total incorporation (c.p.m./127 mg protein*)	Specific activity (c.p.m./ μ mole)	CO_2 incorporated (μ mole/24 h†)
Arginine	4.26	28,007	903	44.8
Aspartic acid	20.22	20,379	106	32.6
Glutamic acid	13.04	14,522	94	23.3

* Incorporation into protein from 1 g tissue during a 4.67 h incubation.

† No corrections were made for dilution due to non-radioactive metabolic CO_2 formed during the incubation.

Table 2. INCORPORATION OF $^{14}\text{CO}_2$ INTO FREE AMINO-ACIDS AND UREA OF *Bipalium*

Compound	Tissue content (μ mole/g)	Total incorporation (c.p.m./g)	Specific activity (c.p.m./ μ mole)	CO_2 incorporated (μ mole/24 h)
Alanine	5.6	54,520	9,692	87.1
Arginine	<0.1	806	~8,850	1.3
'Argininosuccinate compounds B + C'	~1.3	3,742	2,917	5.9
Aspartic acid	9.0	112,604	12,512	180.6
Citrulline	<0.1	17,730	~209,500	28.3
Glutamine	0.5	1,521	3,073	2.4
Glutamic acid	11.3	76,462	6,797	122.2
Glycine	0.5	1,080	2,087	1.7
Serine	1.0	1,512	1,493	2.4
Urea	6.0	161,577	26,896	258.2

In addition to the compounds listed above, two unidentified ones also contained radioactivity. One, a total of 2,410 c.p.m., and the other, 1,580 c.p.m.

decomposed with 'Dowex-2(Cl-)'. The guanidino-C was liberated by treating 17 mg of the isolated arginine with 10 mg arginase and 10 mg urease in 0.1 M *tris* sulphate, pH 7.8, containing 0.005 M cobalt sulphate for 4 h at 38° C. Acidification of this reaction mixture liberated the guanidino-C as $^{14}\text{CO}_2$, which was absorbed in sodium hydroxide and precipitated and counted as $\text{Ba}^{14}\text{CO}_3$. Of the total carbon-14 incorporated into arginine, 97.3 per cent was present in the guanidino-C. Ornithine was isolated from the reaction mixture with 'Dowex-50(H^+)' after the arginase-urease treatment and was recrystallized from 80 per cent ethanol. It contained 1.3 per cent of the incorporated carbon-14.

The incorporation of $^{14}\text{CO}_2$ into the free amino-acids and urea is shown in Table 2. These compounds were isolated from the combined TCA supernatant and wash solutions with 'Dowex-50(H^+)' and were separated by two-dimensional chromatography as already mentioned. The amino-acids other than arginine and citrulline were determined with picryl sulphonic acid. Arginine was determined with the Sakaguchi reaction and citrulline as described by Archibald²⁶. The latter two compounds were added as non-labelled carriers to the free amino-acid fraction and their estimation is based on difference plus recovery corrections. The compounds designated as 'argininosuccinate B and C' were chromatographically identical to the two compounds formed when authentic argininosuccinate was carried through the isolation procedure used for the amino-acids. They are probably the two cyclic forms identified by Westall²⁷ and designated by him as compounds B and C, although authentic samples of these were not available for comparison. The estimation of these compounds was with an L-ornithine standard. A portion of the isolated citrulline was arsenolysed with ornithine transcarbamylase¹² and the ureido-C was determined as $\text{Ba}^{14}\text{CO}_3$. This C contained 91 per cent of the incorporated carbon-14. Urea was determined as described by Archibald²⁸ and a portion was also decomposed with urease to determine the urea-C as $\text{Ba}^{14}\text{CO}_3$.

The pattern of incorporation of $^{14}\text{CO}_2$ into citrulline, arginine and urea shown by *Bipalium* has been found repeatedly to be due to the operation of the urea cycle in ureotelic vertebrates. In addition, both ornithine transcarbamylase and arginase occur in this land planarian⁷. Both enzymatic^{29,30} and isotope incorporation data^{30,31} are now available which indicate that arginine and urea are synthesized in all classes of flatworms by the Krebs-Henseleit ornithine-urea cycle. This extends the distribution of this pathway to at least two major invertebrate phyla, the Platyhelminthes and the Annelida, and indicates that it is indeed a primitive pathway which

has persisted in some species of most living groups, while having been lost, in whole or part, from others.

This work was supported by U.S. Public Health Service grant AI 05006. I thank the personnel of the Hans Peterson Nursery, Houston, Texas, for their help in collecting the flatworms.

- ¹ Oparin, A. I., *The Origin of Life on the Earth*, third ed. (Oliver and Boyd, Edinburgh, 1957).
- ² Horowitz, N. H., *Proc. U.S. Nat. Acad. Sci.*, **31**, 153 (1945).
- ³ Cohen, P. P., and Brown, jun., G. W., *Proc. Fifth Internal. Cong. Biochem.*, **8**, 129 (1963).
- ⁴ Baldwin, E., *Biochem. J.*, **29**, 252 (1935).
- ⁵ Cohen, S., and Lewis, H. B., *J. Biol. Chem.*, **184**, 479 (1950).
- ⁶ Kilby, B. A., and Neville, E., *J. Exp. Biol.*, **24**, 276 (1951).
- ⁷ Campbell, J. W., and Lee, T. W., *Comp. Biochem. Physiol.*, **8**, 29 (1963).
- ⁸ Bishop, S. H., and Campbell, J. W., *Science*, **142**, 1583 (1963).
- ⁹ Campbell, J. W., and Bishop, S. H., *Abst. Sixth Internal. Cong. Biochem.*, **5**, 394 (1964).
- ¹⁰ Bishop, S. H., and Campbell, J. W., *Comp. Biochem. Physiol.*, **15**, 51 (1965).
- ¹¹ Linton, N. N., and Campbell, J. W., *Arch. Biochem. Biophys.*, **97**, 360 (1962).
- ¹² Campbell, J. W., and Bishop, S. H., *Biochim. Biophys. Acta*, **77**, 149 (1963).
- ¹³ Campbell, J. W., and Gaston, S., *The Physiologist*, **6**, 153 (1963).

- ¹⁴ Brieteux-Grégoire, S., and Florkin, M., *Comp. Biochem. Physiol.*, **12**, 55 (1964).
- ¹⁵ Porembska, Z., and Mochmacka, I., *Acta Biochem. Polon.*, **11**, 113 (1964).
- ¹⁶ Hinton, T., *Ann. N.Y. Acad. Sci.*, **77**, 366 (1959).
- ¹⁷ Tamir, H., and Ratner, S., *Arch. Biochem. Biophys.*, **102**, 249 (1963).
- ¹⁸ See chapters by Hanson, E. D., Remane, A., Hand, C., Steinbock, O., and Hartman, W. D., in *The Lower Metazoa*, edit. by Dougherty, E. C., Brown, Z. N., Hanson, E. D., and Hartman, W. D. (University of California Press, Berkeley, 1963).
- ¹⁹ Hyman, L. H., *Amer. Mus. Novitates*, No. 1241 (1943).
- ²⁰ Smith, P. F., *Ann. N.Y. Acad. Sci.*, **79**, 543 (1960).
- ²¹ McCarty, K. S., Woodson, B., Amstey, M., and Brown, O., *J. Biol. Chem.*, **239**, 644 (1964).
- ²² Varner, J. E., *The Enzymes*, second ed., edit. by Boyer, P. D., Lardy, H., and Myrback, K., **4**, 247 (1960).
- ²³ Lee, T. W. (unpublished observations).
- ²⁴ Satake, K., Okuyama, T., Ohashi, M., and Shinoda, J., *J. Biochem., Tokyo*, **47**, 654 (1960).
- ²⁵ Greenstein, J. P., and Winitz, M., *Chemistry of the Amino Acids*, **3**, 1841 (John Wiley, New York, 1961).
- ²⁶ Archibald, R. M., *J. Biol. Chem.*, **156**, 121 (1944).
- ²⁷ Westall, R. G., *Biochem. J.*, **77**, 135 (1960).
- ²⁸ Archibald, R. M., *J. Biol. Chem.*, **157**, 507 (1945).
- ²⁹ Kurelec, B., *Veterin. Arhiv Zagreb.*, **34**, 193 (1964).
- ³⁰ Campbell, J. W., *Comp. Biochem. Physiol.*, **8**, 13 (1963).
- ³¹ Agosin, M., and Repetto, C., *Comp. Biochem. Physiol.*, **8**, 245 (1963).

CHLORAMPHENICOL-, DIHYDROSTREPTOMYCIN-, AND KANAMYCIN-INACTIVATING ENZYMES FROM MULTIPLE DRUG-RESISTANT *Escherichia coli* CARRYING EPISOME 'R'

By SUEHIKO OKAMOTO and YOSHIKI SUZUKI

Department of Chemistry and Laboratory of Radiation Research, National Institute of Health, Shinagawa-ku, Tokyo, Japan

IN our previous investigations¹, no drug resistance was observed in the cell-free amino-acid incorporation system from chloramphenicol- or tetracycline-resistant strains of *Escherichia coli*. The resistant strains examined in those investigations included chloramphenicol- or tetracycline-resistant strains selected by serial transfer on media containing the drugs (the *in vitro* developed chloramphenicol- or tetracycline-resistant strains) as well as a naturally occurring multiple drug resistant strain carrying episome 'R' (resistant to four drugs: chloramphenicol, tetracycline, dihydrostreptomycin and sulphanilamides).

Recently by a modification of our experimental conditions we obtained from the strain carrying 'R' a cell-free amino-acid incorporation system resistant to chloramphenicol and dihydrostreptomycin.

Further study revealed that components responsible for the cell-free resistance reside in the 100,000g supernatant fraction and not in the ribosomes. The resistance shown in cell-free systems was finally found to be due to drug inactivating enzymes in the 100,000g supernatant fraction. Inactivation of chloramphenicol requires acetyl-coenzyme A, while ATP is required for inactivation of dihydrostreptomycin. The absence of acetate ion in our previous systems clearly explains our failure to detect chloramphenicol resistance on the cell-free level.

A kanamycin inactivating enzyme was also found in the cell-free extract of another 'R' strain which is resistant to kanamycin as well as to the four drugs already mentioned. Acetyl-coenzyme A was shown to be a required co-factor in the system.

In this article, partial purification and some characterization of these drug inactivating enzymes will be reported.

Inactivation of the drugs by the extract of the 'R' strain. The bacterial strains used were: (1) *E. coli* K12 CS-2 (Skaar and Garen²) sensitive to all the drugs tested here; (2) *E. coli* K12 ('R-4'), a strain resistant to four drugs (chloramphenicol, dihydrostreptomycin, tetracycline and sulphanilamides) derived from K12 CS-2 by transmission of an 'R' factor ('R-4') from a naturally isolated drug resistant strain of *Shigella flexneri*³; (3) *E. coli* K12 ('R-5'), a five drug resistant derivative of K12 CS-2 obtained by transmission of an R factor ('R-5') from

another naturally isolated drug resistant strain of *Shigella sonnei*⁴ which was resistant to kanamycin in addition to the four drugs already mentioned. The concentrations of drugs required to produce 50 per cent inhibition of growth of K12 CS-2 in the peptone-glucose medium are 0.6 µg/ml. of chloramphenicol, 3.6 µg/ml. of tetracycline and 0.75 µg/ml. of dihydrostreptomycin, and 90, 25 and 10 µg/ml. for K12 ('R-4') and ('R-5'). The respective 50 per cent inhibition doses of kanamycin for K12 CS-2 and for K12 ('R-5') are about 1 µg and 10 µg/ml. Cells were grown in peptone-glucose medium with aeration to the late log phase (2-3 g of wet cells per litre); the culture was chilled rapidly by pouring on to crushed ice and gathered by centrifugation. Cells were washed and homogenized in an equal volume of 0.02 M *tris* buffer (pH 7.8) containing 0.01 M magnesium acetate, 0.06 M potassium chloride and 0.006 M 2-mercaptoethanol, and disrupted by passage through a French pressure cell. The disrupted suspension was centrifuged at 30,000g for 20 min. This supernatant was dialysed over-night and further fractionated into a supernatant fraction (100 S) and ribosomal fraction by two centrifugations at 100,000g for 90 min.

As already mentioned, the 100 S fraction of the 'R' strain causes cell-free resistance to chloramphenicol or dihydrostreptomycin, but incubation of the drugs with this fraction in the aforementioned buffer does not result in any significant inactivation of the drugs. Then, it was found that the drugs are inactivated when incubated in the complete system of amino-acid incorporation containing the 100 S from K12 ('R-4'). Further investigations on the necessary factors for drug inactivation revealed that chloramphenicol is inactivated by the 'R' 100 S to a limited extent in the presence of ATP; the 'R' D-C fraction (see as follows) freed from low molecular weight substances exhibited an absolute requirement for ATP, coenzyme A and acetate ion as shown in Table 1 (Exp. 1). Dihydrostreptomycin was found to be inactivated by the 'R' D-C fraction in the presence of ATP and Mg⁺⁺ (preferably with ATP generating system) (Table 2). The ATP cannot be replaced by ADP, AMP, GTP, CTP or UTP. Addition of coenzyme A produces

no significant stimulation of the reaction. Extracts from K12 *CS-2* elicited very little or no inactivation of the drugs under the same conditions (Table 3); this seems also to be the case in the *in vitro* developed chloramphenicol-resistant strain since it does not give a resistant cell-free amino-acid incorporation system.

In addition, the *D-C* fraction of K12 ('R-5') was shown to inactivate kanamycin, which was not observed in the case of K12 ('R-4') (Table 4). Inactivation of kanamycin has the same co-factor requirement as that of chloramphenicol (Table 4).

Table 1

Exp. 1. Co-factor requirement for chloramphenicol inactivation by the *D-C* fraction of K12 ('R-4')

System	Growth inhibition by residual chloramphenicol (%)
Complete system	7
" - Coenzyme A	90
" - Acetate ion	89
" - ATP	86

Exp. 2. Co-factor requirement for chloramphenicol inactivation by the A-50 *CM* fraction of K12 ('R-4')

System	Rate of inactivation ($\mu\text{g}/15 \text{ min}$)
No addition	0
+ Acetyl-coenzyme A	17
+ ATP, coenzyme A	0
+ ATP, coenzyme A, 100 S fraction of K12 <i>CS-2</i>	13

Exp. 1. The complete system contains 0.1 mg protein of the 'R-4' *D-C* fraction, 0.10 M *tris* buffer (pH 7.8), 0.01 M magnesium acetate, 0.06 M KCl, 4 μmoles ATP, 0.04 μmoles coenzyme A and 50 μg of chloramphenicol in a total volume of 0.5 ml. The mixtures were incubated at 37° C for 30 min. A 0.05 ml. portion was pipetted into 5 ml. of peptone-glucose medium prewarmed to 80° C, and heated for 5 min. After cooling, each tube was inoculated with about 10^7 cells of *E. coli* K12 *CS-2* and the growth inhibition was measured as described in the text.

Exp. 2. The reaction system contains 50 μg protein of the A-50 *CM* fraction in a solution of: 0.10 M *tris* buffer (pH 7.8), 0.06 M KCl, 0.01 M magnesium acetate and 30 μg of chloramphenicol in a total volume of 0.5 ml. The following co-factors were added: 0.2 μmoles acetyl-coenzyme A; 2 μmoles ATP and 0.02 μmoles coenzyme A; 2 μmoles ATP, 0.02 μmoles coenzyme A and 150 μg protein of the 100 S fraction from K12 *CS-2*. The mixtures were incubated at 37° C for 15 min. A 0.1 ml. aliquot was pipetted into 5 ml. of peptone-glucose medium (at 80° C) and the rate of drug inactivation was estimated as described in the text.

Table 2. CO-FACTOR REQUIREMENT FOR INACTIVATION OF DIHYDROSTREPTOMYCIN BY THE *D-C* FRACTION OF K12 ('R-4')

System	Rate of dihydrostreptomycin inactivation ($\mu\text{g}/30 \text{ min}$)
Complete	25
" + Coenzyme A	28
" - ATP, CrP, CrK	0
" - Mg^{++}	< 7.5

The complete system contains 0.5 mg protein of 'R' *D-C* fraction, 0.10 M *tris* buffer (pH 7.8), 0.01 M magnesium acetate, 0.06 M KCl, 2 μmoles ATP, 4 μmoles creatine phosphate, 60 μg creatine kinase and 60 μg dihydrostreptomycin in a total volume of 0.5 ml. The mixtures were incubated at 37° C for 15 and 30 min. 0.05 ml. portions were pipetted into 5 ml. of peptone-glucose medium prewarmed to 80° C and heated for 5 min. After cooling, the medium was inoculated with 10^7 cells of *E. coli* K12 *CS-2*, and the rate of drug inactivation was estimated as described in the text.

Table 3. ACTIVITIES OF DRUG INACTIVATION OF THE *D-C* FRACTIONS FROM K12 *CS-2* AND K12 ('R-4')

Drugs	K12 <i>CS-2</i>	K12 ('R-4')	Control (no <i>D-C</i> fraction)
Chloramphenicol (Original concentration * 2 $\mu\text{g}/\text{ml.}$)			
After incubation			
For 10 min	80	0	
For 30 min	75	0	84
Dihydrostreptomycin (Original concentration 1 $\mu\text{g}/\text{ml.}$)			
After incubation			
For 45 min	42	8	
For 90 min	43	0	51
(Original concentration 2 $\mu\text{g}/\text{ml.}$)			
After incubation			
For 90 min	92	0	95

The reaction mixture for chloramphenicol inactivation contains 1.2 mg protein of the *D-C* fraction of K12 ('R-4') or 1.7 mg protein of the same fraction of K12 *CS-2*, 0.10 M *tris* buffer (pH 7.8), 0.01 M magnesium acetate, 0.06 M KCl, 4 μmoles ATP, 3 μmoles creatine phosphate, 60 μg of creatine kinase, 0.04 μmoles coenzyme A and 50 μg of chloramphenicol in a total volume of 0.5 ml. The reaction mixture for dihydrostreptomycin inactivation contains the same ingredients except that coenzyme A is omitted and chloramphenicol is replaced by 50 μg dihydrostreptomycin. The mixtures were incubated at 37° C for the indicated time intervals and 0.05 ml. or 0.10 ml. portions were pipetted into 5 ml. of peptone-glucose medium (at 80° C). The rate of drug inactivation was estimated by the method described in the text.

* The original concentration refers to the concentration of the drug present in the peptone-glucose medium when no drug inactivation takes place.

Table 4. INACTIVATION OF KANAMYCIN BY THE ENZYME PREPARATIONS FROM *E. coli* K12 ('R-5'), K12 ('R-4') AND K12 *CS-2*

Exp. 1. Experiments with the *D-C* fractions

Source of <i>D-C</i> fraction	System	Inactivation of kanamycin ($\mu\text{g}/2 \text{ mg protein}/45 \text{ min}$)
'R-5'	Complete	60
'R-5'	" - Coenzyme A	8
'R-5'	" - ATP, CrP, CrK	0
'R-4'	"	0
<i>CS-2</i>	"	0

Exp. 2. Experiments with the A-50 *CM* fraction of K12 ('R-5')

System	Inactivation of kanamycin ($\mu\text{g}/30 \mu\text{g protein}/120 \text{ min}$)
Complete	18
" - ATP	1
" - Acetate ion	0
" - <i>D-C</i> fraction from <i>CS-2</i>	1
" - Mg^{++}	3
Complete system replaced with Acetyl-coenzyme A *	19

Exp. 1. The complete mixture contains 2 mg protein from the *D-C* fraction of either K12 ('R-5'), K12 ('R-4') or K12 *CS-2*, 0.10 M *tris* buffer (pH 7.8), 0.01 M magnesium acetate, 0.06 M KCl, 10 μmoles ATP, 4 μmoles creatine phosphate, 60 μg creatine kinase, 0.04 μmoles coenzyme A and 60 μg of kanamycin in total volume of 1.0 ml. The mixture was incubated at 37° C for 45 min. 0.10 ml. portions were pipetted into 5 ml. of peptone-glucose medium prewarmed to 80° C, and heated for 5 min. After cooling, the medium was inoculated with 10^7 cells of *E. coli* K12 *CS-2*, and the rate of kanamycin inactivation was estimated as described in the text.

Exp. 2. The complete system contains 30 μg protein of the A-50 *CM* fraction of 'R-5', 0.10 M *tris* buffer (pH 7.8), 0.01 M magnesium acetate, 0.1 M KCl, 0.02 μmoles coenzyme A, 0.2 mg protein of the *D-C* fraction from *CS-2* and 30 μg of kanamycin in a total volume of 0.5 ml. The mixtures were incubated at 37° C for 120 min, and further procedures were carried out as described in Exp. 1.

* ATP, creatine phosphate, creatine kinase, coenzyme A and the *D-C* fraction from K12 *CS-2* were replaced with 0.7 μmoles of acetyl-coenzyme A.

The activities of the drug-inactivating enzymes were determined in the following ways:

Assay of inactivation of chloramphenicol. The total reaction mixture for the inactivation of chloramphenicol contains 0.10 M *tris* buffer (pH 7.8), 0.01 M magnesium acetate, 0.06 M potassium chloride, 2 μmoles ATP, 0.02 μmoles coenzyme A, an appropriate amount of chloramphenicol (20–100 μg) and the enzyme preparation in a total volume of 0.5 ml. After incubation at 37° C for various time intervals an aliquot originally containing 5 or 6 μg of chloramphenicol is pipetted into 5 ml. of peptone-glucose medium which was prewarmed at 80° C and heated for 5 min at this temperature to denature the enzyme. The medium is inoculated with 1×10^7 cells of *E. coli* strain K12 *CS-2* and shaken at 37° C for about 3 h. The bacterial growth is determined by turbidity. The amount of chloramphenicol which escaped inactivation can be estimated by comparison with suitable control tubes which contain graded amounts of chloramphenicol and heat denatured reaction mixture. The activity of the enzyme fraction is expressed by the rate of inactivation of the drug.

Assay of inactivation of dihydrostreptomycin. The composition of the reaction mixture is as follows: 0.10 M *tris* buffer (pH 7.8), 0.01 M magnesium acetate, 0.06 M potassium chloride, 2 μmoles ATP, 4 μmoles creatine phosphate, creatine kinase of 60 μg protein, 15–50 μg dihydrostreptomycin and enzyme preparation in a total volume of 0.5 ml. Other procedures are essentially the same as already described here.

Assay of inactivation of kanamycin. This was performed in a similar way to that of dihydrostreptomycin, except that 0.02 μmoles of coenzyme A was included in the reaction mixture.

Separation of Resistant Components

All operations were carried out in the cold. The 'R' 100 S fraction was treated with DNase (4 $\mu\text{g}/\text{ml.}$) and fractionated by ammonium sulphate precipitation. The precipitate formed between 30 and 65 per cent saturation was dissolved in 0.02 M *tris* buffer (pH 7.8) containing 0.05 M potassium chloride, 0.006 M 2-mercaptoethanol and 10^{-4} M ethylenediamine tetraacetate. The solution was passed through a 'Sephadex G-50' column with the same buffer to remove ammonium sulphate. This fraction was adsorbed to a DEAE-cellulose column (1 cm \times 30 cm) and eluted with the same buffer containing 0.3 M potas-

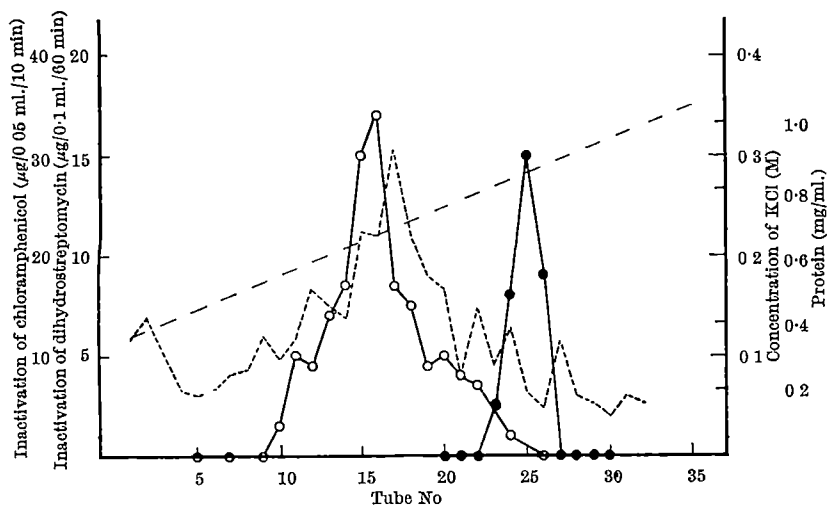


Fig. 1. Fractionation of the drug-inactivating enzymes on DEAE-Sephadex A-50. About 74 mg protein of the *D-C* fraction of K12 ('R-4') was applied to a DEAE-Sephadex A-50 column (1 cm × 25 cm) and eluted with 250 ml. of *tris* buffer (pH 7.8) containing 0.006 M 2-mercaptoethanol, 10⁻⁴ M ethylenediamine tetraacetate and a linearly increasing concentration of KCl (0.12–0.35 M). Seven ml. fractions were collected. Protein concentration was estimated by the method of Lowry ———, Protein; ○—○, *CM* inactivation, ●—●, *DHSM* inactivation

sium chloride to remove nucleic acids. The eluate was precipitated with ammonium sulphate at 65 per cent saturation, and redissolved in the same buffer (KCl 0.1 M) and dialysed over-night against this buffer ('R' *D-C* fraction).

The 'R' *D-C* fraction (about 50 mg protein) was applied to a DEAE-Sephadex A-50 column (1 cm × 25 cm) and eluted with the aforementioned buffer, containing a linearly increasing concentration of potassium chloride from 0.12 to 0.35 M in a total volume of 250 ml. Seven ml. fractions were collected and assayed for their ability to inactivate chloramphenicol, dihydrostreptomycin and/or kanamycin.

As in Fig. 1, activities for chloramphenicol and dihydrostreptomycin inactivation were eluted, showing clearly separated peaks at concentrations of about 0.22 M and 0.28 M, respectively ('A-50 *CM* fraction' and 'A-50 *DHSM* fraction'). The A-50 *DHSM* fraction inactivates dihydrostreptomycin in the presence of ATP and Mg⁺⁺. Inactivation of chloramphenicol by the A-50 *CM* fraction requires the presence of the *D-C* fraction of K12 *CS-2* in addition to ATP, coenzyme A and acetate ion. All these requirements can be replaced by acetyl-coenzyme A alone (Table 1, Exp. 2). Kanamycin inactivating activity was eluted a little behind the A-50 *DHSM* fraction (A-50 *KM* fraction), but the bulk of these two activities overlapped each other. The requirement of A-50 *KM* fraction for kanamycin inactivation is the same as that of A-50 *CM* fraction (Table 4).

Some Properties of Drug-inactivating Enzymes

Under the conditions already described, chloramphenicol, dihydrostreptomycin and kanamycin are respectively inactivated at rates of about 60 μg, 2 μg and 5 μg/min/1 mg protein of the appropriate 'R' *D-C* fraction. Heating of 'R' *D-C* fraction at 60° C for 5 min in 0.10 M *tris* buffer (pH 7.8) containing 0.01 M magnesium acetate and 0.06 M potassium chloride completely destroys dihydrostreptomycin and kanamycin inactivating activities. Chloramphenicol inactivating activity is somewhat more heat stable; more than half its activity is retained after heating at 60° C for 5 min, but it is completely abolished by heating at 80° C for 5 min.

The *D-C* fraction obtained from K12 ('R-4') grown in the presence of 4 μg/ml. of dihydrostreptomycin and 10 μg/ml. of chloramphenicol did not have higher activities of drug inactivation than that of untreated cells. Thus, inducibility of the drug-inactivating enzymes by the drugs was not observed.

Several efforts have been made to see whether tetracycline and sulphanilamides are inactivated by the extract of 'R' cells, but the results have been negative so far.

It seems that such drug-inactivating enzymes are not contained in the extract of the *in vitro* developed chloramphenicol-resistant strain or the high level dihydrostreptomycin-resistant strain selected by a single step mutation *in vitro*, since the results of experiments using cell-free amino-acid incorporating systems by us and other workers^{5,6} indicate that soluble components are not responsible for drug resistance in these cases.

The results of the work recorded here demonstrate that the extract of K12 ('R') contains separate enzymes which can inactivate chloramphenicol, dihydrostreptomycin and kanamycin, respectively; comparable activities are not observed with the extract of the sensitive strain or the *in vitro* developed chloramphenicol-resistant strain. Moreover, kanamycin inactivation is observed only in the case of K12 ('R-5'), but not in the K12 ('R-4'). It is very probable that the resistance of 'R' cells to these drugs is caused by these enzymes which are produced by 'R' genes.

The acetyl-coenzyme A requirement for chloramphenicol and kanamycin inactivation strongly suggests that the reactions may be acetylation of some group(s) in the drug molecules. The mode of dihydrostreptomycin inactivation is not so clear at present.

It was already observed by Miyamura *et al.*⁷ that the culture of the 'R' strain inactivates chloramphenicol in the growth medium more rapidly than that of the drug sensitive strain or the *in vitro* developed chloramphenicol-resistant strain does. Similar findings were recently reported by Tsukamoto *et al.*⁸ on chloramphenicol, dihydrostreptomycin and tetracycline inactivation. Our present results afford the enzymatic basis to these *in vivo* findings except for the case of tetracycline. Although at present we are unable to demonstrate inactivation of tetracycline or sulphanilamides by the extract of the 'R' strain, it is possible that, in these cases, similar inactivating enzymes may be found under appropriate conditions.

The mechanism of drug resistance of the 'R' strain seems to be of some practical interest. The drug inactivating enzymes may serve as tools in clinical and experimental researches. If the precise mode of the inactivation is elucidated, it will be helpful to obtain derivatives of the drugs which are effective against 'R' cells, as was the case of penicillinase-resistant penicillin derivatives⁹.

We thank Drs. J. Tomizawa and T. Komai for their advice. The four drug-resistant strain of *Shigella* is a kind gift of Dr. R. Nakaya of our Institute, and Drs. Sasagawa and Ikemura, of Niigata Prefectural Institute of Health, kindly supplied the five drug-resistant strain of *Shigella*.

¹ Okamoto, S., and Mizuno, D., *J. Gen. Microbiol.*, **35**, 125 (1964).

² Skaar, P. D., and Garen, A., *Proc. U.S. Nat. Acad. Sci.*, **42**, 619 (1956).

³ Nakaya, R., Nakamura, A., and Murata, Y., *Biochem. Biophys. Res. Comm.*, **3**, 654 (1960).

⁴ Kimura, S., Mizuno, T., Akiba, A., Sasakawa, I., and Ikemura, K., *Med. and Biol.*, **68**, 61 (1964) (in Japanese).

⁵ Flaks, J. G., Cox, E. C., Witting, M. L., and White, J. R., *Biochem. Biophys. Res. Comm.*, **7**, 390 (1962).

⁶ Speyer, J. F., Lengyel, P., and Basillo, C., *Proc. U.S. Nat. Acad. Sci.*, **48**, 684 (1962).

⁷ Miyamura, S., Shigeno, N., and Watanabe, T., *Jap. J. Bact.*, **17**, 787 (1962) (in Japanese).

⁸ Tsukamoto, C., Miyake, M., and Sato, H., *J. Pharmaceut. Soc. Japan*, **85**, 41 (1965) (in Japanese).

⁹ Chain, E. B., in *New Perspectives in Biology*, edit. by Sela, M., 205 (Elsevier Publishing Co., 1964).

LETTERS TO THE EDITOR

ASTRONOMY

Stability of Rotating Massive Stars in General Relativity

THE suggestion by Hoyle and Fowler¹ that stars with masses of 10^6 – $10^{10} M_{\odot}$ may provide the energy for radio sources, and the subsequent discovery of quasars, has stimulated considerable interest in the structure of very massive stars². Iben³, using a binding-energy argument, showed that within the framework of general relativity a spherical massive star becomes unstable long before it has contracted to the stage at which nuclear reactions become important. A similar conclusion was obtained by Chandrasekhar⁴, using a detailed stability analysis on the spherically symmetric relativistic equations and calculating the relaxation oscillations from a variational principle. Similar results have been obtained by Fowler⁵ using a virial theorem approach.

In a recent publication, Roxburgh⁶, using a virial theorem approach, found that rotation had a considerable stabilizing effect, changing the radius at which instability occurred by a very large factor. In view of the large change produced by rotation it is desirable to have a proper stability analysis of rotating massive stars in general relativity, since, in the Newtonian theory, it is known that the virial theorem gives results that may be wrong by a factor of four⁷. We have recently undertaken such an investigation which will be published in detail elsewhere⁸; here we wish to report the principal results.

The general relativistic equations have been given by Misner and Sharp⁹ and by Chandrasekhar¹⁰ in a weak field approximation. This is sufficient for our purpose. If we confine our attention to slow rotation and integrate the equations over latitude, so, leaving purely radial variables, the equations that govern the structure of the rotating massive star can be expressed as¹¹:

$$\frac{2}{3}\Omega^2 r - \frac{1}{\rho} \frac{dP}{dr} - \frac{GM_r}{r^2} \left(1 + \frac{P}{\rho c^2} + \frac{2GM_r}{rc^2}\right) - \frac{4\pi GPr}{c^2} = 0 \quad (1)$$

$$\frac{dM_r}{dr} = 4\pi r^2 \rho \quad (2)$$

$$\rho = \rho_0 + \frac{3U}{c^2}, \quad \frac{U}{\rho} = \frac{3(\gamma-1) - \beta(3\gamma-4)}{\gamma-1} \quad (3)$$

$$\frac{d \log P}{d \log T} = \frac{(32 - 24\beta - 3\beta^2)}{(8 - 6\beta)} \simeq 4 - \frac{3\beta^2}{8}, \quad \beta \ll 1 \quad (4)$$

$$P = P_g + P_R = P_g/\beta \quad (5)$$

$$P_g = \frac{R}{\mu} \rho_0 T, \quad P_R = \frac{aT^4}{3}$$

Equation 4 is the adiabatic condition which is used since the star is in convective equilibrium^{1,4}.

With $\beta \ll 1$ these equations are the same as those of the polytrope $n = 3$, and the equations are readily integrated. β is then given by Eddington's¹² equation which approximates to:

$$\beta = 8.6 \left(\frac{M}{M_{\odot}}\right)^{\frac{1}{2}} \quad (6)$$

By introducing suitable dimensionless variables equations (1)–(5) reduce to:

$$\frac{d\sigma}{d\xi} = (1 + 3q\sigma) \left[\frac{1}{3}\alpha\xi - \frac{m}{\xi^2} \left(1 + \frac{q\sigma}{1 + 3q\sigma} + \frac{8mq}{\xi}\right) - q\xi\sigma^4 \right] \quad (7)$$

$$\frac{dm}{d\xi} = \xi^2 \sigma^3 (1 + 3q\sigma) \quad (8)$$

where:

$$\alpha = \frac{\Omega^2}{2\pi G\rho_c}, \quad q = \frac{P_c}{\rho_c c^2} \quad (9)$$

The former measures the degree of rotation, the second the effects of general relativity. These equations are readily solved subject to the boundary conditions:

$$\sigma = 1, m = 0(\xi^3) \text{ at } \xi = 0 \quad (10)$$

To calculate the stability against radial oscillations we introduce an adiabatic displacement $\Delta r = r e^{i\omega t}$ into the general relativistic equations and linearize in the perturbations. After some elimination, this gives an eigenvalue equation to determine ω^2 and η . It can readily be shown that the problem is self-adjoint and a variational principle derived to determine ω^2 . This gives:

$$\begin{aligned} \Sigma^2 \left[\int_0^{\xi_1} B \sigma^3 (1 + 3q\sigma) \left(1 + \frac{4mq}{\xi}\right) \eta^2 \xi^2 d\xi \right] \\ = \int_0^{\xi_1} B \sigma^3 \left\{ \left(1 + \frac{4mq}{\xi}\right) \left[\frac{\Gamma\sigma}{4} (\xi\eta' + 2\eta)^2 + \alpha(1 + 3q\sigma)\eta^2 \xi^2 \right. \right. \\ \left. \left. + 2\eta^2 \left(\sigma' \xi - (1 + 3q\sigma) \frac{m}{\xi} \right) \right] - 2q\Gamma\eta \frac{m}{\xi} \sigma (\eta' \xi + 2\eta) \right. \\ \left. - \eta^2 \frac{mq}{\xi} \left(2\sigma + 20 \frac{m}{\xi} \right) \right\} d\xi \end{aligned} \quad (11)$$

where:

$$B = \frac{e^{-4q\sigma}}{e^{-4q}}, \quad \Sigma_2 = \frac{\omega^2}{4\pi G\rho_c} \quad (12)$$

$$\Gamma = 4/3 + \beta/6$$

To calculate Σ^2 we took a trial function:

$$\eta = \xi + \lambda_1 \xi^2 + \lambda_2 \xi^3 \quad (13)$$

where λ_1 and λ_2 are parameters to be varied so as to obtain the minimum value of Σ^2 . We initially set $\lambda_2 = 0$ and varied λ_1 so as to minimize Σ^2 ; with this value of λ_1 , λ_2 was then varied to obtain an improved value of Σ^2 . The accuracy of our solution was estimated by applying the principle to the non-relativistic case, and comparing with the results of Cowling and Newing⁷. Our trial function gave considerably better results than the linear trial function.

With Γ given by equation (12) and β by equation (6) we evaluated Σ^2 for $M/M_{\odot} = 10^6, 10^7, 10^8, 10^9$ and 10^{10} for different values of q and α . Instability was found to set in when:

$$q_1 = 0.425 \left(\frac{2GM}{Rrc^2} \right) = 0.55 \left(\frac{M}{M_{\odot}} \right)^{\frac{1}{2}} + 0.56 \alpha$$

Since the massive object is like a polytrope of index 3, the maximum possible value of α is 3.95×10^{-3} (ref. 13). With $M/M_{\odot} = 10^{10}$ and α at this maximum value, the radius at which instability sets in is decreased by a factor of 400. Even small values of α can have a consider-

able effect. For sufficiently large masses and maximum α , the star becomes unstable at a radius:

$$R_I = 193 R_g$$

where R_g is the Schwarzschild radius $2GM/c^2$.

This should be compared with the results of the virial theorem analysis of Roxburgh⁶ which gave $R_I = 250 R_g$. The detailed analysis therefore confirms the general conclusions of the virial theorem analysis.

B. DURNÉY
I. W. ROXBURGH

High Altitude Observatory,
Boulder, Colorado,
and
King's College,
University of London.

¹ Hoyle, F., and Fowler, W. A., *Mon. Not. Roy. Astro. Soc.*, **125**, 169 (1963).

² *Proc. First Texas Symp. Relativistic Astrophysics* (University of Chicago Press, 1965).

³ Iben, I., *Astrophys. J.*, **138**, 1090 (1963).

⁴ Chandrasekhar, S., *Phys. Rev. Letters*, **12**, 114, 437 (1964); *Astrophys. J.*, **160**, 417 (1964).

⁵ Fowler, W. A., *Rev. Mod. Phys.*, **36**, 545 (1964), **36**, 1104 (1964).

⁶ Roxburgh, I. W., *Nature*, **207**, 363 (1965).

⁷ Cowling, T. G., and Newing, R. A., *Astrophys. J.*, **109**, 149 (1949).

⁸ Durney, B., and Roxburgh, I. W. (to be published).

⁹ Misner, C. W., and Sharp, D. H., *Phys. Rev.*, **136**, B571 (1964).

¹⁰ Chandrasekhar, S., *Phys. Rev. Letters*, **14**, 241 (1965).

¹¹ Fowler, W. A. (to be published).

¹² Eddington, A. S., *Internal Constitution of the Stars* (Camb. Univ. Press, 1930).

¹³ Monaghan, J., and Roxburgh, I. W., *Mon. Not. Roy. Astro. Soc.*, **131**, 129 (1965).

PHYSICS

Effect of Ultra-violet Irradiation on the Electrical Conductivity of Zinc Oxide Single Crystals

EXTENSIVE examination of ZnO crystals and powder in the past few years has been reported by Heiland *et al.*¹ for its optical and electronic properties. Present measurements have been initiated because of the effect of the solar ultra-violet energy on thermal control coatings of spacecraft which use ZnO as a pigment with an adequate binder. Previous measurement of electrical conductivity of ZnO, with and without doping, indicated that the increase in conductivity with doping is due to the donors as charge carriers. Optical measurements^{1,2} indicated that when the specimens have been heated in ZnO vapour the unresolved peak near the absorption edge is more pronounced, and this was attributed by Scharowsky² to be due to the absorption of zinc ion, while Heiland *et al.*¹ considered this to be due to unspecified lattice defects resulting from the diffusion of atomic zinc. Craeynest *et al.*³ explains a similar effect by reflexion spectra in powders and proposes a complex centre with an unpaired electron, but he could not detect any band at 380 nm by heating ZnO powders in nitrogen or argon atmosphere.

Single crystals of ZnO were obtained from Minnesota Mining and Manufacturing Co., which have less than 55 parts per million impurities other than zinc. A hexagonal bar was cut from the central portion of the crystal rod to give it a constant dimension. The face area and the length of the crystal was measured with a microscope which enabled us to calculate the volume of the crystal. Volume was also calculated by knowing the density and weighing the crystal on a semi-microbalance. The two methods differed by only 1 per cent. For conductivity measurements a thin coating of DuPont silver-conducting paint No. 4317 was used, and the crystal was mounted on a substrate which could withstand temperatures up to 400° C and would sustain a high vacuum. Conductivity was measured at 25° C by measuring the potential drop across a standard high resistance in series with the crystal, with the help of a Kiethly 610B electrometer. To avoid heating the crystal, a potential of 3 V was used. Cur-

rent was allowed to develop through the crystal for 10 min and the potential drop was recorded on a chart recorder.

The resistance measurement of the crystal was carried out in an atmosphere of air and dry nitrogen. Measurements were also taken when the crystal was placed under vacuum and before ultra-violet irradiation was initiated. An Ultek Corporation vacuum system was used, having sorption rough pumping and ion fine pumping so that no contamination occurred on the crystal. The vacuum was maintained at $4-7 \times 10^{-7}$ torr. The ultra-violet irradiation was performed with a BH6 high-pressure mercury arc lamp controlled by a constant intensity device developed by Arnett⁴. The irradiation was filtered using a CS7-37 Corning filter so that the principal illuminating wavelengths were concentrated about 3650 Å. The sample was irradiated for 50 h at 0.013 W/cm². When the irradiation was stopped, the sample was allowed to cool from the irradiation temperature of approximately 60° C to 25° C. The resistance was then measured while still under vacuum. Air was then allowed to enter the vacuum chamber until atmospheric pressure was attained and the resistance was again measured. The results are shown in Table 1.

Electrical conductivity in ohm ⁻¹ cm ⁻¹	Time (min) after potential is applied			
	0	4	8	10
Before irradiation	5.50 $\times 10^{-3}$			4.685 $\times 10^{-3}$
in air				
After irradiation	7.127 $\times 10^{-3}$	9.528 $\times 10^{-3}$	1.003 $\times 10^{-2}$	1.003 $\times 10^{-2}$
in vacuum				
After irradiation	4.685 $\times 10^{-3}$	4.78 $\times 10^{-3}$	4.499 $\times 10^{-3}$	4.499 $\times 10^{-3}$
and letting air				
in vacuum chamber				

The observed increase in conductivity after 50 h of ultra-violet irradiation may result from the loss of oxygen from the sample, while the excess zinc atoms become ionized and diffuse into interstitial position in the lattice, which accommodates them. The oxygen evolved will be absorbed by the ion pumps of the system and so there is little possibility of it being re-absorbed by the crystal itself. Electrons which make possible an electric conduction are removed from zinc, compared in a similar way with thermal excitation, as reported by Scharowsky². The increase in conductivity of the crystal implies that the zinc atoms are not electrically neutral. However, lowering the conductivity by allowing air to enter the vacuum chamber gives an idea of the dependency of conductivity on oxygen pressure and the influence of chemisorption of oxygen as reported by Kokes⁵. Mollow⁶ concludes, on the basis of heating crystals in hydrogen, that the change in conductivity with time is due to a diffusion process. It seems probable that the effect of heating may be comparable with the effect of ultra-violet irradiation, but no definite picture could be drawn. Further measurements of magnetic and optical properties are expected to give interesting results.

One of us (R. B. L.) is a recipient of a post-doctoral research associateship of the U.S. National Research Council-National Academy of Sciences. We thank Mr. William C. Snoddy for his advice; Mr. Max Harper for assistance in preparing the vacuum apparatus; and Mr. Charles Cooper for his help in mounting the crystal.

R. B. LAL
G. M. ARNETT

Space Thermodynamics Branch,
Research Projects Laboratory,
National Aeronautics and Space Administration,
George C. Marshall Space Flight Center,
Huntsville, Alabama.

¹ Heiland, G., Mollow, E., and Stockmann, Z., *Solid State Phys.*, **8**, 191 (1959).

² Scharowsky, E., *Z. Phys.*, **135**, 318 (1953).

³ Craeynest, F., Van Maenhout, W., Van der Vorst, and Deykeyser, W., *Phys. Stat. Sol.*, **8**, 841 (1965).

⁴ Arnett, G. M. (to be published).

⁵ Kokes, R. J., *J. Phys. Chem.*, **66**, 99 (1962).

⁶ Mollow, E., *Z. Phys.*, **138**, 478 (1954).

Tschebychev Approximation to the Equation of Thermogravimetric Data

THE use of approximate expressions rather than tabulations for non-elementary functions, such as the exponential integral, is often predicated on the simplicity of computation and the attainable accuracy. Since approximations receive quite wide usage, it is generally recognized that many expressions of varying degrees of complexity and precision are available. However, it is not so widely realized by workers not directly concerned with numerical analysis that techniques exist which may be used to obtain maximum accuracy from given functional forms by varying appropriate constants within the expressions. Procedures which best achieve the desired result require that the constants be selected such that the maximum deviation of the approximant from the exact function be minimized over the selected range of the independent variable. This is a statement of the Tschebychev criterion (for a brief introduction see ref. 1). It is the purpose of this communication to present a Tschebychev approximation to the function $p(x)$, given by Doyle² as:

$$p(x) = e^{-x} x^{-1} - \int_x^\infty e^{-\tau} \tau^{-1} d\tau = e^{-x} x^{-1} - E_1(x)$$

which occurs in the equation of the thermogravimetric data plot.

Doyle² indicates that the use of truncations of the Schlömilch expansion yields:

$$p(x) \sim f(A, x) = (x + A)^{-1} x^{-1} e^{-x}, x \geq 10$$

where A is 1 for the two-term expansion, and 2 for the three-term expansion.

The functional form, sufficiently simple for computation, possesses maximum relative errors of -7.2 per cent and +1.3 per cent, respectively, for $x \geq 10$. It is clear that some different selection of A should permit a higher accuracy with no increase in complexity.

To accomplish the fit, we seek to minimize (relative deviation):

$$\max_{x \geq 10} \left| \frac{e^{-x} x^{-1} - E_1(x) - (x + A)^{-1} x^{-1} e^{-x}}{(x + A)^{-1} x^{-1} e^{-x}} \right|$$

that is, select A such that:

$$\delta = \min_A \max_{x \geq 10} |1 - x(x + A) e^x E_1(x)|$$

The normal error curve for a problem of this type will possess two extrema, $\pm \delta$, the location of which may be obtained by application of the Remes second algorithm^{3,4}. The algorithm is effected by: (1) assuming a set of abscissae for which extrema might be expected (for example, $x_1 = 10$, $x_2 = 50$); (2) solving for A and δ (deviation of $+\delta$ at x_1 , $-\delta$ at x_2); (3) determining the abscissae of the true extrema of the resultant error curve; (4) using these as elements for a continuing iteration. This can easily be done for the foregoing function yielding:

$$x_1 = 10, x_2 \sim 27$$

$$A = 1.8768$$

$$\delta = 2.0 \times 10^{-3} = 0.2 \text{ per cent, } x \geq 10$$

It should be noted that an improvement of an order of magnitude is obtained over the relative error of the three-term Schlömilch expansion.

Table 1. $p(x)/f(A, x)$

x	Schlömilch three-term ($A = 2$)	Tschebychev fit ($A = 1.8768$)
10	1.013	1.0020
15	1.007	0.9990
20	1.003	0.9982
25	1.002	0.9980
30	1.001	0.9980
35	1.000	0.9981
40	1.000	0.9982
45	1.000	0.9982
50	1.000	0.9983
100	1.000	0.9990
200	1.000	0.9994

I thank Dr. J. Ross Macdonald for directing my attention to this application and Dr. C. D. Doyle for his advice.

ERIC L. JONES

Texas Instruments, Incorporated,
Dallas, Texas.

¹ Hamming, R. W., *Numerical Methods for Scientists and Engineers* (McGraw-Hill Book Co., New York, 1962)

² Doyle, C. D., *Nature*, **207**, 290 (1965).

³ Remes, E., *C.R. Acad. Sci., Paris*, **199**, 337 (1934).

⁴ Murnaghan, F. D., and Wrench, J. W., *Math. Tables Aids Comput.*, **13**, 185 (1959).

Calculations with Two Nearly Equal Quantities

DR. WORRELL¹ has suggested that, when a small signal is to be detected against a large background, more reliable results can be gained by using ratios of observations than by using differences. For example, in a counting experiment where S events in a standard interval are to be detected against a background of B events, he shows that the relative error in $S = (S + B) - B$ is:

$$e_s = \sqrt{\frac{S + 2B}{S^2}}$$

while the relative error in $R = (S + B)/B$ is:

$$e_r = \sqrt{\frac{S + 2B}{B(S + B)}}$$

for large values of B .

For small values of S , e_r is clearly much smaller than e_s . While this result is indeed entirely reliable, its importance is not clear. If $S/B \rightarrow 0$, e_s becomes large compared to 1 for any finite value of B , while

$$e_r \rightarrow \sqrt{\frac{2}{B}}$$

which can be made as small as desired by using long counting times. Hence no indication is given that S even exists, but R can readily be found to high accuracy.

The drawback is that the value of R so accurately found is $1.0 \dots$, which physically leads to the identical result that no indication at all is given that S even exists.

The quantity of physical significance is, of course, not R but $(R - 1)$, since it is only the changes in R from unity which can carry any information as to the existence or magnitude of S .

Now:

$$R - 1 = \frac{B + S}{B} - 1 = \frac{(B + S) - B}{B}$$

The relative error in this is:

$$\sqrt{\frac{S + 2B}{S^2}}$$

or exactly e_s , again for large values of B . The gain in reliability of the value of R is thus of no practical interest.

J. H. FREMLIN

Department of Physics,
University of Birmingham.

¹ Worrell, F. T., *Nature*, **207**, 621 (1965).

WHILE most of the points in the foregoing communication are correct, I believe that Fremlin has misinterpreted my remarks. There seem to have been two points in my letter that were not clearly made. One was the nature of the experimental situation in which the ratio calculation was found to be useful: the other was my statement that "information can be extracted with greater reliability by taking the ratios rather than the differences". This state-

ment was not meant in a precise sense. Indeed, it is clear that if you calculate S from $S = B(R - 1)$, where:

$$R = \frac{S + B}{B}$$

you are bound to end up with the same error as you would have if you have calculated S from $S = (S + B) - B$. The accuracy of S does not depend on the method by which it is calculated. What was meant by the statement quoted may be clearer after the discussion which follows.

The derivation which I presented in my communication had the following basis: (1) it is well known that any experimental procedure which requires calculation of the desired result by subtracting two nearly equal quantities should be avoided if there is an alternative, because of the large errors that will result; (2) in just such an undesirable situation I found that I could get a clear indication that the phenomenon of interest existed by plotting the ratio of the two measured quantities versus time, whereas a plot of the differences versus time showed so much scatter that I hesitated to say that the phenomenon existed.

In our experiments, measurements were being made of the intensity of the 3914 Å radiation in the permanent airglow. This radiation is usually only detectable above the background of scattering from the lower atmosphere at twilight, during which period its intensity is decreasing with time. The signal intensity can be found by taking repeated measurements of signal-plus-background ($S + B$) and background (B) and subtracting corresponding pairs of readings. Under marginal seeing conditions, the experimenter may be confronted with a plot of signal versus time with such a large scatter that it is uncertain whether or not a signal is there. In such circumstances, a plot of the ratio:

$$\frac{S + B}{B}$$

versus time showed considerably less scatter than a plot of S versus time, and there was an evident peak in the ratio plot demonstrating the existence of the signal for a certain period. Thus the information that was "extracted more reliably" was that the signal was there. In my communication I chose not to discuss the question of quantitative interpretations of the ratio-versus-time plot, since that is a matter for more detailed discussions.

The symbolism which I chose in my derivation may have been misleading, since such an equation as.

$$\frac{e_S}{e_R} = \sqrt{\frac{B(S + B)}{S^2}}$$

containing S in isolation, may carry the implication that S is a measured quantity, which it is not. It may be better to state the matter as follows: If we measure two quantities, x and y , and calculate $x - y$ and x/y , then the relative errors in $x - y$ and x/y are:

$$e_{x-y} = \sqrt{\frac{x+y}{(x-y)^2}}$$

and

$$e_{x/y} = \sqrt{\frac{x+y}{xy}}$$

respectively. From these:

$$\frac{e_{x-y}}{e_{x/y}} = \sqrt{\frac{xy}{(x-y)^2}} \quad (1)$$

This ratio clearly becomes large when x and y are about the same magnitude.

In the light of this result, Fremlin's statement that e_R (or $e_{x/y}$) can be made as small as you wish by using longer counting times is seen to apply equally well to e_S

(or e_{x-y}), as I pointed out in my communication. I also pointed out that:

$$\frac{e_S}{e_R} \left(\text{or } \frac{e_{x-y}}{e_{x/y}} \right)$$

is independent of counting time, as will be evident from the foregoing equation (1). In any event, we are not at liberty to go to long counting times, as Fremlin suggests, because of the transient nature of our phenomenon.

In summary, having found a situation where the ratio of two measured quantities was undeniably more useful than their difference, I have shown that this result was predictable from error theory.

FRANCIS T. WORRELL

Lowell Technological Institute Research Foundation,
Lowell, Massachusetts.

METALLURGY

Mechanism of Oxidation of Chromium Diffusion Coatings on Iron

IN recent years, we have carried out detailed investigations of the effects of time, temperature and carbon content of iron on the composition, hardness and metallographic structure of chromium diffusion coatings on iron and plain carbon steels¹⁻³. The interdiffusion process has been investigated and the values of the interdiffusion coefficients obtained. The mechanism of chromizing using a chromium-cryolite medium has been determined⁴ and the effects of high-frequency heating during chromizing have been examined⁵. This communication summarizes recent work on the mechanism of breakdown of chromized iron during oxidation in dry oxygen at temperatures in the range 700°–1,000° C.

A detailed investigation of the oxidation of chromized iron has been carried out using thermogravimetric, metallographic and electron-probe microanalyser techniques to establish the kinetics of the process, the redistribution of iron and chromium within the metal, and to provide detailed information concerning oxide film morphology. The chromized coatings studied were 17 and 25 μ thick and contained 2.08 and 4.85 mg cm⁻² of chromium respectively. Iron specimens for analysis were supplied by the British Iron and Steel Research Association and the coatings were prepared by Metal Diffusions, Ltd., Peterborough.

Overall, the kinetics of oxidation at all temperatures can be represented schematically as shown in Fig. 1. There were three clear stages of oxidation. The first stage AB was parabolic and this was followed by the second stage BC in which the rate of oxidation was much less than that required by the parabolic relationship. Finally, breakaway

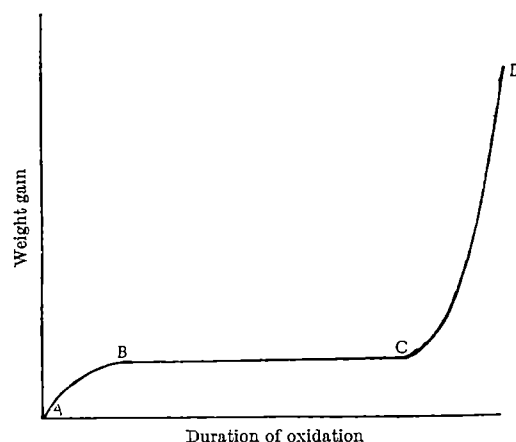


Fig. 1. Schematic curve for the oxidation of chromized iron

Table 1. SPECIMEN LIFE AS A FUNCTION OF TEMPERATURE AND COATING THICKNESS

Oxidation temperature °C	Specimen life (h)	
	17 μ thick coating	25 μ thick coating
< 850	> 400	—
850	191	—
920	84	245
960	18	110
1,000	6.5	24

oxidation occurred and there was a rapid rise in the rate of oxidation *CD*. The useful life of the specimen corresponds to the total period *AC*. The variation of specimen life with temperature and coating thickness is shown in Table 1. It is clear that the specimen life decreased markedly with increasing temperature and that increasing the coating thickness increased coating life by a factor of about four.

At temperatures < 850° C the final breakaway stage was not observed. Chromium sesquioxide was the only phase detected in the oxide scales during exposures of up to 400 h duration at such temperatures, and the amount of iron in solid solution in the Cr_2O_3 was never greater than 3 per cent. This was true despite the fact that the chromium in the surface layer of the coating was depleted to 2 per cent (Fig. 2). At temperatures > 850° C, when the chromium was depleted to between 13 and 20 per cent in the surface layer of the coating, a thin layer of oxide containing 50–60 per cent iron and < 15 per cent chromium was formed between the metal and the Cr_2O_3 layer. Many of these regions of iron-rich oxide were examined in cross-section using electron probe micro-analysis; but the regions were generally less than 4 μ in thickness and accurate quantitative analyses could not be achieved. The ratio of concentrations of iron:chromium was generally greater than 5:1. It was not, therefore, possible to identify the oxide phase containing 50 per cent iron directly from micro-analysis as both a sesquioxide of iron-containing chromium and an iron-rich spinel are possible. However, samples of this iron-rich oxide were detached and subjected to X-ray analysis. It was found that all the lines obtained were characteristic of either chromic or ferric oxide which have similar lattice parameters. This suggested that the second oxide phase to form at the metal- Cr_2O_3 interface was a sesquioxide rather than a spinel. At later stages in the process of oxidation, when the chromium concentration at the metal surface became less than 13 per cent, chromium spinel oxides were present in the scale. It is of considerable interest to note that, in a number of scales, one or other of the sesquioxide phases was found to be missing close to breakaway. This observation implied that one or other of the phases had disappeared in producing spinels of intermediate composition close to breakaway. During the breakaway stage *CD* the oxide richest in chromium was found to contain as little as 19 per cent chromium in some areas of the scale.

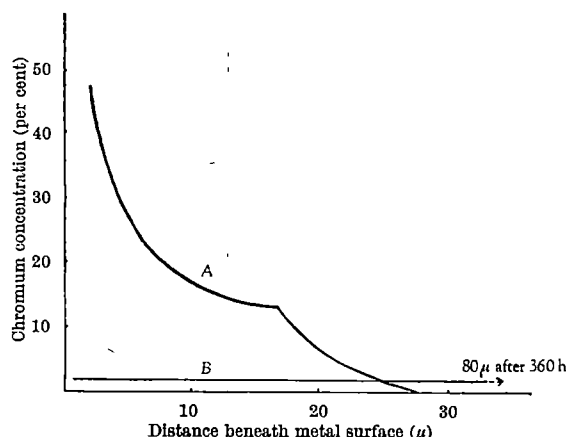


Fig. 2. Secondary diffusion in chromized iron. A, Chromium concentration profile prior to exposure; B, chromium concentration profile after 360 h oxidation at 800° C

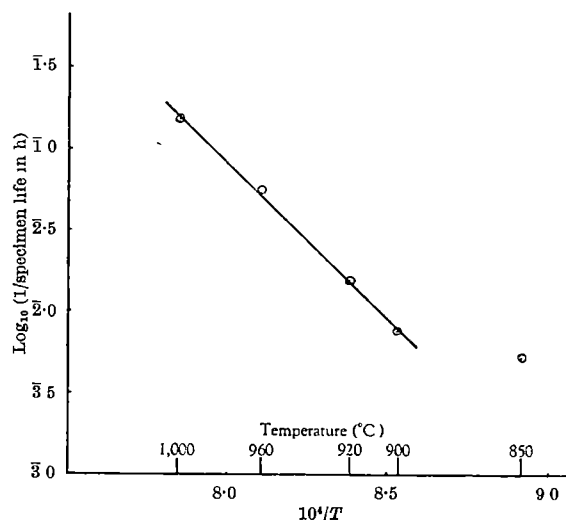


Fig. 3. The relationship between the reciprocals of the specimen life of chromized iron and the temperature of oxidation

In these specimens of chromized iron internal stresses in the oxide layers were largely relieved by creep and the formation of blisters at all temperatures between 700° and 1,000° C. Small amounts of localized cracking of the scale did occur in specimens exposed at temperatures \leq 850° C. This occurred near point B (Fig. 1) at the end of the parabolic period. Nevertheless, a period of significantly reduced oxidation then followed. At 850° C cracking at this stage was at a maximum.

The present work is of considerable interest in view of the controversy with respect to the mechanism of breakdown of protective scales on iron-chromium alloys and stainless steels⁶⁻¹². Some authors favour chemical theories involving sesquioxide-spinel transformations whereas other authors attribute film breakdown to mechanical causes. Our evidence suggests that although there may be some contribution from mechanical factors at the end of the parabolic stage, the formation of spinels is due to interaction between the sesquioxides Fe_2O_3 and Cr_2O_3 . Evidence for this, in addition to the direct evidence from X-ray and electron probe micro-analysis, can also be obtained from the following considerations. If we assume that the overall rate controlling process is diffusion it is possible to consider an overall diffusion coefficient, *D*, for the whole process of oxidation prior to breakaway. It is reasonable to suggest that if the oxidation mechanism does not change significantly over the range of temperature involved, the values of *D* might be inserted in the Arrhenius equation, inasmuch that the value of *D* would increase with increase in temperature. For a given chromium diffusion coating the amount of chromium involved is constant and thus, as *D* increases, the period of oxidation required to produce breakaway would decrease proportionally. Consequently it is also reasonable to replace the values of *D* in the Arrhenius equation by the reciprocal of the oxidation life. It can be seen in Fig. 3 that a good linear relationship is observed between $\log (1/\text{life})$ and the reciprocals of the oxidation temperatures (°K). This suggests that despite this oversimplification the Arrhenius relationship is meaningful, and this is additional evidence in support of an oxidation mechanism based on chemical changes and interdiffusion. If mechanical cracking of the oxide scales were severely to affect the process, then such a relationship would not be expected to hold. Indeed, such cracking of scales was found experimentally to be more noticeable at 850° C and the point corresponding to this temperature does not lie on the straight line in Fig. 3. We therefore take the view that at temperatures greater than 850° C there is conclusive evidence for a chemical breakdown of oxide scales during the oxidation of chromium diffusion coatings on iron.

We thank the British Iron and Steel Research Association for a supply of iron, and Metal Diffusions, Ltd., for preparing the chromized materials. One of us (D. M.) thanks the Ministry of Defence for a research bursary during the tenure of which the work was carried out.

I. A. MENZIES
D. MORTIMER

Corrosion Laboratories,
Department of Chemical Engineering,
Faculty of Technology,
University of Manchester.

- ¹ Menzies, I. A., and Mortimer, D., *Second Intern. Congr. Metallic Corrosion*, New York (1963).
² Menzies, I. A., and Mortimer, D., *Corrosion Sci.*, **5**, 539 (1965).
³ Mortimer, D., M.Sc.Tech. thesis, Univ. Manchester (1962).
⁴ Menzies, I. A., and Mortimer, D., *Proc. Sixth Intern. Congr. Electrodeposition and Metal Finishing*, London (1964); *Trans. Inst. Metal Finishing*, **41**, 15 (1964).
⁵ Menzies, I. A., and Mortimer, D., *Nature*, **196**, 981 (1962).
⁶ Edstrom, J. O., *J. Iron Steel Inst.*, **185**, 450 (1957).
⁷ Rahmel, A., *Z. Electrochem.*, **66**, 363 (1962).
⁸ Yearian, H. J., Derbyshire, W. D., and Radavitch, J. F., *Corrosion*, **13**, 597t (1957).
⁹ Caplan, D., and Cohen, M., *J. Metals*, **4**, 1057 (1952).
¹⁰ Caplan, D., and Cohen, M., *Corrosion*, **15**, 141t (1959).
¹¹ Smeltzer, W. W., *Acta Met.*, **8**, 268 (1960).
¹² Perrow, J. M., and Smeltzer, W. W., *J. Electrochem. Soc.*, **109**, 1023 (1962).

Mechanism of Oxidation of Chromium Diffusion Coatings on Plain Carbon Steels

THE preparation and properties of chromium diffusion coatings on iron and some plain carbon steels have been discussed in detail in earlier publications¹⁻⁴. More recently we have discussed the mechanism of breakdown of chromium diffusion coatings on iron during oxidation at high temperatures⁵. Here it was indicated that the protective oxide films broke down by a chemical diffusion mechanism involving the transformation of iron and chromium sesquioxides to spinels and that extensive chromium depletion occurred in the coatings during oxidation. The present communication summarizes recent work on the mechanism of breakdown of chromized carbon steels during oxidation in dry oxygen at 700°–960° C.

A detailed investigation of the oxidation of chromized plain carbon steels containing 0.35 and 0.78 per cent carbon has been made using thermogravimetric, metallographic and electron-probe micro-analyser techniques to establish the overall pattern of kinetics, the redistribution of iron and chromium, and to provide information concerning oxide film morphology. The chromized coatings studied were 7 and 9 μ thick on the 0.35 and 0.78 per cent carbon steels respectively. The amounts of chromium in the coatings on these two steels were 4.15 and 4.67 mg cm⁻², respectively, to allow some comparison of these materials with chromium diffusion coatings on iron, which contained similar amounts of chromium. The steels used were supplied by the British Iron and Steel Research Association and the diffusion coatings were prepared by Metal Diffusions, Ltd., Peterborough.

Before discussing the results it is worthwhile to point out that the chromized zone on a carbon steel is very much different from that on pure iron. For a given chromium uptake the coating on a carbon steel is thinner and richer in chromium than a coating on pure iron. The surface chromium concentration in the coating may be as high as 80–90 per cent. The maximum hardness of the coatings was about 2,500 Vickers hardness number, and previous work^{1,2} using metallographic and X-ray techniques has shown that these coatings are rich in coarse lenticular carbides, probably (CrFe)₇C₃. This carbide is stable at high temperatures and may be expected to restrict the rate of secondary diffusion of chromium at all temperatures.

The kinetics of oxidation were of the form shown schematically in Fig. 1. As with chromized iron there were three stages of oxidation, but these were less clearly de-

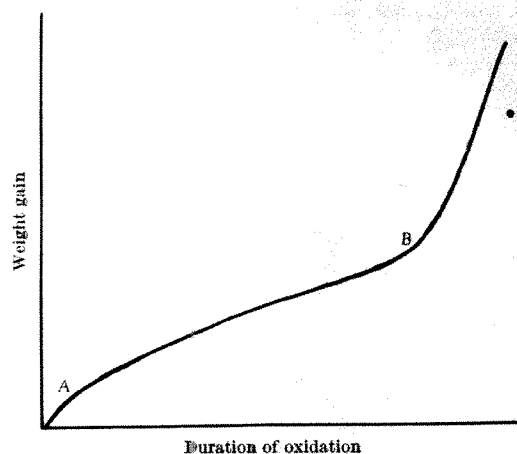


Fig. 1. Schematic kinetic curve for the oxidation of chromized plain carbon steels

fined. The initial stages of oxidation were irregular and weight gains somewhat irreproducible. In some cases slight weight losses were observed. Carbon dioxide was evolved and some decarburization of the coatings occurred. In general, as with chromized iron, the useful specimen life *AB* in Fig. 1 decreased with increasing temperature and in this case also with increasing carbon content of the steel. This is clear from the results in Table 1, where data for chromized iron containing a similar amount of chromium are also given.

Table 1. SPECIMEN LIFE AS A FUNCTION OF TEMPERATURE AND CARBON CONTENT OF THE STEEL

Oxidation temperature °C	chromized iron	Specimen life (h)	
		chromized 0.35% C steel	chromized 0.78% C steel
900	> 400	52	40
930	245	40	20
960	110	22	11

The shortening of the useful life with increasing carbon content of the steel is the most noteworthy feature. Determination of chromium concentration profiles across the chromized coatings after extensive periods of oxidation (compare Fig. 2) indicated that relatively little secondary diffusion had occurred with these materials. The chromium content of the surface layers of the coatings was always greater than 50 per cent, even immediately prior to complete breakdown of the coatings. Secondary diffusion of chromium or excessive depletion of chromium from the diffusion coating was evidently not the cause of oxidative breakdown. Indeed, the coating thickness tended to increase slightly with increasing time of oxidation.

The explanation for breakdown of the coatings was determined by visual and microscopical examination of the coatings and oxide films. Quantitative confirmation was obtained by examination of the coatings and the general features of the specimens after oxidation with the electron-probe micro-analyser. In the early stages of oxidation, Cr₂O₃ films were formed. The surface of the chromized zone at the metal-oxide interface was found to be severely roughened. The evidence available suggests that preferential oxidation of the ferrite occurred, leaving carbon-rich material in the interface. The oxide film was 'pegged-in' at such points and was extremely adherent. As oxidation continued, failure occurred at the rims of the cylindrical specimens (Fig. 3). At this stage cavities were also formed at the core-coating interface. Since the oxide films were in compression, it is believed that tensile stresses were transmitted to the carbide-embrittled chromized zone which cracked at particular points of weakness, for example, specimen rims. The evidence available suggests that cracks in the coatings were in some places transmitted to the Cr₂O₃ film, which in turn cracked, thus allowing gas access to the base of the chromized zone. Electron probe micro-analyses confirmed that oxides

rich in iron formed first underneath the chromized zone, and shortly afterwards nodular growths appeared above this zone on the outer surface of the specimens. As oxidation proceeded, the number and size of such nodules increased. The outermost part of the smaller nodules consisted of chromium-rich spinel whereas the core of the nodule between the base and the outer zone consisted of iron-rich spinel. The spinels are believed to be formed by local interaction between the original chromium-rich oxides and iron oxides growing from the base of the crack. Near to breakaway, cracking of the chromized zone and cavitation at its base were extensive (Fig. 4) and nodules contained large amounts of pure iron oxides.

Samuel and Hoar⁶ suggested that the high initial chromium concentration in the coating together with the retarding effect of the carbide on secondary diffusion was responsible for the relatively low rate of oxidation of a 1.0 per cent carbon steel. The performance of a 0.4 per cent carbon steel was, however, relatively poor; this was attributed to the fracture of the chromized layer due to stresses arising from the $\alpha \rightleftharpoons \gamma$ phase changes in the core on thermal cycling. This explanation seemed unlikely to us since we have observed cracking in specimens exposed at 700° C, that is below the $\alpha \rightleftharpoons \gamma$ transition temperature. It was thought, however, that stresses might arise from differing thermal expansion characteristics of the chromized zone and the carbon steel core. Such an explanation would not, of course, depend on oxidation but merely on thermal treatment. We have found that specimens of both steels heated in argon and subjected to rapid cooling developed some degree of cavitation at the core-coating interface but no cracking was found to occur.

It is clear from the present work that the mechanism of breakaway oxidation in carbon steels is distinctly

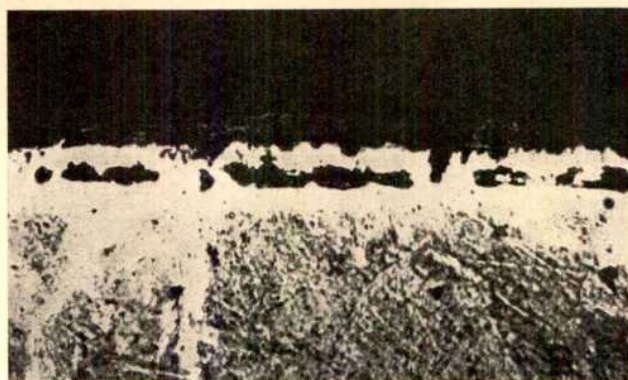


Fig. 4. Cavitation on a specimen of chromized 0.78 per cent carbon steel oxidized almost to breakaway after 11-13 h at 960° C ($\times 400$)

mechanical in origin. Breakaway is associated with pitting, cracking and basal cavitation of the coatings, which is followed by localized breakdown of the Cr_2O_3 oxide films. Nodular growths first appear in regions of potentially high stress, that is, specimen rims, and finally over the entire specimen surfaces until breakdown of oxidation resistance is complete.

We thank the British Iron and Steel Research Association for a supply of carbon steels and Metal Diffusions, Ltd., Peterborough, for preparation of the diffusion coatings. One of us (D. M.) thanks the Ministry of Defence for the award of a research bursary during the tenure of which the work was carried out.

I. A. MENZIES
D. MORTIMER

Corrosion Laboratories,
Department of Chemical Engineering,
Faculty of Technology,
University of Manchester.

¹ Menzies, I. A., and Mortimer, D., *Second Intern. Congr. Metallic Corrosion*, New York (1963).

² Menzies, I. A., and Mortimer, D., *Corrosion Sci.*, **5**, 539 (1965).

³ Menzies, I. A., and Mortimer, D., *Proc. Sixth Intern. Congr. Electrodeposition and Metal Finishing*, London (1964); *Trans. Inst. Metal Finishing*, **41**, 15 (1964).

⁴ Menzies, I. A., and Mortimer, D., *Nature*, **196**, 981 (1962).

⁵ Menzies, I. A., and Mortimer, D., *Nature* (preceding communication).

⁶ Samuel, R. L., and Hoar, T. P., *Metallurgia (Manchr.)*, **60**, 75 (1959).

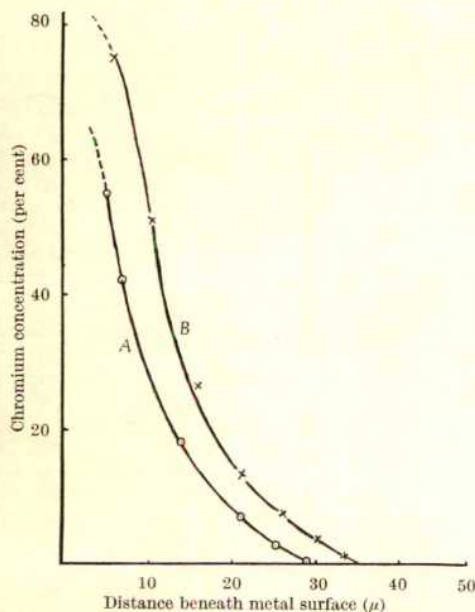


Fig. 2. Chromium concentration profiles in oxidized carbon steels. A, 0.35 per cent carbon steel oxidized 400 h at 700° C; B, 0.68 per cent carbon steel oxidized 400 h at 700° C

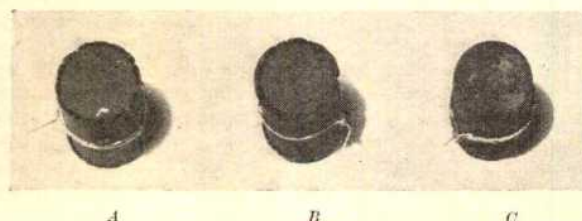


Fig. 3. Cylindrical specimens of chromized materials oxidized for 260 h at 800° C ($\times 2$). A, 0.78 per cent carbon steel; B, 0.35 per cent carbon steel; C, iron

GEOLOGY

Organic Matter in Recent and Ancient Limestones and its Role in their Diagenesis

Hamilton and Greenfield, in their recent investigation of the organic matter associated with the calcareous sediments of Biscayne Bay, Florida, concluded from the anomalous uptake of free amino-acids, and from the difficulties encountered in washing the sediment free of ninhydrin-positive substances, that some other organic compound must be entrapped within the particles of the sediment¹. We are interested in the possibility that the organic substances postulated by Hamilton and Greenfield may be the same material as that which occurs in the Recent carbonate sediments of the Trucial Coast of the Persian Gulf, which is tentatively assumed to be a mucilaginous secretion of blue-green algae.

In all samples which have been examined from the shallow waters along the Trucial Coast, the carbonate sand grains are intimately associated with a transparent mucilaginous substance. This mucilage can easily be seen under a stereoscopic microscope when the carbonate is slowly dissolved with very dilute hydrochloric acid. As the carbonate grains dissolve, their ghosts remain outlined in transparent mucilage which occurs as a film over the surfaces of all grains, including skeletal debris, pellets,

grapestones and oolites. It lines internal surfaces, such as the chamber walls of small gasteropods and the tests of Foraminifera, and pervades the microcrystalline accretionary grains. Small colonies of single-celled blue-green algae are scattered in the mucilage, and minute thread-like tubes of mucilage extend from the algae into the carbonate of the sand grains. The close association between the mucilage and the algae suggests that it is largely a secretion of the algae. A similar mucilaginous substance also occurs in the Recent carbonate sediments of the Bahama Banks^{2,3}.

This mucilage appears to be a very stable material because it can be demonstrated that the mucilage, or a derivative of it, is also present in ancient limestones. Moreover, it plays an important mechanical part during aragonite diagenesis.

By nature of the prevailing on-shore wind and wave approach along the Trucial Coast, the Recent carbonate sediments have built out as a prograding coastal plain, which in places extends inland for up to 10 miles. In cores of sediment collected several miles inland, where the sediments must be several thousands of years old, the mucilage is still present and shows precisely the same distribution relative to the grains as it does in the sediments off-shore.

A similar, acid-insoluble, transparent substance can be seen in cores of partially cemented Pleistocene limestones beneath the floor of the Persian Gulf, some 100 miles off-shore from the Trucial Coast. Here it forms thin envelopes around the grains and also ramifies throughout accretionary grains. In some of the cores, at depths of 40 ft. below the present sea-bed, aragonite has been replaced by calcite by a process of solution of the one and precipitation of the other. It can be demonstrated that the acid-insoluble material acted as a persistent framework during this critical stage in the diagenetic evolution of the rocks and thus preserved the outlines of aragonitic skeletal debris and the internal detail of aragonitic accretionary grains. If, as the evidence suggests, the mucilage of the Recent sediments is a secretion of the algae, then these lowly plants must have played a hitherto unsuspected major role in the diagenesis of limestones.

Careful acid treatment of limestones of all ages, back to the Lower Palaeozoic, reveals the presence of an acid-insoluble transparent substance which often includes minute transparent tubes⁴⁻⁶. This substance can be made more obvious by staining with dyes, such as malachite green or methyl blue, when it becomes apparent that its distribution is analogous to that of the mucilage in the Recent carbonates. Most analyses of ancient limestones record a small proportion of acid-insoluble organic matter, usually classed as 'kerogen'. The possibility arises therefore that the kerogen of the ancient limestones may in fact be mainly a derivative of the ancient algal secretions.

D. J. SHEARMAN

PATRICK A. d'E. SKIPWITH

Department of Geology,
Imperial College of Science and Technology,
London, S.W.7.

¹ Hamilton, R. D., and Greenfield, J. L., *Nature*, **207**, 627 (1965).

² Illing, L. V., *Bull. Amer. Assoc. Petr. Geol.*, **38**, 1 (1954).

³ Newell, N. D., Purdy, E. S., and Imbrie, J., *J. Geol.*, **68**, 481 (1960).

⁴ Wethered, E., *Quart. J. Geol. Soc.*, **47**, 550 (1891).

⁵ Dangeard, L., *Bull. Soc. Geol. France*, Fifth Ser. **6**, 237 (1936).

⁶ Nesteroff, W. D., *C. R. Acad. Sci., Paris*, **242**, 1047 (1956).

GEOCHEMISTRY

Abundances of Rare Earth Elements in Eclogitic Rocks and Basaltic Achondrites

SOME similarities between the chemical compositions of eclogites and the basaltic achondrites have been pointed out by Lovering¹. In a recent neutron-activation investigation of uranium and thorium abundances² a remarkable

similarity was found in the distribution of these two elements in six eclogitic inclusions from basic breccia pipes at Delegate, New South Wales, and eight eucritic achondrites³.

Schmitt *et al.*⁴ have analysed one of the eclogitic inclusions from Delegate (sample No. R 117) and four eucritic achondrites (Juvinas, Nuevo Laredo, Pasamonte and Stannern) for fourteen rare earth elements, yttrium and scandium. They interpreted their results by calculating the absolute abundances in each sample relative to lanthanum, then expressed the relative abundance of each element as a fraction of the relative abundance of that element in chondrites. These 'normalized' values were then plotted as a function of trivalent ionic radius. From these diagrams Schmitt *et al.* concluded that the four eucrites had normalized abundances identical to those observed in the chondrites, though the absolute abundances were about twelve times higher. The eclogitic inclusion, R 117, on the other hand, was considered to show fractionation of these elements relative to chondrites.

An inspection of the analytical results indicates that the fractionation in the eclogitic inclusion of the rare earth elements, yttrium and scandium, relative to the chondritic pattern, is not as extreme as Schmitt *et al.* supposed. The apparent fractionation is due to the normalization against lanthanum, which is depleted in this specimen relative to most of the other rare earth elements. If the abundances are normalized to another element, say, samarium, the eclogite pattern becomes quite similar to that of the chondrites, and is virtually indistinguishable from the eucrites.

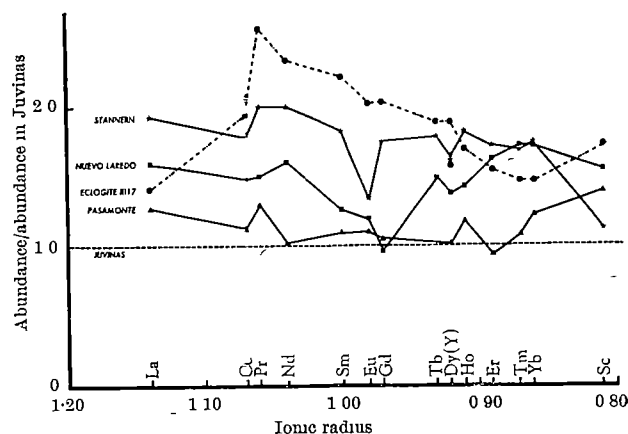


Fig. 1. Ratios of abundances of fourteen rare earth elements, scandium and yttrium in Stannern, Nuevo Laredo and Pasamonte, and R 117 relative to Juvinas, plotted as a function of trivalent ionic radius.

In order to compare the distribution of these elements in the eucrites and the eclogitic inclusion, it is probably safer to deal with absolute abundances, thus obviating the confusion which normalizing to a single element can introduce. To avoid the 'saw tooth' pattern which a plot of absolute abundances would yield, the abundances can then be normalized to the absolute abundances in one of the eucrites. In Fig. 1, values are arbitrarily normalized to the abundances in the eucrite Juvinas, and plotted as a function of trivalent ionic radius in the normal way.

Fig. 1 shows that first there is significant fractionation of the rare earth elements, yttrium and scandium, between the four eucrites. Secondly, the distribution of these elements in the eclogitic inclusion is not greatly different from that in the eucrites, and the differences between R 117 and Nuevo Laredo and Stannern are no greater than those between these two eucrites and Juvinas or Pasamonte.

It is apparent that the similarity between the Australian eclogitic pipe inclusions and the eucrites in their

major element chemistry and uranium and thorium contents can now be tentatively extended to the rare earth elements, yttrium and scandium.

J. W. MORGAN
J. F. LOVERING

Department of Geophysics and Geochemistry,
Institute of Advanced Studies,
Australian National University,
Canberra.

¹ Lovering, J. F., *Trans. Amer. Geophys. Union*, **39**, 947 (1958).

² Morgan, J. W. (unpublished thesis).

³ Lovering, J. F., *J. Geophys. Res.*, **64**, 1073 (1959).

⁴ Schmitt, R. A., Smith, R. H., and Olehy, D. A., *Geochim. Cosmochim. Acta*, **28**, 67 (1964).

CHEMISTRY

Cation Sites in Synthetic Zeolites

SYNTHETIC zeolites¹, especially those with the same structure as natural faujasite, have recently come into prominence as industrial catalysts. Pickert, Rabo, Dempsey, Schomaker and references² have convincingly demonstrated the importance of the positions of the exchangeable cations which compensate for the effective negative charge of aluminium atoms in the aluminosilicate framework. 'Linde' 13X normally has Na⁺ in all the cation positions. Very little attention has been given to the effect of dehydration of the zeolites on the position of the cations, or the position of individual ions when more than one type of cation is present.

We have examined the electron spin resonance spectrum of Mn²⁺ at a concentration of 1 equivalent per cent of exchangeable cations in 'Linde' 13X pre-exchanged with a variety of other ions. The Mn²⁺ ions can occupy different sites; their position depends on which other cations are present and the extent of dehydration. Fig. 1 shows the spectrum of Mn²⁺ in the hexagonal prisms which connect the sodalite cages of the faujasite framework. In these sites the cations are in distorted octahedral co-ordination. This spectrum occurs for hydrated 13X extensively pre-exchanged with Zn²⁺, Mg²⁺ or Li⁺. It is noteworthy that these ions are smaller than Mn²⁺ and have a high charge to radius ratio. They evidently prefer other sites to the hexagonal prisms. The spectra of Mn²⁺ in hydrated 13X pre-exchanged with other ions, (Na⁺), K⁺, Cd²⁺, La³⁺, Ca²⁺ show six peaks but no fine structure (Fig. 2). Pre-exchange with Ca²⁺, which cannot enter the hexagonal prisms or the sodalite cages, causes a little Mn²⁺ to enter the prisms, but most seems to remain in the cages while Na⁺ fills the prisms.

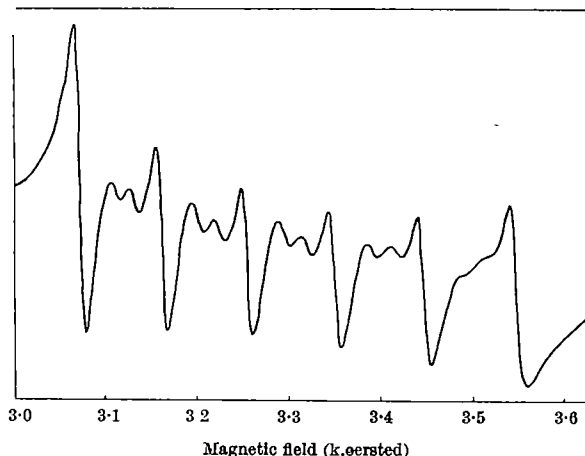


Fig. 1. The first derivative electron spin resonance absorption spectrum of hydrated 13X approximately 72 per cent exchanged with Zn²⁺ ions and 1 per cent Mn²⁺ ions, measured at 3,270 Mc/s

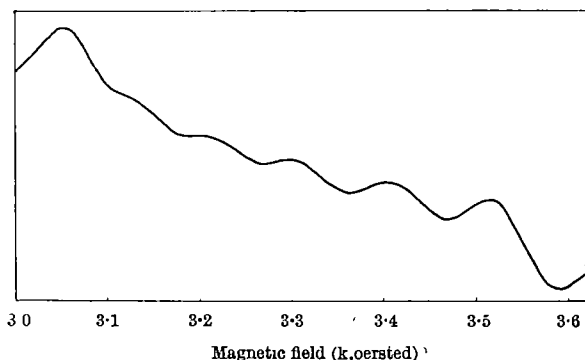


Fig. 2. The electron spin resonance spectrum of 13X treated with sodium acetate/acetic acid buffer, pH 5.5, and 1 per cent exchanged with Mn²⁺ ions, measured at 9,270 Mc/s

During dehydration of 13X exchanged with Zn²⁺ the spectrum of Fig. 1 remains unchanged, indicating that the Mn²⁺ remains in the hexagonal prisms but, in 13X pre-exchanged with Li⁺, Mn²⁺ leaves the prisms. Mg²⁺ gives an intermediate result. On the other hand, when 13X is pre-exchanged with Ca²⁺, Mn²⁺ enters the prisms only after dehydration at 200° C, while further dehydration at 400° C progressively releases the Mn²⁺ probably to the sodalite cages.

In our observations only the transitions between the $+\frac{1}{2}$ and $-\frac{1}{2}$ electron spin levels of Mn²⁺ can be resolved; the basic six line spectrum is due to coupling with the ⁵⁵Mn nucleus, spin $\frac{5}{2}$. The fine structure of Fig. 1 arises from transitions in which the nuclear spin also changes. The fine structure must disappear when the Mn²⁺ or its environment is in rapid motion. Thus Fig. 2 suggests that Mn²⁺ in hydrated 13X exchanged with large ions is at least partly co-ordinated to water. The proof that it is Mn²⁺ in hexagonal prisms which generates the spectrum of Fig. 1 comes both from theoretical examination and from the fact that the spectrum does not change with dehydration. The hexagonal prisms are the only sites not associated with water.

T. I. BARRY
L. A. LAY

Division of Molecular Science,
National Physical Laboratory,
Teddington.

¹ Breck, D. W., *J. Chem. Educ.*, **41**, 878 (1964). Broussard, L., and Shoemaker, D. P., *J. Amer. Chem. Soc.*, **82**, 1041 (1960).

² Pickert, P. E., Rabo, J. A., Dempsey, B., and Schomaker, V., *Proc. Third Intern. Cong. Catalysis*, Amsterdam 1964 (North Holland Publishing Co., Amsterdam).

Quantitative Chromatographic Analysis of Organic Acids by Tritiating Methylation

IN fatty-acid analysis, thin-layer chromatography is a valuable technique for the separation of complex mixtures into specific groups and for separations of *cis* and *trans* isomers and certain positional isomers of unsaturated fatty acids, which cannot easily be achieved by gas-liquid chromatography.

Isotope derivative methods may be a valuable tool for quantitative evaluation of thin-layer chromatograms. Esterification with ¹⁴C-diazomethane¹ has been applied for this purpose, but the preparation of the reagent is rather complicated and expensive. We therefore applied a method according to which samples of organic acids are esterified with inactive diazomethane after exchange with tritiated water. This 'tritiated methylation', developed by Melander² and Verly *et al.*³ for the preparation of labelled methanol, was used before for the analysis of gibberellins by Baumgartner *et al.*⁴

An ethereal solution (0.2 ml.) of tritiated water, containing 6.67 μ c. in 1 μ l. water per ml. dry diethyl ether,

is added to the sample of organic acids which may be dissolved in small amounts of anhydrous diethyl ether, acetone or dioxan. The weight of the sample can amount to as much as 10 mg which corresponds to a threefold molar excess of acids with respect to the tritiated water, the average molecular weight of the acids being 300. For larger samples it is advisable to use greater amounts of tritiated water. After an exchange period of 15 min, which is found to be sufficient, the acids are quantitatively esterified by addition of an excess of an ethereal solution of diazomethane.

Afterwards, the sample is brought on to the thin-layer plate and the chromatogram developed. Spraying with 1 per cent 'Ultraphor' (BASF) in ethanol is applied for silica gel plates and 0.1 per cent 2,7-dichlorofluorescein in ethanol for silicagel plates impregnated with 20 per cent silver nitrate. The amount of spraying agent in the latter case must be as small as possible to avoid quenching in the tritium counting. The components are marked in ultra-violet light, the appropriate zones scraped off and subsequently extracted in a percolator with diethyl ether-hexane 7 : 3 (v/v) for 4 h. The solvent is evaporated and the samples are transferred to vials by rinsing with 10 ml. liquid scintillator, containing 4 g PPO and 0.1 g dimethyl-POPOP per litre toluene, and counted with a Tricarb liquid scintillation counter. If quenching is observed, corrections are made by using the channel ratio method⁵.

The essential point in the procedure is the quantitative extraction from the adsorbent. In all cases shown in Table 1, the amounts of the samples were chosen in such a way that recoveries greater than 95 per cent were ensured. For fatty acids, amounts of at least 1–5 mg of the different components in a mixture are sufficient for this purpose. Smaller amounts of the sample can be analysed by adding inactive methyl esters of the components to the esterified mixture before chromatography is applied, which is an advantage of this method. In this way, satisfactory results could be obtained in the microgram range as well. This was illustrated by analysis of a known mixture of stearic, oleic and linoleic acid with decreasing sample size. After tritiation and esterification of the samples, 10 mg of an equimolar inactive mixture of the esterified acids was added. For samples of 1, 0.1, 0.5 and 0.01 mg, the same composition was found with a standard deviation of the mean of ± 2 per cent. A number of applications of this method are given in Table 1.

Table 1. APPLICATIONS OF THE TRITIATING ESTERIFICATION PROCEDURE*†

No. of sample	Components	Molar equivalent composition (per cent)	
		Known	Found
1	Elaidic acid	49.2	47.2 \pm 1.1
	Oleic acid	50.8	52.8 \pm 1.1
2	9,12- <i>cis,cis</i> Octadecadienoic acid	31.4	32.2 \pm 1.0
	11,15- <i>cis,cis</i> Octadecadienoic acid	68.6	67.8 \pm 1.0
3‡	Monomeric acid	49 \pm 2	49.7 \pm 0.8
	Dimeric acid	22 \pm 1	25.8 \pm 1.7
	Trimeric acid	29 \pm 2	24.6 \pm 0.8

* Medium of tritiation is diethyl ether. Total sample weight is 10 mg. For thin-layer chromatography 'Silicagel G' (Macherey Nagel) was used for sample 3 and 'Silicagel G'-20 per cent AgNO₃ for samples 1 and 2.

† Eluents: benzene-light petroleum 7 : 3 (v/v) for sample 1, benzene-5 per cent diethyl ether for sample 2; iso octane-diethyl ether 7 : 3 (v/v) for sample 3.

‡ This sample was a commercial mixture of polymerized C₁₈-acids of known composition.

The total yield of incorporation of tritium per sample was small and variable (1–7 per cent of the total activity of the tritiated water). This is not due to an incomplete reaction, since no significant difference in the incorporated tritium activity was found when the time of the tritium exchange of stearic acid was varied from 2 to 30 min. Moreover, no apparent effect of the medium on the efficiency of tritium incorporation was found. The variations in the total incorporated activity limit the direct applicability of the method to relative figures for the composition of a given sample. When absolute information is required, a known amount of an internal standard

has to be added before tritiation is applied. The reproducibility of this method has been found to be within 5 per cent.

The analytical applicability of the method, which is demonstrated by the data of Table 1, is due to non-discriminating incorporation of tritium in the acids. On studying a number of test mixtures, it appeared that the applicability is not limited to acids with comparable dissociation constants, but that much stronger acids like dicarboxylic acids, tartaric acid, benzoic acid and phthalic acid can be directly compared with stearic acid (Table 2). On the other hand, there is an indication that phenolic compounds can also be treated in the same way. The reason for this unexpected finding may be that not only exchange of the acids with tritiated water occurs, as was supposed by Melander and Värly^{2,3}, but that also a direct acid catalysed exchange of tritium between water and diazomethane takes place, as is suggested by the experiments of Van der Merwe *et al.*⁶.

Table 2. EXAMPLES OF THE APPLICABILITY OF THE TRITIATION PROCEDURE*

No. of sample	Components	Molar equivalent composition (per cent)	
		Known	Found
1	Stearic acid	12.4	12.1
	1,12-Dodecane dicarboxylic acid	87.6	87.9
2	Stearic acid	27.0	28.3
	12-Ketostearic acid	51.0	50.8
3	12-Hydroxystearic acid	22.0	20.9
	Stearic acid	15.7	14.3
4	Benzoic acid	35.8	33.8
	Phthalic acid	48.5	51.9
	Stearic acid	21.0	21.3
	dl-Tartaric acid	79.0	78.7

* Medium of tritiation is diethyl ether or acetone. Total sample weight is 10 mg.

For thin-layer chromatography 'Silicagel G' (Macherey Nagel) was used. Eluents: iso octane-diethyl ether 6 : 4 (v/v) for samples 1, 2 and 3. Iso octane-diethyl ether 8 : 2 (v/v) for sample 4.

Special precautions had to be taken in the isolation of the more polar compounds. For quantitative extraction of *dl*-tartaric acid dimethylester, 50 mg inactive ester was added before chromatography was applied. In the case of benzoic and phthalic acid, direct counting of suspended silicagel⁷ was applied to avoid incomplete extraction. The counting efficiency, however, was low (about 3 per cent). Reliable results were obtained by adding extra silicagel to a constant weight for all components. Suspension counting cannot be applied to silicagel-AgNO₃ due to severe quenching by silver nitrate. The reliability of the tritiation procedure for a mixture of benzoic and stearic acid was also checked by tritiating known amounts of the ¹⁴C-labelled acids. After esterification, chromatographic separation and extraction, the ³H- and ¹⁴C-activities of the doubly labelled products were determined with a dual channel Tricarb liquid scintillation counter. The ³H/¹⁴C-ratios for equal molar specific ¹⁴C-activities of the two acids were the same within the experimental error of about 10 per cent.

We consider this method to be a valuable tool in the quantitative chromatographic analysis of many types of acidic organic compounds.

G. K. KOCH
[The late] G. JURRIENS

Unilever Research Laboratory,
Mercatorweg 2,
Vlaardingen,
The Netherlands.

¹ Mangold, H. K., *J. Amer. Oil Chemists' Soc.*, **38**, 708 (1961); *Fette, Seifen, Anstrichmittel*, **61**, 877 (1959).

² Melander, L., *Arkiv Kem.*, **3**, 525 (1951).

³ Verly, W. G., Rachele, J. R., du Vigneaud, V., Eidinof, M. L., and Knoll, J. E., *J. Amer. Chem. Soc.*, **74**, 5941 (1952).

⁴ Baumgartner, W. E., Lazer, L., and Dalziel, A., *Adv. Tracer Methodology*, **1**, 257 (Plenum Press, New York, 1963).

⁵ Ballie, L. A., *Intern. J. exp. Rad. and Isotopes*, **8**, 1 (1960).

⁶ Van der Merwe, K. J., Steyn, P. S., and Eggers, S. H., *Tet. Letters*, **52**, 3923 (1964).

⁷ Snyder, F., and Stephens, N., *Anal. Biochem.*, **4**, 128 (1962).

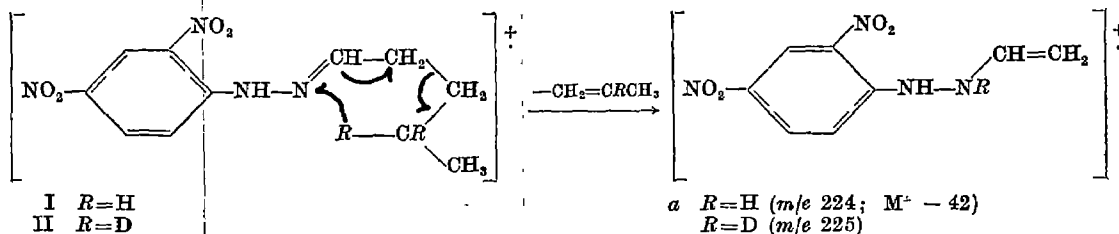
Mass Spectrometric Fragmentation of Nitrophenylhydrazones

In a recent communication¹ Kleipool and Heins reported the principal mass spectral features of 2,4-dinitrophenylhydrazones (DNP) of aliphatic aldehydes and ketones. Independent work in our laboratory² utilizing the identical instrument ('Atlas CH-4') confirmed their results. Particularly noteworthy are the intense molecular ion peaks, which should lend themselves to the convenient characterization of the parent carbonyl compound especially when combined with the recently described³ "in situ regeneration" technique.

Table 1. PERCENTAGE OF TOTAL IONIZATION ($\Sigma_{140}^{M^+}$) OF SELECTED FRAGMENTS OF VALERALDEHYDE NITROPHENYLHYDRAZONES

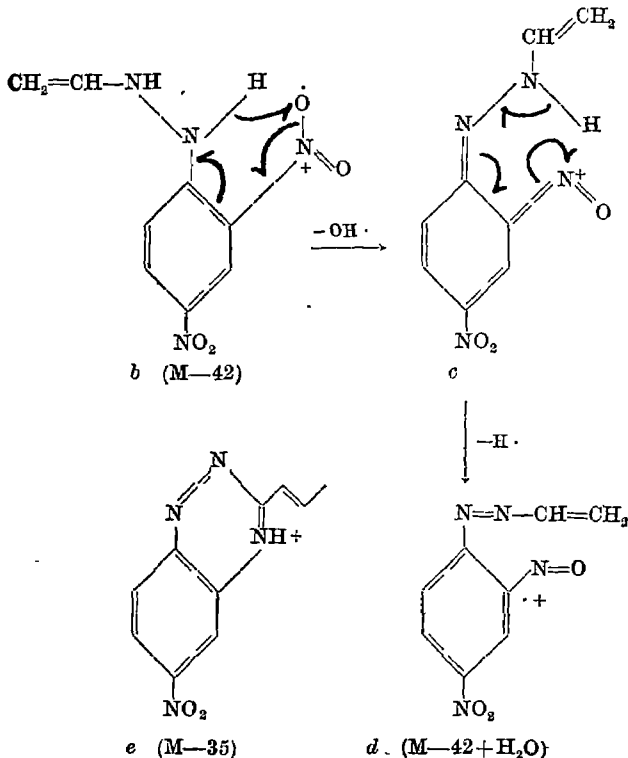
Valeraldehyde nitrophenylhydrazones	M^+	$M^+ - OH$	$M^+ - H_2O$	$M^+ - 35$	$M^+ - 42$	$M^+ - 42 + H_2O$
2,4-dinitro	19.8	—	—	0.8	7.2	19.0
<i>o</i> -nitro	6.65	1.75	12.6	35.0	3.15	2.80
<i>p</i> -nitro	32.0	—	—	—	28.16	0.96

The present report records certain experimental observations, which in part lead to a revision of the mechanistic conclusions of the Dutch workers¹ and in part shed additional light on others. Kleipool and Heins¹ noted that valeraldehyde (I) and higher aldehyde DNP derivatives display an intense peak at m/e 224 which they attribute (correctly) to β -fission of the alkyl chain with accompanying hydrogen rearrangement. Since butyraldehyde DNP does not exhibit an m/e 224 peak, they assume that a hydrogen atom in the δ -position is required which is transferred to the *p*-nitro group bearing the positive charge. In the light of the large body of information that is now available⁴ about hydrogen rearrangements this explanation seemed to us improbable. In fact, when 4,4-*d*₂-pentanol⁵ was oxidized by Barton's procedure⁶ to the aldehyde and then transformed into the deuterium-labelled DNP (II), a quantitative shift of the m/e 224 peak to m/e 225 was observed. The mechanism of this fragmentation can therefore be depicted in terms of I \rightarrow a and proceeds by the well-known⁴ rearrangement of the γ - rather than δ -hydrogen atom analogous to the McLafferty rearrangement of ketones. In contrast to the latter, hydrogen from a primary carbon cannot be abstracted in this rearrangement of DNP derivatives and thus explains the failure¹ to observe an m/e 224 peak in the spectrum of butyraldehyde DNP.



Kleipool and Heins¹ noted that the m/e 224 peak is accompanied by an even more intense one at m/e 206 which they ascribe to the further loss of water. Since aromatic nitro compounds are known⁷ to lose NO, the m/e 206 peak could also partly or totally correspond to $M^+ - 2NO$. That this process does not occur has now been demonstrated by high-resolution measurements with an A.E.I. MS-9 mass spectrometer. Kleipool and Heins¹ suggest that the aldehyde hydrogen and the *ortho* nitro group are required for this process to occur, since aldehyde *p*-nitrophenylhydrazones and ketone DNP derivatives do not exhibit this loss of water. Mechanistically, we depict this sequence in terms of b \rightarrow c \rightarrow d. This is supported further by the data for valeraldehyde DNP and its *o*- and *p*-nitrophenylhydrazones summarized in Table 1 in terms of the percentage of total ionization. It will be noted that

the sum of the $M^+ - 42$ and $M^+ - 42 + H_2O$ columns is very similar for the *p*-nitro and 2,4-dinitro derivatives, but very much lower in the *o*-nitro analogue. Evidently, this is due to a competing reaction—the extensive loss of 35 mass units, which we have shown by high-resolution mass measurements to correspond to the combined expulsion of the elements of OH and H_2O . In fact, the mass spectrum of valeraldehyde *o*-nitrophenylhydrazone also displays (see Table 1) peaks corresponding to $M^+ - OH$ and $M^+ - H_2O$. The driving force for the combined loss of OH and H_2O is



probably the great stability of the resulting ion which may be represented by e.

We thank the National Science Foundation for a predoctorate fellowship (to S. D. S.), the National Aeronautics and Space Agency for funds (grant NsG 81-60) for

the purchase of the 'Atlas CH-4' mass spectrometer, and Dr. A. M. Duffield and Dr. D. Becher for mass spectral measurements.

CARL DJERASSI
SHELIA D. SAMPLE

Department of Chemistry,
Stanford University,
Stanford, California.

¹ Kleipool, R. J. C., and Heins, J. T., *Nature*, **203**, 1280 (1964).

² Beugelmans, R. (unpublished results).

³ Mason, M. E., Johnson, B., and Hamming, M. C., *Anal. Chem.*, **37**, 761 (1965).

⁴ Budzikiewicz, H., Djerassi, C., and Williams, D. H., *Interpretation of Mass Spectra of Organic Compounds* (Holden-Day, Inc., San Francisco, 1964).

⁵ Duffield, A. M., Beugelmans, R., Budzikiewicz, H., Lightner, D. A., Williams, D. H., and Djerassi, C., *J. Amer. Chem. Soc.*, **87**, 805 (1965).

⁶ Barton, D. H. R., Garner, B. J., and Wightman, R. H., *J. Chem. Soc.*, 1855 (1964).

Electronic Absorption Spectrum of the $\text{CH}_3\text{O}\cdot$ -radical

In flash photolysis experiments involving methyl nitrite, transient absorption bands at 1999, 2036 and 2064 Å were seen¹ faintly at 50 μsec, increasing in intensity slowly to a maximum at 2.5 msec and almost disappearing by 25 msec. These bands did not appear for any other nitrite tested. The reasons¹ for these bands being attributed to $\text{CH}_3\text{O}\cdot$ (being produced originally in a metastable excited state) were that this free radical, together with $\text{NO}\cdot$, was expected to result from the alkyl nitrites which are in fact accepted sources of $\text{RO}\cdot$ (despite the early investigations² in which the primary photochemical process in CH_3ONO was deduced to be dissociation to the molecules HNO and H_2CO), the observation of the expected partner fragment NO at the smallest delay times (50 μsec), and the virtual elimination of HNO , HCO , and formaldehyde in an excited state as the appropriate carrier.

Because methoxyl is the first alkyl homologue of hydroxyl, a consideration of the known electronic structure and transitions of the latter may be pertinent to a discussion of the possible transitions for $\text{CH}_3\text{O}\cdot$, as the replacement of the hydrogen atom by the methyl group (being completely saturated, with no easily excited electrons) should act only as a small perturbation of the electronic energy-levels resulting from hyperconjugation effects. The ground state of $\cdot\text{OH}$ is $^2\Pi$ and the first transition observed in absorption is to the $^2\Sigma^+$ state near 3100 Å. The transition may be described as one in which the unpaired electron in the $2p\pi$ orbital in the ground state is promoted to the σ^* anti-bonding molecular orbital in the $^2\Sigma^+$ state, consistent with the 20 per cent decrease of the vibrational interval in the upper state. The $^4\Sigma^+$ state in which there is one electron in each of the $2p\sigma$ and two $2p\pi$ orbitals would be the most probable low-lying metastable state. By analogy then, the methoxyl radical would also have an allowed transition to a level about 4 eV above the ground state in which the C—O stretching frequency should be distinctly smaller than the value of 1,050 cm^{-1} common for this vibration in infra-red spectra of methoxyl-containing compounds. This vibration should be readily excited in the upper state, making a progression in this vibrational interval observable, because of the increase in bond-length accompanying the occupancy of the anti-bonding orbital. In fact the observed frequency difference of approximately 800 cm^{-1} is fully consistent with the predicted spectrum, but the electronic transition itself lies at 6.3 eV, far above the corresponding $\cdot\text{OH}$ state. The vibrational interval of 800 cm^{-1} is also common as a bond-stretching frequency of first-row atoms, so that the coincidence with the theoretical prediction may be entirely fortuitous.

The energy absorbed at the maximum of the 2200 Å continuum of methyl nitrite is about 130 kcal/mole, sufficient to leave less than 90 kcal/mole to the methoxyl radical as excess electronic, vibrational and kinetic energy, after the N—O single bond is broken (36 kcal/mole (ref. 3) required) and the nitric oxide molecule may well have a small amount of vibrational excitation in the ground state (5 kcal/mole). Although there might well exist a state of $\text{CH}_3\text{O}\cdot$ similar to the $^4\Sigma^+$ state of $\text{OH}\cdot$ and lying less than 90 kcal/mole or 4 eV above the ground state, there is no reason to believe that the methoxyl radical can be formed in such a state because only a single σ -bond between N and O atoms is broken in the dissociation process. Thus $\text{CH}_3\text{O}\cdot$ would be formed in either a vibrationally or an electronically excited doublet state; if conversion to the metastable state were readily possible by some obscure mechanism, then the reverse reaction would also occur, and the life-time of the metastable state would not be nearly so great as 2.5 msec.

Following the previous assignment of these bands, the deduction was drawn¹ that the radical is reasonably stable under the conditions of temperature and pressure used.

However, published kinetic investigations of methoxyl radicals have demonstrated⁴ that there is only a small activation energy required for abstraction by methoxyl radicals, and further that there is no activation energy for disproportionation between methoxyl radicals to form methanol and formaldehyde. In other words, methoxyl radicals ought to destroy one another very efficiently, at every collision (apart from a possible steric factor). Again the analogy of hydroxyl proves illuminating: in the flash photolysis of water the hydroxyl radical lasts about 200 μsec (ref. 5) in the presence of only water or inert gases; if other hydrogen-containing gases are present the life-time is drastically reduced because of abstraction reactions. In fact this abstracting property of hydroxyl radicals has often been used⁶ to prepare other radicals trapped in low-temperature solutions. Therefore, for methoxyl radicals to last 100 times as long as hydroxyl radicals under similar experimental conditions is exceedingly difficult to reconcile with available experimental evidence.

In summary, both spectroscopic arguments based on the position or the duration of the new bands and kinetic inferences fail to support the assignment of the transient spectrum near 2000 Å (ref. 1) to the methoxyl radical, or indeed to any other small radical. The time duration definitely points to the carrier being a relatively stable molecule with a singlet ground state, HNO and CS being simple instances of this type of chemical entity.

J. F. OGILVIE

Emmanuel College,
Cambridge,
England.

¹ McGrath, W. D., and McGarvey, J. J., *Nature*, **201**, 991 (1964).

² Thompson, H. W., and Purkis, C. H., *Trans. Faraday Soc.*, **32**, 674, 1466 (1936).

³ Cottrell, T. L., *The Strengths of Chemical Bonds*, second ed. (Butterworths, London, 1958).

⁴ Wijnen, M. H. J., *J. Chem. Phys.*, **27**, 710 (1957); **28**, 271 (1958).

⁵ Black, G., and Porter, G., *Proc. Roy. Soc., A*, **266**, 185 (1962).

⁶ Fujimoto, M., and Ingram, D. J. E., *Trans. Farad. Soc.*, **30**, 1304 (1958).

Equation of State of Liquid-vapour Equilibrium with the Same Constants for Both Phases

As is known, the general equation of state of van der Waals, published in the year 1873, as well as its numerous modifications, expresses the relation between pressure, volume and temperature of gases-vapours and liquids qualitatively well, but generally only with a rather rough approximation with the same constants for both phases, especially for the liquid phase. Nevertheless, in view of the average experiences with these equations, several of their results, relatively, could be considered as accurate or satisfactory ones. Recent comparative calculations of Shah and Thodos¹ about the most common fourteen equations of state, based on the critical data of argon and *n*-butane, clearly confirmed anew these circumstances. They have found the formula of Redlich and Kwong² as the relatively best one by stating that "... this simple equation possesses the remarkable ability to represent the gaseous and liquid regions rather accurately"; yet they give no detailed results for the liquid states. Ree³, somewhat earlier, reached a similar conclusion that "... the Berthelot equation of state⁴ gives satisfactory results of liquid and vapour volumes in the vapour-liquid equilibrium for Ar, Kr, Xe, N_2 , O_2 , CO and CH_4 , ...", that is, they examined definitely only the volume values.

It has been recognized for a long time that the best agreements can be reached for states of low up to medium densities, where the volume dependence of pressure is not so abrupt. Incomparably more delicate is the situation at greater densities and especially for the liquid state well below the critical point, where relatively slight volume variations correspond to relatively great pressure changes. Wohl⁵, himself author of a well-known

Table 1. SOME EXPERIMENTAL AND CALCULATED DATA OF LIQUID ARGON, THE LATTER OBTAINED WITH THE REDLICH-KWONG EQUATION. THE CONSTANTS ARE CALCULATED IN CASE A FROM THE CRITICAL STATE ALONE: $a = 1.68797 \times 10^{-12}$, $b = 22.2037 \text{ cm}^3/\text{mole}$; IN CASE B FROM THE 84.15° K AND CRITICAL STATES: $a = 1.72115 \times 10^{-12}$, $b = 22.7510 \text{ cm}^3/\text{mole}$

Temp. (°K)	Experimental volume press. (cm^3/mole) (atm.)	v_{calc} cm ³ /mole calc. with p_{exp}	v_{calc} cm ³ /mole calc. with p_{exp}	p_{calc} atm. calc. with v_{exp}	p_{calc} atm. calc. with v_{exp}	p_{calc} atm. calc. with v_{exp}	p_{calc} atm. calc. with v_{exp}
83.78 Trp.							
84.15	28.067	0.704	27.356	0.975	-109.37	-155.37	0.7039
93.02	29.341	1.800	28.623	0.975	-72.61	-40.34	5.995
107.52	31.831	4.500	31.408	0.987	-17.59	-3.91	28.83
116.820	33.670	10.14	33.875	1.006	+16.80	+1.66	50.66
125.054	35.836	15.668	36.874	1.029	+36.54	+2.33	60.84
136.775	40.476	27.238	43.755	1.081	+52.80	+1.94	65.68
144.445	46.226	37.552	52.678	1.140	+55.24	+1.47	61.67
150.86 crit.	74.560	48.34	74.56	1	+48.46	+1	48.34

equation of state, had already characterized in the year 1914 the difficulties in this respect by writing: "A somewhat exact calculation of the vapour pressure from the volume of the liquid by any general equation of state is, of course, excluded, as far as lower pressure regions are concerned".

A numerical presentation of these difficulties is shown in Table 1, where for liquid argon some experimental and calculated data are given, the latter obtained with the Redlich-Kwong equation which, according to Shah and Thodos, is the relatively best one (see foregoing).

We see that in case A (Table 1) the v_{calc} values obtained with the p_{exp} values, relatively, are really in fairly good agreement with the experimental values, in sharp contrast to the p_{calc} values obtained with the v_{exp} values. For temperatures nearer the triple-point the p_{calc} values are negative and, for example, for 84.15° K the absolute value of p_{calc} is even higher than p_{crit} itself. The p_{calc} values have also a positive maximum toward the critical point and higher than p_{crit} . The p_{calc} values are in part better when the constants of the equation are obtained from two extreme states of the liquid (Table 1, case B), without being satisfactory ones in this case either, and similarly having a positive maximum higher than p_{crit} itself.

For this, and other two-constant equations of similar structure, this result appears to be no surprise, and is for any liquids unavoidable, because the p_{calc} values are simple differences of two different terms (= functions) of generally much higher values than those of p_{exp} itself. For instance, in the case of argon at 84.15° K the two terms are higher than p_{exp} by about four orders of magnitudes, in that of water at 293.15° K by about six orders of magnitudes. It seems to be hardly possible that two such different and relatively simple functions could follow accurately enough the courses necessary for their differences to result in the expected p_{exp} values for broader ranges of liquid states. Thus even the Redlich-Kwong formula, considered as the relatively best simple equation of state, seems to be unsuitable for the representation of the vapour pressure values of liquids. Very probably, it is on such and further grounds (see following) that any more complete and satisfactory functional representation can scarcely be found in the literature for the pressure behaviour of liquids.

These difficulties first seemed to be consequences of the only rather approximate suppositions about the short-range molecular forces, raising hopes of better results by finding more appropriate representations of these forces. Such hopes have now been really fulfilled, although in a form notably different from that expected.

As is well known, the van der Waals equation can also be expressed for the pressure in three terms—the kinetic, attractive and repulsive ones. This structure of the expression, really, could hardly be more logical, clear and simple for the first but complete representation of all possible intermolecular forces. The unification of the

terms of attraction and repulsion into a single attractive term, as, for example, in the van der Waals and Redlich-Kwong equations, seemed to be a certain suppression of the specific course of the repulsive forces and hence to be a certain over-simplification, which really proved to be one cause, although not the only cause, of the serious difficulties of such equations.

These three separated terms could be more conveniently modified in searching for their more appropriate forms to fit better the experimental data, when the equation, originally for one mole, was reduced to that for a single molecule. Simultaneously its three-dimensional parameters v and $b = v_{\text{min}}$ were replaced by the corresponding linear parameters d and d_{min} , respectively, and the expression of the 'free volume' $v - b$ by that of the 'average free intermolecular distance' $d - d_{\text{min}}$, here denoted by λ . In recent theoretical studies the use of molecular and linear parameters is not new when dealing with problems of gases, vapours and liquids.

An extensive numerical examination of this latter type of the equation resulted in numerous cases in real solutions also for broader ranges of the liquid state, even without the presence of λ in the kinetic and attractive terms. Yet all these solutions gave a kinetic constant considerably below its normal value, clearly indicating that the solution depends also on some completely unusual modification of the equation. Really, with the normal value of the gas constant, solutions are possible with the unusual position of the λ variable specifically in the numerator of the kinetic term, in which, in order to obtain more accurate results, an exponential dependence of λ on d is required. A more generally applicable form of the equation of state was thus found as:

$$p = \frac{k}{d^3} \left(\frac{\lambda}{d} \right)^{(a/d)^n} T - \frac{f_2}{d^3} + \frac{f_3}{d^4 \lambda^2} \quad (1)$$

where k is the constant of Boltzmann, while a and n determine the height and somewhat also the course of the exponential λ -dependence. With increasing value of d the equation evidently converges into that of ideal gases.

The figure, in the customary $\log p$ versus $\log v$ representation, gives the experimental curves and some calculated data of such different substances as the strongly dipolar water and the non-dipolar carbon dioxide. The constants of the equation were calculated with the data of such wide temperature intervals of the liquid states as 20°–220° C for water and –50°–0° C for carbon dioxide, respectively (in Fig. 1 marked with \times), their values being those shown in Table 2.

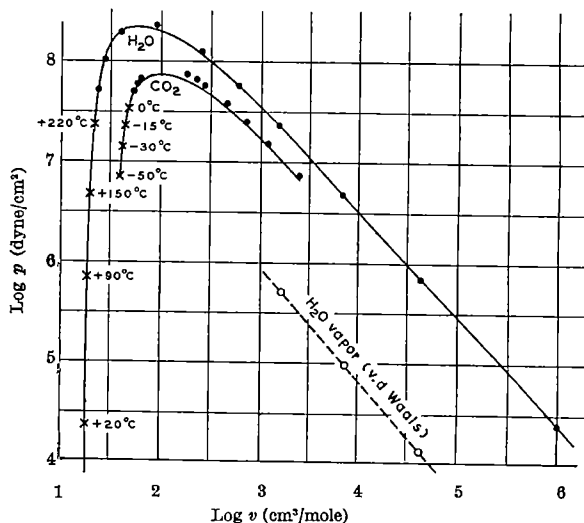


Fig. 1. Experimental curves (—) and some calculated data (●) of liquid H₂O and CO₂ and that of their saturated vapour, the calculated data obtained with the given equation. (×), Basic points of calculation, (○), data of saturated vapour calculated with a van der Waals-type equation by the use of the same basic points of calculation

Table 2. CONSTANTS OF THE GIVEN EQUATION FOR H_2O AND CO_2 . THE CALCULATIONS WERE MADE WITH EIGHT NUMBERS IN ORDER TO OBTAIN SUITABLE RESULTS ALSO FOR LOWER PRESSURES

Const.	H_2O	CO_2
k	$1.3805864 \times 10^{-16}$	$1.3805470 \times 10^{-16}$
f_1	$1.8866444 \times 10^{-37}$	7.744948×10^{-37}
f_2	$5.2299157 \times 10^{-39}$	7.682670×10^{-38}
σ	7.8447519×10^{-3}	6.8015995×10^{-3}
n	0.9	1.4229
d_{min}	2.5087914×10^{-8}	2.5295745×10^{-8}

The values of the constants of Table 2 were calculated by the given form of the equation which, of course, is only an approximate one on several grounds. We will always be forced to use approximate expressions for such complicated processes of short-range molecular forces, the values of the constants being dependent therefore not only on the conceptual approximation of the problem, and even on the selected points of the liquid state used for the calculation, but also on the selected form of simplification.

The position of the points on the experimental curves used for the calculation of the constants (Fig. 1) leaves no doubt that the equation gives good to excellent results for these broad ranges of liquid states. This surprising fact means that the given relatively simple three-term equation, in comparison with the two-constant—two-term and even the more complicated formulae mentioned here, seems to contain some appropriate new step as to equations of state. Also the calculated points of the saturated vapour state, obtained, of course, with the same constants, are favourable or acceptable ones; this is especially so when it is taken into account that this is only the first stage in a new direction. The greatest divergence of the calculated pressure values is about 10 per cent for water and about 20 per cent for carbon dioxide near the critical point, generally rapidly decreasing at greater vapour volumes, and finally scarcely differing from the experimental data. The same equation, evidently, can represent the corresponding volume values of liquids with a considerably increased precision.

The essentially increased applicability of the formula presented here gives the impression that the modifications incorporated could also be of theoretical significance. Improvements and applications of the solution given and the possible or probable theoretical background will be reported separately.

I thank Prof. P. H. Emmett of the Johns Hopkins University, Baltimore, Maryland, for discussions. I also thank the Petroleum Research Fund of the American Chemical Society and the Cabot Co., Boston, for financial support.

ISTVÁN KÜHN*

Department of Chemistry,
The Johns Hopkins University,
Baltimore, Maryland.

* On leave from the Department of Physics of the Engineering Faculty of the Buenos Aires University, Buenos Aires, Argentina.

¹ Shah, K. K., and Thodos, G., *Indust. Eng. Chem.*, **57** (3), 30 (1965).

² Redlich, O., and Kwong, J. N. S., *Chem. Rev.*, **44**, 233 (1949).

³ Ree, F. H., *J. Chem. Phys.*, **36**, 3373 (1962).

⁴ Berthelot, D. J., *J. Phys.*, **8**, 263 (1899).

⁵ Wohl, A., *Z. physik. Chem. (Leipzig)*, **87**, 1 (1914).

Organochlorine Pesticides in the Atmospheric Environment

POLLUTION of the environment has long been a subject of public concern and much attention has been paid recently to the new ecological factors introduced by the wide agricultural usage of pesticidal chemicals. Contamination of soil and crops has often featured in environmental studies of these pesticides, but so far little attention has been given to atmospheric pollution, although, as long ago as 1961, Harris and Lichtenstein¹ showed that the loss by volatilization of aldrin, dieldrin, heptachlor and gamma-benzene hexachloride was a major factor in their disappearance from soils treated with these compounds. Later,

in the United States, the President's Science Advisory Committee drew attention to the fact that inhalation of air contaminated with pesticides might present a hazard to man² and recommended that air should be continuously monitored for pesticide residue levels. In 1965, an Advisory Committee of the United States Food and Drug Administration referred to studies which showed the presence of up to 0.075 p.p.m. of dieldrin in total diets³ and considered the possibility that half as much again could be absorbed from "air or other exposures".

Little practical work seems to have been undertaken to determine pesticide residues in the atmospheric environment, but in the United Kingdom, Wheatley and Hardman⁴ recently reported indications of the presence of organochlorine insecticides in rain-water collected in an agricultural area at Wellesbourne in Warwickshire. Steps were therefore taken at this Laboratory to examine rain-water collected in the Metropolitan area.

Two collecting stations were set up in Central London, one being on the roof of the Laboratory of the Government Chemist in Lambeth, S.E.1, and approximately 20 m above ground-level. The other was set up, with the co-operation of the Meteorological Office, at the London Weather Centre's station on the roof of State House, High Holborn, W.C.1, some 53 m above ground-level and about 1.45 km distant from the other station, across the River Thames. At these two stations, rain was collected in all-glass apparatus, with amber-coloured receiving vessels to reduce the incidence of photochemical degradation⁵. Samples were removed monthly for examination.

The organochlorine pesticides in the water were determined by a gas-liquid chromatographic method using electron-capture detection and silicone and 'Apiezon' columns as described by de Faubert Maunier *et al.*⁶. The limits of detection by this method were 5 parts per million-million for the isomers of benzene hexachloride (BHC) and 10 parts per million-million for the other organochlorine pesticides in general use in the United Kingdom.

The results for the months of February to July 1965, expressed in parts per million-million, are given in Table 1. Because of the small amounts of rain collected and the low levels of the pesticides found, it was not possible to confirm the results obtained for the individual samples by an alternative technique. However, at the end of the 6 months, the combined residues from each station were examined by thin-layer chromatography on silica-gel plates with hexane:acetone, 99:1, as mobile phase.

The appropriate areas of the chromatograms were eluted and the separate eluates individually re-injected on to the gas-liquid chromatograph in columns. In this way, the presence of dieldrin, *pp'*-DDT and the α - and γ -isomers of BHC was confirmed for both stations. The identity of the *pp'*-DDT from both stations was further confirmed by conversion to *pp'*-DDE by treatment with ethanolic potassium hydroxide⁷. The *pp'*-DDE formed by this reaction was identified and determined by gas-liquid chromatography.

Insufficient quantities of the residues were available to confirm the presence of β -BHC, *pp'*-TDE and *pp'*-DDE

Table 1. ORGANOCHLORINE PESTICIDE RESIDUE LEVELS IN LONDON RAIN-WATER (Parts per million-million)

Station—Cornwall House, London, S.E.1

Month 1965	α -BHC	β -BHC	γ -BHC	HEOD (dieldrin)	<i>pp'</i> -DDE	<i>pp'</i> -TDE	<i>pp'</i> -DDT
Feb.	40	90	90	50	70	—	400
Mar.	15	—	80	60	20	—	115
Apr.	30	—	80	50	—	—	300
May	20	65	70	95	—	15	70
June	30	—	55	25	—	—	—
July	25	—	40	10	15	85	85

Station—State House, High Holborn, London, W.C.1

Month 1965	Rain (mm)	α -BHC	β -BHC	γ -BHC	HEOD (dieldrin)	<i>pp'</i> -DDE	<i>pp'</i> -TDE	<i>pp'</i> -DDT
Mar.	33.4	10	—	20	20	—	—	—
Apr.	37.3	20	—	70	70	—	100	140
May	36.6	20	25	55	50	—	—	220
June	36.2	20	—	155	20	—	50	125
July	78.0	30	—	70	15	10	20	80

by alternative techniques or to make an infra-red study of any of the residues⁸.

Some individual rain-water samples which had been taken between September 1964 and January 1965 at a number of other places in the British Isles were also examined. The collecting sites ranged from Stornaway in the Outer Hebrides to Camborne in Cornwall, and the samples showed residues of organochlorine pesticides in amounts similar to those found in London rain-water. Samples of snow collected in different suburban areas within 25 km of London gave similar results. The size of these other samples, and the low levels found, again precluded the application of any confirmatory technique to the individual samples, but by appropriate bulking of the residues it was possible to confirm, by thin-layer chromatography, the presence, in the rain-water and in the snow, of dieldrin, *pp'*-DDT and the α - and γ -isomers of BHC.

The levels at which these pesticides have been detected are extremely low, near to the limits of sensitivity of the delicate electron-capture detection system used. Strict precautions were taken throughout to ensure that no fortuitous contamination of samples or extracts occurred within the Laboratory. Two samples taken from remote areas of Scotland during periods of heavy rainfall showed practically no residue content and these results suggest that adventitious contamination of samples in the Laboratory was negligible.

Present results suggest that the atmosphere carries, either as vapour or by occlusion on dust particles, small amounts of the organochlorine pesticides in common use in the United Kingdom and that they are 'scrubbed out' by rain and snow.

Work is now proceeding to determine the residue levels in air samples, and preliminary results show the presence of organochlorine pesticides in the range of 10–20 parts per million-million. Most of these residues appear to be associated with particulate matter. These experiments may eventually make it possible to determine the respiratory intake by man and to compare the result with his intake from certain foods of known pesticide-levels⁹.

We thank the Director-General of the Meteorological Office for permission to quote from the London Weather Centre's records of the rainfall on the roof of State House.

D. C. ABBOTT
R. B. HARRISON
J. O'G. TATTON
J. THOMSON

Laboratory of the Government Chemist,
Cornwall House, London, S.E.1.

¹ Harris, C. R., and Lichtenstein, E. P., *J. Econ. Entomol.*, **54**, 1038 (1961).

² *Use of Pesticides, Rep. President's Sci. Advis. Comm.* (U.S. Government Printing Office, Washington, 1963).

³ *Rep. Food and Drug Admin. Advis. Comm. appointed to Review Established Tolerances for Residues of Aldrin and Dieldrin on Raw Agricultural Commodities* (U.S. Government Printing Office, Washington, 1965).

⁴ Wheatley, G. A., and Hardman, J. A., *Nature*, **207**, 486 (1965).

⁵ Roburn, J., *Chem. and Ind.*, 1555 (1963).

⁶ de Faubert Maunder, M. J., Egan, H., and Roburn, J., *Analyst*, **89**, 157 (1964).

⁷ Hamence, J. H., Hall, P. S., and Caverly, D. J., *Analyst*, **90**, 649 (1965).

⁸ Abbott, D. C., Crosby, N. T., and Thomson, J., *Proc. Soc. Anal. Chem. Cong.* (in the press).

⁹ *Review of the Persistent Organochlorine Pesticides, Appendix F* (H.M.S.O., London, 1964).

BIOCHEMISTRY

Separation of High-molecular Deoxyribonucleic Acid and Ribonucleic Acid

So far there has been no really satisfactory method for the quantitative separation in high-molecular form of a mixture of DNA and RNA isolated from cells. The procedure based on the different solubility of DNA and RNA in salt solutions of varying concentration^{1–3} is quantitatively unsatisfactory. Another method uses chromatographic separation on methylated albumin^{4–7},

but involves a series of steps which entail the danger of degradation in the recovery of the macromolecule. Specifically, single-stranded DNA cannot be quantitatively recovered without loss of its high-molecular character, because complete elution occurs only at strongly alkaline pH. Nygaard and Hall⁸ have recently described a micro-method for the detection of DNA/RNA hybrids based on their adsorption on membrane filters of nitrocellulose containing material.

On the basis of the selective adsorption of single-stranded DNA on nitrocellulose fibres of a certain nitration degree (the most satisfactory preparation proved to be 'Nitrocell', supplied by the Serva Laboratories, Heidelberg), we have developed a simple method for the quantitative separation, in high-molecular form, of the two components from a nucleic acid mixture. While RNA and double-stranded DNA (dissolved in $2 \times$ standard saline citrate (SSC), pH 6.7) pass through such a column (Fig. 1), single-stranded DNA is quantitatively retained. Elution occurs only with very diluted solutions at pH > 9 (Fig. 2). The fact that the sedimentation constant ($S_{20} = 23.8$) of a single-stranded DNA from bacteriophage *FD* (courtesy of Prof. H. Hoffmann-Berling, Max-Planck Institut für Medizinische Forschung, Heidelberg) remained unchanged ($S_{20} = 22.1$) proves that the molecule size is not altered during the adsorption and elution steps.

For the isolation of the nucleic acids, approximately 6×10^6 HeLa cells, cultivated in Hanks's solution with the addition of lactalbumin hydrolysate and calf serum, were washed and gently disintegrated by repeated freezing and thawing; during the last thawing, dodecylsulphate *tris*-buffered to pH 7 was added (final concentration 0.5 per cent). The resulting viscous solution was diluted and deproteinized in the usual way by treating it twice with phenol⁹. After two-fold precipitation by 67 per cent alcohol, the nucleic acids were dissolved in $0.1 \times$ SSC, dialysed against SSC, and centrifuged for 10 min at

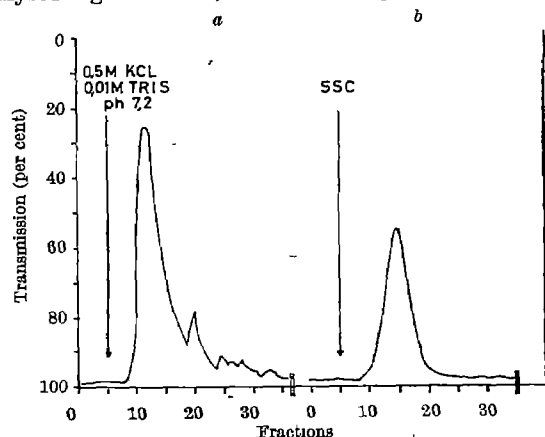


Fig. 1. *a*, Elution diagram of high-molecular RNA from yeast; *b*, that of a double-stranded DNA isolated from thymus. Optical densities were measured with the Uvicord (LKB, Stockholm).

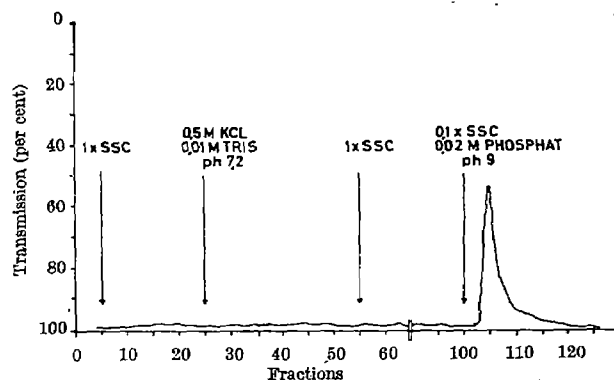


Fig. 2. Elution of a circular form of the single-stranded DNA from phage, dependent on the eluting agent.

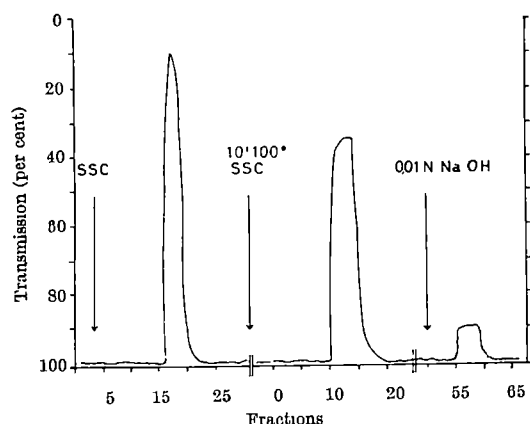


Fig. 3. Elution pattern of a mixture of RNA and DNA isolated from HeLa cells before and after transformation of the double-stranded DNA into the single-stranded form by heating

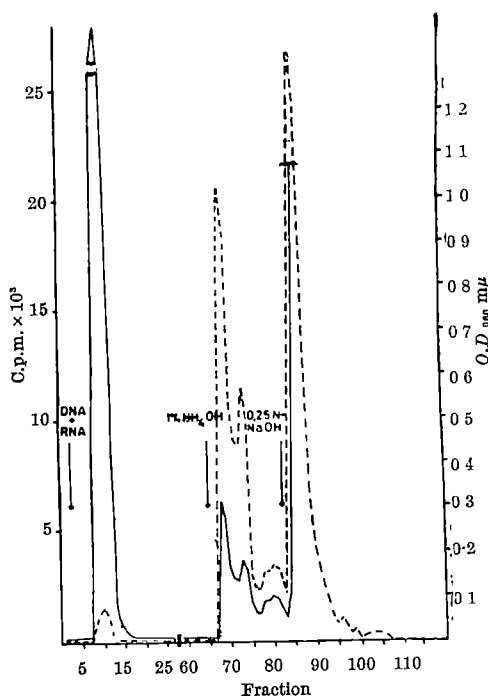


Fig. 4. 443 $O.D._{260}$ of a preheated and rapidly cooled mixture of RNA and DNA isolated from HeLa cells, which had been incubated with 3H -thymidine ($0.5 \mu Ci/ml$ medium) 24 h before collecting, were applied on top of a column of nitrocellulose (1×16 cm) and eluted as indicated in the graph. The total activity amounted to 738×10^3 and 755×10^3 c.p.m., the recoveries were 724.5×10^3 and 751×10^3 c.p.m., of which the RNA fraction contained less than 2 per cent

15,000 r.p.m. to remove any impurities. The yield amounted to 268 $O.D._{260}$. $O.D._{260}/O.D._{280} = 2.03$.

The mixture of the two nucleic acids in SSC was heated to 100° for 10–15 min to render the DNA single-stranded, and cooled immediately by pouring it into $1/3$ volume of $3 \times$ SSC, frozen at -40° . To eliminate any low-molecular fragments, the solution can be filtered through 'Sephadex G 100' and evaporated to a small volume under reduced pressure before it is applied to a firmly packed column of nitrocellulose ($1 \text{ cm} \times 12 \text{ cm}$) which has been previously washed with 0.1 M tris , pH 7, and soaked with $2 \times$ SSC. The presence of orthophosphate ions is detrimental to the adsorption step. Elution of RNA is performed with $2 \times$ SSC, pH 6–7; of the single-stranded DNA with $0.03 \times$ SSC, pH 9–9.5, in some cases with 0.01 M tris pH 11.5 or 0.01 N sodium hydroxide (Fig. 3).

The results obtained with nucleic acids from different sources are summarized in Table 1.

Preliminary experiments seem to indicate that a separation of adsorbed DNA according to molecular size but

Nucleic acid	$O.D._{260}$ applied	$O.D._{260}$ eluted with $2 \times$ SSC pH 6.7	$O.D._{260}$ eluted with $0.1 \times$ SSC pH > 9
DNA (thymus)	22.7	20.1	2.0
DNA (single-stranded, circular) (phage)	8.25	—	7.92*
DNA (native) + DNA (denatured)	33.84 + 19.42	34.9	18.13
DNA (partially renatured) (thymus)	7.69	3.75	3.20
RNA (high-molecular) (yeast)	14.9	14.6	—
RNA + DNA (HeLa)	47.2	48.4	—
RNA + DNA (HeLa) $10' 100^\circ$	54.8†	41.0‡	12.8

* Eluted at pH 11.

† Containing 630 μg DNA.

‡ Containing 34–40 μg DNA (ref. 10).

not to G + C content can be accomplished. The elution pattern of a high-molecular DNA, rendered single-stranded, shows several distinct peaks when the elution is performed step-wise with increasing pH (Fig. 4). Further experiments are in progress.

OLAF KLAMERTH*

Institut für Virusforschung,
Heidelberg, Germany.

* Present address. Institut für Biologie am Reaktorzentrum Seibersdorf, Vienna.

¹ Aldridge, W. G., *Nature*, **187**, 323 (1960), 192, 284 (1962). See below (ref. 10).

² Colter, J. S., Brown, R. A., and Ellem, K. A. O., *Biochim. Biophys. Acta*, **55**, 31 (1962).

³ Jones, A. S., *Nature*, **199**, 283 (1963).

⁴ Mandell, J. D., and Hershey, A. D., *Anal. Biochem.*, **1**, 66 (1960).

⁵ Hershey, A. D., and Burgi, D., *J. Mol. Biol.*, **2**, 143 (1960).

⁶ Suoeka, N., and Cheng, T. Y., *J. Mol. Biol.*, **4**, 161 (1962).

⁷ Ishihama, A., Mizuno, N., Takai, M., Otaka, E., and Osawa, S., *J. Mol. Biol.*, **5**, 251 (1962).

⁸ Nygaard, A. P., and Hall, B. D., *Biochem. Biophys. Res. Comm.*, **12**, 98 (1963).

⁹ Kirby, K. S., *Biochem. J.*, **64**, 405 (1956).

¹⁰ Burton, K., *Biochem. J.*, **62**, 315 (1956).

Optical Rotatory Dispersion and Secondary Structure of Ribonucleic Acid in Mammalian Ribosomes

MACROMOLECULAR single-stranded RNA from viruses and ribosomes is now generally thought to contain short double-helical regions¹ formed when the polynucleotide chain bends back on itself. The existence of such regions was largely inferred from the changes in ultra-violet absorption which occurred on heating solutions of macromolecular RNA. What, however, is the relation between the configuration of macromolecular ribosomal RNA in solution and in the original ribosome? Yeast ribosomes have been reported to have an optical density equal to the sum of the optical densities of the separated RNA and protein². Other evidence has been obtained by measuring the changes in ultra-violet absorption of ribosomes when these are heated (and from X-ray diffraction investigations³). The increases in ultra-violet absorption which were observed were the same⁴ or about two-thirds^{5,6} of those of the isolated ribosomal RNA. Measurement of increases in ultra-violet absorption on heating mixed systems of nucleic acids and proteins is not always very satisfactory on account of turbidity changes (for example, ref. 5) and independent evidence has therefore been sought by measuring the optical rotatory dispersion curves of intact ribosomes and of the RNA and protein isolated from them.

Rabbit reticulocyte ribosomes were prepared and supplied to us by Dr. R. A. Cox of the National Institute for Medical Research, Mill Hill, London. The ribosomes were washed to remove sucrose. The protein/RNA ratio by weight in these ribosomes was 1.17. The protein and RNA were separated by a modification of the method of Cox and Arnstein⁶, which entailed treating the ribosomes with 1.5 M guanidine hydrochloride and precipitating the RNA with ethanol. The guanidine hydrochloride was removed by dialysis. During dialysis a precipitate of insoluble protein separated; this was removed by centrifugation and

optical rotation studies were made on the remaining solution. The quantities of soluble protein and of RNA isolated were 24 and 63 per cent, respectively, of the amounts originally present in the whole ribosomes. The optical rotatory dispersion curves were determined on solutions containing 30 $\mu\text{g/ml}$. RNA or 260 $\mu\text{g/ml}$. protein, using a continuously recording spectropolarimeter (the Polarmatic 62, Bellingham and Stanley, London). Below wave-lengths of 230 $m\mu$ the reproducibility was low because of uncertainties in the base line, coupled with the high value of the Verdet correction factor in this range.

Fig. 1 shows the dispersion curve of the RNA component. The features of this curve are almost identical with those of rat liver RNA⁷. The optical rotatory dispersion curve of the protein component (Fig. 2) was that typical of many proteins and gave a linear Drude plot, which indicated a comparatively low helical content. When the results were plotted according to the Moffitt equation using a mean residue weight of 130, a straight line resulted and the b_0 value at pH 6.9 was 62; this suggests a helical content of only 10 per cent. On increasing the pH to 9.0, b_0 increased to 144, implying a helical content of about 23 per cent. A similar increase in helical content with increasing pH has been observed⁸ with the histone fraction 1b of calf thymus nucleohistone and is the result expected on discharging the basic amino-acid groups.

The dispersion curve of a solution of ribosomes is shown in Fig. 3. No change was observed in the shape of this curve when 10 mM EDTA was present, which implied that its features were independent of dissociation into sub-units. Fig. 3 (crosses) also shows the result of summing

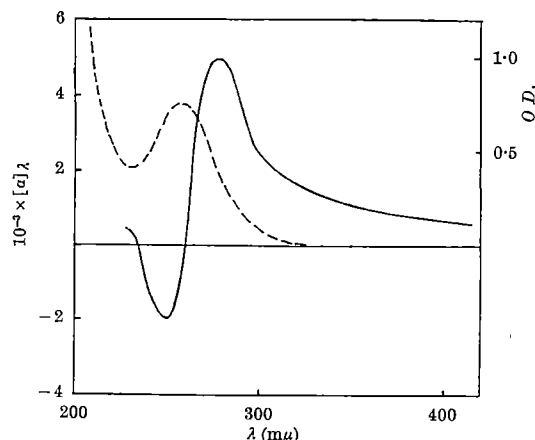


Fig. 1. Optical rotatory dispersion curve of ribosomal RNA (30 $\mu\text{g/ml}$.) in 0.1 M NaCl 10 mM tris HCl pH 7.0 (—), optical density of the same solution (-----).

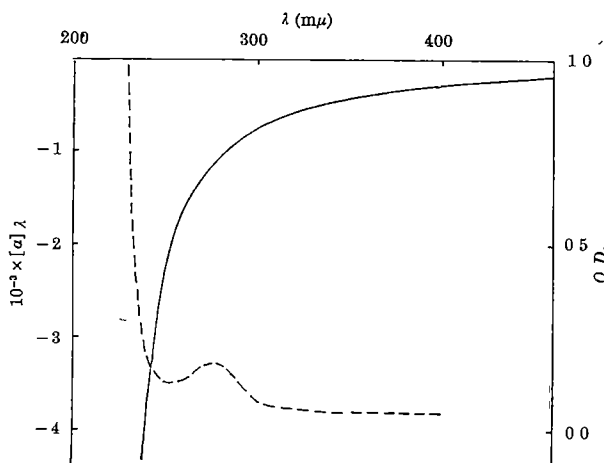


Fig. 2. Optical rotatory dispersion curve of 'soluble' ribosomal protein (260 $\mu\text{g/ml}$.) in 0.1 M NaCl 10 mM tris HCl pH 7.0 (—), optical density of the same solution (-----).

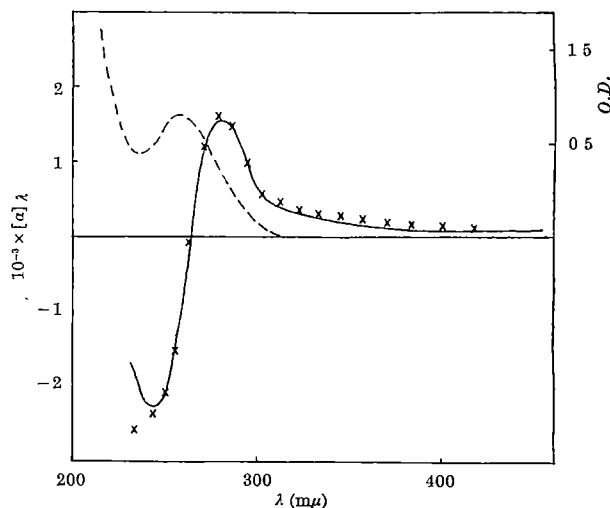


Fig. 3. Optical rotatory dispersion curve of rabbit reticulocyte ribosomes (71 $\mu\text{g/ml}$.) suspended in 0.1 M NaCl, 10 mM tris HCl, 1 mM MgCl_2 , (—), optical density of the same suspension of ribosomes (-----), x represents the summation, at each λ , of $(0.46 [\alpha]_{\lambda, \text{RNA}} + 0.54 [\alpha]_{\lambda, \text{protein}})$ where the $[\alpha]_{\lambda}$'s are for the separated components

the specific rotations of the protein and RNA components on the basis of 46 per cent RNA and 54 per cent protein (that is, $[\alpha]_{\lambda} = 0.46 [\alpha]_{\lambda, \text{RNA}} + 0.54 [\alpha]_{\lambda, \text{protein}}$). Within experimental error, the sum of the specific rotations of the separated components was equal to the specific rotation of the whole ribosomes. The only deviation occurred at the lowest wave-lengths when the measurements on the protein solution were least accurate.

The form of the Cotton effect and the actual rotation displayed by RNA at a given wave-length appears to be closely related to its secondary structure, and we therefore conclude that the secondary structure of RNA within mammalian ribosomes is the same as that which the RNA possesses in free solution. More precisely, if there is any internal conformation within the ribosomes which is not present in the separated RNA and protein, then this conformation does not appreciably affect the optical activity in the wave-length range corresponding to the absorption band of the bases of the RNA.

A. BLAKE

A. R. PEACOCKE

Nuffield Department of Clinical Biochemistry,
University of Oxford.

- ¹ (a) Hall, B. D., and Doty, P., *J. Mol. Biol.*, **1**, 11 (1959). (b) Doty, P., Boettcher, H., Fresco, J. R., Haselkorn, R., and Litt, M., *Proc. U.S. Nat. Acad. Sci.*, **45**, 482 (1959). (c) Doty, P., *Biochem. Soc. Symp.*, No. 21, 8 (1962). (d) Fresco, J. R., Alberts, B. M., and Doty, P., *Nature*, **185**, 98 (1960).
- ² Bonhoeffer, F., and Schachman, H. K., *Biochim. Biophys. Res. Comm.*, **2**, 366 (1960).
- ³ Zubay, G., and Wilkins, M. H. F., *J. Mol. Biol.*, **2**, 105 (1960). Klug, A., Holmes, K. C., and Finch, J. T., *J. Mol. Biol.*, **3**, 87 (1961).
- ⁴ Schlessinger, D., *J. Mol. Biol.*, **2**, 92 (1960).
- ⁵ Lee, M. F., Walker, I. O., and Peacocke, A. R., *Biochem. Biophys. Acta*, **72**, 310 (1963).
- ⁶ Cox, R. A., and Arnstein, H., *Biochem. J.*, **80**, 574 (1963).
- ⁷ Samejima, T., and Yang, J. T., *Biochemistry*, **3**, 613 (1963).
- ⁸ Haydon, A. J., and Peacocke, A. R. (unpublished observations).
- ⁹ Samejima, T., and Yang, J. T., *J. Biol. Chem.*, **240**, 2094 (1965).

Sulphocysteine in the Urine of the Blotched Kenya Genet

THE urinary amino-acid excretion of the blotched Kenya genet was first shown to be unusual by Datta and Harris¹. In a survey of the urinary amino-acid excretion of various animals from the London Zoo, they found that the genet had an exceptionally high excretion of cystine. This was determined by paper chromatographic and polarographic analysis. A large quantity of cysteine acid was formed on oxidation of the urinary amino-acids, and it was reported that the cystine excretion was 1.0–1.5 mg

Table 1. ANALYSIS OF URINE FROM SOME GENETS

Species of <i>Genetta</i>	Vol. urine (ml./24 h)	Creatinine excretion (mg/24 h)	Total thiol after reduction (μ mol/24 h)	Total thiol after reduction of water eluate off 'Dowex 50' (μ mol/24 h)	Automatic amino-acid analysis	
					Sulphocysteine (μ mol/24 h)	1/2 cystine (μ mol/24 h)
(1) <i>genetta neumanii</i> (male)	95	101	317	279	165	46
(2) <i>genetta neumanii</i> (female)	50	92.5	230	200	100	47
	70	182	290	320	139	33
	66	70	420	270	121	48
(3) <i>tigrina</i> (male)	33	105	218	59	54	8
	62*	63*			84*	118*

Urine was reduced in an electrolytic desalter (6 min, 0.6 amp). Free thiol was then estimated by Ellman's reagent (5,5'-dithiobis-(2-nitrobenzoic acid)). Sodium cysteine sulphonate yields one molar equivalent of thiol under these conditions. The urine was passed down a column of 'Dowex 50' (10 \times 0.9 cm), which was then washed with water. The principal ninhydrin-positive materials in this fraction were sulphocysteine and taurine. Analysis was carried out using a Beckman amino-acid analyser. Sodium cysteine sulphonate 1 H₂O gave a colour yield of 61 per cent compared with leucine in this system.

* These values were obtained after allowing the animal to feed *ad libitum* on horse meat and the excretion of several other amino-acids was also increased. Other studies were made on a limited meat diet.

Urine was collected while these animals were in metabolic cages at the National Institutes of Health and was stored at 4° prior to analysis.

Table 2. EFFECT OF DIETARY VARIATIONS ON THE URINARY AMINO-ACIDS OF *Genetta neumanii* (MALE) NO. 4

Diet	Vol. urine (ml./24 h)	Creatinine excretion (mg/24 h)	Total thiol after reduction (μ mol/24 h)	Total thiol after reduction of water eluate off 'Dowex 50' (μ mol/24 h)	Automatic amino-acid analysis	
					Sulphocysteine (μ mol/24 h)	1/2 cystine (μ mol/24 h)
Control period (limited meat diet)	44	125	152	112	34	45
Oral neomycin supplement to the above diet 30 mg/day	23.5	41	204	146	97	17
	24	50	132	130	92	13
Eighty per cent cereal diet	38		255	119	78	53
Fasting for 24 h	7.6	30	18	3.5	7.4	5.3

per ml. This was unusual as it represented a greater solubility than that obtained by dissolving known cystine in a solution containing an equivalent concentration of salts and urea². In cystinuria, a human inherited metabolic disorder, cystine is excreted at this concentration but gives rise to frequent calculus formation, and there was no evidence that the genet ever formed calculi. The other interesting feature was that in human cystinuria there was a failure of tubular reabsorption of lysine, arginine and ornithine as well as cystine, whereas the genet had no disorder of basic amino-acid excretion.

In an attempt to resolve some of the anomalies, we have submitted the urine of the Kenya genet to automatic amino-acid analysis. We have found that the amount of cystine excreted by the genet was not so great as had previously been reported but was greater than that seen in a normal healthy man. However, analysis of the urine by electrolytic reduction followed by estimation of the thiol groups by Ellman's reagent, 5,5'-dithiobis-(2-nitrobenzoic acid) (ref. 3), showed that the amount of thiol liberated was greatly in excess of the amount of cystine found by automatic amino-acid analysis (Table 1).

It was then observed that there was another ninhydrin-positive substance in the urine that was eluted from the ion-exchange column ahead of taurine which had not previously been observed in urinary amino-acid analyses. It was characterized by having a ratio of light absorption at 440 m μ : 570 m μ , which was greater than that found for most aliphatic amino-acids and comparable with that of cystine. After the urine was hydrolysed (6 N hydrochloric acid for 18 h at 100° under nitrogen) this peak disappeared. It was also destroyed by electrolytic reduction. A partial purification was obtained by passing the urine through a cation-exchange resin, and eluting with water. This eluate was shown to contain only the genet substance and taurine as ninhydrin-positive material. The eluate after concentration was oxidized with bromine water followed by acid hydrolysis as before. Automatic amino-acid analysis now showed a new fast-running peak with a low ratio of light absorption at 440 m μ : 570 m μ . This was shown by high-voltage electrophoresis (Whatman 3 MM, 4,000 V, 6.8 per cent formic acid) and thin-layer chromatography (silica-gel G, Merck, solvent CHCl₃, MeOH, 17 per cent NH₃ - 2:2:1) to be cysteic acid. On the basis of these observations it was thought that the new amino-acid excreted by the genet was *S*-sulphocysteine

(cysteine-*S*-sulphonate). This substance was synthesized by the method originally described by Clarke⁴. The unknown substance was found to be identical with *S*-sulphocysteine by thin-layer chromatography and by high-voltage electrophoresis in the systems described here. Using the latter method a small quantity of the unknown substance was isolated and shown to have an infra-red spectrum identical with *S*-sulphocysteine after lyophilization with potassium bromide.

Genets belong to the family of Viverridae, but the classification of genets into individual species is confusing. Setzer⁵ describes two species of genet, *Genetta genetta* and *Genetta tigrina*, though Walker⁶ thinks that as many as six species exist. The species most readily available to us was *Genetta genetta neumanii*, which had been bred for several generations at the Washington Zoo. The urinary amino-acids from three members of this species have been investigated by us (Tables 1 and 2) and sulphocysteine was isolated from the urine of this species by the method already described. The original publication of Datta and Harris¹ referred to the high urinary excretion of cystine in *Genetta tigrina* obtained principally from the London Zoo. We have been able to study the urinary amino-acids from one of this species, which was kindly loaned to us by the Baltimore Zoo. Analysis by all the methods described above showed no significant variation from our findings with *Genetta genetta neumanii* (Table 1).

In order to investigate why the genet species in particular should have such a high sulphocysteine excretion, we measured fasting plasma amino-acid levels in one of the *genetta neumanii* by automatic amino-acid analysis, but no sulphocysteine was detected. This result indicates that the urinary sulphocysteine does not arise as a result of a renal overflow phenomenon. The urinary excretion of sulphocysteine was not significantly altered after supplementation of the genet's diet for one week with neomycin nor after altering his diet from predominantly meat to a diet containing 80 per cent cereal (Table 2). It is, therefore, thought that the sulphocysteine was not absorbed directly from the diet, nor was it the result of intestinal microbial metabolism. It was also observed that a high dietary intake of raw horse meat increased the urinary excretion of cystine and some non-sulphur containing amino-acids. Fasting the animal generally resulted in a reduction of urine output as well as in a reduction of urinary amino-acids and creatinine.

There are only a few reports of the occurrence of sulphocysteine in mammals, though it is thought to be a key intermediate in the sulphur metabolism of *Aspergillus nidulans*⁷. Sulphocysteine has been reported in small quantities in the urine of the rat⁸. Oral administration of sulphocysteine to rats caused an increase of urinary thiosulphate⁹. Of considerable interest is the isolation of *S*-sulphoglutathione from the ox lens¹⁰ and from the rat intestine¹¹. It is not known what significance *S*-sulphoglutathione has in general metabolism, but compounds of this type could be regarded as potential thiols which would not be so vulnerable to oxidation as the parent thiol itself. Biological synthesis of *S*-sulpho compounds has been shown to occur from sulphate in the rat¹¹, presumably by reduction to sulphite which then condenses with thiol groups to form *S*-sulpho compounds¹². In *Aspergillus nidulans*, the probable pathway of synthesis is by the condensation of thiosulphate with serine¹³.

These results indicate that among the genets which we have examined, the urinary cystine concentration did not exceed the solubility of cystine in urine as determined by Dent and Senior² and it is unlikely that cystine calculus formation would occur. Sulphocysteine can be estimated by polarographic analysis¹⁴ and it is possible that the values for cystine excretion by the genet as determined polarographically by Datta and Harris¹ could have appeared higher than those which we now observe by automatic amino-acid analysis because of interference from sulphocysteine and possibly other related substances present in the urine. Finally, it can be seen from Table 1 that the sum of cystine and sulphocysteine excretion is less than the total thiol detected by Ellman's reagent after electrolytic reduction. It remains possible that additional compounds of this type may be present which do not give a colour with the ninhydrin reagent.

We thank the Director of the Washington Zoo for supplying us with *Genetta genetta neumanii* and the Director of the Baltimore Zoo for loan of a *Genetta tigrina* for these investigations. We also thank Dr. Calvin E. Sevey of these Institutes for his care of the animals and Mrs. Katherine Bradley for her skilled technical assistance. This work was carried out while one of us (J. C. C.) was a visiting scientist at the National Institutes of Health, on leave from the Medical Professorial Unit, St. Bartholomew's Hospital, London.

JOHN C. CRAWHALL
STANTON SEGAL

National Institutes of Health,
Bethesda, Maryland.

¹ Datta, S. P., and Harris, H., *Ann. Eugen.*, **18**, 107 (1953).

² Dent, C. E., and Senior, B., *Brit. J. Urol.*, **27**, 317 (1955).

³ Crawhall, J. C., Saunders, E. P., and Thompson, C. J., *First Meet. Fed. Europ. Biochem. Soc.*, Abst. A117 (1964).

⁴ Clarke, H. T., *J. Biol. Chem.*, **97**, 235 (1932).

⁵ Setzer, H. W., *Proc. U.S. Nat. Mus.*, **106**, 447 (1956).

⁶ Walker, E. P., *Mammals of the World* (Johns Hopkins Press, Baltimore, 1964).

⁷ Nakamura, T., and Sato, R., *Nature*, **193**, 481 (1962).

⁸ Cavallini, D., DeMarco, C., Mondovi, B., and Tentori, L., *J. Chromatog.*, **3**, 20 (1960).

⁹ DeMarco, C., Coletta, M., Mondovi, B., and Cavallini, D., *G. Biochim.*, **9**, 1 (1960).

¹⁰ Waley, S. G., *Biochem. J.*, **71**, 132 (1959).

¹¹ Robinson, H. C., and Pasternak, C. A., *Biochem. J.*, **93**, 487 (1964).

¹² Segal, I. H., and Johnson, M. J., *Analyt. Biochem.*, **5**, 330 (1963).

¹³ Nakamura, T., and Sato, R., *Nature*, **185**, 163 (1960).

¹⁴ Kolthoff, I. M., and Stricks, W., *J. Amer. Chem. Soc.*, **73**, 1728 (1951).

Biogenesis of Ethylene in *Penicillium digitatum*

A NUMBER of compounds were compared to glucose as substrates for the biogenesis of ethylene in *Penicillium digitatum*. Several compounds of low molecular weight gave a greater production of ethylene by the organism than did an equimolar amount of glucose. D,L-Serine, L-serine and glycine proved to be better substrates for this purpose, with D,L-serine being the best.

NRRL strain 786 of *Penicillium digitatum* was obtained from the American Type Culture Collection and propagated on potato-dextrose-agar slants containing a small amount of water extract of orange peel. A luxuriant, sporulating mat of the fungus was grown according to the method of Wooster and Cheldelin¹ in a stationary medium. The tissue mat was removed from the medium, washed several times with glass-distilled water, and stored at -5° C under a nitrogen atmosphere. For testing substrates, the mats were thawed, homogenized in a Waring blender, and 15-ml. portions were pipetted into 50 ml. of a modified Wooster and Cheldelin medium containing 1 mmole of the substrate. Because casein hydrolysate, ammonium tartrate and asparagine were excluded from the medium the substrate served as the sole external source of carbon. The flasks were kept in a water-bath shaker for 48-72 h while the atmosphere above the media was flushed and passed through a mercuric perchlorate solution to trap the ethylene produced by the cultures. The ethylene was then released from the perchlorate solution into a suitable analysis system by adding lithium chloride to the ethylene-mercuric perchlorate complex².

Quantitative measurements of the released ethylene were made on a Perkin-Elmer model 154 vapour fractometer equipped with a two-meter activated alumina column 0.25 in. diam. The gas was initially trapped in a 5-ml. external column, packed with silica gel and immersed in a dry ice-acetone bath. The system was then purged with the helium carrier gas to remove excess nitrogen and the temperature of the external trapping column was raised to 40° C before releasing the sample into the fractometer under a pressure of 15 mm mercury. A 'thermistor'-type sensing chamber was used with a sensitivity of 1 mV.

The radioactivity of the ethylene produced from specifically labelled serine, glycine, glyoxylate and betaine was determined with a Nuclear-Chicago model 6000 'Dynacon' electrometer fitted with a 100-ml. ion chamber. The radioactivity of the ethylene produced from D,L-serine-3-¹⁴C, to which various amounts of aminopterin had been added, was determined with a Packard 'Tri-Carb' liquid scintillation counter, model 314 EX. No effort was made to determine the influence of mercuric perchlorate on the phosphorescence of the scintillating liquid, if any, as the object of this experiment was to determine the relative effects of various concentrations of aminopterin on the production of ethylene from D,L-serine-3-¹⁴C by *Penicillium digitatum*.

It can be seen from Table 1 that D,L-serine is a better substrate than glucose for the biogenesis of ethylene in *Penicillium digitatum*. These data are statistically significant at the 0.01 level.

Serine and glycine both occur in the choline cycle of the nitrogen pool. It was therefore desirable to use some specifically labelled compounds from this cycle as substrates for *Penicillium digitatum*. That radioactively labelled ethylene was produced from glyoxylate-1-¹⁴C, glycine-2-¹⁴C, D,L-serine-3-¹⁴C and betaine-methyl-¹⁴C, during a 48-h incubation period indicated that all three carbon atoms of serine can become a component of the ethylene molecule (Table 2). However, the conversion efficiency for the C-2 of glycine and C-1 of glyoxylate is quite low. Indeed, the unexpectedly high conversion efficiency of C-3 of serine indicates that both carbon atoms of ethylene are derived from C-3 of serine, and this would appear to be possible only through one-carbon metabolism.

Table 1. ETHYLENE PRODUCED BY *Penicillium digitatum*, USING D-GLUCOSE AND D,L-SERINE AS SUBSTRATES OVER A 60-H PERIOD OF INCUBATION

Culture No.	Substrate	Ethylene collected (μl.)	*Average
(1)	D-Glucose	9	
(2)	D-Glucose	12	
(3)	D-Glucose	6	
(4)	D,L-Serine	28	
(5)	D,L-Serine	33	
(6)	D,L-Serine	38	
			93

* Significant at 0.01 level.

Table 2. RADIOACTIVITY OF ETHYLENE PRODUCED BY *Penicillium digitatum* FROM 1 MMOLE OF SPECIFICALLY LABELLED SUBSTRATES DURING 48-H INCUBATION

Substrate	Substrate activity (mc./mmole)	¹⁴ C-Ethylene (c.p.m.)	Ethylene yield (μl.)	Ethylene activity (mc./mmole)	Conversion efficiency*
Glyoxylate-1- ¹⁴ C	7.35 × 10 ⁻²	2,710	2.5	0.011	0.151
Betaine-methyl- ¹⁴ C	1.0 × 10 ⁻¹	3,478	5.8	0.006	0.060
Glycine-2- ¹⁴ C	1.0 × 10 ⁻¹	12,537	10.9	0.012	0.116
D,L-Serine-3- ¹⁴ C	1.0 × 10 ⁻¹	23,600	1.2	0.197	1.965

* Ratio of specific activity of ethylene to specific activity of substrate.

Table 3. EFFECT OF AMINOPTERIN ON ETHYLENE PRODUCTION BY *Penicillium digitatum* FROM 1.0 MMOLE D,L-SERINE-3-¹⁴C HAVING A SPECIFIC ACTIVITY OF 4.95 × 10⁻³ MC./MMOLE

Concentration of aminopterine (mM)	¹⁴ C-Ethylene yield (c.p.m.) Experiment 1*	Experiment 2†
40	792	809
20	949	689
10	1,050	805
2	462	794
1	835	576
0	904	601

* Incubation period, 70 h

† Incubation period, 86 h

In support of the reported unusually small volume of ethylene obtained from D,L-serine-3-¹⁴C, the following considerations are offered: the 23,600 c.p.m. in the product ethylene is only 1.06 × 10⁻⁴ of the activity of the substrate serine. If 1 mole of serine is required to produce 1 mole of ethylene, then only 1.06 × 10⁻⁴ mmole of ethylene was produced. The temperature of the fractionating column in the gas fractometer was 40° C and the pressure under which the gas was introduced into the fractometer was 15 mm mercury. However, as there was a pressure drop between the gas inlet and the sensing chamber, a pressure of 10 mm is assumed.

$$V = \frac{nRT}{P} =$$

$$\frac{(1.06 \times 10^{-7} \text{ moles}) (0.0821 \text{ l. atm. deg.}^{-1} \text{ mole}^{-1}) (313 \text{ deg.})}{770/760 \text{ atmospheres}} = 26.8 \times 10^{-7} \text{ l.} = 2.68 \mu\text{l.}$$

Here the specific activity of the ¹⁴C-ethylene would be 8.9 × 10⁻² mc./mmole and the conversion efficiency would be 0.89. Likewise, if we were to assume that both carbon atoms of the ethylene molecule were derived from C-3 of serine, similar calculations would show the yield of ethylene to be 1.34 μl., which would have a specific activity of 0.178 mc./mmole. The conversion efficiency, in this case, would be 1.78 and these values are very close to those reported in Table 2.

Since all the labelled carbon atoms of the compounds used in the foregoing experiments enter transmethylation reactions, it was thought that perhaps the biogenesis of ethylene was related to transmethylation. Attempts were made to corroborate this hypothesis by conducting experiments with aminopterine, which is a specific inhibitor of tetrahydrofolic acid³. Table 3 shows that there was no correlation between the aminopterine concentration and the ethylene produced when D,L-serine-3-¹⁴C was used as the substrate. This would indicate that the tetrahydrofolic acid system does not take part, in the biogenesis of ethylene in this organism.

Concerning the metabolic route to ethylene, the present data support those of Wang and Persyn⁴, who found that the C-1 and C-6 of glucose were equally efficient in leading to the formation of ethylene in *Penicillium digitatum*. This indicates that glucose must be converted to triose phosphates before it is converted to ethylene. It is at this stage of glycolysis that the C-1 and C-6 of glucose are equilibrated through the action of triose phosphate isomerase. Burg and Thimann⁵ at first found ¹⁴C-glucose to be the only compound to yield labelled ethylene, but Burg and Burg^{6,7} have since found that C-2 and C-3 of trioses are incorporated into ethylene in apple tissue. However, Burg and Burg's results⁷ show that C-2 and C-3 of pyruvate, which are equivalent to C-2 and C-3 of serine, are equally effective in forming ethylene in apple tissue. The present work is in agreement with Gibson⁸, who found that C-3 of pyruvate is converted to

ethylene in *Penicillium digitatum* with a much greater efficiency than that of C-2 or C-1. It also supports the earlier contention of Burg and Burg⁶, and of Wang⁴, that triose phosphates are involved in the pathway from glucose to ethylene.

This work was supported in part by the U.S. Atomic Energy Commission grant AT-(40-1)-2456 and by the National Science Foundation grant YB-1889. We thank Mrs. E. W. Carnes for her assistance.

B. A. SPRAYBERRY*
W. C. HALL
C. S. MILLER

Department of Plant Sciences,
Texas A. and M. University,
College Station, Texas.

* Present address: 5002 Grace Avenue, North Olmsted, Ohio.

¹ Wooster, R. C., and Cheldelin, V. H., *Arch. Biochem.*, **8**, 311 (1945).

² Young, R. E., Pratt, H. K., and Blale, J. B., *Anal. Chem.*, **24**, 551 (1952).

³ Huennkens, F. M., and Osborn, M. J., *Adv. Enzymol.*, **21**, 375 (1959).

⁴ Wang, C. H., and Persyn, A. A., *Plant Physiol.*, **37** (Suppl.) (August, 1962).

⁵ Burg, S. P., and Thimann, K. V., *Arch. Biochem. Biophys.*, **95**, 450 (1961).

⁶ Burg, S. P., and Burg, E. A., *Plant Physiol.*, **37** (Suppl.) (August, 1962).

⁷ Burg, S. P., and Burg, E. A., *Nature*, **203**, 869 (1964).

⁸ Gibson, M. S., *The Biogenesis of Ethylene*, a dissertation (Purdue University, 1963).

Characterization of Glucose-6-phosphate Dehydrogenase among Chinese

VARIATIONS in the physico-chemical characteristics of glucose-6-phosphate dehydrogenase (G-6-PD) have been described both in individuals with normal¹⁻⁴ and deficient⁵⁻¹¹ enzyme activity. Recently, Kirkman, McCurdy and Naiman¹² have reported on the characteristics in three unrelated Chinese males with G-6-PD deficiency. They found that the G-6-PD migrated slightly faster than normal G-6-PD but the migration was not quite as fast as that of (A-) G-6-PD observed in Negroes. The enzyme had slightly more thermolability, a bimodal pH optimum curve similar to that of the Mediterranean variant, abnormally low *K*_ms for G-6-P and TPN and relatively greater utilization of G-6-P analogues.

This communication reports on the character of G-6-PD in a group of normal and deficient Chinese subjects. For these investigations, blood was collected in ACD (formula A, U.S.P.) solution. G-6-PD activity was determined by the method of Glock and McHean as modified by Zinkham¹³. Purification of erythrocyte G-6-PD was performed according to the method of Kirkman *et al.*⁸. Starch-gel electrophoresis was performed as described by Shows *et al.*¹⁰.

Starch-gel electrophoresis was performed on haemolysate of 38 normal males and 29 normal females of Chinese origin. Of this group, 36 were Cantonese, 21 were Fukianese, and one each came from Shanghai, Chieking, Shangtung, Hupei and Hunan. The remaining five subjects came from the Chinese mainland, but their place of origin is unknown. All 67 were found to show the single band normally seen in Caucasians.

Detailed investigations were carried out in four normal and four G-6-PD-deficient Chinese subjects from Hong Kong. Patient 1 is a healthy 2-year-old male who has a haemoglobin level of 14-15 g per cent. The deficiency of G-6-PD was detected only following a haemolytic episode after the use of drugs. Patients 2, 3 and 4 have the typical clinical picture of congenital non-spherocytic haemolytic disease, with persistent reticulocytosis and a haemoglobin level of 6-8 g per cent.

Erythrocyte G-6-PD (expressed as units/100 ml. red blood cell) was decreased in all four deficient subjects, ranging from 0 to 15 units as compared with an average value of 228 units in Chinese controls and 210 units in Caucasian controls. Leucocyte G-6-PD (expressed as μM TPN reduced/h/10⁶ white blood cell) was also decreased. In patients 1 and 2 the values were 0.35 and 0.61 as compared with 1.05 in Chinese controls and 1.02 in Caucasian

controls. Starch-gel electrophoresis performed on purified enzyme showed patients 1, 2 and 3 having a single band which migrated 100 per cent (corresponding to that normally seen in Caucasians), while patient 4 showed a single band at 106 per cent (corresponding to that usually seen in Pamaquin-sensitive Negro males (Fig. 1)). In all four patients the K_m for G-6-P was reduced to half the normal values seen in both Chinese and Caucasian controls, but the K_m for TPN was only slightly reduced in patient 4 and not reduced in the other three. Investigations of pH optima were carried out twice on purified enzyme from each patient. Patients 1, 2 and 3 showed a single peak at pH 8, while patient 4 showed a bimodal distribution (Fig. 2). Relative thermostability studies were carried out at 41° C and there was no significant difference between the patients and controls. Finally, substrate specificity was tested with G-6-P analogues. There was a slight but definite increase of activity using both galactose-6-phosphate and 2-desoxy-glucose-6-phosphate as substrates. The increase was more marked in patient 4.

These results indicate that several variants of G-6-PD exist among Chinese. One variant (patient 4) shows a persistent non-spherocytic haemolytic anaemia associated with G-6-PD deficiency. This variant is characterized by a fast band on electrophoresis, a decrease of K_m for both G-6-P and TPN, and a bimodal pH optimum curve. This corresponds closely to the patients described by Kirkman, McCurdy and Naiman¹². Another variant (patients 2

and 3) shows a non-spherocytic haemolytic anaemia with a normal electrophoretic pattern and a single pH optimum peak at pH 8.0. A third variant (patient 1) shows a G-6-PD deficiency without spontaneous haemolytic anaemia, where the haemolytic process can be induced by toxic drug ingestion. Other variants are likely to be found as individuals with normal and deficient enzyme activity are studied more extensively from various parts of China and South-East Asia.

This work was supported by grants from the Chicago Community Trust, the Kettering Foundation and the U.S. Public Health Service (T1-HD-00036 and T1-AM-05186).

PAUL W. K. WONG
LING-YU SHIH
DAVID YI-YUNG HSIA

Genetic Clinic,
Children's Memorial Hospital,
Department of Paediatrics,
Northwestern University Medical School,
Chicago, Illinois.

Y. C. TSAO

Department of Paediatrics,
University of Hong Kong.

- ¹ Boyer, S. H., Porter, I. H., and Weillbacher, R. G., *Proc. U.S. Nat. Acad. Sci.*, **48**, 1868 (1962).
- ² Porter, I. H., *et al.*, *Lancet*, **1**, 895 (1964).
- ³ Nance, W. E., and Uchida, I., *Amer. J. Human Genet.*, **16**, 380 (1964).
- ⁴ Long, W. K., Kirkman, H. N., and Sutton, H. E., *J. Lab. Clin. Med.*, **65**, 81 (1965).
- ⁵ Kirkman, H. N., Riley, H. D., and Crowell, B. B., *Proc. U.S. Nat. Acad. Sci.*, **46**, 938 (1960).
- ⁶ Marks, P. A., Banks, J., and Gross, R. T., *Nature*, **194**, 454 (1962).
- ⁷ Kirkman, H. N., Schettini, F., and Pickard, B. M., *J. Lab. Clin. Med.*, **63**, 726 (1964).
- ⁸ Kirkman, H. N., *et al.*, *J. Lab. Clin. Med.*, **63**, 715 (1964).
- ⁹ Ramot, B., *et al.*, *J. Lab. Clin. Med.*, **64**, 895 (1964).
- ¹⁰ Shows, jun., T. B., Tashian, R. E., Brewer, G. J., and Dern, R. J., *Science*, **145**, 1056 (1964).
- ¹¹ Grossman, A., *et al.*, *Paediatrics* (in the press).
- ¹² Kirkman, H. N., McCurdy, P. R., and Naiman, J. L., *Cold Spring Harbor. Symp. Quant. Biol.*, **29**, 391 (1964).
- ¹³ Zinkham, W. H., *Paediatrics*, **23**, 18 (1959).

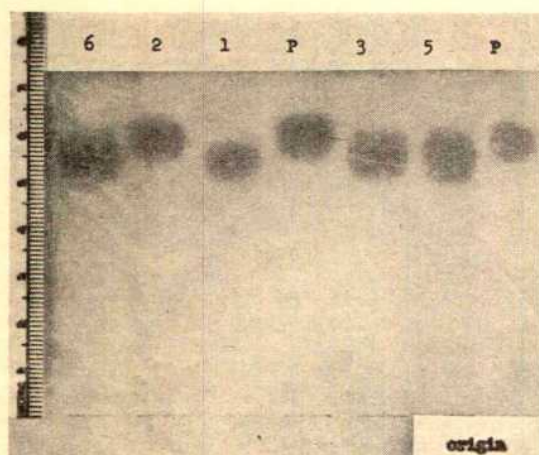


Fig. 1. Vertical starch-gel electrophoresis patterns of red blood cells G-6-PD. Slots 6, 3 and 5 contain preparations from Caucasians. Slots P contain preparations from pamaquin-sensitive Negroes. Slot 1 contains preparation from patient No. 3 and slot 2 contains preparation from patient No. 4

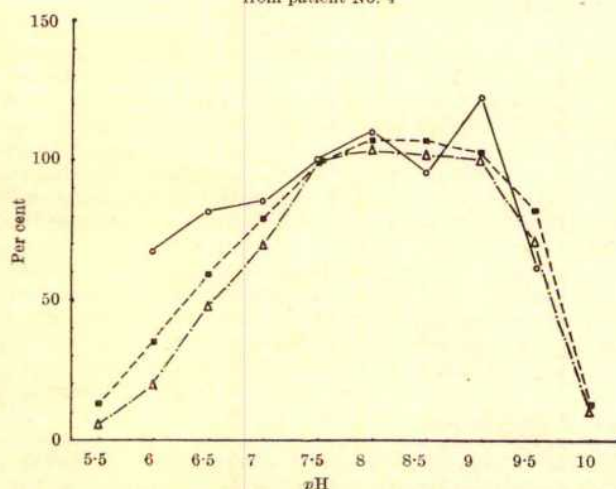


Fig. 2. pH optimum curves for Chinese patients and a Caucasian control. Activity is expressed as percentage of that at pH 7.5. ■, Caucasian control; △, patient No. 3; ○, patient No. 4

Separation Method for Melatonin in Pineal Extracts

MELATONIN, isolated by Lerner *et al.* in 1958¹ from bovine pineal glands, is the most potent agent known that makes the amphibian melanocytes lighter in colour². The physiological role of this compound in mammals^{3,4}, as well as its presence in the epiphysis of lower vertebrates⁵, has been the subject of many recent investigations. It is hard to prove the presence of melatonin in pineal tissues of various origin because it is usually present in these glands only in extremely small quantities. Investigations of the biosynthesis of melatonin offered new possibilities to demonstrate this compound in an indirect way. The presence of the synthesizing enzyme, 5-hydroxyindole-O-methyl transferase, in homogenates of pineal glands of the rat⁶ and the hen⁷ would indirectly indicate the presence of melatonin in the epiphyses of these species. Another indirect method for demonstrating melatonin in Amphibia has been described by Charlton⁸, who demonstrated an accumulation of labelled precursors of this compound in the epiphysis of *Xenopus laevis*.

Other, more direct, methods for the demonstration of melatonin have been described. The original method of Lerner *et al.*¹ requires large quantities of starting material and, for this reason, is scarcely suitable for most purposes. Prop and Ariens Kappers⁹, applying a paper-chromatographic separation method, were able to demonstrate melatonin in the pineal gland of a single rat. This procedure, however, brought about considerable pigment concentration in fractions which did not contain melatonin. The authors ascribed this phenomenon to traces of organic solvents. Quay⁹ developed a separation procedure for a number of indolic compounds, including melatonin. His

assay method, based on the measurement of the ultra-violet fluorescence spectrum, depends strongly on the presence of quenching substances. Since these are known to be present in pineal tissue¹⁰, the applicability of this method to the detection of melatonin in pineal organs is doubtful. Moreover, measurement of the fluorescence is not so sensitive as the blanching reaction of larvae of *Xenopus laevis*^{11,12}.

This report deals with a method of separating melatonin from extracts of pineal tissue. The separation is carried out by filtering the aqueous extract over 'Sephadex G 25', followed by elution with distilled water. The aforementioned blanching reaction of larvae of *Xenopus*^{11,12} was used as a bioassay for the various fractions of the eluate. Since no organic solvents were used in the procedure, 'background lightening' is absent in fractions that do not contain melatonin. The required quantity of starting material is dependent on the amount of melatonin in the gland, but it is fairly small; the recovery of added melatonin is complete. A comparative separation procedure for a number of other indolic compounds from urine has been described by Schlossberger *et al.*¹³.

The upper half of Fig. 1 is an elution diagram of a synthetic mixture consisting of the macromolecule 'blue dextran' with a molecular weight of about 2,000,000, sodium chloride as a representative of a small molecule, and melatonin. The distribution coefficients of the blue dextran and sodium chloride are 0 and 1 respectively, and the apparent K_A of melatonin is about 2.8. This is due to the adsorption of the indole nucleus of melatonin. Other concentrating agents¹⁴, which may occur in pineal tissue^{15,16}, do not interfere. The amine-containing substances serotonin, noradrenaline and adrenaline are retarded even more on the 'Sephadex'. This is due to the small carbonyl content of 'Sephadex' which, if distilled water is used as the elution medium, shows a weak ion exchange capacity.

The lower half of Fig. 1 shows the result of the *Xenopus* larvae blanching test on 'Sephadex' fractions of pineal extracts of various origin. The amount of pineal and control tissue (from other parts of the brain), used in the experiments, was 20–50 mg dry weight: this represented the pineal organs of (approximately) one cow, four lambs, ninety rats, or fifty hens, respectively. In the experiment with hen pineal glands, the bioassay remained positive after a ten-fold dilution of the fractions. This indicates that melatonin could be demonstrated in the pineal organs from only 5 hens. The amount of melatonin in the brain, however, is not constant but varies with the environmental conditions of the animal. This report only deals with the separation procedure, and not with details on the exact localization of melatonin or the factors that influence the quantities found in the brain of various vertebrates in different circumstances. Investigations are being carried

out of the localization of melatonin in various ependymal parts of the roof of the brain (for example, pineal and subcommissural organs) and of the part played by melatonin in the lightening of larvae of *Xenopus* after adaptation to complete darkness.

I thank Prof. J. C. van de Kamer for his advice, and Miss A. W. van Wijngaarden and Mr. C. W. A. C. M. Broekman for assistance.

F. C. G. VAN DE VEERDONK

Zoological Laboratory,
University of Utrecht,
The Netherlands.

- ¹ Lerner, A. B., Case, J. D., Takahashi, Y., Lee, T. H., and Mori, W., *J. Amer. Chem. Soc.*, **80**, 2587 (1958).
- ² Quay, W. B., and Bagnara, J. T., *Arch. Intern. Pharmacodyn.*, **150**, 137 (1964).
- ³ Chu, E. W., Wurtman, R. J., and Axelrod, J., *Endocrinology*, **75**, 238 (1962).
- ⁴ Wurtman, R. J., Axelrod, J., and Chu, E. W., *Science*, **141**, 277 (1963).
- ⁵ Charlton, H. M., *Nature*, **204**, 1093 (1964).
- ⁶ Wurtman, R. J., Axelrod, J., and Phillips, L. S., *Science*, **142**, 1071 (1963).
- ⁷ Axelrod, J., Wurtman, R. J., and Winget, C. M., *Nature*, **201**, 1134 (1964).
- ⁸ Prop, N., and Arlens Kappers, J., *Acta Anat.*, **45**, 90 (1961).
- ⁹ Quay, W. B., *Anal. Biochem.*, **5**, 51 (1963).
- ¹⁰ Lerner, A. B., Case, J. D., and Takahashi, Y., *J. Biol. Chem.*, **235**, 1992 (1960).
- ¹¹ Bagnara, J. T., *Science*, **132**, 1481 (1960).
- ¹² Burgers, A. C. J., and van Dorst, G. J., *Gen. Comp. Endocrinol.*, Supp. 1, 99 (1962).
- ¹³ Schlossberger, H. G., Kuch, H., and Buhrow, I., *Z. Physiol. Chem.*, **333**, 152 (1963).
- ¹⁴ Lerner, A. B., and Case, J. D., *J. Invest. Dermatol.*, **32**, 211 (1959).
- ¹⁵ Giarman, N. J., Freedman, D. X., and Picard-Ami, L., *Nature*, **186**, 480 (1960).
- ¹⁶ Owman, Ch., *Intern. J. Neuropharmacol.*, **2**, 105 (1964).

Distribution of Collagenase in Rat Tissues

THE demonstration, recently reported from this laboratory¹⁻³, of a collagenolytic system in rat bone-cell homogenates raised several questions regarding its importance in the physiology and pathology of connective tissues. Since the activity was largely confined to a subcellular particulate fraction exhibiting a number of the features of lysosomes^{2,3}, it became important to know whether collagenase should be included in the growing list of hydrolases which seem to be characteristically contained in these bodies. By the same token it could be asked whether particle-bound collagenase was to be found equally in all tissues in which collagen turnover occurs or whether it was confined to bone-cells and thus had to be regarded as a part of a remodelling mechanism unique to bone.

Two tissues immediately suggested themselves for examination in seeking answers to these questions: liver, because of the abundant lysosomes which it contains⁴; and polymorphonuclear leucocytes the granules of which have been shown to contain many acid-hydrolases and the efficiency of which as phagocytes in inflammatory processes suggested that a collagenase might be included in their roster of catheptic enzymes^{5,7}. Preparations of these cells were accordingly assayed for collagenolytic activity along with preparations from kidney and brain which were taken as typical examples of parenchymatous organs. These were then compared with bone from the same animals.

Liver, kidney, brain and bone were collected from male Sprague-Dawley rats approximately 55 days old and immediately chilled in ice-cold Krebs-Ringer bicarbonate buffered medium, pH 7.4. Soft tissues were macerated in a mortar at 4° C and homogenized for 3 min in 0.25 M sucrose containing 1.0×10^{-3} M $MgCl_2$ using a glass homogenizer with motor-driven 'Teflon' pestle. Suspensions of bone cells, isolated as previously described¹⁻³, and suspensions of guinea-pig

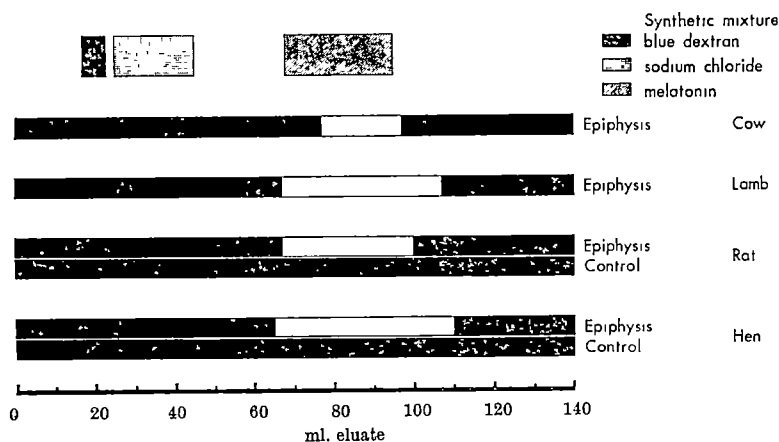


Fig. 1. The upper part shows the effluent volume of synthetic melatonin eluted from a 'Sephadex' column. The lower part of the diagram shows the blanching reaction of black-background-adapted larvae of *Xenopus* after 20 min swimming in the eluates of extracts separated on the same column. ■, Larvae remain dark, □, larvae become lightened

polymorphonuclear leucocytes prepared as described by Oren *et al.*⁸ were similarly treated. Cell fractions from these tissues were isolated by centrifugation as in previous experiments^{2,3}. The 'large granule' fraction sedimenting between 700 and 15,000*g* was of special interest since it had been shown to contain most of the collagenolytic activity of bone cells^{2,3}. In addition a 'nuclear and debris' fraction (0–700*g*), a 'small granule' fraction (15,000–105,000*g*) and a 'supernate' fraction were prepared.

Substrate for the assay was rat bone collagen previously labelled *in vitro* with proline-¹⁴C using recently developed methods which yield material of satisfactory purity and high resistance to degradation by trypsin^{2,3}. Cell fractions were suspended in Krebs–Ringer bicarbonate medium pH 7.3 and promptly incubated with collagen substrate for 40 min at 37° C. Saponin, 1.5 per cent, was present to solubilize all particle-bound activity. The reaction was stopped by diluting the incubation flask contents with 3 volumes of ice-cold medium, after which the entire flask contents were ultrafiltered through 'Cellophane' under vacuum at 4° C. When ultrafiltration was complete (16 h was ordinarily sufficient) the filtrate was evaporated to dryness, taken up in concentrated formic acid, and counted in a liquid scintillation system^{1–3}. The net collagen radioactivity rendered ultrafilterable in 40 min under these conditions has been shown to reflect collagenolysis.

The significant results derived from these experiments are shown in Fig. 1 in which the relative collagenase activity in the large granule fractions derived from the various tissues is compared. It is clear from these data that collagenolytic activity was far larger in bone than any other tissue studied. Indeed, reproducibly measurable activity was found in only one other tissue, namely kidney; even that was at the borderline of the sensitivity of the method. Although other fractions from bone have been shown to contain smaller amounts of collagenase activity^{2,3}, none was found in such fractions derived from the soft tissue cells and leucocytes.

Two important conclusions can be drawn from these observations. First, it seems clear that significant collagenase-like activity is not to be found in solution or in subcellular particles of the lysosome type in several tissues which are known to contain such bodies. Particularly interesting, in view of the large variety of highly active acid-hydrolases found in them, was its absence from leucocyte granules⁶ prepared as noted here, or as in other experiments by Cagan and Karnovsky⁹. High acid phosphatase activity was found in aliquots of the same leucocyte granule preparations, thus ruling out non-specific inactivation as a cause of this finding.

Secondly, significant collagenase activity was to be found only in the large-granule fraction of bone, a tissue which not only contains large amounts of collagen but is, in addition, constantly being remodelled at a measurable rate as part of its normal physiology. Thus it would seem that significant lysosomal collagenase activity must be regarded, at least at present, as being a specific characteristic of the remodelling system of bone. Whether it is unique to bone cannot be determined from data at present available, but several lines of evidence suggest that it is probably a characteristic of remodelling connective tissues in general. While Gross has been unable to extract preformed collagenase activity from skin¹⁰, and attempts in this laboratory to find a collagenase-containing subcellular particle in this tissue have also been unsuccessful so far (unpublished observations), these failures seem as likely to reflect defects in methodology as absence of the enzyme³. Moreover, collagenolytic activity seems to increase in both skin and bone in situations where remodelling is increased—in skin in healing wounds^{10,11}, and in bone following injection of parathyroid extract⁴, or in the increased bone destruction and osteoporosis induced by heparin¹².

This work was supported by U.S. Public Health Service grant AM 00854-9.

We thank Dr. R. H. Cagan and Dr. M. L. Karnovsky for the supplies of guinea-pig leucocytes and leucocyte granules prepared by their methods used in these experiments, and Miss L. Bunde for assistance.

JOHN F. WOODS
GEORGE NICHOLS, JUN.

Department of Medicine,
Harvard Medical School,
and
Peter Bent Brigham Hospital,
Boston, Massachusetts.

¹ Woods, J. F., and Nichols, G., jun., *Science*, **42**, 386 (1963).

² Woods, J. F., and Nichols, G., jun., *Fed. Proc.*, **23**, 550 (1964).

³ Woods, J. F., and Nichols, G., jun., *J. Cell Biol.*, **26**, 747 (1965).

⁴ Nichols, G., jun., Flanagan, B., and Woods, J. F., in *The Parathyroid Glands*, edit. by Gallard, P. J., Talmage, R. V., and Budy, A. M. (Univ of Chicago Press, Chicago, 1965).

⁵ deDuve, C., in *Lysosomes*, edit. by de Reuck, A. V. S., and Cameron, M. P. (Little, Brown and Company, Boston, 1963).

⁶ Cohen, Z. A., and Hirsch, J. G., *J. Exp. Med.*, **112**, 983 (1960).

⁷ Dannenberg, A., jun., Benstone, M., Walter, P., and Kinsky, J., *J. Cell Biol.*, **17**, 465 (1963).

⁸ Oren, R., Farnum, A. E., Saito, K., Milofsky, E., and Karnovsky, M. L., *J. Cell Biol.*, **17**, 487 (1963).

⁹ Cagan, R. H., and Karnovsky, M. L., *Nature*, **204**, 255 (1964).

¹⁰ Gross, J., in *Proc. NATO Advanced Study Conf. Structure and Function Conn. Skel. Tissues*, 1964 (in the press).

¹¹ Grillo, H. C., and Gross, J., *J. Cell Biol.*, **23**, 39A (1964).

¹² Asher, J. D., and Nichols, G., jun., *Fed. Proc.*, **24**, 211 (1965).

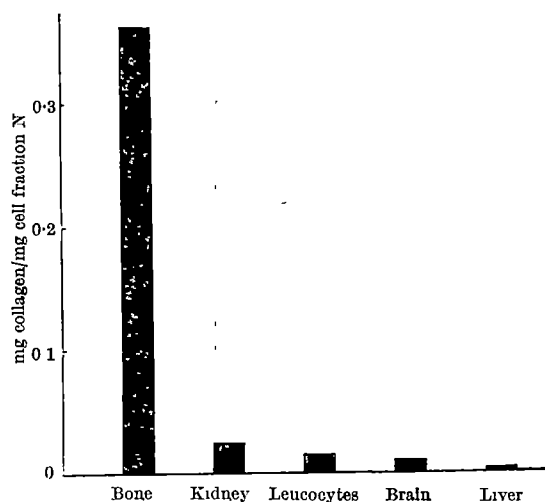
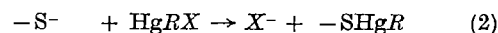
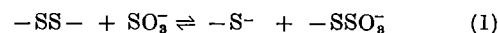


Fig. 1

Cystine Content of Cattle Hair

ORGANIC mercurials react with thiols or with disulphides (following their conversion to thiols by reagents such as sulphite), according to the following equations^{1–3}:



A convenient way of following the reaction in hair is to expose a known amount (10–15 mg) to an approximately four-fold excess of ²⁰³Hg-labelled mercurial such as phenyl-mercuric acetate (25 μmoles, 0.5 μc., in 5 ml.) in a 20 per cent (v/v) dimethyl-formamide solution at pH 9 containing ammonium nitrate (0.400 M), ammonium hydroxide (0.100 M), sodium sulphate (0.625 M) and sodium nitrate (1.250 M) (ref. 4). The mercurial bound to the insoluble keratin is calculated from the amount of radioactivity lost from solution after 24 h at 37° C.

One hair sample was taken from the last intercostal space on the mid-side of each of 19 and 54 animals in March and October 1961 respectively. The numbers of two-year-old Brahman, British, Brahman × British and

Africander × British crossbred steers and heifers sampled are shown in Table 1. Animals were weighed, clipped and coat scored⁵ by Messrs. H. G. Turner and A. V. Schleger. All hair was first washed with several lots of petrol ether, ethanol, and finally water, before overnight drying at 37° C. Assays were carried out on duplicate sub-samples of each hair sample, while a third sub-sample was used for dry-weight determinations. As the weighted mean mercurial binding was higher in March than in October ($P < 0.05$), the two sets of results are treated separately. The March clippings were also associated with sleeker (lower score⁵) coats. On the other hand, the difference between male and female means was not significant. This finding is in disagreement with a previous claim⁶ that female cattle hair had a higher cystine content.

The mean value for mercurial binding in each breed for March and October is given in Table 1. It is at once apparent that the mercurial binding by Brahman hair is lower than that of other breeds. An analysis of variance (Table 2) reveals that the differences between breeds are highly significant. If the Brahman hair is not taken into account, this breed difference is much reduced and indeed loses significance in March samples. The differences between samples are validated by being far greater than those between duplicates.

The breed mean mercurial binding values rank in the same order as the tolerance of a tropical environment. On the other hand, mercurial binding between individual animals within breeds and months was not significantly correlated with either the coat score ($r = -0.078$, d.f. 60), or with the October body weight ($r = +0.214$, d.f. 58).

The specificity of the reaction between mercurials and thiols^{2,3} is such that mercurial binding may be taken as an index of the cystine content of the hair samples⁴. Indeed, the range of cystine contents estimated from mercurial binding values gives figures for British cattle (Hereford × Shorthorn) of between 10.8 and 12.6 per cent. These values are reasonably close to those reported by earlier workers (11.0–14.6 per cent) (refs. 6 and 7). Exact comparison is unfortunately impossible, not only because of different environmental, nutritional and other factors, but also because different breeds are involved.

In samples selected to give the widest possible range of values, the correlation between total sulphur and mercurial binding is high ($r = +0.810$, d.f. 30, $P < 0.001$). Mercurial binding accounts for an average of 78.6 per cent ($S.E.$, 1.3) of the total sulphur (analysis by the C.S.I.R.O.

Microanalytical Service). By analogy with hair from other sources⁸, the methionine content of cattle hair is almost certainly insufficient to account for the 'missing' sulphur. Schöberl and Bisping⁷ showed that there is some cystine destruction in hair and wool as a result of solar, ultra-violet irradiation under the warm humid conditions prevailing close to the skin surface. The location of our animals at 'Belmont' near Rockhampton, just within the tropical zone, and the hair sampling in the exposed intercostal region, would both tend to favour the formation of cystine degradation products. A further possibility is that all the disulphide bonds may not be accessible to the reagent under our conditions. The fact that the reaction had apparently come to a standstill is of course no guarantee of complete accessibility.

The results on Brahman hair are compatible with a greater medullary content. It is known that medullary protein is deficient in sulphur⁹ and that Brahman type hair contains a higher proportion of medullated fibres than other breeds of cattle¹⁰. Unfortunately information on the percentage of medullary protein in cattle hair is lacking. The question as to whether the lower sulphur content and the lower mercurial binding capacity of Brahman hair is of metabolic significance is being investigated.

We thank Mr. H. G. Turner for advice and Mr. G. J. Buch and Miss C. I. Seer for assistance.

P. H. SPRINGELL

C.S.I.R.O. Division of Animal Genetics,
Cattle Research Laboratory, Rockhampton,
Queensland.

S. J. LEACH

C.S.I.R.O. Division of Protein Chemistry,
Wool Research Laboratories, Parkville,
Victoria.

¹ Stricks, W., Kolthoff, I. M., and Tanaka, N., *Anal. Chem.*, **26**, 299 (1954).

² Leach, S. J., *Austral. J. Chem.*, **13**, 520 and 547 (1960).

³ Leach, S. J., in *A Laboratory Manual of Analytical Methods of Protein Chemistry*, edit. by Alexander, P., and Lundgren, H. P., 4 (Pergamon Press, Oxford, 1966).

⁴ Leach, S. J., Meschers, A., and Springell, P. H., *Anal. Biochem.* (in the press).

⁵ Turner, H. G., and Schleger, A. V., *Austral. J. Agric. Res.*, **11**, 645 (1960).

⁶ Okuda, Y., and Katal, K., *J. Biochem. (Tokyo)*, **24**, 207 (1956).

⁷ Schöberl, A., and Bisping, N., *Deut. Tierarz. Wochenschr.*, **65**, 599 (1958).

⁸ Blackburn, S., in *Biochemistry Handbook*, edit. by Long, C., 701 (E. and C. M. Spon, Ltd., London, 1961).

⁹ Matoltsy, A. G., *Exp. Cell Res.*, **5**, 98 (1953).

¹⁰ Schleger, A. V., and Turner, H. G., *Austral. J. Agric. Res.*, **11**, 875 (1960).

Table 1. BINDING OF MERCURIAL BY HAIR DERIVED FROM VARIOUS BREEDS OF CATTLE

Breed*	March		October	
	Mean†	n‡	Mean	n
B	387.0	3	357.1	7
BS	437.7	3	442.2	6
BH	498.3	2	456.9	8
AS	473.7	3	474.1	14
AH	510.8	2	479.1	11
H and S	505.8	6	492.9	8
All	470.9	19	456.6	54
All except B	486.7	16	471.5	47

* Abbreviations: B, Brahman; A, Africander; H, Hereford; S, Shorthorn.

† Expressed in μ moles mercury/g dry hair.

‡ No. of animals.

Table 2. ANALYSIS OF VARIANCE OF MERCURIAL BOUND BY HAIR IN VARIOUS BREEDS OF CATTLE

Source of variation	All breeds			All breeds except Brahman		
	d.f.	m.s.	F	d.f.	m.s.	F
March						
Between breeds	5	14,579	5.76*	4	5,671	2.64
Between animals	13	2,532	9.66†	11	2,182	8.49†
Within breeds						
Between duplicates	19	262		16	257	
Within animals						
October						
Between breeds	5	36,397	19.82†	4	5,623	4.03*
Between animals	48	1,836	4.37†	42	1,395	9.49†
Within breeds						
Between duplicates	54	420		47	147	
Within animals						

* $P < 0.01$

† $P < 0.001$

PHYSIOLOGY

Energy Requirements for Relaxation from Tonic Contractions ('Catch') in an Invertebrate Muscle

THE anterior byssus retractor muscle (ABRM) of the edible mussel, *Mytilus edulis*, responds to pulses of alternating current with a rapid phasic contraction. Stimulation with direct current or acetyl choline produces a prolonged tonic contraction or 'catch'¹, and a rapid relaxation from this catch follows the addition of 5-hydroxytryptamine (serotonin)².

Investigations of the physical properties of paramyosin isolated from molluscan muscles have led to the idea that tension is maintained during a catch through a rigid network of paramyosin crystals³. An opposing view suggested by Lowy, Millman and Hanson is that the holding capacity of the muscle depends on a sliding filament system with long-lasting cross-links⁴.

In the latter case, bonds between myosin and actin would have to be broken when the catch is released with serotonin. According to a recent theory of muscle contraction⁵ these bonds contain calcium and ATP and are cleaved by the calcium-activated actomyosin ATPase.

Thus this theory predicts that a cleavage of these bonds, following the addition of serotonin to a muscle in catch, would result in liberation of both inorganic phosphate and calcium on relaxation. Some of this calcium would be transiently available to the actomyosin system, but would soon be pumped from the sarcoplasm by a process requiring further usage of ATP.

On the basis of the number of thick filaments and the dimensions of paramyosin^{4,6}, it can be estimated that the ABRM contains approximately 3×10^{16} cross-bridges per ml. If each cross-bridge is associated with one ATP then 0.05 μ mole of ATP per gram of muscle should be split if all the cross-bridges break once. However, since the muscle lengthens steadily under constant load following the addition of serotonin, it seems likely that new transient bonds must be formed and broken a few times. Thus the ATP breakage should be in the range of 0.10–0.15 μ mole per gram. The operation of the calcium pump would lead to an additional breakdown of about 0.05 μ mole/g of ATP⁷. In addition to the increase of inorganic phosphate from the breakdown of ATP, free arginine would also increase due to the subsequent action of ATP:L-arginine phosphotransferase. Experiments were, therefore, carried out to see if the predicted breakdown of ATP occurred in association with relaxation on the release of catch.

Paired ABRM were dissected according to the method of Hoyle and Lowy⁸ and loaded with 10-g weights in oxygenated sea-water at 23° C for 3 h before the experiment. The muscles were frozen in a mixture of dichlorodifluoromethane and chlorotrifluoromethane (30:70) at -176° C, and extracted for one week in 48 per cent (v/v) methanol, 0.00125 M ethylenediaminetetraacetic acid at -35° C. Inorganic phosphate was estimated by a modification of the method of Wahler and Wollenberger⁹, and free arginine determined by the method of Rosenberg, Ennor, and Morrison¹⁰ after evaporation of the methanol.

In the first experiment both muscles of a pair were preincubated in a 1 μ M serotonin sea-water solution for 30 min before stimulation with direct current. A comparison of 26 control muscles which were frozen at the peak of contraction, and the paired experimental muscles which were frozen 15 sec after the end of stimulation, showed an increase of inorganic phosphate of 0.31 ± 0.06 μ mole/g, associated with relaxation at 18° C.

In another series of experiments, relaxation by serotonin from a heavily loaded maintained catch at 10° C caused an average increase of inorganic phosphate of 0.11 ± 0.06 μ mole/g in 21 pairs of muscles.

Fig. 1 shows the results of experiments redesigned to eliminate the variance introduced by the large amount of work performed by these muscles. An unloaded muscle was stimulated with acetyl choline at 8° C and allowed to contract 30 per cent from its rest length. Further shortening was prevented by a stop. After one minute of stimulation the muscle was washed with sea-water and loaded with a 25-g weight. This was added to see if the muscle was in a good 'catch'. The criterion of a good 'catch' was taken as the ability of the muscle to hold a 25-g load for 3 min without lengthening. After 3 min the stops were removed and 30 sec later the 25-g loads were lifted from both muscles. The muscles shortened slightly during these operations due to their elastic elements. Serotonin was then added to the experimental muscle but not to the control, and after 1 min in serotonin a 10-g load was added to each muscle to see if it was in a 'catch'. The control muscle was able to bear the 10-g load but the experimental muscle returned to its rest length within 1 min. The average inorganic phosphate increase of 24 muscles which relaxed, compared with their pairs which remained in 'catch', was $+0.19 \pm 0.06$ μ mole/g ($P < 0.004$). In 8 control experiments the addition of serotonin to resting muscles caused an insignificant change in inorganic phosphate of -0.01 ± 0.05 within 2 min.

Thus there are three separate series of experiments which give the same result; namely, that about 0.2 μ mole/g

of phosphorylarginine are broken down during relaxation from the 'catch'. This value corresponds to the predicted value for the amount of ATP which should be broken down if cross-links between actin and myosin, which involve ATP and calcium, are responsible for the maintenance of tension during a 'catch'.

Investigations have also been made of the behaviour of calcium during a tonic contraction of ABRM by studying the rate of efflux of calcium-45 from four separate muscles. When the muscles were stimulated with 0.5 mM acetyl choline they developed about 200 g tension and calcium was rapidly released into the bathing solution.

After the addition of 10^{-4} M serotonin the tension fell to zero within 10 sec and there was an increased rate of calcium efflux, but the calcium was observed outside the muscle at least 5 min after the fall in tension. Therefore it seems that calcium close to the surface of the muscle is being mobilized during stimulation, but the breaking of the catch releases some calcium which is deeper inside the muscle structure and the observed efflux represents the liberated calcium which was not captured immediately by the calcium pumping mechanism. It is interesting that Woolley¹¹ found a substance in many tissues which could form a fat-soluble complex with calcium and serotonin. Thus the action of serotonin might be to sequester calcium causing a breakdown of the hypothetical links.

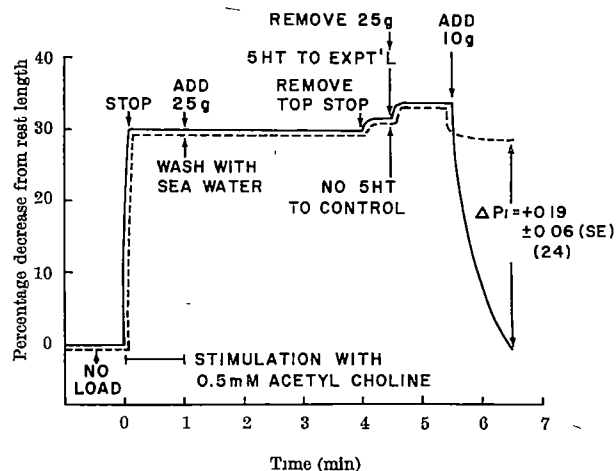


Fig. 1. Liberation of inorganic phosphate during release of a catch of anterior byssus retractor muscle (ABRM) following the addition of 10^{-4} M serotonin (5-HT) at 8° C. The isolated ABRM was mounted on an unloaded isotonic lever which moved between two stops. The muscle was cooled to 8° C for 5 min in sea-water gassed with nitrogen. The muscle was stimulated for 1 min with 0.5 mM acetyl choline and allowed to contract 30 per cent from its rest length. Further shortening was prevented by a stop. A 25-g load was added to the muscle and the acetyl choline removed by washing the muscle with sea-water (8° C). After a 3-min wash period the top stop was removed and the muscle contracted slightly due to its elastic elements. Thirty sec later, the 25-g load was removed and the experimental muscle surrounded by a solution of 10^{-4} M serotonin in sea-water while the control muscle was kept in sea-water. After 1 min of this treatment, a 10-g load was added to each muscle and, 60 sec later, the muscles were frozen in a mixture of chlorotrifluoromethane: dichlorodifluoromethane (70:30) at -176° C. The dashed line represents a typical kymograph tracing for the control muscle. The solid line refers to the experimental muscle.

The finding that there is a release of inorganic phosphate, arginine, and calcium on sudden relaxation from a tonic contraction is consistent with the hypothesis that a specific type of cross-link between actin and myosin is responsible for the prolonged maintenance of tension in the ABRM. Loss of tension requires the cleavage of those links with liberation of inorganic phosphate and calcium. These findings would not be expected from a phase transition of paramyosin³ or if the linkage were of the type suggested by Perry¹² for the interaction of actin and myosin.

We thank Mrs. Constance J. Kick for her assistance and Dr. Jean Hanson for advice.

This work was supported in part by U.S. Public Health Service research grant 2 R01 HE02520-09 from the National Heart Institute.

KATHLEEN MINIHAN
R. E. DAVIES

Laboratories of Biochemistry,
Department of Animal Biology,
School of Veterinary Medicine,
University of Pennsylvania,
Philadelphia.

- ¹ Winton, F. R., *J. Physiol.*, **88**, 492 (1937).
- ² Twarog, B. M., *J. Cell. Comp. Physiol.*, **44**, 141 (1954).
- ³ Johnson, W. H., Kahn, J. S., and Szent-Gyorgyi, A. G., *Science*, **130**, 160 (1959). Rugg, J. C., *Proc. Roy. Soc. B*, **160**, 536 (1964).
- ⁴ Lowy, J., and Millman, B. M., *J. Physiol.*, **149**, 68 P (1959). Lowy, J., Millman, B. M., and Hanson, Jean, *Proc. Roy. Soc. B*, **160**, 525 (1964).
- ⁵ Davies, R. E., *Nature*, **199**, 1068 (1963).
- ⁶ Elliott, G. F., *Proc. Roy. Soc. B*, **160**, 467 (1964).
- ⁷ Hasselbach, W., and Makinose, M., *Biochem. Z.*, **339**, 94 (1963).
- ⁸ Hoyle, G., and Lowy, J., *J. Exp. Biol.*, **33**, 295 (1956).
- ⁹ Wahler, B., and Wollenberger, A., *Biochem. Z.*, **329**, 508 (1958).
- ¹⁰ Rosenberg, H., Ennor, A. H., and Morrison, J. F., *Biochem. J.*, **63**, 153 (1958).
- ¹¹ Woolley, D. W., *Proc. U.S. Nat. Acad. Sci.*, **44**, 1202 (1958).
- ¹² Perry, S. V., in *Muscle*, edit. by Paul, W. M., Daniel, E. E., Kay, C. M., and Monckton, G. (Pergamon Press, Oxford, 1965). Perry, S. V., and Cotterill, Jennifer, *Nature*, **206**, 161 (1965).

HAEMATOLOGY

Hereditary Ovalocytosis and Haemoglobin E-Ovalocytosis in Malayan Aborigines

THE red blood cells of mammalian vertebrates, including man, are normally round, except those of the camel, which are characteristically oval in shape. Less than 15 per cent of the red blood corpuscles in normal healthy men are oval, but in severe anaemias the number of oval cells in the peripheral blood may become much higher. This symptomatic ovalocytosis in severe anaemias is to be distinguished from hereditary ovalocytosis, or elliptocytosis, first described by Dresbach¹ in 1904. In the hereditary condition, more than 25 per cent of the red blood cells are oval or elliptic, and elliptic rod-shaped cells are more common than in the symptomatic condition. The two conditions can usually be distinguished, with experience, but the diagnosis can only be confirmed by demonstration of a family occurrence. Incidence of the hereditary condition in the general population has previously been estimated at approximately 0.04 per cent^{2,3}, although more prevalent in Dutch, German and Italian families^{4,5}. A focus of high frequency of hereditary ovalocytosis (elliptocytosis) has been reported from central Celebes⁶. I now report another focus of high frequency of hereditary ovalocytosis (elliptocytosis) in Malayan aborigines.

Blood smears of 440 persons comprising two groups of aborigines, mostly from the Senoi tribe, were examined. A group of 152 healthy males was made up of 110 jungle fighters of the Senoi Praak regiment and 42 personnel of the Ulu Gombak Aboriginal Hospital. The other group consisted of 288 patients from the same Aboriginal Hospital. In the healthy group, 15 soldiers and 6 Aboriginal Hospital personnel were found to have ovalocytosis with many elliptic rod-shaped/red cells, while 33 of the 288 hospital patients had ovalocytosis. The total frequency was 12.3 per cent among the 440 persons.

Although, except in 2 cases, no confirmation of the family trait could be obtained, as all relatives lived in the deep jungle, this condition was believed to be hereditary, as none of the healthy group was anaemic, while many elliptic cells, rather than oval cells, were seen in the blood. Frequency of this abnormality in the aborigines was in marked contrast to findings among 150 healthy individuals of different non-aboriginal races in Malaya, none of whom had ovalocytosis of the blood. Among patients from the General Hospital at Kuala Lumpur referred to the Haematology Division of the Institute, mostly for anaemia, 990 were examined for their peripheral

blood picture and only three (two Malays and one Chinese) were found to have hereditary ovalocytosis, confirmed by study of the family trait. This hospital serves Chinese, Indians and Malays. Although many of the patients suffering from anaemia were found to have numerous oval cells in the blood, these were readily recognized as symptomatic and could be differentiated from the aboriginal ovalocytosis.

Since the aborigine has a high frequency of the gene for abnormal haemoglobin E, ranging between 8 and 50 per cent in different groups⁷, it is to be expected that the new genetic combination of the gene for ovalocytosis with the gene for haemoglobin E, not reported until now, is not rare in this population. Indeed, of 18 cases of ovalocytosis among healthy aborigines, whose haemoglobins could be analysed, I found 2 associated with haemoglobin E in the blood, and of 22 cases of ovalocytosis among Aboriginal Hospital patients whose blood was examined for abnormal haemoglobin, 4 carried haemoglobin E. Attempts are being made to examine the families, and results of full haematological studies will be reported elsewhere in collaboration with Dr. J. M. Bolton of the Aboriginal Hospital, Ulu Gombak.

I thank Mr. Mohamed Ruslan bin Abdullah and Mr. Abdullah bin Sain of the Senoi Praak Regiment for their co-operation, and Dr. J. M. Bolton of the Aboriginal Hospital for his assistance.

This work was supported in part by the University of California International Center for Medical Research and Training (Hooper Foundation, San Francisco School of Medicine) with research grant GM-11329 from the Office of International Research, National Institutes of Health, U.S. Public Health Service.

LIE-INO LUAN ENG

Institute for Medical Research,
Kuala Lumpur,
Malaysia;
and

The Hooper Foundation
and Haematology Unit,
University of California Medical Center,
San Francisco.

- ¹ Dresbach, M., *Science*, **19**, 469 (1904).
- ² Miller, J. K., and Lucas, A. A., *Amer. J. Clin. Path.*, **8**, 391 (1938).
- ³ Wyandt, H., Bancroft, P. M., and Winship, T. O., *Arch. Intern. Med.*, **68**, 1043 (1941).
- ⁴ Wintrobe, M. M., *Clinical Hematology* (Lea and Febiger, Philadelphia, 1961).
- ⁵ Conti, C., Torlonato, G. and Muzzoloni, M., *Policlinico sez. prat.*, **69**, 1477 (1962).
- ⁶ Bonne, C., and Sandground, J. H., *Gen. Tydschr. Ned. Indre*, **79**, 3016 (1939).
- ⁷ Lie-Injo Luan Eng and Chin, J., *Nature*, **204**, 291 (1964).

I Antigen in Leukaemic Patients

P. J. SCHMIDT *et al.* have observed 20 diminutions or disappearances of I antigen in 20 patients out of 116 investigated cases¹. Among these, 15 were suffering from various forms of chronic or acute leukaemia.

We for our part have observed, following many authors, ABO modifications in various leukaemic patients^{2,3}. We have also observed a decrease of inhibiting power of Gm factors in these diseases. Table 1 shows our results. We should point out, however, that these modifications are almost always observed in patients suffering from acute leukaemia.

Table 1. BLOOD GROUP MODIFICATIONS IN LEUKAEMIAS

System	No. of cases	No. of modifications
Gm	79	6
ABO	66	1 (A) and 3 (H)
Rh	62	0

We have also investigated I antigen in healthy subjects and leukaemic patients. Our results (Table 2) indicate that we have not observed a decrease of I antigen in the

patients which we investigated. The anti-I antibody which we used was obtained from a subject who, to all appearances, was healthy and was I-positive. The research on I antigen was made possible by the use of the technique of tube agglutination; for all our leukaemic patients, a control was effected by a titration method which enabled us to determine quantitatively any variations in the I antigen.

Table 2. DISTRIBUTION OF I ANTIGEN

	No. of cases	I-positive	I-negative
Blood donors	10,090	10,089	1
Patients	2,250	2,250	0
Leukaemias	56	56	0

Our results differ from those of P. J. Schmidt *et al.*

We are still carrying out our investigations and hope to throw some light on the origin of these differences.

We thank Dr. A. E. Mourant (Lister Institute, London) for controlling the specificity of this anti-I serum.

J. DUCOS
J. RUFFIE
P. COLOMBIES
Y. MARTY
E. OHAYON

Centre de Transfusion Sanguine
et d'Hématologie, Toulouse.

¹ Schmidt, P. J., Yokoyama, M., McGinniss, M. H., and Carbone, P. P., *Proc. Tenth Intern. Cong. Blood Trans.*, Stockholm (1964).

² Ruffie, J., Bierme, R., Colombies, P., Ducos, J., Salles-Mourlan, A. M., and Bierme, S., *Comm. Soc. Hémat.* (March 15, 1965).

³ Ruffie, J., Ducos, J., Bierme, R., Marty, Y., Salles-Mourlan, A. M., and Colombies, P., *Nouv. Rev. Franç. Hémat.*, 5, 3, 501 (1965).

⁴ Ruffie, J., Ducos, J., Varsi, M., and Colombies, P., *Nouv. Rev. Franç. Hémat.*, 4, 5, 599 (1964).

Involvement of the Adrenal Cortex in the Appearance of Rat Slow Alpha₂-Globulin

SLOW α_2 -globulin (SA₂G) is a protein which can be demonstrated by vertical starch-gel electrophoresis to be present in the blood serum of the rat in certain physiological, pathological and experimental situations: during pregnancy¹⁻³ and lactation³, in the foetal^{1,3,4} and neonatal¹⁻⁴ states; in rats bearing growing neoplasms^{1,2,5}; in the presence of infections or inflammations⁶; after partial hepatectomy^{7,8}, splenectomy or unilateral renal-ectomy⁹; and following the administration of carbon tetrachloride⁷, various bacterial lipopolysaccharides (endotoxins)^{10,11}, complete or incomplete Freund's adjuvant¹¹, turpentine¹², or certain metallic salts¹³. It generally cannot be demonstrated by this technique, however, in healthy, non-pregnant adult rats¹⁻⁴, nor after clean laparotomies⁷, partial limb amputations⁹, or heavy doses of ionizing radiation¹⁴. Consequently, we define the SA₂G effect as being the appearance of demonstrable levels of SA₂G in response to a competent stimulus. In view of the broad range of competent stimuli for the SA₂G effect, a search for a common factor or controlling agent was undertaken. The present work indicates that the adrenal cortex is intimately involved in the appearance of the SA₂G effect provoked by at least one of the competent stimuli and may be a controlling factor.

Animals used, general procedures and vertical starch-gel electrophoresis were as previously described³. Lipopolysaccharide (endotoxin) of *Salmonella abortus equi*¹¹ (Westphal method, Difco Laboratories, Detroit, Michigan) was used as the provoking agent. Bilateral adrenalectomy was performed through a single skin and double muscle incision under ether anaesthesia. Immediately after the operation the animals were given 0.8 mg of corticosterone (Nutritional Biochemicals Corporation, Cleveland, Ohio) subcutaneously. The sham operation (group 9, Table 1) was performed in a manner analogous to the adrenalectomy. As a general protocol, no further treatment was given until the 20th day after the operation except that all animals were given 1 per cent sodium chloride solution in place of drinking water from the operation onward. The same substitu-

tion was also made for all unoperated animals at least 21 days prior to the injection of lipopolysaccharide or the control dose of saline solution. Animals in groups 2, 3, and 7 were given 0.5 mg lipopolysaccharide in 0.05 c.c. physiological saline intraperitoneally on the 21st post-operative day. Animals in groups 1 and 6 were given the same dose, but not subjected to operation. Animals in groups 4, 5, 8, and 9, serving as controls, were injected intraperitoneally with 0.05 ml. of plain physiological saline. In groups 3, 4, 5, and 6, 1.0 mg corticosterone in 0.5 ml. of physiological saline was injected twice daily, beginning one day prior to the injection of lipopolysaccharide or saline and continuing through the 5th day after injection of lipopolysaccharide or saline. Animals in group 7 were given daily injections of 0.5 ml. physiological saline in the same manner as those treated with corticosterone. Serum samples were obtained from all animals and examined for SA₂G content 2 days after injection of the lipopolysaccharide or the corresponding saline control injection. Some samples were obtained and examined at 4 or 6 days after injection and the results obtained were in consonance with those from the second post-injection day.

Table 1. EFFECT OF ADRENALECTOMY AND REPLACEMENT THERAPY ON THE ABILITY OF BACTERIAL LIPOLYSACCHARIDE TO PROVOKE DEMONSTRABLE SLOW α_2 -GLOBULIN

Group	Adrenal-ectomy	0.5 mg lipopolysaccharide injected (a)	0.05 ml. saline injected (a)	Corticosterone injected (b)	Saline injected (c)	No. of rats examined	% of rats showing SA ₂ G response
1	-	+	-	-	-	16	81.3
2	+	+	-	-	-	10	0
3	+	+	-	+	-	10	70.0
4	+	-	+	+	-	7	14.3
5	-	-	+	+	-	8	0
6	-	+	-	+	-	8	37.5
7	+	+	-	-	+	8	25.0
8	+	-	+	-	-	7	0
9	(d)	-	+	-	-	10	0

(a) Injected 21st post-operative day into adrenalectomized animals, and 1st day into control animals.

(b) 1 mg corticosterone in 0.5 ml. saline injected twice daily beginning one day prior to injection of lipopolysaccharide or saline.

(c) 0.5 ml. saline injected twice daily beginning one day prior to injection of lipopolysaccharide.

(d) This group was sham operated.

Because adrenalectomized animals have very little resistance to the effects of bacterial lipopolysaccharides, it was necessary to use a much smaller dose than in previous work¹¹. Nevertheless, the 0.5-mg dose used here (group 1) is a competent stimulus for the SA₂G reaction in approximately 81 per cent of the animals. In none of these animals was SA₂G still demonstrable 6 days after injection. The results of group 2 show that, in the absence of the adrenal gland, the effectiveness of the lipopolysaccharide as a stimulus for the SA₂G reaction is abolished. This loss of responsiveness following adrenalectomy is largely reversible by the administration of corticosterone as shown by the results of group 3 where 70 per cent of the animals had demonstrable levels of SA₂G. Corticosterone itself is not an effective stimulus for the SA₂G reaction whether administered to adrenalectomized (group 4) or non-adrenalectomized (group 5) animals. The administration of both corticosterone and lipopolysaccharide to non-adrenalectomized animals (group 6) leads to an equivocal result. The number of animals reacting is considerably below that of non-adrenalectomized animals given only lipopolysaccharide (group 1) but above that of adrenalectomized rats treated with the lipopolysaccharide (group 2). That the re-appearance of the SA₂G reaction after administration of corticosterone is not a spurious effect, due to the sodium chloride injected with it, is shown in the results of group 7 where, despite the administration of equivalent amounts of sodium chloride, substantially no restoration of the SA₂G effect was found. The results obtained with this group of animals reinforce those obtained in group 2. Finally, groups 8 and 9 indicate that the mere injection of saline into either an adrenalectomized or a sham-operated animal does not provoke the SA₂G reaction.

It would appear, therefore, that:

(1) Adrenalectomy greatly reduces or abolishes the SA₂G reaction.

(2) This reaction can be restored, at least in part, by replacement therapy using corticosterone.

(3) Corticosterone, while having some effect on the SA₂G reaction, does not itself provoke the reaction.

We conclude from this that the adrenal gland, and presumably the adrenal cortex, is intimately involved in the SA₂G reaction to bacterial lipopolysaccharide, that is, that response of the rat to the administration of bacterial lipopolysaccharide which results in the production of demonstrable levels of SA₂G. We are now attempting to determine whether the involvement of the adrenal gland is a special case in relation to the stimulus used here or whether it represents a common factor in the SA₂G reaction regardless of the nature of the stimulus used.

This work was supported in part by grant GB-2863 from the U.S. National Science Foundation.

WERNER G. HEIM
SANDRA R. ELLENSON

Department of Biology,
Wayne State University,
Detroit, Michigan.

¹ Darcy, D. A., *Brit. J. Cancer*, **11**, 137 (1957).

² Beaton, G. H., Selby, A. E., Veen, M. J., and Wright, A. M., *J. Biol. Chem.*, **236**, 2005 (1963).

³ Heim, W. G., *Nature*, **193**, 491 (1962).

⁴ Wise, R. W., Ballard, F. J., and Esekiele, E., *Comp. Biochem. Physiol.*, **9**, 23 (1963).

⁵ Boffa, G., Fine, J. M., and Zajdela, F., *C.R. Acad. Sci., Paris*, **225**, 802 (1962).

⁶ Heim, W. G., and Lane, P. H., *Nature*, **203**, 1077 (1964).

⁷ Heim, W. G., and Kerrigan, J. M., *Nature*, **199**, 1100 (1963).

⁸ Boffa, G. A., Nadal, C., Zajdela, F., and Fine, J. M., *Nature*, **203**, 1182 (1964).

⁹ Heim, W. G., Kerrigan, J. M., and Lane, P. H., *Nature*, **200**, 688 (1963).

¹⁰ Lawford, D. J., and White, R. G., *Nature*, **201**, 705 (1964).

¹¹ Heim, W. G., and Lane, P. H., *Nature*, **203**, 1077 (1964).

¹² Weimer, H. E., and Benjamin, D. C., *Fed. Proc.*, **23**, 475 (1964).

¹³ Lawford, D. J., *Biochem. Pharmacol.*, **7**, 109 (1961).

¹⁴ Heim, W. G., *Nature*, **207**, 1403 (1965).

HISTOCHEMISTRY

Vital Staining of the Granulated Juxtaglomerular Cells in the Mouse Kidney

THE first reports on vital staining of the granulated cells of the juxtaglomerular system (JG) came from Japanese research workers. Sugiyama¹ used neutral red intravitaly to demonstrate these cells in the kidney of the mouse. Harada² used neutral red and trypan-blue intraperitoneally; paraffin-embedded sections of the kidney were examined after treatment with a bichromate containing fixative. Of the two dyes studied, only the neutral red stained the JG cells.

No data are available as to whether or not other dyes are suitable for staining these cells vitally. In squashed sections of mouse kidney the granulated cells of the preglomerular arteriolar walls are easily detected because of the brilliance of their granules (Fig. 1). Vital staining of these cells can be studied thus in native specimens, without using fixatives, embedding or sectioning.

Adult albino mice of both sexes were used for the experiments. Thirty-eight dyes related to azine, oxazine, thiazine, azo, diazo, phenylmethane and xanthen were used. These were used as 1 per cent solutions, dissolved in physiological saline, and given intraperitoneally and intravenously in 1 ml. volumes. Animals were killed immediately after intravenous injections and 30–60 min after intraperitoneal administration. After killing, squash specimens were immediately prepared from the kidneys; they were examined under a light microscope, and after treatment with acridine-orange by means of fluorescence microscopy (Fig. 3).

Cells of the reticulo-endothelial system and of renal tubuli take up and store acid dyes preferentially³. The JG cells do not take up vitally acid dyes. Positive vital staining was achieved of the JG cells by neutral red-brilliant-cresyl-blue, Nile-blue-sulphate and acriflavine, orange. Staining was most pronounced when the dyes were immediately applied intravenously and also when they were applied intraperitoneally after 1 h (Fig. 2). Some of the dyes tested proved to be toxic. For example, intravenous applications of brilliant-cresyl-blue killed the animals instantly, but the JG cells stained well.

As regards any vital staining mechanism, two factors have to be taken into account: (i) the membrane permeability of the dye; (ii) the physical or chemical affinity of the dye to the granular material.

The membrane permeability of a dye depends on its molecular weight, lipid solubility, and the number of the free methyl groups⁴. The aforementioned four dyes are of basic character, lipid-soluble and possess 2–4 free methyl groups, while the molecular weights vary between 288.7 and 732.8. Seven further basic, lipid-soluble dyes (namely, neutral violet, safranin, azure II, Janus green, gentian violet, crystal violet, Victoria blue) were also attempted. They possess 3–6 free methyl-groups, and their molecular weights vary between 364.8 and 511. As regards membrane permeability, on the whole, they are similar to the aforementioned four dyes; nevertheless they were un-

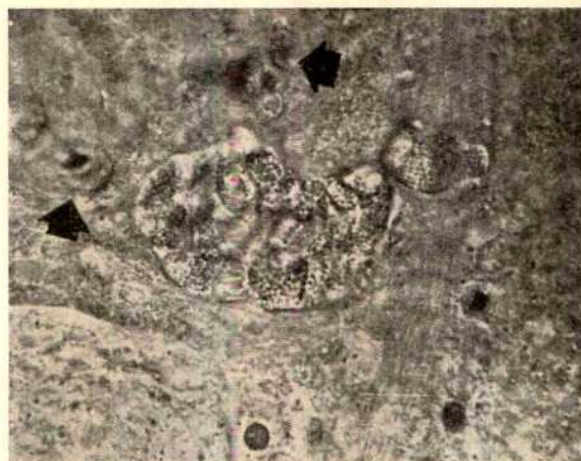


Fig. 1. Squash section of a mouse kidney. The epithelioid cells of the arteriolar wall contain strongly refractile granules. The glomerulus is indicated by arrows. ($\times 1,000$)

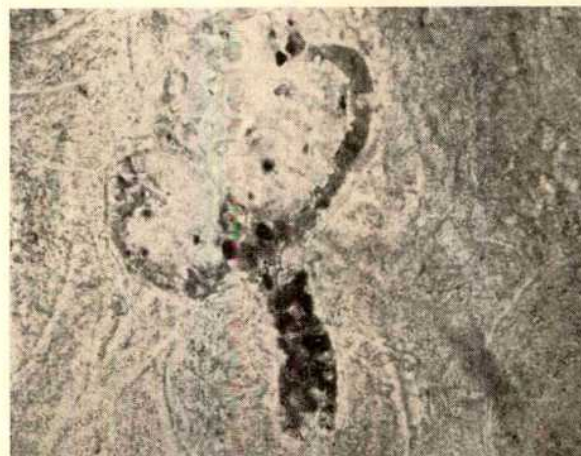


Fig. 2. Squash section prepared immediately after intravenous injection of neutral red. The epithelioid cells contain a large amount of red granules. ($\times 600$)

suitable for inducing vital staining in the granules of the JG cell.

As regards the affinity of the dyes for the JG cells, we have few data to rely on. The staining ability of neutral red was explained by Harada² as being due to the presence of a polysaccharide-sulphate-ester-lipid complex in the granules (Feyrter's chromotrope lipid).

According to Gomba, Soltész and Endes^{5,6} in the granules, neither lipids nor acid polysaccharides were demonstrated histochemically, while large amounts of aromatic amino-acids were found (especially tryptophan and tyrosine). In staining plant cells with neutral red, Boyer⁷ directed attention to the importance of trihydroxyphenols in binding the dye. In animals, similar compounds do not occur, and the connexion between staining of the JG granulated cells and their content of aromatic amino-acids is questionable.



Fig. 3. Squash section prepared 1 h after intraperitoneal injection of acridine orange. The granulated JG cells show a bright yellowish fluorescence. ($\times 200$)

The mechanism of vital staining by alkaline dyes may be explained by the production of salt-bonds between the basic dye and acid cellular parts. With the four dyes, besides the granulated JG cells mast cells were also stained. These cells are known to contain a high concentration of acid groups. According to Strugger⁸ the relation of the isoelectric point of the staining structure to the pH of the medium is important. This relation determines dissociation, that is, the reactive condition of the acid groups in the staining structure. In fixed specimens, the granules of the JG cells are intensively basophilic, which suggests the presence of acid groups. No explanation can be offered, however, for the inability of other alkaline, lipid-soluble dyes, similar in molecular weight and number of free methyl groups to the aforementioned four successful ones, to stain vitally.

V. SZOKOLY
SZ. GOMBA
M. B. SOLTÉSZ

Department of Pathology,
University Medical School,
Debrecen,
Hungary.

¹ Sugiyama, S., cited by Harada in *Stain Technology*, **31**, 206 (1956).

² Harada, K., *Rev. Belge Path.*, **23**, 313 (1954).

³ Jancsó, M., *Speicherung*, Akadémiai Kiadó, Budapest (1955).

⁴ Danielli, J. F., *Cell Physiology and Pharmacology* (Elsevier Publishing Co., New York, Amsterdam, London and Brussels, 1950).

⁵ Gomba, Sz., Soltész, M. B., and Endes, P., *Acta Morph. Acad. Sci. Hung.*, **11**, 189 (1962).

⁶ Gomba, Sz., Soltész, M. B., and Endes, P., *Acta Morph. Acad. Sci. Hung.*, **12**, 239 (1963).

⁷ Boyer, M. G., *Stain Technol.*, **38**, 117 (1963).

⁸ Stragger, S., cited by Frey-Wyssling in *Submicroscopic Morphology of Protoplasm* (Elsevier Publishing Co., Amsterdam, 1953).

IMMUNOLOGY

Thymus and the Production of Antibody-plaque-forming Cells

ALTHOUGH the thymus itself does not play an active part in immune responses¹, its presence is essential for the normal development of immunological faculties. Neonatal thymectomy in many species considerably impairs the capacity of an animal to produce some types of immune responses². Thymectomy in adult life has no immediate effect but, after a period of 6-9 months, reduces the capacity to react to a newly encountered antigen³. The possible mechanisms by which the thymus exerts its influence on the immunological system have been discussed elsewhere⁴, and it has been concluded that the immunological defects encountered after thymectomy are primary and not secondary to infection or auto-immunity⁵. Evidence is presented here to show that thymectomy practically inhibits the development of the capacity to produce antibody-plaque-forming cells following the injection of sheep erythrocytes. This impairment is evident in clinically healthy suckling baby mice thymectomized at birth.

Mice of the inbred strain CBA, F_1 hybrids from crosses between T6 and Ak mice, and non-inbred Swiss (SWS) mice were thymectomized on the day of birth. A sham operation involving thoracotomy, but not removal of the thymus, was performed in litter-mates. The mice were given an intraperitoneal injection of 0.15 ml. of a 5 per cent suspension of sheep erythrocytes in saline on the 10th day of life and killed at intervals of 2 days-3 weeks after injection. Cell suspensions were prepared from their spleens and assayed for the number of antibody-plaque-forming cells by plating out the suspension in agar gel containing sheep erythrocytes according to the technique described by Jerne *et al.*⁶. Routine histological investigations were performed on the thymus area in all thymectomized mice to ensure that thymectomy had

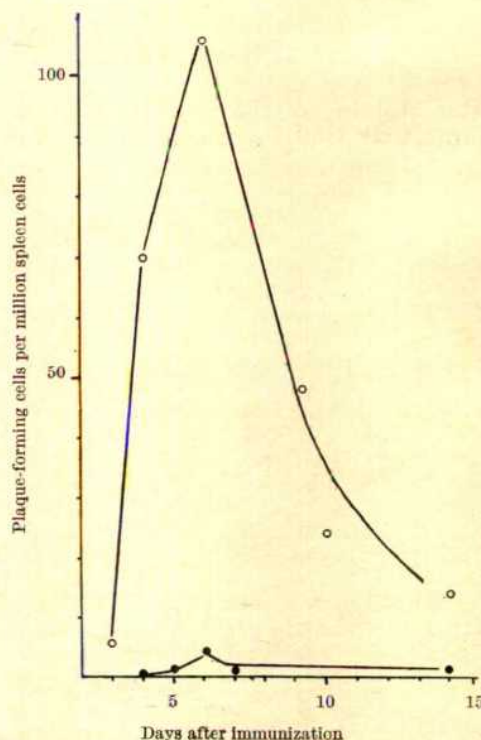


Fig. 1. Number of antibody-plaque-forming cells in spleens of CBA mice at various intervals after immunization with sheep erythrocytes given at 10 days of age. Each point represents the average value of assays on 2-5 mice. ○, Mice sham-thymectomized at birth; ●, mice thymectomized at birth.

Table 1. ANTIBODY PLAQUE FORMATION BY SPLEENS AND THYMUSES OF NORMAL, SHAM-OPERATED AND THYMECTOMIZED MICE

Strain	Treatment given	Cells plated	No. of mice	Age at immunization	Age at death	Antibody-plaque-forming cells per 10^6 cells	
						(Peak) level \pm S.E.	P values
(A \times X76) F_1	Sham-thymectomy at birth	Spleen	8	10 days	14 days	204 \pm 27	<0.001
	Thymectomy at birth	Spleen	10	10 days	14 days	5 \pm 2	
CBA	Sham-thymectomy at birth	Spleen	5	10 days	18 days	106 \pm 6	<0.001
	Thymectomy at birth	Spleen	5	10 days	18 days	4 \pm 2	
SWS	Sham-thymectomy at birth	Spleen	6	10 days	15 days	276 \pm 53	<0.001
	Thymectomy at birth	Spleen	8	10 days	15 days	15 \pm 2	
SWS	None	Spleen	6	56 days*	61 days*	945 \pm 82	Not significant
	Thymectomy at 42 days*	Spleen	6	56 days*	61 days*	901 \pm 65	
	None	Thymus	6	56 days*	61 days*	6 \pm 1	

* Average age.

been complete. Mice with thymus remnants were discarded from the experiments.

The spleens of normal or operated mice aged between 1 week and 4 months and not challenged with sheep erythrocytes gave usually less than 1 antibody-plaque-forming cell per million spleen cells. The results obtained in immunized mice are shown in Figs. 1, 2 and 3 and analysed statistically in Table 1. In sham-thymectomized mice, the number of antibody-plaque-forming cells rose sharply from 2 to 3 days after immunization to reach a peak level at 4, 5 or 6 days, depending on the strain. Thereafter, the number fell rapidly to reach a low, yet significant, level at about 10 days after immunization. By contrast, neonatally thymectomized mice produced very few antibody-plaque-forming cells, the assays being made at intervals from 2 days to 3 weeks after challenge. The difference between the mean peak levels of the thymectomized and control group was highly statistically significant for the three strains of mice used (Table 1).

Thymectomy of 6-weeks-old SWS mice did not interfere with their capacity to produce antibody-plaque-forming cells when immunized at about 9 weeks of age (Table 1). In contrast to the spleens of normal SWS mice immunized with sheep erythrocytes, very few antibody-plaque-forming cells were present in the thymuses of the same mice (Table 1).

The results demonstrate that, in the absence of the thymus from birth, the capacity to produce antibody-plaque-forming cells in response to an injection of sheep erythrocytes has failed to develop. Very few antibody-plaque-forming cells appeared in the spleens during the first 3 weeks after immunization of neonatally thy-

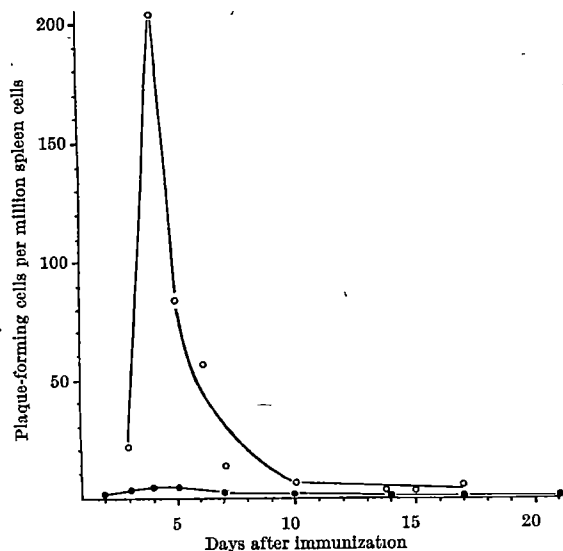


Fig. 2. Number of antibody-plaque-forming cells in spleens of (A \times X76) F_1 mice at various intervals after immunization with sheep erythrocytes given at 10 days of age. Each point represents the average value of assays on 2-10 mice. \circ , Mice sham-thymectomized at birth; \bullet , mice thymectomized at birth.

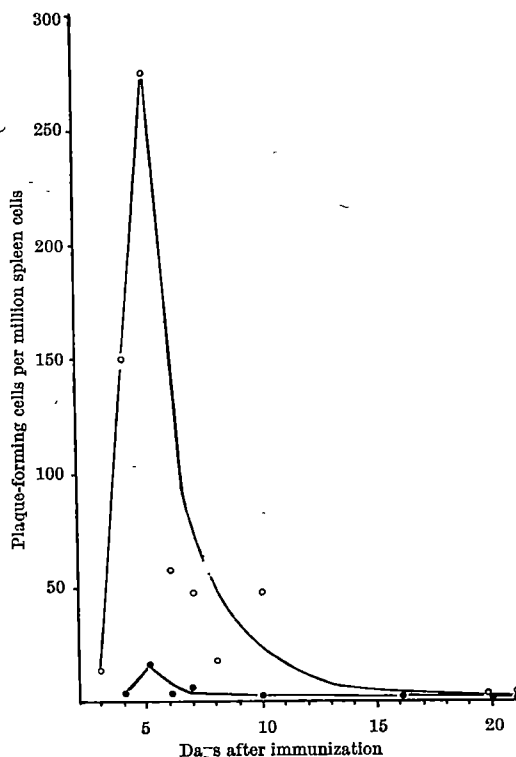


Fig. 3. Number of antibody-plaque-forming cells in spleens of SWS mice at various intervals after immunization with sheep erythrocytes given at 10 days of age. Each point represents the average value of assays on 2-8 mice. \circ , Mice sham-thymectomized at birth; \bullet , mice thymectomized at birth.

mectomized mice of two inbred strains and one non-inbred stock. This deficiency is presumably a primary effect of neonatal thymectomy and not secondary to infection or ill-health: it was evident in very early life in suckling baby mice the growth rate of which was identical to that of litter-mate controls. In a similar experiment it has been reported that colony-bred mice thymectomized at birth also failed to produce normal numbers of antibody-plaque-forming cells when challenged at 4 weeks of age⁷. Thymectomy in adult mice, by contrast, had no immediate effect on the response to sheep erythrocytes although a delayed effect was observed as reported elsewhere⁸. The amount of antibody produced per plaque-forming cells was judged to be within normal limits in the thymectomized mice as estimated by the size of the plaques in our experiments and in those of Takeya *et al.*⁷.

It can be seen that the thymus itself produced only very few antibody-plaque-forming cells (Table 1) after immunization of adult mice. This observation has also been made in other strains of mice⁸. By contrast, specific antibody-plaque-forming cells have been demonstrated in the thymus of rabbits 5 days after a single systemic injection of 5 μ g of somatic polysaccharide of *Salmonella enteritidis*⁹. Since significant numbers of plaque-forming

cells have been detected in the peripheral blood in these rabbits¹⁰, and since radical alterations in the structure of the thymus have been reported after administration of endotoxin¹¹, it is conceivable that circulating antibody-forming cells may have penetrated into the thymus of rabbits immunized with somatic polysaccharide.

In conclusion, it seems that the thymus itself fails to produce significant numbers of antibody-producing cells in response to an antigenic stimulus, but its presence from birth is essential to ensure that such cells will develop in the periphery. Whether the initial development of these cells takes place within the thymus and their final maturation occurs after emigration from the organ cannot be decided on the basis of present evidence. Experiments using thymus tissue enclosed in cell-impenetrable chambers in neonatally thymectomized mice have suggested that a humoral thymus factor plays a part in the maturation of potentially immunologically competent cells¹².

The investigations were supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research, Royal Cancer Hospital) from the Medical Research Council and the British Empire Cancer Campaign for Research, from the Tobacco Manufacturers Standing Committee, and Public Health Service grant CA-03188-08 from the National Cancer Institute, U.S. Public Health Service.

Note added in proof. Since this paper was submitted, similar results obtained by Friedman have been published¹³.

J. F. A. P. MILLER
P. M. DE BURGH
G. A. GRANT

Chester Beatty Research Institute,
Institute of Cancer Research,
Royal Cancer Hospital,
Fulham Road, London, S.W.3.

- ¹ Fagraeus, A., *Acta Med. Scand.*, **130**, Suppl. 204, 3 (1948). Bjorneboe, M., Gormsen, H., and Lundquist, T., *J. Immunol.*, **55**, 121 (1947). Harris, S., and Harris, T. N., *J. Exp. Med.*, **100**, 269 (1954).
- ² Miller, J. F. A. P., *Lancet*, **ii**, 748 (1961). *Proc. Roy. Soc. B*, **156**, 415 (1962). Archer, O. K., Pierce, J. C., Papermaster, B. W., and Good, R. A., *Nature*, **185**, 191 (1962). Jankovic, B. D., Waksman, B. H., and Arnason, B. G., *J. Exp. Med.*, **116**, 159 (1962). Sherman, J. D., Adner, M. M., and Dameshek, W., *Blood*, **23**, 375 (1964).
- ³ Taylor, R. B., *Nature* (following communication). Metcalf, D., *Nature* (following communication). Miller, J. F. A. P., *Nature* (following communication).
- ⁴ Miller, J. F. A. P., *Science*, **144**, 1544 (1964); *Brit. Med. Bull.*, **21**, 111 (1965).
- ⁵ McIntire, K. R., Sell, S., and Miller, J. F. A. P., *Nature*, **204**, 151 (1964). Miller, J. F. A. P., and Howard, J. G., *J. Reticuloendothelial Soc.*, **1**, 369 (1964).
- ⁶ Jerne, N. K., Nordin, A. A., and Henry, C., in *Cell-Bound Antibodies*, edit. by Amos, B., and Koprowski, H., 109 (Wistar Institute Press, Philadelphia, 1963).
- ⁷ Takeya, K., Mori, R., and Nomoto, K., *Proc. Jap. Acad.*, **40**, 572 (1964).
- ⁸ Friedman, H., *Proc. Soc. Exp. Biol.*, **117**, 526 (1964).
- ⁹ Landy, M., Sanderson, R. P., Bernstein, M. T., and Lerner, E. M., *Science*, **147**, 1591 (1965).
- ¹⁰ Landy, M., Sanderson, R. P., Bernstein, M. T., and Jackson, A. L., *Nature*, **204**, 1320 (1964).
- ¹¹ Rowlands, D. T., Claman, H. N., and Kind, P. D., *Amer. J. Path.*, **46**, 165 (1965).
- ¹² Osoba, D., and Miller, J. F. A. P., *Nature*, **199**, 653 (1963); *J. Exp. Med.*, **119**, 177 (1964). Osoba, D., *J. Exp. Med.*, **122**, 633 (1965).
- ¹³ Friedman, H., *Proc. Soc. Exp. Biol.*, **118**, 1176 (1965).

Decay of Immunological Responsiveness after Thymectomy in Adult Life

THE remarkable effects of neonatal thymectomy are by now well documented¹. Primary among these is a failure of the lymphoid tissues to develop properly, which is marked particularly by a deficiency of small lymphocytes. Other features, probably resulting from this, include a defective capacity to perform all kinds of immune responses, and a progressive wasting disease ending in early death.

By contrast, only minimal effects have been found to follow from thymectomy in adult life. In mice, the operation had no effect on growth rate, breeding behaviour, longevity or susceptibility to common infections², although blood lymphocyte counts and lymphoid organ

weights were somewhat depressed³. A wasting disease has been reported in guinea-pigs thymectomized at 150–160 g body-weight⁴. No significant depression of antibody response has been detected after thymectomy of adult rabbits^{5,6}. These results have led to the assumptions that once the lymphoid tissues have been formed, the thymus ceases to perform in its developmental role, and that the function of lymphopoiesis is then taken over by the other lymphoid tissues⁷. Yet no distinct morphological change has been described in the thymus on the attainment of immunological maturity, and it continues its high rate of cell production even in adult life. Indeed, it is still necessary for the recovery of immunological responsiveness after this has been depressed by irradiation⁸, and thus it must be able to resume its lymphopoietic function, if only in response to a stimulus such as might be provided by destruction of lymphoid tissues. Even without this stimulus, however, the thymus must play some part in normal turnover of lymphoid cells, since thymectomy in adult life largely prevents recovery from immunological paralysis^{9,10}. It therefore seemed probable that the lymphoid tissues might not be able to maintain themselves indefinitely in the absence of the thymus, and that if mice were left for a sufficiently long time after thymectomy in adult life they should eventually show a decline in their primary immune responsiveness.

In the first experiment the immune responsiveness of CBA mice was assessed by the ability of their lymphoid cells to cause a graft-versus-host (GVH) reaction after transfer to young (C57BL × CBA)_F₁ hybrid mice. The strength of the GVH response was estimated by the degree of spleen enlargement in the hosts. The 4-point assay procedure outlined by Simonsen¹¹ and developed by Michie¹² was then used to relate the spleen enlargement response to the dose of lymphoid cells, so that a quantitative comparison could be made of the immune potency of cell suspensions made from thymectomized and control mice. CBA female mice were thymectomized (or sham-operated) at 2–3 months of age. Groups of 8–16 mice were tested by GVH assay at intervals up to one year after thymectomy. In each assay the pooled lymph node (cervical, axillary, brachial, inguinal and mesenteric) and spleen cells of one 'test' mouse were compared for immune potency against those from one 'control' mouse. At the intervals 4, 9, 25 and 29 weeks the thymectomized mice were compared with sham-operated controls of the same age. By 52 weeks the potency of these controls might be expected to have declined somewhat through age alone¹³; therefore both thymectomized and sham-operated mice were compared with untreated controls aged 4–6 months. To obtain a measure of the overall responsiveness of the whole 'test' animal as a percentage of the control, each value for the relative potency of a cell suspension was multiplied by the fraction: total number of lymphoid cells obtained from the 'test' mouse/total number of lymphoid cells obtained from the 'control' mouse.

The results of the assays are shown in Fig. 1. The responsiveness of the thymectomized mice remained level with controls for about 25 weeks, then dropped fairly sharply, and thereafter remained about 15 per cent of normal. This fall in responsiveness was due mainly to a fall in the immune potency of the cell suspensions, but also to a fall in the numbers of cells recovered. The responsiveness of sham-operated mice fell only slightly, and at 52 weeks after operation was still about 75 per cent of the 4–6-month-old controls.

In another experiment immune responsiveness was tested by the ability to produce circulating antibody to bovine serum albumin (BSA). The results have been collated from four experiments which were set up with other primary objects in mind. CBA mice of both sexes were thymectomized or sham-operated either at 4 weeks or at 10–14 weeks of age. They were challenged at intervals thereafter by subcutaneous injection of BSA in Freund's adjuvant, and the serum antibody titres estimated 20

and 40 days later by a modification¹⁴ of Farr's antigen-binding-capacity method¹⁵. Since the absolute titres varied from one experiment to another, the titres from thymectomized mice have been presented as a percentage of the controls in each group (Fig. 2). The decay of responsiveness was less obvious than it was in the GVH experiment, but cell-transfer experiments indicate that a small difference in anti-BSA titre can reflect a larger difference in the number of competent cells¹⁶. However, the general pattern was similar in that the decline did not reach its most rapid phase for a considerable time after thymectomy. (The apparent initial fall which can be seen in the mice thymectomized at 4 weeks old reflects an increase in the responsiveness of controls, due to normal growth processes, rather than a decrease in the thymectomized mice.) This rapid phase can be seen at about 10 weeks after thymectomy in the mice thymectomized at 4 weeks old. In those thymectomized at 10–14 weeks old the time varied: in one experiment the responsiveness was still normal at 16 weeks, but in another it had already reached its lower level by 12 weeks after thymectomy (indicated by asterisk) and showed no further fall at 32 weeks. An effect of thymectomy was also seen in the general condition of the mice. While not amounting to a serious wasting disease, it appeared as a distinct ruffled condition of the fur, most obvious on the underside, and was associated with a slight loss of weight.

It may be concluded that even in the adult the thymus has a part to play in the maintenance of the numbers of competent cells in the body. If the effect of thymectomy was to cut off entirely the supply of new competent cells, then the ensuing decay of responsiveness should reflect the rate of loss of the competent cells originally present. According to two simple hypotheses their numbers might be expected to decay in linear fashion if they had a definite life-span, or exponentially if their life depended on chance events—such as encounter with antigens. Support for the latter possibility may be drawn from investigations of irradiated human patients¹⁷ in whom certain of the circulating lymphocytes were distinguishable by the presence of acentric chromosome fragments, which could be seen after stimulation of mitosis *in vitro* with phytohaemagglutinin. During division these fragments fail to become attached to the mitotic spindle, and thus are not transmitted to the daughter cells. The half-time between irradiation and either division or death of the cells was 366 days.

In the present experiments, however, the decay curve of immune responsiveness after thymectomy did not readily fit with either expectation (Fig. 1). In particular,

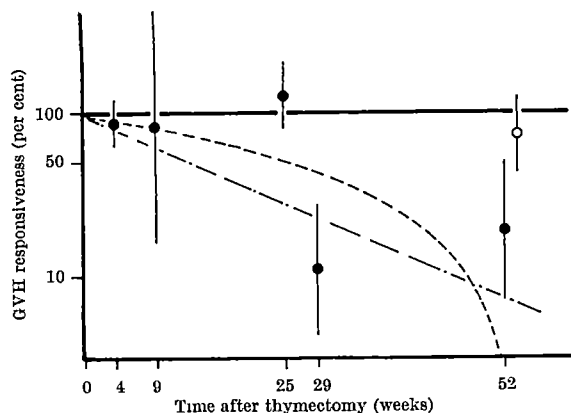


Fig. 1. Results of GVH assays. Each point represents the mean and standard deviation of the responsiveness of thymectomized relative to control mice (computed on log-transformed data). ●, Thymectomized versus sham-operated controls of the same age (4–29 week groups) or versus 4–6 month untreated mice (52-week group); ○, sham-operated (14 months old) versus 4–6 month untreated mice. $P < 0.01$ for difference between 25 and 29 week groups; $P < 0.001$ for difference between thymectomized and sham-operated groups at 52 weeks. Hypothetical linear (—) and exponential (---) decay curves are shown.

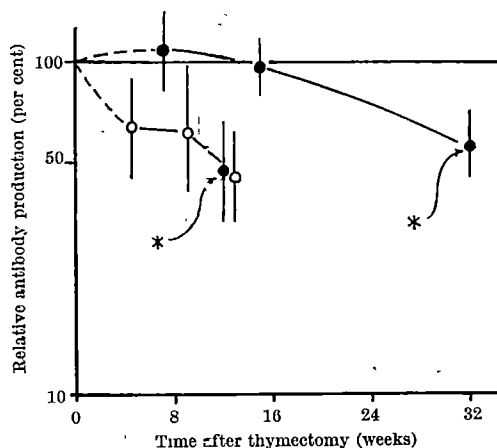


Fig. 2. Results of antibody titrations. Each point represents the mean antibody titre in thymectomized mice as a percentage of that in controls, with standard deviation (computed on log-transformed data). Results from 20 and 40 day bleedings have been combined. Symbols represent four separate experiments: ○, thymectomized at 4 weeks old; ●, thymectomized at 10–14 weeks old. * See text.

although the responsiveness fell to a sub-normal level, it appeared to attain equilibrium by about 7 months after thymectomy, and showed no further decline towards the levels seen after neonatal thymectomy or irradiation. The same was shown by at least one of the BSA experiments (Fig. 2, asterisk). This suggests either that there is a distinct population of cells with a very long life, or that the production of competent cells can continue at a sub-normal rate in the absence of the thymus. In view of the much more severe and permanent depression of responsiveness which can be obtained after neonatal thymectomy, or adult thymectomy followed by irradiation, this latter process would presumably take place only in mature, unirradiated lymphoid tissue.

Although the early part of the curve approximates the linear, rather than the exponential mode of decay, it might still be consistent with an exponential loss of cells if these were being replaced by differentiation from some other cell type. This could be an intermediate form which had already undergone the thymus-dependent phase in its maturation. The eventual exhaustion of such cells could then account for the more rapid fall between 25 and 29 weeks. In conclusion, it is suggested that the thymus continues in adult life to exert a stimulatory influence on the production of competent cells, but that a low rate of production may still occur after thymectomy. It will be of interest to see if this residual responsiveness differs qualitatively from the major fraction, which decays more rapidly after thymectomy.

R. B. TAYLOR

Division of Experimental Biology,
National Institute for Medical
Research,
Mill Hill, London, N.W.7.

- ¹ Good, R. A., and Gabrielsen, A. E., edit. by, *The Thymus in Immunobiology* (Harper and Row, New York, 1964).
- ² Miller, J. F. A. P., *Adv. Cancer Res.*, **6**, 291 (1961).
- ³ Metcalf, D., *Brit. J. Haematol.*, **6**, 324 (1960).
- ⁴ Comsa, C., *Acta Endocr. (Kbh.)*, **26**, 261 (1957).
- ⁵ Harris, T. N., Rhoads, J., and Stokes, J., *J. Immunol.*, **58**, 27 (1948).
- ⁶ Maclean, L. D., Zak, S. J., Varco, R. L., and Good, R. A., *Transpl. Bull.*, **4**, 21 (1957).
- ⁷ Leading article, *Brit. Med. J.*, **ii**, 840 (1962).
- ⁸ Miller, J. F. A. P., *Nature*, **195**, 1318 (1962).
- ⁹ Claman, H. N., and Talmage, D. W., *Science*, **141**, 1193 (1963).
- ¹⁰ Taylor, R. B., *Immunology*, **7**, 595 (1964).
- ¹¹ Simonsen, M., *Prog. Allergy*, **6**, 349 (1962).
- ¹² Michle, D. (personal communication).
- ¹³ Makinodan, T., and Peterson, W. J., *Proc. U.S. Nat. Acad. Sci.*, **48**, 234 (1962).
- ¹⁴ Mitchison, N. A., *Proc. Roy. Soc.*, **B161**, 275 (1964).
- ¹⁵ Farr, R. S., *J. Infect. Dis.*, **109**, 239 (1958).
- ¹⁶ Taylor, R. B. (unpublished observations).
- ¹⁷ Norman, A., Sasaki, M. S., Ostman, R. T., and Fingerhut, A. H., *Science*, **147**, 745 (1965).

Delayed Effect of Thymectomy in Adult Life on Immunological Competence

NEONATAL thymectomy leads to the rapid development of lymphoid organ atrophy and well-characterized immunological deficiencies¹⁻³, but thymectomy performed in adult life leads only to the slow development of a moderate degree of lymphoid atrophy⁴ with no loss of immunological competence when the animals are tested immediately after operation^{5,6}. However, adult thymectomy combined with whole-body irradiation does lead to immunological deficiencies of the same general nature as those following neonatal thymectomy⁷. Recent investigations have shown that continuous repopulation of normal haemopoietic organs occurs in normal life^{8,9}. This suggests that whole-body irradiation by causing cell damage and repopulation in haemopoietic organs may merely accelerate a process which occurs continually throughout life, albeit at a much slower rate. These considerations prompted a re-examination of the long-term effects of adult thymectomy on immunological competence.

Six-week-old mice of the long-lived (*AKR* × *C57BL*)_F₁ strain were thymectomized or sham-operated and challenged 1 week, 11 months or 18 months after operation with intraperitoneal sheep red cells.

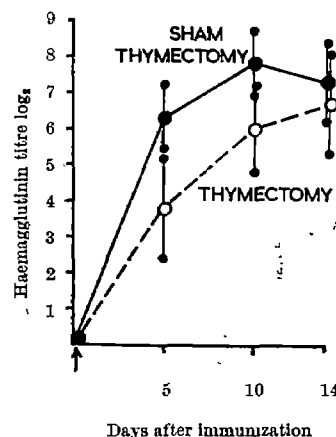


Fig. 1. Haemagglutinin titres in (*AKR* × *C57BL*)_F₁ mice tested 11 months after thymectomy or sham-operation at six weeks of age. No. of mice: thymectomy 23, sham-thymectomy 31. Bars represent standard deviations.

The present results re-emphasize that the thymus has a continuing influence on immunological responsiveness throughout adult life, but do not indicate the processes

Table 1. PRIMARY HAEMAGGLUTININ RESPONSE OF (*AKR* × *C57BL*)_F₁ MICE TO SHEEP RED BLOOD CELLS

Type of mouse	No. of animals	Haemagglutinin titres (log ₁₀)										
		0	1	2	3	4	5	6	7	8	9	10
Normal 2 months old. No antigen	14	12		1			1					
Normal 18-20 months old. No antigen	18	18										
Thymectomy + antigen. 1 week after operation	13											
Sham-thymectomy + antigen. 1 week after operation	14											
Thymectomy + antigen. 11 months after operation	23			1		3	2	6	10	1	6	3
Sham-thymectomy + antigen. 11 months after operation	31											
Thymectomy + antigen. 18 months after operation	13	6	1				2	1	13	13	3	1
Sham-thymectomy + antigen. 18 months after operation	48				4	4	4	7	11	12	3	2

Immunizing dose: 0.2 ml. of 20 per cent R.B.C. i.p. Sera titrated on day 10 following immunization.

Haemagglutinin titres in mice challenged immediately after thymectomy did not differ from those in control mice (Table 1). However, in mice tested 11 months after thymectomy, some lower titres were found, particularly in the early (19S) phase of the response (Fig. 1). In mice tested 18 months after operation, approximately half the thymectomized mice produced no detectable haemagglutinins (Table 1), the remainder producing titres within the control range. The titres in the thymectomized mice challenged 18 months after operation corresponded almost exactly with those observed by Miller⁷ in young adult mice subjected to thymectomy and 350-r. whole-body irradiation. The spleen is the major source of haemagglutinin-production (Metcalf, unpublished results) and in the immunized thymectomized mice challenged 18 months after operation, a general disorganization of the structure of the spleen lymphoid nodules was observed with an obvious decrease in the numbers of germinal centres and small lymphocytes and a diminished number of pyroninophilic mitotic cells.

The observations recorded here suggest that simple thymectomy performed on adult mice does lead to demonstrable immunological deficiencies in at least some mice, provided sufficient time is allowed to elapse before the animals are tested. The present results are only preliminary and need confirmation with other mouse strains and other antigens. The irregular nature of the loss of immunological competence in individual mice following thymectomy, suggests a deletion or loss of responsiveness in some mice of clones of cells capable of responding to the antigen used, this loss apparently being on a random basis and time dependent.

The thymus is known to produce the humoral factor L.S.F. throughout life¹⁰ and a humoral influence of the thymus has been shown to be necessary for the full responsiveness of competent cells following antigenic stimulation^{11,12}. Since there is continuous spleen and lymph node cell repopulation throughout life, the thymus could also serve as a production site for immunologically competent cells, continually reseeding the peripheral organs.

This work was supported by the Carden Fellowship Fund of the Anti-Cancer Council of Victoria.

DONALD METCALF

Cancer Research Laboratory,
Walter and Eliza Hall Institute,
Post Office Royal Melbourne Hospital,
Victoria, Australia.

¹ Miller, J. F. A. P., *Lancet*, ii, 748 (1961).

² Miller, J. F. A. P., *Science*, 144, 1544 (1964).

³ Martinez, C., Kersey, J., Papermaster, B. W., and Good, R. A., *Proc. Soc. Exp. Biol.*, 109, 193 (1962).

⁴ Metcalf, D., *Brit. J. Haematol.*, 6, 324 (1960).

⁵ Fichtelberg, K. E., Laurell, G., and Philipsson, L., *Acta Pathol. Microbiol. Scand.*, 51, 81 (1961).

⁶ MacLean, L. D., Zak, S. J., Varco, R. L., and Good, R. A., *Transpl. Bull.*, 4, 21 (1957).

⁷ Miller, J. F. A. P., *Nature*, 195, 1318 (1962). Miller, J. F. A. P., Doak, S. M. A., and Cross, A. M., *Proc. Soc. Exp. Biol.*, 112, 785 (1963).

⁸ Harris, J. E., Barnes, D. W. H., Ford, C. E., and Evans, E. P., *Nature*, 201, 884 (1964).

⁹ Metcalf, D., and Wakonig-Vaartaja, R., *Proc. Soc. Exp. Biol.*, 115, 731 (1964). Metcalf, D., and Wakonig-Vaartaja, R., *Lancet*, i, 1012 (1964).

¹⁰ Metcalf, D., *Brit. J. Cancer*, 10, 442 (1956).

¹¹ Osoba, D., and Miller, J. F. A. P., *Nature*, 199, 653 (1963).

¹² Levey, R. H., Trainin, N., and Law, L. W., *J. Nat. Cancer Inst.*, 31, 199 (1963).

Effect of Thymectomy in Adult Mice on Immunological Responsiveness

THYMECTOMY in adult animals has been associated with a lowering of the population of lymphocytes in blood, thoracic duct lymph, lymph nodes and spleen^{1,2}. However, no significant defects in the capacity for rejecting allogeneic skin homografts² or for producing serum antibodies^{2,3} have been observed in animals thymectomized in adult life and challenged within 1-2 months after thymectomy. This is in marked contrast to the severe immunological defects which occur following neonatal thymectomy⁴. These observations have suggested that, during early life, the thymus is essential for the complete and normal development of some immunological faculties. Evidence that the function of the thymus in initiating immunogenesis is not necessarily restricted to early life has, however, been produced. Adult mice thymectomized and afterwards exposed to total body irradiation have shown severe deficiencies in immunological functions^{5,6} indicating that the thymus in the adult is still essential to re-establish immune mechanisms when the immunological apparatus has been damaged or destroyed. It has been shown, furthermore, that thymectomy in adult immunologically tolerant mice prevented the reappearance of reactivity with respect to that antigen⁶. The thymus in the adult would thus appear to be essential for the correction of specific immunological defects. The work described here gives further evidence for a continuing immunological function of the thymus through adult life.

from a given donor to produce a graft-versus-host (GVH) reaction was determined by Simonsen's method of spleen assay⁸. The relative spleen weight (mg spleen/10 g body-weight) of the injected baby mice was determined at 10 days of age. Spleen indices were calculated by dividing the average relative spleen weights of the cell-injected mice by the relative spleen weights of the litter-mate controls. The average spleen index of 36 control mice was 1.00 ± 0.13 and animals with indices ≥ 1.5 were considered to show definite evidence of a GVH reaction. The results shown in Table 2 indicate that lymphoid cells from thymectomized mice are as effective as cells from control mice in producing a GVH reaction when collected up to 6 months after thymectomy. After a period of 6-9 months, cells from only 4 of 14 thymectomized mice were effective. Cells from 3 of 8 sham-thymectomized mice aged more than 1 year were ineffective in producing a GVH reaction possibly as a result of the decline of immunological capacity which occurs with advancing age⁹. In the thymectomized group, cells from only 2 of 16 mice killed 9-14 months after thymectomy produced signs of GVH reaction.

Histological examination of the spleen and lymph nodes of old thymectomized animals revealed a striking depletion of cells in the periarteriolar lymphocytic sheaths and primary lymphoid follicles.

It is now well established that some small lymphocytes are long-lived cells¹⁰ capable of initiating graft-versus-host reactions and possibly other types of immunological reactions¹¹. The results presented here demonstrate that thymectomy of adult mice is associated with a decline in

Table 1. EFFECT OF THYMECTOMY IN ADULT LIFE ON THE PRODUCTION OF ANTIBODY-PLAQUE-FORMING CELLS IN THE SPLEEN

Strain	Treatment at 2-3 months	Age (months) at immunization	No. of mice	No. of mice * showing following No. of antibody-plaque-forming cells per 1,000,000 spleen cells:				
				0-100	101-300	301-500	501-800	801-1,200
<i>(Ak × T6)F₁</i>	Sham-thymectomy	4	5					5
	Thymectomy	4	6					6
<i>CBA</i>	Sham-thymectomy	9	6					6
	Thymectomy	9	8			6	2	4
	Sham-thymectomy	4	4					4
	Thymectomy	4	4					4
	Sham-thymectomy	18	5			1	4	
	Thymectomy	18	7	1	4	1	1	
	Sham-thymectomy	24	3			2		
	Thymectomy	24	3	3				

* Killed at time of peak titre after immunization.

In one experiment inbred *CBA* mice and *(Ak × T6)F₁* mice were thymectomized at 2-3 months of age and challenged at various intervals up to 2 years after thymectomy with 0.3 ml. of a 5 per cent suspension of sheep erythrocytes in saline. 4-6 days after challenge, the mice were killed and their spleens removed to assay the number of antibody-plaque-forming cells per million spleen cells, according to the technique described by Jerne *et al.*⁷. Lymph nodes, a small part of the spleen and the thymus area were also removed and kept for histological examination. The results of the plaque assays are shown in Table 1. There was no difference in the capacity of thymectomized and sham-thymectomized mice to produce antibody-plaque-forming cells when injected with sheep erythrocytes 2 months after the operation. However, thymectomized mice challenged 9 or more months after operation produced less antibody-plaque-forming cells than controls.

In another experiment, *C3Hf/BI* mice were thymectomized or sham-operated at 2-4 months of age and killed from 4 to 60 weeks later. Cell suspensions were prepared from the spleens and mesenteric lymph nodes and 5-6 million cells in 0.05 ml. buffered Ringer phosphate solution were injected intravenously into new-born (*C3Hf × C57BL*)*F₁* mice. Half of each litter (about 3-4 mice) received the cells and the other half received only the buffered solution. The capacity of the cell suspensions

immunological capacity which becomes significant after a period of 6-9 months. They suggest that the thymus continues to function during adult life in order to maintain an adequate pool of immunologically competent cells. Thymectomy in the adult, unlike thymectomy in the new-born, has no immediate effect on immunological capacity, presumably because an adequate pool of competent cells has already been constructed. Only when the pool becomes depleted, owing to the limited life span of its cells, do immunological defects with respect to newly encountered antigens become evident.

Table 2. EFFECT OF THYMECTOMY IN ADULT LIFE ON THE CAPACITY OF LYMPHOID CELLS TO PRODUCE GRAFT-VERSUS-HOST REACTIONS

Treatment at 2-4 months	No. of mice with lymphoid cells capable of producing GVH reactions* at following periods (weeks) after operation			
Sham-thymectomy	4-25	26-40	41-60	
Thymectomy	5/8	7/8	5/8	
	12/13	4/14	2/16	

* Expressed as fraction of mice with cells producing, in new-born recipients, an average spleen index ≥ 1.5 .

This work was supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research, Royal Cancer Hospital) from the Medical Research Council and the British Empire Cancer Campaign for Research, from the Tobacco Manufacturers Standing Committee,

and from the National Cancer Institute, U.S. Public Health Service (grant CA-03188-08).

J. F. A. P. MILLER

Chester Beatty Research Institute,
Institute of Cancer Research,
Royal Cancer Hospital,
Fulham Road, London, S.W.3.

¹ Metcalf, D., *Brit. J. Haematol.*, **6**, 324 (1960). Bierring, F., *Ciba Found. Symp. on Haemopoiesis: Cell Production and its Regulation*, edit. by Wolstenholme, G. E. W., and O'Connor, M., 185 (Churchill, London, 1960).

² Miller, J. F. A. P., Doak, S. M. A., and Cross, A. M., *Proc. Soc. Exp. Biol.*, **112**, 785 (1963).

³ Harris, T. N., Rhoads, J., and Stokes, J. A., *J. Immunol.*, **58**, 27 (1948). MacLean, L. D., Zak, S. J., Varco, R. L., and Good, R. A., *Transplant. Bull.*, **4**, 21 (1957). Fichtelius, K. E., Laurell, G., and Philipsson, L., *Acta Path. Microbiol. Scand.*, **51**, 81 (1961).

⁴ Miller, J. F. A. P., *Lancet*, **ii**, 748 (1961). *Proc. Roy. Soc. B*, **156**, 415 (1962). Good, R. A., Dalmasso, A. P., Martinez, C., Archer, O. K., Pierce, J. O., and Papermaster, B. W., *J. Exp. Med.*, **116**, 773 (1962).

⁵ Miller, J. F. A. P., *Nature*, **195**, 1318 (1962). Globerson, A., Fiore-Donati, L., and Feldman, M., *Exp. Cell. Res.*, **28**, 455 (1962).

⁶ Claman, H. N., and Talmage, D. W., *Science*, **141**, 1193 (1963). Taylor, R. B., *Immunology*, **7**, 595 (1964).

⁷ Jerne, N. K., Nordin, A. A., and Henry, C., in *Cell-Bound Antibodies*, edit. by Amos, B., and Koprowski, H., 109 (Wistar Institute Press, Philadelphia, 1963).

⁸ Simonsen, M., *Prog. Allergy*, **6**, 349 (1962).

⁹ Makinodan, T., and Peterson, W. J., *Proc. U.S. Nat. Acad. Sci.*, **48**, 234 (1962).

¹⁰ Little, J. R., Brecher, G., Bradley, T. R., and Rose, S., *Blood*, **19**, 236 (1962).

¹¹ Gowans, J. L., McGregor, D. D., Cowen, D. M., and Ford, C., *Nature*, **196**, 651 (1962).

Rapid Detection and Titration of Antihuman Serum Albumin Antibody

HUMAN serum albumin (HSA) is an antigen frequently used for the examination of the synthesis of antibody in various species. The techniques usually utilized are variants of either precipitin or haemagglutinating methods. Although these procedures are extremely sensitive, both suffer from the disadvantage of requiring a time-interval between the addition of reagents and final reading of the results. The haemagglutinating techniques, furthermore, require absorption, frequently multiple times, of the sera with the red cells which are used in the testing method. The following technique, a slight modification of a method described¹, has proved to be reproducible, rapid, and to have the sensitivity of tanned red cell haemagglutination. Ten ml. of bentonite stock solution prepared according to the method of Bozicevich *et al.*¹ is centrifuged at 1,800 r.p.m. for 15 min. The pellet is re-suspended in 1 ml. of distilled water and an equal volume of a 1 per cent saline solution of human serum albumin is then added. The mixture is shaken gently and permitted to incubate at room temperature for 15 min. One ml. of 0.1 per cent aqueous methylene blue and 15 ml. of distilled water is added, the suspension shaken, and after 10 min recentrifuged for 10 min. The pellet is re-suspended and washed twice in 10 ml. of distilled water. The final suspension is made up with 5 ml. with distilled water. A suspension so prepared and stored at 4° C is shaken gently before use. The flocculating characteristics are retained for periods of two weeks with no change in titre.

The agglutination procedure is performed by adding one drop of the test serum to one drop of the bentonite suspension and centrifuging for 2 min in a 'Sero-fuge'. A positive test is observed as varying degrees of clumping after gently flicking the tube, and is most easily read over a fluorescent white lamp. Titrations may be carried out using a calibrated loop containing as little as 0.025 ml. with serial transfer of the serum to be tested into 10 × 75 ml. test-tubes containing saline. A fixed volume of bentonite, for example, 0.025 ml., is added and the centrifugation carried out as previously noted. Inhibition investigations with 1 per cent albumin added first to the serum followed by the bentonite have been successful. The serum

may be tested for the presence of macroglobulin and the titre of 19 and 7S antibody estimated by the addition of 2-mercaptoethanol followed by incubation for 30 min at 37° C before adding the bentonite.

Equivalent titres of antibody are obtained when the same dilutions of antisera are compared utilizing the foregoing technique and the tanned red cell haemagglutination procedure with a microtitrator².

The technique is recommended for its simplicity and rapidity of results. Once the bentonite has been sensitized the entire titration may be performed and results obtained within a matter of minutes, thus avoiding the tedious and technical difficulties of precipitin and haemagglutination procedures. Both rat and rabbit serum have been tested and yielded satisfactory results. Other substances such as bovine and human γ -globulins have not resulted in satisfactory specific agglutination patterns. It is thus likely that the success of this simplified procedure results from propitious physico-chemical properties of human serum albumin.

This work was supported by the Health Research Council of the City of New York (I-286 and U-1426).

MARK S. TOBIN

Department of Medicine,
New York Medical College,
Flower and Fifth Avenue Hospitals,
New York.

¹ Bozicevich, J., Bunim, J. J., Freund, J., and Ward, S. B., *Proc. Soc. Exp. Biol. and Med.*, **97**, 180 (1958).

² Sever, J. L., *J. Immunol.*, **88**, 320 (1962).

PATHOLOGY

Virus-like Particles in Sub-acute Sclerosing Encephalitis

SUB-ACUTE sclerosing encephalitis (van Bogaert) is a fatal disease of the central nervous system of uncertain origin, usually affecting children and adolescents. The pathological features of this disease are diffuse inflammatory cell infiltrates in the white and grey matter, gliosis, and demyelination¹. Frequently, Type A intranuclear inclusion bodies have been observed in neurones of cases running a short clinical course (Dawson's inclusion body encephalitis) while in the more chronic cases these bodies are less frequently found. Although often a viral aetiology has been suggested, the cause of this disease remains obscure, since animal inoculations of brain tissue have been unsuccessful in transmitting the disease¹.

To our knowledge, ultrastructural investigations in this disease have not been made. This electron microscopic examination of a cortical biopsy from a case of sub-acute sclerosing encephalitis has revealed a large number of virus-like particles in the cytoplasm of astrocytes in the white matter; similar virus-like particles in the cytoplasm of neurones in the grey matter were not seen.

The patient was a nine-year-old Negro female with a fifteen-month history of progressive mental deterioration, difficulty in holding objects with her hands, difficulty with gait, slurring of speech, fine tremors, enuresis, a twelve-month history of generalized seizures and a ten-month history of precocious puberty. These were followed by progressive motor and mental deterioration and transitory migratory seizures involving various extremities and facial muscles. Her history had been that of progressive deterioration with double incontinence, inability to recognize her surroundings, and inability to control arms and hands. On physical examination she was unresponsive to all but painful stimuli. There was gross cachexia, an indwelling Foley catheter, and a naso-gastric feeding tube in place. Gross postural contractures of all four extremities were present. The head was normal size, and there was increased rigidity of the neck with no adenopathy. The pupils were equal, but did not react to light. There was

bilateral optic atrophy. The maculae were within normal limits. There was no response to threat or visual stimuli. There was early breast development and pubic hair was present.

There was gross wasting and atrophy of all four extremities with an increased spastic rigidity, right greater than left. At this time, no spontaneous movements or fasciculations of the extremities were noted. The biceps and triceps deep tendon reflexes were hyperactive and equal. The patellar and ankle reflexes were absent. The Babinski responses were positive bilaterally. Gross myoclonic movements were noted on afferent stimuli.

In the absence of macular changes and the presence of myoclonus seizures, and a fifteen-month history, a diagnosis of sub-acute inclusion body encephalitis was entertained.

A cerebral biopsy, including grey and white matter, obtained through an open craniotomy, was immediately fixed in buffered formalin and osmium tetroxide. Neuropathological examination revealed extensive loss of neurones, and infiltrates of round cells in the Virchow-Robin spaces in grey and white matter (Fig. 1). Proliferation of fibrous

and protoplasmic astrocytes was noted in the deep layers of the cortex and in the white matter (Fig. 2). In the white matter, the astroglial proliferation was extensive, the number of oligodendroglial cells was reduced, and myelin sheaths were practically absent; numerous histiocytes containing sudanophilic material were also noted. Intracellular or intracytoplasmic inclusion bodies were not observed. The leptomeninges were unremarkable.

Under the electron microscope, numerous virus-like particles were seen in the cytoplasm of astrocytes (Fig. 3). The particles were made of a central dense osmiophilic core or nucleoid, about 300 Å in diameter, surrounded by a clear halo 50 Å thick, which in turn is enclosed by a single membrane also 50 Å in thickness. The diameter of the entire particle was 500 Å which is somewhat larger than the size of Coxsackie and poliomyelitis virus (300 Å), and in the size range of the equine encephalomyelitis virus (500 Å)².

The ultrastructural features of these particles are quite similar to that of known viruses; for example, there is a striking resemblance between the fine structure of the virus of the avian sarcoma-leukosis complex as revealed by Haguenau and Beard and the bodies seen in this biopsy³. The fibrillar material in the cytoplasm of the astrocytes probably is not related to the virus-like particles since it has already been identified in other cortical biopsies where astrocytosis is prominent⁴.

The characterization of these virus-like particles, and their role in the pathogenesis of the disease, are now being investigated.

The demonstration of particles of probable viral nature in a human disease of the central nervous system of sub-acute or chronic course is of interest since recovery of viruses has been achieved in animals suffering from chronic central nervous system diseases of degenerative or demyelinating nature, such as scrapie, visna and Aleutian disease of mink⁵. The probable role of a slow virus in the pathogenesis of chronic disease of the central nervous system of man should be re-investigated in view of the findings recorded here⁵.

This work was supported by U.S. Public Health Service grants NB 05572-01 and NB 04613-02.

NICHOLAS K. GONATAS
G. MILTON SHY

Departments of Neurology and Pathology
(Neuropathology),
University of Pennsylvania,
Philadelphia.

¹ Greenfield's *Neuropathology*, 206 (Williams and Wilkins Co., 1963).

² Rivers, M. T., and Horsfall, F. L., jun., *Viral and Rickettsial Infections of Man*, 13 (J. B. Lippincott, Philadelphia, 1959).

³ Haguenau, F., and Beard, J. W., *The Avian Sarcoma-Leukosis Complex in Tumours induced by Viruses: Ultrastructural Studies*, edit. by Dalton, A. J., and Haguenau, F., 45 (Academic Press, New York and London, 1962).

⁴ Gonatas, N. K., Terry, R. D., and Weiss, M., *Trans. Amer. Neurological Assoc.*, 89, 13 (Springer, Inc., New York, 1964).

⁵ Brody, A. J., Hadlow, W. J., Hotchin, J., Johnson, R. T., Koprowski, H., and Kurland, L. T., *Science*, 147, 1114 (1965).

Differential Inhibition of Deoxyribonucleic Acid and Ribonucleic Acid Synthesis induced in Cultured Mammalian Cells by 7:12-Dimethylbenzanthracene

THE mammalian cell genome has frequently been implicated as the specific site of chemical carcinogen-cell interaction. Notable were the findings of Brookes and Lawley¹, who demonstrated a positive correlation between the degree of binding of aromatic hydrocarbons to mouse skin DNA *in vivo* and the carcinogenic potency of these agents. Certain hydrocarbon carcinogens suppress interferon synthesis in virus-infected cells² and appear to be similar in activity to actinomycin D (ref. 3) in their ability to inhibit the replication of DNA rather than RNA viruses. The work recorded here concerns the probable specific

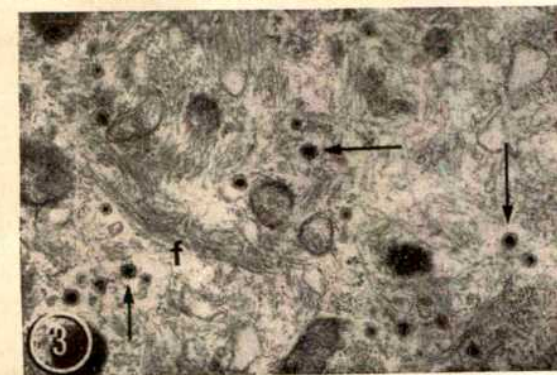
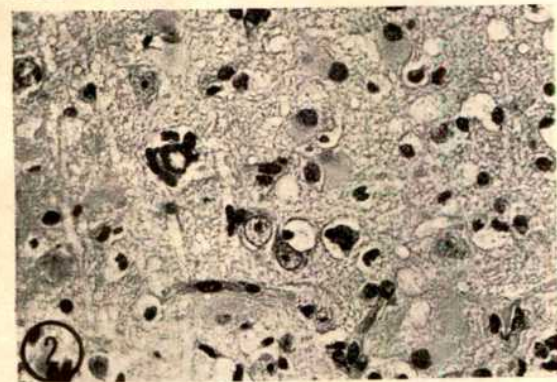


Fig. 1. Perivascular infiltrates of round cells in white matter. ($\times 300$)

Fig. 2. White matter. Protoplasmic astrocytes and histiocytes (pale cytoplasm). ($\times 300$)

Fig. 3. White matter. Electron micrograph from cytoplasm of an astrocyte. Numerous virus-like particles are seen, three indicated by arrows. Bundles of 70 Å thick fibrils are also seen in amplex numbers. ($\times 28,000$)

- alterations in DNA replication induced in cultured normal cells treated with low concentrations of 7:12-dimethylbenzanthracene (DMBA) soluble in the culture medium. The rates of incorporation of labelled uridine and thymidine were measured in several cell lines and correlated to the cell cycle and total DNA formation.

Unlabelled or tritiated DMBA (0.1 mg; sp. act. = 14,500 mc./mmole) dissolved in acetone was deposited in light-shielded flasks and the solvent was evaporated off over an 80° C water bath. The particles were shaken in a complete medium (CM) consisting of 90 per cent Eagle's essential medium + 10 per cent calf serum at 37° C for 72 h. After the shaking period the insoluble particles were removed by Seitz filtration, and the radioactivity was measured by liquid scintillation (^3H = 7–10.5 $\mu\text{C./ml.}$; DMBA = 0.5–0.75 μM). Cell preparations obtained from 4–5-day-old secondary monolayer cultures of mouse (BALB/c) or hamster embryo tissues were suspended either in CM alone (control) or CM + ^3H -DMBA (1.5 $\mu\text{C./ml.}$ = 0.12 μM). In alternate experiments, cells were suspended in CM + cold DMBA (0.12 μM). Replicate cultures ($2.5\text{--}4.0 \times 10^6$ cells/100-mm plastic dish) were incubated at 37° C in a humidified carbon dioxide incubator. At varying intervals duplicate dishes of cell monolayers were pulse labelled with serum free Eagle's medium, containing 1 $\mu\text{C./ml.}$ of either uridine-2- ^{14}C (sp. act. = 1 mc./mmole), thymidine-2- ^{14}C (sp. act. = 30 mc./mmole) or thymidine- ^3H (sp. act. = 13,000 mc./mmole) according to Levine *et al.*⁴. Radioactivity was determined by liquid scintillation and DNA was analysed by the diphenylamine action⁵.

In previous experiments⁶, phenol isolated DNA from ^3H -3:4-benzo(a)pyrene treated hamster kidney cells in culture had a specific activity of 37.5 $\mu\text{C./g.}$ This activity corresponded to 0.8–1.0 molecule of the hydrocarbon/DNA molecule of 20,000 nucleotide units, a value which closely approximated the binding level of ^3H -3:4-benzo(a)pyrene to mouse skin DNA *in vivo*¹. Control and ^3H -DMBA-treated mouse embryo cells pulsed with uridine- ^{14}C gave very similar incorporation rates of this nucleoside. In fact, the rate of incorporation of uridine- ^{14}C by carcinogen-treated cells (Fig. 1) was slightly elevated within the first 8 h following treatment. During this period the cell division mechanism was significantly impaired, although some cells apparently did divide and the survivors were viable, as evidenced by outgrowth on sub-culture in carcinogen-free CM.

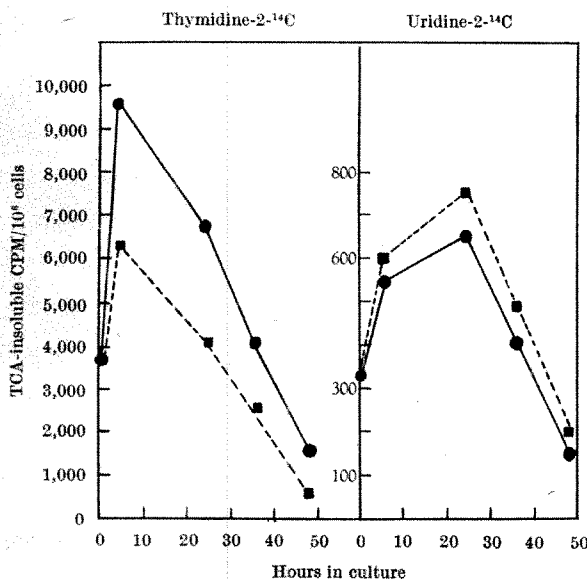


Fig. 1. The effect of ^3H -DMBA (1.5 $\mu\text{C./ml.}$; 0.12 μM) on the incorporation of uridine- ^{14}C and of thymidine- ^{14}C by mouse embryo cell cultures. Time zero = time at which CM (alone) was removed from the monolayers (2 h) and the cultures then overlaid with either CM alone or CM + DMBA. —, CM alone; ---, CM + DMBA.

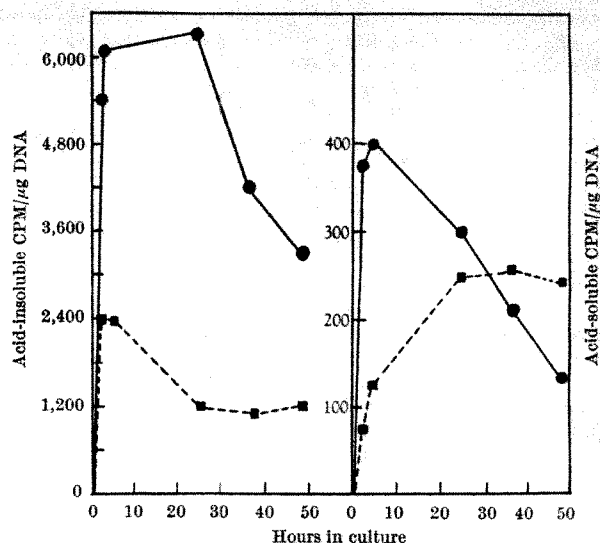


Fig. 2. Effect of DMBA (0.12 μM) on the incorporation of thymidine- ^3H by secondary hamster embryo cell cultures. —, CM alone; ---, CM + unlabelled DMBA.

In sharp contrast to a lack of inhibition of uridine incorporation DMBA induced a marked suppression on the rate of thymidine- ^{14}C incorporation (Fig. 2) within 2 h following mouse cell-carcinogen interaction. A measurement of the rate of incorporation of thymidine- ^3H into the acid-soluble and acid-insoluble fraction of hamster embryo cells growing in the presence of cold DMBA (0.12 μM) again demonstrated the very early inhibition of DNA synthesis. On the other hand, the DNA content per cell (corrected for viability by vital staining with 0.02 per cent nigrosin) was usually about 30–33 per cent higher in DMBA treated cells than in control cultures. The summarized results of three separate experiments are listed in Table 1.

Table 1. EFFECT OF ^3H -DMBA (1.5 $\mu\text{C./ML.}$) ON THE RATE OF FORMATION OF HOT TCA-EXTRACTABLE DNA BY MOUSE EMBRYO CELLS

Culture medium	Hours in culture	Cell No. $\times 10^6$ /dish	CPM ^3H -DMBA per 10^6 cells	$\mu\text{g DNA per } 10^6$ cells
CM alone	0	2.5	—	7.5
"	24	3.5	—	12.0
"	48	4.0	—	13.5
"	72	6.5	—	5.5
CM + ^3H -DMBA	0	2.5	—	7.5
"	24	1.6	3.5×10^5	16.0
"	48	1.5	2.0×10^5	19.0
"	72	1.8	2.1×10^5	8.0

A correlation can be drawn between the time of maximum uptake of chemical carcinogen in culture⁷ and the suppression of labelled thymidine incorporation in these experiments. These observations are in agreement with the findings on the *in vivo* depression of DNA synthesis by DMBA⁸. As cell division was inhibited by DMBA during the first 24 h of growth, the increase in DNA per cell suggests that the depressive activity of this carcinogen occurred in the pre-mitotic phase of growth. It is very likely that the surviving 'DMBA-cells' lose the control mechanism for DNA replication at some point between the S (DNA synthesis) and the G₂ phases of the cell cycle. It is possible that this carcinogen, in complexing with DNA, induces the formation of new primer sites which may be active in further DNA replication without cell division. The foregoing mechanism was recently proposed for the action of nitrogen mustard on cultured cells⁹.

The lack of inhibition of uridine- ^{14}C incorporation by DMBA during the first 24 h of culture does not rule out the possibility that synthesis of later formed DNA-dependent RNA would be suppressed, as shown by *in vivo* experiments¹⁰. The major questions provoked by these results are whether or not the early alteration induced by DMBA at the DNA level is involved in a

specific manner with neoplastic transformation, and whether this DMBA-induced increase in DNA per cent is related to polyoma virus induced cellular DNA synthesis¹¹. It is well known that cells of chemically induced tumours contain a mosaic of surface antigenic properties¹² acquired during transformation. The system described here in which the concentration of carcinogen can be limited in its reaction with single cells might prove useful in the induction and identification of intracellular antigens as well as a defined number of phenotypically expressed surface antigens.

This work was supported by a postdoctorate fellowship (C-9717), National Cancer Institute, U.S. National Institutes of Health.

LAWRENCE J. ALFRED*

Lawrence Radiation Laboratory,
University of California,
Berkeley.

* Present address: Cancer Research Institute, University of California, Medical Centre, San Francisco.

¹ Brookes, P., and Lawley, P. D., *Nature*, **202**, 781 (1964).

² De Maeyer-Guignard, J., and De Maeyer, E., *Nature*, **205**, 985 (1965).

³ De Maeyer-Guignard, J., and De Maeyer, E., *Science*, **146**, 650 (1964).

⁴ Levine, E. M., Becker, Y., Boone, C. W., and Eagle, H., *Proc. U.S. Nat. Acad. Sci.*, **53**, 350 (1965).

⁵ Schneider, W. C., *Method in Enzymol.*, **3**, 680 (1957).

⁶ Alfred, L., and Brookes, P. (unpublished results).

⁷ Alfred, L., *British J. Cancer*, **18**, 564 (1964).

⁸ Jensen, E. V., Ford, E., and Huggins, C., *Proc. U.S. Nat. Acad. Sci.*, **50**, 454 (1963).

⁹ Lewis, A. G., Danielli, G. A., and Piccinini, E., *Nature*, **207**, 608 (1965).

¹⁰ Gelboin, H. V., Klein, M., and Bates, R. R., *Proc. U.S. Nat. Acad. Sci.*, **53**, 1353 (1965).

¹¹ Dulbecco, R., Hartwell, L. H., and Vogt, M., *Proc. U.S. Nat. Acad. Sci.*, **53**, 403 (1965).

¹² Klein, G., in *Cellular Control Mechanisms and Cancer*, 236 (Elsevier Publishing Co., 1964).

RADIOBIOLOGY

Effect of Sodium Alginate in inhibiting Uptake of Radiostrontium by the Human Body

THE selective inhibition of radiostrontium uptake by rats following the administration of doses of sodium alginate has been reported recently¹⁻³. We have performed a similar experiment with a normal healthy adult human male volunteer, in order to see whether sodium alginate was effective in inhibiting uptake of radiostrontium from the human gastro-intestinal tract.

In the first stage of the experiment, 10 g sodium alginate was administered orally to the subject, during the morning, 20 min prior to an oral administration of 0.36 μ c. strontium-85 (0.51 MeV γ -rays; half-life, 65 days). No food had been consumed since the previous evening. Regular measurements of body radioactivity were made by means of the Windscale whole-body counter⁴ in order to determine the time pattern of retention of strontium-85 in the human body. All urine and faeces excreted during the 9 days following administration were collected and analysed for strontium-85 and calcium. When after 23 days the amount of strontium-85 retained in the body had dropped below the limit of detection (5 nc. strontium-85), the first stage of the experiment was completed.

The second stage involved the oral administration of 0.48 μ c. strontium-85 to the same subject, under conditions identical with those of the first stage, except that no sodium alginate was administered. Similar body radioactivity measurements of strontium-85 were again made and excreta collected and analysed for strontium-85 and calcium.

The γ -ray spectrum obtained from each measurement of body radioactivity was corrected for the subject's body content of the naturally occurring potassium-40 and fall-out calcium-137. The net counting rates in the strontium-85 photopeak, corrected for physical decay, are pre-

sented in Fig. 1 for each stage of the experiment. It can be seen (Fig. 1) that after day 5, the amount of strontium-85 retained in the body was lowered by a factor of approximately 8 when sodium alginate was administered.

The amount of strontium-85 excreted in urine was also lower in the first stage of the experiment (Fig. 2) than in the second stage, by a factor of approximately 10.4 averaged over the 7 days following administration.

In each stage of the experiment, one blood sample was taken from the subject at about 2.5 h after administration. Assessments were made of the amounts of strontium-85 and calcium in serum, and the results showed that the ratio of strontium-85 to calcium in serum in stage one of the experiment was lower by a factor of about 9.2 than that in the second stage.

Although the second stage of the experiment has not yet been completed, these preliminary results clearly show that sodium alginate has been effective in reducing

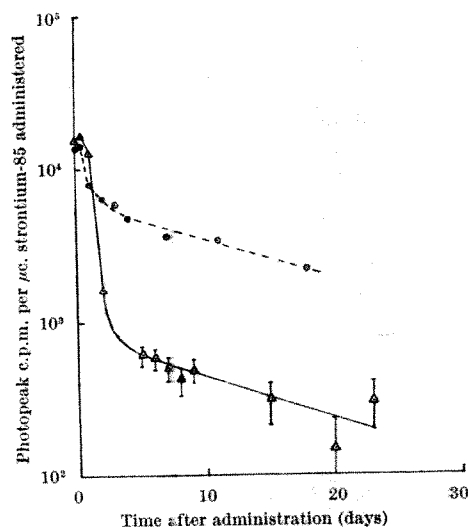


Fig. 1. Body retention of strontium. ----, Without sodium alginate; —, with sodium alginate

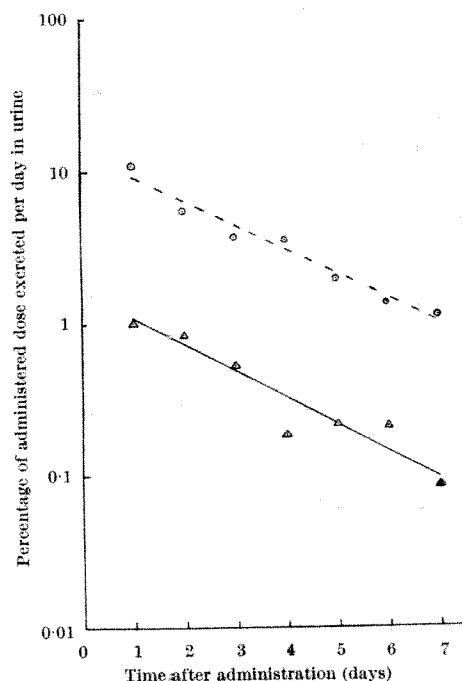


Fig. 2. Daily excretion of strontium in urine. ----, Without sodium alginate; —, with sodium alginate

- the amount of strontium-85 transferred from the human gastro-intestinal tract to blood and whole body (and presumably bone) by a factor in the range 8–10.4.

Sodium alginate is non-toxic at the level used in this experiment, and selectively inhibits the uptake of radiostrontium without significantly interfering with the uptake of calcium by the body^{1,2}. The ability to inhibit selectively the uptake of radiostrontium from the gastro-intestinal tract would seem to be of definite value in the event of contamination of food or water with radiostrontium. The effect of food in the stomach at the time of administration of sodium alginate, however, has not as yet been worked out. After an accidental inhalation of radiostrontium, quite a large proportion of material may be cleared from the respiratory tract to the gut; in this event there may also be a place for alginate therapy.

Our present programme of experiments is designed to assess the effect of sodium alginate administered before, with or after an intake of radiostrontium, and also to assess the optimum doses and type of sodium alginate. It is also proposed to see whether the degree of inhibition is affected by the contents of the stomach.

A low viscosity form of sodium alginate ('Manucol SS/LD', supplied by Alginate Industries, Ltd.) was used in this experiment.

We thank Dr. C. T. Blood of Alginate Industries Ltd., Dr. G. Dolphin of the Authority Health and Safety Branch, Mr. W. L. Templeton, Dr. J. C. Dalton of Windscale Technical Section and our other colleagues of the U.K. Atomic Energy Authority, Windscale and Calder Works, for their co-operation and advice.

R. HESP
B. RAMSBOTTOM

U.K. Atomic Energy Authority,
Windscale and Calder Works,
Sellafield, Seascale,
Cumberland.

¹ Waldron, Edward D., Paul, T. M., and Skoryna, S. C., *Nature*, **205**, 1117 (1965).

² Skoryna, S. C., Paul, T. M., and Waldron, Edward D., *Canad. Med. Assoc. J.*, **91**, 285 (1964).

³ Paul, T. M., Waldron, Edward D., and Skoryna, S. C., *Canad. Med. Assoc. J.*, **91**, 553 (1964).

⁴ *Directory of Whole Body Counters*, Intern. Atomic Energy Agency (1964).

BIOLOGY

Production of Carotenoid by a Green Alga

THE first observations on carotenoid production by the chlorophycean alga *Dictyococcus cinnabarinus* were made by Wenzinger¹, Haag² and recently Kol³. From experiments carried out on static culture and for periods of a few months of growth, these authors reported that when sugars were present in the medium the cultures changed colour from green to red. The production of redness, they observed, was due to the prevalence of carotenoids in the chlorophyll formed.

Starting from these observations we decided to investigate the behaviour of *Dictyococcus cinnabarinus* and the eventual carotenoid production in submerged culture using different sugars, with and without addition of carbon dioxide, and in different conditions of temperature and light.

The experiments were performed in 500-ml. Erlenmeyer flasks containing 100 ml. of culture broth, on a rotatory shaker duly thermostated and illuminated. Illumination was supplied by fluorescent tubes ('warm white' type) which gave a light intensity of 3,500–4,000 lux. The strain used was *Dictyococcus cinnabarinus* 280 ('Kol-F. Chodat') (obtained from the algal collection of the Botanical Institute of the University of Geneva) and as culture broth, Detmer medium, diluted three times, was used. In the experiments with addition of carbon dioxide, a mixture of

air containing 3 per cent carbon dioxide was supplied to each flask at a rate of 0.5 v/v medium/min.

Each experiment lasted about 2 weeks and the following sugars were tested: glucose, fructose, galactose, lactose and sucrose, at a concentration of 2 per cent w/v of the culture medium.

200,000 cells/ml. grown in a seed flask containing Detmer/3 medium without sugar were inoculated into the 'fermentation' flask. It must be emphasized that in the absence of sugars the culture remained green, while in the presence of sugars, namely glucose, the culture changed colour gradually from green to yellow, to pink up to an intense orange colour by the end of the fermentation, which indicated accumulation of carotenoids.

The experiments showed that in the presence of glucose a good growth is obtained with a sugar utilization of 40–50 per cent, while other sugars are not utilized and the growth is quite poor; that supply of carbon dioxide does not interfere with the utilization of carbohydrates. Furthermore, the results have shown that the optimum for inoculum is 200,000 cells/ml. of green culture, each cell having a diameter of 5 μ , and in the red culture 10–12 to 20–25 μ (the latter are aplanosporangia, that is, mother cells in sporogony); that the optimum temperature is 25°; and that the best growth is obtained when 12 h of light is alternated with 12 h of dark.

For pigment determination, the cells collected by centrifugation were ground in a mortar with Na₂CO₃ and 'Celite', adding gradually 80 per cent aqueous acetone; the acetone solution was then extracted with light petroleum. The absorption spectrum of the ethereal extract was determined between 400 and 700 m μ using a Cary spectrophotometer.

Thin-layer chromatography was used for the preliminary characterization of the pigments. Amounts sufficient for their identification were obtained by column chromatography on 'Florisil' or alumina. Elution was effected with a gradient of acetone in light petroleum (b.p. 40°–70°). Coloured fractions belonging to each band were pooled, evaporated and examined in the spectrophotometer.

Six fractions of colour ranging from yellow to red were separated and identified as follows:

- (1) β -Carotene (epiphasic), 5–6 per cent of the total; m μ 478,450 (425) in light petroleum, m μ 515,485 (458) in carbon disulphide—plates yellow or orange—yellow—m.p. 174°.
- (2) Echinenone (90 per cent epiphasic); m μ 456–458 in light petroleum, m μ 488 in carbon disulphide; by reduction with LiAlH₄ gives isocryptoxanthin.
- (3) Unidentified (epiphasic); m μ 462 in light petroleum, m μ 500 in carbon disulphide.
- (4) Unidentified (epiphasic); m μ 470 in light petroleum.
- (5) Xanthophyll (hypophasic); traces—m μ 471, 441, 420 in light petroleum.
- (6) Unidentified (hypophasic); traces.

The spectra of fractions I and II can be superimposed on those taken with pure samples.

It is interesting to emphasize finally that in the cultural conditions which we describe, no chlorophyll could be detected; echinenone does not seem to have been previously demonstrated in the Chlorophyceae.

F. DENTICE DI ACCADIA
OLGA GRIBANOVSKI-SASSU
A. ROMAGNOLI
L. TUTTOBELLO

International Centre for Chemical Microbiology,
Istituto Superiore di Sanità,
Rome.

¹ Wenzinger, F., thesis, Geneva (1940).

² Haag, E., thesis, Geneva (1942).

³ Kol, E., *Acta Botanica Acad. Sci. Hung.*, **7**, 303 (1961).

A Functional Relationship between Neurosecretory Fibres and Pituicytes in the Eel

SYNAPTIC contacts between neurosecretory fibres and pituicytes have been demonstrated in the eel, and the suggestion made that this association might form part of a mechanism for feed-back of information to the hypothalamus¹. Recent experimental investigations support this view.

Fifty eels were kept in darkness for 3–5 weeks and then transferred to illuminated white or black backgrounds for 4 h. The pituitaries of the white background animals showed the following features in contrast to those of eels kept on a black background:

(1) Many synapses between neurosecretory fibres (with elementary neurosecretory vesicles of 1200 Å diameter) and pituicytes.

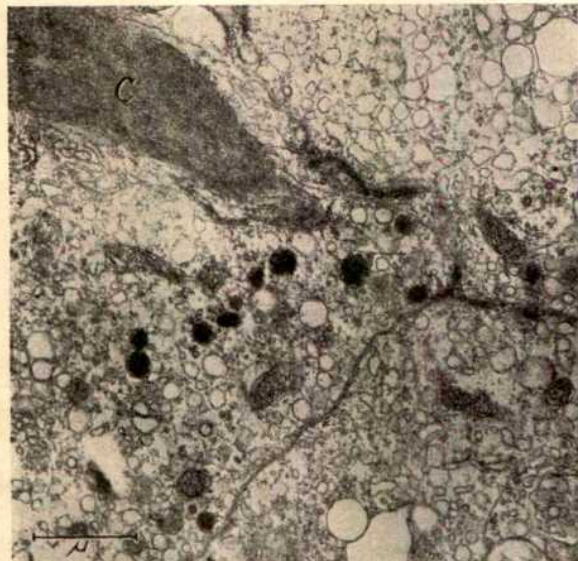


Fig. 1. An electron micrograph showing part of a group of pituicytes in the pituitary of an eel maintained on an illuminated white background. Membrane-bound secretory inclusions may be seen. C, Colloid

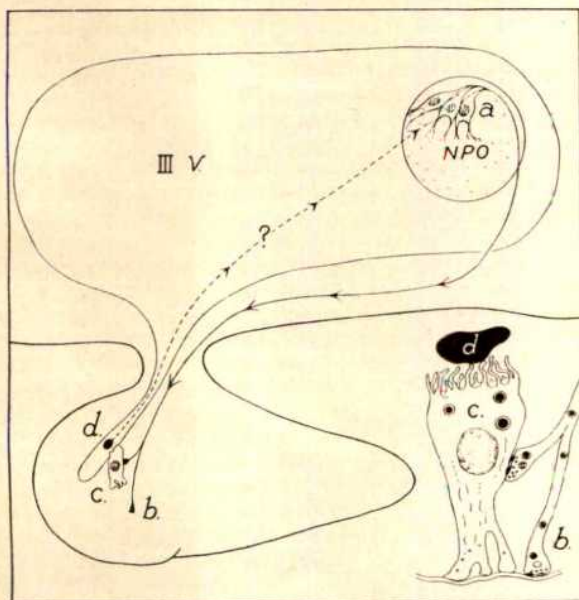


Fig. 2. A diagram depicting a possible feed-back mechanism linking the proximal and distal parts of the hypothalamic-hypophysial neurosecretory system of the eel. a, Preoptic nucleus (NPO); b, synapse; c, pituicyte; d, periodic acid-Schiff-positive colloid. III V, third ventricle of brain

(2) A great number of membrane-bound secretory droplets, about 4000 Å in diameter, in the pituicytes.

(3) Much periodic acid-Schiff-positive material in the central canals (Fig. 1), surrounded by pituicytes; these canals appear to be in communication with the ventricles of the brain.

All these features were rare in control animals and black background animals.

These observations are consistent with a view that in the eel neurosecretory fibres may stimulate pituicytes to release their products into the cerebrospinal fluid. By so doing they might affect the preoptic nuclei since it has been shown that dendrites of the perikarya of the neurosecretory neurones project into the ventricles of the brain in the eel², frog³ and toad⁴.

An association of neurosecretory fibres and ependymal cells has been demonstrated in a bird⁵, and recently it has been shown that ependymal cells in the median eminence of the rat appear to have a glandular function and secrete into the cerebrospinal fluid, though no synaptic contacts between these cells and nerve fibres were observed⁶.

The cerebrospinal fluid could act as a means of transport between the pituitary and the hypothalamic nuclei and thus provide a channel for the transfer of information from the distal to the proximal part of this neurosecretory system. The work recorded here suggests a possibility that pituicytes or other glial elements might play an important part in such a process.

FRANCIS KNOWLES
LUTZ VOLLRAH

Department of Anatomy,
Medical School,
University of Birmingham.

¹ Knowles, Sir F., and Vollrath, L., *Nature*, **206**, 1168 (1965).

² Stutinsky, F., *Z. Zellforsch.*, **39**, 276 (1953).

³ Dierckx, K., *Arch. Intern. Pharm.*, **140**, 708 (1962).

⁴ Rodriguez, Gen. Comp. Endocrinol., **4**, 684 (1964).

⁵ Nishioka, R. S., Bern, H. A., and Mewaldt, L. R., *Gen. Comp. Endocrinol.*, **4**, 304 (1964).

⁶ Lévêque, T. F., Stutinsky, F., Stoeckel, M. E., and Porte, A., *C.R. Acad. Sci., Paris*, **260**, 4621 (1965).

A Dual Neurosecretory Innervation of the Pars Distalis of the Pituitary of the Eel

INVESTIGATIONS of the innervation of the eel pituitary with the light microscope demonstrated in the pars distalis a few nerve fibres which stained with the Gomori technique and many others which did not¹. The latter, which appeared to originate in the nucleus lateralis tuberis, do not seem to stain with any of the common 'neurosecretory' stains², and therefore under the light microscope the degree of neurosecretory control of the pars distalis appears to be slight.

Under the electron microscope, however, an extensive neurosecretory innervation of the pars distalis can be demonstrated. Most of the fibres which invade the anterior lobes of the pituitary (proximal and rostral pars distalis) are seen to contain vesicles, irregular in outline, each with a central electron-dense granule about 700 Å in diameter. These fibres are found also, with their perikarya, in the nucleus lateralis tuberis; they resemble the Type B neurosecretory fibres described in the neuro-intermediate lobe of the elasmobranch pituitary³. They do not stain with neurosecretory stains, but nevertheless should be termed neurosecretory⁴; they discharge into perivascular and intervascular spaces which permeate the pars distalis.

Material with affinity for neurosecretory stains appears in abundance only in mature eels in which there is evidence of an increased activity of GTH and TSH cells⁵. In these animals many typical neurosecretory fibres containing Type A elementary neurosecretory vesicles⁶, about 1400 Å in diameter, are found. These fibres were extremely rare in elvers or young eels.

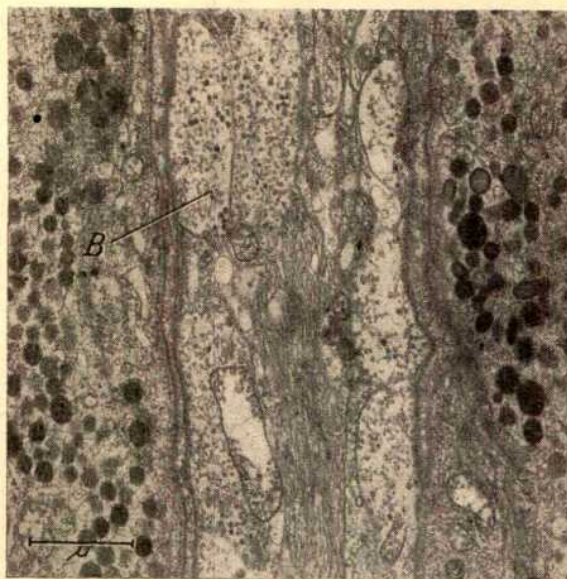


Fig. 1. Electron micrograph of a portion of the rostral pars distalis of an eel pituitary, showing a neurosecretory fibre tract lying between groups of ACTH cells. B, Type B fibre containing electron-dense granules, each about 700 Å in diameter.

There is evidence, therefore, at the level of ultra-structure for a neurosecretory innervation of the pars distalis of the eel by at least two kinds of neurosecretory fibre, and that the activity of one fibre type (Type A) may be correlated to stages of the life-cycle.

FRANCIS KNOWLES
LUTZ VOLLRATH

Department of Anatomy,
Medical School,
University of Birmingham.

¹ Bargmann, W., *Z. Zellforsch.*, **38**, 285 (1953).

² Knowles, Sir F., and Vollrath, L., *Z. Zellforsch.*, **69**, 474 (1966).

³ Knowles, Sir F., *Phil. Trans. Roy. Soc., B*, **249**, 435 (1965).

⁴ Bern, H. A., and Knowles, Sir F., in *Neuroendocrinology*, edit. by Martini, L., and Ganong, W. F. (Academic Press, New York, 1966).

Control of Furunculosis in Impounded Adult Salmon

FROM July 27 to August 17, 1964, 17 salmon were found dead among about 40 fish impounded at Ceannacroc on the Moriston, a tributary of the River Ness. Eight salmon examined between August 1 and 14 showed typical symptoms of furunculosis, and pure cultures of the causative organism, *Bacterium salmonicida*, were isolated from the internal organs and muscle. As a serious outbreak of furunculosis had occurred in this impoundment and at another impoundment on the Ness system in 1963, an attempt was made to control the outbreak which seemed to threaten this year.

It was decided to use an intramuscular depot injection, so as to maintain the highest blood levels of antibiotic for the longest time after a single injection. Chloramphenicol was chosen as the antibiotic since it is known to be effective against *B. salmonicida* both *in vitro* and *in vivo*¹. Chloramphenicol has a low solubility in water so that an aqueous suspension would provide a depot in the muscle which would be dissipated slowly. 'Intramycin', a veterinary preparation containing 15 per cent chloramphenicol in aqueous suspension (Parke Davis and Co.), was used at a rate of 1 ml./15 lb. of fish. At this rate, a single intramuscular injection of this preparation should supply effective blood levels of chloramphenicol for 24–48 h.

All the impounded fish were treated on August 17. The fish were injected on the left side slightly anterior to the dorsal fin and towards the heart and coelom, the

needle being inserted to its full extent. Sterile disposable plastic syringes were used, with needles 21 gauge, 1.5 in. long. A total of 44 fish was treated, including seven fish which were being transferred to the impoundment from the trap nearby.

Two fish died within a few hours after the injection. These were in a decidedly poor condition before treatment, and handling and injection may have hastened their death. Two more fish died later, one on September 6 and one on October 9. Unfortunately it was not possible to examine any of these fish after death. Following the treatment given on August 17, ten more fish were added to the impoundment from the trap. All these were injected before being placed in the impoundment and they, and the other fish impounded, have apparently remained healthy and normal.

It should be emphasized that this treatment was devised to meet an emergency, but the results obtained suggest that a rigid experimental investigation would confirm the results of this *ad hoc* test. If this should be so, the treatment has a number of advantages: it is easily carried out and can readily be taught to non-technical staff; it seems to have no adverse effect on the fish; and it is inexpensive, as it is estimated that 50 fish could be treated for less than £5.

I thank Mr. J. Wood, superintendent to the Ness District Fishery Board, and his staff, for their help.

ANGELA C. LEAMAN*

Department of Agriculture and
Fisheries for Scotland,
Marine Laboratory,
Victoria Road, Aberdeen.

* Present address: Botany Department, University College of Wales, Aberystwyth.

¹ Snieszko, S. F. United States Department of the Interior, Fishery Leaflet 467.

Action of Gibberellic Acid on Auxin Transport

THE polar transport of β -indolylacetic acid (IAA) in plant organs has been known since the observations of Went¹ and van der Weij². Most of the direct investigations of IAA movement have used bioassays for detecting auxin³. But these analyses have been limited by the sensitivity of the method⁴. The recent use of IAA labelled with carbon-14 (IAA*) facilitates the study of the auxin translocation⁵.

It has been previously observed that, in the stem sections of *Lens culinaris*, there is a predominantly basipetal movement of carbon-14 from applied IAA*. It has been assumed that gibberellic acid (GA) increases the uptake of IAA* and changes the auxin transport in these sections⁶. In the present communication, the action of GA on the velocity of IAA* translocated in the section of the stem of *Lens culinaris* Med. will be discussed.

The movement of IAA* (¹⁴C-labelled in the carboxyl group: specific activity of 8 mc./mmole) has been examined in 6-mm sections, using the technique of McCready⁷ and Pilet⁸. The agar (1.5 per cent) blocks were 16.6 mm³ in volume. The donors contained 5 or 50 μ moles of IAA*. At the end of each experiment, each set of forty replicate blocks was pooled for the analysis of their radioactivity—these were expressed in counts per min (c.p.m.) per mg of dry weight.

Seeds were soaked for 4 h and then placed in Petri dishes on filter paper with de-ionized water for 24 h. Seedlings were then selected (4 mm roots) and planted under the same conditions⁹. After 72 h, when used for experimental purposes, the stems were 10 mm \pm 2 mm long. All experiments were carried out at 25.0° C, in complete darkness. During manipulation only green light was used.

The principles involved in the assay are shown diagrammatically in Fig. 1. After an incubation of 2 h in Ringer's solution containing (or not) GA, the sections (40 for 25 ml.)

Table 1. EFFECT OF A PRETREATMENT ON THE TRANSLOCATION OF ^{14}C FROM IAA*

Diffusion time, 6 h; donor blocks, IAA* (specific activity, 8 mc./mmole; conc., $50\ \mu\text{M}$; standard (D_0), $11,080 \pm 160$ c.p.m.); pretreatment, 40 sections for 25 ml.; radioactivity in c.p.m./mg of dry weight; each result: average of 40 blocks

	No pre-treatment (NP)	Ringer	Pretreatment (P)		
			10 $\mu\text{g}/\text{ml}$.	50 $\mu\text{g}/\text{ml}$.	100 $\mu\text{g}/\text{ml}$.
Donors					
D_0	6,042	6,268	6,004	5,898	5,242
Loss from donors					
$AD = D_0 - D_6$	5,038	4,812	5,076	5,182	5,838
$(AD/D_0) \cdot 100$ (per cent)	45.4	43.4	45.8	46.9	52.6
Receivers					
R_0	410	380	495	580	612
$(R_0/AD) \cdot 100$ (per cent)	8.1	7.8	9.7	11.2	10.5
Sections					
$Ar = AD - R_0$	4,628	4,432	4,581	4,602	5,226
$(Ar/AD) \cdot 100$ (per cent)	91.8	92.4	90.8	88.8	89.5

are placed, for 6 h, in the horizontal position. Table 1 shows the results of a typical experiment using, as a control, sections without pretreatment in Ringer's solution. It can be observed: (a) The Ringer's solution reduced the uptake of carbon-14 from IAA*. (b) For

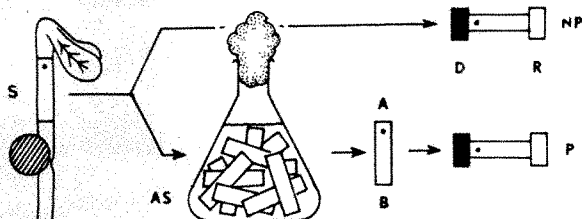


Fig. 1. Diagram showing the principles of the method used to study the action of gibberellic acid on the transport of carbon-14 from IAA* in the donor blocks. S, Section of the stem; apical (A) and basal (B) cut surfaces; donor (D) and receiver (R) blocks; NP, not pretreated; P, pretreated

the GA-pretreatment the radioactivity in the sections was much reduced. (c) These effects increased when the GA concentration was increased.

The velocity of carbon-14 translocation was estimated in the manner introduced by van der Weij, incorporating later improvements by Jacobs¹⁰ and McCready and Jacobs¹¹. The method involves plotting the amount of IAA* translocated through the sections (length: L) against time. The presumed line is then extrapolated back to 'zero IAA*', and the time-intercept so determined is taken as the time (T) required for auxin to be transported in the section. Assuming (Pilet⁶) that the regression is:

$$y = mx + h$$

(y , radioactivity in receivers; x , diffusion time), the velocity (V) of movement can be calculated from:

$$V = L/T \text{ and } T = -h/m$$

The time course of transport of carbon-14 with IAA* in donors was first analysed (Fig. 2). For each experiment, the net increase of radioactivity in the receivers follows an apparently linear course over the period 1–6 h. The calculated intercepts are given in Table 2. From these results it can be concluded that: (a) The velocities for 5 and $50\ \mu\text{M}$, respectively, are not significantly different from each other according to statistical tests. (b) The mean calculated velocity is, for the control (A, C, E), 5.69 mm/h and, for the treated sections (B, D) with GA ($50\ \mu\text{g}/\text{ml}$), 9.83 mm/h.

Table 2. CALCULATION OF THE VELOCITY OF TRANSPORT OF ^{14}C FROM IAA* (see Fig. 2)

Donor blocks, IAA* (specific activity, 8 mc./mmole; standard (D_0), $5\ \mu\text{M}$ $1,160 \pm 27$ c.p.m. and $50\ \mu\text{M}$ $11,080 \pm 160$ c.p.m.); diffusion time, 2–6 h; pretreatment (see Table 1); concentration of GA, $50\ \mu\text{g}/\text{ml}$.

Assays	Length	Regression equation $y =$	T (h)	V (mm/h)
Initial concentration				
5 μM				
No pretreatment (NP) A	6.30	$(20.6) \cdot x - (24.3)$	1.18	5.34
Pretreatment (P)				
Ringer + GA B	6.48	$(30.9) \cdot x - (19.8)$	0.64	10.05
Initial concentration				
50 μM				
No pretreatment (NP) C	6.42	$(80.7) \cdot x - (80.1)$	0.99	6.48
Pretreatment (P)				
Ringer's E	6.50	$(63.7) \cdot x - (79.0)$	1.24	5.24
Ringer's + GA D	6.24	$(105.2) \cdot x - (68.4)$	0.65	9.60

$y = mx + h$. $T = -h/m$. $V = L/T = L/(-h/m)$. L , Length of sections. T , Time required for carbon-14 to be transported through the section.

Consequently, gibberellic acid causes an increase of both uptake and movement (velocity) of auxin applied, in donor blocks, on the cut apical surface of the sections of *Lens* stem.

P. E. PILET

Laboratory of Plant Physiology,
University of Lausanne.

¹ Went, F. W., *Rec. Trav. Bot. Néerl.*, **25**, 1 (1928).

² Weij, H. G. van der, *Rec. Trav. Bot. Néerl.*, **29**, 379 (1932).

³ Pilet, P. E., *Phytohormones de croissance*, 426 (1961).

⁴ Jacobs, W. P., *Plant Growth Regulation*, 397 (1961).

⁵ Goldsmith, M. H., and Thimann, K. V., *Plant Physiol.*, **37**, 492 (1961).

⁶ Pilet, P. E., *Physiol. Plant.*, **18**, 687 (1965).

⁷ McCready, C. C., *New Phytol.*, **62**, 3 (1963).

⁸ Pilet, P. E., *Méthode de Mesure du Transport d'Auxines Radioactives*, 7 (1964).

⁹ Pilet, P. E., and Went, F. W., *Amer. J. Bot.*, **43**, 190 (1956).

¹⁰ Jacobs, W. P., *Amer. J. Bot.*, **38**, 307 (1951).

¹¹ McCready, C. C., and Jacobs, W. P., *New Phytol.*, **62**, 19 (1963).

Growth Responses of Mature Endosperm in Cultures

THE endosperm is usually a triploid tissue which supports the growth and germination of the embryo, and stores reserve food materials in mature seeds.

The immature endosperm of several plants undergoes proliferation on synthetic media, but only in two plants—*Santalum album*¹ and *Ricinus communis*²—has the mature endosperm yielded a continuously growing undiffer-

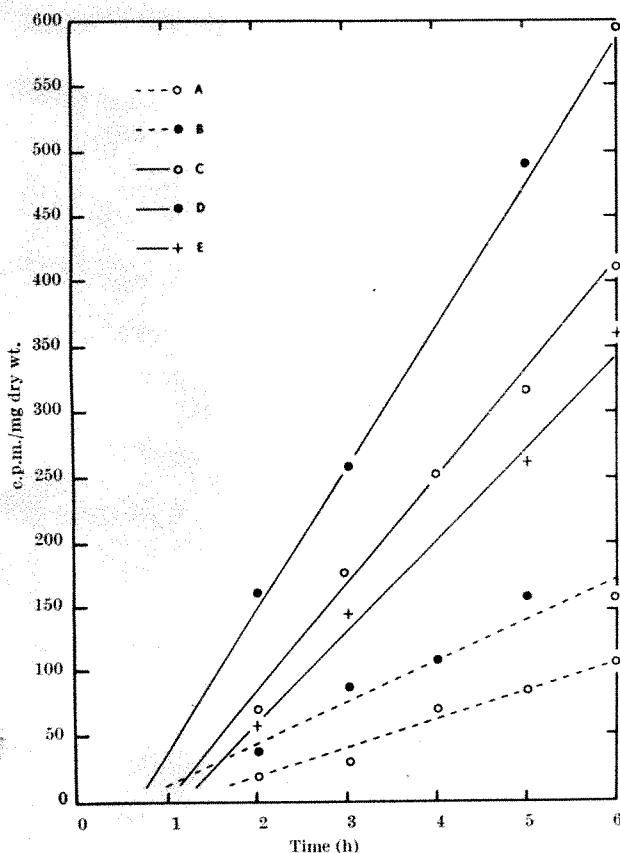


Fig. 2. The early stages of the time course of transport of carbon-14 from IAA* supplied at an initial concentration of $5\ \mu\text{M}$ (---) and $50\ \mu\text{M}$ (—) in the donors. A calculation of the velocity of movement of carbon-14 from IAA* (see Table 2). Radioactivity in the receivers. ○, No pretreatment; ●, pretreatment with Ringer's solution and gibberellic acid ($50\ \mu\text{g}/\text{ml}$); +, pretreatment with Ringer's solution

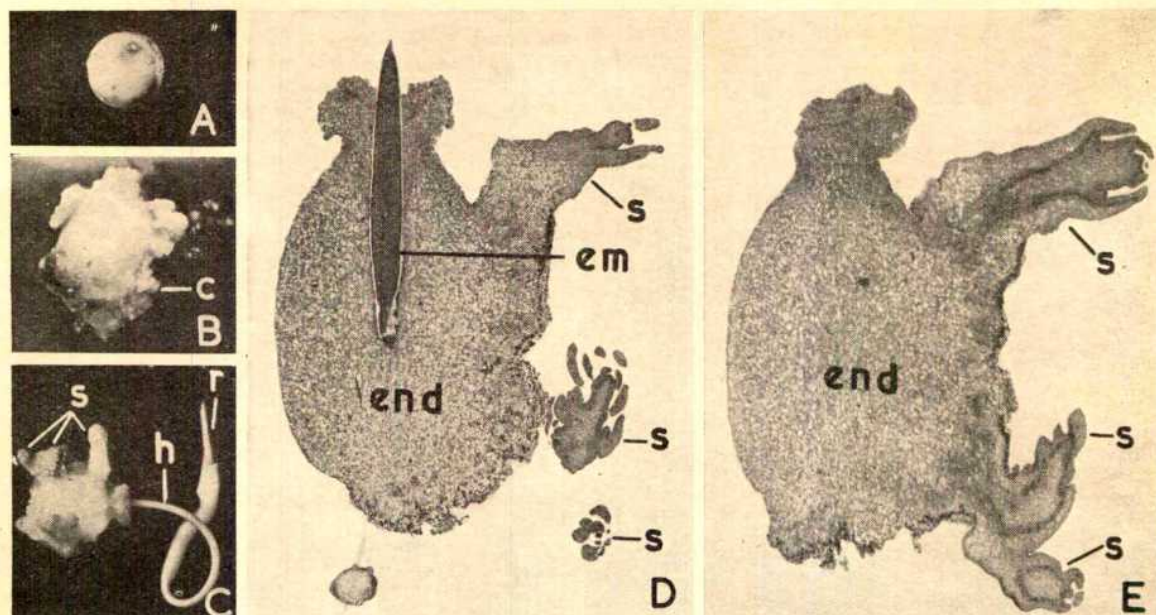


Fig. 1. *Exocarpus cupressiformis*. A, Entire endosperm with intact embryo; stage of inoculation ($\times c. 2.8$); B, 11-week-old culture on BM+IAA (1 p.p.m.)+K (1 p.p.m.)+CH (400 p.p.m.) showing surface callusing of endosperm ($\times c. 2.2$); C, 5-week-old culture on same medium; note differentiation of shoot buds on the endosperm and germination of embryo ($\times c. 2.5$); D, E, sections of endosperm shown in C; note the unaffected embryo in D and differentiation of shoot buds in D and E ($\times c. 10$). c, Callus; em, embryo; end, endosperm; h, hypocotyl; r, root; s, shoot

entiated tissue. Both these are under investigation in this laboratory.

We have recently obtained a differentiation of shoots in cultures of the mature endosperm of *Exocarpus cupressiformis*, a member of the Santalaceae. The mature entire endosperm with intact embryo (Fig. 1A) was surface-sterilized with chlorine water for about 15 min, repeatedly washed with sterile water, and inoculated on a semi-solid modified White's medium containing 2 per cent sucrose (BM). IAA; 2,4-D; CH (casein hydrolysate); K (kinetin) and YE (yeast extract) were used as supplements. The cultures were maintained at $25^{\circ} \pm 2^{\circ} \text{C}$ and 50–60 per cent relative humidity under diffuse light.

There was no response on BM+CH (500 p.p.m.). If the concentration of CH was increased to 1,000 p.p.m., 20 per cent of the cultures showed germination of the embryo. However, on BM+IAA (1 p.p.m.)+K (1 p.p.m.)+CH (400 p.p.m.), after three weeks, surface callusing of endosperm occurred in 29 per cent cultures (Fig. 1B) and germination in 25 per cent (Fig. 1C). Occasionally, the same cultures showed both these features, and in some of them shoot buds also differentiated from the endosperm (Fig. 1C).

Figs. 1D and E represent microtome sections of the endosperm shown in 1C. It may be noted that in Fig. 1D the embryo is completely unaffected and shoot buds have developed superficially from the endosperm itself (Fig. 1E). They have a well-differentiated vascular supply and several foliar primordia which were chlorophyllous. In between the shoot buds, the peripheral region of endosperm shows distinct patches of vascular tissue. Some of the shoot buds appeared on the lower side and were embedded in the medium; others developed on the upper side and had no direct contact with the medium.

We have also cultured the endosperm of another member of the Santalaceae, *Osyris wightiana* (Fig. 2A). There was some callusing of the endosperm (Fig. 2B) in 20-week old cultures on BM+2,4-D (5 p.p.m.)+CH (1,000 p.p.m.). If the concentration of CH was reduced to 500 p.p.m., callusing was much more profuse (Fig. 2C). Squashes of callus showed cells of various sizes and shapes, and patches of tracheidal cells. When BM was supplemented with IAA (2 p.p.m.) and K (5 p.p.m.), instead of callusing the endosperm produced superficial outgrowths which showed distinct patches of vascular tissue (Figs.

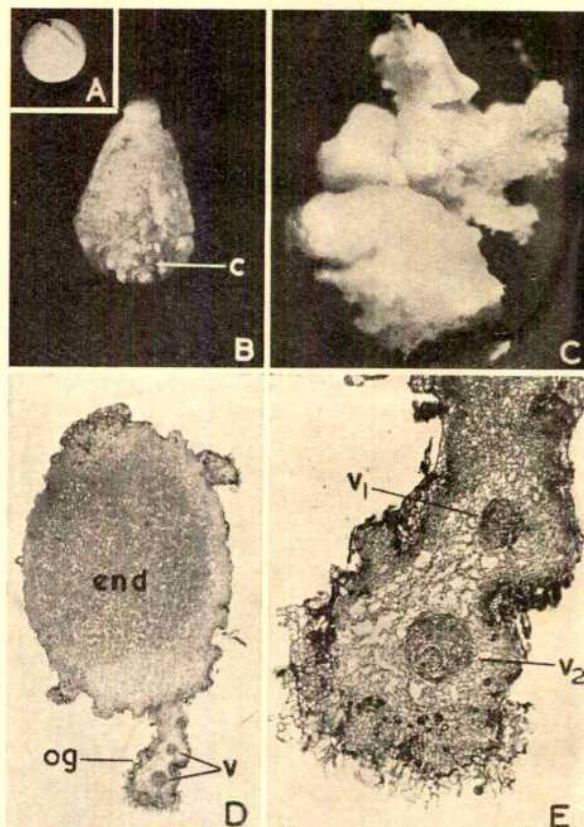


Fig. 2. *Osyris wightiana*. A, Entire endosperm with intact embryo; stage of inoculation ($\times c. 2.1$); B, surface callusing of endosperm on BM + 2,4-D (5 p.p.m.)+CH (1,000 p.p.m.) in 20-week-old culture ($\times c. 1.8$); C, 22-week-old culture on BM+2,4-D (5 p.p.m.)+CH (500 p.p.m.) showing profuse proliferation of endosperm ($\times c. 1.5$); D, 12-week-old culture on BM+IAA (2 p.p.m.)+K (5 p.p.m.); note development of outgrowths with well-differentiated vascular tissue ($\times c. 9$); E, enlarged view of one outgrowth ($\times c. 72$). c, Callus; end, endosperm; og, outgrowth; v, vascular bundle

2D and E). Germination of the embryo occurred in 75 per cent cultures. In contrast to *E. cupressiformis*, the vascular tissue did not develop on the periphery of the endosperm proper.

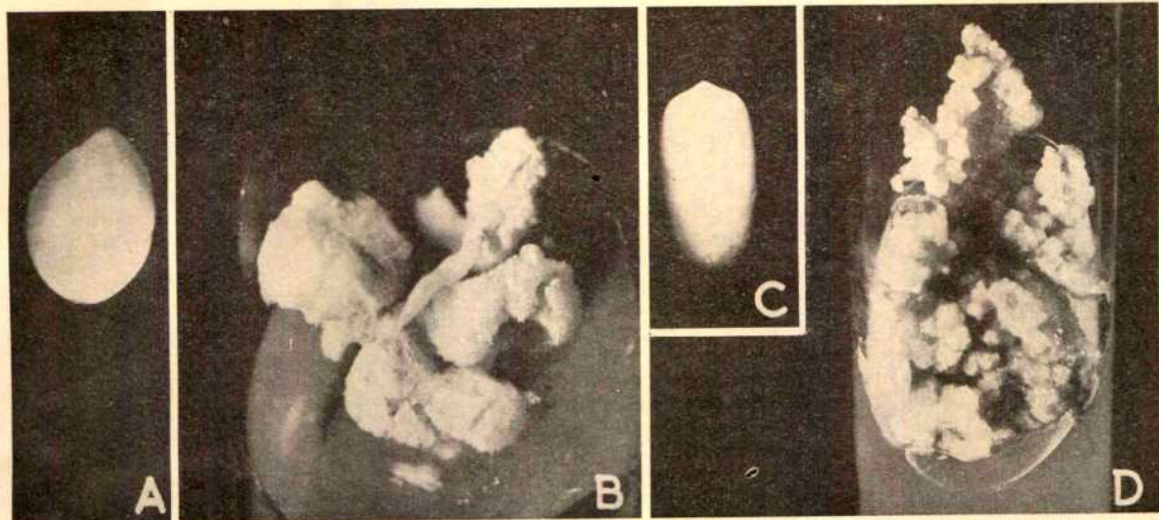


Fig. 3. A, B, *Putranjiva roxburghii*; C, D, *Jatropha pandurafolia*. A, C, Entire endosperm with intact embryo; stage of inoculation ($\times c. 3.5$); B, D, 7-week-old (B) and 13-week-old (D) cultures on BM+2,4-D (2 p.p.m.)+K (5 p.p.m.)+YE (2,500 p.p.m.) showing proliferation of endosperm (B $\times c. 2.5$; D $\times c. 1.5$)

Results of endosperm culture of two members of the Euphorbiaceae—*Putranjiva roxburghii* and *Jatropha pandurafolia*—may also be mentioned. The stage of inoculation is shown in Figs. 3A and C. There was excessive proliferation (Figs. 3B and D) of endosperm in 7- to 13-week-old cultures on BM+2,4-D (2 p.p.m.)+K (5 p.p.m.)+YE (2,500 p.p.m.). The callus consists of cells of variable shapes and sizes, and differentiation of vascular tissue is quite common. In this or in any other treatment none of the cultures has so far shown any differentiation of shoot buds as in *E. cupressiformis*, or vascularized outgrowths as in *O. wightiana*.

There is no previous report of the *in vitro* differentiation of endosperm into roots and shoots. In *E. cupressiformis* the shoots clearly differentiate from the endosperm. The work is being further extended to obtain fully differentiated plants from this and other endosperms.

We thank Prof. P. Maheshwari for his advice; Prof. G. L. Davis (Armidale, Australia) for providing fresh fruits of *Exocarpus cupressiformis*; and Prof. R. D. Adatia (Bombay) and Dr. S. P. Kapoor (Poona) for providing fresh fruits of *Osyris wightiana*.

B. M. JOHRI
S. S. BHOJWANI

Department of Botany,
University of Delhi, Delhi 7, India.

¹ Rangaswamy, N. S., and Rao, P. S., *Phytomorphol.*, **13**, 450 (1963).

² Satsangi, Asha, and Mohan Ram, H. Y., *Phytomorphol.*, **15**, 26 (1965).

Ascent of Sap in Trees: Effect of Negative Absolute Pressure on Flow Resistance

IN considering the ascent of sap in trees, it is generally accepted¹ that the transpiration process at the leaves somehow 'pulls' the sap up through the xylem vessels in the tree trunk during active transpiration periods. This 'cohesion theory' postulates that negative absolute pressure (liquid tension) must be present in trees more than 34 ft. high, or in any plant where the combined effects of hydrostatic head and flow resistance produce a pressure drop of more than one atmosphere. Investigators have had little success, however, in applying traditional resistance laws to the calculation of viscous pressure drop. Theoretical estimates of flow resistance to sap movement have varied by several orders of magnitude² and experimental checks of flow resistance through sections of trees frequently show the flow rate decreasing with time at constant pressure³. It appears that the present resistance 'law' is incorrect, is being misinterpreted, or the viscosity of sap is not what it is assumed

to be from measurements made at positive pressures. This communication is relevant to the latter possibility and reports, in particular, on whether negative absolute pressures have any detectable effect on flow resistance.

Although water is used as the primary viscosity standard for Newtonian liquids⁴, several reports of anomalous behaviour can be found in the literature⁵. Also, since 1945, the investigations of flow resistance from a continuum mechanics point of view have shown that many fluids exhibit anomalous behaviour when subjected to special flow conditions. Truesdell⁶ discusses this possibility in defining a dimensionless parameter called the 'truncation number' for a 'Stokesian fluid' which is a simple generalization of the Newtonian fluid. He suggests that the classical linear theories become inadequate if the truncation number approaches unity, that is, the ratio of the viscosity times deformation rate divided by the pressure is of order of magnitude one or greater. As an example of the possible non-linear behaviour, Truesdell cites the deformation rate required for water.

Since this truncation number goes to infinity when the pressure is reduced to zero absolute, it was postulated that the flow resistance might be affected and have some bearing on the ascent of sap in trees. Further motivation for the experiment arose from the absence of data in the literature on viscosity measurements at negative absolute pressures.

In order to measure the flow resistance of water and a sap-like substance (an aqueous solution of corn syrup), a falling-head viscometer was constructed which placed the test liquid in tension by hanging dual columns of mercury from the liquid in the capillary viscometer (Fig. 1). The absolute pressure was varied from plus one atmosphere absolute to minus one atmosphere absolute by varying the height of the mercury in the barometer column relative to the height of the mercury-test liquid interface in the tension column. Viscometric measurements were made by displacing the interface from its equilibrium position and measuring the time required for the displaced interface to fall a given distance as determined by a cathetometer⁷.

Operation of the falling-head viscometer was as follows: With the entire system evacuated to 10^{-3} mm mercury, degassed water at 95° C was admitted to the capillary tube, tension columns, and main reservoirs through the Kern stopcocks. More hot water was flushed through the system to remove any remaining gas pockets. The valves were closed, and the system pressurized to two atmospheres by raising the mercury reservoirs. After allowing several days for the smallest bubbles to go into solution, water was removed through the Kern stop-

cocks, allowing the mercury to rise to near the top of the tension column. The absolute pressure of the water in the capillary was reduced to zero absolute or less by lowering the mercury reservoirs and observing the level of the mercury in the barometer columns relative to that in the tension columns. The viscometer was operated by closing the cross-connexions and raising one reservoir slightly to displace the mercury interface from its equilibrium position. The cross-connexion was opened, and the time required for the displaced interface to fall a predetermined distance was measured.

The results of the experiments on degassed, distilled water indicate that the viscosity of distilled water remains essentially unchanged in the range of pressures from plus one to minus one atmosphere absolute. All observed values of viscosity were well within 1 per cent of the arithmetic mean value. Since the experimental uncertainty was estimated to be about 1 per cent, it was concluded that any change in viscosity with pressure could not be detected with this apparatus. The same result was obtained for aqueous solution of Karo syrup up to five times as viscous as water, so it would appear that negative absolute pressure has no detectable effect on the resistance to sap movement in trees.

The results of the tests at pressures near zero absolute indicate that the previously mentioned 'truncation number' is not a similarity parameter for the flow of water. Since no non-linear effects were observed, even with a truncation number of infinity, it appears that the past use of water as an example of a Stokesian fluid with zero natural time is questionable since the purpose of such an example was to show how certain abnormal conditions might result in non-linear effects. In a forthcoming article⁸, Truesdell considers only gases in his discussion of the Stokesian fluid. He also eliminates the pressure from the denominator of the truncation number by using the perfect gas law. This removes the possibility of an infinite truncation number for any condition involving actual materials which are assumed to be continuous media.

Pressures below minus one atmosphere absolute could not be attained with the apparatus used because of nucleation problems at the mercury interface. The pressures achieved are comparable with those attained by others using the mercury column technique⁹, but do not approach the tensile strength of 280 atmospheres recorded using a centrifuge¹⁰. Contrary to the nucleation criteria set up by Weyl and Marboe¹¹, a solid surface does not seem to be required for nucleation. In this experiment, nucleation always occurred at the test fluid-mercury interface. Since mercury has an atomic structure quite different from water (they share no hydrogen bonds as water and glass do), this type of structural discontinuity is evidently sufficient to provide nucleation sites at the interface.

Unique to this experiment was the use of the O-ring glass joints and hydrophilic stopcocks (glass stem and

rubber diaphragm) in contact with the water under tension. When nucleation occurred at the mercury interface, the stopcocks were used to remove the resulting gas bubble. Nucleation was never observed to occur in the joints or in the stopcocks. Parts of this type should be useful in future attempts to measure negative absolute pressure in living trees.

The apparatus also made possible approximate estimates of the bulk modulus of water and it was found that degassed water at pressures near zero absolute has about a 100 per cent increase in isothermal compressibility over the commonly quoted value for 15 atmospheres. This trend agrees with the variation in bulk modulus for pressures between 1 and 15 atmospheres reported by Kerr¹².

We thank Profs. J. B. Jones and W. B. Cottingham for their advice. This work was supported by the Purdue University Research Foundation.

BRUCE JOHNSON

Engineering Department,
U.S. Naval Academy,
Annapolis, Maryland.

JOHN R. DIXON

Division of Engineering,
Swarthmore College,
Pennsylvania.

¹ Scholander, P. F., Hemmingsen, E., and Garey, W., *Science*, **134**, 1835 (1961). Zimmermann, M. H., *Sci. Amer.*, **208**, 133 (1963). Zimmermann, M. H., *Biorheology*, **2**, 15 (1964).

² Curtis, O. F., and Clark, D. G., *An Introduction to Plant Physiology*, 179 (McGraw-Hill, New York, 1950). Levitt, J., *Plant Physiol.*, **31**, 248 (1956).

³ Dixon, H. H., *Transpiration and the Ascent of Sap in Plants* (Macmillan, London, 1914). Kelso, W. C., Gertjeansen, R. O., and Hossfeld, R. L., *U. Minn. Agric. Exp. Sta. Tech. Bull.*, **242** (1963).

⁴ Swindells, J. F., Coe, J. R., and Godfrey, T. B., *J. Res. Nat. Bur. Stand.*, **48**, 1 (1952). Dorsey, N. E., *Properties of Ordinary Water-Substance* (Reinhold, New York, 1940).

⁵ Lindgren, E. R., *Ark. Fysik*, **22**, 503 (1962). Low, P. F., in *Eighth Nat. Conf. Clays and Clay Minerals*, 170 (Pergamon, London, 1960). Derjaguin, B. V., *Dokl. Akad. Nauk S.S.S.R.*, **147**, 403 (1962).

⁶ Truesdell, C. A., *J. Rat. Mech. and Anal.*, **1**, 234 (1952); *J. Math. Phys. Appl.*, **29**, 228 (1950).

⁷ Johnson, B., thesis, Purdue Univ. (1965).

⁸ Truesdell, C. A., *Handbuch der Phys.*, **3-3** (in the press).

⁹ Otis, C. H., *Plant Physiol.*, **5**, 419 (1930). Thut, H. F., *Ohio J. Sci.*, **28**, 292 (1928).

¹⁰ Briggs, L. J., *J. App. Phys.*, **21**, 721 (1950).

¹¹ Weyl, W. A., and Marboe, E. C., *Research*, **2**, 19 (1949). Marboe, E. C., *Chem. and Eng. News*, **27**, 2198 (1949).

¹² Kerr, S. L., Kessler, L. H., and Gamet, M. D., *A.S.M.E. Trans.*, **72**, 1413 (1950).

Respiratory Changes in the Cacao Seed Cotyledon coincident with Seed Death

CACAO seed sensitivity to cold was first demonstrated by Boroughs and Hunter¹. Previous investigations by Ibáñez^{2,3} have shown that a treatment of 4° C for 10 min will destroy viability in these seeds. Such a viability loss, however, was reversible if the seeds thus chilled were immersed for 10 min in a water bath at 37° C. Under such conditions, at least 85 per cent of the seeds remained viable and grew into healthy plants. After 15 min of such a cold treatment, however, no amount of post heat treatment could prevent ultimate death of the seed.

Subsequent work by Ibáñez⁴ has shown that the area of the seed most affected by the cold was the cotyledon and not the white embryonic tissue. Respiration rates in radicular tissue remained unchanged regardless of temperature treatment, while cotyledonary tissue showed a large increase in endogenous respiration after cold treatment. In addition, chilled embryonic tissue, growing in a sterile medium, independent of cotyledon material, developed into a small plant, as did normally treated material.

In this investigation we have attempted to relate the onset of cold-death irreversibility in the cacao seed with increases in endogenous respiration in the cotyledonary tissue following cold treatment.

Seeds of *Theobroma cacao*, clone UF-613, were taken from mature pods and submitted to the following treatments:

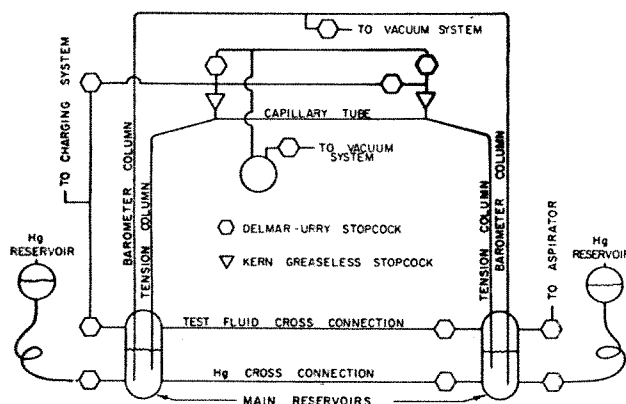


Fig. 1. Falling-head viscometer

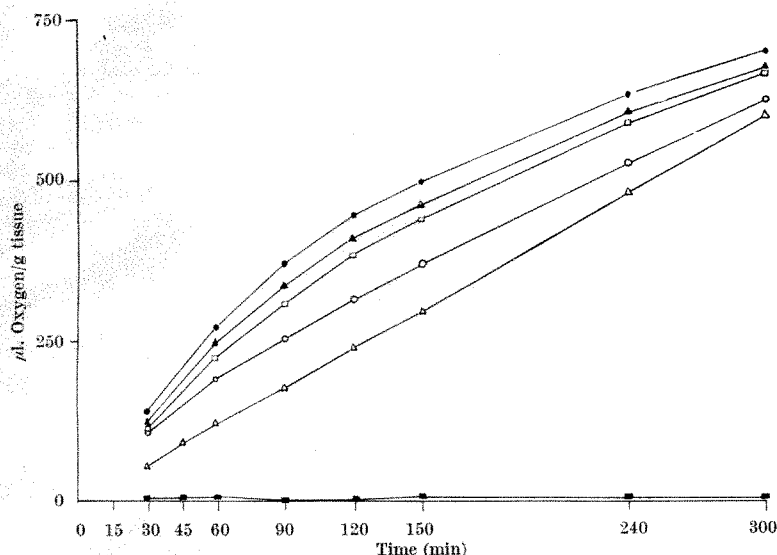


Fig. 1. Respiration in cacao cotyledon tissue following various cold treatments. Δ , Normal; \circ , 5 min at 4°C ; \square , 10 min at 4°C ; \blacktriangle , 15 min at 4°C ; \bullet , 20 min at 4°C ; \blacksquare , boiled control.

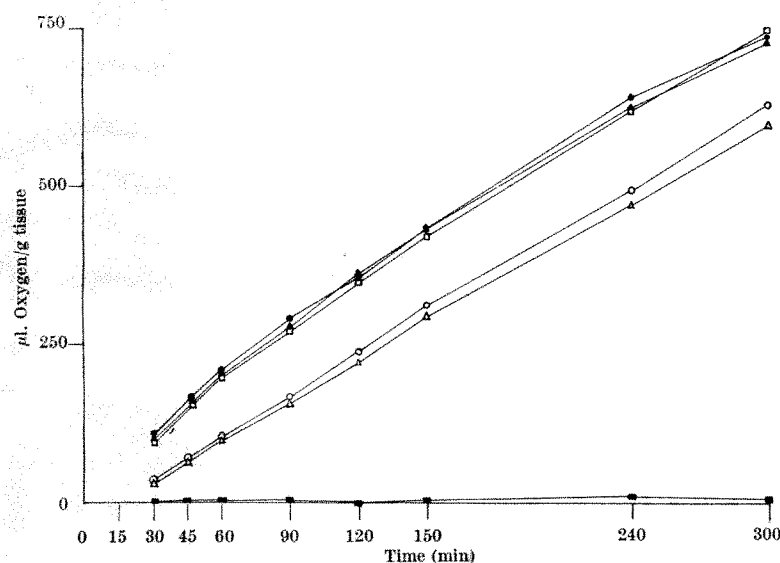


Fig. 2. Respiration in chilled cacao cotyledon following incubation at 37°C . Δ , Normal; \circ , 5 min at 4°C ; \square , 10 min at 4°C ; \blacktriangle , 15 min at 4°C ; \bullet , 20 min at 4°C ; \blacksquare , boiled control.

Normal control seeds were immersed in water at room temperature (25°C) for 5, 10, 15 and 20 min. Seeds subjected to cold treatment were immersed in water at 4°C for periods equal to those given here for the normal control. In addition, a group of cold-treated seeds received post heat treatment in water at 37°C for periods equal to the individual treatment periods given here.

The exposure times were chosen because of previous work³, which showed that a treatment of 5 min at 4°C had an appreciable killing effect on seed viability while a 10-min treatment was almost 100 per cent effective in killing the seed. These processes, however, were reversible by post heat treatment at 37°C . A treatment of 15 min at 4°C killed the seed and post treatment at 37°C did not reverse this effect. The chilling process was carried further in the 20-min treatment.

After cold treatment, the radicular tissue was removed from the seeds and the cotyledonary material was cut with a cork borer of small diameter to yield pieces of approximately 200 mg in weight. These samples were then added to Warburg flasks containing the following ingredients: 0.1 ml. potassium phosphate buffer pH 7.0, 0.1 M; 200 mg cotyledonary material; 0.2 ml. 10 per cent KOH (in the centre well); water to a final volume of 3 ml. The final

pH of the reaction was 7.0. The endogenous respiration reactions were measured at 30°C . The assay method used has already been described².

Fig. 1 demonstrates the respiration of the cotyledonary material after the various cold treatments and under normal conditions. These results represent the averages of five individual experiments. While there is some difference in respiration between normal tissue and tissue chilled for 5 min, and there is an appreciable increase in respiration of tissues chilled for 10, 15 and 20 min, over the respiration of the normal system, a boiled control showed no activity.

Fig. 2 outlines respiration in cotyledonary tissue after the various cold treatments, and post treatments at 37°C . Normal and 5 min at 4°C are practically indistinguishable from one another, while 10, 15 and 20 min at 4°C show an appreciable increase in respiration over the normal system. A boiled control showed no activity.

It is apparent that there is some relationship between the increase of respiration following chilling of cotyledonary material and the irreversible death point of the seed after cold treatment. This is supported by the fact that restoration of viability by post heat treatment results in a decrease in respiration of the seeds which have been chilled in water for 5 min at 4°C . This lower rate of respiration is practically indistinguishable from that of the normal system. However, the respiration of the seed following chilling in water at 4°C for 10 min remained at a relatively high level after post heat treatment, even though such treatment results in a restoration of viability of the seed. This indicates that increase in respiration in cotyledonary material has some relation to seed death, but it is a manifestation of processes occurring in the cell due to cold and not a direct factor in seed death.

Previous work with cacao seed^{3,4} has indicated that the major effect of irreversible cold-induced death occurs in the cotyledonary tissue during the period of 10–15 min in water at 4°C . The increase of respiration with chilling time suggests a progressive effect of cold treatment on the cotyledon. Such an effect could be attributed to a gradual change in the physiological properties of the cotyledon, possibly a solid-state change of membrane lipids. Restoration of viability following heat treatment could then be due, at least in part, to a reversal of such physiological change. While these data suggest that membrane damage in cotyledonary tissue is a direct result of cold treatment, how the process could occur is, as yet, unknown.

This investigation was supported by the U.S. Atomic Energy Commission under contract AT(30-1)-2043.

IVÁN A. CASAS
EDWARD S. REDSHAW
MANUEL L. IBÁÑEZ

Nuclear Energy Program,
Interamerican Institute of Agricultural Sciences,
Training and Research Centre,
Turrialba, Costa Rica.

¹ Borroughs, H., and Hunter J. R., *Turrialba*, **11**, 160 (1961).

² Ibáñez, M. L., *Turrialba*, **13**, 31 (1963).

³ Ibáñez, M. L., *Turrialba*, **23**, 127 (1963).

⁴ Ibáñez, M. L., *Nature*, **201**, 414 (1954).

⁵ Ibáñez, M. L., and Casas, I. A., *Turrialba*, **13**, 209 (1963).

VIROLOGY

Mixed Infection of African Green Monkey Kidney Cells by Adenovirus 7 and SV 40

It has been shown that mixed infection with adenovirus 12 and SV 40 can occur in African green monkey kidney cells (AGMK)¹; moreover, the double infection results in an enhancement of adenovirus growth². In both the foregoing investigations it was demonstrated with electron microscopy that less than 1 per cent of the cells contained adenovirus 12 particles 72 h after infection with adenovirus alone, yet with adenovirus plus SV 40, 75 per cent of the cells contained adenovirus. Determinations of adenovirus infectivity in human embryonic kidney cell cultures using limiting dilution titrations also consistently demonstrated enhancement of adenovirus growth by SV 40. Similar results were obtained with adenovirus 5 by infectivity titrations and electron microscopy.

To investigate further this phenomenon, we measured haemagglutinin production as another parameter of adenovirus growth. Adenovirus 7 was chosen because of its capacity to agglutinate rhesus monkey erythrocytes. The prototype strain of adenovirus 7 was obtained from Dr. J. Kasel (National Institute of Allergy and Infectious Diseases, National Institutes of Health). It was serially passed in HEP-2 cells and titrated in primary human embryo kidney cells³. Primary AGMK tube cultures were inoculated with adenovirus 7 only and with adenovirus 7 plus SV 40 simultaneously using methods previously described^{1,2}. The tubes were incubated at 37°C on a roller drum for 72 h, frozen and thawed three times, and titrated. Haemagglutinin titres for rhesus monkey erythrocytes were determined using a micro-titre method³. To two-fold serial dilutions of 0.025 ml. tissue culture fluid, were added 0.025 ml. saline and 0.025 ml. 1 per cent erythrocytes in each micro-titre well. The titre plates were incubated for 1 h at 37°C and the end-point was read as the last dilution (of the original two-fold) in which a definite agglutination pattern was obtained. All experiments were performed in duplicate, and haemagglutination observations of each tube also were performed in duplicate. The quadruplicate haemagglutination results were the same for all titrations in this investigation. Infectivity titrations were carried out in roller tube cultures of primary human embryo kidney cells. Identical cultures with single and double infection were fixed, sectioned and examined with the electron microscope¹. The results are summarized in Table 1.

Table 1. INFECTION OF AFRICAN GREEN MONKEY KIDNEY CELL CULTURES WITH ADENOVIRUS 7 ONLY AND ADENOVIRUS 7 PLUS SV 40

Inoculum	Haemagglutinin titre*	Cells with adenovirus particles by electron microscopy*	Infectivity titre*
Adenovirus 7 (10 ^{5.2} TCID ₅₀)	0	0	10 ^{6.2} TCID ₅₀ /ml.
Adenovirus 7 (10 ^{5.2} TCID ₅₀) plus SV 40 (10 ^{6.4} TCID ₅₀)	1:32	20 per cent	10 ^{7.9} TCID ₅₀ /ml.

* 72 h after inoculation.

Although approximately 20 per cent of the cells examined electron-microscopically had intranuclear adenovirus particles, in a few cells there was morphological evidence of mixed infection with SV 40 particles and adenovirus particles within the same nucleus. The close association of the two viruses provides a morphological basis for the serological evidence of 'hybridization' of adenovirus 7 and SV 40 presented by Huebner *et al.*⁴.

Our findings show that the development of adenovirus haemagglutinin as well as mature infectious virus particles is enhanced by mixed infection with SV 40. This is consistent with the work of Brandon and his associates⁵, who report good evidence that haemagglutinin is an intrinsic part of the infective particle. In their experiments adenovirus 3 was adsorbed and eluted on monkey erythro-

cytes through three cycles, and more than 90 per cent of the infective particles accompanied the haemagglutinin. Thus, our investigation has provided additional affirmative evidence that adenovirus growth is enhanced by SV 40. Moreover, the quantitative haemagglutination technique is much less cumbersome for determining this phenomenon than infectivity titrations or electron microscopy.

RICHARD K. WERTZ

CYNTHIA C. O'CONNOR

Clinical Pathology Department,

ALAN S. RABSON

GREGORY T. O'CONNOR

Pathologic Anatomy Department,
National Institutes of Health,
Bethesda, Maryland.

¹ O'Connor, G. F., Rabson, A. S., Berezsky, I. K., and Paul, F. J., *J. Nat. Cancer Inst.*, **31**, 903 (1963).

² Rabson, A. S., O'Connor, G. F., Berezsky, I. K., and Paul, F. J., *Proc. Soc. Exp. Biol. and Med.*, **116**, 187 (1964).

³ Sever, J. L., *J. Immunol.*, **88**, 320 (1962).

⁴ Huebner, R. J., Chanock, R. M., Rubin, E. A., and Casey, M. J., *Proc. U.S. Nat. Acad. Sci.* (in the press).

⁵ Brandon, F. B., and McLean, I. W., jun., *Adv. Virus Res.*, **9**, 157 (1962).

CYTOLOGY

Isolation of Macronuclei from the Ciliate *Tetrahymena pyriformis* GL

SEVERAL different non-ionic detergents have recently been used for the isolation of nuclei from mammalian cells¹⁻⁵. One of these detergents, 'Triton X-100', was found to solubilize readily cytoplasmic components, without affecting the structural integrity (at the light-microscope level) of nuclei from rat liver cells and mouse plasma cell tumours⁵. 'Triton'-isolated nuclei contained more than 90 per cent of the DNA in the original cell homogenate, retained rapidly labelled RNA and NAD-pyrophosphorylase activity⁵. The detergent digitonin has been used with some success for the isolation of macronuclei from stationary phase *Tetrahymena pyriformis*, mating type II, var. 1 (ref. 6).

This communication describes a simple method for the isolation of macronuclei from the ciliate *Tetrahymena* after cell lysis in 'Triton X-100'.

Growth conditions for *T. pyriformis* GL and the method for collecting cells were the same as reported earlier⁷. Macronuclei were isolated from organisms in the following growth stages: (1) exponential multiplication at the optimum temperature for growth (29°C); (2) exponential cultures incubated for 2 h at 34°C; and (3) maximum stationary phase. Brief periods of incubation at 34°C were also used to induce synchronized cell division^{8,9}.

The gathered organisms were washed once at room temperature in a diluted Ringer's sodium phosphate (RSP) buffer (final concentration: 0.047 M sodium chloride, 0.002 M potassium chloride, 0.001 M magnesium sulphate, 0.0125 M sodium phosphate buffer, pH 7.3) and resuspended in the RSP-buffer to give a final density not exceeding 4×10^6 cells/ml. The suspension was gently shaken to avoid cell aggregation. From this suspension, 100 ml. was removed with a plastic pipette in 10-ml. portions and slowly added to 100 ml. of medium A (0.5 per cent v/v 'Triton X-100' and 0.5 M sucrose in RSP) in a 500-ml. Erlenmeyer flask kept in crushed ice. An L-shaped glass rod (2 × 27 cm, area of blade approximately 15 mm²) rotating at 200 r.p.m. in medium A was used for mixing. Five min after addition of the last aliquot of the cell suspension to medium A, almost all cells were completely lysed. From this time on, 200 ml. of medium B (2 per cent w/v proteose peptone, Difco, and 0.1 per cent w/v liver fraction L, Wilson Laboratories, in distilled water) were slowly added to the mixture while stirring continued.

Frequent examination of samples under a phase-contrast microscope showed that lysis in medium A started at the anterior part of the cell and swept along the long

axis to the posterior part, releasing cytoplasmic particles and the macronuclei. Medium B, a part of the growth medium of *Tetrahymena*, was found to stabilize the nuclei and to prevent their clumping and attachment to cytoplasmic fragments. More than 95 per cent of the nuclei were observed intact and their surface was found free from cytoplasmic contamination (by phase contrast microscopy). Such successful lysis could be obtained only with stationary-phase and heat treated cells. Less than 50 per cent of log-phase cells could be lysed by this method.

The lysed cell suspension was slowly passed through a cotton filter at room temperature, but collected in an ice bath. The filter was prepared by taking sterilized absorbent cotton (4-oz. packages, Parke, Davis and Co.), covering it on both sides with cheese cloth and cutting 6 x 6 in. squares, which were put into the bottom part of a polyethylene Buchner funnel (diam. 11 cm). The filtrate was then layered in 40-ml. portions on top of the following two layers in a 90-ml. round bottom, plastic centrifuge tube: bottom, 10 ml. of 1 M sucrose in RSP; intermediate layer, 30 ml. 2 per cent (v/v) 'Triton X-100' and 0.5 M sucrose in RSP. Centrifugation (PR-2 International Centrifuge, head No. 240) was carried out in three 5-min steps at 70, 250 and 800g. Deceleration was manually controlled to avoid mixing of the three layers. Mitochondria and small cell particles remained in the upper layer, inorganic particles (resistant to digestion in 1 N acid or alkali at 25° C) were found in the middle layer, while most nuclei formed a creamy membranous pellet at the bottom of the tube. The supernate was rapidly removed by suction, except 5 ml. covering the pellet. The remaining fluid was poured out gently and 1 ml. 1 M sucrose was then added to the pellet and repeatedly sucked back gently into a plastic pipette until the material of the pellet was in homogeneous suspension. This suspension was used for phase contrast microscopy and further purification in the following way. An equal amount of 2 M sucrose in RSP buffer was added to the suspension, carefully mixed, and a 2-ml. portion was layered over 2 ml. of 1.75 M sucrose RSP buffer in a 5-ml. cellulose tube and centrifuged for 20 min at 10,000 r.p.m. in an SW-rotor of the Spinco ultracentrifuge (model L). After decantation of the supernate, 1 ml. of 0.5 M sucrose was added to the gelatinous pellet and the nuclei were resuspended by gently pipetting as already described.

Phase contrast photomicrographs of macronuclei prior to the final purification step are shown in Fig. 1A. This fraction was found to be contaminated with small particles (diam. about 5 μ). These particles are virtually all removed by ultracentrifugation during the last purification. Examination of the final nuclear fractions in the electron microscope also failed to reveal contamination by cytoplasmic structures (Fig. 1B). The structure of these nuclei appears to be identical to the ultrastructure described by Elliott *et al.*¹⁰ in whole cell preparations of

T. pyriformis, mating type I, variety 1. It is of interest to note that the nuclear membrane was found to be present in all preparations examined. Nuclei isolated with 'Triton X-100' from rat liver, however, had lost most of their outer membrane⁶.

A preliminary investigation of DNA isolated from *Tetrahymena* nuclei indicated it to be fibrous and a good primer in an RNA-polymerase assay¹¹.

The work is being continued to adapt the present method to the isolation of nuclei from *Tetrahymena* cells in the exponential growth phase.

This investigation was supported in part by grant RG 06461-05 from the National Institutes of Health, Bethesda, Maryland.

Y. C. LEE

O. H. SCHERBAUM

Department of Zoology,
University of California,
Los Angeles.

¹ Zalta, J. P., Rosencwajg, R., Carasso, N., and Favard, P., *C.R. Acad. Sci., Paris*, **255**, 412 (1962).

² Hubert, M. T., Favard, P., Carasso, N., Rosencwajg, R., and Zalta, J. P., *J. Microsc.*, **1**, 435 (1962).

³ Fisher, H. W., and Harris, H., *Proc. Roy. Soc., B*, **156**, 521 (1962).

⁴ Hymer, W. C., *Fed. Proc.*, **21**, 104 (1963).

⁵ Hymer, W. C., and Kuff, E. L., *J. Histochem. Cytochem.*, **12**, 359 (1964).

⁶ Rosenbaum, J. L., Ph.D. thesis, Syracuse University, New York (1964).

⁷ Scherbaum, O. H., James, T. W., and Jahn, T. L., *J. Cell Comp. Physiol.*, **53**, 119 (1959).

⁸ Scherbaum, O. H., and Zeuthen, E., *Exp. Cell Res.*, **6**, 221 (1954).

⁹ Zeuthen, E., and Scherbaum, O. H., in *Recent Developments in Cell Physiology*, edit. by Kitching, J. A., 141 (Butterworth's Scientific Publications, London, 1954).

¹⁰ Elliott, A. M., Kennedy, J. R., and Bak, I. J., *J. Cell Biol.*, **12**, 515 (1962).

¹¹ Lazarus, L., and Scherbaum, O. H. (to be published).

GENETICS

Aggressive Behaviour, Mental Sub-normality and the XYY Male

It is well known that 1 per cent of males in institutions for the mentally sub-normal are chromatin-positive and that the majority of these have an XXY sex chromosome constitution¹. In 1963 Forssman and Lambert² reported on a survey of the nuclear sex of 760 male patients in three Swedish institutions for criminal and 'hard-to-manage' males of sub-normal intelligence. They found 2 per cent to be chromatin positive, but the chromosome constitution of these patients was not reported. Casey (personal communication) surveyed 942 males in two comparable institutions in England, and found 2.2 per cent of them to be chromatin-positive. He also studied the chromosomes of these 21 chromatin-positive males and found 7 to have either an XXY sex chromosome constitution or to be mosaics in which the XXY cells formed the major cell line. This finding of a third of the chromatin-positive males to have an XXY cell line contrasted sharply with that of Maclean and his colleagues, who studied 2,607 ordinary mentally sub-normal males, and found only 2 of the 28 chromatin-positive patients to have an XXY constitution. This contrast led us to wonder whether an extra Y chromosome predisposes its carriers to unusually aggressive behaviour. We decided that if this were the case, then we might expect an increased frequency of XYY males among those of a violent nature. The purpose of this communication is to report our findings in a survey of mentally sub-normal male patients with dangerous, violent or criminal propensities in an institution where they are treated under conditions of special security.

In the course of routine physical and haematological examination a blood sample was obtained for chromosome studies. Of

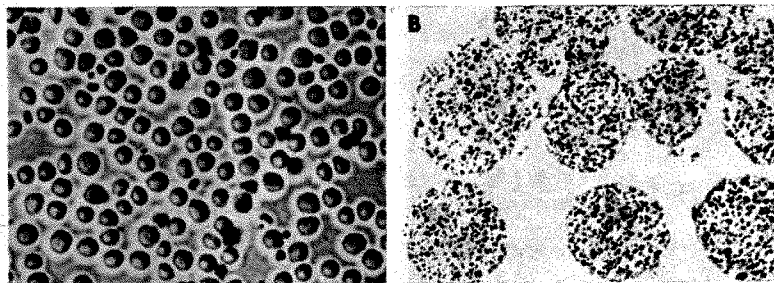


Fig. 1. Macronuclei isolated from *Tetrahymena pyriformis* GL in the stationary phase of growth. A, Phase contrast photomicrograph (scale = 10 μ); B, electron-micrograph. After final purification, the nuclei were resuspended in 0.5 M sucrose Ringer's phosphate buffer and fixed with 6 per cent glutaraldehyde for 30 min. The nuclei were then washed once with phosphate buffer (0.1 M, pH 7.4), post fixed with 1 per cent osmium tetroxide for 30 min, washed three times with distilled water and stained with 2 per cent uranyl acetate for 60 min. After quick dehydration, the samples were embedded in epon resin and sectioned 750 Å thick. A Hitachi HU 11 A electron microscope was used (\times c. 4,000).

the total of 203 patients available, six declined to give a blood sample so that chromosome observations were made on the remaining 197. We found that twelve had a chromosome abnormality; seven had 47 chromosomes and an XYY sex chromosome constitution, one had 48 chromosomes and an XYY sex chromosome constitution, and one was an XY/XXY mosaic. The remaining three had structural abnormalities of the autosomes; one had 45 chromosomes and a translocation between two chromosomes in group 13-15, one had 46 chromosomes with a reciprocal translocation between an autosome number one and an autosome number 16, and one had an apparent deletion of part of the long arm of an autosome in group 21-22.

The most important finding in this group is that eight individuals have an additional Y chromosome. Very little is known about the XYY male, as only a few cases have so far been described, and these refer to a heterogeneous group of children and adults from whose description no clear picture of the XYY male has emerged³⁻⁷. However, the finding that 3.5 per cent of the population we studied were XYY males must represent a marked increase in frequency by comparison with the frequency of such males at birth. On theoretical grounds XYY males at birth must be less common than XXY males, who form approximately 0.2 per cent of the new-born male population⁸. We have examined 266 randomly selected new-born male babies without finding an XYY individual, and we have also examined 209 randomly selected adult males, again without finding one with an XYY constitution. In addition, we have examined the chromosomes of approximately 1,500 males for a variety of reasons and only one of these was found to have an XYY sex chromosome constitution.

Further clinical investigations have yet to be undertaken on these abnormal patients, but it is already apparent that they are unusually tall. Among the 197 men we studied the difference between the mean height of the males with one Y chromosome (67.0 in.) and that of those with two Y chromosomes (73.1 in.) is highly significant ($P < 0.00001$). In fact in this particular group a man more than 72 in. in height has an approximately 1 in 2 chance of having an XYY constitution.

At present it is not clear whether the increased frequency of XYY males found in this institution is related to their aggressive behaviour or to their mental deficiency or to a combination of these factors. We are attempting to elucidate this problem.

We thank Dr. W. M. Court Brown for his help and encouragement, and Mr. Peter Smith for statistical advice.

PATRICIA A. JACOBS
MURIEL BRUNTON
MARIE M. MELVILLE

Medical Research Council,
Clinical Effects of Radiation Research Unit,
Western General Hospital,
Edinburgh, 4.

R. P. BRITAIN
W. F. MCCLEMONT

State Hospital,
Carstairs.

¹ Maclean, N., Mitchell, J. M., Harnden, D. G., Williams, J., Jacobs, P. A., Buckton, K. E., Baikie, A. G., Court Brown, W. M., McBride, J. A., Strong, J. A., Close, H. G., and Jones, D. C., *Lancet*, i, 293 (1962).

² Forsman, H., and Hambert, G., *Lancet*, i, 1327 (1963).

³ Hauschka, T. S., Hasson, J. E., Goldstein, M. N., Koepf, G. F., and Sandberg, A. A., *Amer. J. Hum. Genet.*, 14, 22 (1962).

⁴ Fraccaro, M., Glen Bott, M., Davies, P., and Schutt, W., *Folia Hered. Path.*, 11, 211 (1962).

⁵ Sandberg, A. A., Ishihara, T., Crosswhite, L. H., and Koepf, G. F., *New England J. Med.*, 268, 585 (1963).

⁶ Court Brown, W. M., Harnden, D. G., Jacobs, P. A., Maclean, N., and Mantle, D. J., Privy Council, *M.R.C. Special Report Series*, No. 305, H.M.S.O. (1964).

⁷ Townes, P. L., Ziegler, N. A., and Lenhard, L. W., *Lancet*, i, 1041 (1965).

⁸ Maclean, N., Harnden, D. G., Court Brown, W. M., Bond, J., and Mantle, D. J., *Lancet*, i, 286 (1964).

PSYCHOLOGY

Tactile-vision: Thermal and Texture Cues in the Discrimination of Black and White

TEMPERATURE and texture have both been suggested as possible normal explanations of the 'skin-vision' phenomenon¹. Neither of them explains Russian claims of perception at a distance (claims not supported by Barrett and Rice-Evans² using a low level of illumination); however, they do provide potential cues in situations where touch is involved. Colourants may give surfaces with characteristic textures and the colour of a surface will affect the amount of heat it absorbs. Recently, Buckhout³ found that, even with illumination provided by a 40-W bulb (a good source of infra-red radiation), out of 80 subjects not one could distinguish between 10 colours in 90 presentations. He controlled texture by covering the colours with a thin layer of plastic. In the experiment reported here I investigated what part both texture and temperature might play in a very simple situation: that of a black-white discrimination.

The apparatus used was a 9 in. × 7 in. × 20 in. light-proof box comprising two compartments separated by a sheet of frosted glass. In one was a 60-W lamp and in the other a stack of 72 stimulus cards: 24 each of black, white, and half black and half white photographs of size 2.5 in. × 3.5 in. With one hand inside the box the subject went through the pack of cards, feeling each in turn and then guessing its type. After each guess he posted the card out through a chute in the side of the box, providing himself with knowledge of results. The subjects were given two sessions: one with the light on and one with it off. They were not aware of this difference and the order of the two conditions was alternated between them. The cards were shuffled in between sessions.

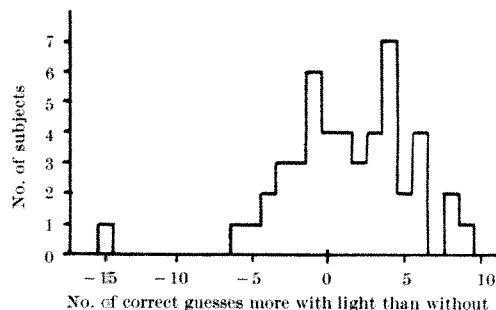


Fig. 1

Forty-eight students were tested. The mean number of correct guesses expected by chance is 24/72 for each condition. The observed means were 25.3 for light on and 24.2 for light off. Clearly texture was not used as a cue. Subtracting the dark from their corresponding light scores gave the results shown in Fig. 1. A Wilcoxon matched-pairs signed-ranks test gave a significance of $P = 0.014$, which can be seen to be due not to a few high scorers but rather to the group as a whole: discriminative ability being spread thin among the subjects. Under the hypothesis black and white is the easiest colour discrimination task, and yet subjects score only one or two correct guesses more than is expected by chance. It remains possible that with a lengthy series of trials this skill could be developed: subjects often reported holding a number of different hypotheses one after the other, and it might be that here they find difficulty in selecting the relevant aspects of their sensations.

C. N. FRENCH

Department of Psychology,
University of Aberdeen.

¹ Liddle, D., *Discovery*, 25, 22 (1964).

² Barrett, S. M., and Rice-Evans, P., *Nature*, 203, 993 (1964).

³ Buckhout, R., *Percept. Mot. Skills*, 20, 191 (1965).

FORTHCOMING EVENTS

Tuesday, December 28

INSTITUTE OF BIOLOGY (in the Lecture Hall of the Natural History Museum, Cromwell Road, London, S.W.7), at 3 p.m.—Dr. N. E. Hickin. "Being a Biologist" (Christmas Lecture for Children).

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 3 p.m.—Sir Bernard Lovell, O.B.E., F.R.S., Prof. F. Graham Smith, Prof. Martin Ryle, F.R.S., and Dr. A. Hewish. "The Exploration of the Universe". (The One Hundred and Thirty-Sixth Course of Six Lectures Adapted to a Juvenile Auditory. Further Lectures on December 30, January 1, 4, 6 and 8.)

Wednesday, December 29

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 2.30 p.m.—Dr. J. K. S. Joseph. "Discovering Britain's Past from the Air" (Christmas Lecture for Children).

Monday, January 3

PLASTICS INSTITUTE (at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 2.30 p.m.—Mr. J. E. Proctor. Young People's Lecture.

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 3 p.m.—Sir Dudley Stamp, C.B.E. "Philatelic Cartography".

INSTITUTION OF MECHANICAL ENGINEERS, MEDICAL ENGINEERING WORKING PARTY (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Informal Discussion on "Human Co-ordination and Engineering Control".

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. A. P. Prosser. "The Chemistry of Mineral Processing".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned

LECTURER IN THE DEPARTMENT OF PATHOLOGY—The Professor of Pathology, Department of Pathology, University of Edinburgh Medical School, Teviot Place, Edinburgh, 8 (December 31).

LECTURER or ASSISTANT LECTURER IN PURE MATHEMATICS—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (December 31).

LECTURER (with an Honours B.Sc. degree in biochemistry or equivalent qualification) in DENTAL BIOCHEMISTRY—The Secretary of the University Court, The University, Glasgow (December 31).

PROFESSOR OF SOCIOLOGY at the University of New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, December 31).

SENIOR LECTURER IN MATHEMATICS; a LECTURER or ASSISTANT LECTURER IN MATHEMATICS; a LECTURER or ASSISTANT LECTURER IN STATISTICS, a LECTURER or ASSISTANT LECTURER IN OPERATIONAL RESEARCH, and a LECTURER or ASSISTANT LECTURER IN NUMERICAL ANALYSIS—The Registrar, Bradford Institute of Technology, Bradford, 7 (December 31).

ASSISTANT LECTURER IN SOCIAL PSYCHOLOGY—The Registrar, The University, Nottingham (January 3).

DIRECTOR (highly qualified scientifically with an aptitude for administration and negotiation with public bodies and others) OF THE NATURE CONSERVANCY at the London Headquarters, Belgrave Square, S.W.1—The Natural Environment Research Council, Establishments Division, State House, High Holborn, London, W.C.1 (January 7).

PROGRAMMER (with a degree or equivalent qualification, at least two years programming experience, and preferably a knowledge of Algol, Fortran or Elliott 803 machine code) in THE COMPUTING LABORATORY, to help in the provision of a computing service, by advising on the use of programmes and writing new programmes—The Registrar, University College of North Wales, Bangor, North Wales (January 7).

DEMONSTRATOR (graduate qualified in palaeontology, and preferably with at least an honours degree) in GEOLOGY at the University of Newcastle, New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (January 8).

LECTURER or ASSISTANT LECTURER in THE PHILOSOPHY OF SCIENCE in St. Salvator's College—The Secretary, University of St. Andrews, College Gate, St. Andrews, Fife, Scotland (January 8).

LECTURER (with a distinguished academic record in mathematics, and preferably with interests in a branch of applied mathematics and some experience in computing) in MATHEMATICS at Wollongong University College, University of New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 10).

WILLIAM JULIEN COURTAULD CHAIR OF HELMINTHOLOGY at the London School of Hygiene and Tropical Medicine—The Academic Registrar, University of London, Senate House, London, W.C.1 (January 10).

LECTURER (Grade II) in METALLURGY within the DEPARTMENT OF MECHANICAL ENGINEERING—The Registrar and Secretary, The University of Bristol, Bristol (January 12).

ASSISTANT LECTURER or LECTURER (preferably with qualifications in the philosophy of science) in the DEPARTMENT OF PHILOSOPHY—The Registrar, The University, Hull (January 14).

CHEMIST (with a degree or equivalent qualification) to assist in work on crop nutrition, especially with analysis of plants and soils from field experiments—Head, Broom's Barn Experimental Station, Higham, Bury St. Edmunds, Suffolk (January 14).

ASSISTANT LECTURER or LECTURER (registered member of the veterinary profession, preferably with previous experience in pathology) in VETERINARY PATHOLOGY, Royal (Dick) School of Veterinary Studies—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (January 15).

LECTURER in HYDROLOGY in the DEPARTMENT OF FORESTRY and NATURAL RESOURCES—The Secretary, The University, Edinburgh (January 15).

LECTURER or ASSISTANT LECTURER in BOTANY at the University of Singapore—The Inter-University Council, 33 Bedford Place, London, W.C.1 (January 15).

LECTURER (with a higher degree, and preferably previous university teaching experience) in GEOGRAPHY at the University of Newcastle, New South

Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 15).

ASSISTANT LECTURER or LECTURER (qualified to teach special aspects of either Russian or Latin American sociology in addition to other fields of sociology) in SOCIOLOGY—The Registrar, University of Essex, Wivenhoe Park, Colchester, Essex (January 17).

CHAIR OF STATISTICS—The Secretary of the University Court, University of Glasgow, Glasgow (January 17).

LECTURERS in GEOLOGY, a LECTURER in BIOLOGY; a SENIOR LECTURER in CHEMISTRY, an ASSOCIATE PROFESSOR/SENIOR LECTURER in PSYCHOLOGY; a SENIOR LECTURER/LECTURER in PHILOSOPHY, a SENIOR LECTURER/LECTURER in PHYSICS; and a SENIOR LECTURER/LECTURER in MATHEMATICS at Macquarie University, Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1; or The Registrar, Macquarie University, P.O. Box 458, North Sydney, Australia (January 17).

SENIOR LECTURER or READER in SOCIOLOGY, with special responsibility for mathematical sociology—The Registrar, University of Essex, Wivenhoe Park, Colchester, Essex (January 17).

SENIOR LECTURER in the DEPARTMENT OF PATHOLOGY—The Registrar, The University, Sheffield (January 22).

LECTURER (with a superior academic record in mechanical, civil or electrical engineering) in ENGINEERING at the University of New South Wales, Broken Hill Division—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 24).

ASSISTANT LECTURER (with interests in genetics or cytology) in BOTANY—The Registrar, The University, Hull (January 25).

BIOCHEMIST (with M.Sc. or Ph.D. in biochemistry or physiological chemistry or equivalent, and at least three years experience of biochemistry or clinical pathology) for the Cork Health Authority—The Secretary, Local Appointments Commission, 45 Upper O'Connell Street, Dublin 1, Republic of Ireland (January 25).

RESEARCH ASSISTANT in the GEOGRAPHY of the MIDDLE EAST—The Registrar and Secretary, University of Durham, Old Shire Hall, Durham (January 28).

CHAIR of MATHEMATICAL STATISTICS—The Registrar, The University, Hull (January 28).

LECTURER in VETERINARY PHYSIOLOGY at the University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 28).

PROFESSOR of the ARCHAEOLOGY of the ROMAN EMPIRE—The Registrar, University Registry, Oxford (January 29).

ASSISTANT LECTURER in the DEPARTMENT of ZOOLOGY (duties will include teaching in the field of vertebrate zoology)—The Secretary, University College, Gower Street, London, W.C.1 (January 31).

EMBRYOLOGIST (scientist with research experience in embryology or developmental genetics)—The Director, Poultry Research Centre A.R.C., West Mains Road, Edinburgh, 9 (January 31).

JAMES WATT CHAIR of MECHANICAL ENGINEERING—The Secretary of the University Court, The University, Glasgow (January 31).

LECTURER in NUCLEAR PHYSICS, and a LECTURER in THEORETICAL PHYSICS—The Secretary, The University of Lancaster, Bailrigg House, Lancaster (January 31).

LECTURER in PURE MATHEMATICS—The Secretary, The Queen's University, Belfast, Northern Ireland (January 31).

LECTURER (with a good honours degree in engineering or an equivalent qualification, and preferably some industrial experience) in the STRENGTH of MATERIALS SECTION of the DEPARTMENT of MECHANICAL ENGINEERING—The Registrar, University of Strathclyde, George Street, Glasgow, C.1 (January 31).

SENIOR LECTURER in MECHANICAL ENGINEERING—The Secretary, The Queen's University, Belfast Northern Ireland (January 31).

SENIOR LECTURER in METEOROLOGY in the DEPARTMENT of MATHEMATICS, Victoria University of Wellington, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, January 31).

CHAIR of GEOLOGY and MINERALOGY—Secretary to the College, Trinity College (University of Dublin), Dublin, Republic of Ireland (February 1).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS in VARIOUS BRANCHES of CHEMISTRY, including INORGANIC CHEMISTRY, CHEMICAL ENGINEERING, ENGINEERING, TECHNOLOGY, PHYSICS and ALLIED SUBJECTS—The Registrar, The University, Manchester, 13 (February 14).

TURNER and NEWALL RESEARCH FELLOWS in ENGINEERING, INORGANIC CHEMISTRY, PHYSICS and ALLIED SUBJECTS—The Registrar, The University, Manchester, 13 (February 14).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS (preferably with the degree of Ph.D. or equivalent research experience) in APPLIED MATHEMATICS, BIOCHEMISTRY, ENGINEERING CHEMISTRY, PHARMACOLOGY; PHYSICS or CHEMICAL ENGINEERING—The Secretary, The University, Edinburgh (February 15).

CHAIR of PURE MATHEMATICS at the University of Otago, Dunedin, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1; or The Registrar of the University (New Zealand and London, February 28).

LECTURER (with a degree in pure science or agricultural science) in ENTOMOLOGY at Lincoln College University of Canterbury, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, March 31).

POST-DOCTORAL RESEARCH FELLOW in TREE PHYSIOLOGY in the SCHOOL of FORESTRY, University of Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, March 31).

BIOCHEMIST (Basic Grade) to work with and assist the Senior Biochemist—The Secretary, City General Hospital, Stoke-on-Trent, Staffordshire

POSTGRADUATE RESEARCH ASSISTANT in the DEPARTMENT of BOTANY for work on British ashwoods—The Registrar, The University of Sheffield, Sheffield.

RESEARCH ASSISTANT (pharmacology, physiology or zoology graduate) for comparative work on the control of heat production in newborn and adult animals—Dr. R. E. Moore, Department of Physiology, Royal Free Hospital School of Medicine, 8 Hunter Street, London, W.C.1.

RESEARCH ASSISTANT (preferably with a degree in agriculture, agricultural botany, botany or equivalent qualifications) in the POTATO BRANCH to assist in investigations into the effects of herbicides on varieties of potatoes—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge.

SCIENTIFIC INFORMATION OFFICER (with a degree in agriculture or related subjects and a flair for languages, including English) to join a team engaged in publishing the latest research findings on temperate and tropical grasslands

and annual field crops in two journals of international repute—The Director, Commonwealth Bureau of Pastures and Field Crops, Hurley, near Maidenhead, Berks.

TECHNICAL OFFICER FOR HERBAGE SEED CROPS, and a TECHNICAL OFFICER FOR CEREAL SEED CROPS (candidates should have a sound basic training in agriculture and possess a recognized diploma)—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

University of Oxford. Lord Nuffield's Benefaction for the Advancement of Medicine—Report for 1963-64. Pp. 28. (Supplement No. 1 to the *University Gazette*, Vol. 96, October 1965.) 2s. 6d. Report of the Committee on the Disciplinary Powers of the Vice-Chancellor and Proctors. Pp. 9 (Supplement No. 2 to the *University Gazette*, Vol. 96, October 1965.) 2s. 6d. (Oxford: The University, 1965.) [311]

Water Resources Board. Estimating Frequencies Using Probability Paper. By R. B. W. Bannerman. Pp. 17. (T.N.4) (Reading: Water Resources Board, 1965.) [311]

Column, Vol. 1, No. 1 (Autumn 1965). Edited by D. Barnes (The W. G. Pye Journal of Gas Chromatography) Pp. 8. (Cambridge: W. G. Pye and Co. Ltd., 1965.) Gratic. [311]

Space, No. 2 (May 1965). Edited by Miles Henslow. Pp. 49-96. Subscription rate for 12 numbers: 40s.; 6 dollars. (London: Cockatrice Press, Ltd., 1965.) [311]

Ministry of Technology. Forest Products Research Laboratory Leaflet No. 47: The Movement of Tumbers. Pp. 7. (London: H.M. Stationery Office, 1965.) 9d. net. [311]

The World Land Use Survey. Occasional Papers No. 3: Land Use Statistics of the Countries of Europe. By Sir Dudley Stamp. Pp. vi+42. (Bude, Cornwall: Geographical Publications, Ltd., 1965.) 10s. [311]

Planning, Vol. 31, No. 491 (October 1965): Reforming the Commons. By Members of the Study of Parliament Group. Pp. 287-312. 6s. *Planning*, Vol. 31, No. 492 (November 1965) Public Patronage of the Arts. By John Higgins. Pp. 313-332. 5s. (London: Political and Economic Planning, 1965.) [311]

The Royal Society. Provisional United Kingdom Contribution to Phase-Of the International Biological Programme. Prepared by British National Committee for the International Biological Programme. Pp. 65. (London: The Royal Society, 1965.) [311]

Report of the Agricultural Research Council for the years 1963-65. (Cmd. 2792) Pp. vi+121. (London: H.M. Stationery Office, 1965.) 8s. net. [311]

University of Birmingham: Faculty of Science and Engineering. Postgraduate Prospectus 1966. Pp. 75+8 photographs. (Birmingham: University of Birmingham, 1965.) [311]

Bulletins of Marine Ecology, Vol. 6, Part 5 Continuous Plankton Records Atlantic and the North Sea. By Dr. J. M. Colebrook and G. A. Robinson. Continuous Plankton Records—Contribution Towards a Plankton Atlas of the North Atlantic and the North Sea. Part 10: *Navicula planamembranacea* Hendey. By G. A. Robinson. Pp. 123-145+plates 62-66. (Edinburgh: The Scottish Marine Biological Association, 1965.) 11s. 6d. [311]

University of Oxford. Oration by the Vice-Chancellor and Annual Report, 1964-1965. Pp. 37-56. (Supplement to *Oxford University Gazette*, No. 3249.) (Oxford: The University, 1965.) 2s. [311]

Building Research Station Digest (Second Series), No. 63. Soils and Foundations—I. Pp. 6. (London: H.M. Stationery Office, 1965.) 4d. [311]

Ministry of Housing and Local Government. River Wye Basin: Hydrological Survey (Hydrometric Area, No. 55). Pp. v+57. (London: H.M. Stationery Office, 1965.) 12s. 6d. net. [311]

Philosophical Transactions of the Royal Society of London. Series A: Mathematical and Physical Sciences. No. 1088, Vol. 258 (28 October 1965): A Symposium on Continental Drift. Organized for the Royal Society by Prof. P. M. S. Blackett, F.R.S., Sir Edward Bullard, F.R.S., and Prof. S. K. Runcorn, F.R.S. Pp. xi+1-323+A1-A6 140s.; 21 dollars. Series B: Biological Sciences. No. 759, Vol. 249 (7 October 1965): The Biological Report of the Royal Society Expedition to Tristan da Cunha, 1962. By D. B. Baird, J. H. Dickson, M. W. Holdgate, and N. M. Wace. Pp. 257-434+plates 32-47. 85s.; 12.75 dollars. No. 760, Vol. 249 (7 October 1965) Evidence for a Dual Control, by Neurosecretion, of Hormone Synthesis and Hormone Release in the Pituitary of the Dogfish *Scyliorhinus stellaris*. By Sir Francis Knowles. Pp. 435-456+plates 48-59. 26s.; 3.90 dollars. (London: The Royal Society, 1965.) [311]

New Materials and Processes in Instrument Manufacture. Edited by P. J. Geary. (Proceedings of the SIRA Conference, Eastbourne, England, 13-14 May 1965.) Pp. 108. (Chislehurst, Kent: British Scientific Instrument Research Association, 1965.) 80s.; 12 dollars. [311]

Royal Observatory Bulletin, No. 99: Studies of the Globular Cluster ω Centauri. II. Radial Velocities of Bright Members. By G. A. Harding. Pp. E65-E78+2 plates. (London: H.M. Stationery Office, 1965.) 3s. net. [311]

National Foundation for Educational Research in England and Wales. Nineteenth Annual Report, 1964-1965. Pp. 55. (Slough: National Foundation for Educational Research in England and Wales, 1965.) [411]

Report of the Medical Research Council, October 1963-March 1965. (Cmd. 2787) Pp. ix+326+4 plates. (London: H.M. Stationery Office, 1965.) 20s. net. [411]

General Register Office. The Registrar General's Quarterly Return for England and Wales—Births, Deaths and Marriages, Infectious Diseases, Weather; Population Estimates, Quarter ended 30th June 1965. (No. 466, 2nd Quarter 1965) Pp. 40. (London: H.M. Stationery Office, 1965.) 2s. 6d. net. [411]

Imperial College of Science and Technology (University of London). Propulsion Without Wheels. By Prof. E. R. Laithwaite (Inaugural Lecture, 16 February, 1965.) Pp. 95-107. (London: Imperial College of Science and Technology, 1965.) 5s. [411]

Other Countries

Commonwealth of Australia. Department of External Affairs. Australian National Antarctic Research Expeditions. ANARE Interim Reports. Series A (4): Glaciology. Publication No. 79: The Photoelectric Metering of Wind-Blown Snow. By I. H. Landon Smith and B. Woodberry. A New Photoelectric Drift Snow Gauge. By E. R. Wishart. Pp. 26. (Melbourne: Antarctic Division, Department of External Affairs, 1965.) [511]

Canada. Department of Mines and Technical Surveys. Geological Survey of Canada. Paper 64-39: Groundwater Hydrology and Water Supply in the District of Mackenzie, Yukon Territory, and Adjoining Parts of British

Columbia. By L. V. Brandon. Pp. vii+102 (3 plates). 75 cents. Paper 64-43: The Design of a Spinner-Type Remanent Magnetometer. By A. Laroche. Pp. v+25+plate 1. 35 cents. Paper 65-4: Geological Survey of Canada—Radiocarbon Dates IV. By W. Dyck, J. G. Fyles and W. Blake Jr. Pp. 23. 35 cents. Paper 65-10: Rocks and Minerals for the Collector—Northeastern Nova Scotia, Cape Breton, and Prince Edward Island. By Ann P. Sabina. Pp. vii+76 (6 plates). 75 cents. Paper 65-15: A Depositional Study of the Harbour Seam, Sydney Coalfield, Nova Scotia. By P. A. Hachebard, A. R. Cameron and J. R. Donaldson. Pp. iv+81. 75 cents. Paper 65-16: Structural Analysis of Three Extension Faults Number 4 Mine, Canmore, Alberta. By D. K. Norris. Pp. iii+11. 75 cents. Paper 65-18: Field and Laboratory Methods Used by the Geological Survey of Canada in Geochemical Surveys. No. 8. Preparation of Geological Materials for Chemical and Spectrographic Analysis. By P. J. Lavery. Pp. iv+23 (6 plates). 75 cents. (Ottawa: Queen's Printer, 1965.) [511]

Merentutkimuslaitoksen Julkaisu. Havforskningsinstituttet Skrift. No. 208 The Gulf of Bothnia in Winter Time. I. Data from Winter Cruises 1959-63. Pp. 91. No. 214 The Baltic Cruises with R/V *Aranda* 1961-Hydrographical Data. Pp. vii+68. (Helsinki: Merentutkimuslaitoksen Havforskningsinstituttet, 1965.) [511]

South Africa Council for Scientific and Industrial Research. C.S.I.R. Research Report No. 208: Principles of Mass Housing. (Based on a series of lectures on housing delivered to post-graduate students in Town and Regional Planning, 1962) Pp. xx+118. C.S.I.R. Research Report 207: Technical High Schools. Recommendations of the Vocational School Buildings Committee. Pp. viii+67. (Pretoria: Council for Scientific and Industrial Research, 1965.) [511]

World Meteorological Organization. Technical Note No. 68: Meteorological Aspects of Atmospheric Radioactivity. (Papers presented by WMO experts during the 13th Session of the United Nations Scientific Committee on the Effects of Atomic Radiation) Pp. 194. (WMO-No. 169 TP.83) (Geneva: World Meteorological Organization, 1965.) 18 Sw. francs. [511]

Bibliography of Vitamin E, Vol. 6: 1960-1964. Compiled by Wilma F. Kujawski. Pp. 166. (Rochester, New York: Distillation Products Industries, 1965.) [511]

Australia. Commonwealth Scientific and Industrial Research Organization Soil Publication No. 22: Soil Development Associated with Stranded Beach Ridges in South-East South Australia. By G. Blackburn, R. D. Bond and A. R. P. Clarke. With an Appendix by N. H. Ludbrook. Pp. 66+4 maps. (Melbourne: Commonwealth Scientific and Industrial Research Organization, 1965.) [511]

South Australia. Institute of Medical and Veterinary Science. 26th Annual Report of the Council, July 1963-June 1964. Pp. 83. (Adelaide: Government Printer, 1965.) [511]

National Museum of Canada. Natural History Papers, No. 30 (August 18, 1965). New Restoration of Hadrosaurian Dinosaur. By C. M. Sternberg. Pp. 5. Anthropology Papers, No. 10: Preliminary Excavations at a Cobble Tool Site (D.R. 7) in the Fraser Canyon, British Columbia. By Donald H. Mitchell. Pp. 20+11 plates. (Ottawa: Queen's Printer, 1965.) [511]

Sandia Corporation. Fallout Report Beta-Gamma Radioactivity in Air at Sandia Laboratory. January through June 1965. By R. E. Womelsdoff. Pp. 12. (Albuquerque, New Mexico: Sandia Corporation, 1965. Available from the Clearinghouse for Federal Scientific and Technical Information National Bureau of Standards, U.S. Department of Commerce, Springfield, Virginia.) [511]

Institut Royal Météorologique de Belgique. Annuaire-Rayonnement Solaire, 1964. Pp. vi+60. Bulletin Mensuel—Observations Ionosphériques Août 1965. Pp. 26. (Bruxelles: Institut Royal Météorologique de Belgique, 1965.) [511]

United States Department of the Interior. Geological Survey. Geophysical Abstracts, No. 224 (September, 1965). By James W. Clarke, Dorothy B. Vitalano, Virginia S. Neuschel and others. Pp. iii+695-781. (Washington: D.C.: Government Printing Office, 1965.) 35 cents. [511]

United States Department of the Interior. Geological Survey Bulletin 1198-C Bryophytes Associated with Mineral Deposits and Solutions in Alaska. By Hansford T. Shacklette. Pp. iii+18. 15 cents. Bulletin 1201-B Quaternary Geology of the Mount Chamberlain Area, Brooks Range, Alaska. By G. W. Holmes and Charles R. Lewis. Pp. iv+32+plate 1. Professional Paper 399-A: Geology of Ishigaki-shima, Ryukyu-retto. By Helen L. Foster. Pp. vi+119+plates 1-4. Professional Paper 433-C Transport and Dispersion of Labelled Bed Material, North Loup River, Nebraska. By W. W. Sayre and D. W. Hubbell. Pp. vi+48. 45 cents. Professional Paper 450-B: Diabase Sheets of the Taylor Glacier Region, Victoria Land, Antarctica. By Warren Hamilton. Pp. iv+71+plate 1. Professional Paper 470: Geology and Tungsten Mineralization of the Bishop District, California. By Paul C. Bateman. Pp. vii+208+plates 1-11. Professional Paper 476-A: Geology and Uranium Deposits in the Cave Hills Area, Harding County, South Dakota. By G. N. Piripingos, W. A. Chisholm and R. C. Kerpfer. Pp. iv+64+plates 1-4. Professional Paper 492: Thermal Springs of the United States and Other Countries of the World—a Summary. By Gerald A. Waring. Pp. ix+383. 2.75 dollars. Professional Paper 504-A: Glacial Reconnaissance of Sequoia National Park, California. By F. E. Matthes. Pp. x+58+plates 1 and 2. Professional Paper 604-B Postglacial Drainage Evolution and Stream Geometry in the Ontonagon Area, Michigan. By John T. Hack. Pp. iii+40+plate 1. (Washington: D.C.: Government Printing Office, 1965.) [511]

United States Department of the Interior. Geological Survey. Professional Paper 604-C Geology and Petrogenesis of the Island Park Caldera of Rhyolite and Basalt, Eastern Idaho. By Warren Hamilton. Pp. iv+37+plate 1. Professional Paper 525-C Geological Survey Research 1965, Chapter C. Pp. v+219. 2 dollars. (Washington, D.C.: Government Printing Office, 1965.) [511]

Editorial and Publishing Offices of "NATURE"

MACMILLAN (JOURNALS) LIMITED

4 LITTLE ESSEX STREET, LONDON, W.C.2

Telephone Number. Temple Bar 6633. Telegrams. Phusis Lond W.C.2

Annual subscription: Inland £14, Overseas £13 15s., payable in advance, postage paid to any part of the world.

Advertisements only should be addressed to:

T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2

Telephone Number. Holborn 4743

Registered as a newspaper at the General Post Office

Copyright © Macmillan (Journals) Limited, December 25, 1965